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CHAPTER 4

Female Genital System

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PATHOLOGY OF THE GENITAL SYSTEM OF THE NONGRAVID FEMALE

GENERAL INTRODUCTION

Unique challenges presented by the female reproductive system

In no other system does one commonly encounter so great a variety of gross and microscopic changes in "normal" tissues as those that are routinely encountered in reproductive organs in the normal cycling and gravid female. The complex physiologic events that are associated within the estrus cycle, in pregnancy, or during the postpartum periods are accompanied by dramatic tissue changes in the gonads, tubular, and external genitalia, such as marked hyperplasia, atrophy, necrosis, hemorrhage, and tissue invasion. These are changes that pathologists usually associate with pathologic processes. Consequently, when presented with reproductive tissues, an examining pathologist must start with a firm understanding of the normal embryology, anatomy, and reproductive physiology of the species under study. Additionally, the reproductive tract co-opts cytokines, immunologic and inflammatory components for physiologic functions complicating interpretation. Awareness of these is essential.

Remarkable species variations exist, which limits the usefulness of broad generalizations and makes casual extrapolations fraught with error. A good clinical history that includes stage of cycle, prior reproductive status, and supporting endocrinology is especially important; if not provided, it should be aggressively sought!

It must also be appreciated that, to a significant extent, the commercial value of individual animals can depend on the pathologist's report (e.g., endometrial biopsy from a broodmare). Not only do the descriptive and diagnostic sections of the report have to be accurate and precise, clinicians and owners will likely place great weight on the pathologist's interpretation and assessment of clinical significance. This part of the report must be carefully formulated.

Pathology of the reproductive system is unquestionably both fascinating and challenging.

DISORDERS OF SEXUAL DEVELOPMENT

Disorders of sexual development are common and affect all domestic animal species. Most are incidental, of a minor nature, and do not affect reproductive performance. Some are confused with serious disease during clinical examinations (e.g., detection of cysts in or near the ovary during ultrasound examination) and during routine surgery or postmortem examinations. Basic knowledge of the normal process of sexual development, including a full appreciation of the anatomic changes that occur, is essential for the pathologist. The first section of this chapter provides a brief overview of normal sexual development. This is followed by a discussion of frequently encountered lesions and anomalies of the reproductive organs.

Normal sexual differentiation

Knowledge of the essential elements of the developmental biology of the reproductive organs is important for appreciation of the anatomy and histology of the gonads—tubular and external genitalia of the female—and is essential for comprehension and interpretation of anomalies, the phenotype of neoplasms, or the pathogenesis of many diseases.

There are 3 sequential series of events that occur during normal sexual development:

1. The initial establishment of sex chromosomes (at fertilization).
2. The subsequent modeling of embryonic gonadal tissue as ovarian tissue, thereby establishing gonadal type.
3. Controlled, programmed regression and growth of different parts of the indifferent tubular and external genital tissues of the early embryo to form those definitive structures of the normal female reproductive pattern (the female phenotype). This is referred to as the establishment of sex phenotype.

Establishment of sex chromosomes

The determination of sex chromosomes is fixed at the time of fertilization when egg and sperm unite. The expression of genes imposes on the primitive bipotent gonad developmental changes that will result in formation of the definitive gonadal type (ovary or testis), a key step in control of subsequent stages in sexual development.

Each normal embryo will have either XX (in females) or XY (in males) sex chromosomes. Aberrations in the multiple genes of sexual development can lead to a broad variety of developmental anomalies. Knowledge gained through study of spontaneous cases of abnormal sexual development in animals and humans has greatly expanded through work using engineered mutations in mice. However, the precise mechanisms through which genes control each aspect of sexual development are complex and only superficially understood.

When the sex chromosomes are XX, there is no *SRY* and therefore no testis-determining factors (for detailed development of the male, see Vol. 3, Male genital system). The gonads await activation of female specific genes. Upregulation of many genes occurs early in female development and at the same time or before *SRY* is expressed in males. *WNT4* is an important gene in ovarian and therefore female development. It is active in the primitive bipotent gonad and is downregulated in males. *WNT4* upregulates the folistatin *(FST)* gene. Nuclear receptor subfamily 0, group B, member 1 *(NROB1)*, also known as *DAX1*, is an important gene in the development of females, and it is female specific. It is upregulated by *WNT4*, and it is inhibited by *SRY*. It inhibits *SOX9*, the gene that is important in male development. It is not, by itself, an ovary-determining gene, however. Forkhead/winged helix transcription factor 2 *(FOXL2)* is also female specific, like *NROB1* (*DAX1*). In combination with bone morphogenetic protein 2 gene *(BMP2)*, it upregulates *FST* expression.

Establishment of gonad phenotype

Growth and development of the gonads require a complex series of steps. The ovary arises from paired primordial primitive bipotent gonads called the *genital ridges*, located between the mesonephros and attachment of the mesentery. Primordial germ cells migrate to the developing indifferent gonad in vessels of the wall of the yolk sac and the mesentery to the sex cords. *These sex cords go on later to organize as follicles containing the germ cells.*

In a normal XX female embryo, the outer cortical layer of the primitive bipotent gonad becomes the ovary, whereas the medullary region develops as the testis in normal male (XY) embryos. The typical ovotestis has ovary at the periphery and testis in the medulla. *Gonadal* factors control *subsequent differentiation of the rest of the reproductive organs.* Hormones produced by Sertoli cells and interstitial endocrine cells in males act to promote male and inhibit female tubular and external genitalia. Female sexual differentiation does not require products from the fetal ovary, but does require activation of genes such as *WNT4*, *DAX1*, and *SF1*.

Phenotypic sexual development

Female tubular and external genital development progresses in genetic female embryos only in the absence of hormones, whereas in the male, *anti-Müllerian hormone (AMH) and testosterone function to develop male structures.* These hormones influence the regression of female embryonic tubular structures and support the formation of male tubular organs (epididymis, deferent duct, accessory genital glands) and stimulate the progressive development of the genital tubercle to become the penis, the genital folds to close as the prepuce, and the genital sinus to differentiate into the pelvic structures.

Modeling of the tubular genitalia involves growth, fusion, and extension of the embryonic paramesonephric ducts to form the internal female tubular organs (uterus, cervix, and cranial vagina) and changes in the urogenital recess, genital tubercle, and genital folds to form the vulva, clitoris, vestibule, and caudal vagina. The presence of androgens, either endogenous (from testicular tissue, e.g., in individuals with ovotestes) or from exogenous sources (androgens administered during pregnancy) can influence this development, leading to partially masculinized females.

Development of the tubular genitalia

Even as the primitive bipotent gonads are developing from the genital ridge, in embryos destined to become females, cylindric (ductal and tubular) elements are forming in the abdomen that will further differentiate into the tubular genitalia.

During the early stages, male embryos and female embryos contain the same structures and cannot be differentiated. Paired **mesonephric (Wolffian) ducts** and paired **paramesonephric (Müllerian) ducts** form de novo from longitudinal folds along the lateral inner abdominal wall (Fig. 4-1).

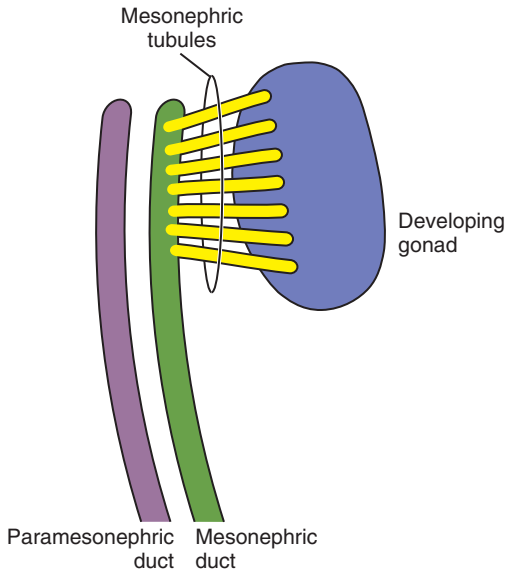

Figure 4-1 Embryonic development of the female genital system. Initial development of the indifferent ducts, tubules, and external genitalia is the same in male and female embryos. Anatomically, the internal organs in the "indifferent" stage consist of 2 bilaterally symmetrical ductal systems—the mesonephric (Wolffian; male) and paramesonephric (Müllerian; female) ducts—and the developing, but uncommitted, gonad. Additionally, the mesonephric duct develops tubular extensions at its cranial end that extend into the hilar area of the gonad. Although the uterine tubes, uterine horns, body, cervix, and cranial vagina develop from the paramesonephric duct, the mesonephric tubes become the rete and contribute to the sex cords of the ovary. Normally the ducts of the opposite sex regress; however, their embryonic remnants are frequently encountered in otherwise normal adult animals, appearing as grossly detectable cystic structures in or near the ovary, uterus, cervix, or vagina. (Illustration prepared with the help of Michael Simmons.)

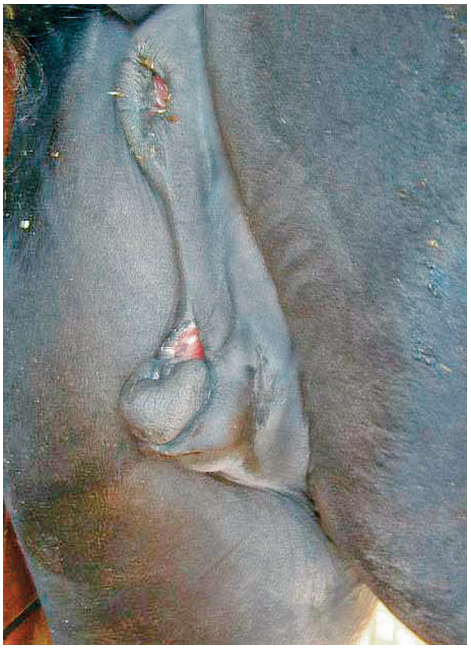

Figure 4-2 Abnormal external genitalia of a horse with a ***disorder of sexual development.*** There is clitoromegaly and partial fusion of the vulvar lips. The external female genitalia were partially masculinized from exposure to testosterone released from testicular tissue (interstitial cells) present in its ovotestes during embryonic development.

Mesonephric tubules branching from the cranial segment of the mesonephric ducts contribute to development of the rete and sex cords.

The mesonephric and paramesonephric ducts run parallel to one another. Both ductal systems are symmetrically paired and extend caudally toward the urogenital sinus. The cranial ends of the developing paramesonephric ducts do not close and open directly into the peritoneal cavity, corresponding to the tissue that will become the fimbrial part of the uterine tube. In domestic animals, the paramesonephric ducts fuse at their caudal ends to form the body of the uterus, the cervix, and the cranial vagina, each with a single lumen.

Abnormal sexual development

Disorders of sexual development (DSD) occur in all domestic species. They result from abnormalities of sex chromosome origin or inappropriate product; hormone; or receptor upregulation, downregulation, or exposure. The mechanisms involved in the production of many abnormalities are not known, and a condition cannot often be classified precisely. *The classification of DSD is now based on as complete a description of the underlying abnormality as possible, beginning with the sex chromosome type, presence of SRY, gonad type, tubular genitalia, and external genital phenotype.* As sex chromosome typing becomes more readily available, complete classification becomes possible.

The main categories begin with the sex chromosome complement. Disorders are therefore either sex chromosome, XX, or XY DSD. The presence of the *SRY* gene is listed next. The gonadal type (ovary, testis, ovotestis, or dysgenesis) follows. The tubular genitalia and the external appearance are listed. In completion, a specific syndrome is included. Terms such as intersex, ambiguous development, hermaphrodite, or pseudohermaphrodite are therefore avoided.

Abnormal sexual development commonly results in abnormal-appearing external genitalia. As demonstrated in Figure 4-2, a photograph of a mare with bilateral ovotestes, the external genitalia have features that are partially female and partially male. Testosterone produced by testicular tissues (interstitial endocrine cells) of her ovotestes masculinized her external genitalia. The vulva is located more ventrally than in a normal female, the vulvar lips are variably fused, and there is clitoromegaly. Clitoral enlargement is an important indicator of an underlying disorder. The spectrum of changes ranges from nearly normal-appearing females, with grossly normal ovaries and uteri, to males with small genitalia, perhaps hypospadias, and gonads contained in scrotal sacs that look like testes with epididymides, but histologically containing ovarian tissue. It is not uncommon to find normal-appearing testes attached to complete and well-formed uterine horns (Fig. 4-3).

Classification of gonads can be challenging, and pathologists often have difficulty in identifying ovotestes. *Ovotestes contain both ovarian and testicular tissues* (Fig. 4-4). Careful examination of the connective tissue of the outer part of the gonad for ovarian structures is required. Follicles can develop here and can ovulate, and there may be corpora lutea in

Figure 4-3 Abnormal internal reproductive organs from a **bitch,** revealing a near-normally formed, but **hypoplastic, uterus** (paramesonephric duct derived). There are **bilateral ovotestes** that are predominantly testicular and an attached epididymis (derived from mesonephric tubules).

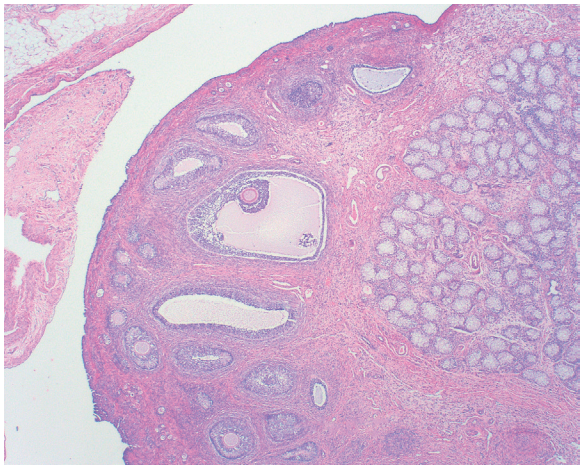

Figure 4-4 Section of an **ovotestis from a bitch.** The gonad is contained within a bursa (visible on the left) and has both ovarian tissue (with primary and antral follicles on the left) and lobules of hypoplastic testicular tissue in the medullary area (on the right) containing seminiferous tubules. Even relatively small areas of testicular tissue in ovotestes produce sufficient amounts of 2 hormones: testosterone from the interstitial cells and anti-Müllerian hormone from Sertoli cells, which act during early gestation to masculinize the developing mesonephric and paramesonephric ducts, tubules, and the external genitalia.

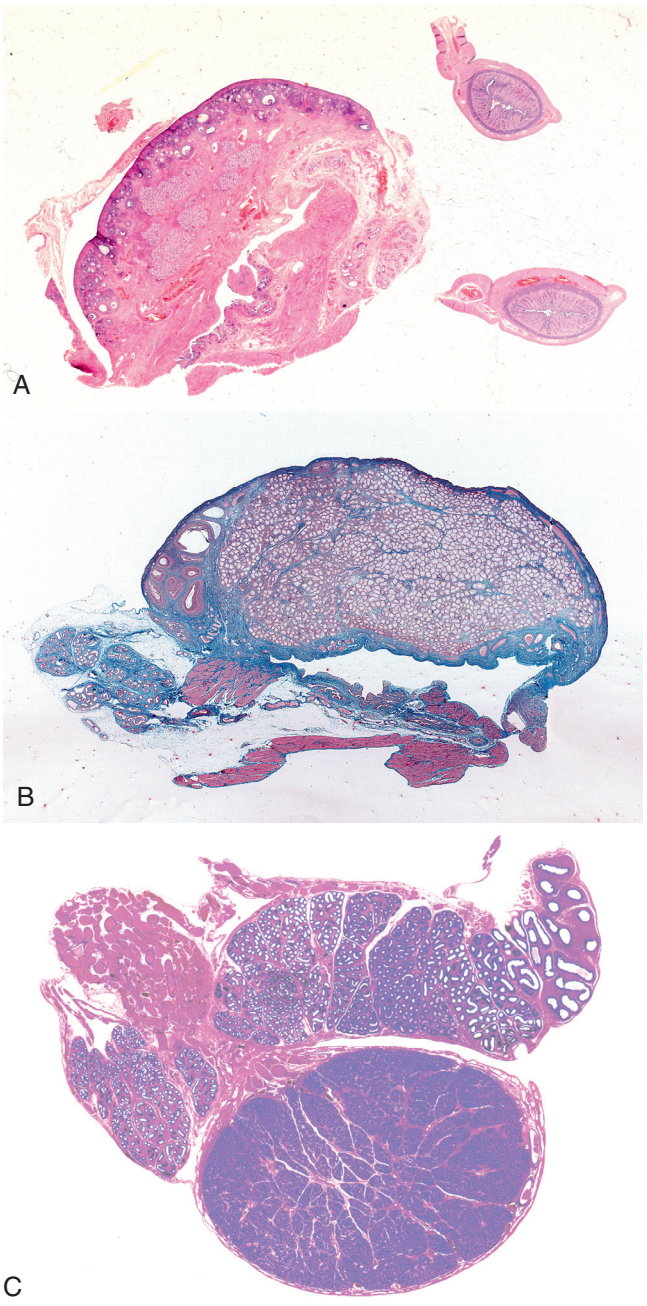

Figure 4-5 Ovotestes from bitches. These 3 subgross photographs show **the variability in amounts of ovarian and testicular tissue that occur in ovotestes.** Microscopically, ovarian tissues are found in the cortex, and testicular tissues develop in the medulla. **A.** The ovotestis on the left is mostly ovarian, with only a few lobules of hypoplastic seminiferous tubules in the medulla. **B.** The ovotestis is mostly testicular tissue (seminiferous tubules forming a lobular pattern), but also note the presence of tertiary follicles in the ovarian tissue at the left pole. **C.** The ovotestis is nearly entirely testicular and has a large epididymis attached.

gonads that also contain male (seminiferous and interstitial endocrine) tissue. Ovotestes will vary greatly in both their gross and microscopic appearance, depending on the relative amounts of ovarian and testicular tissue present (Fig. 4-5). Ovotestes composed predominantly of ovarian tissues are usually contained in a bursa. There may be identifiable epididymal tissues at the caudal pole, near the attachment of the proper ligament of the ovary. Ovotestes composed of mostly testicular tissue will look like hypoplastic testes, have large epididymides, and may be near or in the inguinal canal or scrotal sac. They will, however, be attached to uterine horns.

In dogs, ovotestes are commonly contained within a bursa (Fig. 4-6). Associated developmental changes involving the tubular genitalia include persistence of variable amounts of

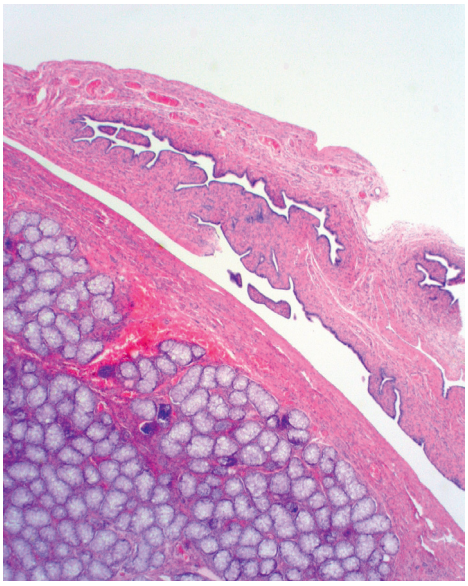

Figure 4-6 Gonadal tissues from a bitch. Although this ovotestis is mostly testicular, it is enclosed in a bursa. Sections of the uterus are shown in Figure 4-7.

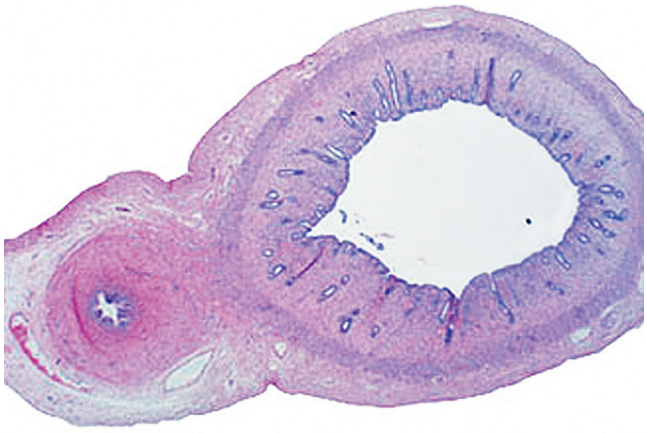

Figure 4-7 Subgross picture of a section of one uterine horn from the bitch in Figure 4-6. Note the presence of derivatives of both the **paramesonephric and mesonephric ductal systems** (uterine horn on right and deferent duct on left, respectively). Hypoplastic deferent ductal tissues also located on the side of the broad ligament (mesometrium).

mesonephric ductal tissues that are found as either cysts or linear segments of the mesonephric duct(s) (Fig. 4-7).

Sex chromosome disorders of sexual development

An abnormal number or structure of sex chromosomes leads to inadequate gene expression and subsequent disorders. These disorders are divided into 2 groups: those with **aneuploidy** and those with **chimerism and mosaicism**. Examples of aneuploidy include X_ (a Turner-like syndrome), XXX, and XXY (Kleinfelter-like syndrome). These mimic the human equivalent of these syndromes. Chimerism and mosaics is most commonly seen as freemartinism, where there is an XX/XY sex chromosome combination. **Chimerism**, known as *freemartinism, occurs most commonly in cattle.* The bovine freemartin is a genetic female born co-twin with a male (twinning occurs in 1-2% of pregnancies in cattle). The twinning is

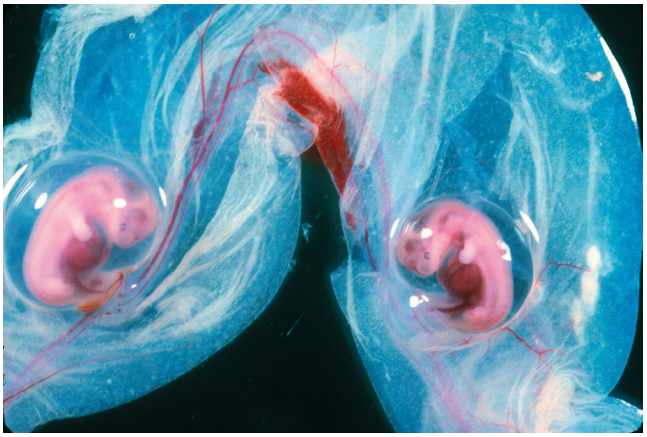

Figure 4-8 Twin bovine embryos contained within their amnions. Note the fusion of branches of their umbilical arteries causing **shared circulation of blood** and establishment of chimerism. When the twins are of opposite sexes, development of the female's reproductive organs will be altered and partially masculinized. The constellation of changes that occur in her developing reproductive organs is known as **freemartinism.**

dizygotic in most cases; anastomoses develop between the placental vascular systems of the 2 fetuses (Fig. 4-8). Normal development of the gonads, tubular, and external genitalia of the female embryo is dramatically altered by hormones and cells received through the common circulation with her male twin. If anastomoses fail to develop, the female is not affected, but *almost all male-female twinning in cattle results in the female becoming a freemartin.*

The effect on the male is always minimal. In the female the gonads are small and frequently contain hypoplastic seminiferous tubules (ovotestes). The uterine horns are hypoplastic, or may be nearly completely absent or lack communication with the vagina (Fig. 4-9). Some parts of the mesonephric ducts are commonly present. The external genitalia commonly have enlargement of the clitoris, a small vulva, and a prominent tuft of hair (Fig. 4-10A). *A consistent feature (and therefore a key diagnostic feature) is the presence of poorly developed vesicular glands attached to the lower fused segment of the paramesonephric ducts* (Fig. 4-10B). The vagina is short and nonpatent.

A critical step in freemartinism is when *blood of the twins is exchanged and leads to permanent colonization by hematopoietic cells,* with the result that each twin develops an acquired tolerance for the blood cells of the other. This condition appears to be confined to hematopoietic cells. The allantoic fusion in bovine twin pregnancies occurs at about the 10-mm crown rump–length stage of development. Approximately 90% of females born co-twin with males are freemartins and sterile. Freemartins occur in bovine triplets, quadruplets, and quintuplets as well as twins.

Exchange of nonhematopoietic cells does not appear to take place in freemartinism. *It is believed that the male fetus sterilizes the female by testis-determining factors carried by the bloodstream via the placental anastomoses to the female gonad, where it inhibits ovarian development.* This inhibition is manifest first functionally; estrogen production by the freemartin ovary is reduced, and androstenedione is elevated as early as 40 days of gestation. This is well before morphologic evidence of damage is recognized.

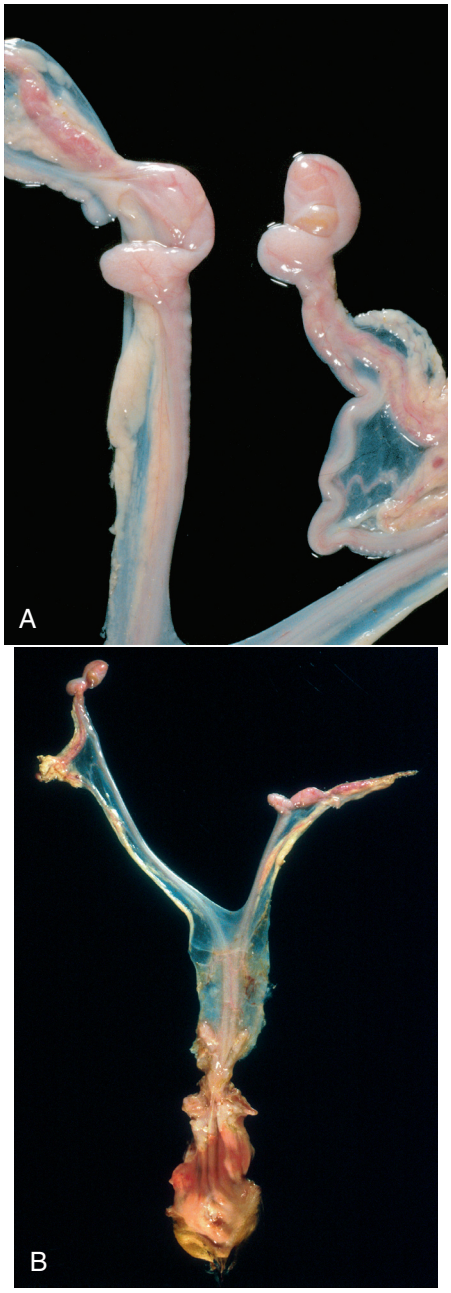

Figure 4-9 A, B. Reproductive organs of a newborn freemartin calf. Note the markedly hypoplastic uterine horns, failure of the paramesonephric ducts to fuse to form a single uterine body, presence of paired vesicular glands, and abnormal gonads. In panel **A,** note that the gonads in this case are also hypoplastic and are sometimes ovotestes.

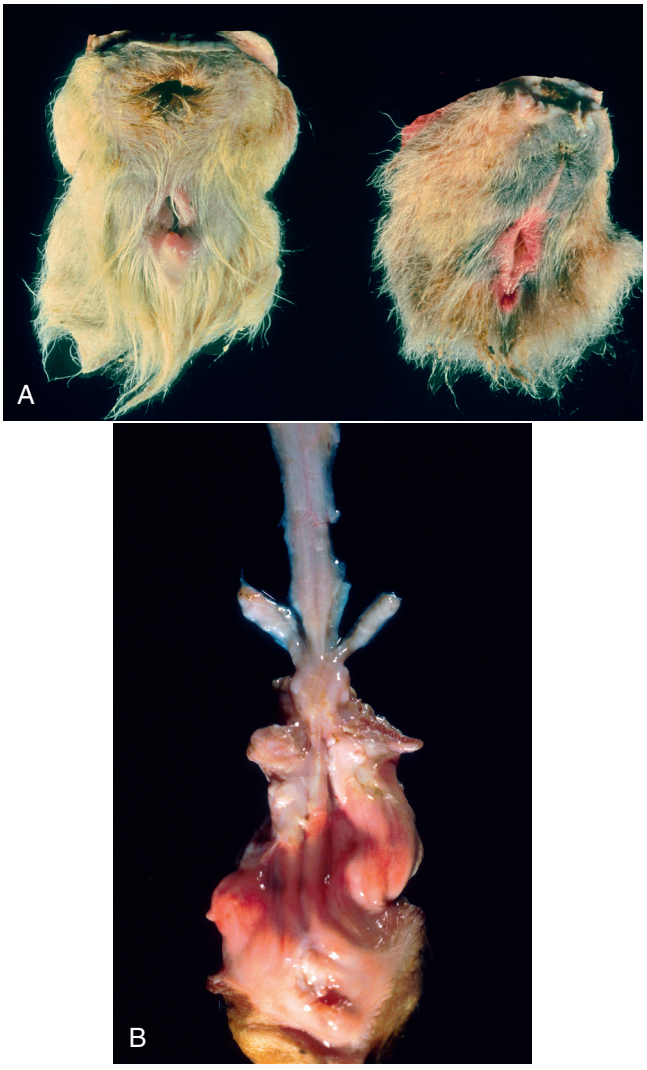

Figure 4-10 A. Abnormal vulva from a **freemartin heifer** (same animals as in Figure 4-9). Comparison of the external genitalia of a normal newborn freemartin calf on the right and the prominent clitoris and a long tuft of hair attached ventrally to the freemartin calf's vulva on the left. The vagina is shortened and not patent in freemartins. **B.** *A key diagnostic feature of the freemartin condition is the presence of paired hypoplastic vesicular glands,* seen in this photograph as paired structures extending from the nonpatent vagina in the middle of the photograph.

The gonad of a freemartin is usually a cordlike thickening in the cranial border of the ovarian ligament. The rete is well developed, but other ovarian structures may be absent. Alternatively, greatly reduced ovarian tissue can be present, but it lacks the normal germ cell complement. The variation of the tubular genitalia is directly linked to the degree of gonadal development. When the gonad is predominantly ovary, there is no epididymis and the deferent duct is poorly developed. With increasing differentiation towards a testis, the deferent duct and epididymis become better developed. The uterus and uterine tube, of paramesonephric duct origin, may vary from cordlike structures without a lumen to well-developed uterine horns with lumen and endometrial glands. *Communication with the vagina is always absent, no matter how well developed the uterus may be.*

Vestigial vesicular glands are always present, the vagina is hypoplastic or nonpatent with a complete hymen, the vulva and vestibule are hypoplastic, and the clitoris is enlarged. Many freemartins have a prominent fold of skin on the median plane of the body, extending from a position ventral to the vulva to an area near the umbilicus. Mammary glands fail to develop.

Twin ovulation is relatively common in *horses.* Most twins do not survive to term, but vascular anastomoses with hematopoietic chimerism occur in equine twins, although freemartinism is not proven. Large anastomoses occur between chorionic sacs in *swine,* and freemartinism results. Because of

the large litter size, it is difficult to identify the neighbor of affected piglets, and proof is lacking. In *sheep*, dizygotic twinning is common and freemartinism does occur, but it is much less common; only 1% of male female co-twins are freemartins. Large-caliber interplacental anastomoses develop infrequently. Freemartinism does occur in *goats*, complete with hematopoietic chimerism, but is rare because, as in sheep, large anastomoses between fetuses are rare. Freemartinism occurs in camelids also, but is not reported in dogs or cats.

XY disorders of sexual development

Any congenital disorder of the genital system in an animal with XY sex chromosomes is called an **XY DSD**. They are subdivided into those with or without an *SRY* gene (XY *SRY*+ and XY *SRY*– DSD). The most extreme is the XY *SRY*+ ovarian DSD with female internal genitalia and phenotype.

Individual examples of all species of domestic mammals with XY sex chromosomes and female phenotype are reported. *The majority are XY, SRY+, testicular DSD with a female phenotype.* These were called XY sex reversal syndrome, androgen insensitivity, or testicular feminization syndrome. Horses have an inherited autosomal sex-limited dominant trait with a Y chromosome mutation with variable expression. There is a considerable range of phenotypic expression in this syndrome. Affected individuals vary from phenotypically normal but sterile mares with inactive ovaries and normal tubular genitalia, to individuals with streak gonads or ovotestes and severely hypoplastic or aplastic tubular genitalia. A family of horses with an XY, *SRY*+ DSD that is an X-linked trait, based on pedigree analysis, is reported. Affected animals were phenotypically normal mares but had hypoplastic testes and uteri.

An XY *SRY*+, testicular DSD, and female phenotype is especially recognized in humans, mice, rats, horses, cattle, and cats. This inherited syndrome has *a controlling mutation identified as TFM*. Affected individuals have XY chromosomes and have testosterone-producing testes. The external genitalia are female in type. The *TFM* mutation produces a deficiency of intracellular androgen receptors, rendering all cells insensitive to androgens. AMH is still produced by the testes of affected animals, and therefore paramesonephric duct derivatives are absent because of their sensitivity to this hormone. Horses with this syndrome have normal-appearing female external genitalia. The vagina ends in a blind sac, with no cervix, uterus, or uterine tubes being present. The gonads are small and clearly testicular, but present in the normal ovarian position (Fig. 4-11). The seminiferous tubules are small and lined by inactive Sertoli cells (Fig. 4-12). Rare spermatogonia can be found, but spermatogenesis does not occur. Well-differentiated interstitial endocrine cells are present (see Fig. 4-12).

Horses with the syndrome have no detectable male accessory genital glands. Cattle with this syndrome have rudimentary vesicular glands and ampullae. Both cattle and horses that lack androgen receptors have normally formed female mammae. Horses are unique as affected individuals show male behavior patterns. This syndrome is distinguished from others by assaying the androgen receptors on target cells, such as fibroblasts from the genital skin. Labial tissue must be used because receptor activity is sharply reduced in nongenital skin. Occasional mammals with XY *SRY*+ DSD and gonadal dysgenesis are reported. They are phenotypically female. Those phenotypically male animals that are XY *SRY*+ but have anomalies are discussed in Vol. 3, Male genital system.

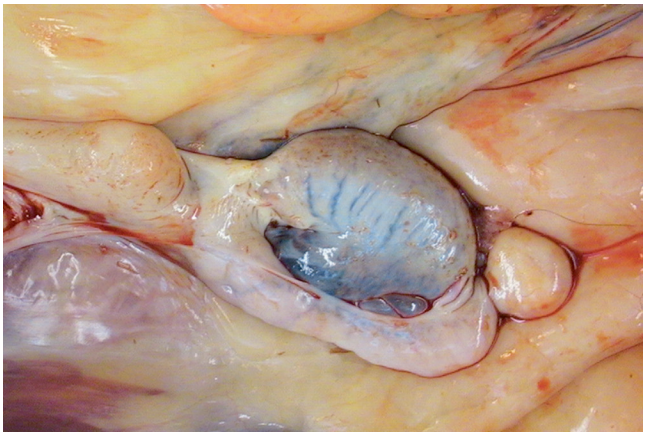

Figure 4-11 **Retained ovotestis** in a young mare that had masculinized external genitalia. The retained gonads have gross features of hypoplastic testes and nearly normal-appearing epididymes.

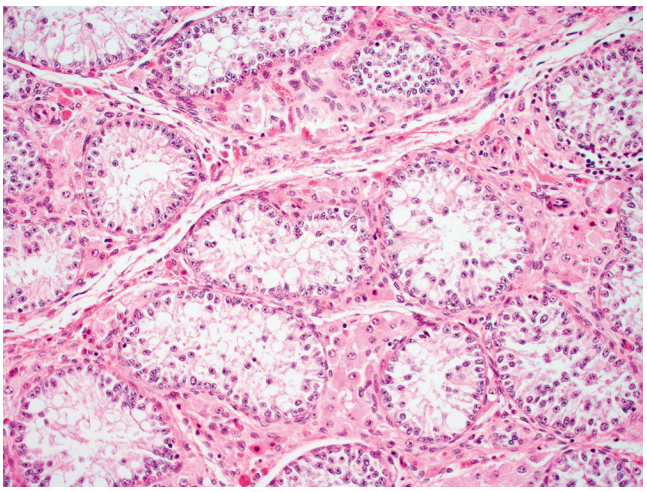

Figure 4-12 Photomicrograph of gonadal tissues from the mare in Figure 4-11 demonstrating the presence of many hypoplastic seminiferous tubules and interstitial cells.

XX disorders of sexual development

Mammals with XX sex chromosomes and abnormal sexual development are myriad. The majority are normal females with a minor anomaly, such as cystic remnants of ducts or tubules. These are the XX *SRY*–ovarian DSD. There are no reports documenting an XX, *SRY*+ DSD in domestic mammals.

An extreme XX *SRY*– DSD is *complete sexual reversal* (XXSR), where the sex chromosomes are XX and the phenotype is male. Much of what is known about this condition is from mice that have an autosomal dominant gene, *Sxr*, which acts like a Y chromosome. Thus XX mice that carry the *Sxr* gene develop testes and male tubular genitalia. A similar condition occurs in dogs, pigs, and goats, although in these species the autosome-associated gene with the Y action functions as a recessive gene.

XX ovotesticular female DSD (*XX sex reversal*) is common in *pigs*. The mode of inheritance is unknown, and some cases are confused with freemartinism. The masculinizing effect is incomplete. The gonads in affected individuals are often ovotestes and, although the testicular tissue lacks germ cells, ovulation and pregnancy are reported. Hematopoietic chimerism does not exist, as would occur in freemartinism.

This condition is studied in goats with the polled intersex syndrome (PIS). Affected goats have an XX testicular or XX ovotesticular disorder of sexual development because they have a deletion of the 11.7 kb PIS region of chromosome 1. The PIS region is a normal region that is 300 kb upstream of and regulates the function of *FOXL2*, an ovary determining gene in goats. *FOXL2* normally inhibits DMRT1 that regulates the testis-determining gene *SOX9*. Deletion of the PIS locus results in an inactive *FOXL2* and a male goat with XX chromosomes. The autosomal gene with the testis-determining region is on the chromosome that controls polling or is closely linked to it. The polled gene is a dominant autosome, a single copy of which produces hornlessness without affecting sexual differentiation. In the homozygous condition, however, there is apparent translocation of a subcritical portion of the testis-determining gene to the autosome that contains the gene for polling, producing the recessive mode of sex determination. There is a considerable range of masculinization. The affected individuals may have largely female appearance with only an enlarged clitoris and abdominal testes. Other animals have the appearance of near-normal, although sterile, males.

XX *SRY*– males caused by male differentiating genes on the X chromosomes are reported in >15 breeds of dogs. The American Cocker Spaniel breed is particularly prone. XX *SRY*– testicular and XX *SRY*– ovotesticular DSD are described. Some XX *SRY*–ovotesticular female DSD are fertile and give birth to pups. The male components of the gonads lack germ cells. The condition has an autosomal recessive inheritance. Genes other than *SRY* are implicated, possibly *SOX9*, in initiating male differentiation in XX female canine embryos.

Ovary—developmental anomalies

Abnormal development of genital components is described in the context of normal sex chromosomes (XX for females). The majority are XX *SRY*– ovarian DSD.

Agenesis of one or both ovaries is rare, but is observed in ruminants, swine, and dogs. In bilateral agenesis, the tubular genitalia may be absent as part of the defect or, if present, are infantile or underdeveloped.

Duplication of ovarian tissues is very rare. Although there are no controlled studies, duplication may just be splitting of ovarian tissue. Ovaries from a young calf with ovarian duplication are shown in Figure 4-13.

Ovarian remnants. Interest in the occurrence of anomalous ovarian duplication commonly arises when either cats or dogs, supposedly surgically neutered, exhibit signs of estrus. This is *ovarian remnant syndrome.* In the bitch, the effects of circulating estrogen are easily detected by serial vaginal cytology. Hormonal studies are confirmatory, and ultrasonographic and exploratory surgical examinations are also done. This is best done when the animal is in estrus and has large follicles or during the luteal phase of the cycle, when corpora lutea would also make ovarian tissue easily identified. Although proposed, there is little information about whether small pieces of ovarian tissue inadvertently released during canine or feline surgery might implant on the peritoneal surface in the abdomen. In some countries, parts of ovaries are surgically placed in the spleen and these subsequently develop ovarian neoplasia. Although frequently discussed, there is little evidence that congenital ectopic ovarian tissues occur in any of the domestic animal species.

Vascular hamartomas of the ovary are described in horses, swine, cattle, and dogs. Because they are often confused with neoplasms, they are described in the section on ovarian tumors.

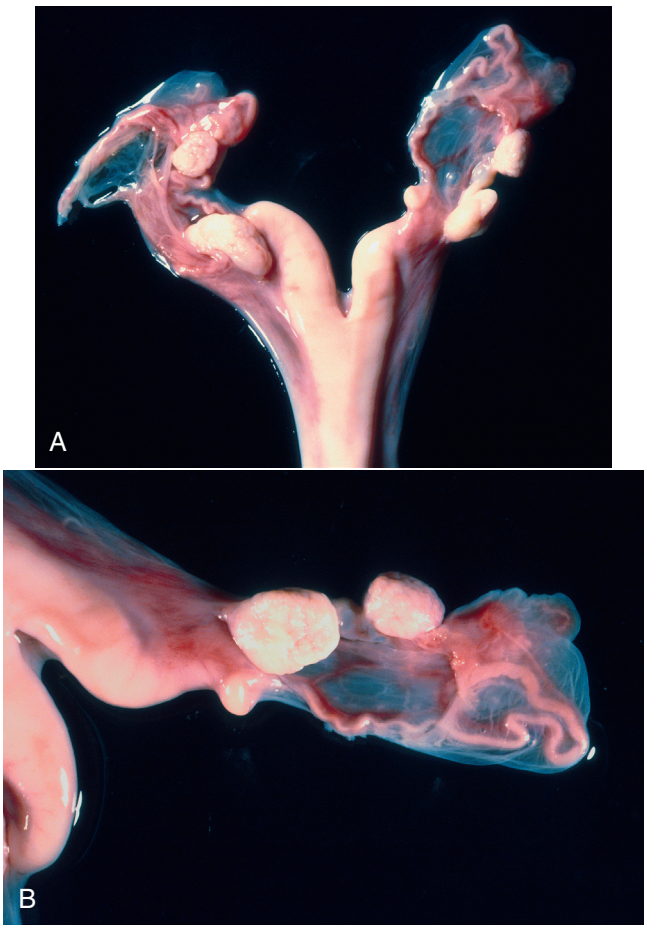

Figure 4-13 Duplication of the ovaries occurs very rarely. **A.** The gonads in this newborn calf were each divided, giving the impression that 2 separate gonads had formed. An isthmus attaches the ovaries on the right. **B.** The 2 separated left gonads are shown more clearly in this photograph.

Hypoplasia of the ovaries is studied primarily in cattle, but occurs in other species. *It is usually bilateral but varies considerably in its severity and symmetry* so that "severe hypoplasia" or "partial hypoplasia" may be applicable to one or both ovaries. In severe hypoplasia, the defective gonad varies in size from a cordlike thickening in the cranial border of the mesovarium to a flat, smooth, firm, bean-shaped structure in the normal position. There are neither follicles nor luteal scars and, microscopically, the ovary is largely composed of medullary connective tissues and blood vessels.

Ectopic adrenal tissue is usually found incidentally during histopathology. These small nodules are composed of adrenal cortex and are most commonly found with the capsule of the ovary of mares or with the ovarian suspensory ligament of the ovary of queens (Fig. 4-14).

Cysts arising from remnants of developmental structures

Cystic structures in and near the ovary and uterine tubes are common, especially in the mare. Figure 4-15 demonstrates the embryonic origin of the most common of these cysts. Cystic remnants are more common and can become large, especially in the mare (Fig. 4-16).

Cysts arising from either the cranial or caudal segments of the mesonephric tubules are thin-walled, movable cysts at either the cranial or caudal pole of the ovary and are called cystic epoophoron or paroophoron, respectively, or just **cystic mesonephric tubule remnants**. **Cystic rete** are common in the cat and dog and arise from the rete ovarii, which are also derived from the mesonephric tubules. These arise in the hilar area of the ovary. All cystic remnants of the mesonephric tubules have smooth muscle in their walls, whereas the cystic rete do not. The epithelial lining is cuboidal in small cysts, tends to become squamous in slightly larger cysts, and is often absent due to pressure atrophy in larger cysts. Only the cystic rete progress to compress ovarian tissues and compromise ovarian function. Hyperplasia of the rete epithelium is common, and cystic adenomas occur, most commonly in the bitch.

Cystic apical segments of the paramesonephric duct or **fimbrial cysts** are common in mares**.** They are 1 or 2 thin-walled cysts, up to 1 cm in diameter, attached to the fimbria of the uterine tube (Fig. 4-17). They may be detected by palpation or by ultrasonography, but do not reduce fertility. The differential diagnosis is a cystic remnant of the mesonephric duct, but these are found adjacent to the uterine tube or along the lateral border of the uterine tube, uterine body, or in the cervix or vagina.

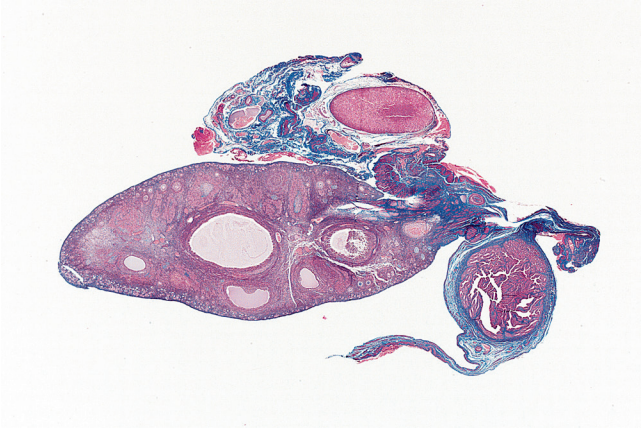

Figure 4-14 Ectopic adrenal tissue is most commonly found adjacent to, or attached to, the tunic of the ovary of the queen and mare. A nodule of ectopic adrenal tissue (upper right) is in the suspensory ligament adjacent to the ovary in this feline tissue. Cross-sections of a uterine tube and ovary are also present.

Mesonephric duct remnants are frequently found as small cysts lying along the lateral side of the uterine tubes, uterine horns, uterine body, cervix, or cranial vagina. They are in or near the attachment of the mesosalpinx or mesometrium of the uterine tube and uterine horns, but those found in the uterine body and cervix tend to be found within the myometrium, and those in the vagina are commonly multiple or linear cystic structures in the submucosa. Animals with disorders of sexual development and a female phenotype often have remnants of mesonephric ducts located in the attachment of the mesometrium and run parallel with the uterine horns (Fig. 4-18). These have the microscopic appearance of the deferent duct of the male and have a lumen lined by epithelial cells and a relatively prominent smooth muscle wall.

Cystic mesonephric duct remnants are common in the cow. The fluid they contain is usually clear, but may be opaque (Fig. 4-19).

Duplication of the paramesonephric duct is occasionally seen in dogs. These have a more complex endometrial type lining, and the cells become vacuolated in the diestral phase of the estrus cycle.

Arrested development of the paramesonephric duct

There are 3 patterns of defect:

1. Failure of segments of the paramesonephric ducts to develop.
2. Failure of the caudal parts of the 2 paramesonephric ducts to fuse appropriately and develop a single lumen.
3. Failure of the caudal ends of the fused paramesonephric ducts to fuse with the invaginated urogenital sinus, leading

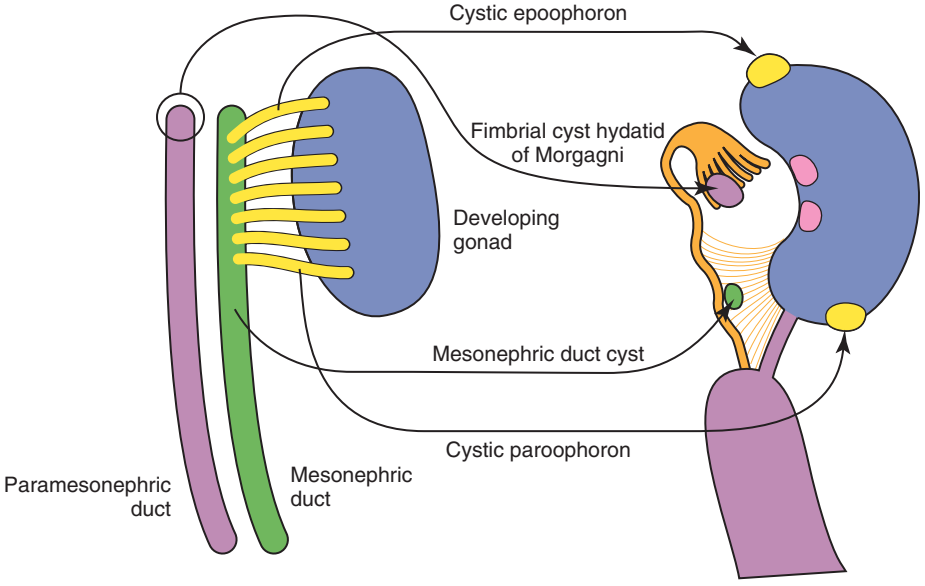

Figure 4-15 Cysts in or near the ovary and along the uterus are common, but usually incidental findings. The **embryonic origin of several common cysts is shown in this drawing.** Details are provided in the text, and examples of some of these cysts as they occur in different species are shown in the following figures. (Michael Simmon, illustrator.)

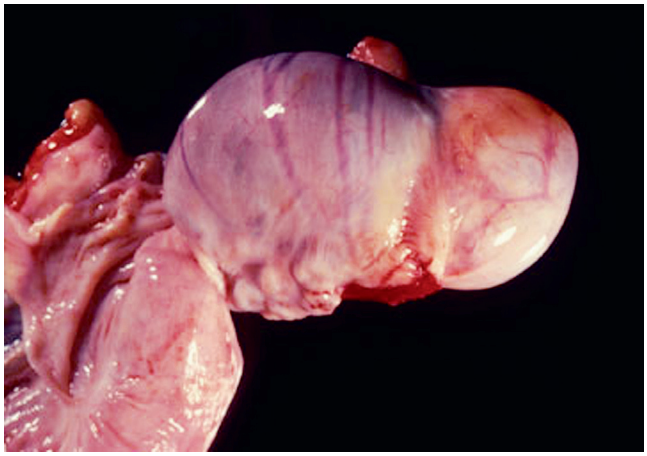

Figure 4-16 A large **cystic epoophoron** extends from the proximal pole of the ovary of a mare. These cysts arise from remnants of the cranial mesonephric tubules, and although they can become large, do not reduce fertility. They are moveable over the surface of the ovary, are frequently palpable per rectum, and can be seen during ovarian ultrasonography.

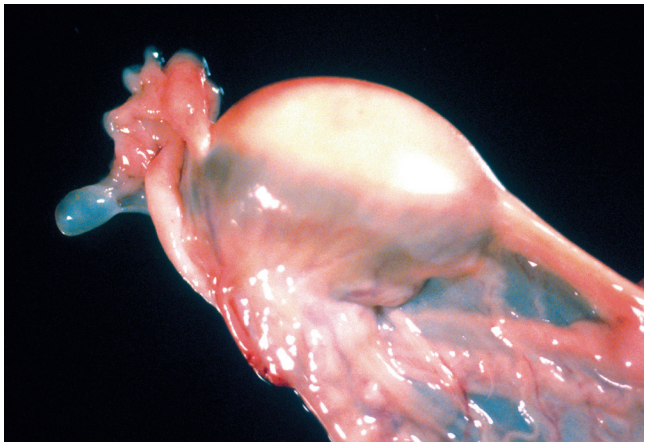

Figure 4-17 **Paramesonephric duct cyst (fimbrial cyst)** extending from the fimbria of the uterine tube of a young filly. These are very common.

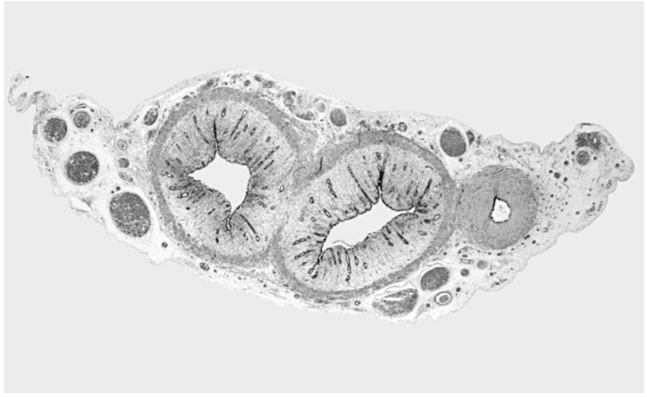

Figure 4-18 Cross-section of **uterine horns** at their bifurcation, with a **unilaterally persistent mesonephric duct** (corresponding to the deferent duct) in the mesometrial attachment on the right side.

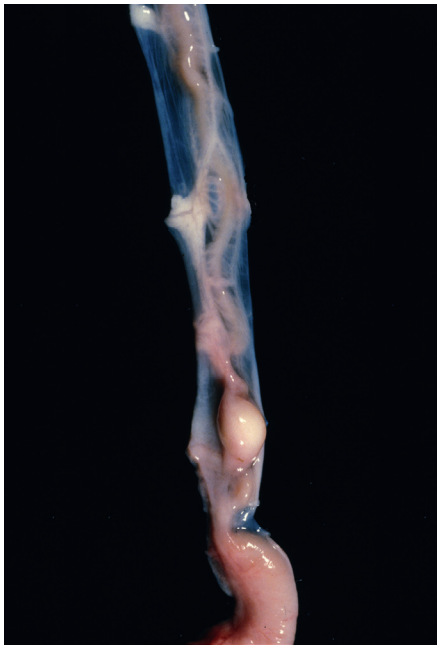

Figure 4-19 **Cystic remnant of a mesonephric duct** adjacent to the uterine tube in a cow.

to failure of the lumen of the fused ducts to become continuous with the caudal vagina.

Segmental aplasia of the paramesonephric duct is found as either failure of short or long segments of the uterine horn to develop (Fig. 4-20). This was commonly found in white Shorthorn cattle and gave rise to the name "white heifer disease" for the syndrome, but no breed is exempt from the condition. The arrested development is thought to be autosomal recessive. Complete absence of an entire horn is called **uterus unicornis.** Within the isolated segments of uterus, secretions and sloughed epithelial cells accumulate and become inspissated to form soft tan concretions within the distended isolated lumen of the uterine horn proximal to the area of segmental aplasia (Fig. 4-21).

Failure of the paramesonephric ducts to fuse is most commonly found in the cow and usually involves the cervix, and less commonly the uterine body. Partial failure of the paramesonephric ducts to fuse with loss of the joining wall to form a single lumen is more common than complete failure to fuse. An example of the more common appearance is shown in the tissues in Figure 4-22. The uterine body and cranial cervix have developed normally, but there is a persistent median septum in the cervix, leaving 2 cervical canals and the appearance of 2 cervices. The apparent septum is the medial walls of the 2 paramesonephric ducts that failed to regress.

Less commonly, there may be failure of the caudal aspects of the paramesonephric ducts to fuse. This occurs in the cow and bitch and results in a longitudinal median band of tissue extending from the mid-dorsal to the mid-ventral walls of the vagina cranial to the urethral orifice.

Failure of fusion of the paramesonephric ducts with the urogenital sinus results in persistence of a tissue band running across the vagina just cranial to the opening of the urethra. This tissue forms an **imperforate hymen** that is complete or, more commonly, is only a small piece of the wall (Fig. 4-23). The remainder of the genital tract may be normal, but if the hymen is complete, in time and with the accumulation of secretions (there may be 10 L or more), the vagina, cervix,

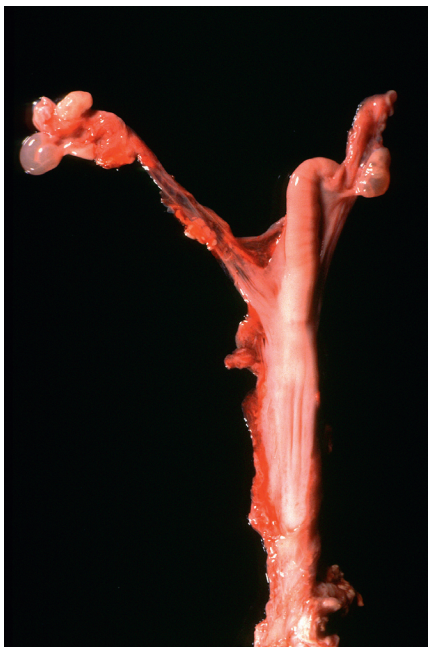

Figure 4-20 **Segmental aplasia** of the left uterine horn of a heifer caused by failure of a segment of the paramesonephric duct of this side to develop normally. Also note the hydrosalpinx.

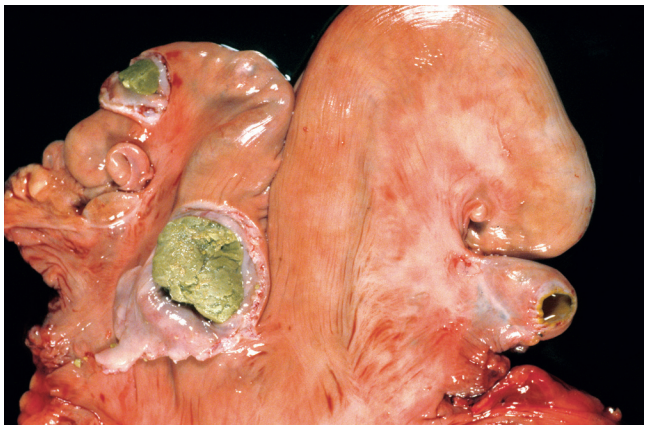

Figure 4-21 **Segmental aplasia** of the left uterine horn of an adult cow. The uterine wall has been opened revealing soft tan **concretions** formed from accumulated uterine secretions and sloughed cells.

and uterus become distended and atonic, and severe dilation leads to atrophy and permanent loss of tone of the walls. If the hymen is perforated and the distension relieved early, the genital tract may function normally. If the hymen is complete, secretions may accumulate and subsequent bacterial infection of the contents can produce pyometra. In cows, moderate **hypoplasia of the cervix**, in which some rugae are absent and the canal widely patent, may prevent closure of the canal and predispose to persistent invasion of the uterus and chronic endometritis. Occasionally, the cervical canal is irregular in its course or even tortuous. Although this may not interfere with conception following natural breeding, insertion of an inseminating catheter may be difficult or impossible. Repeated attempts to pass a pipette can lead to cervical trauma, occasionally complicated by formation of traumatic inclusion cysts or abscesses.

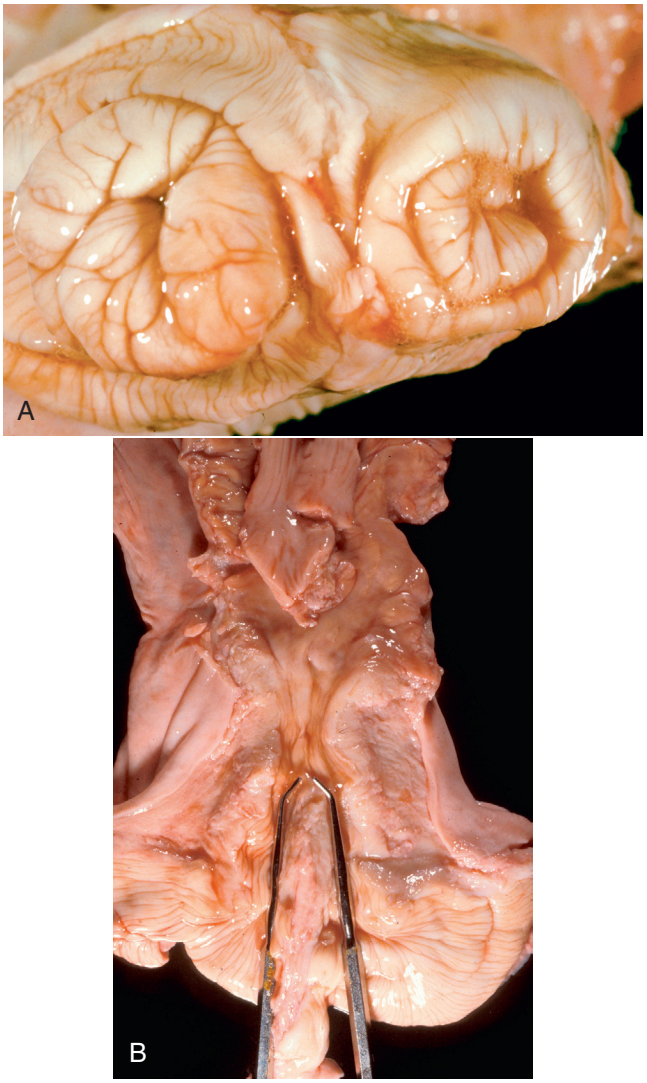

Figure 4-22 **Double external cervical os. A.** As viewed from the vagina—caused by failure of the medial wall of the mesonephric ducts to join and dissipate during embryonic development. **B.** This defect is demonstrated in the dissected bovine cervix. The uterine body is normally formed as a result of fusion of the 2 paramesonephric ducts); however, 2 separate cervical canals (demonstrated by the probes), are present in the distal cervix as a result of failure of the medial wall of the joined paramesonephric ducts to fuse and dissipate. Complete division of both the uterine body and cervix is less common and when present is called *uterus didelphys*.

Dilations and diverticula of the cervix are a cause of infertility in heifers. The malformations occur at the level of the third and fourth rugae, and the cervical canal is constricted caudal to the defect. The dilated areas are usually spherical and located near the internal os. Diverticula are dorsal or dorsolateral and eventually become filled with tenacious mucus. The cause of the condition is unknown, but is assumed to be developmental.

Vaginal anomalies

Vaginal stenosis is uncommon in most species, but occurs to variable degrees in bitches. It may be congenital or acquired. The stenosis in Figure 4-24 was complete and had caused dilation of the proximal vagina.

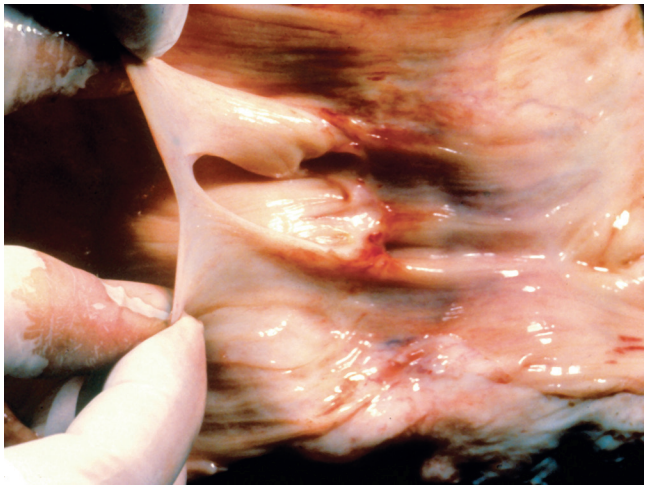

Figure 4-23 **Incomplete hymen** in the caudal vagina of a young heifer. The hymen is a membrane that forms as a tissue band at the point where the invaginating urogenital sinus that will form the caudal vagina meets the caudal aspects of the descending paramesonephric ducts, which will form the cranial vagina. Complete or partial hymens are commonly found in young calves. The vaginal lumen cranial to the hymen will accumulate mucus and cellular debris.

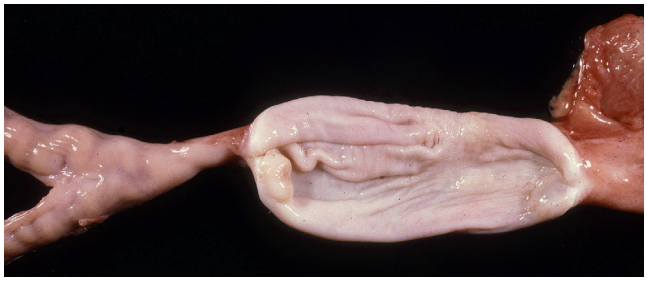

Figure 4-24 **Segmental stenosis** of the tubular genitalia is uncommon. The middle region of the **vagina** of this bitch was completely occluded, and the lumen of the cranial vagina was distended with tan fluids. (From Romagnoli S, Schlafer DH. Disorders of sexual differentiation in puppies and kittens: a diagnostic and clinical approach. Vet Clin North Am Small Anim Pract 2006;36: 573-606.)

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PATHOLOGY OF THE OVARY (NONDEVELOPMENTAL-RELATED CONDITIONS)

Reproductive organs of sexually mature animals normally undergo dramatic changes during the estrous cycle. These morphologic changes associated with follicular development, ovulation, luteinization, and regression of the corpus luteum are referred to as the **ovarian cycle.** Species variations exist that can potentially be very confusing, both during gross and microscopic examination. Albeit beyond the scope of this chapter, the importance of acquiring basic information from the relevant literature, including basic texts on animal reproduction and theriogenology, cannot be overemphasized.

Miscellaneous lesions

Intrafollicular hemorrhage occurs commonly in calves and is also found in follicular cysts in the bitch and occasionally in atretic follicles of cows. Hemorrhage occurs during ovulation in all species of domestic animals, but is usually minimal and largely confined to the cavity of the collapsed follicle. However, it can be severe and occasionally even lethal in the mare. In the mare in the autumn (fall transition), hemorrhage occurs in anovulatory follicles (hemorrhagic anovulatory or "autumn" follicles).

Focal areas of serositis develop adjacent to corpora lutea, indicating that they occur following ovulation. The recently formed strands of tissue, which are referred to as **ovulation tags**, are composed of fibrin, proliferating capillaries, and slight infiltrates of leukocytes. As the lesion regresses, neutrophils are replaced by lymphocytes, macrophages, and plasma cells, and mesothelial cells cover the tags. Fine bursal adhesions may result, but they are too delicate to interfere with ovulation or the passage of ova into the uterine tube. Ovulation tags occur in all species, but most frequently in the cow and mare. Scars that form on the surface of the ovary are small and of no consequence.

A significant form of **ovarian hemorrhage** is that which follows the older clinical practice of manual enucleation of corpora lutea in cattle. The blood loss may vary from 0.5 L to several liters and cause death. Hemorrhage is more profuse in pregnant cows and those with pyometra than in normally cycling cattle. In cases of pyometra and salpingitis, the release of inflammatory detritus into the bursa often results in the formation of extensive adhesions between the uterine tube and ovary. The expressed mass of luteal tissue persists indefinitely as a roughly spherical, flattened soft mass. The mass develops a fibrous capsule enclosing the necrotic luteal cells and can be differentiated from a lipoma.

Postparturient vascular lesions occur frequently in the bovine ovary. The changes consist of intimal proliferation of mucoid tissue in ovarian arteries and hyalinization of the walls of arteries and arterioles. Some of the more severely affected vessels occasionally undergo thrombosis. Degeneration of arterioles also occurs in rapidly regressing corpora lutea associated with abnormally short estrous cycles.

Oophoritis is relatively rare and, when it occurs, it is usually pyogenic (Fig. 4-25). Some cases of lymphocytic

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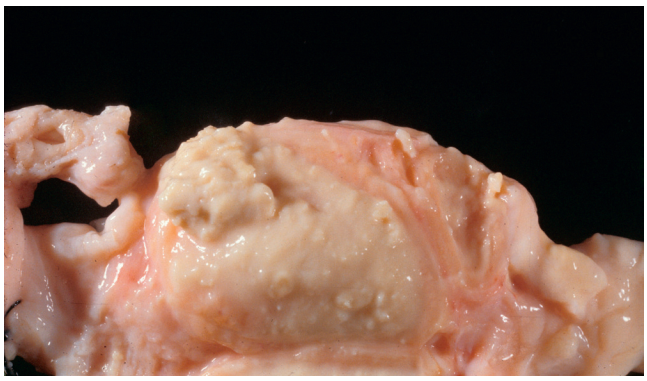

Figure 4-25 **Purulent oophoritis** has destroyed the ovary of this cow. This cow also had suppurative salpingitis and metritis. The uterotubal junction usually prevents ascending infection from an infected uterus.

oophoritis are reported in dogs as a potential cause of ovarian and ovulatory failure.

Ovarian abscesses may follow enucleation of the corpus luteum in cows with pyometra or from oocyte retrieval using needle aspiration. Serosal granulomas on the ovary occur in bovine peritoneal tuberculosis and in brucellosis, visible macroscopically as small red nodules or tags. Similar lesions are usually visible on adjacent serosal surfaces of the genital organs and adnexa. These infective granulomas remain localized to the surface of the ovary and do not penetrate its substance.

Perioophoritis occurs in cats with feline infectious peritonitis and secondary to salpingitis. The characteristic lesions of feline infectious peritonitis should include a plasma cell–rich inflammatory process; confirmation of the presence of the feline coronavirus is by immunohistochemistry.

Age-related degenerative changes

Equine ovarian varicosities

The surface of the equine ovary is richly endowed with veins that, with age, commonly become varicose. Thrombi may form, and if extensive can lead to infarction or ischemic damage to the ovary. Ovarian varicosities are rare in other species. They appear as dark, nearly black serpentine, fluid-filled tissue on the surface of the ovary (Fig. 4-26A, B).

Ovarian cysts

Cysts arise either within or adjacent to the ovary. Several different types of cysts develop from embryonic structures, including cysts of the mesonephric duct, mesonephric tubules, and paramesonephric duct (see Fig. 4-15). These cysts are discussed briefly in this section.

There are 3 types of **cysts that develop from mesonephric tubules**: cystic rete, cystic epoophoron, and cystic paroophoron. A **cystic epoophoron** (see Fig. 4-16) develops from the cranial mesonephric tubules, whereas a **cystic paroophoron** occurs less commonly and is found on the caudal surface of the ovary. Because these cysts are found near the ovary, they are casually referred to as *paraovarian cysts*. More precise identification can be achieved through closer examination (the location relative to the ovary is very helpful) or by histopathology. Cysts of the epoophoron and paroophoron are movable cysts on the cranial surface of the ovary and have a thin wall of connective tissue and muscle fibers, are lined by

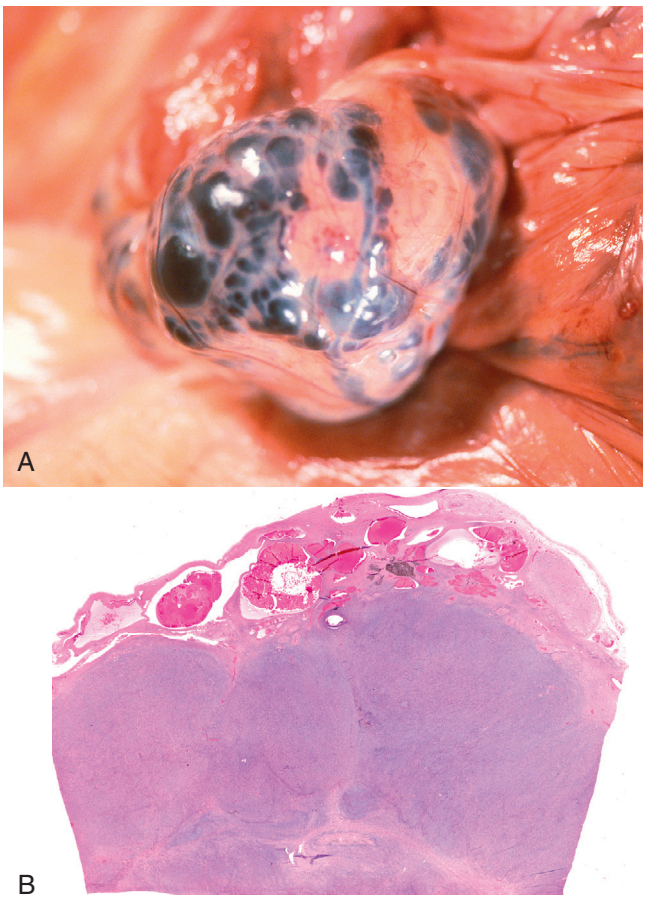

Figure 4-26 **A. Varices** on the surface of the ovary of an older mare. **B.** Subgross photograph showing the distended veins on the surface of this ovary. These vessels commonly thrombose.

low columnar epithelium with clear cytoplasm, and have a basement membrane. Follicles may protrude from the surface of the ovary, but are not movable.

Cystic rete (Fig. 4-27) develop from mesonephric tubules that form the rete ovarii within the ovary. Less commonly, cystic extraovarian rete are found. **Cystic rete tubules** have been observed in the bitch, queen, and cow. Those in the cow seldom attain significant size, but those of the bitch and queen occur commonly and can become large enough to be confused with cystic follicles, cyst adenomas, or cyst adenocarcinomas. Cystic rete are differentiated from cystic remnants of the mesonephric duct by the absence of smooth muscle in their wall. Cystadenomas of the ovary may arise focally in these cysts, but these are exceptionally rare.

Cysts of paramesonephric duct origin are very common in the mare. They are located on the fimbria of the oviduct and are called *fimbrial cysts, hydatids of Morgagni, or accessory oviducts.*

Cystic subsurface epithelial structures (cystic SES) of the bitch. The modified peritoneal cells covering the surface of the ovary of the bitch normally extend into the ovary a short distance, where they are arranged as small, single, epithelial-lined cavities called "subsurface epithelial structures" or SES. Their importance is 2-fold. They frequently give rise to single, or more commonly, multiple cysts extending along the ovarian surface, and SES often undergo papillary hyperplasia and neoplastic transformation. Neoplasms of the SES are usually adenomas, but carcinomas do occur. These tumors tend to

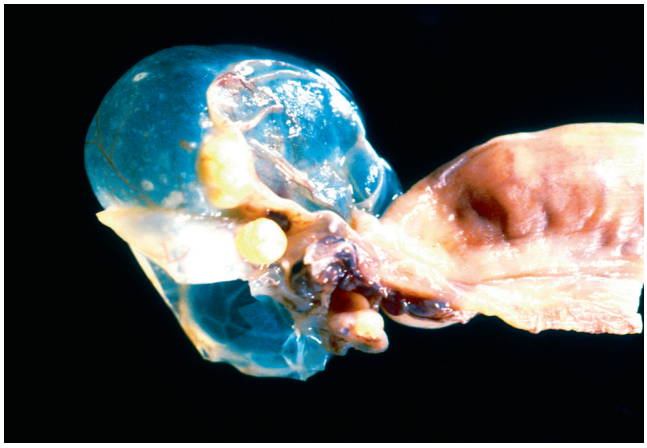

Figure 4-27 Cystic rete ovarii have compressed ovarian tissue in this cat. Cyst rete are common in the ovaries of queens and frequently occur in bitches. They develop in the hilar area of the ovary. They can be single or multiple.

form cysts and have a folded, papillary pattern. They are often multifocal.

The incidence of cysts of the SES increases with age. They are small, seldom larger than 5 mm in diameter, and can be located anywhere within the ovary, but their relationship to the surface is usually obvious. These cysts are lined by cuboidal epithelium. They do not appear to have any effect on fertility, but because the SES are hormonally sensitive, cyst formation may be associated with hormonal dysfunction. The lining of the cysts, like the surface epithelium and the SES, expresses cytokeratin, and this allows differentiation from atretic follicular cysts.

Germinal inclusion cysts of the mare

Entrapment of small segments of peritoneum is associated with ovulation, and in the mare leads to the development of inclusion cysts that are found near the ovulation fossa (Fig. 4-28). They are also referred to as *fossa cysts*. Although rather commonly encountered as one or a few small cysts lined by epithelial cells (small follicles are lined by granulosa cells) near the ovulation fossa, they are rarely numerous or large enough to interfere with ovulation. Occasionally, their expansion can lead to atrophy of compressed ovarian tissue with loss of function.

Cystic follicular disease

Anovulatory cystic ovarian disease occurs in most, if not all, species. Its importance as a disease entity varies greatly among species; in domestic species, *it is only a serious problem in cows and sows.* Cystic ovarian disease occurs infrequently in the bitch and queen and rarely in the mare, ewe, doe, and camelids.

Diagnosis depends in part on differentiating anovulatory cystic follicles from normal antral or tertiary follicles. An important criterion for this differentiation is size. Follicles larger than they should normally be at ovulation, persistence, and possible associated signs of hyperestrogenism are features of cystic follicular disease.

Tertiary ovarian follicles vary greatly in size between species, ranging from about 3 mm in the queen up to 7 cm in the mare! Normal follicular diameters near ovulation are approximately 2 cm in the cow, 1 cm in the sheep and pig, and 0.75 cm in the dog. Knowledge of the stage of the cycle

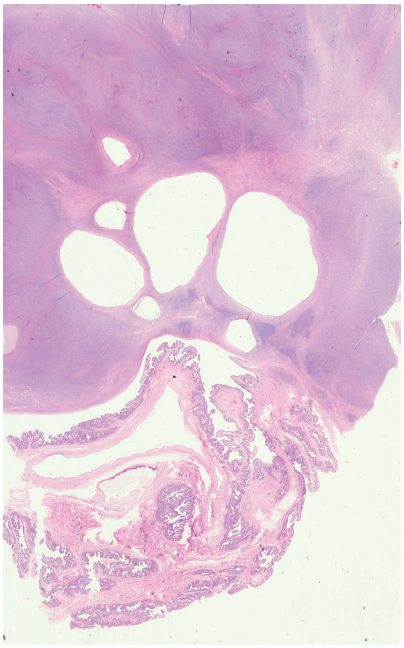

Figure 4-28 Inclusion cysts of various sizes in the ovarian stroma adjacent to the ovulation fossa in a mare. The cysts form from small pieces of surface epithelium (modified peritoneum) that become entrapped after the surface is disrupted at ovulation. They are usually multiple. These persist for long periods of time and slowly increase in size, with resultant atrophy of the ovary when they become very large. Their location adjacent to the ovulation fossa and persistence and slow increase in size are characteristic features of germinal inclusion cysts in the ovary of mares.

is also helpful, but disappointingly is provided inconsistently in the histories accompanying clinical case submissions. The examining pathologist should persist and insist on receiving as complete a reproductive history as possible.

It is only in cattle that the condition has received detailed study. Accordingly, the major consideration here is of the bovine disease.

Cystic ovarian disease in cows

A well-known feature of anovulatory follicular cysts is their relation to nymphomania, but *most ovarian cysts are not associated with signs of persistent estrus.* The behavior of cows with cystic ovaries is variable. The majority of cows with ovarian cysts are anestrous. The disease arises from the failure of mature follicles to ovulate. It occurs most often before the first postpartum ovulation. Approximately 45-60% of animals that develop anovulatory follicular cysts will re-establish normal ovarian cycles spontaneously. Cystic follicles may also develop after postpartum ovarian cycles have been established, and these cysts are more likely to persist if effective treatment is not instituted.

The *cause* of cystic ovarian disease is not understood in any species. The disease in cattle occurs more frequently after parturient or postparturient disease, and there is evidence that *intrauterine infections* play a role in the pathogenesis of the disease. There is clearly a *genetic predisposition* to the disease in certain families. The daughters of cows that have had cystic ovaries have a substantially increased risk of developing the disease as compared to the general population. The disease tends to involve primarily dairy cows, but it can occur in cattle

of any breed if they are withheld from breeding for a prolonged period of time. One of the factors that has made the understanding of the disease difficult is the definition of the disease itself. *Follicular cysts in cattle are usually defined as follicles >2.5 cm in diameter that fail to ovulate and may persist*. By the time a follicle can meet the criteria to be defined as cystic, the conditions that led to its formation have passed and are not available for study. To offset this difficulty, anovulatory ovarian disease has been produced by a variety of experimental techniques. Unfortunately, it is not known whether any, or all, of the experimental manipulations induces anovulatory cystic disease by the mechanisms that operate in the natural disease.

The most widely held theory of the *origin of cystic ovarian disease* has involved aberration of the preovulatory surge of luteinizing hormone, either the absence of the surge or the mistiming of the surge. This hypothesis, or some modification of it, is still the most attractive. Cows that develop cystic ovaries as the result of estrogen and progesterone treatment have an increased mean basal concentration of luteinizing hormone secretion, with increase in the frequency and amplitude of pulses, but the characteristic preovulatory luteinizing hormone surge is deficient. This increase in luteinizing hormone secretion is thought to be due to aberrant hypothalamic function altered experimentally by steroids or naturally by ovarian secretion.

The cysts may be single (Fig. 4-29) or multiple on one or both ovaries. These cysts may persist, but during the course of the disease, additional cysts may be recruited, and some cysts undergo atresia. Patches of luteal tissue can be seen grossly in the wall of some of the cysts and can be recognized histologically in about a quarter of them. The walls of the cysts show the same type of degeneration that occurs in normal atresia. Degeneration of the granulosa cells takes place first, the cells undergoing pyknosis and karyorrhexis and sloughing into the cyst lumen. The oocyte also undergoes degeneration. The changes of the theca interna are variable. The theca is partially luteinized in some cases, whereas in others it degenerates and is infiltrated by fibrous tissue. The luteal tissue may occur in patches or form a crescent of variable thickness.

Luteinized cyst

This type of cyst develops when ovulation fails to occur and the theca undergoes luteinization. There is no ovulation papilla, and the luteal mass is smooth and rounded (Fig. 4-30). The cavity of the cyst is spherical and lined by a layer of fibrous tissue adjacent to the zone of luteinized cells. Luteinized cysts occur more frequently in *cattle and swine* than in other species of domestic animals. Partial luteinization of the wall of follicular cysts is a common feature in the bitch. In cattle they usually occur as single cysts. Single luteinized cysts may occur in pregnant sows, but multiple cysts are associated with infertility.

Anovulatory luteinized cysts should not be confused with cystic corpora lutea. A cystic corpus luteum is a corpus luteum that has formed after ovulation and in which a central cavity has persisted in a mass of developing luteal tissue. Cystic corpora lutea are not evidence of ovarian malfunction. They form after ovulation, and they do not affect the length of the estrous cycle. If the cow has been successfully bred, the cavity will slowly become obliterated. Large central cysts may occasionally persist for 30-40 days after conception. *Cystic corpora lutea can be distinguished from luteinized cysts by the ovulation papilla that distorts the outline of the cyst at the point of ovulation*. Additionally, their size is greater reflecting their development from cystic follicles.

Ovarian epidermoid cysts have been recognized in cattle. These are reported to be multiple and small and have a squamous epithelial lining that forms keratin.

Extra-ovarian lesions associated with cystic ovarian degeneration in cows

Changes in other organs develop only if the ovarian cysts persist. Because most cases are responsive to treatment,

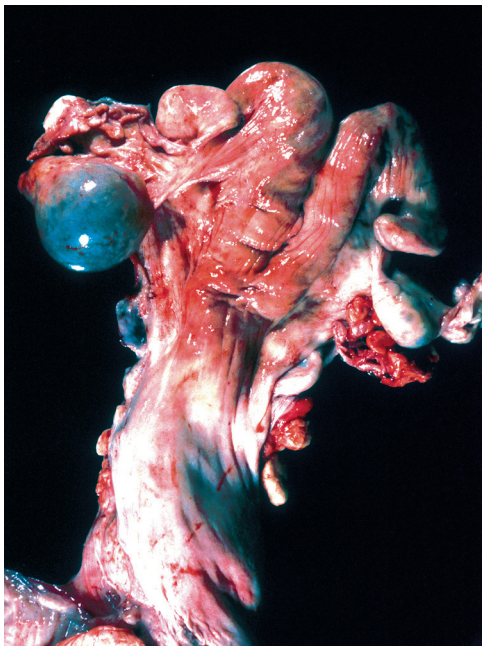

Figure 4-29 Cystic follicle in the ovary of a cow. This condition is thought to be due to inadequate luteinizing hormone release, resulting in development of an **anovulatory follicle,** the lining of which may luteinize to form a luteinized follicular cyst.

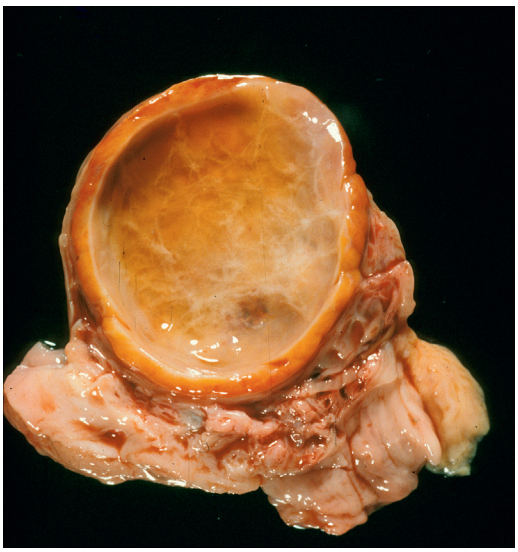

Figure 4-30 Luteinized follicular cyst from a cow. Note the uniform layer of luteinized cells that line the transected cyst. Because these develop from follicles that have not ovulated, there is no ovulation papilla.

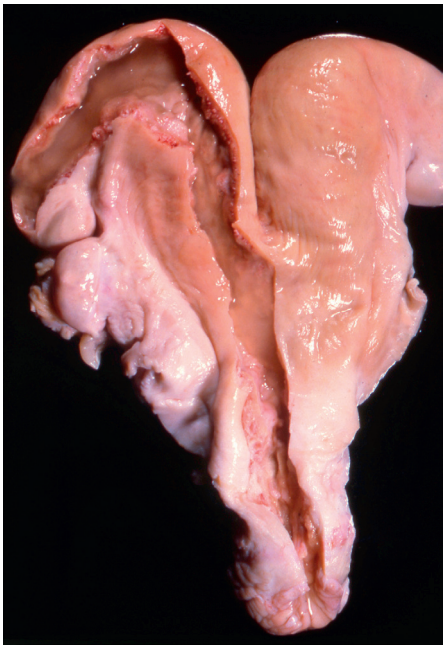

Figure 4-31 **Mucometra** in the uterus of a cow. The endometrium undergoes hyperplasia caused by the long-term release of estrogens usually in cows with follicular cysts. In cases where the accumulated secretions are less viscous, the condition is call *hydrometra*. If the accumulations are large, the resulting pressure will lead to atrophy of the endometrium.

this is now the exception. It has been shown that in cystic ovarian disease the hormone limits are within normal concentrations; however, the toxicity of ovarian hormones depends not on the absolute levels, but in the loss of cyclicity and on persistence.

In established cases of cystic ovarian disease, *the uterus is usually altered*, the differences in degree being related to the duration of the condition. In association with functionally active follicular cysts, the uterus is enlarged and the wall is edematous (Fig. 4-31).

The *cervix* is enlarged, the external os patent, and the plicae edematous. The endometrium may appear grossly normal, being smooth, semitransparent, gray-pink, and moist. There may be some degree of *cystic endometrial hyperplasia* detectable by the naked eye as tiny gray-white elevated blisters in the surface of the endometrium, which is overlain by a thin layer of clear viscous mucus. The accumulation of mucus *(mucometra)* and the development of cystic endometrial hyperplasia progress with time and are most striking in those animals that have been affected long enough for atrophy of the ovarian cysts to have occurred and the animal to have become anestrous. By then, the endometrium has become very cystic and the volume of mucus accumulated to 100-1,000 mL or more.

The cervix may be hypertrophic or atrophic, as described previously. It usually contains thick, viscid, gray-white, and cellular mucus. The epithelium undergoes squamous nonkeratinizing metaplasia of mild degree. The vagina is edematous when the cysts are active, but otherwise the most striking change is the formation of *cysts in Gartner's ducts*. These mesonephric duct vestiges that lie in the wall of the vagina, one on each side of its floor, are normally visible only microscopically as continuous or discontinuous ducts lined by a simple cuboidal epithelium. When chronically stimulated by estrogen, the epithelium becomes squamous and the ducts cystic and visible, or palpable, as a series of blebs or as tubules up to 1 cm in diameter on the floor of the vagina.

Cystic Gartner's ducts are usually accompanied by *cystic Bartholin's glands*. Either may become abscessed. Bartholin's glands, one on each side of the floor of the vulva, undergo cyclic secretory changes during the estrous cycle, and a squamous epithelium on the ducts is normal in estrus. Exaggeration of this change following long-term exposure to estrogens causes occlusion of the ducts and the formation of retention cysts. The vulva may be edematous when the cysts are active, and the clitoris may be enlarged in long-standing cases.

Cystic ovarian degeneration in other species

In **swine**, *cystic ovaries are common and are an important cause of infertility*. Failure of a single follicle to ovulate need not interfere with pregnancy and single large cysts, 2-3 cm in diameter, may be found in pregnant animals. They are presumed to represent mature follicles that did not ovulate, but single anovulatory cysts may also be associated with irregularity of the estrous cycle. The association is more clearly manifested with multiple cysts. The cysts vary in type from follicular, which are lined by normal granulosa cells, to luteinized cysts. Multiple luteinized cysts, with some cysts up to 5 cm in diameter, are a distinctive feature of cystic ovarian disease in swine. The luteal tissue may form a complete rim or be present in patches. The endometrium in cases of multiple luteal cysts shows hyperplasia of progestational type, and in long-standing cases the clitoris is enlarged.

Cystic degeneration of the ovarian follicle, with loss of cyclicity and infertility comparable to that of the cow and the sow, does not appear to occur in the **mare**, but the matter is not clear because anovulatory follicular cysts do develop during the winter anestrous period, and some of these mares will show irregular signs of estrus. The number and size of these anovulatory cysts vary considerably. There is no evidence that development of these cysts, regardless of their size, is indicative of reproductive disease.

In the **bitch**, anovulatory cysts of both follicular and luteinized type tend to occur in older age groups. Grossly, they tend to be multiple and commonly have partial luteinization of cyst walls (Fig. 4-32). The incidence of cystic ovarian disease is low in the bitch, but there is some confusion on this point because cystic rete tubules, cysts of SES, paraovarian cysts, and cystadenomas have been confused with follicular cysts.

Polycystic ovarian disease in the bitch is rare, but because it can cause hyperestrogenism, it carries a special risk in the dog because of the species' sensitivity to either endogenous or exogenous estrogen, which can induce lethal bone marrow suppression. The condition can involve either one or both ovaries, the affected ovary being greatly enlarged by multiple, thin-walled cysts that vary from 1-12 mm in diameter (see Fig. 4-32). The disease develops in mature animals that may be either nulliparous or multiparous. If clinical signs occur, they are apt to be associated with *hyperestrogenism* and include persistent estrus with cornification of the vagina and swelling of the mammary glands or the toxic effects of hyperestrogenism on the bone marrow, with resultant pancytopenia. This pancytopenia can result in anemia, thrombocytopenia with bleeding, most often occurring as epistaxis, or neutropenia with increased susceptibility to infection. The mechanism of the inhibitory action of estrogen on the hematopoietic tissues

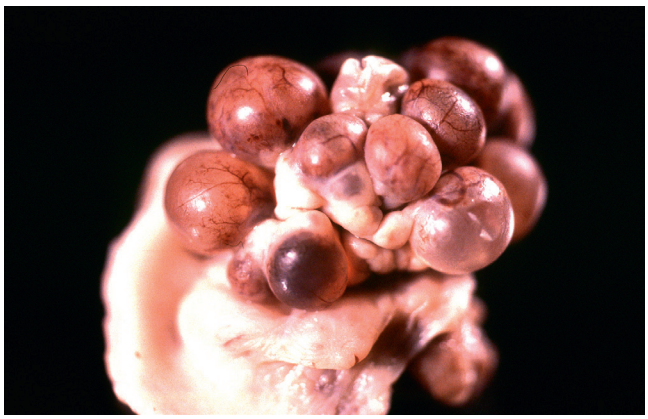

Figure 4-32 Multiple follicular cysts in a bitch. Externally, many of the nodular structures have the appearance of corpora lutea. Others have thin clear walls and look like enlarged follicles that contain clear fluid. These anovulatory follicles are lined by granulosa cells that frequently undergo partial luteinization. The amount of luteinization accounts for the difference in appearance. This is analogous to the luteinization that occurs in cattle with the persistent anovulatory follicles shown in Figure 4-30.

in estrogen-sensitive species is not known. Estrogen appears to inhibit the differentiation of pluripotent stem cells while stimulating the differentiation and maturation of committed stem cells.

Most of the cysts are obviously of follicular origin but in various stages of atresia. Degenerating oocytes can be identified in some of the cysts, but the disease is not just one of anovulation because in some cases hundreds of follicles have been recruited. The granulosa and theca lining of the cysts is attenuated in the larger ones. Patchy areas of luteinization form in the walls of some of the cysts. This luteinization may involve individual cells or sheets of cells and either the theca or the granulosa.

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NEOPLASTIC CONDITIONS OF THE OVARY

The differential diagnoses for enlargement of the ovary include a number of non-neoplastic conditions:

- Normal cyclical changes (tertiary follicles, developing corpora lutea)
- Anovulatory follicles
- Ovarian hematoma
- Oophoritis
- Ovotestes
- Cystic conditions of ovarian structures

The types of ovarian cysts vary between species. For example, common ovarian cysts found in the ovaries of bitches include cystic rete and cysts arising from the subsurface epithelial structures; cysts found in the equine ovary include epithelial inclusion cysts arising in the area of the ovulation fossa and hemorrhagic anovulatory follicular (HAF) cysts. These conditions must be differentiated from primary ovarian neoplasms that occur with variable frequency in all species of domestic animals.

In general, ovarian neoplasms tend to occur in mature or older animals and may be associated with behavioral changes or changes in target tissues if the tumor is endocrinologically functional (e.g., stallion-like behavior in mares with granulosa cell tumors that are producing androgens). Although rare, metastases from neoplasms in other sites may also be found in ovaries (in cattle, lymphosarcoma can be found within corpora lutea; in dogs, mammary gland carcinomas can metastasize to many organs, including the ovaries).

Tumors with a phenotype of specifically ovarian tissue can be divided into 3 broad categories: tumors of the **surface celomic epithelium**, tumors of the **sex cords** and **gonadal stroma**, and tumors of **germ cells**. Tumors developing from nongonadal supporting tissues of the ovary can be of the usual variety of fibroblastic, smooth muscle, and vascular tumors, but are, in fact, uncommon in domestic animals.

- Sex cord–stromal tumors
  - Granulosa-theca cell tumors
  - Thecoma or luteoma
- Tumors of the surface epithelium and the SES
  - Papillary and cystic adenomas
  - Papillary adenocarcinomas
- Germ cell tumors
  - Dysgerminoma
  - Teratoma

Primary ovarian tumors occur most frequently in the bitch, mare, and cow. In the bitch, they are often bilateral, especially those arising from the surface epithelium and the SES. In the cow, ewe, and mare, they are usually unilateral and of the gonadal-stromal type. In cows, there is a tendency for the tumor to occur in the daughters of affected dams and, in these animals, removal of the affected ovary may be followed by the development of a tumor in the other ovary. Very few ovarian neoplasms have been reported in the feline and porcine species. The tumors of the cat are similar to those of the bitch.

Sex cord–stromal tumors

These are commonly tumors of granulosa and theca cells and their luteinized counterparts. The vast majority are mixtures of phenotypes with *granulosa cells and theca cells predominating and often coexisting in the same tumor* (e.g., *granulosa-theca cell tumors)*. Some tumors histologically resemble testicular tumors of the Sertoli cell and interstitial endocrine cell types. *This group of tumors frequently produces hormones.*

The **granulosa-theca cell tumor** is the most common tumor in this group. They are *generally unilateral, usually nonmalignant tumors* in any species. Although they may be observed in young animals, the incidence increases with age. Part of their interest lies in the production of steroids by these tumors.

In *mares*, in which species the tumors are common and have been studied most carefully, 3 behavioral patterns have been recognized: *anestrous, continuous or intermittent estrus, and male behavior.* Surprisingly, testosterone levels in the peripheral plasma are elevated in most cases. Male behavior is usually only seen in those cases in which the testosterone levels are >100 pg/mL plasma. Some mares with granulosa-theca cell tumors also have elevated estrogen levels, but these

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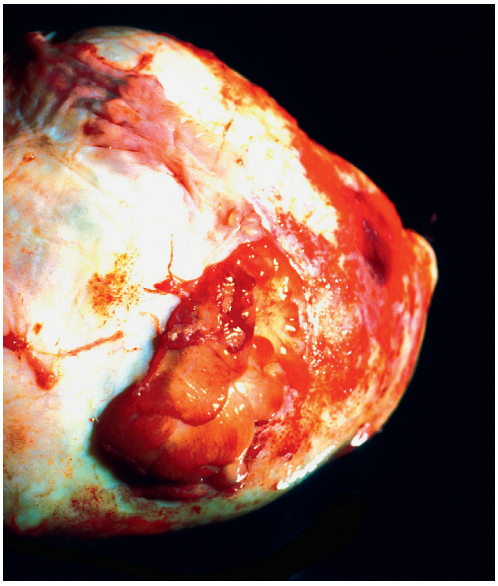


Figure 4-33 Enlarged ovary from a mare that has been nearly completely replaced by a large expanding **granulosa cell tumor.** The red tissue on the surface of the mass in the center is the displaced congested fimbria. The ovulation fossa is lost due to expansion of the tumor.

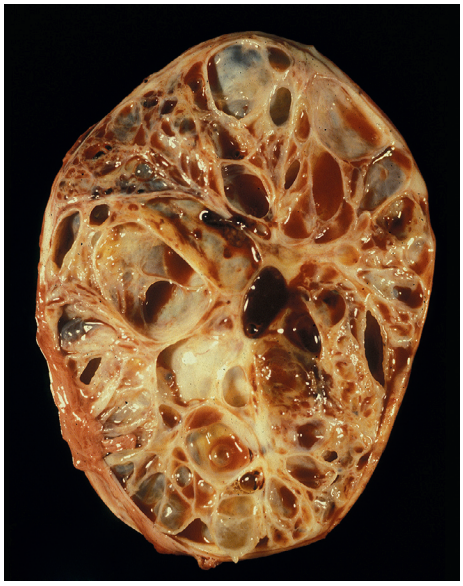


Figure 4-34 Granulosa cell tumor from a mare. The tumor has been bisected and reveals a very characteristic gross appearance. Neoplastic granulosa cells form follicle-like cavities lined by neoplastic granulosa cells. Solid forms of this tumor occur less commonly.

elevations are less clearly related to behavioral patterns. Even in cases in which hormone production associated with the tumor is low, *atrophy of the opposite ovary usually occurs*. The cause of this atrophy has not been determined. It was thought to be the result of testosterone production by the tumor, but convincing association between testosterone levels and ovarian atrophy has not been established, and androgen production by tumors other than the granulosa-theca group has not caused atrophy of the contralateral ovary. Granulosa cells produce the peptide hormone, *inhibin*, which inhibits release of follicle-stimulating hormone. This hormone has been demonstrated to be elevated in granulosa cell tumors, and it may be that the production of inhibin produces the characteristic ovarian atrophy seen in this disease. Use of serum anti-Müllerian hormone has recently been shown to be a sensitive indicator for diagnosis of granulosa cell tumors in cows and mares.

Normal function of the atrophic ovary usually returns within a year of removal of the tumor. In bitches with sex cord–stromal tumors, *cystic endometrial hyperplasia and pyometra* are common. Vaginal changes can be used to monitor estrogenic influences.

The surface of the granulosa-theca cell tumor is smooth (Fig. 4-33), and the cut surface may be solid or cystic (Figs. 4-34, 4-35). The solid portions of the tumor are white or yellow, depending on the lipid content. The cells in these tumors resemble their counterparts in normal follicles, but their histologic arrangement is quite varied. These tumors commonly contain areas with characteristic gland-like or rosette patterns of abortive follicles, some of which may contain a secretory globule resembling an ovum and called a *Call-Exner body*.

This type of differentiation is frequently observed in the early stages of development of bovine neoplasms, but is less common in other species and in large tumors. Instead, the arrangement of cells is usually diffuse with, in some tumors

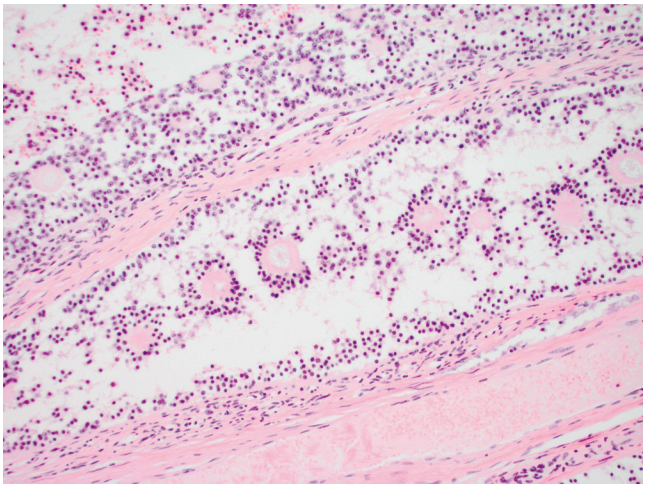


Figure 4-35 Photomicrograph showing characteristic aggregates of neoplastic granulosa cells surrounding protein globules **(Call-Exner bodies)** in an equine **granulosa cell tumor**.

or parts of tumors, a pseudoalveolar pattern, depending on the disposition of the stroma. There is a tendency for some granulosa-theca cell tumors to develop a tubular pattern similar to that of the Sertoli cell tumor of the testis (see Fig. 4-35). The stroma consists of broad irregular bands of dense collagen. Cyst formation and hemorrhage are common. The more thecomatous portions of such tumors may resemble normal theca cells and, at the other extreme, be distinguishable from plump fibroblasts only by the demonstration of sudanophilic droplets in the cytoplasm or by histochemical techniques for steroids.

The cysts that often make up the bulk of the tumor are lined by granulosa cells that are surrounded by a variable population of thecal cells (Fig. 4-36). In some equine tumors,

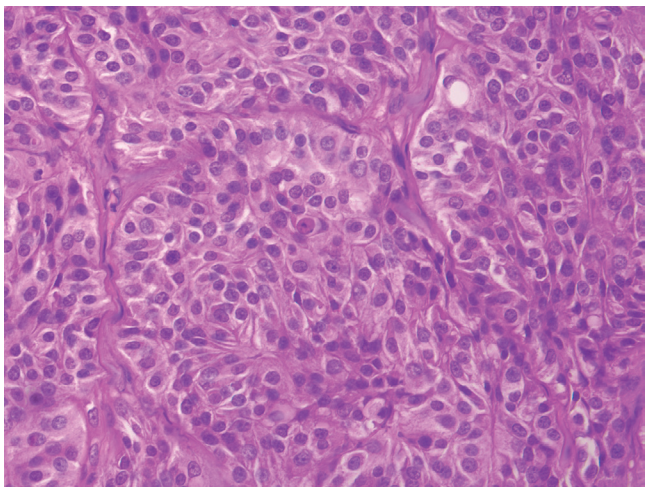


Figure 4-36 Photomicrograph of a **granulosa cell tumor** from a mare. Neoplastic cells are aligned perpendicular to the fibrous septa.

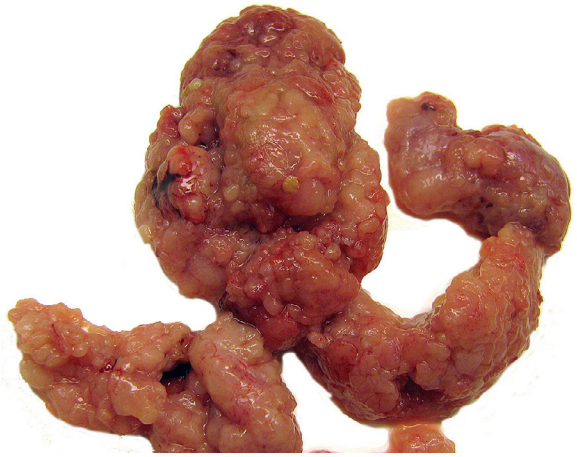


Figure 4-37 **Papillary cystadenocarcinoma** of the ovary of a bitch. The ovary is enlarged, and its surface is very irregular. In adenocarcinomas, neoplastic cells frequently penetrate the surface of the ovary and metastasize to the lining of the bursa and throughout the abdomen. Papillary cystadenomas are quite common in older bitches, tend to develop multifocally, and usually develop in both ovaries.

particularly those associated with high testosterone levels, embedded in the thecal cell layer are large polyhedral eosinophilic cells resembling the testosterone-producing Leydig cells. Ancillary diagnostic testing includes endocrinology. Testosterone and estradiol concentrations tend to be variable, but α-inhibin concentrations have been shown to correlate to tumor mass and to be more consistently elevated.

Tumors composed only of theca cells, **thecoma** or **luteoma**, are much less common in all domestic animals than those arising from granulosa cells or containing a mixture of theca and granulosa cells. The few tumors reported have been firm, white-to-orange, and composed of streaming oval or spindle cells that resembled the cells of the theca interna. The demonstration of lipid in the cytoplasm allows differentiation from fibromas.

Sex cord–stromal tumors often develop within ovarian remnants.

Tumors of the surface celomic epithelium

Neoplasms arising from the epithelium covering the surface of the ovary are very important tumors in women, but in nonhuman mammals, they occur commonly only in the bitch as *papillary cystadenomas and cystadenocarcinomas.* It appears that the tumor, irrespective of type, may occasionally stimulate the ovarian stroma, with concomitant production of steroid hormones.

Grossly, the papillary structures, when extending from the surface of the ovary, produce a cauliflower-like appearance (Fig. 4-37). Once free of the bursa, peritoneal implantation occurs readily. Papillary cystadenocarcinomas should be considered in the differential diagnosis of *ascites* in adult entire bitches. The ascites develops from obstruction of the diaphragmatic lymphatics by permeating tumor fragments, with perhaps a contribution by secretion by the tumor epithelium. Histologically, the epithelium varies from low cuboidal to columnar, with stratification in some areas, and mitotic figures are scant, even in the malignant form. The tumor appears to develop from the surface and from tubular structures (cortical tubes, SES) of the ovarian cortex. Many of the tubules are continuous with the surface epithelium and responsive to estrogenic stimulation.

Papillary tumors have been induced in the bitch by prolonged administration of diethylstilbestrol. The experimentally induced tumors regress following withdrawal of hormone treatment. *Tumors of the surface epithelium can occur in association with sex cord–stromal tumors*, possibly by hormonal induction by the granulosa-theca tumors. *The separate identification of the 2 tumors can be difficult, particularly in areas where the tumors join, but is aided by immunohistochemical staining.* Cytokeratin intermediate filaments, especially CK7, are expressed in the surface epithelium and the SES. On the basis of limited investigation, it appears that this specificity has been conserved during tumor development and aids in easy differentiation of the above from CK7-negative tumors of the sex cord–stromal group.

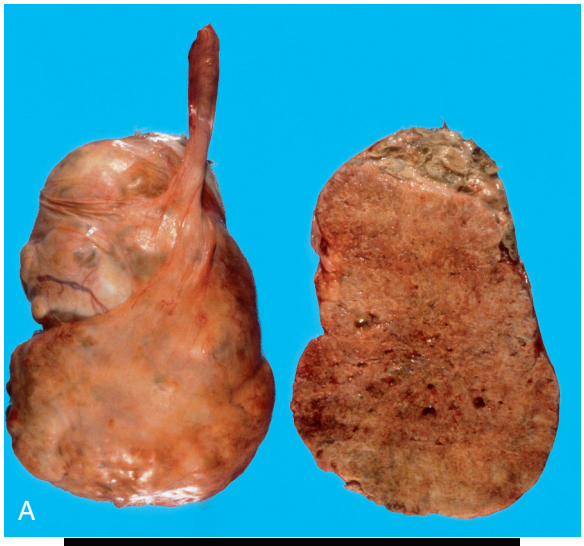
Tumors of germ cells

Germ cell tumors of the ovary, like their testicular counterparts, arise from germ cells and are usually divided into 2 main categories in domestic animals: the **dysgerminoma** and the **teratoma.**

*The **dysgerminoma** is composed of cells that show morphologic similarity to primordial germ cells and resemble their testicular homolog, the seminoma. These are relatively rare. It is usually unilateral and has been observed in the bitch, queen, cow, mare, and sow.* Grossly, the tumor is smooth, or nearly so, and relatively soft. The cut surface is gray and may have areas of hemorrhage or yellow patches of necrosis. It is composed of a uniform population of large rounded cells with large central chromatic nuclei. Mitotic figures and giant cells are frequent. The pattern of growth is diffuse; the stroma is always scanty. Just as in the canine testicular seminoma, accumulations of lymphocytes are present within the tumors.

*In **teratomas**, totipotential germ cells have undergone somatic differentiation, giving rise to 2 or more germinal layers with a variety of tissues present in the tumors.*

Ovarian germ cell tumors are rare in domestic species. In addition to the germ cell tumors that show no somatic differentiation (dysgerminomas) and tumors that have extensive



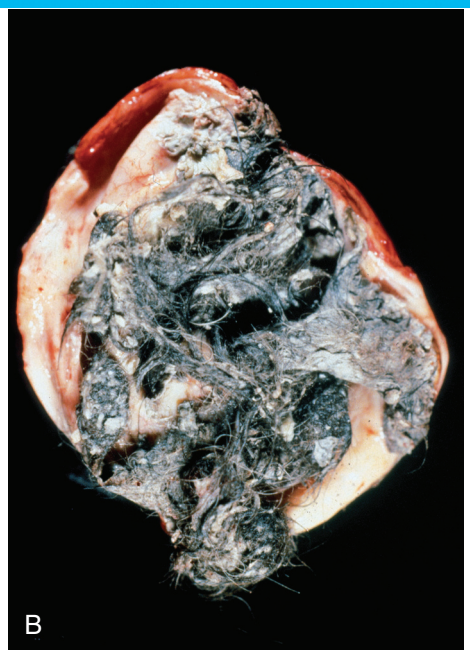


Figure 4-38 A. Teratoma in the ovary of a bitch. The ovary is enlarged, effaced and contains hard areas (bone and cartilage) and cystic spaces. **B. Equine ovarian teratoma.** The large cystic mass containing mats of hair. A variety of tissues can often be identified in these tumors, including bone, teeth, nervous tissue, intestine, and muscle.

differentiation into tissues of multiple germinal layers (teratomas), there are reports of other rare tumors such as endodermal sinus tumors (yolk sac tumors), choriocarcinomas, embryonal carcinomas, and tumors from single germ layers (epidermoid cyst). This variability is to be expected in tumors arising from pluripotential germ cells.

Teratomas usually have solid and cystic areas containing sebaceous material and hair (Fig. 4-38). Various other tissues are often present, including neural tissue, adipose tissue, bone, teeth, and respiratory epithelium (Fig. 4-39).

There were several theories on the histogenesis of teratomas, but it is now believed that the benign cystic ovarian teratomas are *parthenogenic tumors* that develop from a single germ cell that has completed its first meiotic division, but not its second. The evidence for this has come from a series of

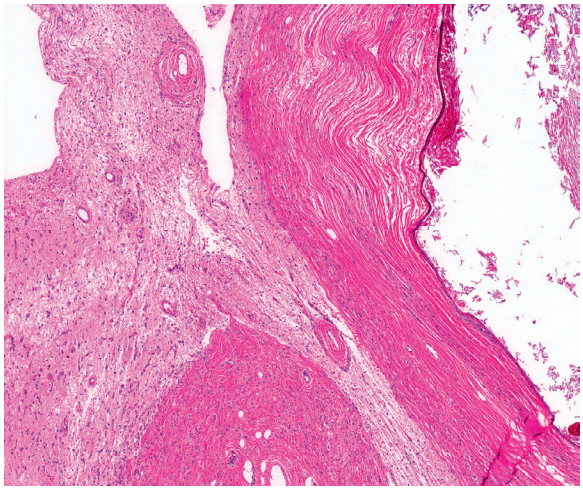


Figure 4-39 Photomicrograph of a typical teratoma with multiple tissue types.

elegant cytogenetic and biochemical studies that have shown that tissues from ovarian teratomas are unique in that they are XX diploid cells, but are homozygous at chromosomal loci for which the host is heterozygous. This lack of heterozygosity is most reasonably explained as being the result of meiotic division.

Tumors of nongonadal tissues

Ovarian hemangioma is rare in all domestic animals except the pig. Ovarian hemangiomas are globular, well circumscribed, and tan to red-brown. Their surface is smooth and glistening and contains prominent vessels. The tumors arise in the ovarian cortex, and they are occasionally bilateral. The tumor is composed of well-differentiated endothelial cells that line vascular clefts and spaces.

Ovarian tumors of other supporting tissues are very infrequent. **Leiomyomas** developing from the smooth muscle in the mesovarium have been reported in the bitch, queen, and sow. **Lipomas**, **fibromas**, and **rhabdomyosarcoma** occur in the bursa of the dog. Pathologists unfamiliar with the appearance of the ovarian stroma, particularly in the mare, sometimes mistake normal tissue for fibroma or leiomyoma. **Lymphoma** can affect the ovary of cattle, dogs, and cats. In cattle neoplastic foci tend to develop within corpora lutea (Fig. 4-40).

Metastatic tumors

Secondary tumors of the ovary are probably not less rare than primary ones, the relatively high incidence largely being attributable to secondary deposits of lymphomas. Mammary carcinomas, endometrial carcinoma and canine transmissible venereal tumor in the bitch, and intestinal carcinomas in the cow may metastasize to the ovary and apparently have an affinity for corpora lutea.

Vascular hamartomas

Vascular hamartomas of the ovary are included with tumors because they may be confused with neoplasms and because granulosa-theca cell tumors occasionally occur in the same gonad. *Hamartomas are tumor-like malformations that may be present at birth* and cease to grow after the animal reaches maturity unless the mass is subjected to trauma, infection, or vascular embarrassment. They have been observed in the cow, mare, sow, and bitch. The mass of malformed vessels may vary

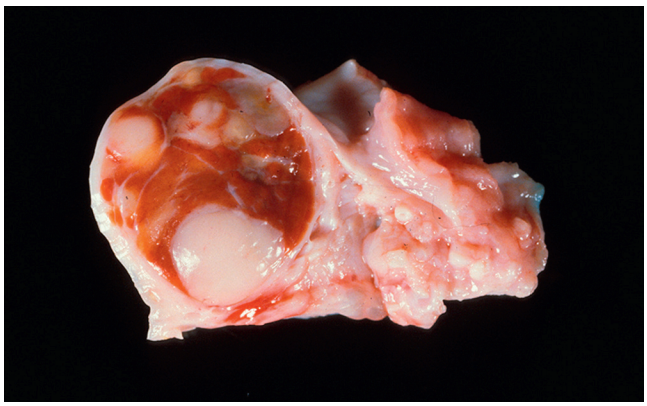


Figure 4-40 Bovine ovarian lymphosarcoma. Several soft pale masses of neoplastic lymphocytes are present within a corpus luteum. Although the uterus is a predilection site for bovine lymphosarcoma, ovarian tissues, especially corpora lutea, are frequently involved.

in size from those barely visible on gross examination to those weighing several kilograms. The smaller hamartomas are composed principally of mature-appearing, tortuous arteries and veins with relatively little intervascular connective tissue. In the mature individual, the increase in size of the hamartoma is due to thrombosis, with subsequent edema, hemorrhage, and necrosis, followed by proliferation of fibrous connective tissue. Small hamartomas are clearly demarcated from the adjacent ovarian tissue, but the ovary is gradually replaced as the mass enlarges. *The distinction between hemangiomas and vascular hamartomas is a difficult one*, and it may be that some of the reported ovarian hemangiomas are, in fact, vascular hamartomas.

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PATHOLOGY OF THE UTERINE (FALLOPIAN) TUBES

Primary lesions in the uterine tubes are uncommon. *Hydrosalpinx, pyosalpinx, and salpingitis are most important*, and these are usually secondary to disease of the uterus or to

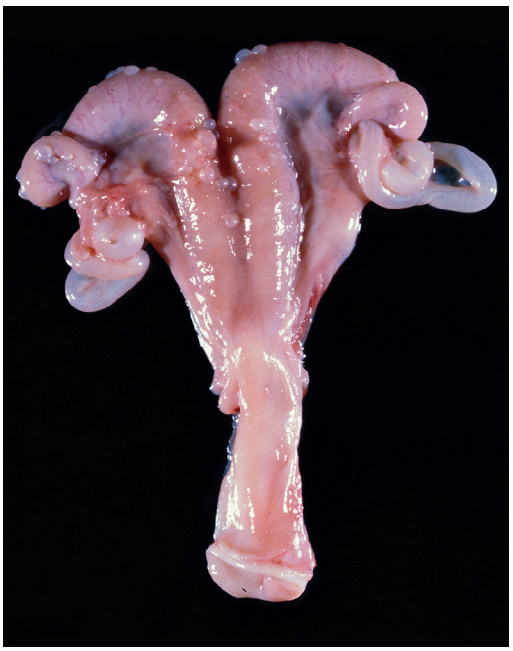

Figure 4-41 Bilateral hydrosalpinx in a sheep. Also note the small cluster of serosal inclusion cysts on the surface of the uterine horns at the top of the photograph.

manual manipulation of the ovary. As regards incidence of salpingeal lesions, the only agreement is that they are much more common than is their diagnosis. They are recognized to be important in the cow and sow, but not in other species.

Hydrosalpinx

Hydrosalpinx is so called because the uterine tube is distended, uniformly or irregularly, up to 1.5 cm or so, with clear watery mucus that fluctuates. The tube appears to be increased in length and tortuosity and is thin walled. Histologically, there may be extensive multilocular cyst formation in the mucosa with obliteration of the lumen, and in some chronically inflamed uterine tubes, there is extensive interstitial fibrosis.

Distension of the uterine tube by fluid follows loss of patency of the lumen (Fig. 4-41). *The obstruction may have a congenital or inflammatory basis.* Congenital anomalies involving the tubes are very rare except in freemartins, but secondary hydrosalpinx can be associated with obstructions and segmental aplasias of the uterine horns. In the latter instances, the apex of the horn is usually distended with fluid also. Acute septic inflammations are more likely to produce *pyosalpinx*; chronic infective inflammations tend to produce loculations and stenosis of the lumen with *secondary hydrosalpinx*. As almost all infections are of ascending type from coexisting uterine inflammation, the most severe changes and the obstruction in such cases are usually at or near the uterine end of the tube.

A common form of inflammation that results in hydrosalpinx in cattle is at least in part *traumatic in origin, following manual manipulation of the ovary.* Uterine irrigations are somewhat less important, but in either event, it is the adhesion between the tube and adnexa (usually the ovary with partial or complete obliteration of the bursal cavity) that causes obstruction of the abdominal ostium and secondary hydrosalpinx. Squashing of the normal diestrual corpus luteum (there

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is at best only partial expression of the body) is not difficult, but because of its vascularity, there is always some hemorrhage, varying in extent from very slight to fatal. Small clots, which are usually retained in the bursa, can be completely resorbed. Larger clots must be organized, and this results in the formation of adhesions within the bursa and the fimbriated portion of the tube. With more extensive hemorrhage, adhesions may form to adjacent abdominal viscera and other genital tissues.

Salpingitis

The uterine tube is a rather simple structure histologically, but even minor inflammatory changes, evidenced by slight congestion or the presence of a few plasma cells, appear to be important because of the readiness with which the epithelial cells desquamate or lose their cilia. The proper function of the living epithelium is necessary for the propulsion of the ovum, for the dissolution of the cumulus oophorus prior to fertilization, and for the maintenance of a luminal environment suitable to survival of the ovum. The salpingeal mucosa has much less capacity for restitution than does the endometrium.

Inflammation of the uterine (fallopian) tubes without significant enlargement is the most common and most important tubal lesion. It is usually bilateral; is usually not detectable macroscopically; and may show serous, catarrhal, or fibrinous inflammation. In the mildest forms of salpingitis, the mucosa alone is affected, and changes of functional significance may be slight enough to be overlooked histologically. Congestion of the mucosal vessels, mononuclear cell infiltration, loss of epithelial cilia, and some desquamation of epithelium may be the only changes detectable. With more severe infections, catarrhal exudate collects in the lumen, the mucosal folds are thickened by cellular infiltration and congestion, and the epithelium is in large part destroyed. Loss of epithelium occurs first in the free edges of the mucosal folds, and these denuded areas tend to fuse and adhere to produce intramucosal cysts. Alternatively, in chronic catarrhal salpingitis, the mucosa is virtually destroyed and replaced by proliferated connective tissue and cellular infiltrations with partial or complete occlusion of the lumen.

Salpingitis is a common lesion in animals, with both *Mycoplasma* and *Ureaplasma* infections. Nonspecific infections causing salpingitis almost invariably do so following spread from the uterus. *There is probably 70-75% association between uterine and salpingeal inflammation* when diagnosis of the latter is based on histologic evidence. In some cases there will be perimetritis with adhesions, pyosalpinx, or bursal abscess. *Adhesions* of the infundibulum of the mare are very common. The cause of these adhesions is unknown. Some are associated with perimetritis, but most are not. It has been suggested that they may develop as a result of ovulatory hemorrhage. However, this explanation does not easily account for the substantial predominance of adhesions of the right infundibulum.

Granulomatous salpingitis is uncommon. When it does occur, the uterine tube will be firm and distended.

Pyosalpinx

This is less common than hydrosalpinx and typically follows metritis in the same manner as do other forms of salpingitis. *The significant anatomic difference is the accumulation of pus in the tube following obstruction of the lumen*. The obstruction may be produced by inspissated exudate, inflammatory thickening, and fusion of the mucosal folds, or chronic granulation tissue. The length of the tube is usually not uniformly involved by the inflammatory process; rather, there are segments in which the reaction is more acute or more advanced so that the obstruction tends to involve irregular segments with the intervening portions distended with exudate.

The entire thickness of the wall of the duct is infiltrated with neutrophils, lymphocytes, and plasma cells, and the same cells collect in the lumen and in the mucosal cysts formed by adhesions between the denuded epithelial folds. Surviving epithelium may be partly squamous. Eventually, the bacteria will be destroyed and the exudate converted to a watery fluid (hydrosalpinx). Frequently, accompanying pyosalpinx are the *bursal adhesions* and *local peritonitis* described earlier.

Among the organisms that may be found in inflammatory diseases of the uterine tube are streptococci, staphylococci, *Escherichia coli*, and *Trueperella pyogenes*, with the latter as the most common and important. *Brucella suis* in swine and *Mycobacterium tuberculosis* in cattle are responsible for specific forms of salpingitis; the lesions are as described for these infections in the uterus.

Miscellaneous uncommon diseases of the uterine tubes such as neoplasms are reported, for instance, leiomyoma, fibroadenoma, adenoma, and adenocarcinoma. Metastases from ovarian neoplasms also implant within the uterine tubes. Cystic remnants of mesonephric and paramesonephric duct occur.

PATHOLOGY OF THE UTERUS

Abnormalities of position or location

Torsion *of the uterus is uncommon except in the cow and mare.* In almost all cases, such twisted uteri are pregnant (Figs. 4-42, 4-43), but torsion may also occur with pyometra, hydrometra, endometrial polyps, and endometrial neoplasia. *The torsion is of the same nature as an intestinal volvulus and occurs about the transverse axis of the organ, with the mesovarium as one fixed point*. In uniparous species (cow) in which a well-developed intercornual ligament does not permit much independent movement of the horns, the entire organ is involved in the torsion, which is about the mesovarium and vagina or cervix as fixed points. In multiparous species (bitch, cat) with long horns and no intercornual ligament, the torsion will involve part of one horn or the entire horn, the fixed points in the latter instance being the mesovarium and the site of

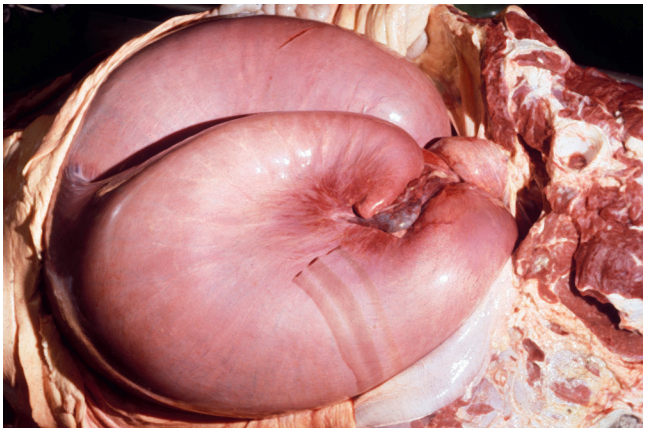

Figure 4-42 **Torsion of a gravid bovine uterus.**

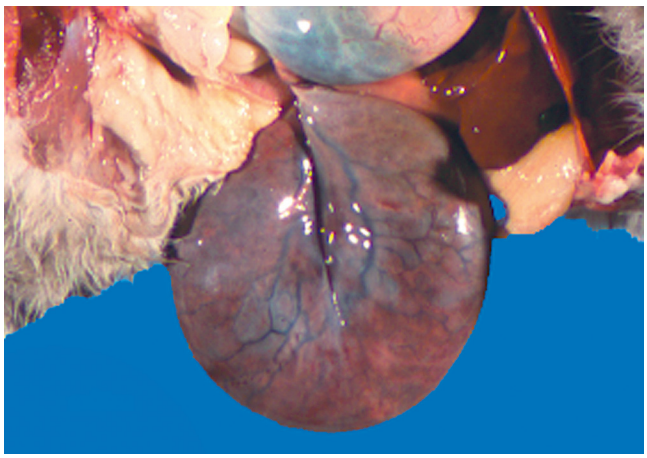

Figure 4-43 Torsion of one gravid uterine horn in a cat.

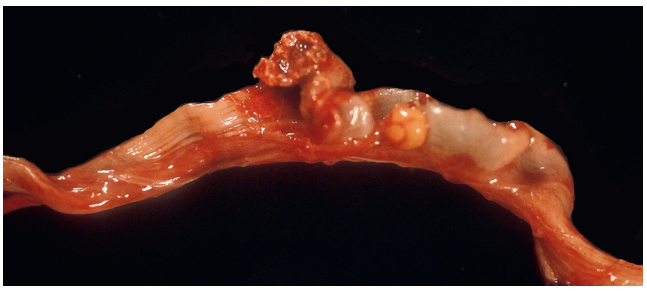

Figure 4-44 Uterine artery rupture in a mare. The resulting hematoma can dissect along fascial planes in the broad ligament or extend into the abdominal cavity, where they continue to bleed and frequently lead to exsanguination.

attachment of the horn to the uterine body. There seem to be no rules governing the direction of the twist. Minor degrees of torsion (up to 90°) are fairly common in cows and apparently resolve themselves. *The condition becomes of importance only when the torsion is 180° or more and results in dystocia*. Any twist in excess of 180° may also result in local circulatory embarrassment. The thinner-walled veins are obstructed before the arteries, and the uterus becomes congested and edematous, with edema of the placenta and death of the fetus. The devitalized uterine wall becomes friable and susceptible to rupture or, if cesarean section is performed, the friability of the wall makes suturing difficult. Death of the fetus may be followed by mummification if the cervix remains closed; if air and bacteria enter the uterus, the fetus putrefies. In the bitch and cat, transverse rupture of the twisted segment near parturition can release the dead fetuses into the peritoneal cavity, which can be misdiagnosed as evidence of ectopic pregnancy.

The fetuses within the peritoneal cavity (sometimes called *ectopic pregnancy*) undergo mummification; attach to the omentum, liver, or intestine; and become covered by a rather thin membrane. Occasionally a fetus becomes dismembered, and fetal bones may be scattered throughout the omentum. In some cases the mummified fetuses remain in the peritoneal cavity for months or years without causing clinical signs of disease. If the horn does not rupture, the fetus may die, become mummified, and the uterus will shrink.

Prolapse *of the vagina, cervix, and/or uterus occurs commonly in ruminants and pigs* and exceptionally in other species. Predisposing causes in the cow are essentially those that cause, or are associated with, uterine hypotony and probably also with dysrhythmia of involutionary contractions. Among the most common associations in the cow are *prolonged dystocia relieved by forced traction, retained placenta, and postparturient hypocalcemia*. Probably the same sorts of influences operate in ewes, and in addition, uterine prolapse after parturition is a common complication of the *hyperestrogenism* that results from the ingestion of legumes with a high content of estrogens.

In any species, usually only the previously gravid horn prolapses. In the cow and ewe, the nongravid horn, and sometimes intestine and bladder also, may be present within the everted horn. The pathologic sequelae of prolapse are comparable to those of intestinal intussusception, with the added factor of trauma. Congestion and edema are followed by hemorrhage, necrosis, and sepsis. Gangrene may supervene.

Rupture of the uterus may occur spontaneously, but is usually a result of *obstetrical manipulations*. Most ruptures occur in the body of the uterus adjacent to the pelvic brim as irregular tears that may involve the full width of the wall or only the mucosa. Mucosal ruptures are of little consequence. Complete ruptures are often fatal either by virtue of hemorrhage, spread of uterine inflammation to the peritoneum, or displacement of retained membranes into the abdominal cavity. The majority of ruptures occur in uteri that are devitalized as a result of torsion or prolonged dystocia.

Rupture may also follow acute distension of the uterus produced by *infusion fluids*. This is not an uncommon accident. The rupture occurs on the lesser curvature along the line of attachment of the mesometrium, and the irrigating fluids spread into the ligament.

Circulatory disturbances

Endometrial hyperemia and edema occur normally at *estrus* and reach the greatest relative development in the bitch in proestrus. The resulting diapedesis and endometrial exfoliation account for the uterine hemorrhage at proestrus in this species. A small amount of mucosal hemorrhage is common in heifers, less common in older cows, and occurs immediately after estrus. The source of the hemorrhage is the endometrial capillary bed immediately cranial to the cervix. It is probably an estrogen withdrawal effect and the nearest thing to menstruation in domestic animals. Punctate hemorrhages occur in the uterine serosa in heifers in estrus.

Hemorrhage of importance follows *torsion and eversion*, by obvious mechanisms. Perhaps the most common association of abnormal bleeding is *endometrial hyperplasia* in the bitch, especially if there is superimposed infection. *Subinvolution of placental sites* also produces uterine hemorrhage. A less common cause in this species is *uterine neoplasia*. Vaginal hemorrhage from uterine leiomyosarcomas was a fairly common clinical feature in Saanen goats.

Rupture of the uterine artery with hemorrhage into the broad ligament, with or without extension into the abdominal cavity, occurs in the mare and rarely in other species. The latter situation commonly results in the death of the mare from exsanguination. The uterine artery near the rupture commonly contains pre-existing chronic degenerative changes (Fig. 4-44).

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PATHOLOGY OF THE ENDOMETRIUM

Irregularities of endometrial growth

Atrophy

Atrophy of the endometrium results from loss of trophic ovarian function. Senile atrophy is not important in large domestic animals. Atrophy is common after ovariectomy, may reflect hypopituitarism of chronic inanition or wasting disease, or a primary hypophyseal lesion. The more superficial portions of the endometrium are the more atrophic, and in advanced atrophy, the lining mucosa covers a thin layer of condensed stroma in the depths of which are the inactive glandular remnants that are sometimes cystic.

The endometrium of those species that have a seasonal period of anestrus undergoes a normal atrophic change. In mares, in which these changes have been studied by endometrial biopsies, the luminal and glandular epithelium becomes cuboidal, and the glands are straight during the winter anestrous period, but there is considerable individual variation in the degree of atrophy mares develop.

Hyperplastic conditions of the endometrium

General considerations. Endometrial hyperplasia in the *bitch* is common and usually involves cystic distension of endometrial glands. There are 2 discrete patterns: *generalized cystic endometrial hyperplasia* (CEH) and *pseudoplacentational endometrial hyperplasia* (PEH) or localized endometrial hyperplasia of pseudopregnancy. Both frequently result in accumulation within the uterine lumen of endometrial secretions. In PEH, cellular debris from associated superficial endometrial necrosis will also be present, which causes the distended uterus to have features that can be confused as being pyometra. In either CEH or PEH, infection of the uterus may follow.

This association between development of endometrial hyperplasia and subsequent infection has been recognized for many years and is known as the *cystic endometrial hyperplasia–pyometra syndrome.* Bacteria might also provide appropriate stimulation that could drive the endometrium to undergo hyperplasia and hypertrophy. An alternative plausible pathogenesis for cystic endometrial hyperplasia–pyometra is that an initial event is the establishment of a *low-grade, subclinical infection* that, during the luteal phase (also referred to as the secretory or progestational phase), causes the endometrium to proliferate. Changes in the uterine environment, accumulation of secretions, and other progestational effects could then lead to massive proliferation of bacteria and inflammatory cell within the endometrium and accumulation of purulent exudate in the uterine lumen.

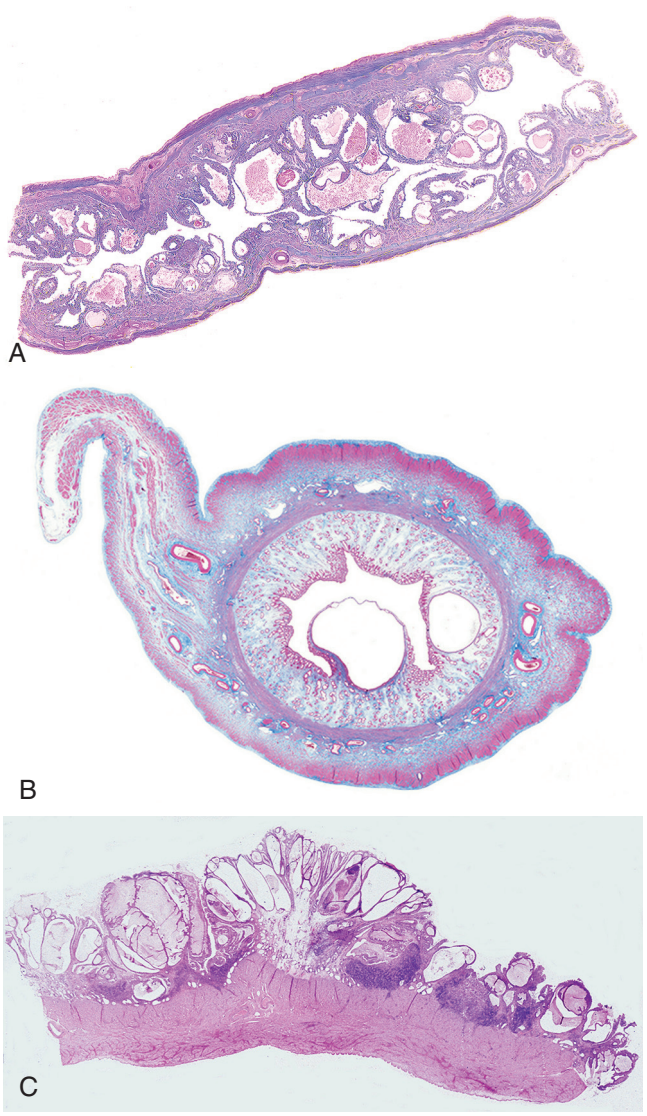

Figure 4-45 Cystic endometrial hyperplasia (CEH) in uterine sections from 3 bitches. The hyperplasia can **involve many glands, as in A, or involve only one or a few glandular units, as in B.** Occasionally, the entire endometrium is involved. The hyperplastic endometrium in the latter cases tends to secrete large amounts of fluid. CEH predisposes the bitch to development of endometritis and pyometra. **C.** The darker areas in the endometrium are mononuclear cellular infiltrates that will change to become predominantly neutrophilic when bacteria proliferate and will result in pyometra.

Cystic endometrial hyperplasia can involve a single or a few glands, or endometrial glands extending along segments of endometrium (Fig. 4-45). Sometimes the entire endometrial surface is involved. Individual endometrial glandular cysts can become quite large, up to 1 cm. More diffuse involvement can leave the endometrium thickened and, on cross-section, effaced by variably dilated glands (see Fig. 4-45).

Endometrial hyperplasia can also be attributed in some species to *excessive and prolonged estrogenic stimulation*. Sources of estrogen can be endogenous, for example, from sex cord–stromal tumors, such as granulosa cell tumor; exogenous, as in ingestion of certain plants; or iatrogenic, from inadvertent exposure to creams containing estrogens. Secretions from a

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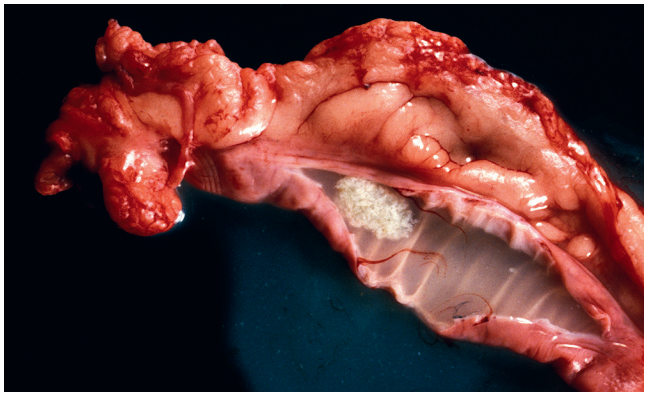

Figure 4-46 Mucometra distending the uterus of a bitch. The physical pressure placed on the endometrium leads to endometrial atrophy over time.

markedly chronically stimulated endometrium can lead to gross accumulation of mucoid fluid in the uterus, a condition called *mucometra* (Fig. 4-46).

Progesterone plays the major role in the induction of endometrial hyperplasia in the dog and cat, but even here the endometrial response to progesterone depends upon *estrogen priming*. Estrogens act by binding to the estrogen receptors that are present in the endometrial cells and act on these susceptible cells to induce the synthesis of intracellular receptors for progesterone. The progestational effect of conversion of the endometrium to its secretory mode depends on this estrogenic priming, and it is likely that in some cases the disturbances of endometrial growth giving rise to endometrial hyperplasia are to be found in the disturbances of the timing and duration of the priming, although cystic endometrial hyperplasia can be produced in the bitch in the normal early diestral uterus by mild trauma (see also Pyometra, in the bitch and queen, later). Many cases of cystic hyperplasia of the endometrium have developed following the use of long-acting progestational compounds to delay the onset of estrus in bitches and queens. Cystic endometrial hyperplasia in the *cow* is associated with ovarian follicular cysts or granulosa cell tumors, both of which can produce prolonged hyperestrogenism. Cystic endometrial hyperplasia is very uncommon in the *mare and camelids*. It has not been associated with granulosa-theca cell tumors in these species.

Ovarian tumors, especially sex cord–stromal tumors and papillary cystadenocarcinomas, are present in some cases of endometrial hyperplasia of the bitch, but in the majority of clinically recognizable cases, the ovaries contain apparently normal corpora lutea. The hyperplasia develops during the long luteal phase that is normal in the bitch. Often paraovarian cysts are present in cases of endometrial hyperplasia.

Noncystic endometrial hyperplasia is not recognizable macroscopically except as an equivocal thickening of the endometrium. The thickening is due to increased size and number of glands, which are irregular in their distribution and course, the normal parallel alignment being lost. The stroma is not hyperplastic but is edematous. The glands may show both proliferative and secretory activity. In the bitch, the glands are tortuous and secretory; the mucosal epithelial cells are typically progestational, being hypertrophied with clear cytoplasm; the glands of the basal endometrium are also active; and there is little evidence of the normal partitioning of the endometrium into layers. There is usually some degree of adenomyosis. Cystic hyperplasia, the so-called "Swiss-cheese" endometrium, is the histologic extreme of the condition and is irreversible. *It is probable that endometrial hyperplasia is but an exaggeration of the normal proliferative activity of the endometrium in response to ovarian hormones.*

Endometrial hyperplasia is a significant precancerous lesion in women. In domestic animals, this is not so. Endometrial hyperplasia is very common in the bitch, but uterine cancer is extremely rare.

Canine pseudoplacentational endometrial hyperplasia. The endometrium of the bitch occasionally undergoes an unusual, but very characteristic, endometrial segmental hyperplasia consisting of localized proliferation of the endometrium that *closely resembles pregnancy implantation sites* (Fig. 4-47). Pseudoplacentational endometrial hyperplasia (PEH) is a frequent component of pseudopregnancy. The unopened uterus may have one or more ovoid distended areas that may resemble pregnancy sites. Prior to opening the uterus, other differential diagnoses would include endometrial polyp, chronic pyometra, leiomyoma, or fetal mummification.

The pathogenesis of this very specific form of endometrial hyperplasia has been investigated. Experimental studies have shown that a variety of *sterile substances*, such as sterile silk or autogenous tissue, when placed in the lumen of the uterus of a bitch in the luteal phase of the estrous cycle, will result in dramatic endometrial proliferation and remodeling. These studies demonstrated how sensitive and responsive the canine endometrium is during the period of the estrous cycle, when it would be an intimate part of placental structures if the bitch were pregnant.

Segmental endometrial hyperplasia can occur in virgin bitches and is part of pseudopregnancy. Similar segmental areas of hyperplasia have been experimentally produced by placing various sterile materials in the lumen of bitches that are in the luteal phase of their cycles. It has been suggested that these are florid decidual reactions and have been referred to as *deciduomas*, but use of this term is discouraged.

The microscopic features of PEH are characteristic (Fig. 4-48). The hyperplastic endometrium may appear as a broad-based polypoid mass extending into the uterine lumen (see Fig. 4-47) or may form a continuous band of protruding tissue (see Fig. 4-48A, B). The endometrium is organized in 3 discrete layers, corresponding to the deep glandular zone, condensed connective tissue band, and folded luminal epithelial junctional zones. The luminal surface is almost always necrotic, and no fetal placental tissues are present.

Estrogenic plants. Pasture legumes as sources of estrogenic activity have claimed attention as a cause of a spectacular syndrome of *infertility in* **sheep**, and as a cause of less obvious depression of fertility in sheep. Although many estrogenic substances are known to occur in plants, pasture plants that have been found to produce estrogenic effects are mainly some varieties of *Trifolium subterraneum*, *T. pratense*, *Medicago sativa*, and *M. truncatula*. The estrogens of the clover species are usually *isoflavones*; alfalfa and the barrel medic contain *coumestans*.

Extensive metabolism of phytoestrogens occurs in the rumen. During such metabolism, compounds of very different estrogenic potency from those in the plant can be produced. Among the isoflavones, formononetin, a compound with little estrogenic activity in itself, is the main compound producing histologic effects in sheep after its conversion in the rumen to the potent estrogenic metabolite, equol.

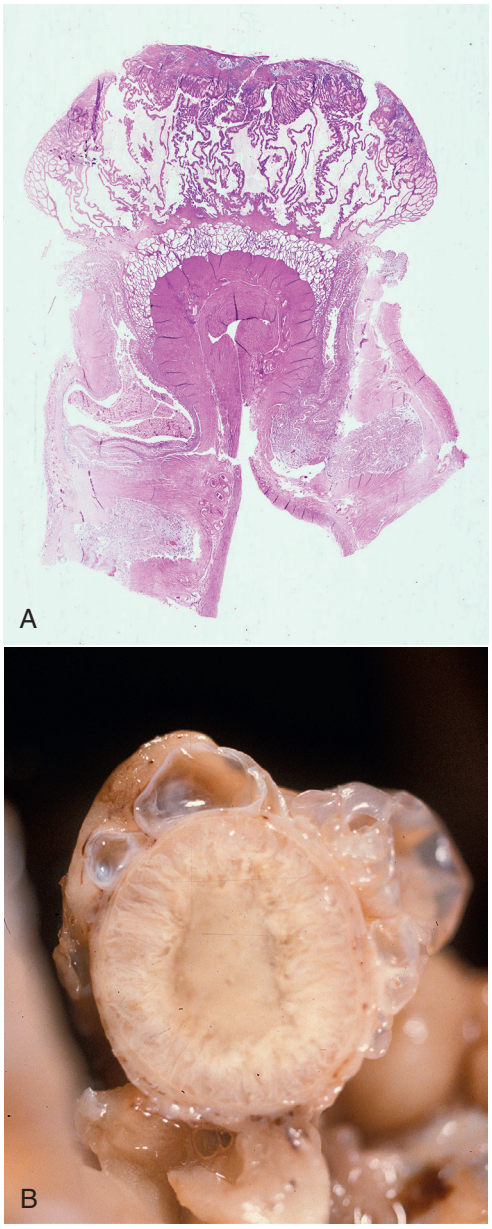

Figure 4-47 Pseudoplacentational endometrial hyperplasia (PEH) in a bitch. A less common form of endometrial hyperplasia occurs during the luteal phase in some bitches. **A.** The endometrium undergoes dramatic highly organized hyperplasia that replicates the endometrial tissue growth and remodeling that occurs at placentation sites during normal pregnancy. The uterine horn is distended and, frequently when opened, the proliferative band of endometrial tissue protrudes from the endometrial surface. **B.** PEH involving the uterus of another bitch. The cross-section of a uterine horn in the area of segmental enlargement with dramatic endometrial hyperplasia is shown. (Also note the presence of several serosal inclusion cysts on the surface of the uterus.)

There is variation in estrogenic potency between strains of clover and also with the season or stage of growth, activity being greatest in winter and early spring. Potency is diminished if the clover is allowed to wilt and dry or if it is made into hay, but the activity is retained if the clover is artificially dried.

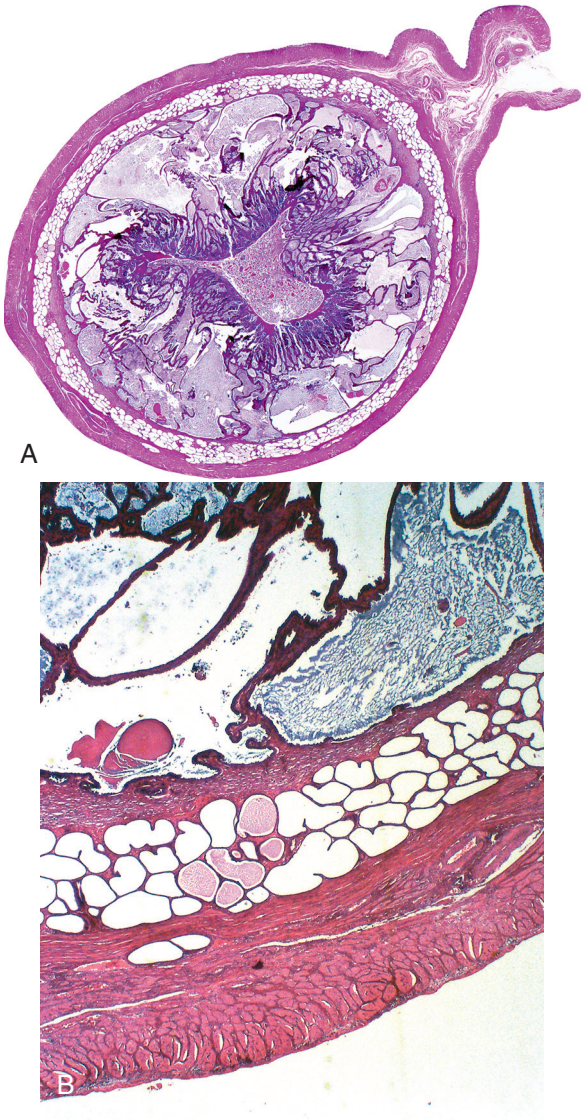

Figure 4-48 Pseudoplacentational endometrial hyperplasia (PEH) in the bitch. Note the uniform distention of the deeper areas of endometrial glands (the deep glandular zone). The luminal epithelium has many thin folds, lined by secretory epithelial cells (equivalent of the junctional zone in a normal pregnancy site). The luminal ends of these folds usually undergo coagulative necrosis. Necrotic debris is usually found admixed with mucoid endometrial secretions. **A.** The subgross photograph in **A** corresponds to the tissues in Figure 4-47B. **B.** Higher magnification.

Estrogenic pastures grazed during the breeding season may temporarily impair fertility, and ewes so affected may have a very low lambing rate and a high incidence of dystocia and uterine prolapse. Wethers on such pastures develop enlargement of the bulbourethral glands (see Vol. 3, Male genital system). The fertility of affected ewes returns after removal from the offending pastures if the exposure is short, but infertility can be permanent if there is prolonged exposure. The infertility is in large part attributable to failure of transport of sperm through the cervix because of changes in the cervical mucus. Estral cycles are normal, and normal ovulation apparently occurs. The dystocia that is common is attributed to

reduced myometrial tone at parturition; it results in a high rate of maternal and neonatal death. Uterine prolapse occurs in maiden and nonpregnant ewes as well as postpartum ewes; mammary development and lactation occur in nonpregnant ewes.

The principal morphologic changes in the cervices of affected ewes consist of greater glandular development, so it is more like uterus; reduced numbers of goblet cells; and a reduced amount of stratified epithelium. These changes are associated with an increased incidence of cervicitis. The endometrium is hyperplastic, and macroscopic cysts are often present. Hydrometra and pyometra are occasionally seen. These are the signs and lesions that were described in the original reports of infertility in ewes caused by estrogenic plants, commonly known as *clover disease*.

The development of varieties of clover low in formononetin has made it possible through changes in management and agronomic measures to control the most severe forms of the disease. But, as the original syndrome has been brought under control, *a much more insidious form of estrogenic infertility in ewes has emerged as the result of chronic or repeated ingestion of low levels of phytoestrogens*. This syndrome causes less severe reproductive losses, but the infertility tends to be permanent. Its anatomic basis consists primarily of subtle cervical changes that also impair sperm transport. The cervical lesions are those of blunting of the cervical folds, with a reduction of the number of folds and crypts associated with an increase in the stroma of the lamina propria. There is an increase in coiled tubular glands of the endometrial type in the cervical lamina propria. This change is most severe at the cranial end of the cervix. This glandular induction is the reason the disease has been referred to by the awkward name of *estrogen-induced transdifferentiation*. The persistent effects of estrogen also bring about modification of sexual behavior; some ewes develop a more male behavior, clitoromegaly, and fusion of the ventral labia. These changes are mild, and the disease can be best identified by careful cervical histologic examination, determining the number of folds, and quantifying the area of the lamina propria. Because this is unknown territory for most pathologists, adequate control material is essential.

The effects of estrogenic pastures or of the various isoflavones on **cows** have not been conclusively examined, but there is little doubt from information accrued in Israel and Tasmania that the effects are important; alfalfa (lucerne) and the clovers listed previously have been blamed. The principal features of the syndromes reported are similar to those produced by cystic follicles. The infertility rate is high and is associated with persistent cystic ovaries, aberrations of the estrous cycle with abnormally long estrus; swelling of the vulva independent of estrus; and sometimes enlargement of the clitoris, increase in size of the uterus and cervix with cystic endometrial hyperplasia, and enlargement and function of the virgin mammary gland.

Adenomyosis

This term applies to the presence of endometrial glands and stroma between the muscle bundles of myometrium (Figs. 4-49, 4-50).

In some cases it is a malformation, and in others it arises by hyperplastic overgrowth of the endometrium. It is not a common lesion in any domestic species, but is seen in the bitch with cystic endometrial hyperplasia. Adenomyosis is occasionally observed in cows as part of the local disarray of segmental aplasia. It may also be present as a malformation of the tips of the uterine horns in cows.

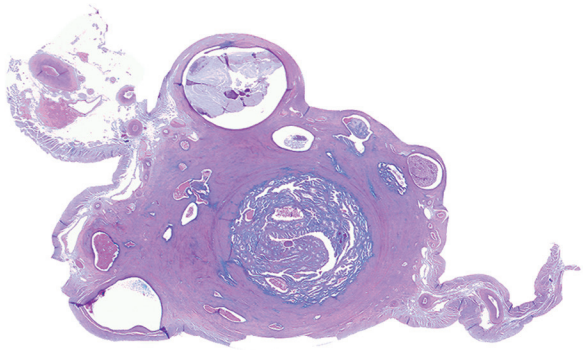

Figure 4-49 Canine adenomyosis. Adenomyosis can occur as single small endometrial glands within the myometrium. They can become more extensive, as demonstrated in this subgross section. The epithelial lining of these ectopic glands ranges from normal appearing to attenuated in the larger cysts.

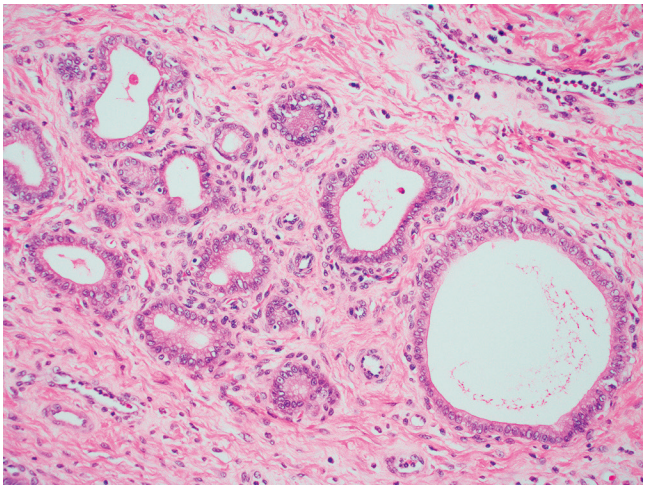

Figure 4-50 Photomicrograph of **canine adenomyosis.** These slightly irregular endometrial glands are present within the myometrium.

Adenomyosis as seen in domestic animals shares features with *endometriosis* of menstruating primates only when the aberrant site is within the myometrium; in this site the 2 conditions are histologically similar.

Endometrial polyp

This lesion is seen most commonly in the bitch and queen and is striking when the polyps are large (Fig. 4-51). Segmental distension of the uterine horn(s) can be confused with tumors of the myometrium (leiomyomas and leiomyomatosis), chronic pyometra, pregnancy or sites of fetal resorption, or segmental cystic endometrial hyperplasia. In contrast to the polypoid form of endometrial hyperplasia, *the true polyp contains substantial connective tissue stroma in addition to dilated glands and is pedunculated* (Fig. 4-52). Polyps may be multiple or isolated, and their shape is molded to the uterine lumen.

Polyps may provide sufficient mass that uterine contractions can mechanically force prolapse of the affected horn. Endometrial polyps may be observed protruding through the cervix into, and sometimes even from, the vagina.

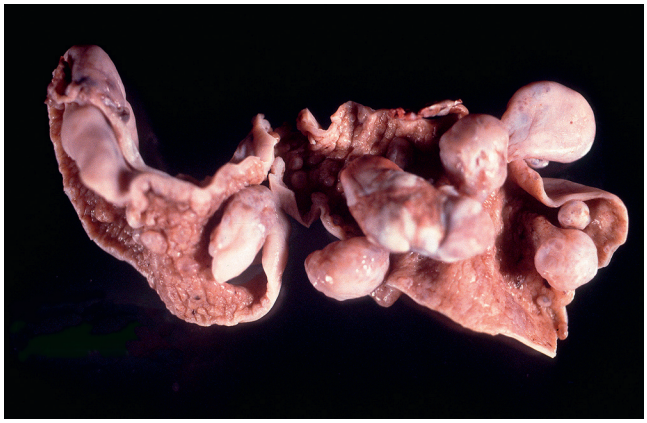

Figure 4-51 Multiple **endometrial polyps** in a queen. This queen had several firm segmental enlargements involving both uterine horns. Endometrial polyps can be single or multiple and occur most commonly in older queens and bitches.

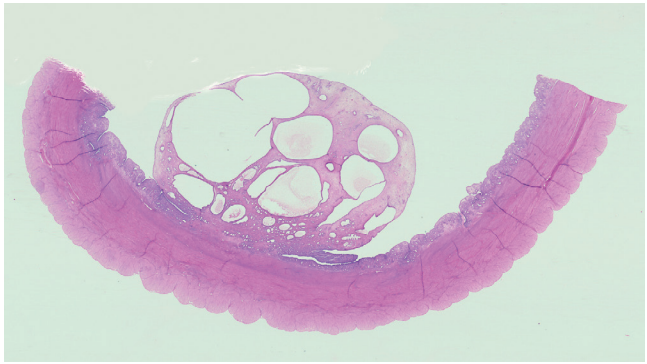

Figure 4-52 Canine **endometrial polyp** extending into the uterine lumen. Note the cystic spaces that are dilated endometrial glands. These polyps contain fibrous connective tissue bands and progress from locally extensive areas of cystic hyperplasia that become fibrotic. Larger polyps assume the shape of the lumen.

Uterine accumulation of secretory or inflammatory exudates

Hydrometra and mucometra

The 2 conditions are considered together as the difference is probably only in physical properties and depends on the degree of hydration of the mucin, which in turn may be related to the relative activity of estrogenic hormones. *The accumulation of thin or viscid fluid in the uterus is concurrent with the development of endometrial hyperplasia or is proximal to an obstruction of the lumen of the uterus, cervix, or vagina.* Grossly, there is uniform or segmental distension of the uterus (Fig. 4-53). If hydrometra or mucometra persists for a long time, the endometrium becomes markedly attenuated and will atrophy (Fig. 4-54).

In cases of congenital or acquired obstruction of the lumen of the tubular genitalia, the volume of fluid that accumulates proximal to the obstruction depends on the site of the obstruction. In uterus unicornis in cattle, there may be 500 mL; in imperforate hymen, there may be 10 L or more. The fluid is slightly cloudy and watery, but in some cases of segmental aplasia where the volume of retained secretion is not great, it may be very viscid, ocher colored, and sometimes inspissated to rubbery masses of mucin and cellular detritus. In these cows

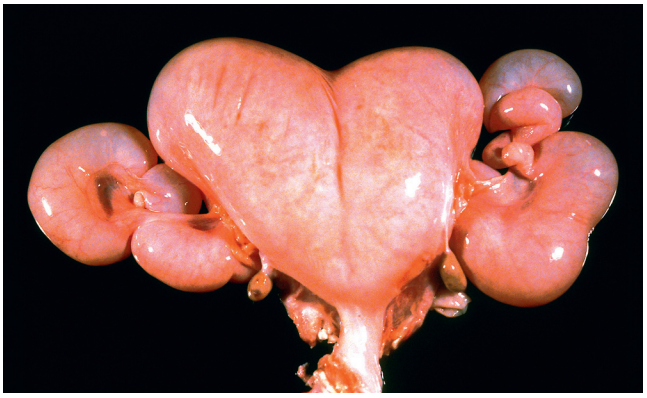

Figure 4-53 **Hydrometra** in a goat. The uterine wall becomes thinner as fluids accumulate. The accumulation of viscous fluid within the uterus is called *mucometra*.

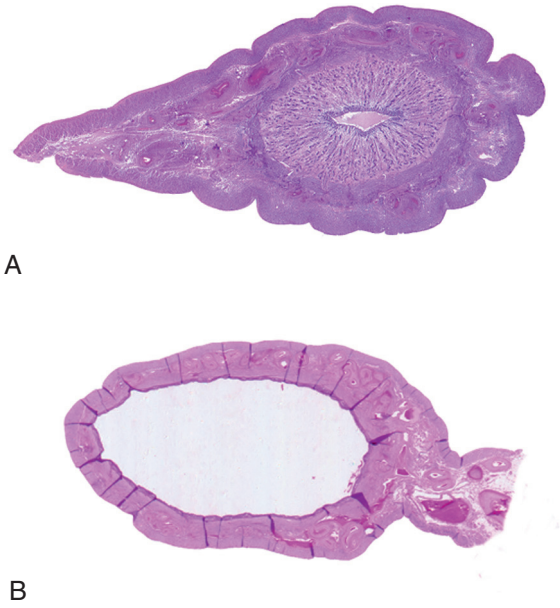

Figure 4-54 **A.** Subgross photograph of a cross-section of a uterine horn from a normal bitch for comparison to **B**, the distended uterine lumen from a bitch with **hydrometra.** Long-standing hydrometra or mucometra can lead to atrophy of the endometrium.

the ovaries are normal. If affected uteri become infected, intractable pyometra results. An abnormally long and tortuous cervix may result in a form of mucometra caused by the retention of uterine secretions.

Serosal inclusion cysts

Uterine serosal inclusion cysts are thought to arise by pinching off of surface epithelial indentations. They are occasionally observed in the aged pluriparous bitch and less commonly in ruminants, and appear as thin-walled cysts containing clear watery fluid. Serosal inclusion cysts form from small folds of peritoneum that adhere and form entrapped pieces of serosal epithelium that slowly accumulate secreted fluids. They tend to occur either during uterine involution or in association with perimetritis; however, inflammatory cellular infiltrates are not usually present in or near these cysts.

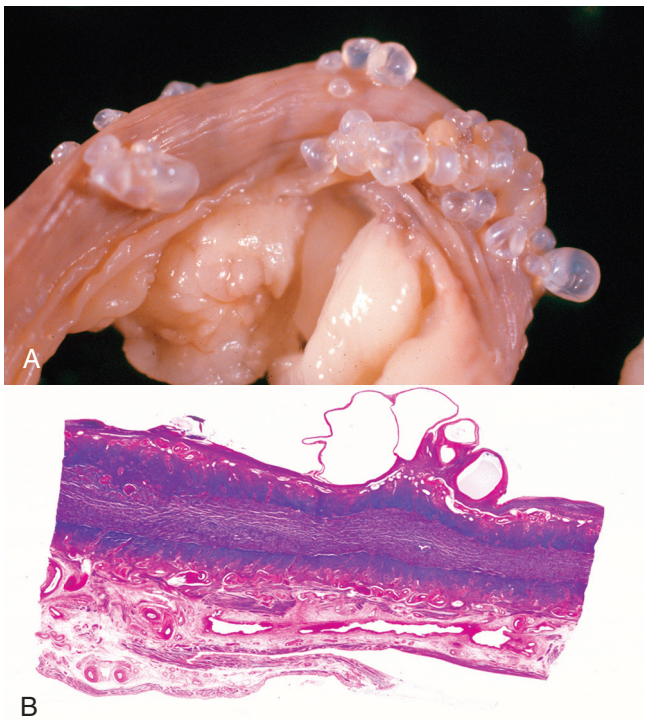

Figure 4-55 **A. Serosal inclusion cysts** on the surface of a canine uterus. These cysts develop following adhesion of the serosa to itself in areas of linear folds. **B.** Subgross photomicrograph revealing both the superficial location and very thin walls that are key features of this condition.

The gross appearance of these cysts can be striking. Most appear to lie on the surface of the uterus (Fig. 4-55). They contain clear fluid and have thin translucent walls. They may vary from a few millimeters to 2 cm or more in diameter. Serosal inclusion cysts can be distinguished from paraovarian cysts by their location. Similarly, although a differential diagnosis would also include mesonephric duct cyst, the latter are located along the mesometrium, tend not to be multiple, are not as superficially located, and microscopically would have smooth muscle in their wall.

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INFLAMMATORY DISEASES OF THE UTERUS

General considerations

Inflammation limited in extent to the endometrium is termed **endometritis**; involvement of the entire thickness of the wall is **metritis**; of the serosa, **perimetritis**; and of the mesometrium, **parametritis**. The classification is to some extent a useful index of the severity of reaction and of the pathogenesis. The great majority of inflammatory conditions of the uterus begin in the endometrium and are in some manner associated with the reproductive process. The predisposing factors are to be sought then at either end of the gestation period.

The normal nonpregnant uterus is endowed with a high degree of resistance to infection, and even in the case of the specific genital diseases, brucellosis, trichomoniasis, and campylobacteriosis, is incapable of supporting bacterial growth or even the persistence of bacteria for any extended period. Something is known of factors that render the uterus susceptible, at least temporarily, to infection, and more and more is being discovered about innate mechanisms of resistance. The self-limiting nature of most infections, other than those associated with pyometra, has long been recognized and formed the basis for the recommendation of a period of sexual rest for animals with uterine infections. Even the specific genital infections are self-limited in duration; active infection of a uterus with *Campylobacter fetus* or *Tritrichomonas foetus*, in the absence of pregnancy, survives only for 2-3 estrus cycles. *Brucella abortus* causes infection of the pregnant uterus but does not persist well in the nonpregnant uterus, although it is apparently capable of persisting in the mammary gland and lymph nodes of infected cows.

Uterine resistance varies during the estrous cycle, susceptibility being greatest during the luteal phase of the cycle. Several factors may be involved. *Uterine motility* is increased during estrus, and this aids in the physical clearance of microorganisms. *Innate immune mechanisms,* including pathogen-associated molecular patterns such as Toll-like receptors and defensins, operate in the endometrium. The epithelium has a cytokine profile that varies with the cycle and exposure to infectious agents. *Adaptive immune mechanisms* include uterine synthesis and secretion of immunoglobulin G (IgG) and IgA; these vary during the cycle, but the variations are not great, and the timing varies between species. The functional activity of the neutrophils migrating into the uterus may also be affected by the stage of the cycle, being more active during estrus than diestrus, but much of the reduced uterine resistance during diestrus and pregnancy is related to secretion into the uterine lumen of progesterone-induced immunosuppressants that are capable of inhibiting lymphocyte proliferation. It has been well demonstrated that *the uterus that is under the influence of progesterone (which includes the pregnant uterus) is very susceptible to many nonspecific bacteria* and that a uterus not under the influence of progesterone is remarkably resistant to the same organism, even when its expulsion is prevented by ligation of the cervix. This comparison can be carried a step further to show that even greater susceptibility to infection is present at the implantation sites in the pregnant uterus. The

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uterus after ovariectomy is resistant to infection but does not clear infections as promptly as does the uterus under the influence of estrogens. These factors of susceptibility and resistance provide some insight into the pathogenesis of postcoital uterine infections and pyometra in the dog and cat.

A different set of influences operates on the puerperium. *It is well recognized that uterine infections are likely to follow any abnormal parturition, such as an abortion, retained placenta, twin births, dystocia, and traumatic lacerations of the genital canal.* Of the specific abortive agents in the cow, *C. fetus* and *T. foetus* typically cause early death of the conceptus that is not complicated by placental retention or metritis. But with other causes, *B. abortus* especially, abortion may occur later in pregnancy, at which time the well-developed and manifold interdigitations of the cotyledons favor retention of the placenta and the development of acute metritis. But in these instances, much of the uterine disease is the result of secondary invasion by bacteria that gain access to the uterus as the result of an abnormal delivery of a dead or diseased fetus.

Some cases of postpartum metritis are a continuation and exaggeration of a gestational uterine infection, but *most puerperal infections of the uterus can be viewed as analogous to wound infections, with the organisms entering via the cervix*. Probably all mares have uterine infections by streptococci within 1-3 days of parturition, but these do not persist for more than 2-3 days. In the cow, too, it has been observed that 25-30% are infected in the normal puerperium but that most recover spontaneously from these infections with *T. pyogenes, E. coli*, and other bacteria. Clearly, the outcome will be determined as much by the number and virulence of invading organisms as by the environment within the uterus. The recognized predisposing causes outlined previously will all be associated with retarded uterine involution, as will most cases of metritis. With the more virulent anaerobic bacteria, such predisposition is not essential.

The period necessary for normal involution varies with the species and is determined by the nature of placentation (depth and nature of invasion—deciduate versus adeciduate) and the rate of endometrial repair. It is well advanced in the mare within 9 days, as judged by the capacity for fertile mating at that time, in contrast to the involutionary process in the bitch, which involves deposition of material and extensive, slowly progressive remodeling. In the latter case, abnormalities of involution are common and termed *subinvolution of placental sites.* Most of these are probably never recognized clinically and spontaneously regress.

Endometritis

In endometritis, *the endometrium or uterine mucosa is mainly involved*. Almost all uterine infections begin as endometritis, and many such cases progress to involve the myometrium, becoming metritis. The mildest forms are seen postcoitus. In the mare, semen is ejaculated directly into the uterus and induces a transient postcoital endometritis. Such postmating endometritis is recognized in most species. Infectious agents tropic for the uterus include *T. foetus* and *C. fetus* or pyogenic cocci and coliforms of low pathogenicity.

Uterine infection by α-hemolytic streptococci, *Klebsiella pneumoniae, E. coli*, and *Taylorella equigenitalis* (the contagious equine metritis organism) frequently occurs in **mares** both following foaling and after coitus. The endometritis is usually mild, but the impact on fertility can be substantial.

Mild endometritis may not be overtly evident clinically. A slight opacity of the normally crystal-clear estral mucus may be all that is seen. Histologically, the changes are not striking and consist for the most part of a diffuse but light infiltration of neutrophils with slight desquamation of the superficial epithelium and no significant vascular changes. Involvement of the glands is minimal. The significance of a few leukocytes in the stroma is always equivocal in cattle; they follow within 2-3 days of parturition and are present during estrus. However, the presence of neutrophils in the stroma of the endometrium is evidence of inflammation in the mare. *The best indication of endometritis in all species consists of accumulations of plasma cells and foci of lymphocytes in the stroma*. Resolution of this type of endometritis may occur with no more residue than a few cystic glands with periglandular fibrosis (Fig. 4-56), although during its course, it may be responsible for early embryonic mortality. If the endometritis is more severe or persists for a longer time, the cumulative damage to the endometrium may render the mare sterile.

Contagious equine metritis is a *venereal disease of mares* caused by *T. equigenitalis*, a gram-negative, microaerophilic coccobacillus. The disease produced by this organism does not appear to be significantly different from other common infecting organisms of the mare's genitalia. The interest in this disease stems from its apparently abrupt appearance in

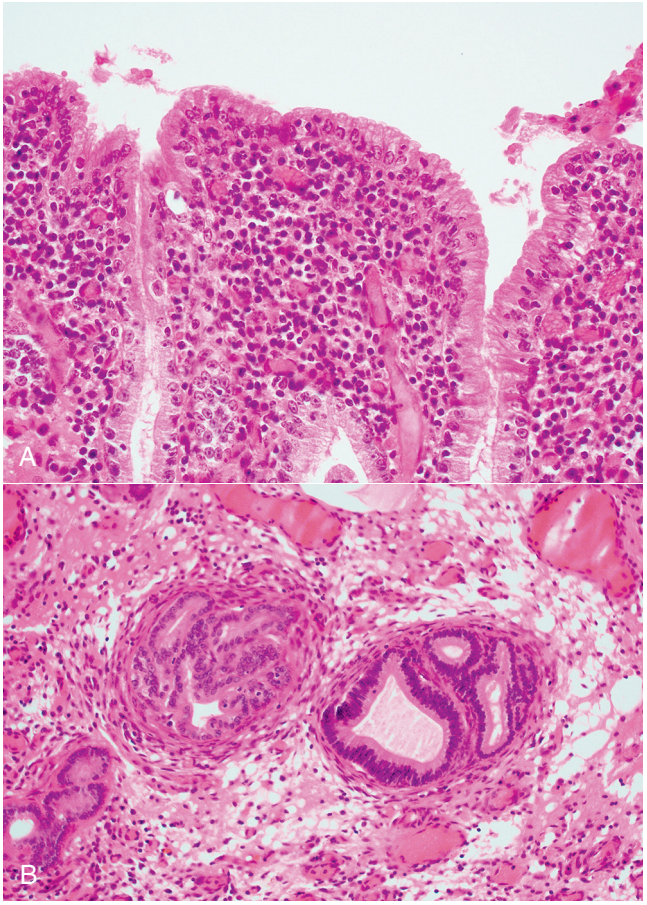

Figure 4-56 **A.** Endometrial tissue from a mare with marked lymphocytic **endometritis**. **B.** Glandular nesting caused by periglandular fibrosis. Inflammation, gland loss, lymphatic distention, and fibrosis are histologic features assessed during evaluation of equine endometrial biopsies for categorization.

horse-breeding establishments in England in 1977 and its rapid spread to equine studs around the world. Strict control measures seem to have limited its spread, and the clinical disease is now rare.

The disease causes temporary infertility in mares and a mucopurulent discharge that lasts 2-3 weeks. The organism can persist in infected mares for several months, and recovered mares represent an important reservoir of infection. Stallions transmit the organism by genital contact but do not develop clinical disease.

The temporary infertility that is the clinical hallmark of the disease is the result of mild to moderate inflammation of the endometrium and adjacent structures. After experimental introduction of the organism into the uterus, and presumably after natural infection, the bacteria can be regularly demonstrated in the uterine tubes, endometrium, cervix, and vagina for a 2-3 week period, during which time the organism elicits a mild to moderate inflammatory response. The endometrial folds are turgid and swollen and covered by a small amount of cloudy viscid exudate. At this stage, the endometrium is edematous and the inflammatory infiltrate consists mainly of neutrophils. As the reaction subsides, the edema disappears and plasma cells predominate. Changes in the cervix parallel those of the endometrium. Salpingitis develops in some cases but is a less regular feature of the infection. Infection produces no gross changes in the vagina or vaginal vestibule, and histologic changes are mild. No lesions develop in the clitoral fossa or sinus, but the organism can be recovered from these sites after it has disappeared elsewhere.

More severe grades of endometritis are common to the puerperium in **cattle**. Nothing of significance may be visible on the serosal surface, but the organ is enlarged and flabby, and collapsed rather than firm and contracted. The lumen contains chocolate-colored lochia that is slightly tenacious and often without foul odor. With the admixture of inflammatory exudate and placental detritus, the uterine content becomes progressively dirty gray-yellow. The endometrium is red and swollen, and the intercotyledonary areas are ragged and tattered with shreds of mucosa free in the lumen. Small hemorrhages are common in the mucosa, and neutrophils are prominent at the surface involving all mucosal elements, including the glands. Where suppuration and superficial necrosis produce the tattered mucosa, the surface is comparable to a pyogenic membrane. The remainder of the genital canal may show nothing more than the traumatic lesions incident to parturition. If the uterus is paretic, there may be no discharge in the vagina.

Sporadic outbreaks of suppurative endometritis associated with bovine herpesvirus 4 have been reported. The histopathology is typical for herpesvirus infections, with focal necrosis, ulceration, and common secondary bacterial infections. Intranuclear viral inclusion bodies are found in endometrial epithelial and endothelial cells.

Metritis

The distinction drawn here between endometritis and metritis for purposes of description is that, *in metritis, all layers of the uterine wall show evidence of acute inflammation*. The uterus is paretic, and there may be little or no vaginal discharge. The wall of the uterus is thickened with suffused blood and edema fluid and is very friable. The serosa is dull and finely granular with "paintbrush" hemorrhages and a thin deposition of fibrin, or the subserosal vessels may be very prominent. Other than traumatic rupture, perforation with secondary peritonitis is not common except in anaerobic infections; death in untreated cases usually occurs first from toxemia or septicemia.

The secretion may be scant or abundant, is fetid, and is dirty yellow to red-black. The microscopic picture is that of purulent inflammation. Subserosal connective tissues are edematous and filled with neutrophils, and the same process surrounds the blood vessels of the myometrium and permeates bundles of, and individual, muscle fibers, which themselves undergo granular degeneration. In metritis, as in acute endometritis, neutrophils are in large numbers on the mucosal surface, and there is extensive hemorrhage, necrosis, and sloughing. Invasion of blood vessels, both arteriolar and venous, intensifies the lesion. Thrombosis may extend to the vessels of mesometrium with the usual sequelae of hemorrhage and infarction.

Chronic endometritis

Recovery from the acute phase of the infection often results in chronic endometrial involvement. With greater or lesser degrees of endometrial destruction and replacement by granulation and scar tissue, the uterus takes on the nature of a fistulous tract. The changes depend on the duration and severity of the inflammation, but *consist essentially of productive fibrosis and leukocytosis in which lymphocytes and plasma cells predominate.* Thickening of the endometrium is by inflammatory tissue; the glands are depleted; those that survive are atrophic, flattened, and attenuated, or cystic because of periglandular fibrosis. The lining mucosa may be intact, denuded in places, or show foci of polypoid hyperplasia or squamous metaplasia, as do any chronically irritated mucous membranes. The exudate in the lumen is not copious and may be serous, catarrhal, or frankly purulent. Much of the endometrial stroma, especially that of caruncles, may be replaced by scar tissue, and dystrophic mineralization of necrotic portions of the endometrium may sometimes be extensive enough that the lining of the uterus feels gritty.

It is estimated that 20% of cows with generalized **tuberculosis** and 4% of all tuberculous cows have involvement of the endometrium. There are 3 routes of infection, namely, hematogenous, via the uterine (fallopian) tubes from the peritoneum, and coital; of these, the last is exceptional. As in tuberculous lesions generally in cattle, *there are 2 anatomic forms of the lesion—miliary tuberculosis and diffuse caseating tuberculosis,* although transitional forms do exist. It is generally accepted that the disseminated miliary lesion is of hematogenous origin during the phase of early dissemination.

In *miliary tuberculosis*, the uterus may appear normal externally. In the early stages, there may be no exudate in the lumen, but later, as the granulomas enlarge and ulcerate, the uterus will contain yellow purulent exudate. The granulomas are visible as few or many nodules in the mucosa, usually the more superficial portions, and microscopically are of typical tuberculoid structure. A common site is near the bifurcation of the uterus or in the caruncles of the pregnant uterus.

Caseous tuberculosis causes thickening and rigidity of the horns with serofibrinous or purulent fluid in the lumen. The endometrium is thickened, dry, and extensively caseous (see Fig. 4-59). There may be intense leukocytic infiltration and marked exudation. The caseated area is usually demarcated by a zone of epithelioid cells from a margin of connective tissue.

In association with the uterine lesion in tuberculosis, there is often involvement of other portions of the genital tract.

There may be multiple red granulomas on the surface of the ovary and its ligaments, or there may be enlargement of the ovaries with parenchymal tubercles. Tuberculous salpingitis or pyosalpinx is probably the rule when the uterine tubes are the portal of uterine infection. Similar lesions may be found infrequently in the lower genital tract. Infection of cows with atypical mycobacteria also produces tuberculous metritis and placentitis with multiple small endometrial granulomas.

Uterine abscess

The formation of single or multiple abscesses is not common. The localization of an infection to one part of the uterine wall is thought to follow severe metritis, localized traumatic injury to the infected endometrium, or adenomyosis. Such an abscess may reach 15 cm in diameter and is usually well encapsulated, although there may be some perimetrial adhesion and, in a few instances, rupture into the peritoneal cavity or an adjacent hollow viscus.

Uterine abscesses are observed more frequently in cattle than in other species. There appears to be a relationship between the frequency of abscesses and uterine manipulations involving the use of instruments. *In cattle, most large abscesses are located in the dorsal wall of the uterine body.* This is the area most subject to trauma during the passage of insemination pipettes and uterine catheters. Abscesses that develop following severe metritis or pyometra are usually small (1-3 cm) and do not have preferential sites.

Parametritis and perimetritis

Chronic adhesive peritonitis involving the genital tract does not usually result from septic metritis because the uterine serosa offers an efficient barrier to the spread of infection, and spontaneous rupture of an infected uterus is not common. Few virulent infections spread to the supporting ligaments. Excluding an origin from an extragenital focus, *perimetritis and parametritis in cattle usually follow manual manipulation of the ovary, pyosalpinx, pyometra, obstetrical operation, removal of retained placenta, or uterine irrigation*. In each of the latter 3 circumstances, there may be accidental perforation or rupture of the uterus. The extent of the adhesion may vary from a few fibrous bands (Fig. 4-57) to dense connective tissue that obscures the contour of the organs and fixes them to adjacent viscera. Abscesses may form in the adhesions of the ovarian bursa and the rectovaginal pouch.

Pyometra

Pyometra is acute or chronic suppurative infection of the uterus with accumulation of pus in the uterine lumen. The escape of the pus is usually prevented by a functionally closed cervix. Drainage from the uterus may also be prevented by an acquired or congenital cervical stenosis, and in mares the gravitational pull of the flaccid, distended uterus over the brim of the pelvis may limit the discharge of pus. Pyometra may occur as a sequel to uterine infections of the types described in the previous sections, but as it is a pathologic entity with a number of factors unique in its pathogenesis, it is considered separately here. Pyometra is an uncommon condition in the sow, ewe, and camelids. *It is relatively common in the bitch and cow, but less so in the mare and queen*. Circumstances under which the disease develops in these species vary.

Pyometra in the bitch and queen. Pyometra in the **bitch** is a disease that characteristically affects older animals, especially those that are not bred. *The condition most often develops a few weeks after estrus.* Affected animals may be depressed and anorexic, frequently vomit, and have polyuria and polydipsia, usually accompanied by a vaginal discharge. The pathologic findings vary with the stage of the disease. In less advanced cases, the uterus may be only slightly enlarged, with mild endometrial hyperplasia and inflammation (Fig. 4-58). In the more advanced stages, there is a remarkable distension of the uterine horns (Fig. 4-59), which may occupy most of the

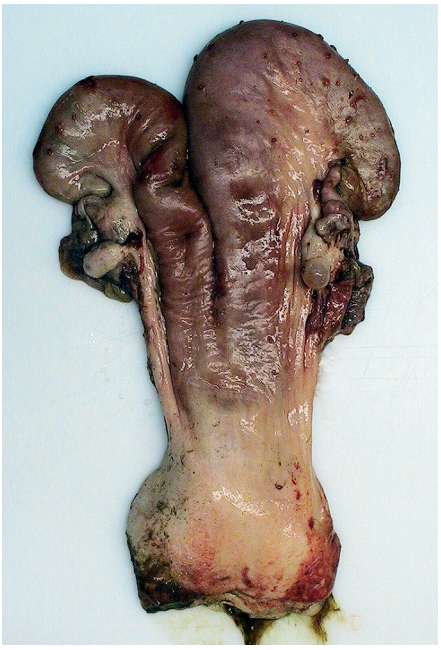

Figure 4-57 Perimetritis of the uterus of a cow with postparturient metritis.

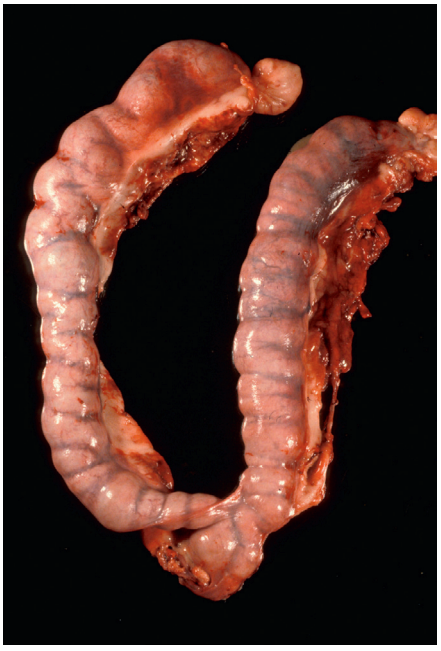

Figure 4-58 Pyometra in a bitch. Note the spiraling of the uterine horns, a change that occurs when the canine uterus is under the influence of progesterone. Both horns are mildly distended by purulent exudate. Cystic endometrial hyperplasia is a common pre-existing lesion.

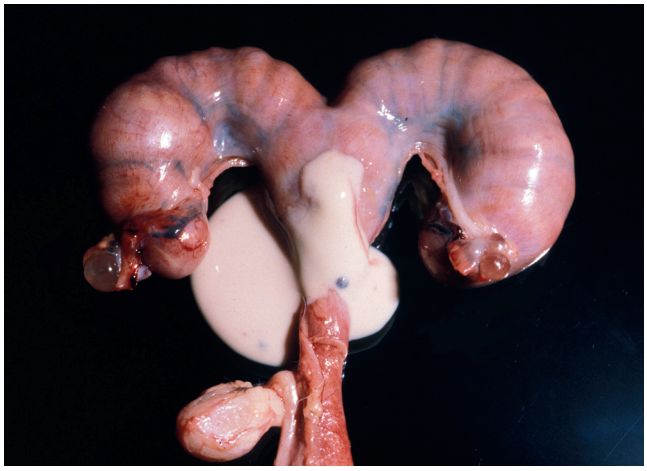

Figure 4-59 Pyometra in a queen.

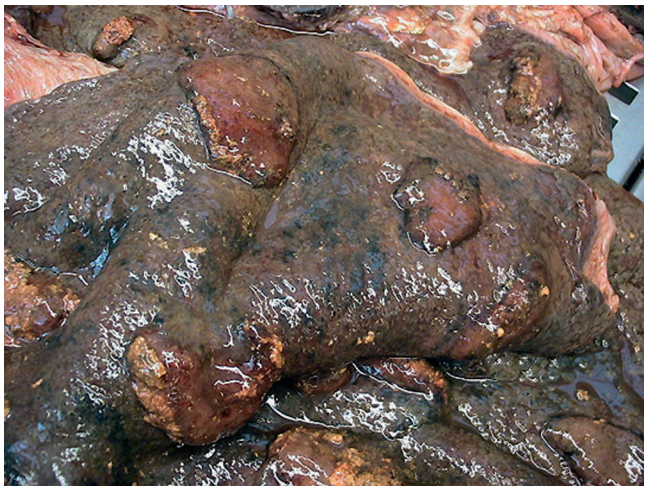

Figure 4-60 Suppurative endometritis and pyometra in a cow. This condition develops most commonly secondary to retained placenta.

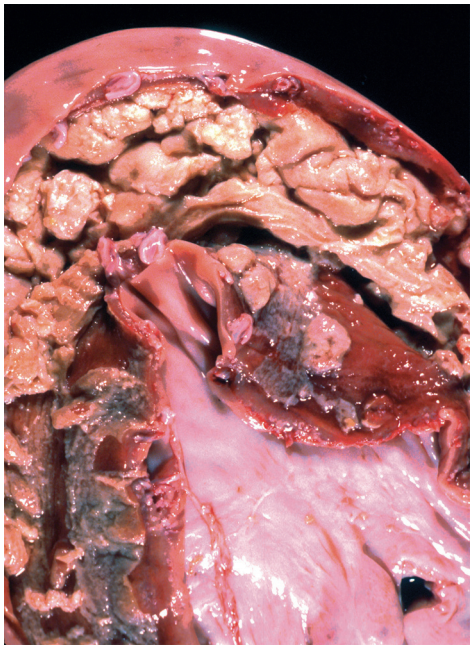

Figure 4-61 Chronic pyometra in a cow. The white material is inspissated purulent exudate.

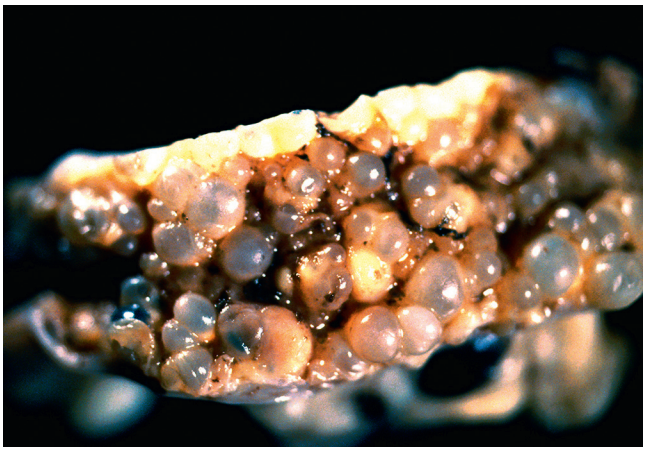

Figure 4-62 Marked cystic endometrial hyperplasia in a bitch. This uterine horn is opened, exposing the endometrial surface. The endometrium is thickened, and there is massive uniform cystic distention of endometrial gland lumens.

peritoneal cavity. In contrast, pyometra in cattle most commonly develops in the postpartum period and is frequently associated with retention of fetal membranes (Fig. 4-60). If the purulent exudate does not drain from the uterus, it can become dehydrated with time, and the inspissated material is found as soft concretions in the uterine lumen (Fig. 4-61).

Distension of the cornua may be symmetric or asymmetric, uniform or ampulla-like dilations, as in the uterus of mid-pregnancy. The cervix is completely or almost completely closed as a functional response to luteal hormones. The serosal surface of the uterus is dark, and the vessels are prominent. The wall is friable, and either rupture or perforation with secondary peritonitis is common. There may be obvious inflammation of the peritoneal serosa and mesometrium, but this is unusual. The nature of the uterine content is variable. In the more severe cases, usually those infected with *E. coli* and *Proteus* spp., the exudate is thick, viscid, tenacious, opaque, red-brown, and with a characteristic fetid odor. In other cases, usually those infected with streptococci and staphylococci, the exudate is more typically suppurative. The mucosa is irregular in thickness, necrotic, and ulcerated in portions with irregular superficial hemorrhages, and in other portions obviously hyperplastic, dull-white, and dry in appearance, with small cysts visible in these hyperplastic areas.

Microscopically, the most significant feature is the remarkable endometrial hyperplasia and progestational proliferation in almost all cases (Figs. 4-62, 4-63). The cells of such progestational epithelium are enlarged, columnar, vacuolated, and have small pyknotic nuclei. In some cases the normal single layer of cells piles up to produce pseudostratification or localized papillary proliferations. Whatever remains of the endometrial lining may show this development, or it may be patchy and alternating with normal epithelium.

These changes are produced by the luteal hormones that inevitably pave the way for the development of this disease. *The histologic changes caused by infection vary with the bacterial cause and time.* Masses of neutrophils collect in the uterine lumen and in the glands, although there is relative sparing of

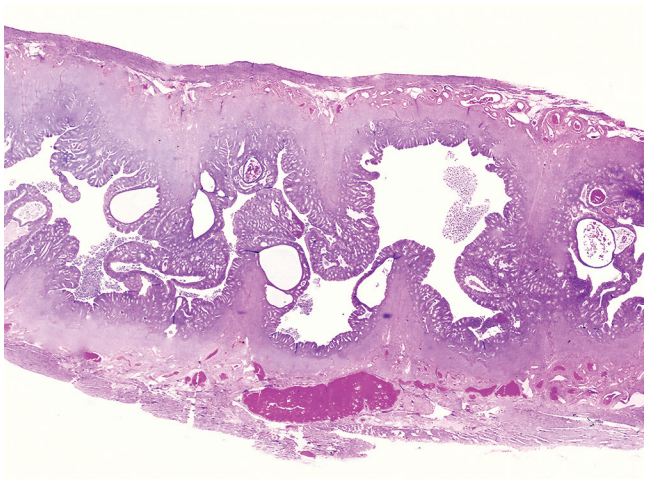

Figure 4-63 A typical case of **cystic endometrial hyperplasia** involving the endometrium of a bitch.

the glands unless they are cystic. Neutrophils collect near the surface and then penetrate the epithelium. Some cases, surprisingly, are dominated by cells resembling eosinophils. This form is not studied. In milder cases there may be a few neutrophils in the endometrial stroma, but they are not many when compared with the numerous plasma cells and lymphocytes. There may not be much vascular reaction over and above that of hormonal origin, although perivascular reaction and leukocytosis of lymphatic vessels are almost constant in the myometrium. Sometimes the reaction is much more severe than that just described, and in the endometrial stroma, there are all the exudative phenomena of acute inflammation accompanied by the early reparative response of granulation tissue. The blood vessels are very congested and some show thrombosis, and about others, there is diapedesis or larger hemorrhage. The stroma is edematous, and bullae often lift off the overlying epithelium. Numerous neutrophils infiltrate the stroma and collect in the lumen. There is little formation of fibrin, and rarely are there microabscesses in the mucosa.

It is clear that most cases of pyometra in the bitch are associated with endometrial hyperplasia. It is usually a disease of the diestral (metestrus) period, a time at which corpora lutea are present and progesterone concentration is high, and because the condition has been produced both experimentally and accidentally by the administration of progestins, it is clear that *progesterone is an essential feature in the pathogenesis.*

Because cystic endometrial hyperplasia had been produced in dogs by administration of high levels of progesterone for extended periods of time, and because cystic endometrial hyperplasia was a hallmark of canine pyometra, it was presumed that cystic endometrial hyperplasia induced by abnormal progesterone secretion or response preceded and paved the way for the bacterial component. Some doubts concerning the validity of this view were generated when hormonal abnormalities could not be demonstrated in cases of pyometra. Now it has been shown that cystic endometrial hyperplasia can be produced in the normal, early diestral uterus by mild trauma to the endometrium. The investigators scratched the endometrium with a thin wire, but endometrial biopsy has caused the same lesion. More to the point, inoculation of appropriate strains of *E. coli* into the uterine lumen, if given 1-2 weeks after estrus, causes a pyometra that has all the features of the natural disease, including cystic endometrial hyperplasia.

It appears that an appropriate stimulus applied to the progesterone-primed endometrium produces cystic endometrial hyperplasia and that one such stimulus can be a bacterial infection. Accordingly, rather than pyometra in the bitch occurring as the result of bacterial infection being superimposed on an abnormal endometrium, *it appears that the bacterial infection of the progesterone-primed endometrium produces both the pyometra and the cystic endometrial hyperplasia*.

The bacterium most commonly present in pyometra in the bitch is *E. coli*, and uterine *E. coli* isolates are typically identical or very similar to isolates from feces of the same bitch. Most of the strains involved are urinary tract pathogens and possess *uropathogenic virulence factors*, including P fimbriae, α-hemolysin, and cytotoxic necrotizing factor 1, that probably enhance the pathogenicity of the strain in the canine genital tract and may facilitate the colonization of the progesterone-primed endometrium. Urinary tract infections are common in bitches with pyometra, and the strains of bacteria infecting both are often identical. It seems probable that the urinary infection predisposes to pyometra and that the uterus is infected at the stage that suitable receptors are developed in the endometrium in response to hormone stimulation. This explanation of the bacterial infection can be only a partial one as not all bitches with pyometra have urinary tract infection and not all infecting organisms are urinary pathogens. In some cases of pyometra, bacteria cannot be recovered.

The clinical signs and extragenital lesions associated with pyometra in the bitch are due to severe toxemia and probably also intermittent bacteremia. Renal signs and lesions are common. Profound hypotension may reduce renal perfusion and lead to prerenal uremia. This is worsened by membranoproliferative glomerulonephritis that often develops as a result of immune complex deposition. Polyuria, which is such a common feature of the disease, is due to impaired tubular ability to concentrate urine. The mechanisms involved in this are not completely understood, but they are thought to be brought about by *E. coli* antigen and have an immunologic basis. Most animals have profound leukocytosis, reflected in the bone marrow as an increased granulocyte to erythroid ratio. There is granulocytic hyperplasia in bone marrow and extramedullary granulopoiesis in liver, spleen, lymph nodes, and adrenals.

Pyometra in the **queen** is roughly comparable to the disease in the bitch, but there are differences, some of which are related to the differences in the estrous cycle of the 2 species. Cats are induced ovulators and usually, if not bred, have repeated estrous periods. However, *pyometra develops most often 2-5 weeks after estrus* and usually after spontaneous ovulation and presumably corpora lutea formation. Unlike the bitch, many queens with pyometra do not have corpora lutea present in their ovaries at death or surgery, but approximately half do, and *there is an obvious correlation between the presence of corpora lutea and the development of pyometra in queens*. The correlation with endometrial hyperplasia is less clear. Most queens with pyometra do have some degree of cystic endometrial hyperplasia, but this change is very common in the age group affected.

Pyometra in the cow. Pyometra in the cow is associated with corpus luteum activity in the ovary. However, in contrast to the situation in the bitch in which the high progesterone levels of early diestrum predisposes to uterine infection, *in the*

cow, uterine disease causes the corpus luteum to persist and maintain a high progesterone level. The retention of the corpus luteum appears to be due to a reduction in or inhibition of the synthesis and release of the luteolytic factor prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) by the diseased endometrium.

Broadly, there are 2 periods in which a uterine infection can lead to retention of a corpus luteum with the accompanying hormonal effects that *convert endometritis to pyometra*. They are during the *early postpartum period*—following dystocia, retained placenta, and metritis—and at *various times after breeding*, as a result of venereal infections, with early embryonic death. Insemination during the luteal phase of the cycle or similar operations that can introduce contamination into the uterus during the luteal phase can mimic venereal infection.

The role of retained luteal tissue in the pathogenesis of pyometra appears clear. The secreted progesterone endows the uterus with a high degree of susceptibility to infection, maintains functional closure of the cervix, and inhibits myometrial contractility.

The amount of pus retained in the uterus of a cow with pyometra varies from a few milliliters to more than several liters and is thick, rather mucinous, and cream or gray-green colored. The cervix has no seal of mucus, so although contracted, there is usually escape of a small amount of pus into the cranial vagina. The wall of the uterus is thick, doughy, and flaccid, but in long-standing cases, especially as complications of mucometra, the walls are thin or fibrosed. The histologic changes do not differ significantly from those of endometritis of comparable duration and severity. The organisms involved are hemolytic streptococci, staphylococci, coliforms, *T. pyogenes*, and *Pseudomonas*. The venereally transmitted protozoan, *T. foetus*, can be the cause of pyometra after breeding.

Rarely, the anomalous developments of the uterus that were described earlier can lead to pyometra. In the segmental aplasias and with imperforate hymen, the lumen proximal to the site of obstruction becomes distended with mucus and cellular detritus. These closed cavities provide a satisfactory environment for bacterial growth, but this is not a frequent complication. When it does occur, the source of infecting organisms is probably hematogenous. Pyometras seldom resolve spontaneously, and although the condition is not usually life threatening, as it is in bitches, the condition will persist unless cyclic activity can be reinstituted.

Pyometra in the mare. Pyometra in the mare differs from the disease in the bitch and the cow in several particulars. Although some cases develop following difficult parturitions with infections, as they do in cattle, many do not. Remarkably, most mares continue to cycle during the disease, and the hormonal influences that are so marked in the bitch, queen, and cow are much less important in the mare.

In some mares, cervical adhesions and closure may lead to pyometra, but in most instances the purulent material collects without demonstrable cervical closure, and in some cases the cervix is fully dilated. Copious amounts of pus can be discharged under such circumstances, particularly during estrus. *Pyometra in the mare seldom leads to evidence of systemic disease*, although some mares develop mild anemia.

The length of the estrous cycle appears to be related to the severity of the endometrial damage. In rare cases, where the endometrial damage is severe, the cycles are prolonged with long luteal phases resulting from delayed or inadequate $PGF_{2\alpha}$ release. Mares with less severe endometritis have normal or shortened cycles.

A variety of organisms may be present in pyometra in the mare: *Streptococcus zooepidemicus* is the most common, but *E. coli*, *Actinomyces* spp., *Pasteurella* spp., and *Pseudomonas* are often present.

Endometrial biopsy. Endometrial biopsy is most firmly established in equine theriogenology. The evaluation system is based on identification and scoring of 4 microscopic lesions plus consideration of the mare's reproductive history. The microscopic features evaluated include stage of cycle, inflammation, presence of fibrosis, dilation of lymphatics, and loss of glands.

Only a small amount of information has been published on endometrial biopsy in other species. Pathologists are asked to evaluate endometrial biopsies for cattle, bitches, and camelids, and as more information is gathered and evaluated, recommendations for scoring will be followed by further validation of their prognostic value.

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PATHOLOGY OF THE GRAVID UTERUS, PLACENTA, AND FETUS

General considerations

It is difficult to generalize on the subject of diseases of pregnancy because of the remarkable variation in the reproductive affairs of the various species. Because the mechanisms by which the gestation period is initiated, the pregnancy maintained, and the delivery triggered have such profound effects on how diseases of the conceptus will be manifest, some review of the special features of reproductive patterns in the various domestic species is given here.

For a pregnancy to proceed, the normal cyclic lysis of the corpus luteum must be prevented. This is achieved in **cattle** by secretion by the blastocyst of interferon-γ, which inhibits synthesis and release of $PGF_{2\alpha}$ from the endometrium. This spares the corpus luteum and allows it to serve as the corpus luteum of pregnancy, and it then provides part or all of the hormonal support of the pregnancy. In cattle, up to 40% of total embryonic loss probably occurs between days 8 and 17 of pregnancy. Early embryonic death will permit release of $PGF_{2\alpha}$ and lysis

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of the corpus luteum and subsequent reinstitution of the estral cycles in these species. The delay between death of the conceptus and the return of the dam to estrus is usually sufficient to allow complete or near-complete autolysis and dissolution of the fragile embryo. As a result, *the early diseased products of conception are rarely available for study.* Venereal infections cause some of the early losses, but on the basis of the few studies that have been done, and on the experience in other species, *it is presumed that chromosomal abnormalities account for most of these early losses.* In monotocous species, embryos that die undergo prompt dissolution, the products are expelled, and the disease is recognized only as infertility with slightly prolonged estral cycles. The death of a single embryo, or even several, does not interrupt the pregnancy in swine, and dead embryos are resorbed or mummified. Rarely, the death of the conceptus may be caused by organisms that convert the pregnancy to a pyometra, the active infection preventing the lysis of the corpus luteum. Trichomoniasis of cattle is an example of a venereal infection that can lead to pyometra.

In the **mare,** gonadotropic hormone (equine chorionic gonadotropin) is produced in linear, slightly dome-shaped structures within the endometrium, *the endometrial cups* (Fig. 4-64). These structures form early in pregnancy. Originally thought to be maternal in origin, "cup" cells are fetal in origin and arise from a band of specialized trophoblast cells that form the chorionic girdle on the expanded blastocyst. These cells become detached from the fetal chorion on about day 35 of gestation and burrow into the stroma of the endometrium. There the cells enlarge and appear as large binucleated cells.

The cup tissue becomes visible at about day 40 of gestation as a horseshoe or ring-shaped band in the endometrium of the pregnant horn (see Fig. 4-64). The cups increase in size until day 60 of gestation, at which time their hormone production is at a maximum. Hormone concentration falls after day 60, and the cups become pale and slough between days 75 and 100 of gestation. The degeneration of the cups resembles graft rejection. The cells of the fetal chorion are surrounded by an intense maternal accumulation of lymphocytes and undergo prompt coagulative necrosis. The necrotic slough is detached and lies free between the endometrium and chorion, and then the necrotic cup cells become contained in a pouch formed by the enclosure in an area of chorioallantois. These are called *chorioallantoic pouches* and appear as teardrop-shaped small extensions from the allantoic surface on the inner surface of the chorioallantoic membrane near where the umbilical cord attaches.

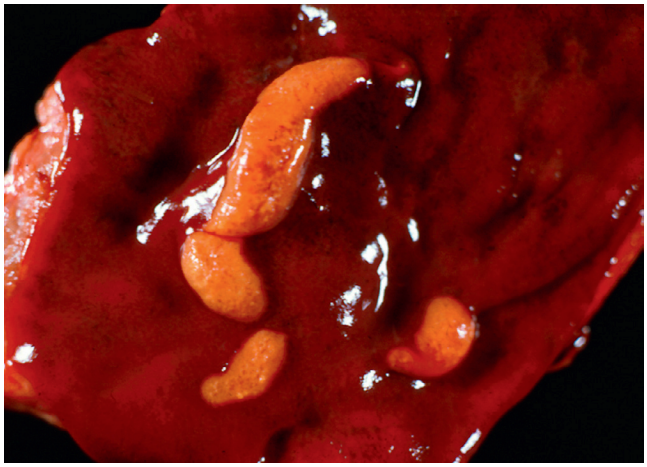

Figure 4-64 Endometrial cups in the endometrium of a mare. These transplanted trophoblast cells produce the hormone *equine chorionic gonadotropin*. This tissue dies and becomes necrotic about 100-120 days of gestation as a result of a marked maternal lymphocytic response.

This potent chorionic gonadotropin in mares stimulates ovarian corpora lutea to develop, and these maintain the pregnancy until approximately 126-140 days, when the fetal chorion takes over the production of progesterone. If the conceptus dies after day 35 of gestation, the cups persist and continue to secrete chorionic gonadotropin, accessory corpora lutea are produced, and the mare fails to return to estrus and continues for a variable period in a state of pseudopregnancy.

Domestic carnivores that are pregnant have corpora lutea that persist during the gestation period. Prolonged luteal presence is also a feature of the relatively long luteal phase of the cycle in bitches and queens. Corpora lutea develop after ovulation, spontaneously in the case of the bitch and induced by coitus in the cat. Once formed, their lifespan appears to be inherent and the hormone production by them to be independent of a pregnancy until the last stages of gestation. As a result, death of the embryos or death or sickness of the fetuses shortens the length of the gestation period only slightly. Fetal death before the terminal stage does not usually lead to premature delivery; rather, the process of autolysis and resorption or mummification begins, and the products are expelled at or near term. *As a consequence, it is very rare to obtain diseased fetuses from these species in a satisfactory state for study.* Most studies of fetal diseases in carnivores have been based on experimental investigation.

The corpus luteum is essential for the maintenance of pregnancy during the entire gestation period in *swine* and *goats*, as it is in dogs and cats, before term. The corpus luteum is only essential during the first half of pregnancy in the *mare* and *ewe*, and during all but the end of pregnancy in the *cow*. During this time, the effects of fetal death are unpredictable. In some cases the corpus luteum may undergo luteolysis, and the dead fetus or fetuses are expelled, usually severely autolysed, but in some cases the corpus luteum will not be lysed and will persist, and the fetus will be resorbed or more likely expelled. If the fetus is older, and particularly in cattle, it may be mummified. During the last third of gestation in these species, the pregnancy requires hormonal support from the fetus, and fetal death at this stage leads to expulsion within a few days. Even a few days allow time for autolysis to be evident; the fetal tissues are pale, the red cells lysed, and pleural and peritoneal cavities are filled with hemoglobin-stained fluid.

The mechanisms by which dead fetuses are expelled in cattle and sheep are not known, but it is probable that they share features with normal parturition. They must involve $PGF_{2\alpha}$ release, cervical compliance, and coordinated myometrial contraction. Chronic fetal disease in cattle and sheep results in premature delivery of live but diseased fetuses. Because fetal stress activates the same hypothalamic-pituitary-adrenal endocrine chain used to initiate normal parturition, it is probable that chronic fetal disease operates through the same chain to cause abortions of live fetuses. Equid herpesviral infection regularly produces abortion of live, diseased, equine fetuses.

Embryonic death

There is little that can be said with profit of the pathology of the ovum, zygote, or early embryo, but a brief consideration is included here for purposes of orientation.

When fertilized ova die, they undergo progressive cytolysis within the zona pellucida, which often remains intact after death. The whole structure then disintegrates and is resorbed or discharged at the next estrus. It is rare to recover abnormal ova from the bovine uterine tube during the first 72 hours postestrus. Presumably, in those cases in which estrus occurs after a normal cycle, the ova die most commonly between days 4 and 10 of the cycle. Most embryonic deaths in cows, however, are associated with prolongation of the interestral period. In the embryonic stage, the embryonic tissue proper disappears first, and the trophoblast remains for a while before degenerating.

The incidence of zygotic and embryonic mortality is remarkably high in the species that have been studied and probably is in all species. Humans and the domestic species studied suffer a zygotic loss of 15-30%. The causes are presumably diverse and may vary somewhat from species to species. However, the demonstration of abnormal karyotypes in early bovine and porcine zygotes and in a high percentage of unselected spontaneous human abortions *suggest that chromosomal or other genetic abnormalities are an important cause of this mortality.* The frequency with which chromosomal abnormalities are found varies with the stage of pregnancy; they are most common in the early stages of pregnancy. This is the pattern one would anticipate because some of the most severe anomalies are not compatible with attachment or implantation. Most of these chromosomal abnormalities represent numerical changes such as monosomy, polysomy, polyploidy, and mixoploidy. Only a small percentage is structural. The monosomies and polysomies are the result of nondysjunction during meiosis or early cleavage stages. Triploidy can be caused either by 2 sperm fertilizing one egg or by the suppression of the second polar body and one sperm fertilizing a diploid egg; both possibilities increase with increased age of the gametes. Tetraploidy occurs as a result of suppression of the first cleavage division of the zygote. Mixoploids develop as a consequence of mitotic error during cleavage division of the zygote. Structural anomalies are caused by spontaneous breakage and reunion. Viruses, drugs, and radiation are known to cause this type of chromosome damage.

Fetal death

A degenerate zygote or early embryo may be resorbed or expelled from the uterus. At a later stage in development, the dead fetus may be mummified, macerated, or aborted; an **abortion** is defined as the expulsion of a fetus prior to the time of expected viability. A dead fetus delivered within the period of expected viability is arbitrarily referred to as **stillborn**. Perinatal mortality is a natural extension and refers to death from causes at or about the time of birth. It should be appreciated that these different terms might be applied to fetuses suffering from the same basic disease.

In uniparous domestic animals, death of the fetus in late pregnancy is usually followed by abortion. In multiparous species, if most of the fetuses die at the same time, all are likely to be aborted, but it is more usual for one or several dead fetuses to be retained with the remaining viable ones and delivered at parturition. It is often apparent that the deaths have occurred at different ages, the dead fetuses being of different sizes and degrees of mummification or maceration.

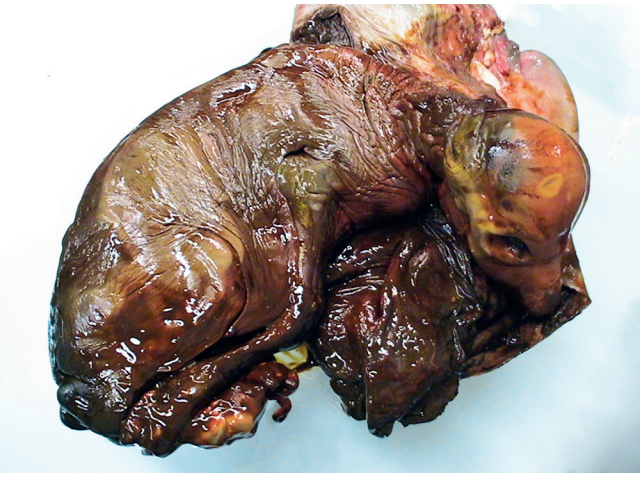

Figure 4-65 A **mummified bovine fetus.** Fetal and placental tissues undergo necrosis and dehydration in the absence of bacteria.

Mummification of fetus

Mummification of a dead fetus is seen occasionally in any, but usually multiparous, species and most commonly in the sow. In the mare, it is typically one of twin fetuses that is mummified. Mummification may also occur in mares receiving progesterone in the early stages of gestation to prevent abortion associated with failure of endometrial cup development and subsequent failure of accessory corpora lutea formation and thereby progesterone production. The exogenously administered progesterone inhibits contractions of the uterus and, if the fetus dies, prevents abortion, so the fetus mummifies. *A prerequisite for mummification is that bacterial infection is not present*.

The fluids are resorbed, and the membranes become closely applied to the desiccated fetus. The whole mass becomes brown or black and rather leathery, moist on the surface with sticky mucus, but without odor or exudate (Fig. 4-65). The time required for complete mummification will depend to some extent on the size of the fetus. Dehydration is advanced by 7 days in sheep fetuses; however, complete mummification probably requires as long as 6-8 months in the case of a 6-month bovine fetus. All stages may be observed—from the earliest, of beginning separation of the placenta with hemoglobin staining of the tissues, to the latest, when all that remains is a firm and shrunken remnant consisting almost wholly of dried skin and bones. Until the time of expulsion, which on occasion occurs spontaneously, or parturition in multipara that still carry some viable fetuses, the cervix remains closed and sealed. In uniparous animals, the mummified fetus may be retained indefinitely. Animals that have had and delivered a mummified fetus usually breed normally on subsequent occasions, so there cannot be any serious uterine lesion accompanying the fetal death. Genetic diseases (both inherited and chromosomal abnormalities), viral and protozoan infections, and placental insufficiencies have been proposed as the causes of fetal death leading to mummification, but the cause is rarely firmly established in any particular case. Very likely all the proposed causes and others can produce fetal death at an appropriate time, and mummification can

result. *The critical features for mummification appear to be the retention of the dead fetus within the uterus through the action of a functional corpus luteum and fetal skin mature enough to resist autolysis.*

Fetal maceration and emphysema

Maceration and emphysema of the fetus require the presence of an infection in the uterus. If the early embryo succumbs to uterine or embryonic infection, maceration is usually followed by resorption within the uterus or expulsion along with a small amount of purulent exudate. This is the usual course of events in venereal infections by *Campylobacter fetus* and *Tritrichomonas foetus* in cows, but is also to be expected with any sort of nonspecific endometrial infection. The causes of infectious death of the conceptus during the period of the fetus are the same as previously, but the consequences, pyometra or acute endometritis, are usually more severe because of the presence of decomposing fetal remnants. The differences between pyometra and endometritis are as discussed earlier; in pyometra the corpus luteum may persist, the cervix remains sealed, and the uterus withholds its contents. If the corpus luteum and cervical seal break down, there is purulent discharge of the contents, and except for the presence of fetal remnants, this endometritis does not differ much from that of the puerperium. After about the third month of pregnancy in the cow, complete fetal maceration does not occur. Fetal bones, and to a degree, fetal hair resist maceration (Fig. 4-66). They may be discharged or retained in the purulent exudate (pyometra) or remain in the uterine lumen indefinitely. The uterine pus is usually intensely fetid until maceration is complete.

The usual infectious causes of fetal death and endometritis are not potent gas-formers; the development of fetal emphysema almost invariably depends on patency of the cervix and on invasion of the uterus and dead fetus by putrefactive organisms from the vagina. There are 2 common antecedents to emphysema: dystocia at or near term, and incomplete abortion. In incomplete abortion, the cervix is open but not completely dilated, and the fetus may be delivered into the cervix or cranial vagina, or because of malpresentation, uterine inertia, or inadequate dilation of the cervix, it may be retained in the uterus. The fetus putrefies, becomes distended with foul gas, and crepitates. If the fetus is small, the dam may survive the initial acute episode of fetal emphysema, after which maceration occurs in a chronic, foul purulent metritis. The cervix is not sealed, but because of uterine paresis, the pus is retained in the flaccid, dependent organ.

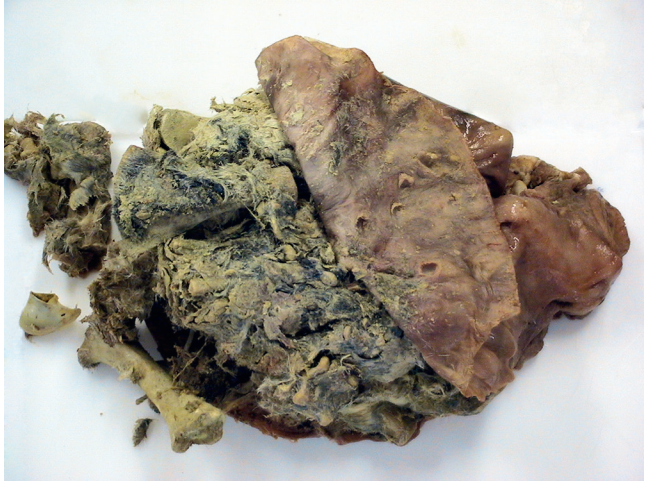

Figure 4-66 A **macerated bovine fetus.** The presence of bacteria causes putrefaction of fetal and placental tissues. Soft tissues slowly undergo fetid disintegration, which leads to loss of all soft tissues, and only fetal bones remain.

Advanced uterine lesions accompany the macerated fetus. The uterine wall is thickened, and the reaction within it varies from the acute exudative inflammation of pyometra to more-or-less complete sclerosis and replacement by granulation tissue in long-standing cases. In these the uterus closes firmly about the bones, and the bones may cause perforation.

Fetal emphysema, at or near term, complicating dystocia is fatal unless treated, and maceration is not an expected sequel. In some cases of uterine torsion, however, the twisted cervix and vagina produce an adequate seal against bacterial invasion of the dead fetus, and mummification, rather than maceration or emphysema, results.

A special form of fetal emphysema occurs in ewes, caused by *Clostridium chauvoei*, the organism responsible for blackleg. It affects fetuses near full term, causing acute tympanitic distension of the uterus, typical hemorrhagic and necrotizing lesions of the fetus, and the accumulation of dark thin discharge in small amounts in the uterus. The pathogenesis of this special infection is not entirely clear, but there is usually a relation to rough handling, such as at shearing.

Embryonic death with persistence of membranes (cystic placental mole)

This is an uncommon development that can follow embryonic death. In the period of the embryo, the fetal membranes are in volume and mass the greater part of the products of conception, and *it is possible for the embryo to die and to be resorbed while the membranes persist and continue to grow. The remaining empty cyst has been referred to as a* **cystic placental mole**. Most empty placental cysts correspond in size to fetal membranes at 3-4 months of gestation. There is usually no patent lumen to the cyst. It is filled with a mass of clear-gelled fluid together with placental stroma. In those cases with placentary development, the allantoic and amniotic epithelia may be present. The condition may become infected and converted to a pyometra or undergo necrosis and be discharged. Infections that result in rapid death of the fetus (e.g., bovine herpesvirus, *Trueperella pyogenes, Histophilus somni*) usually result in a severely autolysed fetus, as time is required for the corpus luteum to lyse, the cervix to dilate, and for prostaglandin to contract the uterus and expel the fetus. In infections that are chronic and take time to kill the fetus (e.g., *B. abortus*, most mycotic abortions, *Yersinia pseudotuberculosis*), the preparations for abortion may be complete by the time the fetus dies, and therefore the fetus will be aborted in a relatively well-preserved condition. In infections that are largely confined to the amnion, such as *Ureaplasma diversum*, the probable mechanism involves macrophage activation, resulting in cytokine-induced production of prostaglandin by the endometrium, lysis of the corpus luteum, and premature delivery of a well-preserved or slightly autolysed fetus, depending on how quickly death follows the infection.

Adventitial placentation (semiplacenta diffusa)

The development of intercotyledonary placentation in cattle is a mechanism of compensation for inadequate development

of placentomes. The inadequate number of placentomes is primarily endometrial and may be congenital or acquired. There are normally 75-120 caruncles in the cow and 40-125 in the ewe and goat. Not all of them are fully used in a normal single pregnancy. Occasionally, the numbers are much less as a congenital disorder of endometrial organization; more commonly, the reduction in number is acquired by inflammatory destruction of portions of the endometrium. Compensation consists of a great increase in the size of remaining caruncles during pregnancy, many of which may fuse, and by the development of a more primitive villus placentation between the placentomes. *The adventitious placenta tissues usually develop first adjacent to the placentomes;* the process may remain localized or involve virtually the entire intercotyledonary placenta, chiefly along the floor of the uterus. In the latter instances, pregnancy is insecure and may not proceed beyond midterm. Hydrallantois is a complication. Adventitial placentation has not been observed in the sheep or goat placenta.

Hydramnios and hydrallantois

Excessive accumulations of fluid in the amniotic and allantoic sacs are infrequent diseases of pregnancy. They occur most often in cattle and are rare in other species. Excess fluid may accumulate in both sacs, but this is the exception. The source, nature, and control of the fluid in the 2 sacs are different, and the conditions that give rise to excess fluid in each sac tend to be different. The total amount of fetal fluid increases progressively throughout pregnancy, and the volume at term in cattle is between 15 and 20 liters. This represents a 5-fold increase during the last 4 months of gestation. The relative quantity of fluid in each sac varies during the gestation period. During the first and third trimesters, the amount of allantoic fluid is greater. The nature of the fluid in each sac varies during gestation. At term, the amniotic fluid is glary, slightly viscous, and consists largely of fetal salivary secretion, the volume being largely controlled by fetal swallowing. The allantoic fluid is thin and watery and is derived from the fetal kidney via the urachus, but regulation of the quantity of fluid is by the membranes themselves.

Hydramnios, or hydrops of the amnion, is usually associated with malformation of the fetus. The malformations may be either inherited or acquired. A variety of inherited diseases can cause hydramnios. There is a high incidence of the condition: in bison-cattle hybrids, in association with chondrodystrophic and muscle contracture malformations of bovine and ovine fetuses, as well as in other types of fetal malformation, particularly those involving gross facial anomalies.

Hydrallantois in cattle is most often associated with uterine disease with inadequate numbers of caruncles and the development of adventitial placentation. It also occurs with increased frequency in cows bearing twins and in pregnancy of cloned embryos. The quantity of excess allantoic fluid may be as much as 170 L, but it is not notably different in quality from normal. The fetal membranes are only slightly thickened, but may be tough and rupture with difficulty. Dystocia with uterine paresis, retention of placenta, and metritis are sequelae of those few cases that do not abort earlier in gestation. The fetuses are usually dead when aborted or delivered at term and small for their age, but may have anasarca and ascites.

The disease occurs but rarely in *mares*. The conditions that predispose in this species are not known.

Amniotic plaques, placental mineralization, and avascular chorion

In cattle these are normal grossly apparent features, and their inclusion here is warranted only to avoid confusion to anyone seeing them for the first time. *Amniotic plaques are foci of squamous epithelium on the internal surface of the amnion*. They may or may not be keratinized. They are ~2-4 mm in diameter, flat, and resemble lesions of the poxes. They are especially concentrated on the umbilical stump, where they are taller, cylindrical, or papilliform. They seem to be constantly present on the bovine amnion during the middle trimester, and they do also occur in other species but have not received much attention.

Deposits of mineral are visible in some chorioallantoic membranes as white streaks and spots. These occur from about the end of the first to the middle of the second trimester. The degree of mineral deposition is quite variable and is more extensive in the allantois than in the amnion.

Metaplastic ossification complete with large multinucleated osteoclast-like cells occurs commonly in the placenta of sows and goats giving birth to normal litters but is rarely seen in other species.

In ruminants and pigs, it is common to find areas of *necrotic chorioallantois*, commonly called the "necrotic tips," at the apical tips of both horns. It is almost invariably present from the period of elongation to term. Sometimes the region may extend for several centimeters.

Prolonged gestation

Syndromes characterized by abnormally long gestation periods occur in cattle and sheep. Some of these syndromes have been only partially defined, but in all cases adequately documented, *evidence of fetal anomaly, either anatomic or functional, has been present*. Two of the syndromes in **cattle** have a genetic basis, the trait being governed by autosomal recessive genes, the defective fetus being homozygous.

- The first of these, which is most common in the Holstein and Ayrshire breeds, produces a nonviable large calf after a gestation period that is approximately 2 months longer than normal. The parturition is abnormal; the maternal preparations of relaxation of the pelvic ligaments and filling of the udder are minimal, and assistance is usually necessary. The calves are large, but of nearly normal proportions. They suffer from severe respiratory distress. Death is due to either these respiratory difficulties or uncontrollable hypoglycemia. The *adrenals are hypoplastic*, and this is thought to be the lethal defect in the syndrome.
- In the second type of inherited prolonged gestation, which is seen in Guernsey, Jersey, and Swedish Red and White breeds, *fetal monsters* are common. Many of these animals have severe head anomalies of the cyclopian type, but in some the defect is discrete and consists only of the absence of the pituitary. *Adenohypophyseal aplasia* is common to all of them. These fetuses fail to develop after approximately the seventh month of gestation, and the length of gestation appears to be determined by the viability of the fetus. Some gestation periods last 17 months; at this time, presumably, the placenta can no longer adequately provide for the fetus, and the dead fetus, still developmentally immature, is expelled.

Not all syndromes of prolonged gestation in cattle fit this pattern either genetically or anatomically, and sporadic

examples of fetuses unable to terminate their gestation occur. These are usually grossly deformed giants with severe brain anomalies; these animals regularly die in utero.

Prolonged gestation occurs in **sheep** as a result of chemically induced teratogenesis. If ewes ingest *Veratrum californicum* on or about day 14 of pregnancy, they may fail to begin labor at term. Pregnancy then continues for weeks beyond term, leading ultimately to rupture of pelvic ligaments and maternal death. The fetuses damaged by the plant toxin cyclopamine have holoprosencephaly and cyclopian deformities and can attain giant proportions. Originally, the pituitaries were thought to be absent. It appears that in most cases the gland is displaced in the malformed head.

The functional defects that prevent the various types of fetuses from making their timely contribution to their delivery can now be interpreted in the light of experimental studies. These indicate that, for a fetal lamb to initiate parturition, the fetal hypothalamus, its connection to the pituitary, the pituitary, and the adrenals must be functionally competent. If any of these links is defective, prolonged gestation results. Only hypophyseal absence, however, leads to fetal dwarfism.

An additional syndrome of prolonged gestation has been recognized in ewes that are fed the African shrub *Salsola tuberculata*. The mechanisms by which this plant poisoning interferes with parturition are unknown. They appear to be different from previously reported ones in that the effects of the plant are not teratogenic, and the intoxication prevents parturition when fed during the last 50 days of gestation. The plant may inhibit fetal hypothalamic-releasing factors.

S. tuberculata is drought-resistant, and the disease occurs when other feed is lacking. The Karakul breed of sheep is the breed raised where the plant is common and is the breed most often affected, but other breeds are susceptible to the toxic effects of the plant. The affected ewes fail to develop normal preparturient udder enlargement, but show no other signs of intoxication.

The fetuses continue to grow and usually initiate parturition 10-20 days past term. At delivery, the affected fetuses are large, lethargic, and suffer a high mortality rate. The pelts are overgrown and worthless, the hooves long, and the teeth erupted. The adrenals of fetuses are hypoplastic and the pituitaries are small, and normal granulation of the cells of the adenohypophysis is lacking.

Prolonged gestation also occurs with exposure to ergot alkaloids (ergopeptines) in fescue endophyte toxicosis in horses and in exposure of ruminants to ergot alkaloids.

Further reading

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ABORTION AND STILLBIRTH

It will be apparent from the foregoing discussion that *diseases of the conceptus may result in death with resorption, fetal mummification, abortion, or stillbirth*, depending on the age of the conceptus and the species involved, but *not all fetal infections result in fetal death*. Some viral infections appear to cause little harm to fetuses, at least during certain stages of gestation, and in some the effects are subtle. Many fetal diseases, such as brucellosis of cattle and sheep, epizootic bovine abortion, *U. diversum*, and mycotic fetal infections in cattle are chronic and may lead to the premature delivery of small-for-gestational-age and diseased fetuses.

The list of bacterial and mycotic infections known to produce sporadic abortion is so long as to be nearly valueless because *any bacteremia or systemic fungal infection occurring during pregnancy carries with it a great risk of bacterial or mycotic colonization of the fetomaternal interface.* The entire basis of this susceptibility is not known; however, factors within the conceptus, such as isolation from the maternal immune system, decreased oxygen concentration, elevated temperature, sluggish inflammatory and immune responses, and the provision of preferred nutrients for many organisms, must be important. The organisms involved in fetal infection are the same irrespective of the period of gestation. The effects vary greatly and overall, fetal resistance increases with age. There is some evidence suggesting that bacterial colonization of the fetal membranes does not occur as readily before embryo attachment.

Bacterial and mycotic fetal infections are most common in cattle and horses. There are, however, *important differences in the pathogenesis of the infections in the 2 species.* Except for the venereal infections early in gestation, bacterial fetal infections in cattle are probably hematogenous in origin. *Leptospira* and *Ureaplasma* infections of cattle, however, may reside on the zona pellucida of the ovum and in the uterine tube and move to the placenta during pregnancy. Bacterial infections of the equine placenta may arise hematogenously, by direct penetration with setae of ingested processionary caterpillars, or infect the fetal membranes through the less protective cervix. The route of the infection can usually be determined by examination of the placenta. Transcervical infection, the most common route, involves the chorion adjacent to the internal os of the cervix.

The fetal effects of viral infection depend on the virus and the age of the fetus. Where the effects are known in detail, the pattern is that infections early in gestation are more likely to kill or produce serious teratologic effects; later in gestation, the effects are less severe. There are important exceptions, and the details are not known for most viral infections. Frequent mention is made throughout these volumes to abortion in relation to specific viral diseases. The fact that no mention is made of fetal disease should not be implied to mean that the virus in question is incapable of reaching the fetus or harming it. And, alternatively, the fact that fetal disease is associated with a specific viral disease should not be construed to mean that fetal disease is produced at all stages of gestation, or if it is, the resultant fetal lesions are the same throughout the gestation period.

Toxic, nutritional, genetic, and physical diseases are also important causes of reproductive failure and some mention is made of them elsewhere, but often the details are not available. Selected here for description are those infections that

Further reading

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BOX • 4-1

Infectious causes of abortion

Bacterial

Actinomycetes—*Crossiella equi, Streptomyces, Amycolatopsis*
Brucella spp.—*B. abortus, B. suis, B. ovis, B. melitensis, B. canis*
Campylobacter spp.—*C. fetus* subsp. *venerealis, C. fetus* subsp. *fetus, C. jejuni*
Chlamydophila spp.
Coxiella burnetii
Deltaproteobacterium
Flexispira rappini
Histophilus somni
Leptospira spp.
Listeria spp.—*L. monocytogenes, L. ivanovii*
Salmonella spp.
Trueperella pyogenes
Ureaplasma diversum
Yersinia pseudotuberculosis
Miscellaneous—β-hemolytic streptococci, *Escherichia coli, Pseudomonas, Staphylococcus aureus, Klebsiella pneumoniae, Actinobacillus equuli, Rhodococcus equi, Encephalitozoon cuniculi*

Mycotic

Aspergillus fumigatus
Pseudallescheria boydii
Yeast—*Candida, Torulopsis*
Zygomycetes—*Lichtheimia (Absidia), Mortierella, Rhizomucor, Rhizopus*

Protozoan

Neospora—N. caninum, N. hughesi
Sarcocystis spp.
Toxoplasma gondii
Tritrichomonas foetus

Viral

Family *Arteriviridae,* genus *Arterivirus—Equine arteritis virus* (EAV), *Porcine reproductive and respiratory syndrome virus* (PRRSV)
Family *Flaviviridae*
- Genus *Flavivirus—Wesselsbron virus* (WESSV)
- Genus *Pestivirus—Border disease virus* (BDV), *Bovine viral diarrhea virus* (BVDV), *Classical swine fever virus* (CSFV, hog cholera virus)

Family *Parvoviridae*
- Genus *Bocavirus—Bovine parvovirus* (BPV), *Canine minute virus* (CnMV)
- Genus *Parvovirus—Porcine parvovirus* (PPV)

Family *Reoviridae*
- Genus *Orbivirus—Bluetongue virus* (BTV), Chuzan virus (CHUV, *Palyam virus*), *Epizootic hemorrhagic disease virus* (EHDV), Ibaraki virus (IBAV)

Family *Herpesviridae*
- Genus *Cytomegalovirus—Suid herpesvirus 2* (SuHV-2, swine cytomegalovirus)
- Genus *Rhadinovirus—Bovine herpesvirus 4* (BoHV-4, Movar virus)
- Genus *Varicellovirus—Bovine herpesvirus 1* (BoHV-1; infectious bovine rhinotracheitis virus [IBRV]; infectious pustular vulvovaginitis virus [IPVV]); *Canid herpesvirus 1,* (CaHV-1, canine herpesvirus); *Equid herpesvirus 1* (EHV-1, equine abortion virus); *Suid herpesvirus 1* (SuHV-1, pseudorabies virus, Aujeszky's disease virus)

Family *Bunyaviridae*
- Genus *Orthobunyavirus*, Bunyamwera serogroup—Cache Valley virus (CVV, *Bunyamwera virus*)
- Genus *Orthobunyavirus*, Simbu serogroup—Aino virus (AINOV, *Shuni virus*), *Akabane virus* (AKAV), Schmallenberg virus (SBV)
- Genus *Phlebovirus—Rift Valley fever virus* (RVFV)

Family *Circoviridae*
- Genus *Circovirus—Porcine circovirus 2* (PCV-2)

characteristically result in abortion as a major manifestation (Box 4-1).

Diagnosing the infectious causes of abortion

The diagnostic rate on the causes of abortion, based on examination of samples submitted to veterinary laboratories throughout the world, varies from 5-90%, depending on the species and geographic location. The proportion of abortions that are caused by infection in most species, with the exception of the horse, is not known; however, ~90% of those abortions in which the cause is determined are assigned to infection.

Not all of the infectious causes can be recognized by the examination of fetuses and placentas. The effects of infectious and noninfectious causes may be manifest indirectly through the dam or directly through the placenta and fetus. *Conditions in the dam affecting the fetus and placenta and which may result in abortion include hemoconcentration, circulatory failure, anemia, fever, endotoxemia, and respiratory disease.* With these conditions, no lesion of diagnostic value will be found in the fetus or placenta.

Pregnant animals may contact infectious agents by many routes: through the respiratory tract, vagina, mouth, or, if insect-derived, through the circulation. Some agents may be carried into the reproductive tract with the semen or embryo transfer fluids. In cattle, *U. diversum*, bovine herpesvirus 1 (BoHV-1), and bovine viral diarrhea virus (BVDV) may contaminate either of these, and being preserved by freezing and resistant to the antibiotics commonly used, they survive there. In addition, it appears that some agents, such as *Leptospira hardjo*, may be maintained in the uterus or uterine tube over the open period and infect the conceptus as it develops. The role of the changing hormonal influence in the reproductive tract on the multiplication of organisms may be substantial. *Campylobacter fetus* moves from the vagina to the uterus as

pregnancy advances, and the hormonal influence is altered. Some organisms may multiply in the conceptus for a very long period before abortion. *U. diversum*, for example, may remain in amniotic fluid for up to 117 days before abortion occurs. Similarly, *Aspergillus fumigatus* may multiply in the placenta for at least 25 days before producing abortion, and it is suspected that BoHV-1 remains in the placenta for a prolonged period before invading the fetus.

Cooperation among all parties involved (owner, clinician, pathologist, bacteriologist, virologist, molecular biologist) is often necessary to reach a diagnosis. Eventually, other specialists, including nutritionists, toxicologists, botanists, and epidemiologists, may be consulted.

Because the act of abortion rarely points to a definite cause, it is important to collect all information at the first contact. Although the history seldom points directly to the cause of abortion, clues may be found that will indicate what needs to be done to determine the diagnosis. Abortion may occur sometime after the initiating illness, and therefore health, performance, and travel records become important in suggesting the diagnosis.

As the dam, placenta, and fetus may equally be involved, each should be examined and sampled. In addition, and perhaps more important, a representative portion of the remaining animals that have not aborted should be examined.

- Samples should include *serum* from the dam and herd (flock, or kennel), swabs, or samples from the uterus (the caruncle in cattle may be useful), placenta, and fetus.
- If the *placenta and fetus* cannot be quickly and suitably transported in their entirety, samples may be removed and one portion placed in fixative and others in plastic bags on ice for culture and molecular techniques.
- Tissues may, in addition, be *frozen* for future culture or testing for agents by using one of the many immunologically based techniques presently available.
- Samples from the conceptus for *culture* should include placenta, stomach content, lung, and kidney.
- Tissues essential for *histology* are a sample of the endometrium (caruncle), placenta, brain, eyelid (with conjunctiva), thyroid, thymus, lung, heart, liver, spleen, kidney, adrenal, and intestine. To be most useful, tissues for histology must be properly fixed or chilled (not frozen) if transport is to exceed 3 hours.

As with the history, it is important to maximize sample collection initially, thereby permitting the use of all the necessary diagnostic aids as the cause is gradually revealed. Samples not required are easily discarded later. The results of serology are more easily interpreted on a herd basis than on an individual animal and may be particularly useful when samples are collected before pregnancy or at the time of pregnancy diagnosis and again at the time of abortion. This technique has proven very useful in the diagnosis of leptospirosis. Only a representative portion of the group need be sampled to establish a baseline titer. Two samples from a dam that has just aborted may contribute little useful information as many agents that subsequently cause abortion have initiated production of a high antibody titer long before abortion occurs. Serum antibody titers are often within the "normal" range at the time of abortion. Interpretation of the significance of antibody titers may be further complicated in that many animals may not respond to the agent with the production of antibody, and yet the animal may be carrying the organism and abort because of it. Up to 22% of cows aborting because of *Leptospira hardjo* may not have a significant titer (microscopic agglutination test) at the time of abortion.

It is important not to eliminate a disease from the list of possibilities because of a history of vaccination. Many disease agents may cause abortion in spite of previous vaccination, for instance, BVDV, BoHV-1, and equid herpesvirus 1 (EHV-1), and of course, vaccination with certain live vaccines during pregnancy may cause abortion. Vaccination titers may be difficult to distinguish from titers resulting from infection, particularly if exposure has followed inoculation of even a single dose of vaccine. Baseline values need to be established.

Frequently, lesions pointing to the diagnosis may only be present in the placenta. As the placenta is a very large organ, and much of it may be normal, *the more of the placenta that can be examined, the more accurate assessment of it is likely to be.* It rates in importance for examination equal to both the alimentary and respiratory tracts of an animal after birth. Unless the entire placenta is expelled, at least part of the portion retained internally should be examined as it often has the most severe lesion and is less contaminated by *bacteria from the environment. If the placenta cannot be found, endometrial samples* become more crucial and should be requested. Samples may be divided in 4 and used for bacterial culture and histology, a portion frozen for virology, and for molecular techniques, according to the submission guide of the diagnostic laboratory. **The optimal submission includes the fetus and placenta plus serum from the dam**. Serum is best collected at pregnancy diagnosis and then resampled at time of abortion. This method has been found of particular use in the diagnosis of leptospirosis in dairy cows on drylot, where the fetus is often not discovered until trampled. Examination of a "fresh" fetus is 53 times more likely to contribute a diagnosis than an autolysed one. Submissions including the entire fetus, placenta, and serum are 4.28 times more likely to result in a diagnosis than where pieces only of the fetus are submitted. Titers of fetal antibody may also be useful in diagnosis of BVDV, *Neospora*, or leptospiral infections.

Some lesions and normal findings that may be confusing will be described briefly. To examine the **caruncle**, it should be cut sagittally and the cut surface examined for infarction or suppuration. Infarction is most commonly recognized in mycotic infections but may be caused by a variety of bacteria, including *Brucella* and *Salmonella*. Infarction frequently results in retention of caruncular material by the cotyledon. A variety of bacteria, including *T. pyogenes,* may cause suppurative placentitis. Occasionally, fibrosis of caruncles is seen and may represent normal caruncular maturation or be the result of a chronic infection caused by organisms such as *Yersinia pseudotuberculosis.*

To examine the **chorioallantoic membranes**, they should be completely spread out and inspected carefully for lesions on both surfaces. Lesions in the bovine placenta are commonly observed toward the tip of the pregnant horn and should not be confused with the normal avascular chorion in this site. On histologic examination of H&E-stained sections of the chorionic villus, a blue finely granular to amorphous material may be observed in the interstitium. This may be glycosaminoglycans, mineral, bacteria, or DNA, and special stains and even close examination will differentiate it. Glycosaminoglycans are considered normal, as is mineral in the early stages of gestation. In the later stages, however, mineral is commonly observed in areas of necrosis and feels gritty on gross palpation.

Trophoblasts should be thoroughly examined on high power as a variety of bacteria may be phagocytosed, or at least contained, by them. The erythrophagocytic trophoblast has been shown to phagocytose *Brucella*, and the organism has been seen by electron microscopy in the rough endoplasmic reticulum. *Coxiella burnetii* is also found in the trophoblast and can usually be distinguished from *Chlamydophila* and other bacteria in that the cytoplasm of the cell appears foamy compared to the finely granular appearance of bacteria.

Lesions of variable intensity are usually seen in vessels of the placenta. Discrete necrotizing vasculitis of the endothelial cells in small vessels of the villi regularly occurs with BoHV-1 infection. Mycotic infections cause very severe vasculitis, resulting in thrombosis and infarction of the caruncle and cotyledon. *H. somni*, although perhaps uncommon as a cause of abortion, may cause a similar lesion. *Chlamydophila* infections of the placenta may also cause severe vasculitis, but usually without thrombosis. The bacteria-packed vessels in *Salmonella* placentitis are so near the surface that they are easily mistaken for phagocytosed bacteria in trophoblasts. Microthrombi may be present also.

Lesions of hemorrhage, necrosis, mineralization, and fibrosis may also be observed on the chorionic and allantoic surfaces and are commonly seen with a variety of fungi and bacteria, including *U. diversum*.

The inner surface of the **amnion** may be severely stained with meconium, particularly in chronic progressive infections of the chorion. It is frequently the site of lesions associated with *U. diversum* infection and is there characterized by multifocal to confluent areas of necrosis, mineralization, and fibrosis, with marked thickening and mild vasculitis being most common. Mycotic and *Mycoplasma* infections may penetrate the chorion and allantois to the amnion and result in a similar lesion.

Discrimination must be used in interpreting the results of cultures derived from samples of fetus, placenta, or even the stomach contents, as organisms may move or be carried from the vagina to the conceptus when the cervix dilates during an impending abortion resulting from another cause. These organisms may rapidly contaminate the placenta, grow in the fetal fluids, and may even be swallowed by a viable fetus, thereby appearing in the stomach contents. *To avoid confusion, vaginal samples should be taken as soon as a vulvar discharge is seen or impending abortion is suspected*. Vaginal discharges from animals with impending abortions are much less likely to be contaminated by extraneous organisms than are samples from an animal that has aborted several days previously.

Observing the relative state of preservation of the fetus may contribute information about the cause. Acute infections, such as those caused by BoHV-1, usually result in the discharge of a severely autolysed fetus, as the fetus is killed rapidly. Chronic infections, (such as *U. diversum*, *B. abortus*, *Y. pseudotuberculosis*, or *A. fumigatus*), or other persistent stresses, however, allow the fetus to prepare itself for delivery, and hence it is discharged in a fresh state, perhaps small for gestational age, and sometimes alive. *Evidence of the significance of an isolate may only be obtained by histologic examination of certain tissues.*

A few of the less commonly examined tissues that may be useful in making the overall decision about the cause of abortion will be briefly considered. Microscopic examination of the **eyelid** (surface and palpebral conjunctiva) will reveal lesions in about 2/3 of the bovine fetuses aborted because of infectious causes. Lesions are most frequently observed on the conjunctival surface and vary from a slight infiltrate of mononuclear cells to a severe necrotizing reaction. As in other areas, the cellular infiltrate may be determined by the duration of the infection before death of the fetus. In cattle, *Yersinia* spp., *Ureaplasma*, and mycotic infections are commonly associated with a heavy focal mononuclear cell infiltrate just under the conjunctiva, whereas *T. pyogenes* infection may result in complete loss of conjunctival epithelium, and the accumulation of large numbers of bacteria, but few inflammatory cells, on the surface. Many bacteria, including *T. pyogenes* and *Listeria*, continue to multiply after the death of the animal. BVDV infection may initiate perivascular lymphocytes and cause injury to hair follicles. The pattern of follicular hair growth when compared to the age of the fetus may determine the time of infection. Mycotic infections cause folliculitis; however, the lesion is very variable, not only as to its presence but also its severity, and although intracorneal pustules may be seen, frequently either no lesion is observed, or traces of inflammation and fungi are only found in the outer sloughing keratinized debris. This material may be lost unless care is taken in sectioning.

Examination of the **brain**, particularly with the discovery of the importance of protozoan infections as a cause of abortion in ruminants, is an essential part of any postmortem examination. Many viral infections manifest their effects in the brain. Although the brain often appears soft and unworthy of fixation, and some are literally poured into the fixative, interesting and informative lesions are often found only in this site.

The **lung** *ranks among the most common organs in which to see lesions.* Aspiration of meconium into airways, with or without evidence of an agent, is seen in the terminal phases of many bacterial, and less commonly in mycotic infections, and is probably related to the rapidity of the development of the lesion and the degree of impairment of placental function. Frequently, there will be no local reaction in the lung to the agent, and in these the exudate present probably represents aspiration of the agent and inflammatory cells from a placentitis. Many of the inflammatory cells may be maternal in origin. In *U. diversum* and bovine parainfluenza virus 3 infections in bovine fetuses, airways may remain relatively clear, and alveolar ducts become thickened and the lumina pavemented with many macrophages and a few neutrophils. Severe necrotizing bronchitis may be seen with EHV-1 infection in horses, whereas BoHV-1 in cattle usually produces only multifocal necrosis and no visible pneumonia.

Examination of the **intestine** contributes much information in some specimens. Sections should be removed from the small and large intestine, with care taken to preserve the content intact. Bacteria may be seen colonizing the luminal content and are used as an indicator of whether or not the bacterial isolate is significant. Terminal swallowing of an agent may transport it to the stomach; however, finding the agent in the intestinal lumen is evidence that it has been in the animal for at least several hours. The fetus swallows amniotic fluid beginning at a very early age, and bacteria and fungi can be easily seen in meconium. In one survey, lesions were observed in association with a variety of agents in 32% of 50 bovine fetuses examined. Lesions varied from mild with *A. fumigatus* to focal areas of necrosis in the mucosa with BoHV-1, diffuse necrotizing colitis with *Listeria*, cryptal necrosis and Peyer's patch lymphocyte hyperplasia

with *Bacillus* spp., loss of crypts, and large numbers of lamina proprial lymphocytes and plasma cells with *Y. pseudotuberculosis*.

Isolation of different organisms concurrently from the products of abortion makes interpretation difficult. BVDV is commonly detected along with other organisms also considered to be causes of abortion, and in one study was found in 3 of 16 fetuses with *Bacillus* sp., 6 of 30 with *T. pyogenes*, and 17 of 45 with fungal infection. In these animals, immunosuppression associated with BVDV infection may permit the organism to circulate in the dam and localize in the placenta, or it may be that, as just stated, the animal was aborting because of BVDV infection and the other organisms were contaminants. In contrast, abortion may occasionally be classified as infectious because of histologic lesions observed, but where no organism is identified. Failure to grow the organism may be due to improper handling of the samples or destruction of the organism in severely autolysed fetuses.

Portions of *chilled kidney* are useful for the fluorescent antibody detection of BoHV-1, BVDV, and *Leptospira*. *Stomach content* is useful for bacterial or fungal culture and direct examination for bacteria (including *Coxiella*) and fungi. In some diagnostic laboratories, all samples of stomach content and impression smears of placenta are examined using a modified acid-fast stain. Using this stain and others, an experienced microbiologist can distinguish a variety of organisms. Certain organisms are extremely fragile, and care in handling samples becomes very important. Samples to be cultured for *U. diversum*, for example, should be collected aseptically, chilled to 4° C, and delivered to the laboratory immediately. Leptospires may be equally fragile.

Fetal serum or thoracic fluid samples for antibody detection may be collected, but the antibody titers, as in the dam, must be interpreted with caution. The fetus is capable of responding to a variety of agents with antibody production relatively early in gestation, and the presence of specific antibody in fetal fluids may be considered evidence of fetal infection. It has been shown, however, at least in sheep, that placental vascular damage may allow antibodies to cross the placenta from the dam to the fetus, thereby making interpretation difficult. When antibody passage occurs, concentrations in the fetus will presumably be lower than in the dam. Fetal antibody detection has been useful in diagnosing fetal BVDV and leptospiral infections in third-trimester fetuses.

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Bacterial causes of abortion

There are many different bacteria that infect the placenta. In general, the response to infection is similar and stereotyped, with some variation between species. The archetype bacterial cause of a placentitis is *Brucella*, which will be described first.

Brucellosis

Bacteria of the genus *Brucella* are small, gram-negative bacilli or coccobacilli that are strictly parasitic, prefer the intracellular habitat, and produce in animals chronic infections with persistent or recurrent bacteremias typically manifested by abortion.

Three classic species of *Brucella* were described and defined originally largely on the basis of host of origin—***B. melitensis***, goats; ***B. abortus***, cattle; and ***B. suis***, swine—but now by biochemical and serologic reactions. The differences between the species are slight and quantitative rather than qualitative, and the number of biotypes within each species is large. The biological similarities among the various species and biotypes of *Brucella* are in keeping with the remarkable similarities in the diseases produced in the different hosts by the various strains of the organism. Infections are initially systemic, and relapsing bacteremic phases are well-established events in the persisting infections. Localization and persistence of infection may occur in many organs, perhaps to a greater extent with *B. suis* than with the other species. Some organs, however, notably the genitalia and placenta, develop intense persistent foci of infection.

Bovine brucellosis caused by *Brucella abortus*. *Brucellosis occurs in cattle in most parts of the world.* In some countries the incidence of the disease is low, either because of measures taken to prevent its entry or to eradicate it, but where the disease is endemic and uncontrolled, the incidence may approach 20-30%.

The usual source of infection for cattle is an aborted fetus or placenta, or contaminated uterine discharges, and the usual route of infection is alimentary. Infection can also occur per vaginam, via the conjunctiva, or through the broken or unbroken skin. The relative importance of these latter routes is not known. Coital infection can occur but is uncommon, especially if genital infection in the male is long standing, possibly because fewer organisms are excreted in semen from chronic lesions. Infection can be transmitted at artificial insemination if semen from infected bulls is used. Irrespective of the route of infection, the development and establishment of infection are probably comparable and will depend on the age and reproductive status of the animal, its inherent resistance, and on the dose and virulence of the infecting strain of the organism. Young cattle are relatively resistant up to about the age of puberty. They can be infected by the usual routes and means, including the ingestion of milk in which the organisms are intermittently excreted. Calves normally eliminate infection in a few months.

Once infection is established in sexually mature animals, females especially, it tends to persist indefinitely. Some, perhaps many, will recover completely, but which ones will, and when they will, cannot be predicted. The organisms extend quickly to the lymph nodes regional to the point of entry, and there they provoke acute lymphadenitis. The

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inflamed nodes are enlarged, often much so; hyperplastic with no clear corticomedullary distinction; and frequently bear small or large medullary hemorrhages. The sinuses are infiltrated with neutrophils and eosinophils, germinal centers and proliferative activity become obvious, and there is slow but remarkable accumulation of plasma cells in the medullary sinuses. The changes in the regional nodes take some weeks to develop fully, and they persist for a prolonged period. There is no fibrosis or necrosis in the nodes.

The infection may be overcome in the regional nodes, but once established, it is expected to spread during the phase of acute regional lymphadenitis. Spread is chiefly hematogenous, and bacteremia may persist for several months, the duration of persistence apparently depending on the susceptibility or resistance of the host. As the infection becomes chronic, bacteremia becomes intermittent, ceases in some animals, and recurs irregularly for at least 2 years in 5-10% of animals. Also, it tends to recur at parturition. It might be expected that the bacteremic episodes would result in localization and persistence of the organism in many tissues, but curiously, localization is largely restricted to the spleen; mammary glands; mammary lymph nodes; pregnant uterus of the female; and to the lymphoid tissues, testis, and accessory glands in the male, and such localizations occur in the early bacteremic phases. The organism has little or no predilection for the kidney (although microscopic interstitial nephritis may be present), ovary, bone marrow, or mesenteric lymph nodes and appears not to be excreted in the urine or feces. Localization does occasionally occur in synovial structures to produce purulent tenosynovitis, arthritis, or bursitis, but whether localization and persistence occur in synovium that is healthy is not clear; some pre-existing inflammatory changes may be necessary.

Infected animals, almost without exception, excrete *B. abortus* in the colostrum. Thereafter excretion of the organism in milk may cease, but frequently it continues, although intermittently, throughout the period of lactation. Organisms excreted in milk are an important source of infection for children and also for calves, but they recover without significant effect. It is still not clear whether, and to what extent, *B. abortus* causes anatomic changes in the mammary glands. This must be expected, but they must also be mild and difficult to distinguish from the focal inflammatory reactions commonly present in mammary glands. Dense infiltrations of the interstitial tissue by plasma cells, lymphocytes, and histiocytes, and exudation of neutrophils in acini, probably constitute the usual changes. The mastitis is focal and not attended by gross changes. The cellular content of milk is increased.

B. abortus has special affinity for the pregnant endometrium and fetal placenta, to which it spreads hematogenously during the initial or later bacteremia. Experiments using *B. abortus* in goats have shown that the organism is carried by the bloodstream to the periphery of the caruncle, where at the extremities of the maternal villi, capillaries leak into the narrow space (hemophagous organ) adjacent to the fetal erythrophagocytic trophoblast of the chorion. Cells of the erythrophagocytic chorionic trophoblast either phagocytose the *Brucella* organisms or they invade; regardless, they multiply and spread, via the uterine lumen and endocytosis or by cell-to-cell transfer, to the rough endoplasmic reticulum of the adjacent chorionic trophoblast cells. They multiply here and spread to the fetus following ulceration of the trophoblast and invasion of the fetal chorionic villi. The process may be similar in the bovine caruncle, where hematomas appear at the ends of maternal septa late in gestation.

Gross lesions *in the placenta are characteristic but not pathognomonic*; similar lesions of lesser or equal severity may be caused by other bacterial infections, and fungi produce similar lesions, usually of greater severity. There is considerable variation in the severity of the placental lesions, and this is reflected to some extent in the course of the local infection. If the lesion is severe, abortion or premature birth is the likely outcome, and if the lesion is of minor severity, the calf may be delivered normally at term and be viable or nonviable. The intrauterine lesions apparently progress very slowly, because an interval of many months may elapse between infection and abortion or normal birth. Abortion occurs most often in the seventh and eighth months of gestation. Once the infection localizes in a pregnant uterus, it almost certainly remains there and remains active until the fetus and placenta are delivered and for some time thereafter. The nonpregnant uterus is not particularly susceptible to *B. abortus*, and following abortion or parturition, the organism is cleared from the uterus in a few weeks, or longer in some cases.

The external appearance of an infected pregnant uterus is normal. Sometimes the placenta is normal. Typically, *between the endometrium and chorion in the intercotyledonary area, there is more or less abundant exudate* that is odorless, dirty yellow, slightly viscid and slimy, and which contains gray-yellow, pulpy floccules of detritus. The fetal membranes and the umbilical cord are saturated with clear edema fluid, and the membranes may be 1.0 cm or more thick. The fetal fluids are usually normal, although occasionally fluid in the amnion is viscid and stained with meconium as the lesion extends.

The placental lesions are not uniform; some cotyledons may appear more or less normal, and others will be extensively necrotic, whereas still others are diseased to intermediate degrees. Similarly, the intercotyledonary placenta varies in the extent to which it is changed, lesions being most prominent adjacent to the cotyledons. Affected areas of intercotyledonary placenta are thickened with yellow gelatinous fluid, opaque and tough, and the normal smooth glistening surface takes on an appearance resembling yellow-to-gray Morocco leather with, on the surface, a patchy coagulum of inflammatory exudate and desquamated, degenerate epithelial cells. Affected cotyledons or portions of them are necrotic, soft, yellow-gray, and may be covered with the sticky, odorless, brown exudate.

Histologically, the lesions produced within placental tissues by *Brucella* bacterial infection is very similar between species. The chorionic epithelial cells are stuffed with bacteria (Fig. 4-67), and many of them, with their inhabitants, desquamate into the intercotyledonary space. Edematous placental stroma contains increased numbers of leukocytes, largely mononuclear but with some neutrophils. The organisms in intact trophoblasts are cocci, but free in the exudates; they assume a more elongate form even while still contained within the ghosts of dead trophoblasts. Within placentomes, the same sort of placentitis is present, but the infection is not so extensive in the trophoblasts covering the cotyledonary villi except at their base, or in the trophoblasts lining the caruncular crypts, although many of the syncytial trophoblastic cells may be necrotic. The intervillus portions of the placenta, the so-called placental arcades, are quite severely affected, and exudate accumulates between the arcades and the expanded outer extremities of the maternal septa. There is normally some placental exudate and minor hemorrhage in these

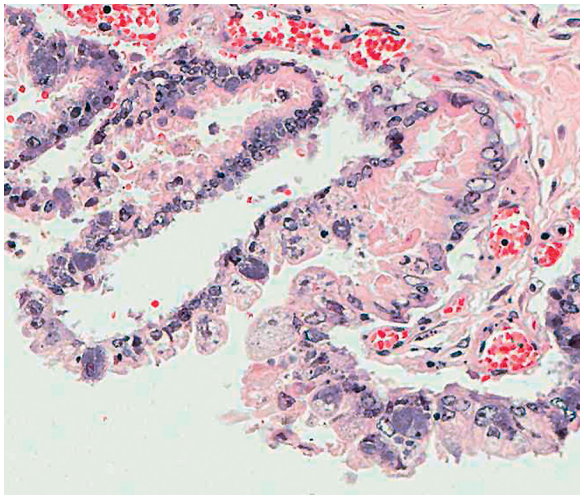

Figure 4-67 Massive proliferation of ***Brucella canis*** **bacteria within the cytoplasm of trophoblast cells,** along the deep edge of a canine placental labyrinth. Vasculitis and neutrophilic placentitis also are features of canine brucellosis.

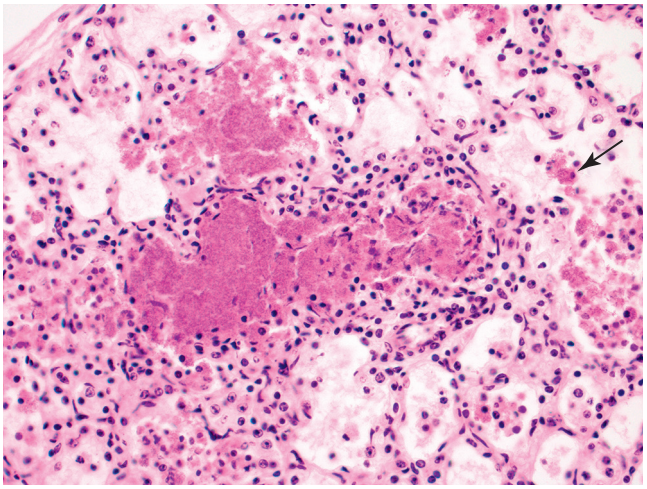

Figure 4-68 **Fetal pneumonia** in a bovine fetus aborted because of in utero infection with *Trueperella pyogenes*. Fetus and placenta infected with *Trueperella* typically contain large numbers of bacteria. The arrow points to an alveolar macrophage with its cytoplasm distended with bacteria.

spaces, but this is greatly exaggerated in placentitis and contains infiltrated leukocytes, epithelial debris, and bacteria. The maternal portions of the placentome are not much involved except for the expanded ends of the maternal septa where these are bathed in the exudate of the placental arcades, and in consequence, become denuded and superficially necrotic. Beneath the necrotic tips of the maternal septa, there are dense aggregates of neutrophils, with granulation and fibrosis extending along the sides of the septa. Inflammatory enlargement of the terminal portions of the maternal septa produces an increased degree of placental interlocking and probably contributes to retention of the placenta. Adhesions, in the usual sense of connective-tissue fusion, between placenta and uterus do not occur. The endometrium is relatively unscathed in the early infections. The zona basalis shows some increase in lymphocytes and plasma cells, and there may be scattered microscopic granulomas of epithelioid cells. Even the intercaruncular epithelium may not be overly disturbed. Later there is severe endometritis.

The **fetus** is usually autolysed and somewhat edematous with blood-tinged subcutaneous fluid. The same fluid is present in excess in the body cavities and the dorsal retroperitoneum. The normal abomasal content of a fetus is clear, translucent, thick, and viscid; in brucellosis, it often becomes very turbid, of a lemon-yellow color, and flaky. *The important fetal lesion in brucellosis is pneumonia, which is present to some degree in most fetuses aborted in the last half of pregnancy.* The lungs may appear grossly normal, but histologic examination reveals scattered microscopic foci of bronchitis and bronchopneumonia. When severely affected, the lungs are enlarged and shaped to the thoracic contour, firm on palpation, reddened on the pleural surface or hemorrhagic, and with fine yellow-white strands of fibrin on the pleura.

Microscopically, there may be any stage from the minor changes mentioned through a well-developed catarrhal bronchopneumonia to the fibrinous variety. The predominant inflammatory cells are mononuclear, although many immature and mature neutrophils may be present in some areas. The septa may be edematous and with perivascular leukocytes. An important differential diagnosis is *Trueperella* abortion, which also produces suppurative placentitis and fetal lung lesions. The pneumonia found in fetuses aborted by *Trueperella* infection is usually accompanied by large colonies of bacteria within airways and alveolar spaces (Fig. 4-68).

In bovine brucellosis, by the time the placentitis has advanced to an extent where abortion is inevitable, acute diffuse endometritis, without histologic specificity, has developed. Variable lesions in the fetus include necrotizing arteritis, especially of pulmonary vessels; focal areas of necrosis; and granulomas with giant cell formation in lymph nodes, liver, spleen, and kidney. The organism may be identified by culture, immunofluorescence, or immunohistochemistry.

Brucellosis in swine, caused by *Brucella suis*. Pigs are susceptible to *B. melitensis* and slightly susceptible to *B. abortus*. The disease is usually caused by *B. suis*, and this organism presents important problems in many countries. There are some differences in the diseases produced by *B. suis* and *B. abortus*, and these depend largely on the frequency with which *B. suis* in swine produces focal granulomatous lesions with coagulative necrosis, the affinity of this organism for the skeleton and joints, and its tendency to remain in granulomatous foci in the nonpregnant endometrium.

The early stages of the pathogenesis of the infection are comparable with the early stages of bovine brucellosis. Infection can occur by the same variety of routes, but in swine, brucellosis is chiefly transmitted by coitus. Boars are as readily infected as sows, and most infected boars develop lesions in the testes or accessory genitalia, from which the organisms are shed in the semen, often for life. They may also be shed in the urine from a focus of infection in the bladder. Infected females may discharge the organism from the uterus for up to 2.5 years. Suckling piglets can be infected, although they are less susceptible than weaners or adults, and although most infected piglets eliminate infection, a few carry the bacterium into adulthood.

With the development of lymphadenitis regional to the point of entry, the infection becomes bacteremic. The bacteremia may be transient or persist for many months or even

for some years. Localization may occur in many organs, but especially in the male and female genitalia; the skeleton, including the vertebral column; synovial structures; mammary glands; lymph nodes; spleen; liver; kidney; bladder; and even in the brain.

B. suis can grow and multiply in phagocytes, and the typical granulomatous lesion begins with the accumulation of histiocytes and epithelioid cells. Perhaps as a response to developing hypersensitivity to the organisms, as the lesion enlarges, caseous necrosis occurs centrally and fibrous tissue forms a capsule. The granulomas enlarge progressively, and the necrotic tissue attracts neutrophils. Giant cells are absent or scarce. Mineral may be deposited in the necrotic foci.

Articular lesions caused by *B. suis* are quite common. They begin as synovitis and chiefly affect the compound and large joints of the limbs. The reaction is purulent or fibrinopurulent. Osteomyelitis in this disease is typically vertebral (or usually observed there). As with other causes of osteomyelitis, localization is typically in the vertebral epiphyses of the lumbar region. There is, however, an unusual tendency to involve and destroy the intervertebral cartilages. The smaller bony lesions are typically granulomatous with dry caseation necrosis, but the necrotic cores of larger lesions may liquefy, and the suppurative reaction extends to the meninges or fistulae to produce paravertebral abscesses.

In the uterus and uterine tubes, there are often conspicuous and characteristic lesions, which are not dependent on an association with pregnancy. They have been referred to as miliary uterine brucellosis and, as the name infers, there are few or very many pale yellow nodules with an average diameter of 2-3 mm seeded in the mucosa. When the nodules are numerous, they may coalesce to form irregular plaques and then are associated with thickening of the uterine wall and stricture of the lumen. The same lesion is usually also present in the uterine tubes, where obstruction results in pyosalpinx. Incised, a small quantity of caseous exudate can be expressed from the nodules. Small, red, flat, and irregular granulomas are often scattered over the surface of the supporting ligaments; grossly, they resemble fetal fat and are easily overlooked. There is some stromal fibrosis and diffuse cellularity in the tubes and endometrium because of plasma cells and lymphocytes. In addition, well-developed and multiple hyperplastic lymphocytic nodules are evident. There are few neutrophils in the stroma, but with the mononuclear cells, they can be seen moving through the epithelium to collect in abundance in the lumen of the uterus and tube and in the more superficial glands. The glands are dilated and neutrophils are enmeshed in strands of mucin and mixed with amorphous globs of mucus. The deeper glands are cystically dilated with attenuated epithelium, serous or thin mucinous content, and few, if any, inflammatory cells. The epithelium lining the lumen and superficial glands is in part retained, in part desquamated, and in part shows the development of a remarkable degree of squamous metaplasia, even to the development of rete pegs and intercellular bridges. The organism is not visible in the lumen.

Abortion usually occurs between the second and third month of pregnancy, but the incidence of abortion is relatively less than in the bovine disease; *there is also a high incidence of stillborn and weak piglets born at term*, and probably also a high incidence of early undetected embryonic deaths. The placenta may be retained. The specific uterine lesions of porcine brucellosis are described previously. Apparently, the miliary lesions may develop during pregnancy to a superimposed diffuse catarrhal type of endometritis with patchy congestion, hemorrhage, and edema and a small amount of creamy-pink catarrhal exudate that contains large numbers of organisms. The fetal placenta may not show conspicuous changes, but as a rule, it is congested with small hemorrhages and patchy edema. Usually too, there is a thin layer of exudate that is slimy, gray-yellow, or gray-brown, like mucopus. It is more copious than the secretion normally present in the interplacental space, and smears from it show numerous free organisms and many epithelioid cells, presumably chorionic, that contain clumps of bacteria. The fetus shows autolysis with subcutaneous edema and blood-stained fluid within body cavities. The stomach contents may appear normal or be slimy, turbid, or yellowish and may contain small flakes like curd.

Brucellosis in sheep, caused by *Brucella ovis*. A *Brucella* mutant, *B. ovis*, causes a specific form of epididymitis in rams in most parts of the world in which sheep are raised. The organism also causes placentitis in pregnant ewes, but epididymitis is the more common and important manifestation of the infection, and the main discussion of the disease is included in Vol. 3, Male genital system.

There are probably as many modes of transmission as there are for the *Brucella* in general, but in this disease, *the coital route is important; infected rams excrete large numbers of the organism in the semen*. There is bacteremia, but no systemic disease. The organism is not very virulent. Even the epididymitis of rams is largely an indirect effect. The epididymal lesion produced by the organism is modest, but it does predispose to spermiostasis and extravasation, which produce the *characteristic spermatic granulomas* by which the disease is recognized. In contrast to the organism's nonpathogenic nature in the nonpregnant ewe, the organism readily parasitizes the placenta and produces abortion; this is not common, although it may be important in individual flocks. Although the manifestations of infection in both ewes and rams are solely genital, the organism can be cultured in large numbers from other organs in which it does not, however, produce lesions.

The *placenta* is grossly edematous, being thickened to 2-5 cm by gelatinous fluid. *Periarteritis and arteritis are features*, as in most forms of placentitis. The intercotyledonary placenta has plaque-like thickenings that may coalesce to resemble yellow-white chamois leather (Fig. 4-69). Diseased cotyledons may become partially detached; they are firmer

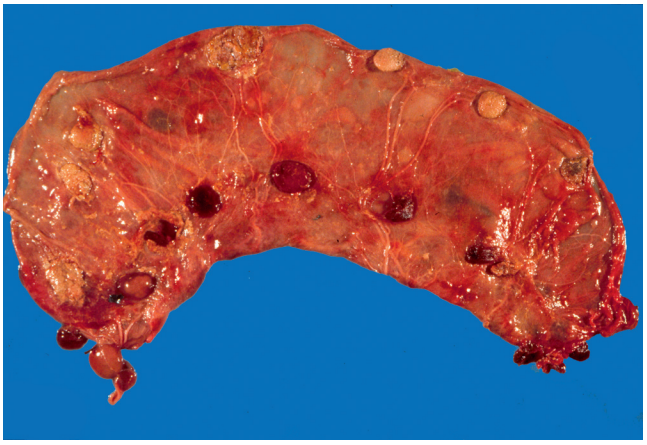

Figure 4-69 Placentitis caused by ***Brucella ovis*** in a sheep.

than normal, enlarged, and pale yellow. There is extensive necrosis of the epithelial elements of the cotyledons, with edema and cellular infiltration of the stroma. Organisms in abundance inhabit the epithelial cells of the chorion.

Most lambs are alive at the commencement of parturition, but mummification or progressive autolysis is also common. As usual, the fetus tends to be autolysed with blood-stained fluid within body cavities. Some may contain flecks or strands of fibrin. When present, mineralized plaques on the walls, soles, or both, of the hooves and accessory digits occur, but these are not pathognomonic. *The fetus shows little histologic evidence of systemic infection*, even though the gastric contents may be heavily infected. Mild pneumonia and lymphadenitis of the tracheobronchial lymph nodes may be present together with the development of germinal centers and plasma cells in other nodes and spleen. Acute interstitial nephritis, particularly about the corticomedullary junction, and inflammatory infiltrates in portal triads are common.

The nonpregnant uterus is not affected by the infection. Infection of the fetus may be produced experimentally at any stage, but following systemic exposure, as per conjunctiva, the period of greatest susceptibility to intrauterine localization is from ~21-90 days of pregnancy.

Following artificial exposure, there is a lag of 2-3 weeks before bacteremia develops, and the bacteremic phase, in rams and probably in ewes, lasts about 2 weeks. Breeding ewes to rams with open epididymal infection is more likely to result in infertility than abortion. The routes of natural exposure of pregnant ewes are not known. Placental localization is followed by slow development of the intrauterine infection, and the pregnancy may survive for 2-3 months after first being infected.

Brucellosis in sheep and goats, caused by *Brucella melitensis*. *B. melitensis* is the principal cause of brucellosis in sheep and goats, although natural infections with *B. abortus* occur occasionally. Brucellosis in goats and sheep is prevalent in countries bordering the Mediterranean, in the Near East, and in South America.

In most respects, *the caprine disease resembles bovine brucellosis*, but the disease in sheep is usually less protracted, and spontaneous recovery is fairly common in a few weeks or months. In the early bacteremic phases, goats may suffer severe illness and even die, but many infections are asymptomatic. The early signs of the disease may be referable to acute mastitis with palpable nodules in the gland and a secretion that is clotted and watery. The organism is excreted in the milk, usually only for a few weeks in sheep, but often for several months or even some years in goats.

As is usual for ruminants, *abortion may be the only sign observed, and it tends to occur late in pregnancy.* The uterine and vaginal discharges after pregnancy or parturition contain large numbers of organisms. Abortion or stillbirth may terminate successive pregnancies.

Brucellosis in dogs, caused by *Brucella canis*. *B. canis* was first isolated and recognized as a cause of abortion and epididymitis in dogs in 1966. The disease was identified in Beagle colonies in North America at that time. Since then, it has been found in many parts of the world and in a variety of breeds. However, accurate incidence rates are not available; in the United States, where the infection is widespread, the reported incidence rates are 0.5-5%.

The disease is caused by a mucoid strain of *Brucella* that shares biochemical features with *B. suis*. Because of the mucoid nature of the organism, it does not possess the smooth (O) antigens of *B. abortus* or *B. melitensis*. As a consequence, the conventional brucellosis test antigens that are used for diagnosis of the disease in other domestic species are ineffective in the diagnosis of *B. canis* infections.

Transmission of the disease can be *by ingestion* of infected vaginal discharge after abortion or *venereally* by infected seminal fluids. The seminal fluids may remain infected for months. Pregnant bitches may abort after 30 days of gestation, but *most abortions occur after 50 days. Testicular degeneration and epididymitis are the usual manifestations of the disease in male dogs.* Affected dogs frequently lick the scrotal skin, producing severe ulceration. Persistent bacteremias are common in many dogs that show no signs of disease. Bacteria have been recovered from the blood for periods as long as a year, even though high agglutinating titers were maintained throughout the bacteremic period. The nongenital lesions consist initially of nonspecific enlargement of the retropharyngeal and mandibular lymph nodes. Later the lymph nodes throughout the body may be enlarged because of diffuse hyperplasia of lymphocytes and macrophages. The aborted fetuses may be dead or alive when expelled, but live fetuses usually die within a few hours or days. Pneumonia, endocarditis, and hepatitis are observed in the infected fetuses.

Focal necrosis is prominent in the chorionic villi and many of the trophoblastic epithelial cells are laden with bacteria (see Fig. 4-67). In the bitch, serosanguineous to gray-green vaginal discharge may be evident for 1-6 weeks following abortion and provides a source of infection for other dogs.

Brucellosis in other domestic species. *Brucella abortus* or *B. suis* is regularly found in the bursal lesions described elsewhere as **poll evil** and **fistulous withers** (see Vol. 1, Bones and joints). There are other isolated reports of suppurative skeletal or synovial lesions in horses caused by these organisms.

Whereas **cats** are resistant to natural infection with *Brucella* spp., **dogs** are relatively resistant to the classic strains of *Brucella*, but natural infections caused by all 3 species of the organism do occur. The majority of such infections are asymptomatic and detected serologically. Orchitis and epididymitis have been observed. Other lesions described in dogs with brucellosis, chiefly minute granulomas in liver, kidney, and lymph nodes, are neither specific nor necessarily related to the infection.

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Campylobacter *infections of the genital tract in sheep and cattle*

Infections caused by *Campylobacter* spp. are common and widespread in humans, cattle, sheep, swine, and chickens and

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also occur occasionally in dogs. In sheep and cattle, diseases caused by these organisms were previously grouped under the title of "vibriosis," given their classification in the genus *Vibrio* at that time. The principal patterns of disease are *genital*, characterized by infertility or abortion, or *intestinal*, characterized by enteritis and diarrhea.

- ***C. fetus* subsp. *venerealis*** causes a true genital infection and is an important cause of infertility in cattle and can cause abortion in cattle and sheep.
- ***C. fetus* subsp. *fetus*** is a common resident of the ovine and bovine intestine and can cause abortion in both species but does so more commonly in sheep.
- C. *jejuni* is a common inhabitant of the intestinal tract in sheep and cattle and may cause enteritis in humans and occasionally animals. It frequently causes abortion in sheep and less often in cattle, although heavy losses in individual cattle herds do occur.

Genital infection of cattle caused by *Campylobacter fetus* subsp. *venerealis*. The disease in cattle caused by C. *fetus* subsp. *venerealis* is a specific venereal disease transmitted by coitus. Infected bulls may carry the organism indefinitely in the preputial cavity, although some recover spontaneously. Bulls do not become permanent carriers until they are at least 4 years of age, and most do not become readily infected until they are 5-6 years of age. Epithelial crypts are more prominent in the penile mucosa with advancing age and provide a favorable habitat for the bacteria. This also appears to be the natural habitat for these organisms, and once the infection is established in these older animals, it tends to be retained permanently as a locally innocuous surface contaminant together with the nonpathogenic C. *sputorum* subsp. *bubulus*.

The organism may also survive for long periods without producing lesions on the surface of the bovine vagina, but not in the uterus. Infected cows develop immunity and control the infection. *The outstanding feature seen with the infection is not abortion but temporary sterility or repeat breeding with prolongation of the interestral periods*. It is probable that the delayed return to estrus is the consequence of early embryonic death following fertilization and embryo attachment. Detectable abortions occur at any time but chiefly about the fourth to sixth month of gestation. The placenta is not usually retained.

The endometrial lesions in cows that are repeat breeders are mild and consist of lymphocytic inflammation with nodules and scattered cystic glands. Aborted placentas are often autolysed, indicating that fetal death occurs before expulsion. *Placental lesions resemble those in brucellosis but are less severe.* The intercotyledonary placenta is edematous, opaque, and may be leathery; there is diffuse inflammation, mainly histiocytic. Diseased cotyledons are yellow and pulpy; many have yellow necrotic villi at the margins, and in others these are scattered throughout. There may be dense accumulations of neutrophils among the denuded villi and in the stroma. The degree of placentitis is quite variable and may be inconspicuous. The parasitism of the chorionic epithelium that is characteristic of *Brucella* occurs but is less evident in placentitis caused by C. *fetus* subsp. *venerealis*. Lesions in the fetus are nonspecific. Blood-stained fluid in the subcutis and body cavities is common, and there may also be fibrin on the serous membranes. The normal colorless thick viscid fluid of the stomach content becomes yellow, very turbid, and flaky.

Genital infection of sheep and cattle caused by *Campylobacter jejuni* or *Campylobacter fetus* subsp. *fetus*. The chief manifestations of these Campylobacter infections in sheep are late abortion, premature birth, and the birth of weak lambs. There is not usually retention of the placenta or sterility. Occasional maternal deaths occur as a result of metritis. Infection is not synonymous with abortion, as infected ewes may deliver infected but clinically normal lambs. The incubation period is 7-60 days, and abortion occurs at any stage of pregnancy. The infection cycles through the flock throughout the season.

Transmission of C. *jejuni* most often occurs by the oral route, and fecal contamination of water supplies is relatively frequent. The organism is carried in the intestinal tract of poultry, and it is a commensal of cattle, sheep, and swine. The organism is also a frequent inhabitant of the intestine in dogs and cats and many rodents.

When strains of C. *fetus* subsp. *fetus* isolated from feces or bile are given orally to susceptible sheep, there is transient bacteremia followed by localization in the gut and bile, and the infection can spread by contact between sheep. In pregnant, nonimmune ewes, localization also occurs in the uterus. Surface (S)-layer proteins appear to be essential for colonization and/or translocation of C. *fetus* subsp. *fetus* to the placenta. With both of these C*ampylobacter* spp., the genital infection is primarily an intestinal infection; transmission is by ingestion, and *uterine localization is an accidental outcome of a brief bacteremic phase in a nonimmune sheep.* The incidence of abortion will depend on the number of ewes pregnant more than 1 month and on their experience with the infecting strain. Immunity following infection or abortion usually lasts for 2 years; however, if other strains are introduced, they may cause abortion, as cross-protection between strains is incomplete. This also applies to vaccination strains.

The abortion rate in natural outbreaks varies from 5-70% but is usually ~25%. *Aborted fetuses may have only nonspecific edematous changes; however, some have rather specific hepatic lesions.* Affected livers have few or many light tan areas, from 1 mm up to 2 cm in diameter, and randomly distributed (Fig. 4-70). Frequently, they have a "target pattern" with a more or less distinct, slightly raised white outer rim and a depressed tan inner zone. The abdomen is frequently distended with fluid, fibrin, and the enlarged liver. If the lamb has lived a while before succumbing, these lesions must be differentiated from the focal necrobacillary hepatitis of umbilical origin. Histologically, *the lesion is multifocal necrotizing hepatitis*, the necrotic region represented by the tan area and the outer rim by cellular infiltrate and necrosis. There is frequently bronchopneumonia with large numbers of neutrophils in major airways and alveolar ducts. Small renal cortical hemorrhages may also be present.

The cotyledons are enlarged, pale yellow to tan, dull and pulpy, and covered with brown exudate. The chorionic stroma is edematous and infiltrated with leukocytes, which are mainly histiocytes. There is frequently vasculitis. There is mild or acute endometritis with rather more prominent inflammatory exudation in the caruncular septa. The placental lesions are more severe over the placentomes than in the intercotyledonary areas and are qualitatively similar to the lesions described in the placenta in bovine brucellosis.

Diagnosis is based on the typical hepatic lesions and demonstration of the organism. Distinctive organisms may be observed in smears of abomasal content or of affected cotyledons and show up well with special stains. Darkfield or phase-contrast microscopy may be used to see the characteristic darting

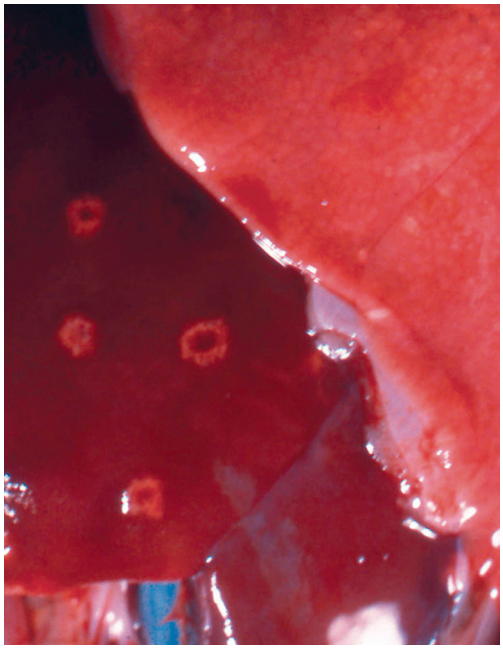

Figure 4-70 Focal hepatitis in a fetal lamb caused by ***Campylobacter fetus*.** These **umbilicated hepatic lesions** are very characteristic lesions of this infection.

motility. Discrimination of the species may be made by culture or application of specific fluorescent antibody or molecular techniques such as PCR tests.

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Flexispira rappini *infections*

Flexispira rappini is a highly motile, anaerobic, rod-shaped bacterium with multiple flagella at both of its tapered ends. Although *F. rappini* is the generally accepted name, it has not been firmly classified. The organism is closely related to organisms in the genus *Helicobacter* but is distinguished from them by its unusual spiral grooves visible on electron microscopic examination. Comparable organisms are frequently found in feces of dogs and pigs and in the stomachs and intestines of a variety of other species.

Flexispira rappini infections in pregnant ewes result in fetal mummification, abortion, and the birth of infected lambs born weak. It is not considered to be the cause of epizootics of abortion, and because the lesions closely resemble those occurring in abortion caused by *Campylobacter* spp. (which do cause epizootics), it is important that it be differentiated.

Naturally or experimentally infected ewes usually abort in the last trimester of pregnancy. The fetus is usually well preserved; however, if it is retained in the uterus, it decomposes rapidly, and the liver may be liquefied. An infected lamb may be born alive, and occasionally only one lamb of a twin may be affected.

Gross and microscopic lesions may be completely absent but, when present, are in the placenta and liver. In the placenta, they consist of a light covering of brown-to-gray exudate on the chorionic surface. In the abdomen, there may be fibrinous peritonitis, with a thin, loosely attached layer covering the liver. The liver may be congested and enlarged or tan and soft. Multifocal to coalescing pale, brown-to-gray, clearly demarcated, irregularly shaped areas (0.5 to several centimeters across) are often visible on the surface and extend into the parenchyma. In both the placenta and liver, lesions not visible grossly may be obvious on microscopic examination. In the placenta, foci of necrosis with accumulations of neutrophils and mononuclear cells may be present on chorionic epithelium. There is heavy infiltration of neutrophils and macrophages in the villus stroma, and severe neutrophil-rich vasculitis may be present in the placental vessels. Capillaries in the villus stroma may be distended with organisms. In the liver, there are multiple, sometimes connecting, foci of necrotic hepatocytes surrounded by large numbers of degenerating neutrophils, macrophages, and lymphocytes.

The condition should be suspected when these lesions are present and *Campylobacter* cannot be isolated. The organism can be seen on dark-field or phase-microscopic examination of a drop of stomach content. It stains weakly gram-negative and is more easily visualized using Giemsa or crystal violet. Special conditions are needed for culture. It is readily identified with 16S rDNA sequencing. The organism also produces abortion in guinea pigs.

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Listeriosis

Infections by ***Listeria monocytogenes*** occur worldwide and in a variety of animals, including humans. In ruminants, infections are traditionally associated with cerebral localization and *encephalitis* (for general discussion of the diseases associated with this organism, see Vol. 1, Nervous system). However, *localization also occurs in the pregnant uterus, and abortion or stillbirth is then the common sign*. The syndromes of encephalitis and abortion may occur together in a herd or flock, but rather surprisingly, this is the exception. More commonly, one or the other syndrome occurs exclusively. *L. monocytogenes* varies greatly antigenically; serovars 1 and 4b are commonly isolated from cattle and serovars 4b and 5 from sheep. Serovar 5 (***L. ivanovii***) seems particularly pathogenic for sheep. *L. ivanovii* as a cause of abortion in sheep and cattle occurs less frequently than *L. monocytogenes* and rarely causes other conditions.

L. monocytogenes is shed in the feces of normal carriers and clinically affected animals alike and has been reported to survive in some soils for as long as 2 years. It is most often *spread by ingestion* of food or water contaminated by feces, urine, placenta, or vaginal discharges from aborting animals. Infection by the oral and other routes has been shown experimentally to produce abortion. *Silage* is often the source of the organism in severe outbreaks of the infection. The aerobic

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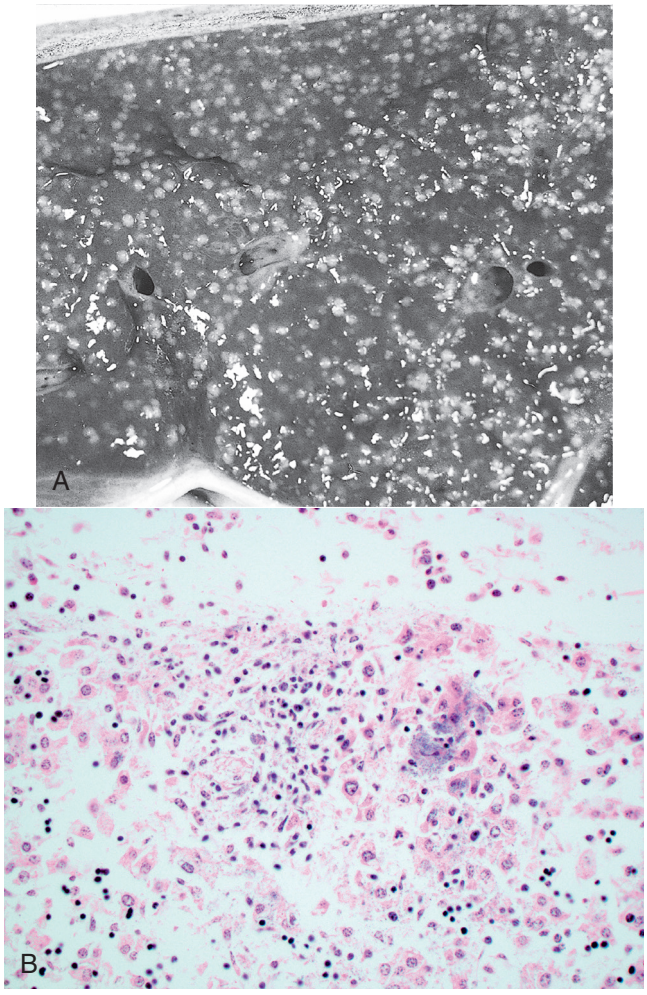

Figure 4-71 **A.** Marked **focal hepatitis** in a fetal calf caused by infection by ***Listeria monocytogenes.*** **B.** Photomicrograph of a focal area of hepatic necrosis and inflammation caused by *Listeria* infection that would correspond to the hepatic foci evident in **A.** Note the characteristic presence of *Listeria* bacteria associated with areas of necrotizing hepatitis.

environment just beneath the surface of a silage bale provides conditions for productive growth of the bacteria. *L. monocytogenes* can multiply in poorly preserved silage with a high pH (6-7.8) but dies at a pH lower than 5.5.

The gravid uterus is highly susceptible to infection, and abortion is experimentally induced by the intravenous injection of pregnant ewes rather than by ingestion. Fetal infection is considered to be by hematogenous spread from the placenta, with an incubation period of about 5-12 days. Abortions in both cattle and sheep caused by *Listeria* occur during the last trimester of pregnancy. The percentage of pregnant animals that abort within any group is variable; usually the abortions produced are sporadic, but sometimes 50% of pregnant animals may abort. If uterine infection develops during the early part of the last trimester, the organism quickly invades the placenta, and the fetus dies as a result of septicemia. The dead fetus is expelled in approximately 5 days; by this time autolytic changes mask minor gross lesions produced by the organism, and all organs teem with bacteria, which continue to multiply after death of the fetus. Abortions at this stage are not usually accompanied by severe systemic disease in the dam. The placenta is typically retained as a result of a mild metritis, but the organisms and associated inflammation are quickly cleared from the nonpregnant uterus.

If the fetus is near term when the infection develops, abnormal parturition is instituted in which dystocia is the rule, severe metritis and septicemia being the most common complications. Lesions in the fetuses and placentas in this group are less likely to be obscured by autolytic changes. Gross lesions consist of *tiny pinpoint yellow foci in the liver* (Fig. 4-71). Bacteria are typically present within these foci of hepatitis and hepatic necrosis. Similar foci, but usually only visible microscopically, are present in the lung, myocardium, kidney, adrenal, spleen, and brain. These foci have a central area of necrosis in which the organism can be visualized along with small numbers of degenerating neutrophils and mononuclear cells. If the fetus is near term, there may be severe diffuse nonsuppurative cerebrospinal meningitis in which the organism can also be seen. In the bovine fetus, in spite of marked autolysis, the most dramatic and distinguishing lesion may be in the mucosa of the colon, where *severe necrotizing colitis* with clusters of colonizing gram-positive bacteria can be seen. The jejunum is autolysed but otherwise normal. Grossly and microscopically, the placental lesion is severe. The necrotic tips of the cotyledonary villi are covered by purulent exudate in which many bacteria are present. There is also focal-to-diffuse red-to-brown *exudative intercotyledonary placentitis.*

Recovery of the organism is sometimes difficult and is probably associated with the advanced autolysis or contamination of some samples. These difficulties can frequently be overcome by refrigerating the specimen (4° C) and reculturing after a period of storage using enriched or selective media.

Clinically affected animals respond well to antibiotic therapy; however, pregnant animals may still abort.

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Leptospira *infections causing abortion in cattle*

Leptospirosis is a zoonotic disease, widespread throughout the world, and is an important cause of abortion and infertility in cattle. Leptospirosis in cattle is caused most commonly by serovar *hardjo*, which occurs as 2 species: ***Leptospira borgpetersenii* serovar *hardjo* type hardjo-bovis** and ***Leptospira interrogans* serovar *hardjo* type hardjoprajitno**. Type hardjobovis is distributed widely and is the only type isolated from cattle in North America. Type hardjoprajitno is a highly virulent type of *Leptospira* and has not been isolated in North America but has mainly been associated with cattle in Europe. Both hardjobovis and hardjoprajitno are spread by urine, postabortion discharges, infected placenta, and sexual contact, or by in utero infection to the fetus. ***L. interrogans* serovar *pomona*** is maintained by swine and some other animals and is a common cause of incidental leptospirosis in cattle.

Exposure of nonvaccinated dairy cows to *L borgpetersenii* serovar *hardjo* type hardjo-bovis has been shown to be associated with increased breedings required per conception. Diagnosis may be confirmed using immunologically based tests to

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reveal and identify the organism and routine testing of fetal cavity fluids for antibody. It has become a relatively common diagnosis (0-40% of bovine fetuses submitted), depending on the geographic location. Infection of cattle by either *L interrogans* serovar *hardjo* or *L interrogans* serovar *pomona* results in bacteremia, followed by damage in various parenchymal organs (especially in the young) and eventually excretion of organisms in milk and urine. Antibody titers develop by 4-6 weeks and may stay up for a year or more. Leptospires then may localize in the proximal renal tubules. Persistence and intensity vary with the organism and the host. *L interrogans* serovar *hardjo* excretion in urine is usually high for 4-6 weeks but may persist at a lower intensity for 6-12 months or life. Cessation is usually associated with a marked increase in urinary antileptospiral IgG and IgA antibodies. *L interrogations* serovar *hardjo* may localize in the uterine tube and descend into the uterus and placenta during pregnancy. Systemic infection may produce a febrile response and "flaccid agalactia" with the udder secretion becoming scant, yellow, and thick. The organism may also localize in the placenta and, in some animals, produces fetal disease and abortion.

Most of the abortions caused by Leptospira *occur in the last trimester of pregnancy* and often follow acute infection of the dam by 1-6 weeks *(*serovar *pomona)* or 4-12 weeks *(*serovar *hardjo)*. *L interrogans* serovar *autumnalis* usually causes abortion in the second trimester. *L. hardjo* may cause early embryonic death. Fetal infection is not invariably fatal, and weak calves may be born with a substantial antibody titer. *Fetuses that die in utero because of Leptospira infection are usually expelled in an autolysed state.* Abortion rates with *L interrogans* serovar *pomona* may be as high as 50%, whereas with *L. hardjo,* they are generally around 3-10%, and infertility is a common observation in infected herds.

Gross and histologic lesions in the fetus and placenta are nonspecific; however, *the presence of* Leptospira*-like organisms in trophoblast cells, nonsuppurative meningitis, and nephritis warrants further testing.*

The placenta may be edematous, but inflammatory changes are modest in spite of the fact that there may be clumps of leptospires in the chorionic epithelium. The fetus is often autolysed, and calves in which leptospiral antigen is detected in the placenta weigh 6-10 kg less than calves with no antigen in the placenta. Histologically, multifocal necrotizing tubular necrosis or interstitial inflammatory infiltrates, usually with plasma cells, can be found in the kidneys of some of the fetuses. Occasionally, the fetus will have nonsuppurative meningitis.

The organism may be demonstrated in smears of homogenates of fetal kidney, lung, liver, and placenta; in fetal aqueous humor by the indirect fluorescent antibody test; or by culture. The organism can also be demonstrated in sections of fixed fetal kidney by immunohistochemistry or in frozen sections by using the fluorescent antibody technique. In stillborn calves, the adrenal glands, lung, and placenta are the most useful organs to examine for leptospiral antigen. Cultures of fetuses can often be misleading. Calves infected with *Leptospira* spp. have been found to be more likely infected by *T. pyogenes* or *Bacillus* spp.; the latter is probably an incidental finding, but more easily demonstrated than the *Leptospira*. Cultures of specimens can be productive but are time consuming and cumbersome, with a long delay before results. Fetuses that die when the infection is generalized may not have formed antibody, but antibody is frequently present in cavity fluids by the time the organism has localized in the fetal kidneys. Cows aborting fetuses infected with *L interrogans* serovar *hardjo* may have high microscopic agglutination titers, but up to 40% are reported as not having a significant titer, and more than half of those may have no detectable titer. Prospective serology on a herd basis has been reported to be of considerable value in sorting out titers resulting from vaccination and either previous or present infections.

Leptospira *infections of the pregnant uterus in swine*

Abortion is often the only evidence of leptospiral infection in a swine herd. There may be no relationship between return to estrus and leptospirosis. Several serovars of ***Leptospira interrogans*** regularly infect swine: *canicola, grippotyphosa, icterohaemorrhagiae, sejroe,* ***pomona***, and ***tarassovi***. The latter 2 serovars, which are adapted to spread in swine, are most commonly associated with fetal disease and abortion. Serologic testing has indicated that infection by serovar *bratislava*, or a closely related serovar, is also widespread; and abortions, stillborn, and weak liveborn piglets may have the organism and antibodies to it. Serovar *bratislava* has also been associated with infertility without abortions. In one report, *L. bratislava* was detected in samples of the uterine tube by means of immunofluorescence. Some of the serum samples from these animals were positive for antibodies, whereas others were negative. In one study, where many animals had a positive titer to *Leptospira*, failure to identify *L interrogans* serovar *bratislava* by immunofluorescence was believed to be associated with transient infections early in life.

Leptospires are passed in the urine, and the organisms can enter through mucous membranes or breaks in the skin. Poor management of effluent from piggeries may result in contamination of drinking water, resulting in transmission of the organism to swine and other animals, inducing leptospirosis in cattle, sheep, horses, and dogs. Infected swine develop bacteremia before the leptospires localize in the kidneys and uterus, where they may persist and can be shed for months. Systemic signs, if they occur at all, develop during the stage of leptospiremia. It is also during this stage that the organism invades the placenta and infects the fetus. *Most of the abortions resulting from leptospirosis occur late in gestation, but stillbirths, sometimes with a few partially mummified fetuses, and births of live but weak pigs are also part of the pattern.* Seropositive dams may have a greater risk of having weak newborn piglets and also of having more weak newborn piglets per litter. Infected fetuses often die in utero and undergo autolysis, which obscures any lesions. Some aborted fetuses have elevated immunoglobulin levels.

The placentas of infected pigs may be edematous, but the most severe lesions are seen in those piglets delivered alive, but sick, at or near term. Some of these will be icteric, have patchy hepatic necrosis, fluid, and fibrin in the peritoneal cavity and inflammatory changes in multiple organs. Focal aggregations of lymphocytes in the renal cortex, medulla, and pelvis are characteristic but inconstant. Leptospires can be demonstrated in the kidneys or lungs on section by using a silver stain. Special stains are much more useful on tissue sections taken from swine than cattle; however, the fluorescent antibody test for the organism on kidney, lung, and placenta homogenates gives a more definitive answer and allows visualization of the lesion with the antigen. In addition, leptospires may frequently be demonstrated by darkfield microscopy,

specific fluorescent antibody test, or detection of leptospiral DNA in the urine of the dam up to 2 weeks after abortion and occasionally in fetal cavity fluids from fresh or autolysed fetuses.

Leptospires are rarely recognized in fluids from fetuses that have been frozen or in fetuses in any condition when aborted because of serovar *bratislava*. Fetal cavity fluids and maternal serum may contain specific antibodies and, along with fluorescent antibody and PCR testing of fetal tissues for antigen, makes a practical diagnostic procedure. As in cattle, maternal antibodies may only be useful in diagnosis if examined on a herd basis.

Leptospira *infections causing abortion or stillbirth in horses*

Until recently, leptospires have not generally been considered to be a major cause of reproductive failure in horses. In Ireland, where tissues from 22 aborted equine fetuses were cultured and examined for leptospires, 9 fetuses were found to contain organisms representing 4 serogroups: *Australis, Hebdomadis, Icterohaemorrhagiae,* and *Pomona*. **Serovars *bratislava, kennewicki,* and *pomona*** are considered to be of major importance in horses, and in one large group of horses tested in Ireland, 89% were found to carry antibody to serovar *bratislava*. The horse is considered to be the maintenance host for serovar *bratislava*.

Foals are usually aborted in the last trimester of pregnancy or stillborn, and gross and microscopic lesions are seldom present. Gross lesions occasionally observed in aborted foals include icterus, yellow mottled liver, mild hepatomegaly, and petechial hemorrhages on mucous membranes. Histopathology may reveal dissociation of hepatocytes, disruption of hepatic cords, multifocal hepatic necrosis, large multinucleated hepatocytes, and renal tubular necrosis. Cerebral perivascular hemorrhage, thrombosis, and severe meningitis with heavy neutrophil and macrophage infiltrates have been observed. Leptospires were identified as the cause of abortion in 4 aborted foals with giant cell hepatitis. For diagnosis, high titers of fetal antibody have been detected in fetal fluid by the microscopic agglutination test, and the organism has been identified in impression smears or histologic sections of kidney and placenta by immunofluorescence.

Leptospira *infections causing abortion in sheep*

A variety of serovars, including ***Leptospira interrogans* serovars *bratislava, ballum,* and *pomona,*** will cause abortion and stillbirths in sheep; however, *the major serovar is hardjo*, and with this serovar, there seems to be a sheep reservoir that does not include cattle. Sheep seem somewhat resistant to leptospirosis but have a vulnerable period for 2 weeks before lambing and 1 week after. Available evidence suggests that, with different serovars, sheep are usually infected from an environment contaminated by other animals. Feeding newborn lambs with colostrum from *Leptospira*-infected cows is believed to cause death in the lamb. Research has also led to the speculation that leptospirosis is generally less common in sheep than in horses, cattle, and pigs; however, *under conditions of intensive management, leptospirosis can cause severe losses in the last 2 weeks of gestation and early postpartum period*. Losses include abortion, stillbirth, and birth of weak lambs. The grazing lands for sheep are usually arid and therefore do not support leptospires, and the drinking habits of sheep are more fastidious in that they do not normally drink out of puddles.

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Ureaplasma diversum *infection*

Ureaplasma diversum is a cause of reproductive failure in cattle. The true prevalence of abortion caused by this organism is unknown, however, as few laboratories attempt detection of the organism. *U. diversum* is a bacterium in the family *Mycoplasmataceae*. The organism is frequently present on the mucous membranes of the nasal passages, vulva, and vagina of the cow, the prepuce of bulls, and in semen and embryo transfer fluids. It remains in these sites in the animal for long periods and can be found in urine, and vaginal and nasal discharges. It may be transmitted to cows (or bulls) during breeding, and as the organism is preserved by freezing, it remains viable in diluted frozen semen.

Virulent strains of *U. diversum* may produce *vulvitis, embryonic death, abortion, or the birth of dead or weak calves at term*. Epidemics of abortion are rare in chronically infected herds, but may occur where large groups of naïve animals are congregated. Abortion usually occurs in the last trimester of gestation, or infection may manifest as premature delivery, with the birth of weak or dead calves near term. The fetal membranes may be retained. On gross examination, *the amnion is frequently the most severely affected portion of the placenta*. It has patchy thickening with fibrosis and, in addition, may have foci of necrosis, hemorrhage, fibrin exudation, mineralization, and areas that are stained with meconium. The chorioallantois may be similarly affected, and if so, the lesion is often more severe on the allantoic surface. On histologic examination of the placenta, fibrosis and ongoing interstitial necrosis are extensive. The inflammatory cell component is mainly mononuclear, with macrophages and plasma cells predominating (Fig. 4-72). Mild arteritis is also present.

The fetus is usually well preserved and may be stained with meconium. The lungs are firm and, even if the calf is born alive, are poorly aerated. There is an erosive conjunctivitis with prominent goblet-cell formation of the palpebral conjunctiva. Foci of lymphocytes, including plasma cells, are present in the lamina propria. The lungs have nonsuppurative alveolitis, and periairway lymphocytic mononuclear cell infiltrates may be prominent. *The lesions in the amnion, chorioallantois, and lung are characteristic, but not pathognomonic,* and require validation by identification by either culture or DNA detection techniques, from placenta, stomach contents, or lung.

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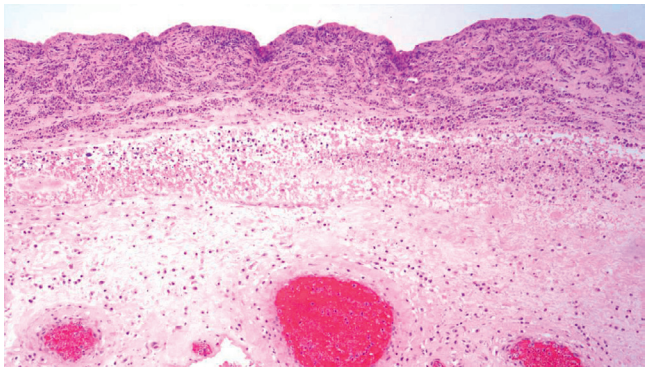

Figure 4-72 ***Ureaplasma diversum*** **chronic necrotizing amnionitis** in the placenta of a bovine fetus.

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Trueperella pyogenes *as a cause of abortion in cattle and sheep*

Trueperella (Arcanobacterium, Actinomyces, Corynebacterium) pyogenes is widespread throughout the world as a common cause of pyogenic infection in a variety of domestic animals. The organism is commonly isolated from aborted fetuses and is fairly consistently diagnosed in 1-15% of bovine fetuses submitted. It also causes abortion in sheep, but this is considered less frequent. *It is believed to be a primary pathogen in the cause of abortion.*

The organism is a common contaminant on the mucous membranes and is found in the tonsils and vagina of healthy cattle. As to the pathogenesis of abortion, it is suspected the organism may penetrate the bloodstream from a mucosal surface, resulting in transient bacteremia, which, in a pregnant animal, results in placental localization and abortion. *Abortion is usually a single event in a herd or flock but is occasionally multiple*. Abortion may occur at any stage of gestation but is usually in the last trimester. Dams may be ill following abortion, and some die with suppurative endometritis, arthritis, or mastitis.

The fetus may be severely autolysed or well preserved, and *gross lesions are visible in the placenta, trachea, and lung.* The placenta is often retained and when submitted may be heavily contaminated. Gross examination of portions submitted reveals marked autolysis and suppurative placentitis with yellow-to-brown exudate over swollen edematous cotyledons. In some fetuses, a hemorrhagic cast is present in the trachea. On gross examination of the lung, it is dark red and swollen with minute yellow foci visible on the surface.

On microscopic examination of the placenta and fetus, lesions may be modest (with large numbers of bacteria but no inflammatory cells in placenta and lung) (see Fig. 4-68) or excessive (with a severe necrotizing, suppurative placentitis and acute fibrinous bronchopneumonia). In addition, bacteria may colonize in vessels, on the skin surface, and on the conjunctiva, with destruction of the epithelium.

The diagnosis may be made based on the typical lesions and isolating the organism, preferably from the placenta, lung, and stomach contents. In the absence of lesions, the diagnosis can be made with less confidence by culture only. It has been suggested that the young fetus may be unable to respond to the organism.

Salmonella *as a cause of abortion in sheep and cattle*

The bacterium *Salmonella* is in the family *Enterobacteriaceae*. It is a facultative anaerobe, a gram-negative rod, and usually motile. There are >1,300 serotypes in the genus *Salmonella*. Some of these are auxotrophic and have an affinity for just one host, whereas others are ubiquitous and found in many animals. *Salmonella enterica* ssp. *enterica* serovar Typhimurium is ubiquitous, whereas *S. enterica* serovar Abortusovis is most prevalent in sheep and therefore considered auxotrophic. Many of the serotypes causing abortion in livestock are ubiquitous. Salmonellosis is of worldwide concern as a human disease and causes major losses in calves. It is also an important cause of abortion in both cattle and sheep and, in some situations, in mares.

Clinically normal hosts may carry *Salmonella*. It is carried by all domestic mammals and birds and is excreted in the saliva, milk, feces, and urine and in the fluids discharged when animals abort. Animals may become infected through their feed, water, bedding, or the equipment used in feeding, handling, and cleaning. *Abortions are often precipitated by a stressful event such as transport, change in diet, or feeding spoiled feed*. It is not completely defined how these events precipitate increased multiplication of the organism, but it may partly be due to disturbance of the normal intestinal flora. Although specific nutrients in the placenta that enhance the growth of *Salmonella* have not been demonstrated, in vivo experiments have shown marked growth enhancement of *Salmonella* following inoculation of whole placental extracts into mice.

The organism may stay in a herd for several years, and *abortions may be sporadic or epizootic, usually occurring in late gestation*. Abortion can occur without illness in the dam or may follow within a week of clinical signs. Cattle may suffer with a variety of clinical manifestations, and in one report, the main manifestations exhibited by *S. enterica* serovar Dublin infection varied from pneumonia, to abortion and enteritis, to polyarthritis, in successive years. Signs of enteritis and abortion appear to be commonly associated. Sheep may have fever and anorexia shortly before aborting.

The following *pathogenesis* of placental infection has been proposed. The organism first localizes in the intestine of the dam, and this is followed by a brief bacteremia with localization in the lymph nodes, spleen, and lung. After a further period of growth, a second bacteremia occurs in which the placentome becomes infected. Excessive growth of the organism leads to almost complete destruction of the fetal villi, which is followed by abortion and may occur without invasion of the fetus. If *S enterica* serovar Dublin infection occurs within a month of term, the calf may be born alive, carrying the organism.

Following abortion, the placenta is often retained and the fetus is usually autolysed. On *gross examination*, the chorioallantois is found to be thickened with amber fibrin-containing fluid, the chorionic surface is diffusely gray to red, and there is yellow exudate on the variably tan and red cotyledons. Portions of the caruncle may remain adherent to the cotyledon. *Histologically*, there is mineralization of individual trophoblast cells, interstitium of the villi, and chorioallantoic arcade. When the chorion is severely affected, mineralization may also be

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extensive on the allantoic surface. Accompanying the mineral, exuberant bacterial growth expands the villi. In some villi, a heavy infiltrate of neutrophils is present and, if the caruncle remains attached to the cotyledon, there is a clear line of demarcation between the affected villus within the maternal crypt and the near-normal maternal septum. Dilated capillaries, immediately under the sloughed trophoblasts in the arcade zone, are filled with bacteria and in cross-section resemble large rounded trophoblasts filled with bacteria. In spite of the severe placentitis, there may be no lesions in the lung; however, when present, lesions consist of a moderate to light accumulation of neutrophils in bronchi, and bacteria may be seen colonizing airway epithelium. In the liver, there can be multifocal suppurative hepatitis.

Diagnosis is based on recovering the organism from placenta and stomach content. Tissues in the fetus are sometimes negative on culture in spite of marked placentitis. Nearly pure cultures may be obtained from samples of vaginal discharge taken just before abortion. Animals have a remarkable increase in titer following abortion, but this is not a commonly used test for diagnosis.

Yersinia pseudotuberculosis *as a cause of abortion in cattle, sheep, and goats*

Yersinia pseudotuberculosis is a gram-negative coccobacillus in the family *Enterobacteriaceae*. The DNA of *Y. pestis*, the cause of human plague, and *Y. pseudotuberculosis* are at least 90% interrelated. Six serogroups of *Y. pseudotuberculosis* are recognized based on immunoreactivity of the O antigens. *Y. pseudotuberculosis* causes ileitis, lymphadenitis, and abscessation in humans, and infects a wide variety of domestic, laboratory, and native animals and birds throughout the world. The organism is frequently isolated from the feces of normal cattle, and from abortions in sheep, goats, and cattle. In sheep, the organism also causes abdominal abscessation and inflammation in the testis and epididymis.

The *pathogenesis* of abortion probably follows invasion of the intestinal epithelium, transient bacteremia, and localization in the maternal caruncle, followed by passage to the chorioallantois and fetus. It is revealing that in the description of the experimental production of disease in sheep that follows, uterine, and specifically caruncular, disease was present as in naturally occurring abortion. It is possible that nutrients in the caruncle favor localization and growth of *Yersinia* that is followed by villus infarction and invasion of the chorion.

Lesions reported are based on limited observations that are generally similar in aborted fetuses and stillborns from sheep, goats, and cattle. The aborted conceptus is usually well preserved and contains a variety of lesions. Placentitis is largely confined to the cotyledons. Cotyledons are red or tan, thickened and relatively devoid of exudate on the surface. Portions of the caruncle may remain attached. The intercotyledonary region is usually translucent, but a frosting of fibrin and some fibrosis may closely surround the affected cotyledons (Fig. 4-73). The thoracic and abdominal cavities contain excess amber fluid with some fibrin. In the fetal liver, there may be pale tan focal areas of necrosis ranging in size from 0.1-1 cm. No other gross lesions are observed.

Histologically in the placenta, there is necrosis of villi and moderate infiltration of granulocytes, macrophages, and mononuclear cells in the interstitium of the chorioallantoic arcade. In placental vessels, there is fibrinoid necrosis of the media

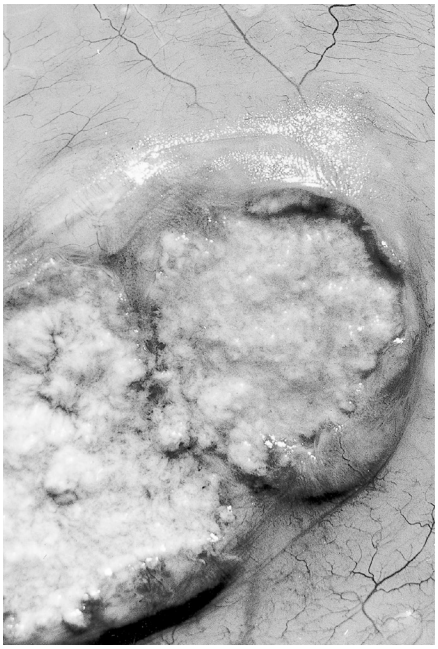

Figure 4-73 *Yersinia pseudotuberculosis* bovine abortion. **Chronic suppurative cotyledonary placentitis** and pericotyledonary fibrin.

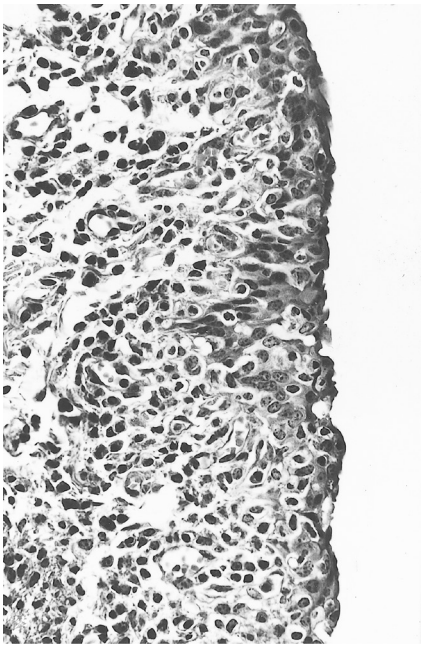

Figure 4-74 *Yersinia pseudotuberculosis* infection. Nonsuppurative **conjunctivitis** in an aborted calf.

accompanied by mononuclear cell and neutrophil infiltrates. Lesions in the attached portions of caruncle consist of thrombosis of septal vessels, hemorrhage, and necrosis, with a heavy diffuse infiltration of neutrophils and mononuclear cells. In the lamina propria of the conjunctiva, there are focal infiltrations of mononuclear cells, plasma cells, and a few neutrophils (Fig. 4-74). In the lung, there are a few mononuclear cells in airways. Hepatic lesions consist of focal areas of necrosis infiltrated by granulocytes and mononuclear cells. Similar foci are visible on microscopic examination of myocardium and lymph

nodes. Peyer's patches are precociously cellular. The lamina propria and submucosa of the colon are heavily populated by plasma cells and mononuclear cells, and within the lumen, colonies of the organism and scattered inflammatory cells can be seen in the meconium.

Extensive lesions in the uterus follow the experimental production of abortion in sheep. They consist of thrombosis of septal vessels, necrosis of septa, and heavy infiltrations of granulocytes in caruncles and intercaruncular regions.

Diagnosis is based on identification of the organism or its DNA from placenta, stomach content, or lung, and the lesions described.

Histophilus somni *infections of the pregnant bovine uterus*

Histophilus somni is the only species of the genus *Histophilus*, family *Pasteurellaceae*, and encompasses bacteria isolated from cattle and previously described as *Haemophilus somnus*, as well as ovine isolates formerly referred to as *Histophilus ovis* and *Haemophilus agni*. *H. somni* is a fastidious gram-negative coccobacillus that is considered normal flora of the male and female bovine genital tract. There are marked differences in the ability of different isolates from the reproductive tract to produce central nervous system (CNS) disease. Preliminary experiments have not shown the reverse to be true.

H. somni is best known for its association with CNS disease and pneumonia in cattle, and these are discussed under those sections. *The organism is often associated with vaginitis, infertility, abortion, and occasionally with fatal endometritis.* Usually abortions induced by *H. somni* involve solitary animals; however, occasionally an entire herd will develop reproductive disease, including vaginitis and infertility attributed to this organism. When this occurs, predisposing factors, in the form of malnutrition, concurrent virus infection, or stress, should be considered.

The organism is commonly resident in the vagina of cows (3-76% of animals sampled), and in the prepuce of bulls (>75% positive). Natural breeding therefore easily spreads it. Most strains of the organism are sensitive to the antibiotics used in the preparation of frozen semen. Sampling of a naturally infected herd revealed >50% of calves infected by 7 months of age. The presence of the organism in the vagina does not imply clinical disease.

The *pathogenesis* of abortion has not been clarified. The organism shows some ability to penetrate the cervical area to infect the placenta; however, lesions in the placenta are usually in cotyledons, which suggests a hematogenous route. Bacteremia following a vaginal or respiratory infection is likely. *Death of the fetus after infection is usually rapid, resulting in the abortion of a severely autolysed fetus.*

Lesions in the placenta are primarily confined to the cotyledon and consist of acute necrotic placentitis, which does not appear to penetrate to either the amnion or allantoic surface. On histologic examination, the most distinctive lesion in the placenta consists of severe fibrinoid necrosis and thrombosis of large and small arteries. The cellular infiltrate in vessel walls and adjacent villi is mainly macrophages with some neutrophils. There is sparing of veins and capillaries. Colonies of bacteria are visible next to the trophoblast cells. Lesions in the fetus are usually sparse; however, acute fibrinous bronchopneumonia is occasionally observed. On histology, airways are seen to contain degenerating mononuclear cells, macrophages, but few neutrophils. Bacteria are frequently observed adjacent to the epithelium of alveolar ducts, and swirling (oat-type) macrophages are numerous in these areas. Fibrin often fills lymphatics, distending interlobular septa.

Diagnosis is based on observing the characteristic lesions in the placenta and lung, and isolation of the organism from the stomach content or placenta.

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Chlamydophila (Chlamydia) *infections causing abortions*

Chlamydophilae (chlamydiae) are obligate intracellular parasites that multiply in membrane-bound vacuoles in a variety of cells. They have 2 distinct forms, the *reticulate body*, which is not infectious, and the *elementary body*, which is infectious and released from the cytoplasm by a mechanism not fully understood but which involves disruption of the cell. The *inclusions*, which can be seen on light microscopy, consist of hundreds of gram-negative *Chlamydophila* cells bound by a membrane in a cytoplasmic vacuole. Based on DNA sequence analysis, the family *Chlamydiaceae* is divided into 2 genera: *Chlamydophila* and *Chlamydia*. The species of *Chlamydophila* and *Chlamydia* of veterinary interest include the redesignated forms of *Chlamydia psittaci*, namely, *Chlamydophila abortus* (former ovine serovar [immunotype] 1), *C. psittaci* (former avian serovar), *C. felis* (feline serovar), *C. caviae* (guinea pig serovar); plus *C. pecorum* (mammals), and *Chlamydia suis* (former porcine serovar of *C. trachomatis*). *C. suis*, *C. pecorum*, and *C. abortus* have all been associated with reproductive failure and abortion. Chlamydiae have also been associated with abortion in horses. Of significance as human pathogens are *C. psittaci*, *C. pneumoniae*, and *C. trachomatis*.

Chlamydophila abortion in sheep, goats, and cattle. *Chlamydophila abortus* is an important cause of in utero infections in sheep and goats, resulting in abortion, stillbirth, and the birth of weak offspring, and can cause abortion in women in close contact with these aborting ruminants. The disease in sheep has been known as "ovine enzootic abortion" and "enzootic abortion of ewes" but will be referred to here as *Chlamydophila* abortion. *C. pecorum*, a fecal organism, may cause sporadic abortion, but this is rare.

The products of abortion, uterine discharge, vaginal fluids, and semen are potential sources of the organism. On exposure of oral, conjunctival or reproductive mucosae, sheep and goats develop chlamydophilemia and may develop interstitial pneumonia or focal hepatitis. Weeks or months later, the organism appears in mononuclear cells of the pregnant uterus and then is found in trophoblasts; the rest of the placenta and the fetus may become infected. Naïve animals, including newly introduced sheep and females pregnant for the first time, are most vulnerable. The incubation period is 50-90 days. *If infected in the early part of gestation, the ewe may abort in the final trimester of pregnancy, or infection may manifest as stillbirths or weak*

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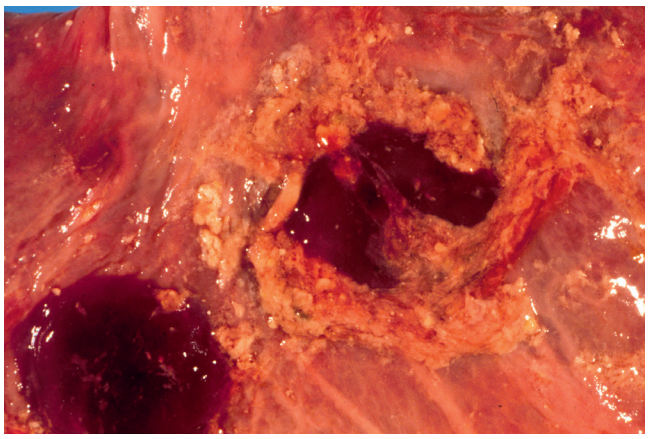

Figure 4-75 Pericotyledonary pattern found in **placentitis** caused by *Chlamydophila abortus* in a sheep.

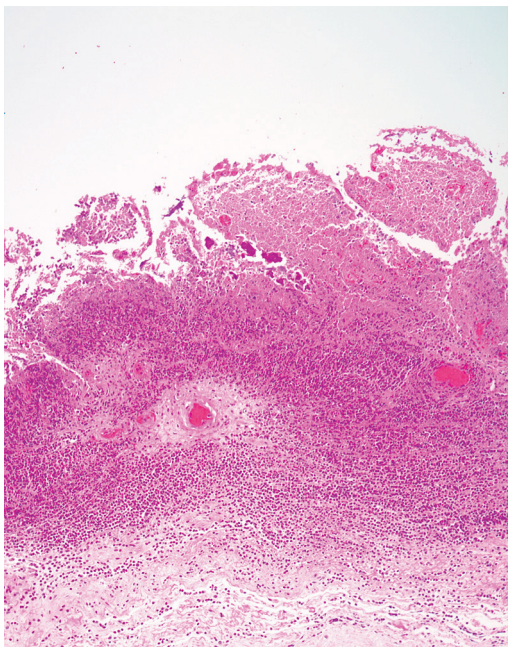

Figure 4-76 **Placentitis** with severe inflammation of the chorionic surface and deeper vasculitis in *Chlamydophila abortus* abortion in a ewe.

lambs. If she is infected in the later part of pregnancy, abortion will occur in the next pregnancy. A ewe that aborts will not abort again, but can carry the organism for several years. Extensive losses (75%) may occur on introduction of the organism to a flock, and yearly abortion rates of 5% are common when it exists in the enzootic form.

Retention of fetal membranes may occur in some animals, and aborting females may occasionally be ill. *The placenta resembles that seen in bovine brucellosis*, and there is patchy, almost equal involvement of cotyledons and intercotyledonary regions. Affected cotyledons are a dull-clay or dark-red color, firm, and matted with a dirty-red exudate (Fig. 4-75). The intercotyledonary region is red to brown and has irregular patches of edema amidst a dry, leathery thickening of the chorioallantois. These thickenings are often concentrated in ridges, which on histology are seen to consist of medium to large vessels prominent because of *marked vasculitis*. The vasculitis is characterized by fibrinoid necrosis of the media with a moderate to heavy infiltrate of neutrophils and some mononuclear cells (Fig. 4-76). In the chorion, there is superficial necrosis, and characteristic inclusions can be seen in the cytoplasm of trophoblast cells. Abortion is likely the result of several factors, including tissue destruction by C. *abortus*, vasculitis, thrombosis, and a fetal inflammatory response. Production of tumor necrosis factor-α (TNF-α) by fetal macrophages that express major histocompatibility complex II molecules may be significant in the pathogenesis of abortion.

The fetus is usually well preserved but may have few gross lesions. These consist of scattered hemorrhages in the subcutis, thymus, lymph nodes, and muscles. The liver is sometimes swollen and has pinpoint yellow foci on the surface. Histologic lesions in the fetus are minimal. There may be focal areas of coagulative necrosis in the liver and spleen. These foci consist of as few as 5 cells, stain pale pink, and are variably surrounded by scant mononuclear cells. Throughout the liver and concentrated in portal areas, there may be an increase in mononuclear cells. In the lung, alveolar septa are thickened by mononuclear cells. Mild meningoencephalitis with vasculitis and hemorrhage has also been reported.

Cattle do carry C. *pecorum* in the intestine, as do sheep, and the organism in the intestine can produce abortion. Abortions in cattle are usually sporadic, occurring throughout the year, with occasional outbreaks in isolated herds. Fetal lesions are similar to those in lambs. *Waddlia chondrophila*, another member of the order Chlamydiales, is also responsible for abortion in cattle. *Chlamydiaceae* may be an under-recognized cause of reproductive failure in swine.

To establish *Chlamydophila* as the cause of an abortion in cattle or sheep, the organism should be demonstrated in a smear (preferably of placenta) stained by a modified Ziehl-Neelsen, Gimenez, or Giemsa method. The organism can also be demonstrated in sections from the diseased placenta by using a specific fluorescent antibody test or immunohistochemistry. The presence of specific antibody in the fetus is confirmative, but antibody is not always present.

Demonstration of antibody in the dam is of little use as a large proportion of cattle and sheep have been exposed and test positive. Specific monoclonal antibodies are available to detect antigens of known pathogenic strains of *Chlamydophila*, but detection of chlamydial DNA is now widely available. The organism can be grown on appropriate cell lines on coverslips, fixed, stained, and examined within 3 days.

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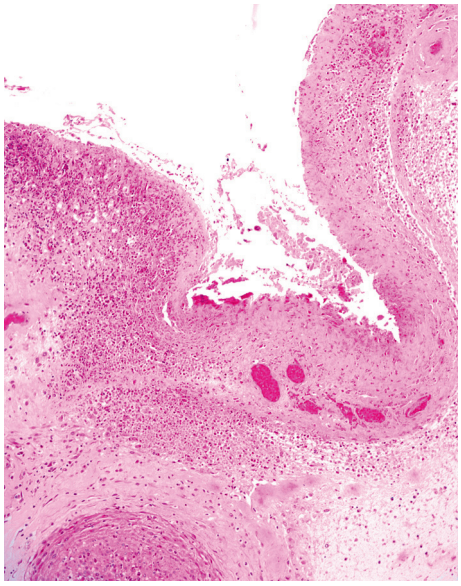

Figure 4-77 Caprine *Coxiella burnetii* abortion. **Suppurative placentitis** primarily involving the intercotyledonary areas of the chorioallantois is shown.

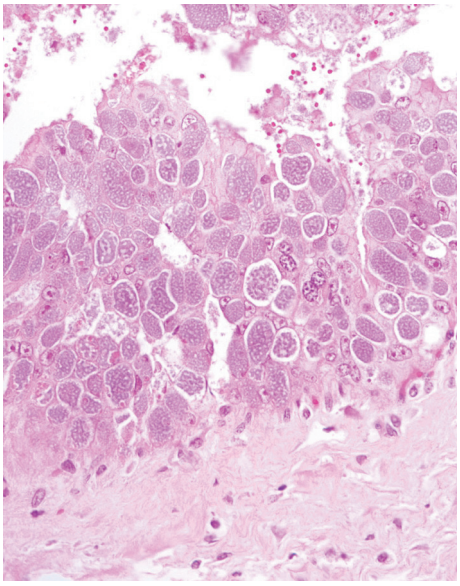

Figure 4-78 Ovine ***Coxiella burnetii*** abortion. Microcolonies of *Coxiella burnetii* distend the cytoplasm of trophoblasts.

Abortion in sheep, goats, and cattle caused by Coxiella burnetii

Coxiella burnetii is in the family *Rickettsiaceae* and is the only member of the genus *Coxiella*. The organism is well known as the cause of Q (query) fever in humans. It is an obligate intracytoplasmic parasite. *Dairy cows, goats, and sheep are the common reservoirs of the organism*, and cats and wildlife may also carry it. Human and domestic animal infection occurs when contaminated dust is inhaled; however, massive contamination of a paddock or pasture facilitates oral transmission. Infection apparently persists indefinitely in sheep and cattle, and organisms are shed at parturition and in the milk and feces. Abortion usually follows initial exposure and may be unusual in endemically infected flocks.

Abortion tends to occur late in the gestation period, and weak lambs and kids may be born during an outbreak. The aborted fetus may be well preserved or autolysed. *Gross lesions are confined to the placenta, which is thickened and leathery*, with multifocal to confluent areas of mineralization. The exudate is copious, off-white, and most obvious in the intercotyledonary region. If the cotyledon is involved, the early lesion appears as a white outer ring with flecks of white scattered in the central region.

Histologically, there is trophoblast hyperplasia and or acute diffuse suppurative placentitis (Fig. 4-77) and extensive necrosis of cotyledonary villi and intercotyledonary trophoblasts (Fig. 4-78). In contrast, the inflammatory cells in the interstitium of the chorioallantoic arcade are largely mononuclear, with a predominance of plasma cells. The lesion, which on gross section appears to be largely confined to the intercotyledonary region, is frequently found on histology to be also extensive in the cotyledons, especially at the periphery. The vasculitis that develops with *Chlamydophila* infections is not usually a feature of placentitis caused by *Coxiella*. *Smears of placental exudate contain large numbers of organisms*, which can be stained by the modified Ziehl-Neelsen or Macchiavello stain. The organism produces microcolonies within trophoblasts, which distend the cytoplasm (see Fig. 4-78). These microcolonies can be seen in conventionally stained sections, but must be distinguished from similar colonies formed by *Chlamydophila* organisms. With H&E stains, *Chlamydophila* inclusions tend to stain poorly and appear more homogeneous, whereas cells containing *Coxiella* frequently have a characteristic foamy appearance with multiple unstained vacuoles within a pale blue cytoplasm. The nucleus, if visible, is usually pushed against the cell wall and assumes a crescent shape.

The histologic lesions in affected fetuses are usually modest. Granulomatous hepatitis and nonsuppurative pneumonia with occasional focal lymphoid accumulations around bronchioles have been described. A few lymphocytes and macrophages may infiltrate the renal medulla and surround the portal vessels of the liver.

Bovine fetuses may also be aborted as a result of *Coxiella* infections. The lesion in the placenta is characteristic and similar to that observed in sheep and goats. Care must be taken in attributing the simple presence of *Coxiella* as sufficient evidence for the cause of abortion. Animals may carry the organism for a prolonged period and, although they appear to abort only once, there may be large numbers of organisms in the placenta in subsequent pregnancies. Assessment of the extent of the placental lesion may be helpful.

Diagnosis is routinely based on the characteristic placental lesions and the appearance of the organism in impression smears stained by a modified acid-fast technique. More definitive methods using direct or indirect fluorescent antibody techniques and tests to detect *Coxiella* DNA are available. Co-infection with *Chlamydophila* and *Toxoplasma* is common in sheep and goats. Cutoff points may assist in determining if the infection is relevant to abortion, but correlation with lesions is always advised.

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Mare reproductive loss syndrome and late-term abortions

A syndrome of abortion reached epidemic proportions in the spring of 2001 and to a lesser extent in 2002. It is of major concern to the equine industry in various states but especially in *Kentucky*. Several hundred late-term fetuses were examined. Most were aborted or stillborn at term or several weeks before term, well preserved, and enclosed within the placenta. There were few or no premonitory signs of illness in the mares. Lesions observed in the fetus included hyphema and little or no inflation in the lungs. Hemorrhages were frequently observed on the chorion, amnion and amniotic segment of the umbilical cord, pleura, and heart. The surface of the amniotic segment of the cord was dull gray to yellow, roughened, and thickened by stromal edema. On microscopic examination, the amniotic portion of the umbilical cord had bacteria on the surface, and where there was loss of epithelium, there were light to heavy infiltrations of neutrophils and macrophages. Similar light infiltrates were seen in the stroma of the chorioallantois.

Cultures of the fetus and placenta yielded non–β-hemolytic *Streptococcus* spp. and/or *Actinobacillus* spp. in 50% and 20% of the specimens cultured, respectively. Similar bacteria were also cultured from lung, stomach content, and placental membranes. Microscopic lesions included funisitis, amnionitis, pneumonia, fetal bacteremia, and sometimes chorionitis. Further investigations supported unusually warm weather and rate of change from cool to warm as major factors, which correlated with increased bacterial growth and *eastern tent caterpillar (ETC)* development. In a controlled study, investigations involving the feeding of ETC to pigs caused abortion. A similar study in mares revealed that abortion occurred only on feeding of the insect exoskeleton and that hair remnants resembling ETC setae were embedded in the submucosa of the digestive tract of aborting gilts and of an aborting mare. Studies of a similar disease caused by processionary caterpillars show that *the setae penetrate the intestine after ingestion and subsequently migrate to the pregnant uterus by direct penetration*. With them go bacteria that induce infection of the pregnant uterus and fetus and placenta.

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Nocardioform placentitis in mares

This disease, caused by actinomycete bacteria, *is found sporadically throughout the world*. All ages and breeds of mares are affected. The mare shows no outward signs of illness except, as with other near-term abortions, premature development of the mammary gland and lactation may occur. Infection is cleared early, and rebreeding has not been accompanied by problems. The bacteria involved are *gram-positive branching filamentous organisms* and include at least 3 different genera of bacteria: ***Crossiella equi***, ***Streptomyces***, and 3 species of ***Amycolatopsis***. No risk factors have been identified, nor has the condition been reproduced.

The lesion in the placenta is somewhat unique in that infection does not seem to start at, nor communicate with, the cervical star. The chorionic surface of the most cranial portion of the body and entrance to the uterine horns is covered with thick brown exudate containing sloughed chorionic cells, neutrophils, and bacteria. The bacteria invade the chorionic epithelium but have not been identified in fetal fluids and organs.

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Miscellaneous infections causing abortion in mares

The *bacterial infections* in foals that produce the syndromes variously known as joint ill, navel ill, foal septicemia, and pyosepticemia neonatorum can also produce abortions and stillbirths. The implication is that these diseases of the newborn can be continuations of intrauterine disease. The organisms involved, in approximately the order of frequency, are: β-hemolytic streptococci, *E. coli*, *Pseudomonas, Staphylococcus aureus, Klebsiella pneumoniae,* and *Actinobacillus equuli*. These organisms may occasionally reach the chorion by way of the bloodstream of the dam, but most ascend through a patent cervix (Fig. 4-79). This produces inflammatory thickening of the cervical star region of placenta adjacent to and radiating from the internal os of the cervix.

Aspergillus fumigatus is the most commonly isolated fungus, but *Lichtheimia* (*Absidia) corymbifera* and other *Aspergillus* spp. have been recovered.

Actinobacillus equuli is an important cause of neonatal death of foals in most countries. Foals may become infected in utero and be aborted, as discussed previously; they may die of septicemia in the early neonatal period; or they may survive for several days and develop foci of localization in many tissues. It is probable that some of these infections are acquired during or after parturition. Aborted foals and those dying acutely of septicemia do not have distinctive lesions. After a course of 3 or 4 days or more, localization of the infection with miliary microabscesses and polyarthritis develops. The

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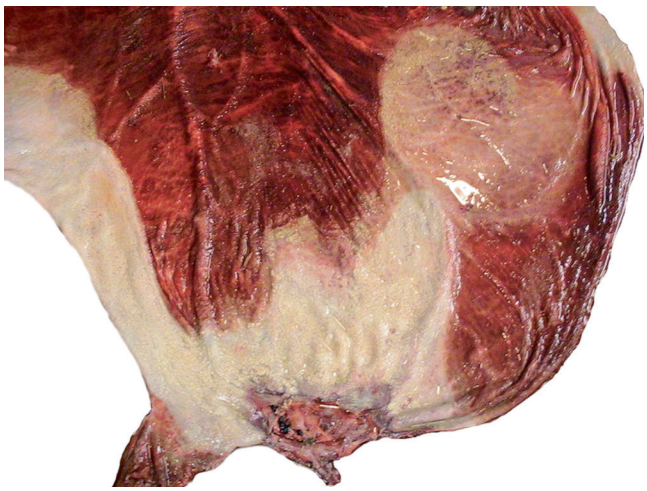

Figure 4-79 **Ascending placentitis** following transcervical infection by *Streptococcus* sp. in a horse.

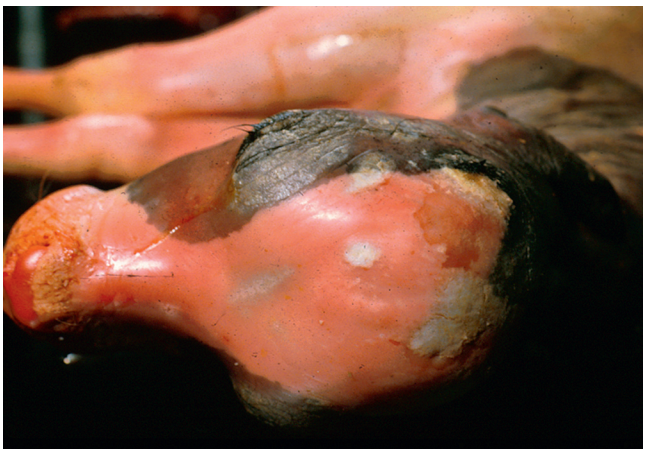

Figure 4-80 Mycotic abortion in a cow with **mycotic dermatitis** in the aborted calf.

microabscesses, which are embolic in origin, may be found in many organs, and they are readily visible to the naked eye in the renal cortices. The renal abscesses are small, being only 1-3 mm in diameter, and numerous. Histologically, bacterial colonies are obvious in the glomeruli and intertubular capillaries and are enclosed in foci of intense suppuration. The arthritis is fibrinopurulent.

There is little information on the epidemiology and pathogenesis of actinobacillosis. Mares, which are caused to abort by this infection, are not ill, and the organism does not persist for long in the uterus after abortion. However, the same mare may abort successive pregnancies, a fact which suggests that the uterus can be reinfected from some endogenous asymptomatic focus. *A. equuli* can occasionally be found in the intestine and tissues of healthy animals and also as an opportunist in pathologic tissues such as verminous thromboses. It is, however, seldom of significance in adult animals, although septicemia has been observed.

Rhodococcus equi is an important cause of pneumonia and colitis in foals <6 months of age, and a rare cause of placentitis and abortion. The fetus may be expelled within the membranes. The cervical star and surrounding region may be red; the lungs are pale and fail to float. *R. equi* may be isolated in pure culture from the placenta, lungs, liver, and stomach content. The lesion in the placenta consists of a perivascular macrophage infiltrate with a few lymphocytes and neutrophils. The macrophages contain numerous gram-positive coccobacilli. Inflammation is most extensive on the allantoic surface. On microscopic examination, there is multifocal hypertrophy and hyperplasia of allantoic epithelial cells, and small numbers of neutrophils and large macrophages are loosely scattered within the amnion. Pulmonary alveoli contain a few granulocytes and many macrophages, some of which are multinucleated.

Encephalitozoon cuniculi is a microsporidial organism that is a rare cause of placentitis and abortion in a wide range of animals, including horses. A Quarter Horse mare aborted a fetus and placenta 6 weeks before term; viscous yellow exudate was scattered over the chorionic surface of the placental body and both horns. In addition, the joints of the foal were swollen and contained tan to red gelatinous material. On microscopic examination, there was necrotizing placentitis, and trophoblasts contained large numbers of intracytoplasmic vacuoles that were filled with elongated gram-positive organisms. These were positively identified by PCR and electron microscopy as *E. cuniculi*. No organisms were identified in the joints.

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Mycotic abortion in cattle

Sporadic cases of bovine abortion result from infection with a variety of fungal species, with *Aspergillus fumigatus* the most frequent isolate in North America. Zygomycetes *(Lichtheimia [Absidia], Mortierella, Rhizomucor, Rhizopus)* are also commonly responsible. Mixed fungal infections are relatively common, in which case both septate and nonseptate hyphae may be observed in the placenta. A small number of abortions result from *Pseudallescheria boydii* and yeast (*Candida*, *Torulopsis*) infections. The portal of entry is not known, but the initial development of lesions in placentomes indicates *hematogenous arrival*. Extension from either respiratory or rumen infections is possible. With the exception of *Rhizopus*, these organisms are well-known secondary pathogens. Abortion occurs late in gestation, between the sixth and eighth month, and the placenta is often firmly retained.

The fetus may appear normal, but often there are characteristic cutaneous lesions in the form of irregular elevated plaques resembling ichthyosis or extensive ringworm. These lesions are seen most commonly about the periorbit, occiput, shoulders, back, and sides (Fig. 4-80). Affected areas are slightly elevated, gray, and irregular in outline and tend to coalesce. Histologically, the infection is seen to be superficial, involving the

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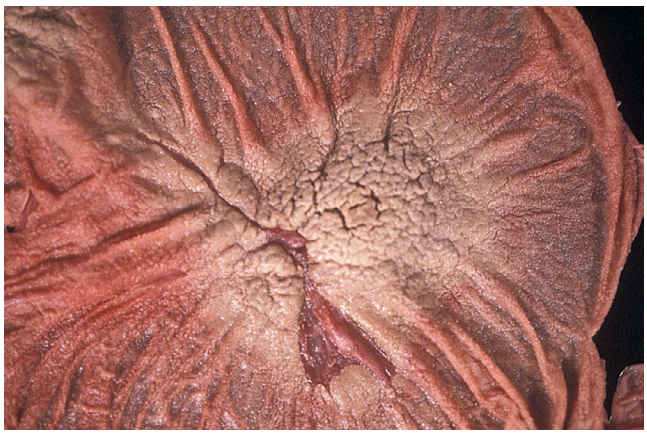

Figure 4-81 **Mycotic placentitis** primarily involving the cervical star area of an equine placenta.

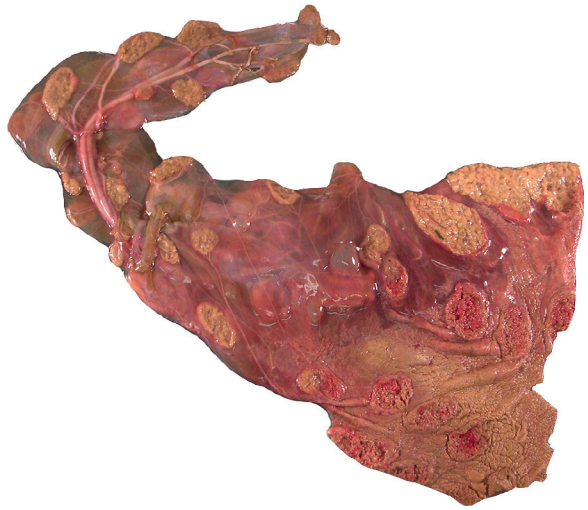

Figure 4-82 **Mycotic placentitis** in a bovine placenta.

epidermis mainly, with parakeratosis, and edema and inflammatory cell infiltration of the underlying dermis. Sometimes the fungus invades the hair follicles and dermis but, with the exception of an occasional case of bronchopneumonia, there are no lesions in the internal organs. The fungus can be isolated from the stomach contents. *The placental lesions are remarkable.* The gross appearance resembles that seen in brucellosis but is often much more severe. The chorioallantois is then leathery with extensive superficial necrosis. Compare the characteristic gross appearance of equine and bovine mycotic placentitis (Figs. 4-81, 4-82). In bovine mycotic placentitis, cotyledons are greatly enlarged and necrotic with swollen margins ("cupping"), and the intercaruncular areas are typically thickened and opaque. The infecting organisms are readily demonstrated in the necrotic tissue, and typically extend along the blood vessels to produce necrotizing vasculitis. They frequently can be demonstrated on cytology using periodic acid–Schiff or Gomori methenamine silver stains.

In contrast to the ascending route of infection of the placenta in mares (see Fig. 4-81), the infection in cattle is hematogenous and begins in the placentomes, with later spread to the intercotyledonary space. In cases with milder placentitis, there are usually no cutaneous lesions in the fetus, although the fungi can be found in the gastric contents. The endometrial lesions are less severe than those in the placenta. Secondary infections may follow retention of the placenta. The majority of cows recover sufficiently after abortion to allow subsequent pregnancies to be carried to term, but in some, endometrial destruction is severe.

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Epizootic bovine abortion

This disease is a tick-borne infection of cattle that produces chronic fetal disease and abortion. The name is a misnomer, as epizootic bovine abortion (EBA) is actually provincial and endemic and also was the original common name for bovine brucellosis. Its distribution is apparently limited by that of the vector, the argasid tick *Ornithodoros coriaceus.* The precise geographic range of the tick is not known. It is known to inhabit brushy foothills of California and adjacent areas of Nevada, Oregon, and northern Mexico, hence the other common name—**foothills abortion.** The most common hosts of the tick are cattle and deer. The tick is presumed to transmit an agent from the deer to cattle, as the infection remains endemic in ranges on which no cattle have been grazed. *The cause of EBA is a* ***novel deltaproteobacterium*** detected in salivary glands and other tissues, in a high percentage of adult *O. coriaceus* ticks in areas endemic for EBA.

Cattle exposed to the ticks for the first time are primarily at risk. The infection produces no recognizable clinical signs in cattle of any age, but if the animals are pregnant, especially at 2-6 months, the infection is passed to their fetuses, wherein a chronic disease develops. The fetus at this stage of gestation has not fully developed a functional immune system. This, coupled with a cow that has not been previously exposed to the agent, allows the agent to cross the placenta to the fetus and initiate chronic infection. There is a 3-month or longer incubation period between the exposure of the dam to ticks, development of the entire spectrum of lesions, and death and abortion of the fetus. Because the lesions gain their specificity only during the latter part of this prolonged period, the disease can only be confidently diagnosed in those animals exposed before the last trimester.

Not all diseased fetuses are aborted. The fetus is likely to have developed a functional immune system by 7-8 months of gestation and may not be aborted if infected by the agent in that time period. Instead, the calf may not die from the infection but be born weak. *Weak calves* are often seen associated with outbreaks of abortion in this disease. Cows that abort because of the disease are not usually ill, and the placenta is shed without difficulty. Cattle develop immunity and do not abort even if they are re-exposed to the ticks and the agent they carry. If there is little movement of animals,

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abortions may be rare in endemic areas. The disease becomes a major cause of abortion in a herd only when pregnant nonimmune animals are moved into an area where the infection is endemic.

The fetuses affected by the disease are most often aborted during the last trimester. Most trigger their own deliveries and die either during delivery or shortly thereafter. Only rarely do they die in utero and undergo autolysis. Many, but not all, affected fetuses have characteristic abdominal distension resulting from *ascites*, which is a striking, but inconstant, lesion. Lymph nodes throughout the carcass are enlarged, usually impressively so. The normal superficial cervical lymph node of a term bovine fetus weighs 3.5-7.0 g. Fetuses aborted in this disease have superficial cervical lymph nodes that weigh 16 g or more, making the node easy to see beneath the skin immediately in front of the scapula. The spleens of affected fetuses are similarly enlarged; the thymus, in contrast, is reduced in size.

Petechial hemorrhages are regularly seen in the conjunctival and oral mucosa as well as in the mucosa of the trachea. The thymus is often embedded in massive hemorrhages and edema. These hemorrhages are, at least in part, traumatic, developing during parturition, the portion of the thymus within the thoracic cavity being spared. The enlarged, coarsely nodular liver is, when present, an impressive gross change in fetuses affected by EBA. However, the lesion is not present in all diseased fetuses, and similar hepatic congestion with ascites occurs in fetuses with cardiac disease. Small gray foci of inflammation can be seen in a variety of tissues, but are most conspicuous in organs such as kidney and heart, which provide a dark background.

The changes that develop in the lymphoid organs are the most specific and diagnostic. The most distinctive fetal lesion is a *remarkable thymic inflammatory change*. The cortical mantle of thymocytes is greatly reduced, and macrophages diffusely infiltrate both the medulla and the septa of the gland. *The enlargement of lymph nodes and spleen consists of remarkable lymphoid and mononuclear cell hyperplasia*. Well-defined secondary follicles develop in the cortical and paracortical regions of the lymph nodes. The sinuses are filled with macrophages that form sheets in the medullary areas. The spleen shows a similar hyperplastic response involving lymphocytes of the follicles and periarteriolar lymphoid sheaths; there is also widespread infiltration of macrophages.

The central veins of the liver are distended and the liver plates thinned. Mononuclear cells are present around portal vessels. Foci may be 100 µm in diameter and are often granulomatous in appearance. Affected fetuses may have vasculitis involving small- and large-caliber vessels. Lesions that are particularly useful in establishing the diagnosis are regularly present in the lung and brain. The alveolar walls of the lung are thickened, and histiocytic inflammatory foci are present in the septa. In the brain, foci of vasculitis are scattered throughout and the meninges are thickened by histiocytic inflammation.

Lesions do develop in the placenta but are usually mild and involve the loose connective tissue. They contrast with the acute severe inflammatory reaction of the chorionic surface seen in bacterial and fungal infections.

Diagnosis of EBA is based on a combination of gross and histologic findings in an aborted fetus in the geographic area endemic for *O. coriaceus* ticks. Identification of the 16S RNA of the deltaproteobacteria is confirmatory.

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Protozoan infections causing abortions

Toxoplasmosis

Infection with ***Toxoplasma gondii*** appears to be very common in many species, but the disease toxoplasmosis is much less common, and its diagnosis is still somewhat of an event. The present discussion is limited to genital manifestations. A more complete discussion of the disease is given with coccidial disease of the intestine (see Vol. 2, Alimentary system).

Toxoplasma gondii is an important abortifacient in sheep and goats; it may occur simultaneously with *Coxiella* and *Chlamydophila* in the same abortion outbreak. Animals that are particularly at risk of *Toxoplasma* abortion are those moved late in pregnancy to areas heavily contaminated with cat feces. This occurs most often when ewes are housed in barns. Under such circumstances abortion rates can be high. Although *T. gondii* can cause abortion, congenital transmission from ewe to lamb also occurs in a high percentage of normal healthy lambings.

The ewes and does show no signs of infection. The aborted lambs and kids show no significant gross lesions; in only a few are lesions and parasites demonstrable histologically in myocardium, lung, and brain. Irregular small or large foci of leukomalacia are common in the cerebral white matter. *The fetal cotyledons of the placenta have characteristic lesions.* The cotyledons are bright to dark red compared with a normal deep purple color; scattered among the fetal villi are *numerous white flecks or small soft white nodules* 1-3 mm in diameter (Figs. 4-83, 4-84A). Histologically the villi are edematous, and there is focal necrosis and desquamation of trophoblastic epithelium. In some there may be caseous and mineralized cotyledonary nodules (Fig. 4-84B). The organism is identified either free or in cysts. The intercotyledonary placenta shows edema only. Encysted organisms can frequently be found in the endometrium. It may be assumed that placental infection can occur

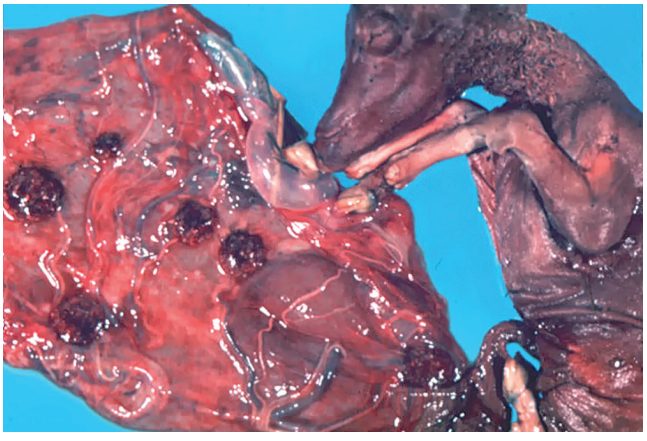

Figure 4-83 Aborted fetal lamb with focal placental necrosis caused by ***Toxoplasma***.

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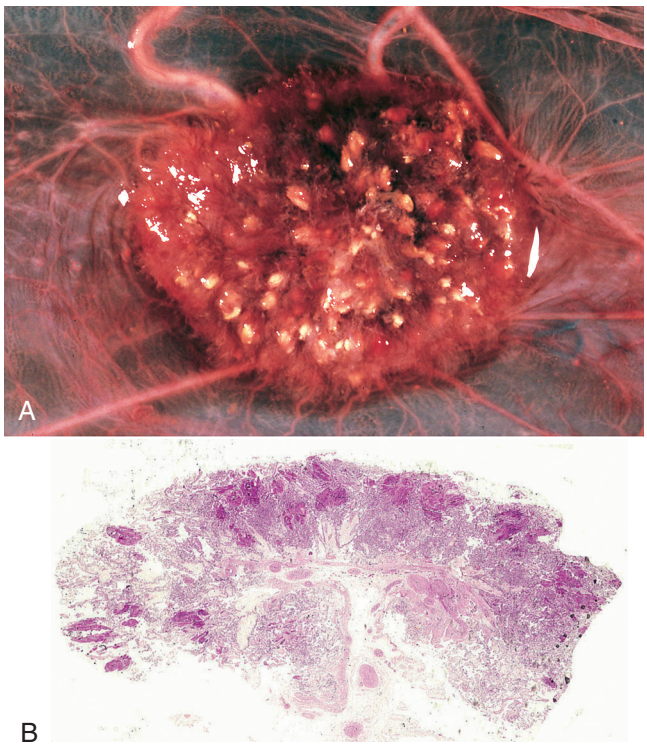

Figure 4-84 **A.** Closer view of an affected cotyledon from aborted lamb in Figure 4-83 showing the discrete foci of necrosis and associated placentitis characteristic of ***Toxoplasma gondii*** infection. **B.** Subgross photomicrograph showing intense foci of necrosis and mineralization.

at any time the infection is active and the organism is in the proliferative phase.

Cattle rarely, if ever, abort because of toxoplasmosis. Although the organism may be present in cattle, it does not appear to cause disease. *Toxoplasma* has been reported to cause abortion in *swine*, with fetal lesions, but this appears to be an uncommon event.

Further reading

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Neospora *infections causing reproductive failure in cattle, sheep, dogs, and cats*

Neospora caninum is a protozoan parasite belonging to the class Apicomplexa. *N. caninum* was first isolated from a litter of puppies, and domestic dogs are the only recognized definitive hosts of the parasite. *It is a major cause of abortion in both dairy and beef cattle throughout the world*. Although observed in tissues from aborted bovine fetuses for many years, the organism was first associated, and in 1987 identified, as the cause of an abortion storm in cattle in New Mexico. It differs morphologically and antigenically from *Toxoplasma* and *Sarcocystis* spp.

The organism can cause severe neuromuscular paralytic disease in dogs and cats. Experimentally induced infections in pregnant dogs and cats lead to in utero death and birth of infected offspring, some of which die, whereas others remain normal. Orally induced infection in nonpregnant cats subsequently bred results in clinically normal young, born infected with the parasite.

In **cattle**, the natural route of infection is probably by consumption of oocysts excreted in the feces of dogs and other canids, or by vertical transmission. Eating infected carcasses or placentas from infected fetuses may infect canids. Most reports of abortion involve dairy cattle and particularly cows on drylot. This is believed to be associated with the density of population and methods of feed storage, providing increased opportunity for contamination, transmission, and spread of the organism. However, beef cows also abort. Conception does not seem to be affected; however, animals have been reported to abort with *N. caninum* in 3 sequential pregnancies. Abortion storms, where fresh or autolysed fetuses are aborted, may be followed by the birth of mummified fetuses for several months. Animals aborting in one year may or may not abort in the next gestation. Herd investigations have shown that 23% of pregnant cows and heifers abort during the first year of infection. Although protective immunity develops in infected cows, this immunity does not prevent transplacental infection, and seropositive cows are more likely to abort than are seronegative cows. Seronegative cows probably always produce seronegative calves. Most *Neospora*-induced abortions occur at 5-6 months of gestation; this mid-gestation abortion frequency is distinctive from other kinds of abortion in dairy cattle. Pregnancy-induced immunosuppression is a possible, but unproven, cause of reactivation of latent *Neospora* infections. The economic impact of *N. caninum* infection in dairy cattle extends beyond that from abortion to include premature culling resulting from the diminished milk production that occurs in seropositive cows. Cows infected with *N. caninum* are usually not clinically ill, nor do they have an increased risk of dying compared to seronegative cows.

Congenitally infected full-term calves may not show signs of disease, but still have a high titer to the organism. *Neospora*-infected calves may be born small-for-gestational-age, unable to rise, ataxic, or have no clinical signs. Fore- and hindlimbs may be flexed or hyperextended; there may be loss of conscious proprioception.

On postmortem, *there are often no useful identifying gross lesions*. Bovine fetuses are often expelled in the second or third trimester and may be fresh, autolysed, or in early stages of mummification. In the placenta, the cotyledons are necrotic and the intercotyledonary region is normal. Rarely, there are scattered tan-to-gray soft foci visible in the brain, brainstem (Fig. 4-85), skeletal muscle, and heart. These consist of *necrosis, mononuclear and glial cells, perivascular cuffing, and sometimes mineralization*. Cysts may be difficult to find or conspicuous, and are probably more common in spinal cord than brain. *N. caninum* invades many different cell types and tissues in the fetus (Fig. 4-86). The zoites proliferate intracellularly by endodyogeny; massive proliferation leads to host cell lysis, and newly formed parasites are released to invade neighboring cells.

N. caninum tachyzoites may be identified in focal brain lesions; however, *free protozoa are difficult to recognize and are most easily identified using immunohistochemistry*. Intracellular

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zoites may be seen in myocytes and Purkinje fibers in myocardium. Less consistent lesions include multifocal hepatic necrosis, focal nonsuppurative interstitial nephritis, interstitial pneumonia, and adrenalitis. Large numbers of *N. caninum*–like zoites are rarely observed in poorly defined cysts within trophoblasts, and more often no significant lesions are seen in the placenta. Mononuclear cells are found in areas of focal necrosis in the cotyledons. *Neospora*, although rarely identified in placenta by immunohistochemistry, may be positive on PCR.

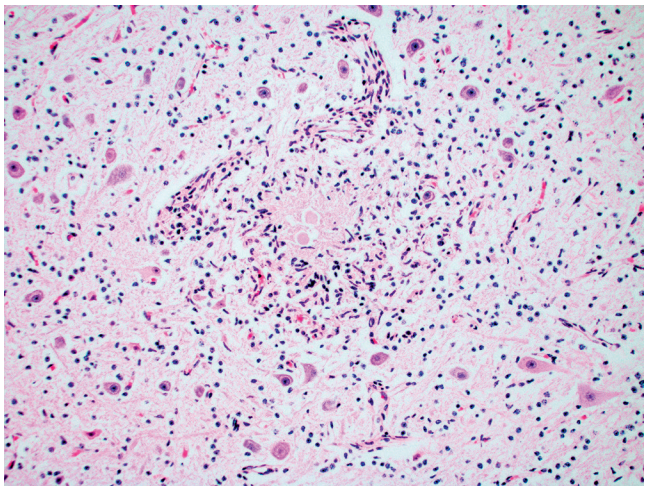

Figure 4-85 ***Neospora*-like organism** in bovine abortion. Focal necrosis is surrounded by a narrow zone of glial cells in brain.

On light microscopy, *N. caninum* can be distinguished from cysts of *Toxoplasma* and *Sarcocystis*, as the cyst wall is slightly thicker. *All stages can be differentiated from other protozoans using immunohistochemistry*, which is used successfully to demonstrate protozoa in brain (Fig. 4-87), spinal cord, lung, kidney, heart, and skeletal muscle of fresh or autolysed fetuses and also, but with more difficulty, in mummified fetuses. Isolation of *N. caninum* from autolysed tissues in cell cultures is rarely successful because of the effects of autolysis. Tissue cysts may be rare in in utero–infected fetuses; however, serology on fetuses aborted at >5 months of gestation has proved useful in detecting antibodies to *N. caninum*. Maternal antibodies to *Neospora* may also be detected but decline within 2-5 months of abortion.

N. caninum has also been identified as a cause of abortion in **sheep** and possibly in **goats.** Lesions appear to be similar in bovine and ovine aborted fetuses, and clinically affected bovine, ovine, canine, and feline neonates. The role of *Neospora* spp. (*N. caninum* or *N. hughesi*) in **equine** abortions is under investigation.

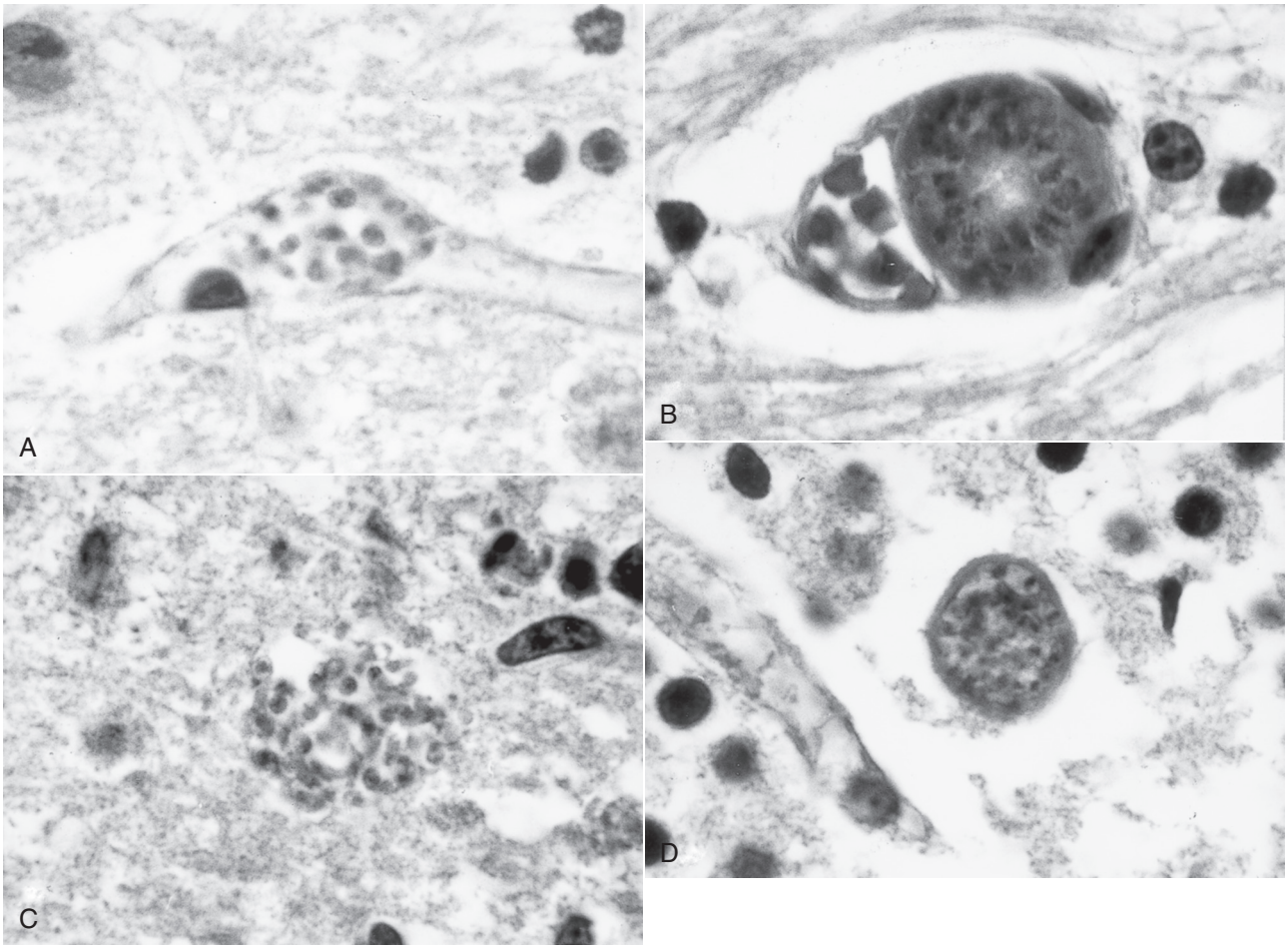

Figure 4-86 ***Neospora*-like organisms** in brain. **A.** Clusters of zoites in vessel. **B.** Clusters of zoites in endothelial cell. **C.** Clusters of zoites lying free in parenchyma. **D.** Cluster of zoites in parenchyma surrounded by cyst-like wall.

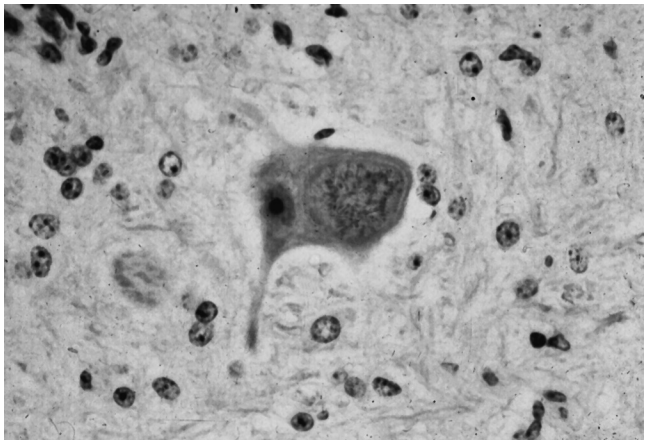

Figure 4-87 Immunohistochemically stained ***Neospora*** zoites.

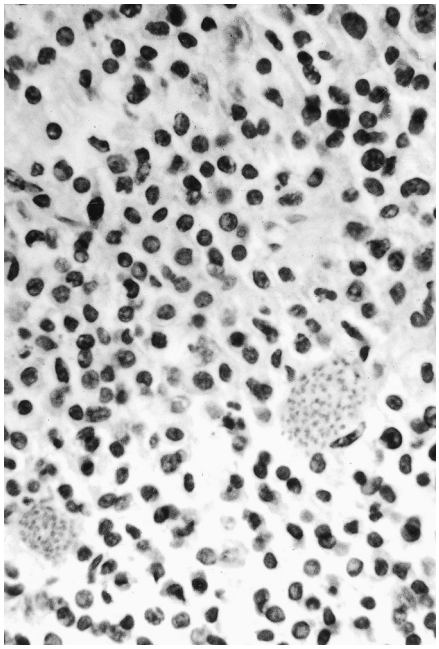

Figure 4-88 **Sarcocyst** in endothelium of small vessel of the Peyer's patch in an aborted bovine fetus.

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Sarcocystis *species causing abortion in cattle, goats, sheep, and pigs*

Sarcocystis spp. cause abortion, stillbirth, and neonatal deaths in cattle, goats, sheep, and pigs. In contrast to some of the other protozoa, they are usually specific for their intermediate host. Transmission, pathogenesis, lesions, and diagnosis are similar in all intermediates, and only lesions observed in cattle will be described. Ingesting food or water contaminated by feces containing oocysts, which are immediately infective to the intermediate host, infects the animal. Definitive hosts include the dog, coyote, wolf, red fox, and raccoon for the pathogenic organism *S. cruzi*.

The *pathogenesis* of *Sarcocystis*-induced abortion is uncertain, but may involve anemia in the dam, leading to fetal hypoxia and death, premature induction of parturition through release of $PGF_{2\alpha}$ from damaged endothelial cells, fever, or direct infection of the fetus. Infection in the dam may result in acute necrotizing endometritis, followed by multiple foci of necrosis in the soft tissues of the fetus. Why the organism is sometimes visible only in the dam but at other times also in the fetus is not known. In the dam, the zoite-containing cysts may be observed in the endometrium or caruncle following abortion, but if it passes to the fetus, in spite of autolysis and near absence of gross lesions, extensive lesions are evident microscopically. In the brain and meninges, there are multifocal areas of necrosis surrounded by undifferentiated mononuclear cells and lymphocytes. Cardiac muscle, kidney, liver, lung, and chorioallantoic membranes may have similar lesions, some of which are mineralized and undergoing fibrosis. Endothelial cells in several soft tissues may contain thin-walled cysts that bulge into the lumen (Fig. 4-88) of the frequently thrombosed vessel. The cysts, packed with elongate zoites, although often difficult to find, are most easily distinguished in nervous tissue, maternal caruncle, and endometrium away from the site of inflammation.

Diagnosis is based on finding typical lesions of nonsuppurative meningoencephalitis, multifocal necrosis in soft tissues, and the presence of cysts containing zoites with a tropism for endothelium. If the organism cannot be found on routine sectioning, it can readily be located using specific fluorescent antibody or immunohistochemistry.

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Genital tritrichomoniasis

This is a specific contagious venereal disease of cattle caused by the flagellated protozoan ***Tritrichomonas foetus*** and transmitted at coitus or artificially via contaminated semen.

Infection in the bull remains in the preputial cavity and must be considered, in the absence of effective treatment, as permanent. In early infections, there is balanoposthitis of moderate severity, with preputial swelling and a slight purulent discharge. As the infection becomes chronic, the inflammatory reaction disappears and the organisms become fewer in number. There is a tendency for them to concentrate on the head of the penis and adjacent areas of the prepuce, and they may be detected by culture or PCR testing of preputial washings.

Females are not readily infected, if at all, except by service or experimentally by placing a culture of the organism in the vagina. A few days after infection, acute vaginitis with swelling of the vulva develops, and there is a moderate amount of mucoid floccular discharge in the vagina. The protozoa may

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be easy or impossible to find in this exudate. The vaginitis resolves quickly, and the infection localizes in the uterus and cervix. Immediately prior to the estrus, which follows the infective service, large numbers of organisms can usually be found in exudate aspirated from the cervix, but as estrus advances, the number of protozoa is greatly reduced.

The manifestations of established trichomoniasis in females are cervicitis and endometritis that result in repeat breeding, abortion, or pyometra. The inflammatory changes in the endometrium and cervix are relatively mild and nonspecific, although the mucopurulent exudate may be rather copious. Discharge of exudate into the vagina may be more or less continuous or intermittent, and the numbers and activity of the trichomonads in the discharge vary considerably over short periods. The discharge may not be apparent at the vulva.

The pattern of repeat breeding in tritrichomoniasis is the same as that in venereal *Campylobacter* infections. Return to service occurs at irregular intervals, a fact that indicates that fertilization and implantation are followed by embryonic death. When the embryo or fetus dies, it may be resorbed, aborted, or retained with the development of pyometra. Trichomonad abortions may occur at any time, but mainly in the first half of pregnancy. *There are no specific fetal lesions, but large numbers of protozoa may be found in the fetal fluids and stomach*. The placenta is not severely altered as in brucellosis; it may be covered by white or yellow flocculent exudate in small amounts, thickened, and slightly tough; hemorrhage without much necrosis may be evident on the cotyledons. Pyometra is one of the remarkable changes of trichomoniasis, but it is a relatively uncommon complication. Its pathogenesis follows the scheme outlined earlier, and its character is remarkable only for the copiousness of the exudate; any volume up to 4 L or more may be present. The exudate may be watery with floccules, colostrum-like, or brown and sticky. It is without odor and swarms with trichomonads.

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Viral infections of the pregnant uterus

Porcine reproductive and respiratory syndrome (PRRS)

The syndrome is caused by species *Porcine reproductive* and *respiratory syndrome virus* (PRRSV), family *Arteriviridae*, genus *Arterivirus*, a positive-strand RNA virus. All ages of nonimmune pigs are affected. Death rates can be high in piglets born with pneumonia or infected before or after weaning. Deaths in piglets are also associated with secondary bacterial infections, including *Salmonella choleraesuis, Streptococcus suis, Actinobacillus pleuropneumoniae*, and *Haemophilus parasuis*. These secondary infections often account for major reproductive losses. Viremia and circulating antibodies may coexist. Strain differences may account for some of the reinfections. Nonimmune animals vaccinated with modified-live PRRSV vaccines may experience increased abortions, stillbirths, and high mortality in newborns.

The effects of PRRSV on reproductive performance are variable between strains, and even though a gilt develops antibodies to one strain, she may be susceptible to another. Similar differences in pathogenicity occur in respiratory disease. Effects also vary greatly depending on the stage of gestation and may result in early farrowing of stillbirths and mummified fetuses.

Initial clinical findings in infected pregnant gilts are anorexia, lethargy, depression, and mild fever; cyanosis of the snout, ears, tails, vulva, and abdomen occurred commonly in European outbreaks but are rarely observed in North America. This is commonly followed by a sudden increase in *early farrowing, late-term abortions, stillbirths, mummified fetuses, infertility*, and sometimes death. Live piglets with respiratory signs, muscle tremors, and splayleg also occur. Transplacental passage may result in fetal antibody production. After an outbreak of reproductive failure, previous levels of reproductive performance may not be achieved. Variable degrees of endometritis and myometritis may be present.

Gross and microscopic lesions in piglets are variable to absent and often obscured by autolysis. "Paintbrush" hemorrhages have been observed on the placenta, and petechial hemorrhages and necrosis occur on umbilical vessels, skin, and renal cortex. Microscopic lesions include lymphoplasmacytic and histiocytic infiltrates within and around small capillaries at the periphery of the tunica adventitia of umbilical arteries. Mild to moderate, multifocal to diffuse, histiocytic and proliferative interstitial pneumonia characterized by mononuclear cell septal infiltration is commonly observed. Hypertrophy and hyperplasia of type 2 pneumocytes is occasionally present. Mild segmental necrotizing and lymphohistiocytic arteritis has been seen in lungs. Mild segmental necrotizing and lymphohistiocytic, suppurative and, less often, eosinophilic hepatitis may be present. Mild multifocal perivascular lymphohistiocytic myocarditis and, rarely, multifocal myocardial hemorrhages are present.

The *diagnosis* is established by isolating the PRRSV, PCR assay, or by in situ hybridization or immunohistochemical (IHC) detection of the virus in formalin-fixed tissues. The virus is rapidly inactivated in aborted, stillborn, or mummified fetuses, and isolation is therefore inconsistent. PCR becomes valuable in diagnosis as there are no consistent gross or microscopic lesions of this syndrome in fetuses, and PCR testing modalities are impacted the least by the adverse effects of autolysis.

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Classical swine fever virus infection of the pregnant uterus in sows

Species *Classical swine fever virus* (CSFV, syn. hog cholera virus), family *Flaviviridae*, genus *Pestivirus*, (single-stranded, positive-sense RNA genome), causes a serious and contagious viral disease of swine; fortunately it has been eradicated from many countries. *Pestiviruses include CSFV, border disease virus (BDV) of sheep, and bovine viral diarrhea virus (BVDV)*. Infection by any of these 3 viruses can have similar results, namely, early embryonic losses resulting from in utero transmission to the fetus, severe congenital abnormalities, and, particularly with BVDV, lifelong persistent infections.

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The virulence of CSFV varies from avirulent to highly virulent. Pigs infected with highly virulent CSFV may have a high fever, depression, incoordination, reddening of the skin, diarrhea, respiratory distress, and death with widespread hemorrhage resulting from severe necrotizing vasculitis. Moderately virulent and virulent strains of CSFV cause subacute and chronic disease with similar but milder signs. During an inapparent viremic phase in a pregnant sow, the virus may be carried to the fetus and result in *embryonic death, malformations, mummification, stillbirth, or birth of live clinically normal pigs or pigs with tremors*. Persistently infected (PI) piglets may be born at term and appear clinically normal or weak but, in either circumstance, eventually succumb to the effects of the virus.

The virus can be transmitted from carrier or clinically affected pigs through their secretions and excretions or through the ingestion of contaminated wastes. Birds, parasites, and fomites have also been identified as the source of virus in outbreaks. At one time, the use of vaccines containing CSFV of low virulence was the cause of severe losses caused by in utero infections in pigs.

After exposure of a pregnant sow, the virus multiplies in the tonsils and spreads to other lymphoid organs, eventually reaching the fetus between 13 and 18 days. During the viremic stage, the virus crosses the uteroplacental interface, infecting all or only some fetuses. In the latter situation, the virus may continue to pass from fetus to fetus in utero, eventually infecting many.

Lesions produced in a fetus following in utero infection are variable and depend on the virulence of the infecting virus, the stage of gestation when the infection occurs, and an apparent variation in the development time of immune competence to CSFV in fetal piglets. In general, and with many exceptions, early infections result in embryonic or fetal death or persistent infections. Early to mid-gestation infections result in *malformations*, including pulmonary hypoplasia, pulmonary artery malformation, micrognathia, arthrogryposis, and CNS malformations (including cerebellar hypoplasia, microcephaly, and defective myelination in brain and spinal cord). Infections during the last trimester may produce no abnormalities, or may result in mummification or stillbirths.

Persistent infections in piglets most commonly follow exposure to the virus from 22-70 days of gestation and, rarely, up to 100 days. These fetuses may or may not die in utero and often show retarded development and runting. Many show no signs until sometime after birth, and PI pigs living to 11 months have been reported. The latter commonly have marked depletion of lymphocytes (especially in the thymic cortex), and B-cell–dependent regions of lymph nodes and spleen. Lesions are also observed in heart (degeneration of endothelium and valvular fibrosis), liver (portal fibrosis), intestine (villus atrophy and ulceration), lungs (interstitial pneumonia), skin (necrosis and degeneration of epithelial cells), and CNS (neuronal degeneration and hydranencephaly). Many PI piglets are born alive with a congenital tremor and CNS dysgenesis, cerebellar hypoplasia, and hypomyelination. Piglets with tremor, which survive for long periods, have reduced clinical signs but continue to excrete CSFV. The similarity of this to the syndrome in border disease virus–infected "shaker lambs" suggests a similar pathogenesis.

Many fetal piglets show some ability to respond immunologically to CSFV between 70 and 90 days of gestation with an immunoglobulin of low specificity and avidity. In accordance with this, fetuses collected from sows infected during this period may show general lymphoid hyperplasia and development of primary follicles. Kidney and brain may have mild lesions with scattered focal mononuclear cells, and spleen and kidney may have foci of necrosis and hemorrhage.

For *diagnosis*, CSFV can be isolated from a variety of fetal tissues, including the placenta and from the serum of PI swine, or detected by various PCR tests. Fetal antibody may be present in late-gestation fetuses, but carrier sows may or may not have antibody. In addition, the virus may be identified by the fluorescent antibody technique in tissue sections. In some laboratories, CSFV is routinely differentiated from BVDV and BDV infections and, in some instances, CSFV vaccine strains from field strains. This may be very important in large eradication and control programs.

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Bovine viral diarrhea virus infection of the pregnant uterus

Species *Bovine viral diarrhea virus* (BVDV), genus *Pestivirus*, family *Flaviviridae*, is closely related to *Border disease virus* of sheep and less closely to *Classical swine fever virus*. For further discussion, see the section on border disease virus infection in sheep and goats, later. *These 3 viruses have been shown to cross infect naturally between species*. The various strains of BVDV, although similar, differ in virulence and can be distinguished antigenically. Two *biotypes* exist, one that is cytopathic for cell cultures (cp-BVDV = CB, cytopathic biotype) and one that is not cytopathic (ncp-BVDV = NCB, noncytopathic biotype). Both cause disease in cattle. In addition, there is *type 2 BVDV*, which can infect fetuses and exists in cytopathic and noncytopathic forms as well.

BVDV and Akabane virus are probably the most important viral pathogens of the bovine fetus. BVDV is distributed worldwide, and persistently infected (PI) cattle are reported to make up about 1% of the general population. They do not develop antibody to the homologous strain to which they are infected, but may respond to heterologous strains used in vaccines. Cattle persistently infected with ncp-BVDV or temporarily infected with cp-BVDV may excrete virus in feces, semen, urine, milk, nasal, ocular, and uterine secretions. Susceptible animals can acquire the virus by inhalation, ingestion or when bred with contaminated semen. Cows may also become infected from contaminated embryo transfer fluids where serum from carrier animals is used. Cytopathic BVDV is derived from mutations of ncp-BVDV. *The ncp biotype may persistently infect the immunologically incapable fetus*. There is no report of a cytopathic biotype being isolated from an infected animal without the noncytopathic biotype also being present; the cp form has no means of maintaining itself in the population of animals without the ncp form.

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Reproductive disease in cattle induced by BVDV includes oophoritis, fertilization failure, embryonic death, absorption or abortion, mummification, stillbirth, birth of calves small for gestational age or with congenital defects, and weak calves or near–normal-looking calves born PI with ncp-BVDV. Abortion storms can occur, and occasionally severe losses resulting from BVDV may follow superovulation and embryo transfer. This may be related to the creation of a population of fetuses of the same gestational age (and therefore equally susceptible to the infection) or contamination of transfer fluids by BVDV. Present evidence suggests that ncp-BVDV infects the bovine fetus following oronasal exposure of the dam and only causes embryo injury when it mutates to the cp form of fetal infection.

Fertilization failure may occur when the virus is introduced in utero to seronegative cows. Conception and pregnancy rates in animals viremic at or shortly after insemination were approximately half of noninfected controls at gestation day 77. Surviving embryos were not infected. These data strongly support BVDV infection as being detrimental to early embryo development. Acutely infected bulls shed the virus in their semen for at least 14 days and may have abnormal semen for several months. Spread of the virus is more likely to occur with natural service than with artificial insemination. Carrier and newly infected cows infect the fetus through the placenta.

In the period from 45 to 125 days gestation, the ncp biotype will probably not cause fetal loss, but if it changes to the cp form, it may result in intrauterine growth retardation. Only the ncp biotype can cause persistent infection. If born alive, these PI animals constitute the main source of virus for other animals. *CNS malformations* follow infections with BVDV between 80 and 150 days of gestation. This is probably associated with the stepwise development of immune capability in the fetus, along with the corresponding gradual development of its nervous system. Abnormalities attributed to BVDV include microencephaly, cerebellar hypoplasia, hydranencephaly, secondary hydrocephalus, hypomyelinogenesis, myelination defects, microphthalmia, cataracts, retinal degeneration, optic neuritis, brachygnathism, thymic aplasia, hypotrichosis, alopecia, pulmonary hypoplasia, and growth retardation with growth-arrest lines in long bones. Porencephaly, hydranencephaly, and secondary hydrocephalus have been described, but the association with BVDV is less clearly documented. It is believed by some that only the cp biotype causes abnormalities. Cerebellar hypoplasia may be inapparent or severe. Normal and severely affected folia may be adjacent. Early lesions consist mainly of edema, hemorrhage, and necrosis of external germinal cells. Remnants of severely affected folia consist of fluid-filled cavities lined by flattened astrocytes surrounded by a thin layer of neuropil containing many hemosiderin-laden macrophages. Some folia have only reduced myelin content. Purkinje cells may be reduced in number and with a disorderly or absent external germinal cell layer. Perivascular mononuclear cells and a few neutrophils may be present in neural parenchyma and meninges in cerebrum and cerebellum. Spongiform alterations of brainstem white matter have been recorded, along with spongiform alteration and liquefactive necrosis of ventral funicular white matter in the spinal cord. Focal malacia of the ventral horns occurs following degeneration of ventral horn neurons along with glial satellitosis. Cataracts and retinal atrophy may be present in the eye, and the latter is presumed to follow focal-to-diffuse necrotizing, nonsuppurative retinitis and usually accompanies the cerebellar lesions. Microphthalmia and optic neuritis with subsequent atrophy also occur.

Other lesions associated with BVDV infections in the fetus include thymic cortical atrophy (with only epithelial stroma and macrophages persisting in the cortex), multifocal myocarditis, peribronchiolar lymphoid hyperplasia, pulmonary hypoplasia, multifocal perivascular dermatitis, and hypotrichosis. Cellular accumulations are usually mononuclear, lymphocytic, and plasma cell rich. Plasmablasts, plasma cells, and lymphoblasts may be prominent in lymphoid tissues.

Antibody in fetal fluids, culture of virus from fluid and tissues, and PCR-based tests are sensitive methods to determine if fetal infection has occurred. The presence of specific antibody in an aborted fetus only indicates the fetus was infected in utero. Fluorescent antibody and immunohistochemical detection of virus are frequently used to indicate the association of the virus with a lesion.

Naturally occurring BVDV infections in pregnant **sows** and **goats** have been reported to pass to the fetus, causing stillbirth and neonatal death similar to chronic swine fever and border disease. Piglets infected in utero can either carry the virus (without antibody), develop antibody later, or become clinically affected and develop intestinal lesions, depending on the stage of gestation when infection occurs. On postmortem, infected piglets may also have nonsuppurative meningitis and choroiditis. **Swine** fetuses dying early in gestation from in utero infections with BVDV may also be absorbed.

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Border disease virus infection in sheep and goats

Species *Border disease virus* (BDV), *Classical swine fever virus* (CSFV), and *Bovine viral diarrhea virus* (BVDV) are in the family *Flaviviridae*, genus *Pestivirus*. All are related antigenically and cause significant disease in animals. BDV and BVDV are closely related, whereas CSFV is distinct. Some strains of BDV appear to be more closely related to certain strains of BVDV than to other strains of BDV, whereas others cannot be differentiated from each other. BDV, BVDV, and CSFV have been shown to cross infect between species, and this may occur naturally. Border disease is the result of in utero infection of pregnant sheep or goats by BDV; prevalence of BDV is variable in sheep and rare in goats. *Infection can result in embryonic or fetal death, abortion, mummification, dysmorphogenesis, early postnatal death, and birth of weak or clinically normal young.* Clinically normal kids or lambs can be persistently infected (PI). Infection in the nonpregnant adult animal may result in fever and leukopenia, but clinical signs are usually absent.

Transmission of BDV is believed to occur mainly between sheep; however, natural and experimental infections of BVDV from cattle to sheep have been reported and can

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cause BDV-like outbreaks. BDV can be transmitted by oral, conjunctival, intranasal, and genital routes. *As congenitally infected sheep can have lifelong viremia, they are probably the main reservoir for the virus.* PI ewes can transmit the virus to their fetus through the placenta (over several pregnancies), and in rams, the virus has been observed in the seminiferous tubules of the testes and can be transmitted in the semen. BDV has been demonstrated in the germinal cells of the ovaries of sheep; the significance of this site in the transmission of the virus is not known.

A wide variety of fetal tissues are affected by BDV infection, and the name *hairy shaker* was coined because of the frequently observed hairy appearance of the wool and the tremor associated with hypomyelinogenesis. Abnormalities induced in the fetus, as in cattle, depend mainly on the stage of gestation and reflect the stepwise development of fetal immunocompetence to the virus. Strains of the virus also vary in virulence, as does the susceptibility of breeds of sheep to the effects of the agent. As the ovine and caprine fetus becomes immunocompetent to BDV by about 90 days, most injury follows infections occurring before this time.

CNS lesions include hypoplasia and dysplasia of the cerebellum, microcephaly, porencephaly, hydranencephaly, leukoencephalomalacia, and reduced weight and length of the spinal cord. The most consistent lesion is *dysmyelination or hypomyelination*, which usually occurs without signs of inflammation.

The mechanisms responsible for hypomyelination have not been completely defined. It has been suggested that BDV has a specific affinity for oligodendrocytes; however, evidence supporting this is lacking. There is strong evidence that the hypomyelination seen in the brain and spinal cord in BD may be due to lowered activity of oligodendrocytes with subnormal thyroxine production. In the tested lambs with BDV, there were significantly decreased concentrations of serum triiodothyronine and thyroxine, and many of the thyroid follicle cells contained BDV. As in many other tissues occupied by BDV in PI animals, little cell death or associated inflammation was present. Further support for the hormonal theory of hypomyelinogenesis came on finding significantly lower concentrations of the thyroid hormone–dependent enzyme 2′,3′-cyclic nucleotide-3′-phosphodiesterase (CNP) in oligodendrocytes from affected animals. CNP is normally found in oligodendrocytes, and its activity, which is dependent on the presence of both thyroxine and growth hormone, increases with myelination. Work in congenitally hypothyroid mice and thyroidectomized rats has shown that growth hormone and thyroxine are necessary for normal myelination of nerve fibers.

Abnormalities in the fleece are commonly seen in fetuses following in utero infections with BDV. In smooth-coated ewes and nannies infected between 50 and 85 days of gestation, the fleece of the fetus may become hairlike and curly. The hairiness is associated with enlargement of primary follicles, increased size of fiber, increased proportion of fibers with a central medulla, and increased diameter of the medulla. Around 90 days of gestation, animals become immunocompetent to the virus, and infection at that time may result in only a diminished number of secondary follicles. The reduction has been suggested to be the result of reduced fetal nutrition because of placentitis. In pigmented breeds, there may be large patches of abnormally pigmented black or brown fleece, especially along the neck. Exactly why these changes are induced has not been determined. However, using immunofluorescence, BDV antigen has been demonstrated in skin follicles of fetuses infected at 45 days of gestation, starting at 40 days postinoculation and continuing in lambs up to 5 months of age.

Intrauterine growth retardation commonly occurs with BDV infection in goats and sheep and was reported to be of similar degree to that seen in fetal starvation, cardiac anomalies, and placentitis, except for lung and brain, which were more severely affected. Growth-arrest lines (unmodelled metaphyseal trabeculae) are commonly observed in the long bones of BDV-infected fetuses and lambs. Arthrogryposis and kyphoscoliosis occur infrequently in conjunction with hydranencephaly, cerebellar dysmorphogenesis, and hypoplasia in BDV-infected fetuses.

BDV infection in sheep and goats causes *placentitis and endometritis* that are particularly evident when infection occurs during the first trimester of gestation. Early in the infection, multiple white 1-2 mm foci of necrosis appear in the caruncles at the base of the crypts. These may fuse into a continuous band of necrosis, which may become mineralized and infiltrated with connective tissue and a few macrophages, or the entire base of the caruncle may be infarcted, necrotic, and infiltrated with neutrophils. The lesion is believed to be associated with endothelial swelling and thrombosis of capillaries in the endometrium and adjacent caruncle. The trophoblast does not usually undergo necrosis but may atrophy.

Definitive *diagnostic tests* can be selected with confidence when several kids or lambs show typical signs of BDV infection. However, diagnosis may be difficult when BDV occurs only as reproductive failure with embryo or fetal death, followed by absorption, abortion, or mummification. Frequently in goats, abortion may be the principal sign. In aborted fetuses with congenital abnormalities, diagnosis requires the detection of the virus, virus-specific antigens, fetal antibodies against the virus, or PCR-based tests in or of cavity fluids. The cotyledons, or preferably the caruncles, are the best source of virus. It is variably recovered from aborted fetuses and almost never from those that are mummified. Although isolates are usually noncytopathic, viral antigen can be demonstrated by direct or indirect immunofluorescence, and immunohistochemistry of selected tissues from affected lambs or the caruncle following abortion.

Sheep have been reported to retain BVDV in the uterus from one estrus to the next and to produce PI offspring when naturally bred or artificially inseminated by semen collected from PI rams.

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Equine arteritis virus infection of the pregnant uterus in mares

Equine viral arteritis (EVA) is a disease of horses caused by species *Equine arteritis virus* (EAV), a positive-stranded RNA virus, family *Arteriviridae*, genus *Arterivirus*. Genetic diversity of the virus is generated in the course of persistent infection of the carrier stallion, and the genetic changes that maintain

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EVA in the stallion's reproductive tract likely affect phenotypic properties of the virus, such as virulence. Strains of the virus vary greatly in virulence. Consequences of infection range from subclinical to mild fever; ocular and nasal discharges; edema around the eyes, legs, and scrotum; skin rash; and, in severe forms, diarrhea, respiratory distress, and rarely death. Embryonic death and resorption rates are permanently increased, and 40-80% of pregnant animals abort or deliver a stillborn foal. Abortion usually occurs 1-4 weeks after the onset of fever and is probably the most grievous manifestation of the disease, although in some outbreaks, severe interstitial pneumonia and necrotizing enteritis may occur in foals.

The virus is spread by aerosolization of respiratory secretions among closely associated animals. *Stallions are probably the most important means of spread*, as they shed the organism in semen for a short or very long period, even for life, and transmit the disease sexually. There are minimal consequences of sexually acquired infection, with little effects on conception rate, unless a mare is infected in the late stages of gestation, in which case she may abort. Stallions that are shedding the organism should not be used for breeding unless they are bred to seropositive mares that have received the organism from either vaccination or natural infection. In contrast, although mares may spread the virus during their illness or shortly after, they generally do not remain carriers, and after recovery, neither mares nor stallions shed the virus in nasal passage secretions, urine, or blood. Most horses develop long-term immunity to EAV after natural infection of the virus.

Aborted fetuses are usually well preserved, but gross and microscopic lesions are rarely observed as the virus seldom produces active infection in the fetus. In foals surviving for more than 24 hours, fever, leukopenia, and/or thrombocytopenia are sometimes observed. Widespread inflammatory lesions in sections of the brain, liver, and spleen have been recorded. On microscopic examination, findings include interstitial pneumonia, lymphocytic periarteritis with fibrinoid necrosis of the tunica media, and renal tubular necrosis. Moderate arteritis with necrosis and mononuclear cell myocarditis has been reported of a few fetuses. In the uterus of the mare, however, there may be acute multifocal necrotizing myometritis and replication of the virus in smooth muscle cells. Uterine infection might be the critical process contributing to abortion.

In the live mare and stallion during the early stages of disease, the presence of EAV may be determined by culture or PCR assay of swabs taken from nasopharynx and conjunctiva, from white blood cells, or from semen. The virus may be recovered from semen or revealed by breeding unexposed mares, which may then develop a fever or become clinically ill. Serologic tests are available to detect previously infected animals. Samples for culture from the conceptus should include placenta, lung, and spleen. Diagnosis can also be obtained by immunohistochemistry, virus isolation, and PCR-based tests.

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Wesselsbron virus infection of the pregnant uterus in sheep and cattle

Species *Wesselsbron virus* (WESSV) is in the family *Flaviviridae*, genus *Flavivirus*, in the yellow fever virus group. It is spread primarily by mosquitoes. Infection is often fatal in the ovine and bovine fetus, in newborn lambs, and pregnant sheep. It may also produce congenital anomalies in bovine and ovine fetuses. Human illness is not fatal and is characterized by fever, chills, muscle pain, hyperesthesia, and a skin rash. Information is largely incomplete with regard to the pathogenesis and pathology of this infection in pregnant animals.

The disease is widespread in the southern and western parts of Africa in sheep and cattle; however, in spite of a high prevalence and high annual infection rate, mortality is low. In a naïve flock of sheep, however, mortality in newborn lambs and pregnant ewes may be high. Pregnant ewes may die before or after abortion, whereas nonpregnant ewes, wethers, and yearlings usually show no signs of illness. C*hanges in the liver caused by WESSV infection may precipitate acute copper toxicity*, thereby inflating the perceived mortality directly caused by this virus.

Pregnant sheep infected with WESSV develop a fever associated with initial viremia and often abort at this stage because of the systemic effects of the virus on the dam. Under these circumstances, neither virus nor antibody is detected in the fetus. If the ewe does not abort during the primary viremia, the virus can invade the placenta and fetus in association with a second growth phase and result in death of the fetus. The fetus may be absorbed, mummified, aborted, or carried to term and be delivered dead. The death rate for lambs infected in utero approaches 100%, and even those born alive die soon after. Both wild and attenuated strains of WESSV have been shown to cause nonsuppurative meningoencephalitis, hydranencephaly, arthrogryposis, and hydrops amnii in fetal sheep; however, this is probably infrequent under field conditions.

In pregnant cattle, WESSV probably only crosses the placenta and infects the fetus in a low percentage of animals, even in the face of prolonged maternal viremia. When the virus does infect the fetus, abortion may occur or the calf may be born weak and die shortly after. These calves have typical liver lesions and precolostral antibody to WESSV. However, virus may not be recovered. Many pregnant cows, even after prolonged viremia, give birth to normal live calves that show no serologic or virologic evidence of infection. In cattle, as described in sheep, certain strains of the virus occasionally produce nonsuppurative meningoencephalomyelitis, porencephaly, and cerebellar hypoplasia in the fetus.

Gross and microscopic descriptions of findings in aborted fetuses, or stillborn lambs and calves, are limited. Experimentally infected newborn lambs have a slight to moderately enlarged, yellow to orange, variably congested liver. On microscopic examination, hepatocyte necrosis is slight to extensive, but is usually single cell and generalized. *Eosinophilic, intranuclear inclusion bodies of various shapes are frequently present*. Separated, individual hepatocytes may be round, shrunken, and have intensely pink-staining cytoplasm and nuclear lysis (Councilman-like bodies). Kupffer cell hyperplasia is prominent, particularly in the central vein region, and Kupffer cells filled with yellow or pink granular material often bulge into sinusoids. The sinusoids also contain large numbers of mononuclear cells and degenerating neutrophils. Bile ducts and canaliculi are frequently distended with bile. In portal areas, there is bile duct hyperplasia, moderate numbers of

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macrophages, and some neutrophils and plasma cells. There are petechial and ecchymotic hemorrhages on the mucosal surface of the abomasum, and the content is dark brown. Other nonspecific lesions, consistent with a viremia, are also described.

The *diagnosis* of WESSV infection in pregnant animals is based on clinical findings, histology, and culture from liver and brain. The lesions produced by Rift Valley fever virus infection may easily be confused with Wesselsbron disease, and, although the liver lesions tend to be different, complications resulting from precipitation of copper toxicity may make the differentiation difficult.

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Porcine parvovirus infection of the pregnant uterus

Species *Porcine parvovirus* (PPV), family *Parvoviridae*, subfamily *Parvovirinae*, genus *Parvovirus*, is an important and common cause of reproductive failure in pigs, and manifests as *embryonic death with reabsorption, mummification, stillbirths, and reduced litter size*. Farrow-to-finish farms are affected nearly twice as often as feeder pig farms, probably because of the maintenance of a continual reservoir of PPV in the finishing pigs. The virus is most commonly spread by the ingestion of contaminated feces and will adhere to the zona pellucida of embryos. Viremia develops in susceptible pigs, and in pregnant pigs the virus enters the placenta and passes to the embryo or fetus 23-32 days following oral inoculation. Up to a fetal age of 70 days, the virus may cause death of the embryo (with absorption) or fetus (with mummification); however, the porcine fetus becomes increasingly resistant to the effects of the virus after 36 days and is immunocompetent by about 70 days. Death of the fetus may be due to the total effect of the virus on rapidly dividing cells, including neurons and capillary endothelium of the cortical laminae in cerebellum and cerebrum, and in cells in the lung, liver, pancreas, and chorioallantois. Movement of virus from one fetus to another may be affected by development of maternal antibody, increasing resistance of fetuses with advancing age, and the variability of the circulation and contact between the placental membranes of adjacent fetuses.

When PPV is introduced by the uterine route, it causes inflammation in the ovaries and degeneration of uterine epithelium, both of which may contribute to reproductive failure but do not otherwise cause clinical signs. Uterine changes may include marked variation in surface epithelial cell height, epithelial cell basophilia, degeneration, and ulceration. Light infiltration of neutrophils, eosinophils, and lymphocytes may be present throughout the endometrium. Corpora lutea contain foci of mononuclear cells, many of which are lymphoplasmacytic. *In the fetus, lesions are most commonly observed in older fetuses and may be present in kidney, liver, brain, and placenta*. In the cerebrum, which is the most dependable site, perivenous accumulations of plasma cells and other mononuclear cells occur in white and gray matter and in meninges. Similar accumulations are observed in the renal interstitium, hepatic portal regions, and in the chorioallantois.

For *diagnosis*, viral antigen is demonstrable by the fluorescent antibody test in affected tissues of embryos and mummified and stillborn fetuses, and a highly sensitive DNA probe, specific for PPV-infected cells, is available. Identification of specific antibody, particularly IgM, in fetal fluids indicates fetal infection, and with other appropriate findings, it is supportive evidence for the cause of reproductive failure. When the only manifestations are small litters or pigs that recycle, the causal relationship of these signs with PPV is difficult to establish, and knowledge of titers of antibody against PPV in the gilt or sow before and after the event may be the only incriminating evidence. Various tests, including an ELISA, have been developed for this purpose.

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Bovine parvovirus infection of the pregnant uterus in cattle

Species *bovine parvovirus* (BPV), family *Parvoviridae*, subfamily *Parvovirinae*, genus *Bocavirus*. It is widely distributed in cattle populations throughout the world. *It is reported to cause enteritis and diarrhea in young calves and abortion and birth of weak calves when infections occur in naïve pregnant cows. The organism is not considered a major cause of abortion*, but this may be because it is not looked for consistently in submissions. Anywhere from 10-100% of the cattle in a population will have antibody to parvovirus, and work in the United States suggests that when reproductive problems, including repeat breeding, embryonic mortality, and abortion, are considered, animals with antibody to BPV had about 3 times as many of these problems as compared to control seronegative animals.

BPV is excreted in the feces of affected and normal animals, and the oral route is important in transmission. Inoculation of parvovirus into pregnant cows can cause in utero fetal death and abortion in the early stages of gestation. Some calves may be born with cerebellar hypoplasia because of lysis associated with the multiplication of BPV in cells of the external granular layer. Cerebellar hypoplasia is believed to follow infection occurring in the period of rapid cell multiplication (107-150 days of gestation) in the cerebellum. *Intranuclear inclusions* are seen in external granular cells, hepatocytes, adrenal cortical cells, and intestinal crypt cells associated with areas of necrosis. Bovine fetuses are capable of forming IgM antibody to the virus by 93 days of gestation; infections in the third trimester usually only result in antibody formation with no abortion.

For *diagnosis*, BPV may be recovered in a variety of tissues, including placenta, amniotic fluid, adrenal glands, and liver. When infection occurs in midgestation, detection of specific antibody in fetal serum may be used as putative evidence of the cause of abortion. The presence of antibody may interfere with recovery of the virus but probably will not completely prevent isolation, as the virus is frequently cell associated.

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Canine minute virus *infection*

Species *Canine minute virus* (CnMV) is a species distinct from *Canine parvovirus 2* and *Feline panleukopenia virus* in the family *Parvoviridae*, subfamily *Parvovirinae*, genus *Bocavirus*, and antibody to it is widespread among dogs. It may occasionally cause mild diarrhea in pups, but its significance is poorly defined. Experimental oral-nasal infection is clinically inapparent, but it may produce inclusion bodies in the cells of the duodenal villi. Transient lymphocytolysis in the cortex of lymph nodes, thymus, and in gut-associated lymphoid tissue has been reported.

The virus seems capable of transplacental transmission to the fetus, and exposure of pregnant dogs has been associated with fetal resorption or birth of dead or weak pups, depending on gestational stage at exposure. Anasarca and myocarditis are reported in dead or weak puppies. Spontaneous reproductive failure associated with CnMV is not reported.

The *pathogenesis* of CnMV and its clinical significance are not yet known, but preliminary findings suggest it may be responsible for a portion of deaths in pups <4 weeks of age, and it may cause reproductive failure. Virus isolation is difficult, possibly because of antibodies in infected dams at the time they resorb their fetuses; however, histologic examination of tissues from dead pups may reveal viral inclusion bodies in small intestinal villus epithelial cells or bronchial epithelial cells. Much more information is necessary to ascertain the role of this virus in canine disease. Reports of deaths and abortions suggest it is more common than currently recognized.

Diagnosis can be confirmed by virus isolation, PCR, immunofluorescence, or immunohistochemistry.

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Bluetongue virus infections of pregnant sheep and cattle

Species *Bluetongue virus* (BTV), family *Reoviridae*, the prototype virus for the genus *Orbivirus*, causes a nearly worldwide viral infection of sheep and other ruminants. The virus distribution is expanding with expansion of the range of insect vectors. In sheep and, with much less severity and frequency in cattle, the disease is characterized by fever, erosive to ulcerative lesions of the upper gastrointestinal mucosa, and focal hemorrhage and necrosis of smooth, skeletal, and cardiac muscles. BTV infections can be fatal, and in cattle, most infections are subclinical. *Infections occurring during pregnancy, however, can cause heavy losses because of early in utero death and absorption, abortion, mummification, or stillbirth with severe fetal growth retardation and multiple congenital anomalies.*

There are several serotypes of BTV, and they vary greatly in virulence. The virus is mainly spread by, and can multiply in, various species of biting insects in the genus *Culicoides*. With many serotypes of BTV, viremia exists for a longer period in cattle than in sheep, and in both, antibody and virus may coexist for several days. The prolonged viremia seen in cattle has led some investigators to designate them as the reservoir host for this virus. The virus has been found in the semen of a few bulls; however, it is usually transient and therefore of less importance than arthropods in transmission.

When an animal becomes infected, primary virus replication occurs in the lymph nodes draining the site of inoculation. This is followed by extension through the lymphatics and bloodstream to the spleen and lymph nodes, where the virus propagates and is carried via the blood to a variety of tissues. The organism appears to be closely associated with erythrocytes during viremia. Species-specific differences in the production and activities of endothelial cell-derived inflammatory and vasoactive mediators appear to contribute to the greater sensitivity of sheep than cattle to BTV-induced microvascular injury.

The consequences of infection during pregnancy depend on the stage of gestation when infection occurs and on the serotype of virus. Infections in sheep up to approximately 50 days of pregnancy may result in death of the conceptus and absorption or abortion. Between 50 and 80 days of gestation, *necrotizing encephalopathy* occurs; and in the last third of gestation, as immunocompetence increases, there may be no obvious disease. On the other hand, infections of near-term and newborn lambs can result in a sluggish immune response, possibly associated with the rise in fetal cortisol at parturition and the ingestion of colostrum, both of which may suppress the active immune response.

Lesions in the brain of the *ovine fetus* infected during the 50-70 day period consist of many mononuclear cells in the meninges and necrosis of the cerebral cortex. The meninges and areas of necrosis gradually become filled with macrophages and, unless the fetus is expelled early, appear as variably sized fluid-filled cavities (porencephaly and hydranencephaly), frequently accompanied by some degree of secondary hydrocephalus in the new or stillborn lamb. Cerebellar dysgenesis, retarded development, and skeletal growth-retardation lines have also been described in fetuses inoculated with BTV. The lungs in fetuses inoculated with virus at 80-100 days become infiltrated with large mononuclear cells within the interstitium of small arterioles. These accumulations eventually form continuous overlapping nodules of large mononuclear cells that have abundant cytoplasm and indented nuclei. Similar lesions are occasionally seen in the renal medulla and hepatic portal areas. Cortical regions of lymph nodes are enlarged by large numbers of primary and secondary lymphoid follicles that contain numerous plasma cells. In the spleen, small white nodules are visible on the cut surface. These consist of greatly expanded periarteriolar lymphoid tissue.

Lesions in the *bovine fetus* develop similarly, and up to 70 days of gestation, BTV infection may result in in utero death and absorption or abortion of the conceptus. Between 70 and 130 days of gestation, serotypes 10, 11, 13, and 17 can cause hydranencephaly and death of the fetus. Two distinct

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processes may be involved in the development of CNS lesions caused by BTV infections in bovine fetuses: *Infarction*, from viral-induced vascular injury, is probably responsible for the large areas of necrosis and cavitation occurring in the rostral regions of the cerebrum, whereas *viral destruction* of clusters of individual cells, including glial cell precursors, results in foci of necrosis and accounts for microcavitation. Collapse of these cavities may facilitate dilation of the lateral ventricles and secondary hydrocephalus. Death of calves with hydranencephaly pre-empts them spreading the virus by an arthropod vector, even if they are viremic. The earliest evidence of antibody activity against BTV group antigen in bovine fetuses occurs at around 145 days of gestation; by 200 days, there is significant neutralizing antibody activity; therefore no virus is detectable at birth.

Diagnosis of in utero infections in cattle and sheep is based on finding the typical lesions and specific fetal antibody to BTV in cavity fluid or blood. Virus isolation after fetal immunocompetence to BTV is unlikely but not invariable. Maternal precipitating antibody (agar gel immunodiffusion test) may be absent at term, whereas neutralizing antibody may be more persistent. The c-ELISA for specific neutralizing antibody is more specific and sensitive. To culture the virus from blood, animal inoculation or washed erythrocytes may need to be used because the organism is closely associated with red cells. BTV and epizootic hemorrhagic disease virus (EHDV) can be detected and differentiated by multiplex RT-PCR.

Epizootic hemorrhagic disease virus infection of the pregnant uterus in cattle, sheep, and white-tailed deer

Species *Epizootic hemorrhagic disease virus* (EHDV) is an arthropod-borne virus in the family *Reoviridae*, genus *Orbivirus*. It exists as at least 2 serotypes, 1 and 2, and is present in Africa, Australia, North America, and Japan. Ibaraki virus (IBAV), first isolated in Japan, is a serotype 2 strain. EHDV is spread by *Culicoides* spp. and *causes fatal hemorrhagic disease of white-tailed deer.* In cattle, the Ibaraki strain can cause severe ulceration of the mucosa of the mouth, tongue, and esophagus, with necrosis of esophageal musculature and marked pain on swallowing. Many animals die. In many respects the disease is similar to BTV infection in sheep, but, in contrast to BTV, *EHDV has very low pathogenicity for sheep.* Although EHDV cross reacts with BTV in some serologic tests, it is considered to represent a separate population of viruses. Experimental inoculation of BTV has been reported to cause abortion and mummification in white-tailed deer. EHDV, particularly the IBAV strain, can cause abortion and stillbirths in cattle.

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Chuzan disease of cattle

Species *Chuzan virus* (CHUV, syn. *Palyam virus*) has been isolated from cattle and the midge *Culicoides oxystoma*. The virus is in the family *Reoviridae*, genus *Orbivirus*, Palyam serogroup, and is most closely related to African horsesickness virus within the orbiviruses. CHUV has been incriminated as the cause of an epizootic in the Kyushu district of Japan, wherein 2,463 calves, mostly beef breeds, were affected. A high percentage of newborn calves were born with *hydranencephaly and cerebellar hypoplasia*. A subsequent seroepidemiologic investigation revealed evidence to suggest the virus had been spread from the most southerly island of Japan to the mainland in the north. Cattle in the region did not have antibody to the virus previous to this epizootic.

Calves involved in the epizootic were born alive and were normal in size and weight. Clinical signs and congenital abnormalities included corneal opacity, blindness, nystagmus, cerebellar ataxia, tremor, and opisthotonos. Postmortem lesions included hydranencephaly, secondary hydrocephalus, microcephaly, and cerebellar hypoplasia. No lesions were seen in other organs, and no virus was isolated from any of the calves; however, 44 of 49 precolostral serum samples checked for neutralizing antibody to CHUV were positive. Experimental infection of 15 cows at 89-150 days of gestation resulted in 14 normal calves and one calf with severe hydranencephaly and cerebellar hypoplasia.

For *diagnosis*, CHUV can be recovered from blood for up to 56 days after inoculation. It is closely associated with red blood cells, even in the presence of antibody. Demonstration of specific antibody in the serum of precolostral calves will be incriminating.

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Canine herpesvirus infection in pups and pregnant bitches

Canine herpesvirus (*Canid herpesvirus 1*, CaHV-1), family *Herpesviridae*, subfamily *Alphaherpesvirinae*, is in the genus *Varicellovirus*. In adults or weaned puppies, CaHV-1 may produce mild upper respiratory infection, and inapparent infections are common. Infection of the neonate is possible by various routes. It may be transmitted from maternal lesions of genital infection to the pup as it traverses the birth canal, from initial maternal infection, or by recrudescence of existing latent virus. Viral recrudescence can be induced in recovered animals by corticosteroids. There is considerable homology between endonuclease fragments of CaHV-1 and felid herpesvirus 1.

Canine herpesvirus infection is regularly fatal for newborn puppies. Resistance to the disease is sharply age related; pups exposed after 2 weeks but up to 8 weeks of age will not develop severe illness. The disease has an incubation period of 3-7 days, after which the affected puppies rapidly sicken and die, usually within 2 days. The clinical signs lack specificity. Affected animals are not usually febrile; most vomit and refuse food. Their breathing becomes shallow and rapid, and shortly before death, the pups give evidence of abdominal pain.

At autopsy, there is usually pleural and peritoneal effusion that may be blood tinged. *Petechial and ecchymotic hemorrhages* are scattered throughout the subserosal tissue, usually representing the most impressive gross feature of the disease. Large

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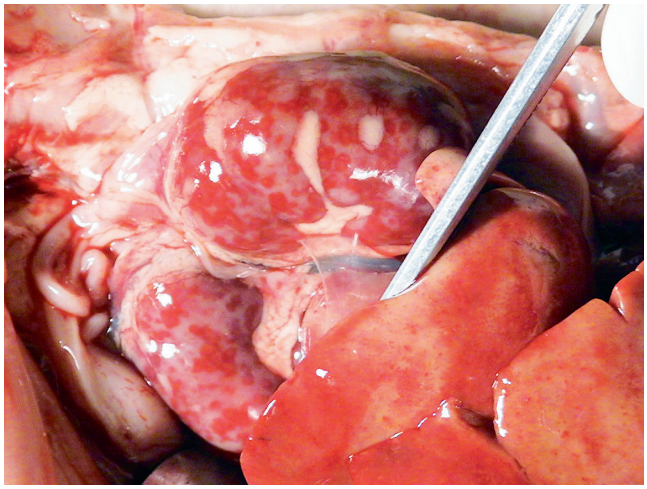

Figure 4-89 Renal hemorrhages in a newborn pup. This lesion is very characteristic for **canine herpesvirus** infection.

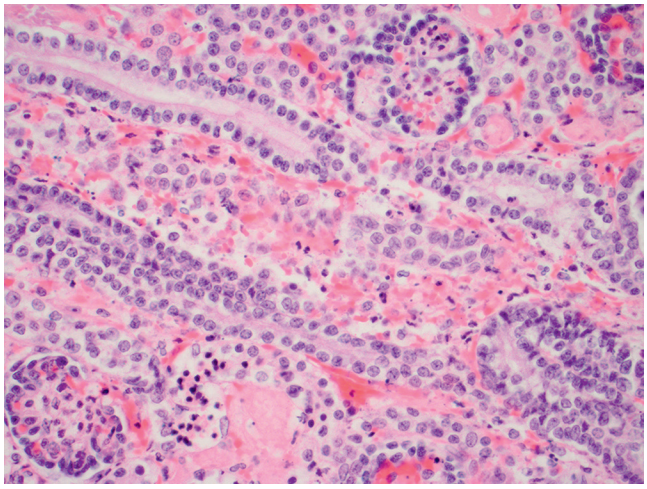

Figure 4-90 Photomicrograph of the renal lesion in Figure 4-89 showing necrosis and hemorrhage caused by **canine herpesvirus.** Herpesviral infections are typically cytolytic, and microscopic lesions in fetal tissues of all species are very characteristic and similar, as shown in Figures 4-91 to 4-94.

hemorrhages are regularly seen in the kidney (Fig. 4-89). The lungs are wet, and frothy fluid fills the bronchi and bronchioles. Lymph nodes are enlarged and reddened, and splenomegaly is present. Tiny red foci, determined microscopically to be foci of necrosis, are occasionally seen in the liver. *Foci of necrosis are characteristic of the disease and may occur in any organ*. Lung, heart, kidney, intestine, pancreas, adrenal, and spleen are common sites (Fig. 4-90). The inflammatory response associated with these disseminated focal areas of necrosis is slight or absent. Viral inclusion bodies are not numerous, but can best be seen adjacent to the areas of necrosis in the liver or lung, or in the kidney. The inclusion bodies are intranuclear; most are basophilic, but some are faintly acidophilic.

Discrete microscopic lesions are present in the brains of the pup as early as 72 hours after experimental infection. These increase in severity as the disease progresses. They consist of nonsuppurative meningoencephalomyelitis, with destruction of gray and white matter; the gray matter is somewhat more severely involved. There are focal and segmental areas of destruction of the cerebral cortices with concurrent microgliosis. Vascular changes vary from endothelial swelling to mononuclear cuffing. Diffuse pial meningitis with infiltrates of mononuclear cells and neutrophils is frequent, as is ganglioneuritis of the Gasserian ganglia.

Canine herpesvirus can cause abortion, stillbirth, and infertility. Depending on the time of gestation, inoculation of bitches with CaHV-1 has been reported to result in death and expulsion of fetuses, mummification, or premature delivery of live puppies. Focal areas of necrosis with intranuclear inclusions were variably observed in liver, spleen, kidney, placental labyrinth, and heart. Endothelium and trophoblast were commonly involved. Virus was recovered from many of the fetuses.

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Suid herpesvirus 1 infection causing abortion in swine

Species *Suid herpesvirus 1* (SuHV-1, pseudorabies virus, Aujeszky's disease virus) is in the family *Herpesviridae*, subfamily *Alphaherpesvirinae*, genus *Varicellovirus*. SuHV-1 is capable of causing *reduced fertility in boars and sows, fetal resorption, mummification, abortion, and stillbirth in sows*, and fatal meningoencephalitis in young pigs, sheep, cattle, and dogs. Strains of SuHV-1 vary in their ability to invade the placenta and fetus; however, abortion without invasion of the conceptus may occur secondary to fever and nervous disease in the sow. When the virus does invade the placenta and fetus, characteristic gross and microscopic lesions are produced. There is a strong correlation between the ability of strains to cause syncytial formation in cell culture and their ability to produce disease in pigs and cattle.

The disease has an almost worldwide distribution, and the carrier sow is the most important source of the virus. Recrudescence and shedding through nasal mucus may occur following stress, and there is some evidence for long-distance airborne transmission, although this would appear not to be a major threat. Boars may carry SuHV-1, spreading it through nasal mucus and semen. The virus can survive in a pen for up to 2 weeks.

The virus enters the nasal passages of the susceptible pig and passes via the pharyngeal and olfactory nerves to the brain, where it rapidly spreads to the rest of the brain, causing nonsuppurative meningoencephalitis. It has affinity for the respiratory tract, and may induce severe rhinitis, tonsillitis, and pneumonia.

Abortion usually occurs about 10 days after the onset of clinical illness in the sow. If the placenta and fetus are invaded, lesions consist of multifocal coagulative necrosis of chorionic villi and focal coagulative necrosis in many organs, including liver, adrenal, and spleen. *Intranuclear inclusions* have been observed in trophoblast and interstitial cells and hepatocytes around areas of necrosis.

Virus may be demonstrated in nasal or vaginal swabs and fetal tissues by isolation and fluorescent antibody or ELISAs; PCR assays are also available.

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Suid herpesvirus 2 (cytomegalovirus) infection of the pregnant uterus of the pig

Species *Suid herpesvirus 2* (SuHV-2, swine cytomegalovirus) is in the family *Herpesviridae*, subfamily *Betaherpesvirinae*, genus *Cytomegalovirus*. Infection by this virus is normally confined to pigs and was first described in England in 1955 as *inclusion body rhinitis* and, subsequently, as a transplacental infection of fetal pigs, in 1961 in Australia.

In neonates, SuHV-2 causes *mild to severe nonsuppurative necrotizing rhinitis* characterized by the presence of *large basophilic intranuclear inclusions* in the epithelium of the mucous glands and ducts in the nasal mucosa. The virus usually affects young pigs (<4 weeks of age) but in naïve herds may cause death in pigs of 4-12 weeks. The virus occasionally causes severe systemic disease in mature pigs. *When infection occurs in pregnant sows, they may deliver small litters, mummified fetuses, or stillborn or weak piglets either before or on the due date.* Pigs infected in utero may be born alive, small for gestational age, and may develop systemic signs and die. Subsequent reproductive performance in infected sows may be reduced and manifested as decreased conception rates and decreased litter size.

Surveys of pig herds for serum antibody to SuHV-2 indicate infection to be widespread globally. Transmission occurs when newly infected pigs excrete the virus in nasal and ocular secretions and urine. Boars may also excrete it in semen and sows in vaginal discharges. The virus has the capacity to remain latent in macrophages and other cells of the monocyte-macrophage system. Animals with latent virus may also excrete it, even in the face of serum antibody, following administration of corticosteroid and probably following periods of stress. Heavy losses are infrequent in commercial herds; however, in gnotobiotic pigs or minimal-disease herds in which pigs have had no previous experience with the virus, the introduction of actively or latently infected animals may prove disastrous.

When a sow is infected with SuHV-2, multiplication occurs first in the nasal mucosa. This is followed by viremia and dissemination of virus to many body organs, including the placenta in pregnant sows. The virus seems to pass through the placenta without inducing injury; experimentally, the virus has not been isolated from the placenta, even when the fetus that has just died is positive. Neither has antibody been detected in piglets that become infected in utero. Fetuses die 4-6 weeks after inoculation of the sow, and the time is independent of the stage of gestation. Casualties of infection are scattered randomly throughout the uterus in various stages of decomposition and mummification, much as with porcine parvovirus infection. Following experimental infection, no gross lesions were seen on autopsy of the autolysed fetuses. However, on microscopic examination there was cytomegaly, and intranuclear inclusions were observed in liver and lung. In a natural outbreak of SuHV-2 infection, pigs born alive with a congenital SuHV-2 infection usually died within a week. Gross lesions in these consisted of pulmonary edema and congestion, hydrothorax and hydropericardium, and multiple firm gray foci in the most ventral portions of the lungs. Mediastinal lymph nodes were enlarged, and petechial hemorrhages were visible on the heart, lungs, intestine, and kidneys. Histologically, there was pulmonary edema and hemorrhage; alveolar septa were thickened, mainly by macrophages, but lymphocytes and neutrophils were also present. Large basophilic intranuclear inclusions were present in capillary endothelial cells and macrophages. Similar inclusions were present in capillaries of the renal medulla and tubular epithelium. Two types of inclusion have been observed: the usual large basophilic type found in pulmonary macrophages and glands and ducts of the nasal mucosa and renal tubular epithelium, and a small herpesvirus-like inclusion found mainly in monocyte-macrophage cells. Nonsuppurative meningoencephalitis has been reported in some pigs, and the lesions are perivascular lymphocytic cuffs and gliosis in the choroid plexus and meningeal vessels outside the cerebral cortex.

Experimental infection of piglets in utero or newborns <2 weeks of age have shown them to be very susceptible and to develop severe debilitating disease, with death a common sequel. This severe infection has been related to viral infection in the cells of the monocyte-macrophage system. This results in disseminated hemorrhage and edema in the lung, kidney, adrenal, liver, and lymph nodes, all of which is related to viral destruction of macrophages and capillary endothelial cells. In the less severe and nonfatal forms of the disease, there is invasion of the mucus-producing cells of the nasal mucosa; the cells of the renal tubules and, less frequently, the epithelium of the salivary glands; Harderian and lacrimal glands; seminiferous epithelium; epithelium of the epididymal duct; mucous glands of the esophagus; duodenum; jejunum; and hepatocytes. Invasion of macrophages into the endothelium or epithelium is not completely exclusive, and both occur often.

The cytomegalovirus inclusions seen on histology, when correlated with appropriate signs, are diagnostic of SuHV-2 infection in pigs. Infection in a herd may be revealed by detecting antibody in the maternal serum by using an indirect immunofluorescent (IIF) antibody test on infected pulmonary macrophages. To detect virus, samples of nasal mucosa, lung, and kidney can be cultured directly on pulmonary macrophages. Viral antigen may be detected in frozen sections of affected lung or kidney by using the IIF test. As stated previously, the organism is not found or seen in the placenta, and antibody is not present in the fetus but may be present in piglets infected after birth.

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Bovine herpesvirus infections of the pregnant uterus in cattle, goats, sheep, and pigs

Bovine herpesviruses cause a wide variety of diseases in several species, including cattle, sheep, goats, wildebeest, and other wild ruminants. They are divided into 6 different groups,

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designated BoHV-1 to BoHV-6. The infections of the pregnant uterus caused by bovine herpesviruses that will be discussed in this section include BoHV-1 (infectious bovine rhinotracheitis virus, IBRV; infectious pustular vulvovaginitis virus, IPVV), and BoHV-4 (Movar virus, a cytomegalovirus).

Species *Bovine herpesvirus 1* is in the family *Herpesviridae*, subfamily *Alphaherpesvirinae*, genus *Varicellovirus*. Based on restriction endonuclease analysis and cross-hybridization, BoHV-1 is subdivided into 3 antigenic variants or subgroups: subgroup 1: IBRV; subgroup 2: IPVV; and subgroup 3: neuropathogenic. *IBRV and IPVV both cause abortion. IBRV is considered to be more virulent than IPVV* and, in addition to abortion, causes severe upper respiratory disease, endometritis, oophoritis, mammillitis, dermatitis, and fatal diarrhea of young calves. IPVV primarily causes inflammation of the penis and prepuce in bulls, inflammation in the vagina and vulva in cows, and abortion in cows.

BoHV-1 is distributed worldwide. Cattle-to-cattle transmission is the principal method of spread, and virus is easily dispatched from naturally infected or vaccinated cattle to susceptible animals through respiratory, ocular, or vaginal discharges. *All BoHV-1 strains*, including thermosensitive and thymidine kinase–negative strains, *are capable of becoming latent* and therefore undergoing recrudescence and shedding from mucous membranes. Virus is more abundant in excretions from primary than from recrudescent infections; however, transmission may occur from either. Live vaccine viruses may become latent, and none of the vaccines can prevent latency by a superinfecting challenge virus, but, if the antibody titer is high in the vaccinate, the incidence of latent superinfection and amount of virus excreted may be lowered. Recrudescence may be induced by superinfections, corticosteroids, transport, parturition, and other stressful situations. Semen may also be an important source of the virus and embryo transfer fluids less so.

Sheep, goats, and pigs often have antibody titers to BoHV-1 and may occasionally show clinical signs of disease. Infection may manifest as an upper respiratory disease, which usually goes unnoticed; however, sheep may develop pneumonia. Abortions, from which BoHV-1 has been recovered, have been described in sheep and pigs. Sheep, goats, pigs, and wild ruminants are rarely the source of BoHV-1 virus for other animals; it is more likely that cattle transmit the virus to them, and disease is an infrequent event.

When the virus enters the mucosa of the respiratory or genital tract in pregnant animals, it multiplies at that site and is carried to the rest of the body in infected leukocytes and blood to the uterus. It has been postulated that, on reaching the caruncle, the virus passes though the endothelium to the interstitium of the villus, therein infecting endothelium, mesenchyme, and then trophoblast. Death is due to tissue destruction in the fetus and the placenta. Early hatched embryos are also susceptible to IBRV infection.

Abortion rates in a herd of cattle may reach 25%; however, the prevalence of abortion in a region declines with a reduction in the naïve population. Most cows infected with BoHV-1 do not abort until 3-6 weeks following the initial infection, and a significantly greater number of cows abort between 5 and 8 months of gestation than earlier. It is speculated the virus can stay in the placenta for a prolonged period before invading the fetus. As the virus invades, the fetus dies quickly with no preparation for delivery. *Expulsion occurs 3-5 days following fetal death, and the carcass is in a state of advanced autolysis.* Rarely, fetal death coincides with the time of normal delivery, and the fetus is discharged well preserved. Vaccination of pregnant cattle with a live vaccine will replicate natural disease.

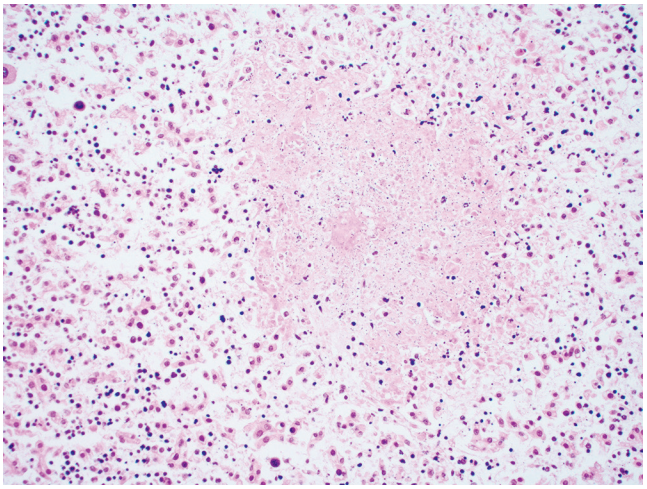

Figure 4-91 Caprine herpesvirus 1 infection. Focal hepatic necrosis is present in an aborted kid.

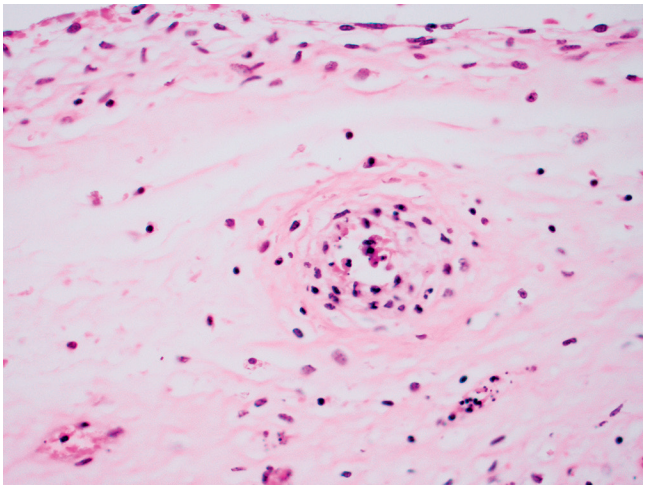

Figure 4-92 Bovine herpesvirus 1 infection. Necrotizing vasculitis in the placenta from an aborted bovine fetus.

Gross lesions in the fetus are usually absent or masked by autolysis, but, when visible, include white to tan, 1-3 mm diameter foci of necrosis under the liver capsule and, more rarely, on the surface of the lung. Perivascular renal hemorrhage may be present in addition to focal hemorrhages at the corticomedullary junction in the kidney.

On microscopic examination, foci of necrosis with minimal cellular infiltrate can be seen in many tissues, including liver, adrenal, kidney, intestine, lymph node, lung, and spleen. Identical lesions are also found in caprine herpes (CpHV-1) abortions (Fig. 4-91). As autolysis advances, *inclusion bodies* are difficult to distinguish but are most profitably sought in the adrenal within the more normal cells surrounding a focus of necrosis. Necrotizing vasculitis is consistently present in the small vessels of placental villi (Fig. 4-92).

To confirm the *diagnosis*, the immunoperoxidase test using monoclonal antibodies for BoHV-1 antigen is specific, highly sensitive, can be done on fixed tissue sections, and requires no

tissue cultures. Results are also available quickly. Fluorescent antibody techniques on frozen tissue sections of kidney, liver, or placenta and tissue cultures of these samples can be used for detection of virus. Viral DNA may also be detected by PCR. To identify antibodies to BoHV-1, the ELISA has a higher validity in differentiating a negative from a positive sample than the serum neutralization test. With BoHV-1, cows abort such a long time after initial infection that most animals have very low titers to the virus at that time. On an individual basis, therefore, antibody titers are of little value in diagnosis. Also, as the fetus dies quickly, there is usually no evidence of fetal antibody production.

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Bovine herpesvirus 4 (cytomegalovirus) infection of the pregnant uterus in cattle

Species *Bovine herpesvirus 4* (BoHV-4, bovine cytomegalovirus, Movar virus) is in the family *Herpesviridae*, subfamily *Gammaherpesvirinae* (cell-associated herpesviruses), genus *Rhadinovirus*. It has worldwide distribution in cattle and has been associated with a wide variety of clinical conditions, including pneumonia, enteritis, metritis, mammillitis, and the disease syndrome "epivag." Epivag is characterized by vaginitis, salpingitis, and oophoritis, or epididymitis.

BoHV-4 is considered an important cause of abortion in cattle; however, experimental evidence confirming a causal relationship is incomplete. The virus is less virulent than BoHV-1, and concurrent infections of BoHV-4 with other pathogens are frequent (up to 75%). It has been suggested that the virus is immunosuppressive and thereby intensifies the effects of other agents. BoHV-4 has been isolated along with bovine viral diarrhea virus and border disease virus from abortions, and with *Mycobacterium paratuberculosis* from animals with Johne's disease.

As the virus is carried within mononuclear cells in blood, grows on mucosal surfaces, and is also considered a poor immunogen, it elicits a low level of neutralizing antibody that is also of low avidity. It is transmitted from cow to cow in oral and nasal secretions and, during viremia, is carried to the placenta, where it multiplies and invades the fetus. The virus may remain latent in mononuclear blood cells and in the trigeminal nerve ganglion. Recrudescence can be induced with dexamethasone. Herds tested for BoHV-4 antibodies vary greatly in the proportion of cattle with antibody to the virus.

Mummification and birth of weak pigs have also been reported in association with BoHV-4 infections in sows. Fetal pigs are capable of developing antibody to the virus by 74 days of gestation. Recrudescence induced by dexamethasone has been reported in sheep and pigs, with spread of virus to other animals.

Descriptions of the lesions seen in the aborted bovine fetus are limited. In one report, no gross lesions were observed in the fetus, but on histologic examination, there was thickening of alveolar septa in the lungs, where many large intranuclear inclusions were observed in large alveolar cells. Similar inclusions were also seen in cells of bile duct epithelium, myocardium, spleen, and epithelium of renal tubules. The virus was not isolated from this animal, but the inclusions seen on light and electron microscopy were typical of cytomegaloviruses.

To confirm the *diagnosis*, fetal lung, liver, and kidney should be cultured for the virus, and fluorescent antibody techniques may also be used to demonstrate its presence. A nested duplex PCR assay has been used to detect BoHV-1 and BoHV-4 in lymph nodes and peripheral blood leukocytes. The virus has been demonstrated in aborted fetuses by in situ hybridization. Neutralization tests are usually unsatisfactory for the detection of antibody as the virus elicits a low antibody response. The ELISA and an IIF test have been found to be more sensitive and may have value on a herd basis. Antibodies may also be detected in cavity fluid of aborted fetuses and are evidence at least of fetal infection.

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Equid herpesvirus 1 abortion in horses

Species *Equid herpesvirus 1* (EHV-1, equine abortion virus) is in the family *Herpesviridae*, subfamily *Alphaherpesvirinae*, genus *Varicellovirus*. It is widespread throughout the world and causes respiratory, neurologic, and generalized neonatal disease, as well as abortion. The virus can be separated into 2 distinct subtypes (1 and 2) on the basis of restriction endonuclease cleavage of the viral DNA. Both subtypes cause respiratory disease, abortion, and neonatal disease. Subtype 1, however, causes a more severe (but less frequently encountered) respiratory disease than subtype 2 and is the more frequent isolate from aborted foals and neonatal disease. Also, it is probably the only type causing neurologic disease. Subtype 2 is commonly isolated from mild upper respiratory disease in horses and less so from abortion. Respiratory disease caused by EHV-1 is common in some areas, whereas abortion is rare; this may be explained to some extent by the presence of a subtype less likely to produce abortion but capable of producing respiratory disease.

EHV-1 is very widespread, and first exposure usually occurs before the foal is 1 year of age. Mild to severe upper respiratory disease is produced, and secondary bacterial infection, frequently with *Streptococcus zooepidemicus* or other streptococci, is common. Because the virus is so widespread and contact so likely at an early age, abortion usually occurs in an animal that has had previous experience with the virus. As with herpesviruses generally, the level of immunity is low, and the virus is transported in leukocytes through the bloodstream to the placenta and hence the fetus. Death of the fetus does not occur until the onset of the usually prompt and uncomplicated abortion. The dam shows no premonitory signs, and the fetus is aborted in a fresh state. The time from exposure, whether by recrudescence of latent virus or reinfection, until abortion varies from 9 days to several months. Respiratory disease in the mare is usually not observed following the infection that results in abortion. It is reported that

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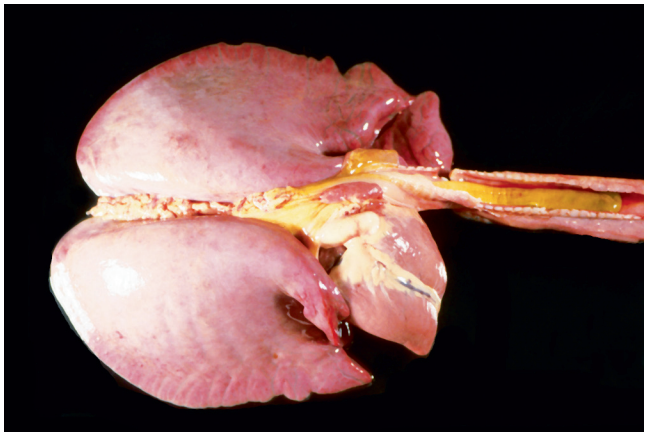

Figure 4-93 Equid herpesvirus 1 pulmonary lesions in an aborted foal. Note the interlobular pulmonary edema and the fibrin cast in the trachea.

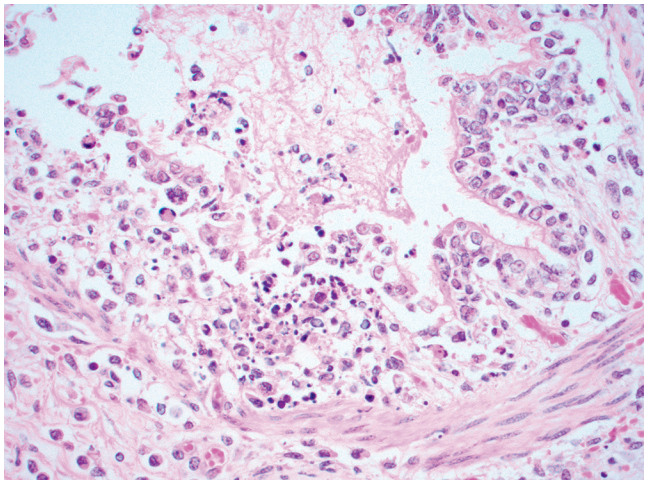

Figure 4-94 Equid herpesvirus 1 abortion. Acute necrotizing bronchiolitis in an aborted equine fetus.

95% of the abortions caused by EHV-1 occur in the last 3 months of pregnancy, and naturally acquired infection has not been observed to produce abortion before 5 months of gestation. Exactly where the virus is, and in what state, during the protracted incubation period has not been determined. Abortion follows endothelial necrosis of the vessels of the endometrium of the pregnant uterus.

The aborted fetuses may show *characteristic and diagnostic lesions* that are variable in their development and may be modest. Edema of the subcutis and fascia and accumulated amber fluid in the body cavities are common in aborted fetuses. There may be slight general icteric discoloration and meconium staining of the eponychia and amnion. The most consistent gross lesion is *severe pulmonary edema*. The lungs are heavy and rubbery, show the impressions of the ribs, and exhibit a pitting response to pressure (Fig. 4-93). There is also edema of the interlobular septa. Their color may be darker or lighter than normal, and tan-to-white foci of necrosis (2-4 mm in diameter) and petechial hemorrhages may be visible on the surface. The bronchial and bronchiolar epithelia are usually necrotic and frequently contain intranuclear inclusion bodies (Fig. 4-94). Casts of fibrin containing sloughed cells are present

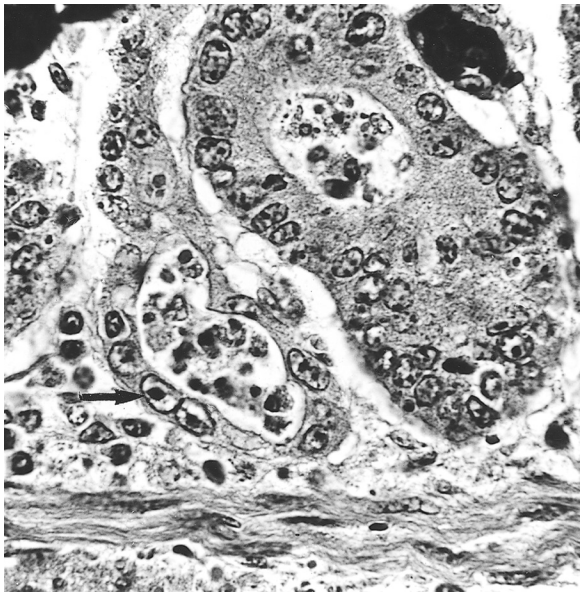

Figure 4-95 Equid herpesvirus 1 infection. Intranuclear inclusions (arrow) and necrosis in intestinal crypts in a 2-day-old foal.

and can occasionally be seen grossly in the bronchi and, rarely, in the trachea (see Fig. 4-93). Beneath the capsule of the liver there are, in about 50% of aborted fetuses, gray-to-white foci of necrosis varying in size from minute up to 5.0 mm in diameter. Such foci may be few or numerous. Petechial or ecchymotic hemorrhages may occur anywhere, but chiefly in the upper respiratory mucosae. Occasionally, there is hemorrhagic necrosis of the renal cortices.

Histologically, the pulmonary interlobular septa are edematous and infiltrated with mononuclear inflammatory cells. The edema and spotty necrosis and hemorrhage involve the whole organ uniformly; there is fibrinous alveolar exudation and necrosis of bronchial and alveolar epithelial cells (see Fig. 4-94). *The acidophilic inclusion bodies found in the nuclei of the bronchial and alveolar epithelium are specific.* The foci of hepatic necrosis are not so common as the changes in the lungs: They are often minute and may be missed in a section. Acidophilic inclusion bodies also form in the nuclei of hepatic parenchymal cells, but they are not constant and are never numerous. If present, they can usually be found around the areas of focal necrosis. There is edema of the liver, and leukocytes in the necrotic foci and portal triads are common. Rarely, there is a diffuse hepatitis without focal necrosis. Lesions of fetal stress, including necrosis of germinal centers, occurs in the splenic follicles and other lymphocytic tissues, including thymus. Intranuclear inclusion bodies may be found in the cells in such foci. There are focal hemorrhages in the splenic white pulp. The placenta is normal.

Foals infected with this virus in utero may be born alive at, or near, term. Whether any survive is not known. Many of them die in the first few days with severe interstitial pneumonia and secondary bacteremia. Focal hepatic necroses are, as a rule, not present in these animals; however, focal necrosis of crypt epithelium with hemorrhage in the intestine is sometimes observed (Fig. 4-95).

The *diagnosis* of abortion can be made on observing typical microscopic lesions, including the presence of inclusions. The demonstration of EHV-1 on cell cultures from samples of

lung, liver, spleen, or thymus or by immunohistochemistry on placenta is definitive. Determination of the specific subtype may be accomplished using monoclonal antibodies and PCR-based tests. Serologic examination for antibody is of little use in the diagnosis of abortion because most animals are exposed to the virus at several intervals in their life, and abortion may occur too long after the last exposure, making interpretation impossible, even on the basis of paired samples.

Equid herpesvirus 4 causes a similar disease, but is less common and is sporadic.

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Akabane virus infection of the pregnant uterus

Species *Akabane virus* (AKAV) is in the family *Bunyaviridae*, genus *Orthobunyavirus*, Simbu serogroup. In Australia, the Simbu serogroup includes Akabane, Tinaroo, Peaton, Aino, Douglas, Thimiri, and Facey's Paddock viruses; Akabane and Tinaroo viruses are naturally occurring reassortants. *Akabane disease*, commonly known as *enzootic bovine arthrogryposis and hydranencephaly* (ag/he), occurs in Australia, Japan, Korea, Israel, and Kenya. Evidence of disease in postnatal cattle, sheep, and goats is minimal; only slight leukopenia in goats and mild keratoconjunctivitis in cattle have been reported, plus one report of encephalomyelitis in cows. In contrast, infection of nonimmune pregnant sheep, goats, and cows has resulted in extensive losses in Japan, Israel, and Australia.

AKAV is transmitted by various insects, including the hematophagous midge *Culicoides brevitarsis* in Australia and various species of mosquitoes, including *Aedes vexans*, *Culex tritaeniorhynchus*, and *C. pipiens*. Losses are seasonal and confined geographically by the range of the insect. Animals beyond this range constitute a naïve population, and if for some reason infected insects invade this area, extensive losses may occur.

Infections by AKAV before 30 days of gestation can produce multiple congenital abnormalities and can result in normal or malformed fetuses that may be aborted; mummified; or delivered alive or stillborn, early, on due date, or late. Many are delivered with difficulty because of the congenital malformations. Anomalies observed include microcephaly, porencephaly, hydranencephaly, hydromyelia, arthrogryposis, kyphosis, lordosis, scoliosis, brachygnathia, and muscle atrophy. Additional abnormalities reported in lambs include spina bifida, cyclops, Arnold-Chiari syndrome, and anal atresia. When the fetus is infected between 41 and 50 days of gestation, thick perivascular cuffing of macrophages, lymphocytes, and plasma cells is observed, coupled with very prominent glial nodules. The large variety of CNS malformations caused by AKAV is attributed to differences in the fetal age at infection, the vulnerability of neurons to direct injury, and the nature of tissue response to such injury. Affected lambs are reported to weigh significantly less than normal lambs, even though gestation length overall is slightly extended. Infection of the fetus after 90 days of gestation is less severe; however, there are inflammatory lesions without malformation in the brainstem.

AKAV is carried by a midge or mosquito from a viremic to a susceptible animal, and at no time does the dam show clinical signs of infection. Viremia lasts for about 4 days, during which time the virus invades the placenta, replicates in trophoblastic cells, and finally crosses the placenta and infects the fetus. If AKAV is inoculated immediately after the integration of the placental cotyledon with the maternal caruncle, viral invasion of the chorioallantoic membrane occurs rapidly. Lesions in the brain and possibly in muscle are probably due to the direct cytopathic effect of the virus on developing neurons. A regular sequence of events follows the primary insult of polioencephalitis; focal necrosis in the brain may progress to malacia and, depending on the extent of the injury and time, to porencephaly and hydranencephaly. Neuronal death in brain and ventral spinal cord motor neurons is followed by denervation atrophy of muscle and arthrogryposis.

Autopsy findings in affected species are similar. *In the brain, gross lesions have a broad range, including microcephaly, porencephaly, and hydranencephaly.* Cystic cavities vary from being visible only microscopically to having almost complete loss of parenchyma. The cerebellum may be normal to markedly atrophic. Microscopically, there is encephalitis with macrophages, plasma cells, and lymphocytes within areas of necrosis and around vessels. Lesions are sometimes most prominent in the brainstem and spinal cord. The cerebral cortex may have multifocal areas of necrosis with gliosis and perivascular mononuclear cells. In the cerebellum, there may be loss and malpositioning of Purkinje cells and reduced size of molecular and granular layers.

Lesions induced in the *brain* early in gestation, because of extensive destruction of cells, may proceed to hydranencephaly with subsequent arthrogryposis. Lesions produced late in the first trimester usually consist of multifocal encephalitis and proceed only to porencephaly. Infections late in the second trimester may produce lesions consisting of multifocal necrosis and gliosis with less development of cavities.

Lesions in the *spinal cord* can be bilateral or unilateral and consist of mineralization of meninges, atrophy of the cord, and hydromyelia. Necrosis and depletion of ventral horn motor neurons may proceed to cavitation. Light or heavy accumulations of perivascular lymphocytes may be present throughout the cord with decreased numbers of axons and hypomyelination in the lateral and ventral tracts.

Joints of the front legs are more commonly affected than those of the hind, and both may be fixed in either flexion or extension. There is slight to marked atrophy of skeletal muscle groups in affected limbs. The muscles are edematous, pale, and flabby to firm. On microscopic examination of muscle, sarcoplasmic nuclei are either swollen or pyknotic. Loss of striations, hypereosinophilia, and empty sarcolemmic cylinders may be present. Replacement of muscle by connective tissue and fat occurs finally. Well-preserved fibers may be present adjacent to areas of complete loss of muscle mass. Polymyositis, from which the virus can be isolated, has been reported in some infected fetuses.

The *diagnosis* of AKAV infection may be made by demonstrating virus or antibody in affected animals. Virus may be recovered from various fetal tissues and fluids, including brain, spinal cord, cerebral fluid, skeletal muscle, chorioallantois, and amnion. It may be grown on hamster lung cells

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and can cause reduced litter size, transplacental infection of the fetus, and birth of live, weak young. AKAV can also grow in the brain of intracranially inoculated suckling mice. As the fetus is capable of producing antibody from an early stage of gestation, inferred evidence of the cause of malformation and abortion may be determined by finding specific antibody to AKAV in fetal cavity fluids, including fluids from stored tissues or precolostral blood samples from affected newborn animals.

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Schmallenberg virus infection of cattle, sheep, and goats

During the summer and fall of 2011, cattle in northwestern Germany and in the Netherlands became ill with a febrile condition that was associated with drop in milk production, hypothermia, and watery diarrhea. Later in the fall, some cattle in these and other European countries aborted or gave birth to calves with neurologic disease and skeletal anomalies. Aborted fetuses had abnormally developed heads, spines, and limbs. Laboratorians were able to identify a novel orthobunyavirus that had the molecular structure of the Shamonda viruses. This virus was named the "Schmallenberg virus" (SBV) after a town in Germany near which a farm had a sick cow from which this virus was isolated. SBV is in the family *Bunyaviridae*, genus *Orthobunyavirus*, Simbu serogroup, and is a reassortant of Sathuperi and Shamonda viruses. An RT-qPCR test was developed which was subsequently used to identify the agent, conduct epidemiologic studies, and to examine the pathogenesis of the disease. Transmission of SBV is associated with exposure to biting midges, mosquitoes, and sandflies. SBV can be transmitted via the semen of infected bulls.

Schmallenberg virus infection of pregnant sheep also results in similar teratogenic lesions, and significant losses have been reported in Europe. Experimental inoculation of pregnant sheep causes subclinical infections in most animals. Infection was established in only about half the ewes inoculated. Affected fetuses and offspring of infected ewes or cows, have hydranencephaly, scoliosis, and/or arthrogryposis—a spectrum of lesions similar to those of other Simbu group bunyaviruses, such as Akabane virus.

Amniotic fluid collected from fetal stomachs, and samples of fetal cerebrum and fetal spinal cords have proven to be good samples from which SBV nucleic acids can be identified by RT-qPCR. ELISAs have been developed for antibody detection, and serologic surveys have been conducted.

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Aino virus infection in cattle

Aino virus (AINOV, syn. *Shuni virus*) is closely related to Akabane virus, and is similarly in the family *Bunyaviridae*, genus *Orthobunyavirus*, Simbu serogroup. As with AKAV, AINOV is mainly transmitted by the midge *Culicoides brevitarsis* but has also been isolated from mosquitoes, including *Culex tritaeniorhynchus* and a mixture of *C. pipiens* and *C. pseudovishnui*. The range of the organism, as determined by serum antibody titers, extends along the northern and eastern coasts of Australia (from just within Western Australia to central New South Wales) and is well within the range of *C. brevitarsis*. The virus is also present in Japan. Using antibody titers as evidence of previous infection, fewer species overall and fewer animals within affected species appear to be infected with AINOV compared to AKAV.

Cattle that are infected do not have clinical signs; however, insofar as precolostral antibodies to the organism are found in deformed newborns, *the organism is capable of crossing the bovine placenta and causing abortion*. Lesions consist of placental edema and necrosis, renal tubular necrosis with interstitial lymphoplasmacytic infiltrates, nonsuppurative meningitis, and interstitial pneumonia with mononuclear cells and some neutrophils. Experiments conducted with sheep indicate that, although the virus can infect the fetus after direct inoculation, it does not cross the placenta after inoculation into the pregnant ewe.

Lesions induced in young calves following intracerebral inoculation of AINOV and AKAV are reported to be different. With AINOV, nonsuppurative encephalomyelitis developed first in the cerebrum adjacent to the ventricles and spread to the medulla and the brainstem, but remained mild in the spinal cord. Using animals inoculated with AKAV as controls, nonsuppurative encephalomyelitis was produced in the brainstem and spinal cord only. Intravenous inoculation of virus into susceptible pregnant cows at various stages of gestation may give a more valuable comparison.

Diagnosis is based on immunohistochemistry or fluorescent antibody testing on frozen sections or smears of homogenates of fetal kidney, lung, and liver. Prospective serology on cows at pregnancy diagnosis and at abortion may be useful. Aino and Akabane bunyaviruses can be differentiated by nested PCR.

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Cache Valley virus infection of sheep

Cache Valley virus (CVV, syn. *Bunyamwera virus*) is in the family *Bunyaviridae*, genus *Orthobunyavirus*, is a serotype of species *Bunyamwera virus*, and is widely distributed in North

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America. Serologic data indicate it infects pigs, horses, raccoons, foxes, and domesticated and wild ruminants, including cattle, sheep, caribou, and deer, as well as humans. It is believed to be spread by insects and has been isolated from a variety of mosquitoes and midges, including *Culiseta inornata, Psorophora columbiae, Aedes sollicitans, Ae. taeniorhynchus, Anopheles grabhamii, An. crucians, An. uadrimaculatus,* and *Culicoides* spp.

There is considerable evidence supporting CVV as the cause of a severe epizootic in a flock of sheep in Texas, where, of 360 lambs delivered, 92 were mummified, stillborn, or born with congenital anomalies. Sixty-nine lambs had *arthrogryposis and hydranencephaly.* All of the ewes bearing affected lambs had neutralizing antibody to CVV, and affected lambs had precolostral antibody to the virus. In the months before pregnancy, 5% of ewes had antibody to the virus and after pregnancy, 63.4% were positive. Following the outbreak, a sentinel sheep was placed in the area, and CVV was eventually recovered from a blood sample.

Examination of 15 lambs stillborn at term during the epizootic revealed that CNS and muscle and joint lesions were frequently present in the same animal, and lesions between lambs mainly differed in severity. Gross lesions in limb joints consisted of articular rigidity with articulations held in the flexed position. The limbs appeared long for the size of the body. In addition, kyphosis, scoliosis, and torticollis were frequently observed. Muscles associated with the affected limbs and spine were described as shrunken, pale, and firm. Lesions in the brain consisted of *hydrocephalus, porencephaly, and hydranencephaly*. The cerebrum, cerebellum, and spinal cord were often markedly reduced in size, and the cerebellum was occasionally mildly lissencephalic.

Microscopic lesions were observed in skeletal muscles, brain, and 2 placentas. In affected muscle, fibers were small, lacked striations, and nuclei were sparse. Fibers were separated by loose connective tissue and adipocytes; rarely, a few neutrophils and mononuclear cells were present. Histologic examination of the brain confirmed gross findings, and cavities not visible grossly were occasionally observed. In regions of marked hydranencephaly, the cerebral cortex was reduced to a few layers of neurons within the meninges. Entire lobes of the cerebellum were often absent, but adjacent lobes were often near normal, with only minor loss of cells in the molecular layer. The density of axons in the spinal cord was markedly reduced, especially in dorsal tracts, and whereas ventral horn neurons were only slightly reduced in the cranial cord, hardly any remained in caudal portions. Inflammatory cells were scant in lesions except in 2 of the 5 placentas examined, wherein a mild perivascular neutrophil infiltrate was evident. Inoculation of 36 ovine fetuses in utero with CVV has added further credibility to the role of CVV as the cause of the epizootic. Following in utero inoculation of the virus, several of the fetuses developed arthrogryposis and hydranencephaly; some were mummified, others died and were absorbed, and a few had oligohydramnios. Lambs infected at 27-35 days of gestation had the greatest mortality rate, whereas lambs infected between days 36 and 45 of gestation had more congenital anomalies. Virus was recovered from fetuses before 69 days but not after 76, regardless of when they were infected, and this was coincident with the development of neutralizing antibody in the fetus. The virus was recovered from allantoic fluid in 11 of 17 fetuses euthanized at <76 days of gestation.

The *diagnosis* of CVV infections resulting in fetal loss and congenital anomalies in lambs is most easily accomplished by examining precolostral serum or cavity fluids of lambs with arthrogryposis and hydranencephaly for antibodies to CVV. CVV antigen may be detected in aborted fetuses by immunohistochemistry, in situ hybridization, or PCR. Differential diagnoses should include infection by Akabane virus, as the lesions are remarkably similar.

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Rift Valley fever virus infection of cattle and sheep

Species *Rift Valley fever virus* (RVFV) is in the family *Bunyaviridae* and is the type species of genus *Phlebovirus* (Zinga virus is a strain of RVFV). RVFV infects many domestic, wild, and laboratory animals and humans. It occurs in at least 18 countries over a 6,700-km north-south range in Africa, plus Saudi Arabia. *Infection by RVFV causes a disease characterized by rapid onset and high mortality in lambs, low mortality in adult sheep and calves, and even lower or no mortality in cattle. There is frequently a high rate of abortion, approaching 100% in sheep and cattle.* People may also become infected, often from contact with body fluids or organs of infected animals, and develop influenza-like illness; mortality is generally low, but morbidity is high and the degree of debility often extreme. *Hepatic necrosis is the prominent lesion and occurs in all affected species and age groups, including the fetus.* Host resistance to fatal hepatic disease has been shown in laboratory animals to be inherited as a simple Mendelian dominant gene. Susceptibility is age and sex related; the young are more susceptible, and males less resistant than castrates and females. Although 2/3 of sheep are genetically resistant to fulminant hepatic disease, *sheep are regarded as the most susceptible domestic animal.*

RVFV is transmitted primarily by mosquitoes, and *Culex pipiens* is a major vector in parts of Africa. Generalized infection in the mosquito is necessary before transmission occurs effectively, and movement of virus from the insect gut is facilitated by the presence of microfilariae in ingested blood. *Aedes* spp. are capable of transovarial transmission. *Aedes albopictus,* a strain of mosquito in the United States, can transmit the virus, and movement of animals either incubating infection or in viremia raises the possibility of international spread of the virus. Biting midges and ticks are also implicated in transmission. The virus probably does not spread from animal to animal in the absence of insects; however, experimentally the virus can be transmitted by just about every route, including aerosols or application into nasal passages, to scarified skin, or on the conjunctiva. *Aborted fetuses and membranes have a very high titer of virus.* Epizootics often follow a period of heavy rain, particularly after drought, and outbreaks may be separated by periods of 10-15 years without a case.

In mature sheep, cattle, or camels, the disease is manifest in *peracute, acute, subacute, chronic, and convalescent forms.* In sheep, the subacute form, in which the animal is febrile for 24-96 hours, is common, and up to 100% of pregnant ewes can abort during this phase or when convalescing. In cattle,

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the peracute form is rare, and abortions occur during the acute or convalescent period in 10-85% of pregnancies. Camels seroconvert and may amplify the virus locally; some may die. Deaths in cows are uncommon. Infertility occurs in both sheep and cattle and may follow retention of fetal membranes and endometritis. Signs are unpredictable in goats. They tend to be less susceptible than sheep, but if infected can abort.

During viremia, the organism is carried to the placenta and fetus, where *the liver appears to be the primary site of multiplication*. Hepatocyte destruction is probably related to the repeated cycles of virus growth and release of virus from infected cells.

Lesions are much the same in cattle or sheep fetuses and, except for one report where experimental inoculation of pregnant ewes with an attenuated RVFV resulted in hydranencephaly and arthrogryposis, *infection by RVFV usually results in death of the fetus in utero*. This occurs at any gestational age and, as it occurs rapidly, the fetus is aborted in an autolysed state. On gross examination, the liver is swollen and discolored orange to brown and occasionally to dark red. Microscopically, the most consistent lesion seen in almost 100% of fetuses examined is *multifocal to massive hepatic necrosis*. A few degenerating neutrophils may be present in these lesions. *Acidophilic intranuclear inclusions* of various shapes (fusiform, round, oval) are frequently present in hepatic cells of ovine fetuses and are helpful in the diagnosis. These are seen less often in bovine fetuses and may be difficult to find when autolysis is advanced. Mineralization of individual or clusters of hepatocytes is also common. Cholestasis may be evident in canaliculi. Other lesions reported include degeneration of lymphocytes in spleen and lymph nodes and degenerative lesions in heart muscle and renal tubules.

The hepatic lesion is considered diagnostic; however, in areas where the condition is not endemic, definitive *diagnosis* depends on identification of the virus. It can be detected early in infection in blood or serum by RT-PCR. It is abundant in fetal tissues and placenta and may be cultured or detected using the indirect fluorescent antibody technique on frozen sections of liver or by immunohistochemistry. Differential diagnoses in sheep include infections by bluetongue virus and Wesselsbron virus.

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Circovirus postweaning multisystemic wasting syndrome

Species *Porcine circovirus 2* (PCV-2), family *Circoviridae*, genus *Circovirus*, is associated with the postweaning multisystemic wasting syndrome (PMWS), and appears to act with other cofactors that cause stimulation of the pig's immune system. Porcine circovirus–associated disease (PDVAD) is associated with wasting and mortality in young pigs and can result in lesions of the respiratory, enteric, reproductive, and renal systems. PMWS was first reported in western Canada in 1991 and has since been found in the United States and in many European and Asian countries. *The disease primarily affects pigs 5-18 weeks of age and is characterized by failure to grow and eventual emaciation*. Affected pigs may have gastric ulcers in addition to a wide variety of lesions, including pneumonia and diarrhea.

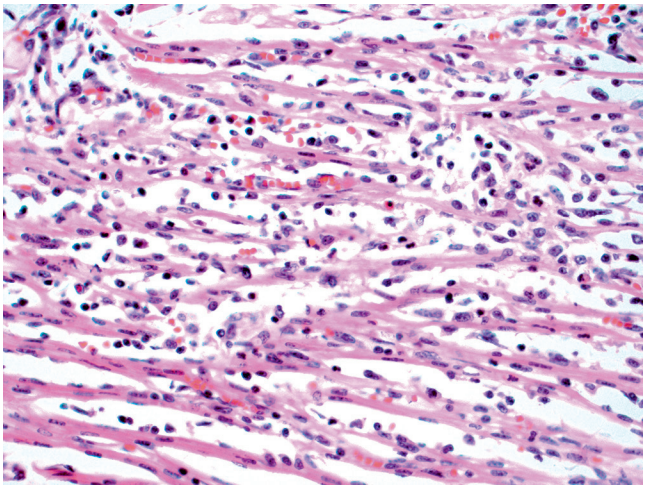

Figure 4-96 Fetal myocarditis in an aborted piglet, caused by **porcine circovirus 2.**

A *reproductive failure syndrome* has been observed in several herds and is characterized by late-term abortions of mummified, macerated, or autolysed piglets, and piglets born weak or stillborn. Lesions in these piglets included enlargement of the liver; congestive heart failure, with excess fluid in the thorax; and abdominal cavities. The heart may be enlarged and irregular in shape, and on histologic examination, extensive areas of myocardial degeneration, edema, and necrosis with mild fibrosis and diffuse infiltrates of lymphocytes and macrophages may be observed (Fig. 4-96). The virus can be demonstrated by immunohistochemistry in heart muscle cells. No other viruses were detected in this investigation. Stress factors and co-infections with other porcine viruses were not detected; however, environmental, husbandry, and genetic factors may be important in the progression of clinical PMWS.

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MISCELLANEOUS LESIONS OF THE POSTPARTUM UTERUS

Endometrial cysts, *adjacent to caruncles, develop in some cows and ewes during the process of uterine involution*. Adhesion of the caruncular stalk to adjacent glandular tissue causes blockage of the underlying glands. The retention cysts enlarge progressively with age, and care must be taken to differentiate them from estrogen-linked cystic hyperplasia.

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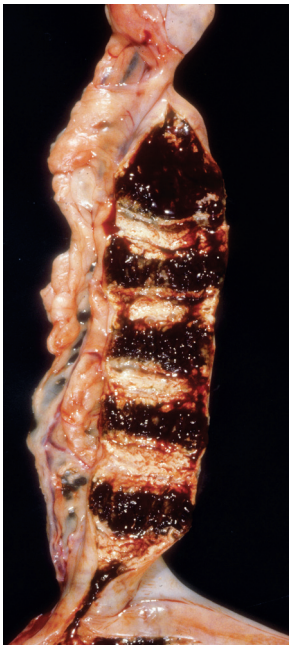

Figure 4-97 Subinvolution of placental sites (SIPS) in a bitch. Fusiform enlargement of uterine horns at sites of failure of involution.

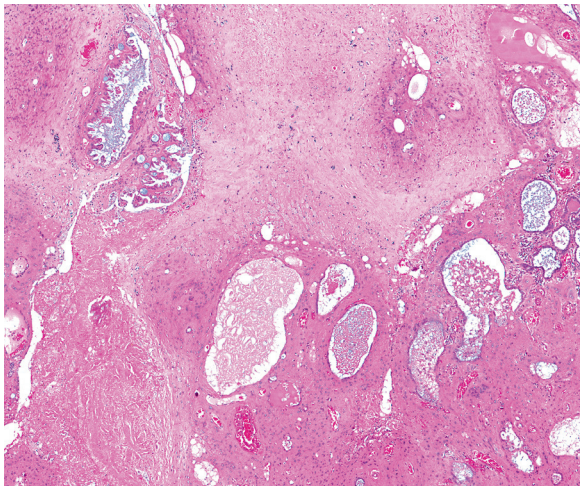

Figure 4-98 Subinvolution of placental sites. Hemorrhage and necrotic debris within the endometrium at placental attachment sites.

Subinvolution of placental sites in the bitch is manifested clinically by a prolonged, blood-tinged vaginal discharge. In the normal bitch, overt uterine bleeding usually ceases within 7-10 days following whelping, but the affected bitch may bleed for several weeks or months. In some cases, the blood loss causes severe anemia and occasionally death. *Ellipsoidal enlargements*, located in the areas of previous placental attachment, are evident in the uterine cornua (Fig. 4-97). The endometrium in the affected areas is hemorrhagic, irregularly thickened, and gray to brown. The endometrium between the enlargements is normal in appearance. The surface epithelium is often detached, but when present, it has heavily vacuolated cytoplasm, indicating progestational stimulation. Corpora lutea are invariably present, but progesterone concentrations are low in the few cases in which the determination was made.

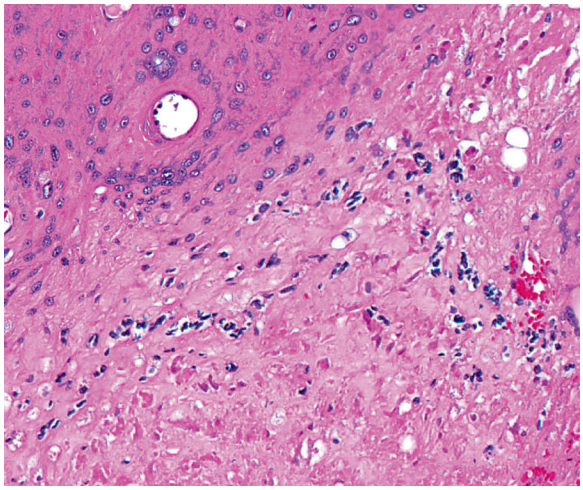

Figure 4-99 Decidual cells, trophoblast cell, and inflammatory cells at a nonhealing placental attachment site in a bitch with **SIPS.**

The uterine mass adjacent to the uterine lumen is composed of an admixture of amorphous eosinophilic debris, fibrin, degenerating placental site tissue, and regenerating endometrium (Fig. 4-98). The deeper tissue contains numerous irregular-shaped cells with large nuclei and abundant vacuolated cytoplasm (Fig. 4-99). They are interpreted as syncytial trophoblasts or alternatively as decidual cells, and are far more numerous than in the normal gravid uterus. Some degree of invasion of the myometrium by these cells is not unusual, and in some cases there is perforation of the serosa, allowing uterine contents to escape into the peritoneal cavity. The condition appears to be more prevalent in young bitches, but *the cause has not been established*.

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PATHOLOGY OF THE CERVIX, VAGINA, AND VULVA

PATHOLOGY OF THE CERVIX

Cysts of the cervix occur in cows, and probably all are *retention cysts* formed by fusion of the rugae. Loss of the original epithelium of the rugae is first necessary and may be incidental to the lacerations of parturition, artificial insemination, or inflammation. The cysts are usually small and not significant. Larger ones may cause partial occlusion of the cervical canal, but this is seldom of importance.

Stenosis of the cervix is extraordinary. It is *acquired* rather than congenital and consists of fusion across epithelial surfaces and scarification. It may follow severe laceration or long-standing inflammation.

Cervicitis is not in itself an entity but, instead, is an *extension of endometritis or vaginitis.* The mucus-secreting

Further reading

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epithelium provides good defense against bacterial invasion and, if the epithelium breaks down, it exposes densely arranged connective and muscular tissues, which are not especially sensitive to the actions of bacteria. In the cow, the epithelium lining the external os and the adjacent few rugae is of the simple vaginal type, and this is more susceptible to irritation than the mucus-secreting surface cranial to it. Most inflammations then are superficial, but there are some exceptions, such as necrobacillosis. The circumstances in which cervicitis occurs have been mentioned in the section on pathology of the uterus or will be mentioned under pathology of the vagina and vulva, later. The usual form of bovine *simple cervicitis* is seen as a swelling of the caudal annular rugae, which are edematous and hyperemic. They soon protrude through the external os into the vagina, and thin mucopurulent exudate accumulates between the folds and collects in the vagina. Histologically, there is epithelial degeneration and desquamation, and inflammatory cells are predominantly mononuclear. Neutrophils penetrate the epithelium and become mixed with mucus, or in more acute cases, there may be frank suppuration. Cervicitis is not usually more severe than this, although in older cows the prolapse and swelling of the rings may be grossly obvious. Although degrees of prolapse result from inflammation, they may also predispose to it. Slight but progressive degrees of eversion of the cervical rings occur with succeeding pregnancies to expose portions of the cervical mucosa to the contaminated environment of the vagina. *Chronic cervicitis* may lead in time to enlargement of the cervix with some stenosis, but enlargement is not a criterion of inflammatory sclerosis. Discrete *cervical abscesses* or *suppurative fistulous tracts* occasionally result from accidental injury acquired during uterine irrigation or artificial insemination.

PATHOLOGY OF THE VAGINA AND VULVA

Cysts in the vagina or vulva are not important in themselves, but they do have some diagnostic significance. They occur as *cystic dilations in Gartner's ducts and Bartholin's glands* in cattle. Gartner's ducts are remnants of the embryonic mesonephric (Wolffian) ducts, which lie, one on each side of the floor of the vagina, beneath the epithelium. They are invariably present to some degree in cattle, although when normal, they are only detectable microscopically as discontinuous ducts lined by simple epithelium and found in the cranial vagina and disappearing caudally. They become cystic in cows poisoned with highly chlorinated naphthalenes, in cows with ovarian follicular cysts, and occasionally following acute vaginitis. With lesser degrees of dilation, the cysts are more readily palpable than visible, but in well-developed instances, they are clearly visible through the elevated and thinned vaginal wall (Fig. 4-100). The cysts may be isolated or they may have a string-of-beads disposition, or the whole duct may be present, dilated to 1-2 cm and tortuous.

Cysts of Bartholin's glands occur under the same stimuli, but chiefly as a consequence of inflammation. The glands lie one on each side of the floor of the vestibule and normally are about 3 × 1.5 cm in dimension. They become visible when enlarged, especially if the vestibular mucosa is slightly everted. These glands are very sensitive to estrogens, responding with the elaboration of thin mucus and hyperplasia of the ductal epithelium. Gross exaggeration of responses in hyperestrogenism accounts for the cystic development. Large retention cysts follow inflammatory stricture of the short excretory ducts. Abscessation may follow localization of infection in the cysts.

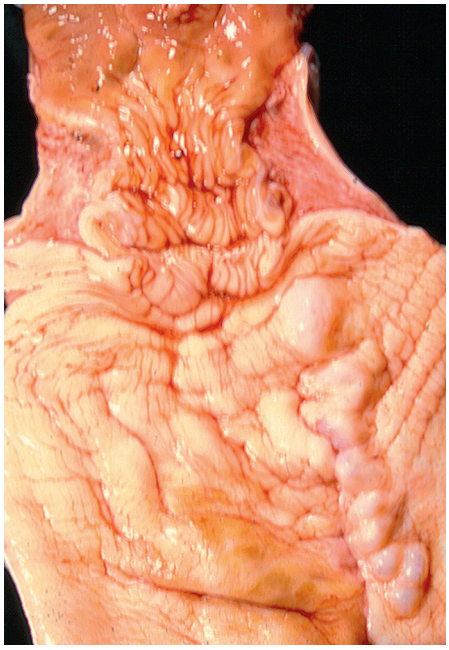

Figure 4-100 Cystic segment of a mesonephric duct in the vagina of a cow (cystic Gartner's duct). These cysts are common in cattle and are found in the ventrolateral area of the floor of the cranial vagina. They are uncommon in other species.

Ruptures of the vagina and vulva are quite frequently acquired as *parturient injuries* and rarely with bull:cow size mismatch at mating. The mucosa alone may be ruptured, or the entire thickness of the vaginal wall. Hemorrhages occur into the vagina or the perivaginal wall from fetal pressure or vascular disruption. *Pelvic fat may herniate into the vagina*; it is often mistaken for a neoplasm. Defects that extend deeper than the mucosa heal with cicatrization, which may, in turn, result in partial stricture. Otherwise, the outcome depends on whether the lacerations become infected and, if so, with what. Occasionally, diffuse cellulitis, abscess, gangrene, or peritonitis are sequelae.

Swelling of the vulva

Vulval swelling is a physiologic response to estrogens. Vulvar enlargement may be marked during the normal estrous period or may be persistent when resulting from exposure to endogenous or exogenous estrogens. Abnormal patterns and concentrations of endogenous estrogens may occur from *cystic follicular disease, estrogen-secreting tumors (granulosa cell tumors), or from exogenous sources such as mycotoxins*. Vulval swelling is most common in the sow and bitch and must be differentiated from vulvitis (bite wounds), physical trauma, or dystocia. Prepubertal pups exposed to creams used in human estrogen therapy will develop vulval development and swelling.

Vulval swelling in **swine** may be due to exposure to mycotoxins. A distinctive syndrome characterized by vulval hyperemia and edema occurs in swine fed moldy grains. The mycotoxin that causes this estrogenic effect is *zearalenone, or F-2*, which is produced by at least 4 different *Fusarium* spp., *F. graminearum* being the most common.

Young gilts are chiefly affected, older animals being more resistant. There is remarkable edematous swelling of the vulva and vagina, which may be severe enough to lead to eversion and prolapse of the mucosa. Prolapse of the vagina may occur in up to 30% of cases, and secondary rectal prolapse occurs in a lesser percentage. The uteri of affected animals may be enlarged by endometrial gland hyperplasia and edema. The ovaries become inactive and atrophic, or polycystic. Gilts may also show mammary gland enlargement with squamous metaplasia of the ductal epithelium. Decreased libido, testicular atrophy, and balanoposthitis have been reported in young boars.

Strains of *Fusarium* vary in toxigenic potency, in regard not only to zearalenone but also to trichothecene toxins, which, if produced in quantity, may broaden the clinical picture to include anorexia and vomition.

Inflammatory diseases of the vagina and vulva

The mucous membrane of the vagina and vulva shares with mucous membranes, in general, a sensitivity to irritants. Although the cranial vagina is of paramesonephric duct origin, the original epithelium is replaced by stratified squamous epithelium from the urogenital sinus. This epithelium proliferates and matures under the influence of estrogen and is then more resistant to infection. This enhanced resistance may be due to mechanical factors in the thickened keratinized epithelium and to local production of lactic acid from the glycogen that is deposited in the epithelium under the influence of estrogens.

Granular vulvitis

Papular eruptions of the vulval mucosa are common in most domestic species but are best known in the *bovine* species by the previous name or as *nodular venereal disease* or *granular vaginitis*. The term vaginitis is a misnomer, as the papules are strictly limited to the vulval mucosa and are not found in the vagina, although in acute cases, there may be an associated nonspecific vaginitis. Vulval granules may be found in any herd, affecting animals of any age, but are usually more prominent in heifers bred naturally. They are much less common in pregnancy and are almost never present about the time of parturition. In severe cases, the papules may be found on all aspects of the vulval mucosa, but they are usually clustered in the ventral commissure about the clitoris as pale or pink elevations a few millimeters in diameter and covered by a normal intact vulval mucosa. When numerous, they are likely also to be more active, larger, often coalescent, congested, and red, with concurrent catarrhal vulvitis and vulval swelling. The overlying epithelium is then easily injured, and bleeding occurs freely from the papules. The resting papules are composed of respectably organized lymphoid follicles (Fig. 4-101).

When the vulval mucosa is irritated, these become red, with small intrafollicular hemorrhages and edema, and hyperplastic, the mitotic frequency being quite high. The same lesions occur on the penis and prepuce of the bull and often persist for many months, as do those of the vulva. These papules have been produced experimentally with *Ureaplasma diversum* and occur commonly in herds with natural *U. diversum* infection but are *not specific for this organism*. The development of subepithelial lymphocytic foci is a characteristic response of mucous membranes to mild persistent or recurrent irritation, and the simplest explanation of granular vulvitis is that *it is the result of mild inflammation of the vulval mucosa*.

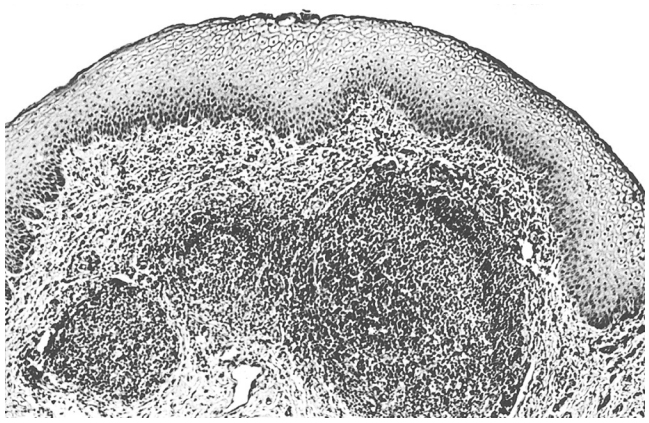

Figure 4-101 Bovine granular vulvitis, with submucosal lymphoid hyperplasia.

Infectious bovine cervicovaginitis and epididymitis ("epivag")

This is a specific infectious disease that has been an important cause of infertility in eastern and southern Africa. A slow-growing *cytomegalovirus of the bovine herpesvirus 4 group* has been recovered from infected animals. In limited studies, these isolates have not reproduced the full spectrum of clinical signs, so there remains some uncertainty about their role in the disease. The slow-growing herpesviral strains are not serologically related to the bovine herpesvirus 1 of infectious bovine rhinotracheitis.

Natural transmission is solely by coitus. Experimental transmission is easy if infective discharges are placed in the vagina or prepuce. After incubating for a few days, diffuse purple inflammatory streaks or patches develop in the cranial vagina, to be followed by the development of copious tenacious creamy discharge in which there are large numbers of neutrophils but few or no organisms. The infection spreads readily from the vagina to the cervix and uterus, also with the production there of the same copious pus. About 25% of infected females are sterile because of the development of *chronic salpingitis with hydrosalpinx and bursal adhesions.* In the bull, there is early but slight palpable enlargement of the deferent duct and epididymis. The disease usually commences in one epididymis but later involves both organs and spreads from the tail of the epididymis to the head of it and ultimately to the testis. The lesion is apparently an *interstitial epididymitis* with the production of excessive peritubular fibrosis and tubular obstruction. Testicular changes are probably secondary to those in the epididymis and to obliterative adhesions in the cavity of the vaginal tunics. Similar productive inflammatory lesions occur in the ampullae and the vesicular glands.

Infectious pustular vulvovaginitis of cattle

We are assuming for this description of infectious pustular vulvovaginitis that it is the disease that for many decades has been variously termed vesicular venereal disease, vesicular vaginitis, coital exanthema, coital vesicular exanthema, and Blaschenausschlag. The assumption is probably valid, but proof is lacking and may no longer be obtainable. Infectious pustular vulvovaginitis is caused by the same herpesvirus (bovine herpesvirus 1) that causes infectious bovine

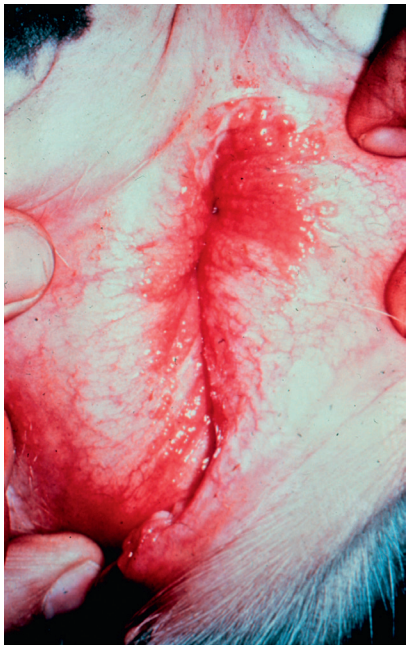

Figure 4-102 Necrotizing vulvitis caused by bovine herpesvirus 1.

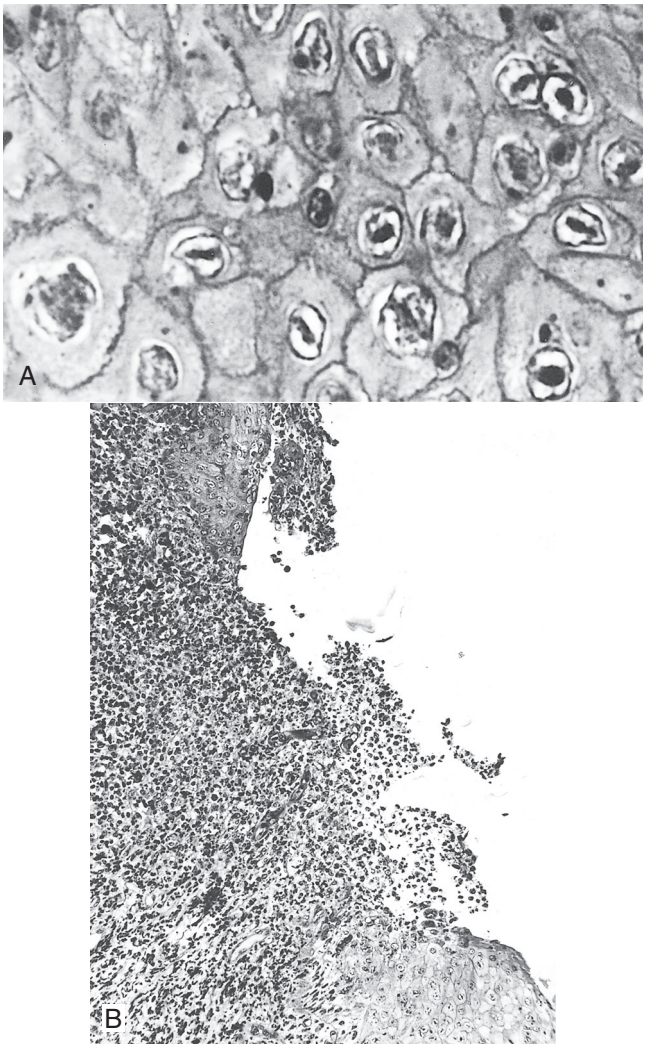

Figure 4-103 A. Intranuclear inclusion bodies in vaginal epithelial cells in bovine herpesvirus 1 infection that has caused necrosis and ulceration of the vaginal wall (**B**).

rhinotracheitis (see Vol. 2, Respiratory system), or at least is caused by a subtype of that virus that is serologically indistinguishable from it. As a rule, nasal and vaginal infections behave epidemiologically as distinct diseases, although occasionally the syndromes occur together in individual animals. The infection can be transmitted to sheep and goats, producing vaginitis.

Infectious pustular vulvovaginitis is *highly contagious.* It is frequently transmitted by coitus, but it can also be transmitted by other mechanical means and is contagious by close contact. It may involve individual or a few animals in a herd, but frequently spreads rapidly to involve all exposed females in a few days. The disease subsides in about 10 days, leaving immunity that is fragile and transient. Reinfection can occur, but early reinfection produces only mild disease.

The incubation period is 1-3 days but may be as brief as 12 hours. The lesions are restricted to the genital tract, but a viremic phase probably occurs because there is early fever and leukopenia. Initially, the vaginal and vulval mucosa is hyperemic with focal hemorrhages in the lymphocytic follicles of the submucosa. *The severity of the vulvovaginitis increases rapidly, and edema of the vulva with hemorrhage develops* (Fig. 4-102). When the damaged tissues become secondarily infected, *mucopurulent vaginal discharge develops.* The focal lesions replace the hemorrhages over the lymphoid follicles and consist of small (2-3 mm) pock-like foci, slightly elevated, pale, soft, and friable.

The focal lesions, being related to the lymphoid follicles, may be in short linear arrangements. The epithelium in the focal lesions erodes or ulcerates so that in a few days the foci are flat, gray, semitransparent plaques the size of the original lesions.

The virus is epitheliotropic, the initial and most severe alterations occurring in the epithelium of the vagina and vulva. There is ballooning degeneration of the epithelial cells, and at about 24 hours, *intranuclear inclusions* can be found in the epithelium (Fig. 4-103). The inclusions are lightly acidophilic or amphophilic and large; they can be found for 3-4 days, by which time the lesion has reached its zenith and is beginning to resolve. The infected cells undergo necrosis, and epithelial disruption and ulceration occur, accompanied by an intense infiltration by neutrophils (see Fig. 4-103). Vesicles and true pustules do not form. Acute inflammation occurs in the lamina propria, with hyperemia and edema and numerous plasma cells and lymphocytes. Many of the small vessels are occluded by adventitial and endothelial swelling. The lymphocytic follicles are remarkably hyperplastic and edematous, and their outlines are obscured by the infiltrating cells in the lamina propria. Resolution occurs in about 8 days, with hyperplastic lymphoid follicles and slight epithelial thickening as residues.

Although most cows that are served naturally by infected bulls do not appear to experience infertility, susceptible heifers that are inseminated with semen containing virus fail to conceive. Intranuclear inclusions may be found in the epithelial cells of the luminal endometrium within 48 hours but are absent by 72 hours after exposure. The surface epithelium and the underlying connective tissue become necrotic and contain many neutrophils. There is pronounced edema of the lamina

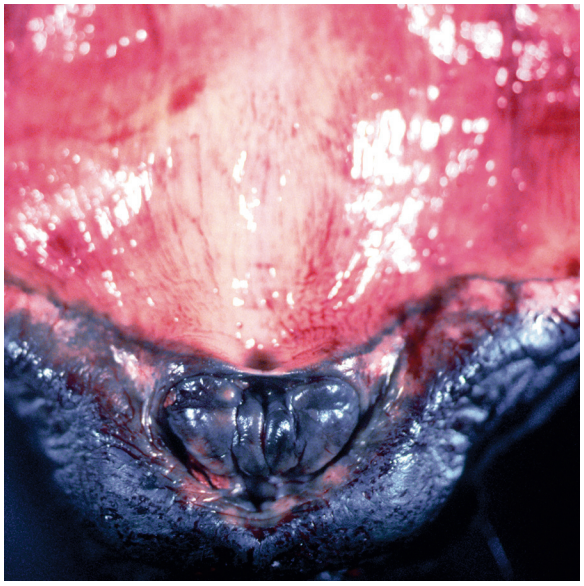

Figure 4-104 Contagious equine metritis (CEM). The clitoris and clitoral fossa of a mare infected with *Taylorella equigenitalis* appear grossly normal, but these are the sites of chronic bacterial infection in mares. Mats of bacteria can be found on the mucosal surface of the sinuses and crypts of the clitoris in CEM.

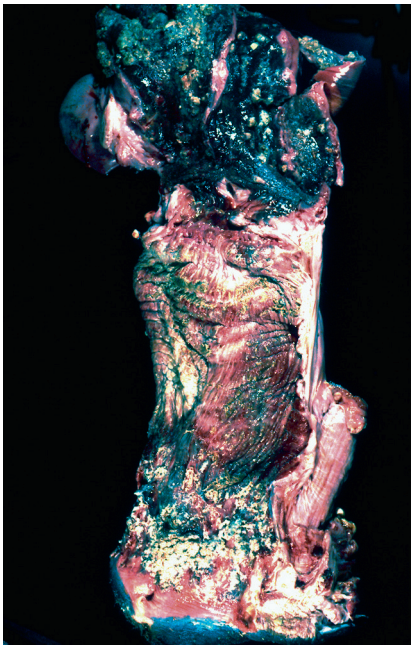

Figure 4-105 Necrotizing vaginitis in a cow, associated with dystocia and associated pressure necrosis.

propria and mononuclear cells; especially, lymphocytes are present. The uterine tubes are involved, but to a less severe degree.

The virus can produce similar lesions on the mucous membrane of the penis of infected bulls. Because recrudescence with viral shedding is a feature of this as well as other herpetic diseases, animals with inapparent infections can also transmit the disease.

A herpesvirus identified as equid herpesvirus 3 produces a comparable genital disease in horses—*equine coital exanthema*. It is discussed with diseases of the penis, in Vol. 3, Male genital system. Caprine herpesvirus infection in goats causes a comparable disease of the vulva also.

Contagious equine metritis

Contagious equine metritis, caused by *Taylorella equigenitalis*, is a sexually transmitted disease of horses. As discussed previously in the section on endometritis, acute infection of mares causes purulent endometritis and cervicitis. In mares that have become carriers, the *Taylorella* organisms reside within the crypts and fossa of and around the clitoris. Although the mare's clitoral area, shown in Figure 4-104, is grossly normal, she had large numbers of *Taylorella* coccobacilli within the clitoral fossa, which was associated with only a mild lymphocytic reaction. An atypical *Taylorella* sp. also capable of causing transient endometritis and endometritis has been isolated from *donkeys* in the United States.

Necrotic vaginitis and vulvitis

Necrotic vaginitis is a deep diphtheritic inflammation of the vaginal mucosa; it occurs in 2 fairly distinct syndromes—either vulvovaginitis or cervicovaginitis. *Necrotic vulvovaginitis* is uncommon, but may involve a number of cows in a herd. It is primarily due to trauma with contamination, often the result of bite wounds by pigs or dogs. *Necrotic cervicovaginitis* (Fig. 4-105) is a complication of parturition and is chiefly observed in ewes and cows as a consequence of dystocia. A number of influences, mainly prolonged pressure necrosis, laceration, and abrasion, usually acting in combination, are responsible for the lesion. Severe necrotic cervicovaginitis is frequently fatal, either with direct extension of the inflammation to the peritoneum or with the uterine complications of prolonged dystocia and fetal emphysema.

Lacerations associated with dystocia commonly occur in mares, sometimes extending to involve the rectum and perineum. *Scarring* of the vulva from previous trauma from delivery is a common finding in large domestic animals (Fig. 4-106). Disfigurement may result from neoplasms that invade the perineal area (Fig. 4-107).

Dourine

Dourine of horses and their relatives is caused by ***Trypanosoma equiperdum*** and is *primarily a venereal disease*. The other trypanosomiases of domestic animals are discussed in Vol. 3, Hematopoietic system, being essentially hemic infections with arthropod vectors. Dourine differs from other trypanosomiases in that the organisms are in the blood only intermittently or in very virulent infections, and transmission is effected not by blood-sucking insects but by contact of infected mucous membranes. *Natural transmission is by coitus*, so the disease is almost exclusively one of stallions and breeding mares. Rare cases occur in unbred animals and in young foals. The disease prevails in the Balkans, much of Africa, Asia, and South America. It has been eradicated from most of Europe and North America.

Strains of *T. equiperdum* vary considerably in their virulence, although not notably in their capacity to infect. Irrespective of the route of experimental infection in horses, the trypanosomes demonstrate some predilection for genital mucosae. Following natural infection, the organisms are frequently numerous in vaginal discharges and the male urethra. There are periods, however, sometimes of several weeks or

Figure 4-106 Disfigured vulva of a sow, resulting from trauma during delivery.

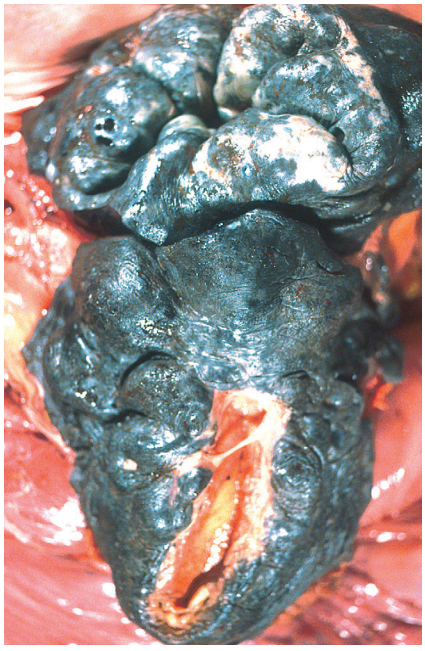

Figure 4-107 Multinodular pigmented masses involving the vulva and perineum of a mare. This **malignant melanoma** had also metastasized to regional lymph nodes. These are common neoplasms that involve the vulva and perineum of the mare. Squamous cell carcinomas also occur in this area.

months, in which the organisms are not present in these sites, so infected animals are not always infective for others at breeding. The organisms penetrate intact mucosa at the site of implantation and proliferate in the submucosal lymph spaces. The incubation period may be several weeks or months, during which time trypanosomes are present in the genital discharges, so there is a possibility that they may proliferate in the lumen of the genital tract for long periods before invading the tissues. From the initial lesions, the organisms are disseminated in the blood to other parts of the body, and edematous swellings occur where they localize. Infection of the blood may be intermittent or continuous, and there may be few organisms or many, depending on the virulence of the strain and the susceptibility of the host. Strains of low virulence may not be demonstrated in the blood by direct smears, but may be demonstrated by centrifuging plasma or directly by transfusing blood in large volumes. In these mild infections, it may also be possible to demonstrate the organisms in genital washes or discharges, but diagnostic problems are simplified by an efficient complement-fixation test.

The signs and course of the infections vary considerably, depending on the virulence of the organism and on the susceptibility of the host. The resistance of the host can be greatly modified by climatic conditions, physical condition, intercurrent disease, and nutritional status, and the South African varieties of the disease can be asymptomatic in animals that are well husbanded. Animals affected with this insidious type of the disease do act as carriers and reservoirs of infection. When the infection is attended by clinical signs, the course is variable; it may be severe and fatal in a few weeks, fatal after a chronic or intermittent course of from several months to 2 years, or clinical recovery may occur.

The signs of dourine can be divided into genital, cutaneous, nervous, and general manifestations, which occur separately or concurrently. The initial signs are usually genital, but may be nervous or cutaneous. The incubation period of the genital signs varies from several days to several months, during which the organisms are present in genital discharges or washings. The external genitalia are swollen and doughy, but characteristically the swellings are neither hot nor painful. The degree of swelling varies considerably from case to case and periodically in an established case, with a tendency to be permanent because of induration in chronic infections. The swelling, when severe, extends to the perineum and ventral abdominal wall. The lymphocytic follicles of the mucosa of the female genitalia become hyperplastic and ulcerate, and at this stage, there may be copious fluid discharge. In males, the swelling involves the head of the penis as well as the prepuce, and may cause prolapse of the urethra and penis. In virulent infections, flat circular ulcers develop in the head of the penis. *Healed ulcers in both males and females often remain as depigmented scars*, and pigmentary atrophy of the skin and genital mucosa may also occur in the absence of ulceration, the loss of pigment being, when present, a very characteristic sign of this disease.

Cutaneous lesions may never occur in the mild disease. In virulent infections, edematous urticaria-like plaques, usually circular but sometimes linear or in rings, occur in the skin, especially on the sides of the body and croup. The swelling may be up to 15 cm in diameter and painless and free of itch. They disappear in a few days and new ones form. There is usually no residuum, but sometimes there are local disturbances of sweating and pigmentation.

Nervous manifestations develop late in the course and usually lead to death. There is acute hyperesthesia initially, which may be generalized or localized to the distribution of particular nerves. Later there is diminished sensitivity or even anesthesia, and this is accompanied by paresis or paralysis of individual motor nerves. The pareses are either unilateral in distribution or asymmetric in severity and most commonly involve the facial nerves and the motor nerves of the hindlimbs. Decubitus follows.

The *general manifestations* are chiefly of continued or remittent fever, emaciation, and severe anemia. Noninflammatory synovial effusions, superficial lymphadenopathy, uveitis, and optic atrophy are described.

Apart from changes in the peripheral nerves that are responsible for the paralytic phenomena, pathologic changes additional to those that are clinically observable are not reported. Degenerative changes occur chiefly in the lumbar and fifth and seventh cranial nerves, being most severe in the roots and also involving the ganglia. The neuropathy is probably preceded by edema and inflammation, and these may still be present at death. The large nerve trunks are transformed into fibrous cords that are fused with the surrounding muscular fascia. Microscopically, there is edema, mononuclear cells, and fibrosis of the perineurium. There is slight endoneural reactivity in fascicles, in which there is extensive fiber degeneration. Sclerosing changes are also present in the ganglia.

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NEOPLASTIC CONDITIONS OF THE TUBULAR GENITALIA

Isolated reports have described both benign and malignant tumors of all histologic components of the tubular genitalia. Some are common in some locales and warrant brief discussion.

Tumors of the vulva are similar to the tumors of the skin. *Squamous cell carcinoma* is well known in the mare, cow, and ewe, and indeed, a high incidence in cows is reported in tropical countries. These bovine cases are analogous to the orbital tumors of cattle, thought to be initiated in a solar dermatosis, their incidence negatively correlated with the degree of local epithelial pigmentation.

Smooth muscle tumors (leiomyoma, leiomyosarcoma)

Leiomyomas are the most common tumors of the tubular genitalia of the bitch. Leiomyomas are less common in other species but, regardless of species, leiomyomas have characteristic gross features. Their location is the smooth muscle in the wall of the uterus, cervix, or vagina, and although they may be solitary, they are sometimes multiple. Most smooth muscle tumors in the bitch are benign regardless of their histological features, and some are *hormone dependent*. It rarely occurs in the bitch earlier than middle age and is frequently associated with ovarian follicular cysts or estrogen-secreting tumors, and often also with endometrial hyperplasia, mammary hyperplasia, and mammary neoplasia. Bitches spayed early in life are usually exempt, and established tumors may regress following neutering. This tumor has been provoked in guinea pigs by continuous low-level doses of estrogen, but for spontaneous smooth muscle tumors as well as endometrial and mammary neoplasms, there is as yet no precise knowledge of the role of estrogen.

Genital smooth muscle tumors may grow to be as large as 10-12 cm in diameter but most are not invasive. The smaller tumors are cellular but, as they enlarge, become firm or hard (hence the clinical term *fibroid*) because of the connective tissue stroma. On cut surface, they have a watered-silk appearance, and the color, whether more cellular or white, depends on relative amounts of muscle and connective tissue. Figure 4-108 shows characteristic gross features of a smooth muscle tumor in the uterus of a cow. These tumors are not encapsulated but are well demarcated, firm, light tan to white, and easily shelled out. In almost all cases, the tumors project as

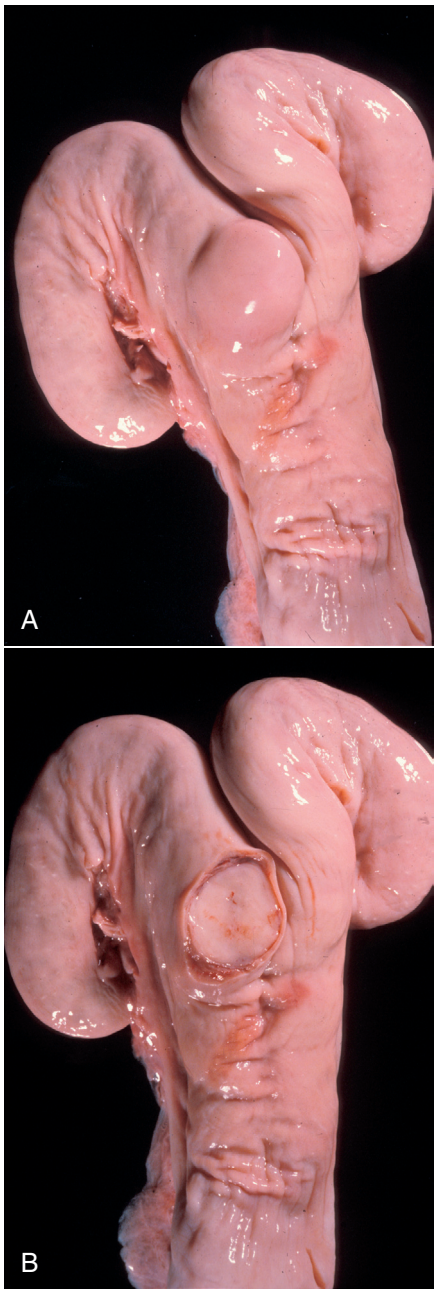

Figure 4-108 **A. Leiomyoma** seen as a single mass in the dorsal wall of the uterus of a cow. **B.** The serosa and myometrium over the mass and outer half of the mass have been removed to reveal the typical firm white appearance of leiomyomas.

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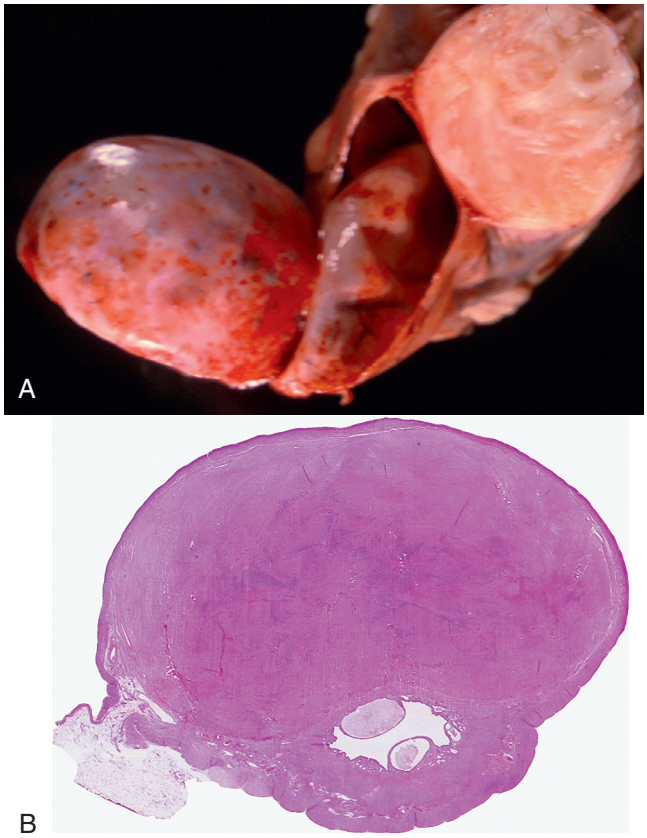

Figure 4-109 A. Leiomyoma arising within the uterine wall of a bitch. This leiomyoma has expanded outwardly from the serosal surface. The tumor has been hemisectioned, and both surfaces are shown. **B.** Subgross section of a uterine leiomyoma from tissues of a different bitch. In this case the tumor has grown within the wall circumferentially. Note the uniform dense nature of the mass.

globose or elliptical masses or as bulbous polyps into the lumen of the vagina, uterus, or cervix. Some extend outward from the serosal surface, and a few are found in the mesometrium. Figure 4-109A shows a canine uterus with 2 smooth muscle tumors in the uterus of a bitch. One extends into the uterine lumen, the other is a mass on the surface of the uterus near the bifurcation. The subgross photograph in Figure 4-109B shows the dense, homogeneous features typical for leiomyomas. Some smooth muscle tumors (called *Leiomyosarcomas*) are much more irregular, invasive, and frequently contain areas of necrosis.

Histologically, the tumor is composed of whorling bundles of smooth muscle cells with abundant stroma but scant intercellular connective tissue. The organization does not depart much from normal, and often the presence of neoplasm is best appreciated by the naked-eye appearance of the tissue. Cytologic features, such as pleomorphism, rate of mitoses, and invasiveness, are used to separate leiomyomas from leiomyosarcomas But there is no correlation between histologic classification and clinical behavior in dogs. Leiomyosarcoma is reported in older Saanen goats (Fig. 4-110), with occurrence reported in siblings, suggesting that in this breed there may be a genetic defect.

Canine transmissible venereal tumor

This tumor is a contagious neoplasm, transmitted most commonly by coitus, and occurs on the external genitalia of either sex. It is also known as Sticker's sarcoma. It has a histiocytic phenotype. This tumor differs from other infectious tumors in that *the infecting cells are transplanted and grow like a graft, thus is a xenograft*. This was the first neoplasm in the history of pathology to be transmitted experimentally; this was accomplished by the Russian veterinarian Novinsky in 1863.

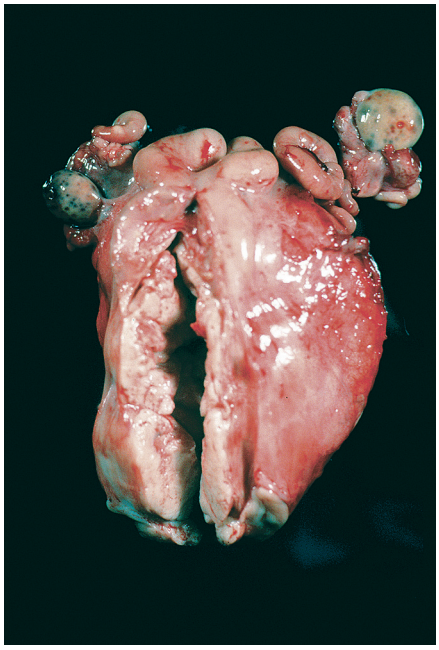

Figure 4-110 Leiomyosarcoma effacing the uterine body, cervix, and cranial vagina in a Saanen goat, a breed that has a predilection for this tumor.

The tumor has been reported worldwide. There are marked fluctuations in its prevalence in endemic areas over the course of a few years. The tumor tends to be common where dogs are allowed to run free and is rare where dogs are controlled. The tumor is not confined to the genitalia, but in some few cases, is found in cutaneous locations. *Metastasis sometimes occurs to regional lymph nodes, but spontaneous regression can occur in <6 months.*

The diploid number of chromosomes in dogs is 78, and normally they are acrocentric except for X and Y. The chromosome complement of tumor cells originating in separated areas of Japan and North America is closely similar, most tumor cells containing ~59 chromosomes; the minor variations in chromosome numbers in some cells and in chromosome morphology are within the variability accepted for stem lines of transplantable tumors and long-term cell cultures. Molecular techniques were used to identify that CTVT from different continents and collected decades apart are clonal, and, while there are 2 subtypes, they have a common origin. The DNA of the CTVT has closely related DNA to wolves and East Asian dog breeds.

In the vulva and vagina, the tumor arises in the epithelium and subjacent stroma as one or more gradually enlarging papular or papillary proliferations (Fig. 4-111). Expansive growth also occurs mainly in the submucosa, and the overlying epithelium becomes stretched and attenuated. With rupture or penetration of the epithelium, the tumor projects into the vagina as an irregular, ulcerated, and friable mass that may protrude from the vulva. Histologically, the tumor varies depending on the stage of growth or regression. During the

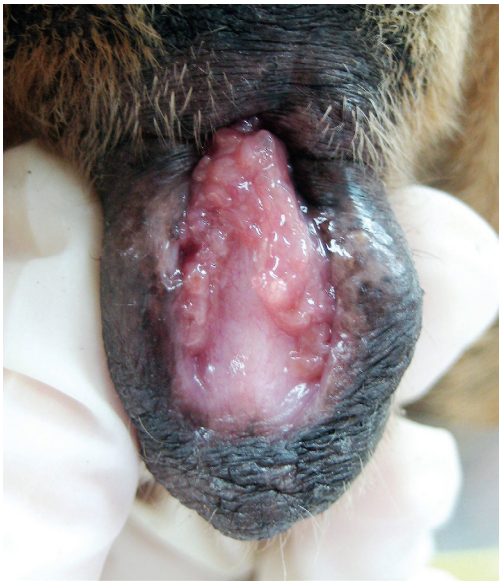

Figure 4-111 Transmissible venereal tumor in the vagina of a bitch. These tumors are transmitted from dog to dog by transplantation of neoplastic cells. (Courtesy Francesca Ivaldi, Brian Butler, St. George's University.)

early stage of growth, the tumor is composed of round, oval, or polyhedral cells with indistinct boundaries and poorly stained or clear cytoplasm. The nuclei are large in proportion to cell size with a single, well-defined nucleolus and many chromatin granules. Variability in the size of the cells is rather characteristic, and mitoses are frequent. The number of intratumoral lymphocytes increases as growth of the tumor slows and the tumor begins to regress. Most of the lymphocytes are T cells.

In the skin also, the tumor seems to arise in the subcutis, and the epidermis is not usually penetrated. It is suggested that natural cutaneous implantation occurs in bite or other wounds. In any locale, the tumor shows a marked tendency to break down after a few months of rapid progression, and on incision, the necrotic tissue resembles pus.

The cells of a CTVT are able to avoid detection by immune cells. They downregulate MHC-1 molecules and there is no MHC-2 activity, because they secrete inhibitory cytokines (TGFβ1 and IL-6). In the initial proliferative phase of CTVT, they express little MHC-1 or -2. After about 12 weeks in an experimental model, MHC expression increased dramatically and was associated with the presence of lymphocytes; and at the same time, the masses stopped growing. It appeared that the lymphocytes stimulated MHC expression and were responsible for regression of the tumors.

Dogs are resistant to challenge after natural regression of the tumor. Secondary tumor challenges begin to regress on day 9 and disappear after about 2 weeks. The secondary tumors are rapidly infiltrated by lymphocytes, most of which are T cells, and undergo degeneration. The tumor is transmissible not only between dogs but also to the fox, coyote, and jackal.

The life history of the tumors as outlined previously, and especially the limited metastatic capability, are not constant, and greater contagiousness and more virulent expressions may be expected in immunosuppressed individuals and in a canine population of suboptimal physiologic status. Such appears to be the case, for example, in the promiscuous, scavenging, malnourished, feral populations. There, metastases are common in both sexes as sequelae to the primary lesions. In the female, extension occurs to the uterus, cervix, and uterine tubes. In both sexes, there is early spread to inguinal nodes, which become large and firm. Cutaneous involvement is also common, with few or many lesions, some up to 6 cm in diameter, often ulcerated and hemorrhagic. Buccal lesions and large periorbital tumors are also described. Ocular lesions are common in this disease, but apart from the sites mentioned, conventional metastatic patterns in internal viscera are rare.

Fibropapilloma of the vulva

This is probably the most common tumor of the bovine vulva (except in tropical countries) and affects young animals primarily. It is, briefly, a papilloma growing on a mucous membrane, the nature of the host tissue apparently determining the reaction to the virus of bovine verrucae (bovine papillomavirus 1). This virus infecting the keratinized bovine skin provokes the typical papilloma of relatively scant connective tissue core and abundant epidermal overgrowth. Transplanted instead to the penile or vulval mucosa, the contribution of the 2 moieties is reversed, the bulk of the tumor being connective tissue with just enough epithelium to cover it. Histologically, *the bulk of the tumor consists of interlacing bundles of fibrocytes.* In the younger tumors, there may be many mitotic figures, and such cases have often been misdiagnosed as fibrosarcomas. Many of the plump spindle cells have large nuclei with bizarre nucleoli and sometimes pale, eosinophilic inclusion-like intranuclear structures. Collagen formation from the stroma is progressive with duration. Surface ulceration is followed by superficial inflammation.

The vulval tumors are usually sessile, rounded growths initially, but become progressively more cauliflower-like. Those attached to the penis are often pedunculated. The natural history of the genital fibropapilloma is the same as that of the common cutaneous papilloma; *spontaneous regression occurs in 1-6 months or so.* Within this period, surgical excision may be followed by recurrence.

Transmissible genital papilloma of the pig

Ordinary cutaneous warts are not described in pigs. A transmissible papilloma occurs in the *preputial diverticulum of the boar.* It is transmissible to the lightly scarified vulval mucosa with an experimental incubation period of ~8 weeks. The lesions are 1-3 cm in size and papular, some of the larger lesions being papillary. The clinical course is not many weeks, after which the lesions begin to regress and are eventually sloughed. Recovered animals are immune to reinfection.

The lesion, histologically, is typically papillomatous with extensive uneven acanthosis and epithelial overgrowth, but with none of the abundant mesodermal reaction that occurs in the bovine disease outlined previously. The lesions have *intracytoplasmic inclusions* that are large, spherical, homogeneous with acidophilic stains, and often surrounded by a halo. A lymphocytic inflammatory response occurs in the underlying dermis.

Carcinoma of the endometrium and cervix

These are *rare neoplasms* in domestic animals, and therein lies their interest. The rarity is real and not, as has been implied, apparent because of inadequate postmortem examination.

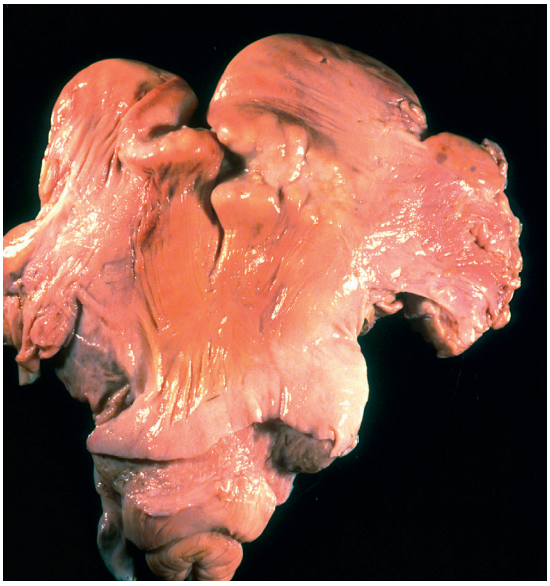

Figure 4-112 Uterine adenocarcinoma in the wall of a bovine uterus. These neoplasms stimulate a marked desmoplastic response, which results in the typical firm, depressed and contracted appearance.

Because cystic endometrial hyperplasia in dogs is common but carcinoma is rare, there does not seem to be any correlation.

Carcinoma of the endometrium appears to occur more frequently in *cattle* than in other domestic animals. The tumors may be single or multiple, hard, nodules of variable size in the uterine wall. Desmoplastic reaction is commonly a prominent feature, which leads to umbilication of the serosal surface (Fig. 4-112). The regional lymph nodes and the lungs are the usual sites of metastatic lesions.

As noted earlier, focal proliferative lesions of the endometrium and normal or prolonged endometrial repair postdelivery may be confused as being neoplastic changes in the endometrium. This is especially true in the bitch and queen, and caution should be exercised before making a diagnosis of endometrial carcinoma in those species.

Primary skin tumors that occur in the perineal area and frequently involve the vulva include squamous cell carcinomas and melanomas in mares (see Fig. 4-107).

Lymphosarcoma

Involvement of the uterus is common in the multicentric form of *bovine* lymphoma but is uncommon in other species. As elsewhere, there are 2 anatomic forms of uterine involvement: diffuse and nodular. In either event, the initial deposition seems to occur in the endometrium. The *diffuse lesion* involves both horns, body, and occasionally the cervix in a more or less uniform thickening, leaving a uterus that can be very large, but retaining its typical shape (Fig. 4-113). The thickened wall loses its elasticity and on cut surface is typically tan and uniform (Fig. 4-114). Gentle pressure may expel small quantities of cloudy, highly cellular fluid. There is patchy ulceration of the endometrial mucosa. There is nothing peculiar to the *nodular form* of the disease. These nodules, few or many, may attain large size and cause corresponding deformity. Central liquefactive necrosis is common in them. Microscopically, there is, with either form of the disease, gradual replacement of the normal uterine structures by infiltrating tumor cells.

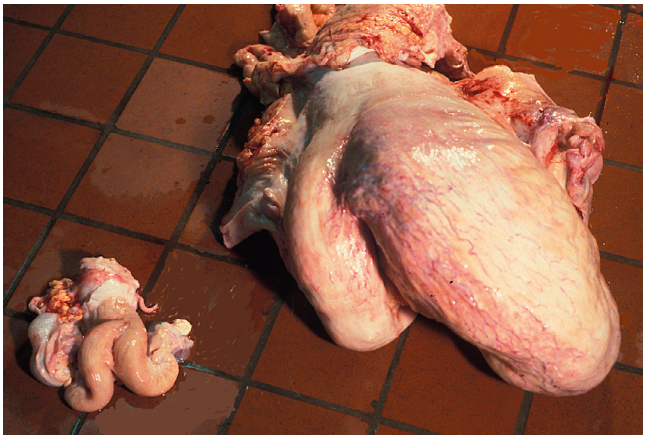

Figure 4-113 Lymphosarcoma diffusely involving the bovine uterus on the right has caused massive enlargement of the uterus. The uterus on the left is from a normal cow.

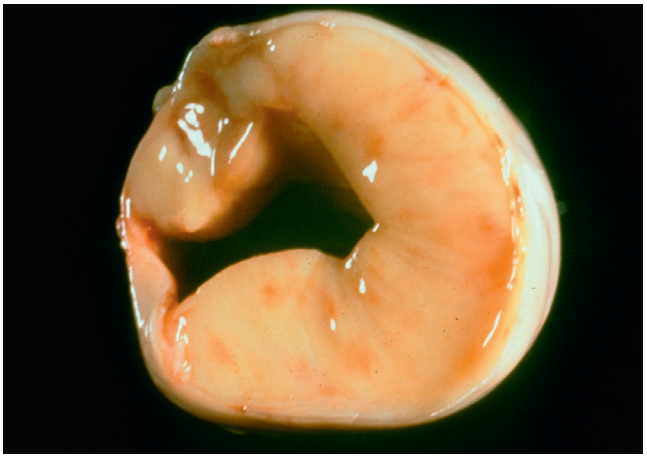

Figure 4-114 Cross-section of a **uterine horn from a cow with lymphosarcoma.** The wall is infiltrated and grossly thickened by the invading neoplasm. Lymphosarcoma has a characteristic tan, slightly soft appearance.

Lesions of corresponding type occur in the vagina, but do so less commonly.

Metastatic tumors

With the exception of lymphoma, secondary neoplastic diseases of the tubular genitalia are rather *rare*. Serosal implantations occur in peritoneal carcinomatosis. There are a few reports of results of immunohistochemical staining of tumors involving either the uterus or ovary in cattle.

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PATHOLOGY OF THE MAMMAE

The mammae provide the newborn with passive immunity and nourishment. They may also be a source of infection with viruses, bacteria, and helminths, either as part of the pathogenesis of the infection or accidentally from mastitis.

Disease of the mammary gland is commonly of 2 types: mastitis of dairy animals, and neoplastic disease of dogs and cats. Diseases of the skin of the mammae, including the papilla (teat, nipple) of the mammary gland, are discussed in Vol. 1, Integumentary system.

Developmental biology

The mammary gland is a secretory unit including the mammary glandular lobules, the lactiferous ducts, lactiferous sinus (when present), and the papillary duct. The papillary duct exits via an ostium, around which is a sphincter. Within lobules are acini surrounded by myoepithelial cells just as occurs in apocrine glands of skin.

The glands form on the mammary line or ridge in the ventrolateral ectoderm of the embryo. This line extends from the urogenital sinus to the mouth. The ectodermal cells of the mammary ridge, which are destined to develop into the mammary glands, congregate in specific areas, of number and location appropriate to the species, to form the mammary buds. From the buds, primary sprouts push into the mesenchyme, the number of sprouts from each bud determining the number of glands that will develop. Only one primary sprout develops from each bud in cattle, sheep, and goats. Secondary sprouts develop from the primary sprout to form the early lactiferous ducts.

Horses, sheep and goats develop 2 mammae, cows and camelids have 4, pigs have 10-13, dogs have 10, and cats have 8 (Table 4-1). Each mamma has one papilla. Cattle, sheep and goats have one mammary gland per mamma. Pigs, horses, and camelids have 2 mammary glands in each mamma and thus 2 papillary ducts per papilla. Cats have 3-7, and dogs have about 14 glands for each mamma. Thus in species with multiple papillary ostia, the mamma is a composite of mammary glands, the number corresponding to the number of papillary ducts, and each is autonomous and separate from its neighbors.

Table • 4-1

Normal mammary tissue arrangement in domestic species

Species	Mammae*	Glands per mamma†
Bovine	4	1
Porcine	12	2
Equine	2	2
Ovine	2	1
Caprine	2	1
Canine	10	Up to 14
Feline	8	3-7
Camelids	4	2

*Previously and commonly called mammary gland, or quarter in cattle.
†Functional unit is the mammary gland. Many species have more than one gland in each mamma.

Mammary development in *males* is similar to females in the embryonic and fetal stages. Male horses (and mice and rats) do not have papillae; the primary sprouts separate from the surface epithelium of the mammary bud under the influence of androgenic hormone and regress. The male mammary gland is susceptible to hormonal stimulation but is not as sensitive as the female. Alveolar structures are not present usually, and the enlargement that occurs under the influence of estrogen is due to ductal hyperplasia. This is seen in dogs with hyperestrogenism syndromes, such as in Sertoli-cell tumors.

Supernumerary papillae (teats, nipples) are common, especially in cattle. Prevalence is up to 30% and probably subject to genetic control. They occur in males, except in male horses, which do not develop papillae. Many supernumerary papilla have functional mammary tissue.

Inflammatory disease of the mammary glands

Mastitis is inflammation of the mammary gland, and this is mostly from bacterial infection. The usual route of invasion is through the papillary ostium and duct (teat canal). Hematogenous spread and localization occurs in mycoplasmosis, tuberculosis, and brucellosis, and it is possible for direct extension from local dermatologic conditions of the skin or papilla. The pathogenesis is complex, with contributing interactions of innate and adaptive immunity, physical damage to the papillary sphincter, and factors associated with mechanical milking. The pathogenesis and mechanisms are intensively studied only in cows. A primary and critical barrier to infection is the ostium and duct of the papilla and its sphincter. The establishment of a bacterial population in the lactiferous sinus is followed by inflammation, but the application of even large numbers of bacteria to the papillary ostium of an otherwise healthy papilla results in infection in only a few cases. The length of the papillary duct seems not to be important. Successful defense systems, both innate and acquired, control entry, proliferation, and tissue invasion and involve a spectrum of tissue, cellular, and cytokine responses.

Mechanical injury

Machine milking of dairy cattle is prone to induce significant damage to the ends of the papilla when milking procedures and the milking equipment are not optimal. This damage, associated with high vacuum, poor liners, ineffective pulsations, and overmilking, leads to partial eversion of the duct, ulceration, fibrosis, and bacterial growth. Papillary injury from overmilking reduces the keratin plug that helps protect from ascending infections.

Innate and acquired resistance of mammae

The major factors in whether an animal develops mastitis are the innate immune response, the pathogen, and the environment. The major focus is the innate immune response, reducing exposure to pathogens, and minimizing the effects of the puerperium.

Reducing contamination of the mammary papilla is the cornerstone of prevention of mastitis as it reduces potential exposure to pathogens. Reducing trauma at milking and chemical injury to the papilla is also well recognized in reducing intramammary infections through reducing "teat end lesions," which are circular or horseshoe-shaped rings around

the opening of the papilla. Mastitis only occurs when bacteria enter the gland; this requires entry through the physical barrier of the papillary duct (streak canal). This opens at parturition when the offspring suckle and during milking. It remains open for 1-2 hours after milking; this is when most mammary infections occur. When closed, the canal seals with keratin and a waxy plug. Mastitis often occurs in the periparturient period when neutrophil migration is inhibited and fewer reactive oxygen species are produced.

Once inside the lactiferous sinus or ducts, bacteria encounter macrophages and neutrophils (called somatic cells) in milk and the lining epithelial cells. These have specific pattern recognition receptors (PRR), especially toll like receptors (TLR), which recognize bacterial pathogen-associated molecular patterns (PAMP) and induce the innate or nonspecific immune response. Activation of intracellular pathways results in release of immune modulating substances, such as TNF-α, interleukin-8 (IL-8),and RANTES (regulated on activation, normal T-cell expressed and secreted) that attract neutrophils and induce inflammation.

Complement (C5a), lactoferrin, and lysozyme facilitate this response. *Complement 5* is present in mammary secretions and is cleaved to C5a, probably by mammary macrophages, the dominant cell in healthy mammary glands. C5a induces the release of proinflammatory cytokines, attracts neutrophils, and enhances phagocytosis. *Lactoferrin* is an iron-binding protein present in secretions and in neutrophils that inhibits multiplication of bacteria with high iron requirements. The high concentration of citrate in normal milk prevents the action of lactoferrin, but it is effective in the secretion of the nonlactating gland. *Lysozyme* is locally synthesized in the gland, and although the concentration in milk is low, there is some correlation between milk titer and susceptibility to infection. The titer may be increased during inflammation. The most common pathogens in cows are *E. coli* and *Staphylococcus aureus*. *E. coli* mediates inflammation through endotoxin and PRR such as TLR-4. *S aureus* mediates inflammation by exotoxins and lipoproteins, peptidoglycans, and lipoteichoic acid. TLR-2 is important. Adaptive immune mechanisms occur but are not as important as the innate system. There are differences between species in the nature of immunoglobulins in colostrum and milk. In the cow, the immunoglobulins are predominantly of the IgG class and are selectively transferred from serum to milk. The levels are low in normal glands, but permeability changes in inflammation allow the titer to increase. The same class of antibody is identified with the concentration of plasma cells in the distal papillary part of the lactiferous sinus and papillary duct, and may affect organisms resident in the papillary duct. *The major role of these antibodies is to promote opsonization of microorganisms*. IgA is also present in milk and may have a defensive effect on bacterial adherence to epithelial surfaces.

Bovine mastitis

Bovine mastitis is typically a response to ascending infection of the gland, and bacteria are the most common pathogens. Potential mammary pathogens are ubiquitous. Modern techniques of microbial classification have identified more than 100 species, subspecies, and serovars isolated from the mammary gland. *Streptococcus agalactiae* and some types of *Staphylococcus aureus* are obligate parasites of the gland and inevitable pathogens, but the great majority of infections are opportunistic.

Mastitis is an inflammatory disease rather than an infectious disease; it is the host response to bacteria that determines if there is local or systemic disease and how severe it is. The process ranges from transient to persistent and from mild or subclinical to severe, peracute, and fatal. Notwithstanding the large number of microbial species that may be isolated from the diseased mammary gland, bovine mastitis is dominated by staphylococci and coliforms; the streptococci are now much less common because of dry cow therapy. Some infections, such as caused by *Cryptococcus* and the atypical mycobacteria, are usually iatrogenic. Some other infections, such as caused by *Pseudomonas* and *Prototheca*, indicate heavy environmental contamination.

Staphylococcal mastitis

Staphylococcal mastitis is predominantly an infection of heifers ("heifer mastitis") at the time of parturition and their first mechanical milking. **Coagulase-negative staphylococci** are the most common cause of heifer mastitis, mastitis around the time of the first parturition, and before the application of mechanical milking. ***S. aureus***, a coagulase-positive bacterium, is a more common cause of mastitis in the older age groups.

Pathogenic strains of staphylococci are always of human or animal origin and persist as permanent inhabitants of the skin and mucous membranes; thus infection of the udder is *contagious*. Little is still known of the pathogenicity of the staphylococci as some strains may produce gangrenous mastitis on some occasions and only a mild disease on others, with little correlation of toxin production to toxigenicity.

Staphylococcal mastitis can be subclinical or clinical; mild or severe and fulminating; and peracute, acute, or chronic. The severe forms of the disease typically occur shortly after parturition, and there is necrosis (gangrene) of affected mammae and high mortality. The affected mammae are swollen and tense, hot and firm, and very painful. There is almost complete stagnation of secretion, and only a few milliliters of brown, blood-stained, or straw-colored watery fluid can be expressed from the papilla. Uninfected mammae are also swollen and tense, and the secretion is reduced but otherwise normal. Gangrene (Fig. 4-115) usually first affects the papilla and adjacent portions of or the whole mamma. The tissues become

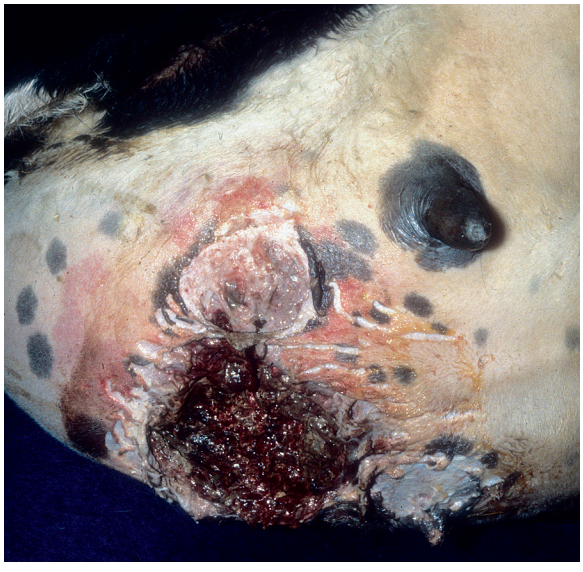

Figure 4-115 **Gangrenous mastitis** in a cow.

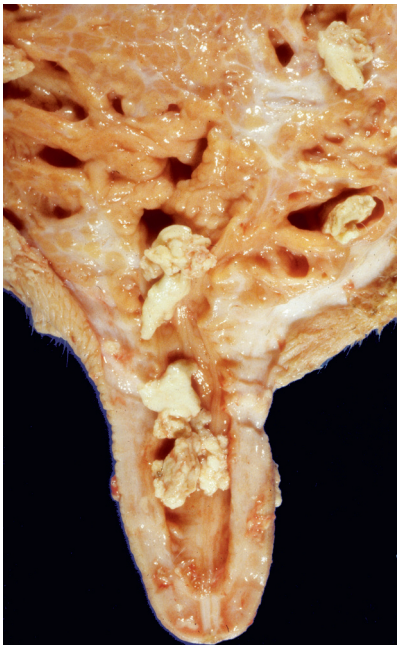

Figure 4-116 Fibrinosuppurative exudate within the lactiferous ducts and sinus of a cow with severe mastitis.

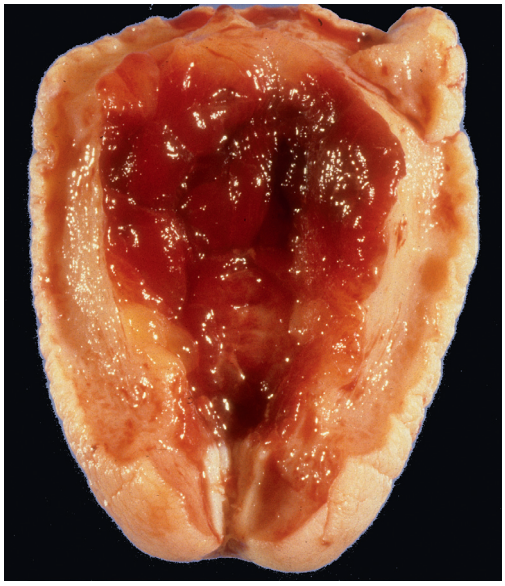

Figure 4-117 Hyperemia of the papillary portion of the lactiferous sinus in a cow with severe mastitis.

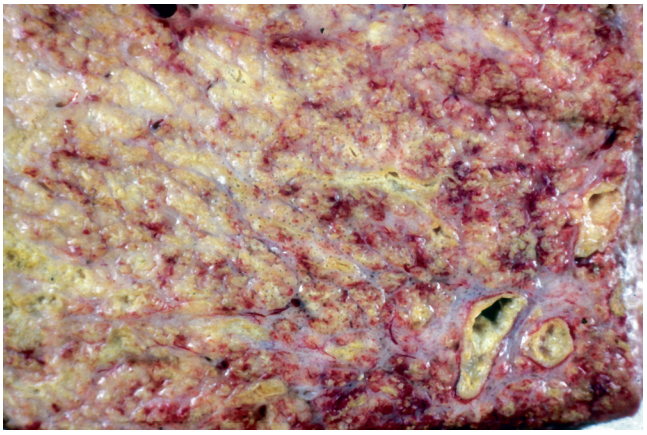

Figure 4-118 Prominent mammary lobules in clinical mastitis in a cow. It is difficult to differentiate involuting gland from inflamed mammary tissue.

blue and eventually black and are softer, insensitive, and cold. There is pitting edema of the inguinal area, flank, and ventrum, and the necrotic skin begins to exude serum and to slough, and gas bubbles develop beneath it. The amount of tissue involved in the gangrenous process is quite variable, and groups of necrotic lobules adjoin others that are near normal. Separation of the gangrenous areas begins a week after onset, proceeds slowly, and the surface is suppurative and fistulae develop.

The changes in glands shedding nonhemolytic, coagulase-negative staphylococci of low pathogenicity without clinical signs of infection are mild progressive fibrosis and lobular atrophy. Hemolytic coagulase-positive staphylococci produced lobular lesions of greater extent and rate of progression.

The nongangrenous and mild forms of the disease are the same for the majority of bacterial mastitides. The gross appearance varies according to severity. The lesions are mostly in the distal portion of the gland about the lactiferous sinus and large ducts. The lactiferous sinus and ducts fill with secretion in the early stage of the disease, the secretion being serous and floccular or distinctly purulent (Fig. 4-116). The epithelial lining of the sinus and papilla may be normal, or it may be red and granular (Fig. 4-117). The adjacent glandular tissue is swollen and turgid (Fig. 4-118). Surface lobulations are distinct because the swollen lobules protrude. There is no recognizable interstitial edema. The affected lobular tissue is gray and often cannot be differentiated from involuting glands, whereas the normal lactating tissue is milky white. With time, the lactiferous sinus and larger ducts have a thickened lining and small rounded polypoid projections into the lumen. The periductal tissues become white and tough with fibrosis and a lack of glandular tissue. The tissues away from the ducts are not as affected. Histologically, the range of changes is very wide, depending on the severity, which depends on invasiveness of the bacteria, toxin production, and the degree of the host response. In subclinical mastitis, neutrophils enter the acinar and ductal lumens of some lobules (Fig. 4-119). There is minimal interstitial vascular change. Neutrophils migrate from venules through the interstitium (Fig. 4-120), and epithelium to contribute to somatic cells in milk. Epithelia of the ducts become hyperplastic and with increasing severity, develop squamous metaplasia. Lymphocytes and plasma cells accumulate in the interstitium, and lymphoid follicles can form, especially around the lactiferous sinus and ducts. With increasing severity, there is death of the epithelium, sloughing of cells into the lumen, and erosion. Granulation tissue will form, and periductal fibrosis develops. In experimental infection, the bacteria enter the tissues and induce a severe reaction with interstitial edema and emigration of neutrophils throughout all the tissues. Stromal lymphatics are widely dilated and contain numerous neutrophils. The acinar epithelium becomes vacuolated and dies. Granulation and fibrosis progress and eventually obliterate many of the acini. The inflammation and retention of milk and exudate causes involution in affected and unaffected acini nearby. The processes of fibrosis and involution continue until the end stages, when some lobules

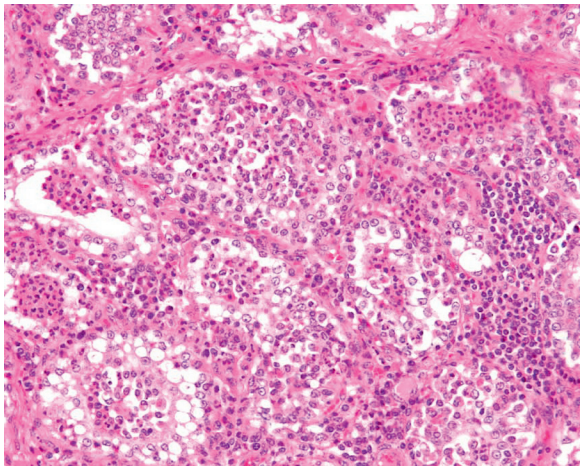

Figure 4-119 Large numbers of neutrophils are within glandular acini of a mammary lobule in subclinical and **clinical suppurative mastitis** in a cow.

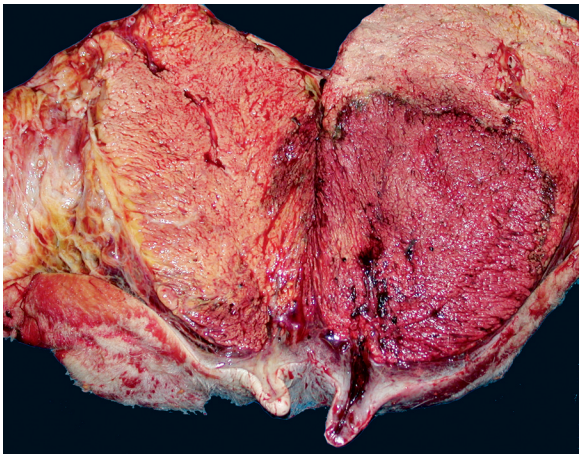

Figure 4-121 Distinct and well-demarcated region of glandular necrosis, and marked subcutaneous edema, typical of bovine **coliform mastitis.**

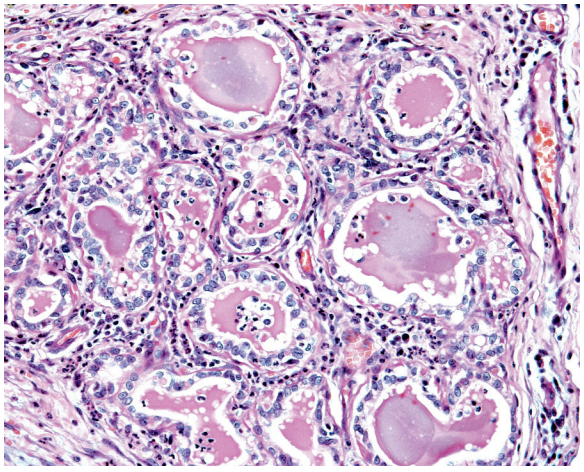

Figure 4-120 Early lesion in **streptococcal mastitis** in a cow. Neutrophils within the interstitium of the gland migrate from venules to the lumen of glandular acini, where the bacteria are present.

show normally involuted tissue, some are obliterated by fibrosis, and others have combinations of the two. Because infection and inflammation progress to involve adjacent tissue, many lobules are out of phase with each other; and there is a succession of events from mild to severe as more gland is involved. Progressive involvement of ducts results in granulation tissue and fibrosis, and obstruction to milk flow. Upstream tissues are dilated and contain stagnant milk, exudate, and numerous organisms. There is concomitant proliferation of periductal fibrous tissue spreading centrifugally to involve and obliterate large amounts of lobular tissue. Such granulation tissue in time cicatrizes, and the duct epithelium can be restored. Similar changes occur in the larger lactiferous ducts and sinus, but they are less exuberant, and the epithelium can become squamous and sometimes keratinized.

Persistent foci of infection transition into a granulomatous reaction previously known as *botryomycosis*. Initially, necrotic foci form and are surrounded by an intense neutrophilic and histiocytic response, and fibrosis develops rapidly. Each granulomatous focus is up to 2 cm in diameter and may be numerous and involve a large proportion of the mamma. The granulomas have colonies of large cocci in the center, a surrounding ring of neutrophils, and sometimes there is a Splendore Hoeppli reaction. Epithelioid macrophages and multinucleated giant cells develop and are subsequently surrounded by fibrosis.

Coliform mastitis

Coliform bacteria, especially ***E. coli***, produce mastitis that is usually severe and in some herds are the major causative agents. Little is known of the pathogenesis, but coliform mastitis is a serious problem in herds where the more common mammary infections are suppressed or eliminated. The other *Enterobacteriaceae* that cause mastitis include bacteria of the genera ***Enterobacter, Klebsiella, Citrobacter, Serratia,*** **and** ***Proteus***. These organisms are part of the *environmental flora*, and they do not have a predilection for the mammary gland; mammary infection may be correlated with the level of environmental exposure. Each usually produces a clinically severe form of the disease with systemic reaction and, especially in the case of *E. coli*, endotoxemia. A milder progressive type of disease can also occur.

It is generally accepted that coliform mastitis is an ascending infection. This infection is often limited to one mamma, and typically there is a distinct region of dead gland sharply demarcated from surrounding normal gland (Figs. 4-121, 4-122). Extensive edema and, if very severe, hemorrhage, is characteristic. The content of the lactiferous sinus and major ducts is often scant and watery or cloudy and blood stained; it contains floccules of fibrin and coagulated casein. Coagulated protein forms plugs in the lactiferous ducts. There is usually severe edema of the subcutis of the gland and ventral abdomen (see Fig. 4-121).

Microscopically, the inflammatory reaction is centered on the ducts. The lining of the larger ducts is missing and replaced by fibrinosuppurative exudate. The lining of the smaller, intralobular ducts is also destroyed and contain plugs of necrotic detritus. The acini are filled with serous fluid in which there are vacuolated desquamated epithelial cells, but leukocytes are few or absent, either in the alveoli or in the septa (Fig. 4-123). The septal tissues, especially the interlobular septa, are widened by edema, and the lymphatics are widely dilated and contain fibrin. This can transition to interstitial fibrosis

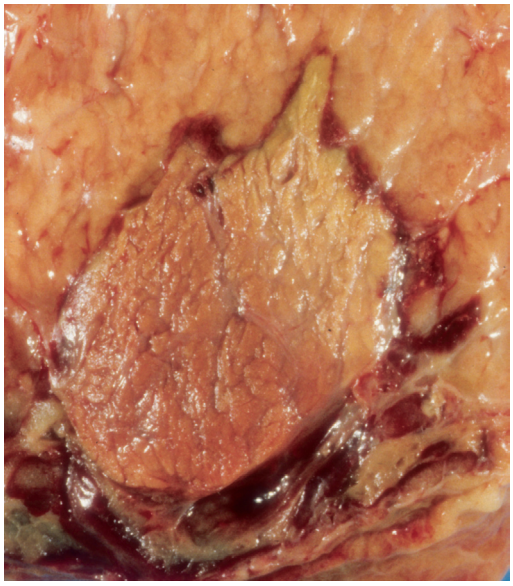

Figure 4-122 A distinct region of mammary glandular necrosis becomes a **sequestrum** over time in bovine coliform mastitis.

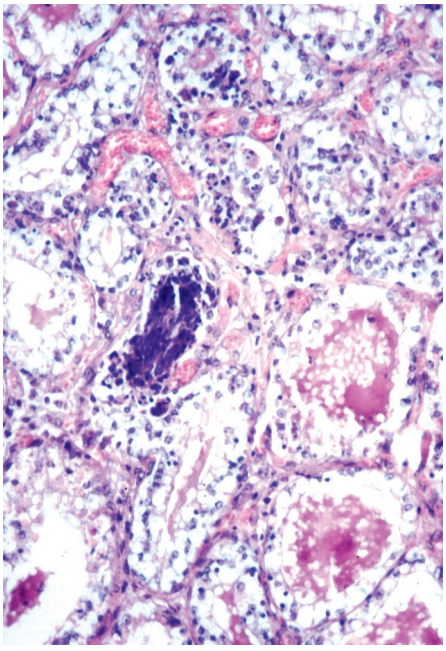

Figure 4-123 **Marked epithelial necrosis** and interstitial edema are features of early bovine coliform mastitis.

(Fig. 4-124). In areas of hemorrhage, red blood cells are throughout the septal connective tissue and within acini.

If the course of the severe inflammation is prolonged beyond 1-2 days, extensive death of tissue may occur. If the animal survives, the necrotic tissue, usually most of one quarter, forms a sequestrum.

Streptococcal mastitis

Bacteria of the family *Streptococcaceae* are important potential causes of bovine mastitis, but modern preventive techniques largely control disease. ***Streptococcus agalactiae, S. dysgalactiae,*** and ***S. uberis*** are the most frequently implicated. The common streptococci have the mammary gland of the cow, goat, and camelid as their natural and sole habitat; environmental factors are important only in the transfer of infection from animal to animal. Less common isolates cause sporadic disease only.

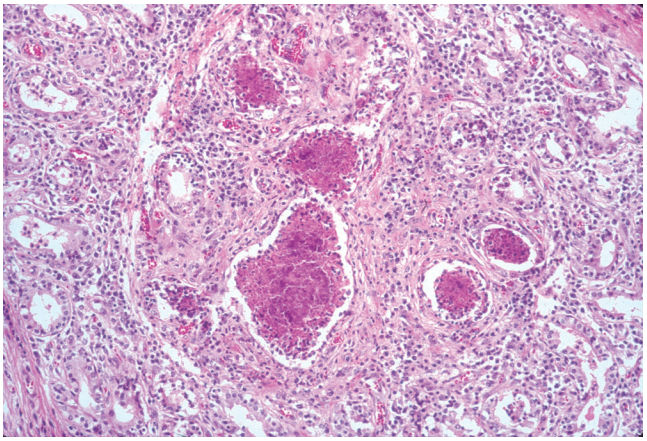

Figure 4-124 **Fibrinonecrotic debris within small interlobular lactiferous ducts** and surrounding fibrosis occurs with time following subclinical or clinical bovine mastitis. (Courtesy Murray Hazlett, University of Guelph.)

The only significant portal of entry of *S. agalactiae* into the mammary gland is through the papillary ostium and duct. *Streptococcal mastitis is usually permanent*. The organism maintains its numbers in the lactiferous sinus and ducts in balance with inflammatory products. Following the establishment of infection, the bacterial population may suddenly increase and penetrate the epithelium, usually for a brief period. The detrimental effects of improper machine milking, including changes in vacuum, rate of pulsation, and period of application, plus individual alternations in susceptibility result in flare-ups of established quiescent infections. Repeated invasion of the epithelium causes inflammatory and reparative reactions, which, if unchecked, culminate in fibrosis and involution of the affected quarter.

As with most bacterial mammary pathogens, the severity of the clinical signs and lesions of streptococcal mastitis vary. The severe cases have systemic disturbances and the milder ones not. With streptococci, which do not penetrate the epithelium readily, toxic products of the organisms or of the inflammation are responsible for necrosis and systemic illness. The period of epithelial penetration is brief, being of only a few hours' duration as the streptococci are rapidly destroyed, and no bacteria may be cultured.

Summer mastitis

"Summer mastitis" or "Holstein udder plague" is mastitis of dairy cows that occurs during the summer months, traditionally the dry cow period. This is usually a **mixed bacterial infection** affecting immature and nonlactating glands of animals at pasture. *Trueperella (Arcanobacterium) pyogenes* is a common isolate along with *S. dysgalactiae, Peptostreptococcus indolicus, Bacteroides melaninogenicus, Fusobacterium necrophorum*, and microaerophilic and unidentified obligate anaerobes. Infection is acquired via the papillary ostium and duct and contamination by flies attracted to pre-existing lesions of the mammary papilla.

The lesion is suppurative, necrotic, and centered on the lactiferous sinus and ducts, thus it is a *necrosuppurative*

galactophoritis. There is minimal involvement of the acinar tissue. Grossly visible abscesses form where the exudate remains stagnant in the ducts. Smaller abscesses occur in the intralobular ducts. Local death of epithelium and neutrophilic inflammation are followed by periductal fibrosis, and there are many lymphocytes and plasma cells within fibrous septa. The smaller intralobular abscesses may heal with obstruction of the ducts and lobular scarring. The large abscesses are centered on the larger ducts, the walls of which are remodeled by exuberant granulation tissue. The ducts are lined in part by hyperplastic and squamous epithelium. Bacteria are numerous in the abscesses and in the lumen of affected ducts. The walls of the lactiferous sinus and papillary duct are thickened by granulation tissue and are narrowed or stenotic.

Mycoplasma *mastitis*

Mycoplasmas are prominent causes of bovine mastitis in some herds. There is rapid spread from an infected mamma to adjoining mammae and to others in the herd. Response to therapy is poor. Shedding of the organisms from infected quarters is inconsistent, adding to the challenge of clinical diagnosis and control.

The diseases produced by *Mycoplasma* spp. and *Acholeplasma* spp. are similar. ***Mycoplasma bovis*** is the most frequent of these infections, although infections by *M. canadense, M. bovigenitalium,* and *M. californicum* are also common.

The disease begins with a *sudden onset of agalactia*. The mamma is initially swollen, firm, and painless. Ordinarily there are no systemic signs, but other manifestations of systemic *Mycoplasma* infection, such as arthritis, may occur. Coexisting mammary infection with bacteria occur, and the clinical signs are worse than with a sole infection. The disease spreads rapidly in a herd; animals in full lactation tend to be affected simultaneously in all glands. The milk from an infected mamma appears as normal, but separates into floccular and clear components. The presence of pus or blood probably indicates coexistent bacterial infection.

Affected mammae are, in the active stages, swollen and firm but later become flaccid as rapid involution occurs. Altered secretion and mammary enlargement may persist for several weeks. Clinical recovery may occur without return to full normal function, and such animals may continue to excrete the organism intermittently for more than a year. It is not clear whether clinical relapses occur or whether over this long period there is cumulative mammary injury.

The route of infection of the mammary gland is not clear. The experimental disease is induced by intramammary or intravenous inoculation and is severe. The usual clinical disease suggests spread from mamma to mamma, but the occurrence of arthritis indicates systemic dissemination.

The histologic lesions in the gland are different from other types of mastitis. Neutrophil migration begins within a few hours of experimental infection as perivascular accumulations in walls of the lactiferous sinus and lobular interstitium, which is edematous. The reaction is patchy at first but quickly involves most of the parenchyma. After several days, lymphocytes, plasma cells, and macrophages appear and become dense focal lymphocytic aggregates in the intralobular and periductal connective tissue (Fig. 4-125). These persist for many months. The epithelial cells of alveoli and ducts develop large lipid vacuoles that are discharged into the lumen. The alveolar epithelium becomes hyperplastic and multilayered. This transitions over several weeks to fibrosis of intralobular

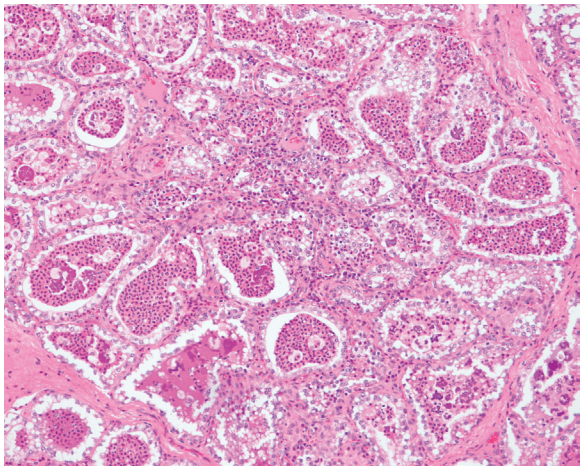

Figure 4-125 ***Mycoplasma bovis*** **mastitis** in cattle differs from other causes of mastitis in having prominent lymphocytes and plasma cells within the glandular interstitium. Neutrophils are within glandular acini.

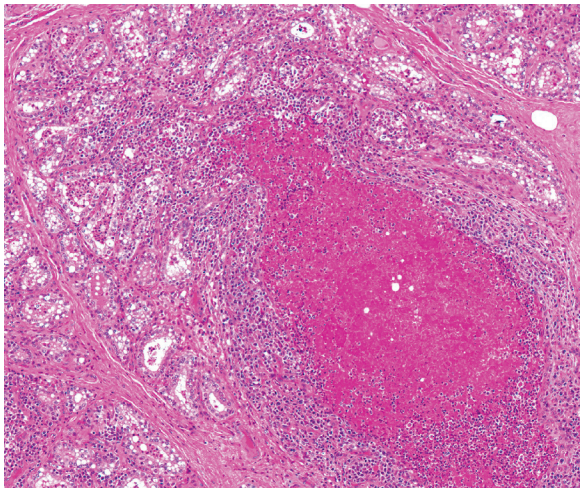

Figure 4-126 Marked dilation of lactiferous ducts with necrotic debris in *Mycoplasma* mastitis. The gross appearance is **caseous exudate.**

septa and subsequent alveolar atrophy. The ductal epithelium becomes hyperplastic too, and squamous metaplasia develops. There is also surrounding progressive fibroplasia. The numbers of interstitial lymphocytes continue to increase. Concurrent epithelial erosion occurs, and exuberant granulation tissue forms. When severe, there are polypoid protrusions into the lactiferous ducts and sinuses. Retention of exudate occurs in small ducts, with subsequent mineralization and granuloma formation (Fig. 4-126).

Miscellaneous infections

There are hundreds of potential mammary pathogens. From a historical perspective, **tuberculous mastitis** is an important classic disease. It is rare in a majority of countries, but is still important in those without a modern dairy industry. Its importance is in its zoonotic potential. Mammary tuberculosis usually develops insidiously without clinical signs of inflammation. The gland progressively increases in size and firmness,

Figure 4-127 Multiple variably sized **abscesses and pyogranulomas** in *Nocardia* mastitis in a cow.

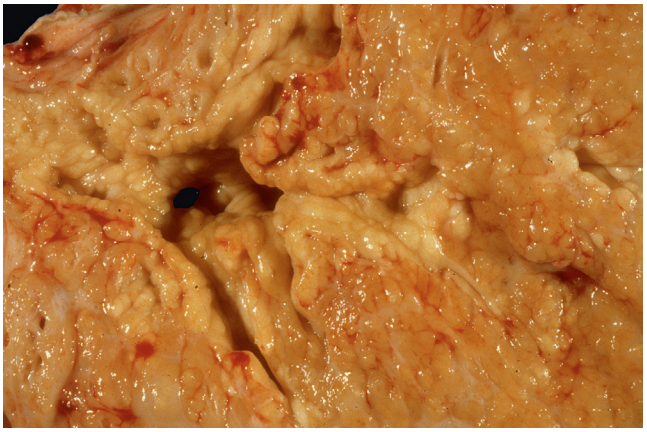

Figure 4-128 **Chronic galactophoritis** results in nodules of lymphoid hyperplasia and epithelial hyperplasia of the lactiferous ducts in bovine mastitis.

and the majority do not have the classic miliary lesions seen in other organs. The caseous inflammation of the lactiferous sinus and ducts (galactophoritis) form also occurs. Lactiferous ducts are especially involved, and bacilli are in the milk before gross lesions of tuberculosis are evident. The milk may be physically normal for a long period after infection, even though it contains the bacilli. In the later stages, there is reduced watery secretion with floccules of caseous exudate and large numbers of bacilli. *Mycobacterium bovis* is the main pathogen. The inflammatory reaction is similar to other locations with granulomatous caseous inflammation, pyogranulomatous inflammation with distinct granulomas, and/or a diffuse histiocytic reaction.

Nocardia spp., other *Mycobacterium* spp., fungi and yeasts (*Cryptococcus neoformans*, *Candida* spp.), and algae (*Prototheca zopfii*) produce **granulomatous mastitis**. These usually develop from environmental contamination or after infusion of contaminated intramammary preparations. Herd outbreaks or sporadic individual cases occur.

These agents may be present in mammary secretions for months without causing clinical signs of mastitis. In other cases, the response to infection is a mild chronic involvement of the mammary gland, whereas in yet other cases, the reaction is of sudden onset and severe clinical course. Severe reactions occur near the time of parturition especially. There may be spread of infection to mammary lymph nodes and lungs in some cases. Affected quarters enlarge rapidly and become firm from fibrosis. In some cases, there is a palpable nodularity, the nodules being 2-5 cm in diameter. These may be sinus tracts discharging on to the skin. In nocardial mastitis, some infections are purulent in small foci (Fig. 4-127), but larger abscesses occur when there is a mixed infection with *Pseudomonas aeruginosa*, *T. pyogenes*, or other organisms. The lesion in the majority of infections is galactophoritis, similar to summer mastitis (Fig. 4-128). The range of inflammatory reactions is identical to infections elsewhere.

Mastitis in swine

E. coli, *S. aureus*, and, to a lesser extent, *Streptococcus* spp. are important mammary pathogens of sows. Little is known of the pathogenesis, but *the range of lesions is identical to bovine mastitis*. In particular, clinical mastitis caused by coliform bacteria occurs as a severe infection shortly after parturition. It affects more than one mamma and is recognized when there is an accompanying severe systemic reaction.

An important syndrome in sows is known as the **mastitis-metritis-agalactia syndrome** or *postpartum dysgalactia syndrome*. Sows become lethargic; anorexic; and develop fever, a vulvovaginal discharge and mammary redness, edema, swelling, firmness, and agalactia. Failure of lactation and systemic signs dominate the syndrome. The syndrome involves a complex interaction between environment, host, and pathogens.

Chronic granulomatous mastitis (previously known as botryomycosis) affecting one or more glands is occasionally observed in old sows, and most cases are caused by *S. aureus*.

Mastitis in horses

Mastitis in mares is rare. They are seldom used as dairy animals and therefore do not have the usual environmental exposure and mechanical injury that accompanies milk harvesting. Bacteria cultured from lactating mares are similar in spectrum to other species and include streptococci and staphylococci. Mastitis is recognized as excessive swelling of a mamma in a lactating mare. Lesions are similar to those in other species.

Mastitis in sheep and goats

Mastitis in the ewe and goat is usually caused by *S. aureus* or *Mannheimia haemolytica*. *Corynebacterium pseudotuberculosis* and *T. pyogenes* also occur. Mastitis in the ewe and doe is especially important in dairy animals and because the case fatality rate can be as high as 50%. Except for infection by *M. agalactiae*, clinical mastitis usually affects only one mamma. The range of organisms causing bacterial disease includes the species pathogenic in the cow.

S. aureus mastitis has a similar course and consequences to the disease in the cow. The morbidity, however, is higher and may approach 25% of a flock. *M. haemolytica* causes mastitis in sheep on summer range at or near the end of lactation and affects ~5% of a flock. This is a severe disease with systemic reaction; if the ewe survives, the systemic reaction subsides within 48 hours. The affected gland, usually one side, is greatly enlarged, tense, blue-black, and the secretion becomes watery and contains flakes. Widespread necrosis of tissue occurs. Animals that do not die develop abscesses in the course of a

week or so; these rupture, fistulate, and eventually most of the affected gland sloughs. Pneumonia in lambs may accompany this form of mastitis.

Contagious agalactia caused by *M. agalactiae* is primarily a disease of goats, and sheep are less susceptible. The disease is endemic in countries bordering the Mediterranean Sea. The organisms are eliminated in the milk and in other discharges for many months in animals that survive the initial stages of the disease. This disease begins with septicemia, during which the organism can be cultured from all tissues and from blood. It is often fatal. If the infected animal survives, there are signs of localization in the eyes, periarticular tissues and the lactating mammary gland. Lactating females and kids are particularly susceptible. Pregnant females may abort or deliver live but infected fetuses. Lameness, keratoconjunctivitis, and changes in the milk occur. Mastitis primarily affects the interstitial tissue with secondary changes in the acini. Mild inflammation causes cessation of lactation, but the more severe reactions may lead to progressive fibrosis with parenchymal atrophy. Lameness is from arthritis and periarthritis, particularly of the carpal and tarsal joints. The synovial membranes may be normal, or they may be red or ulcerated with an increased volume of turbid fluid that may be blood stained. The ocular lesions, which are present in ~50% of cases, consist of mucopurulent conjunctivitis and keratitis that may be complicated by ulceration and staphyloma.

Mastitis affecting both mammae of ewes is a common manifestation of infection by the lentivirus of **maedi-visna** (ovine progressive pneumonia; see Vol. 2, Respiratory system). The mammary disease has an earlier onset and higher prevalence than neurologic or pulmonary disease, although the expression of the various syndromes may be strongly influenced by breed and genotype of the hosts. The presenting signs are reduced milk yield, poor preweaning growth of lambs, and progressive atrophy of both mammae. The virus is present in mammae shortly after exposure. The severity of the lesions increases with the duration of infection. The virus replicates in circulating monocytes and more freely in tissue macrophages. The presence of macrophages in the mammary interstitium encourages a local inflammatory response consisting largely of lymphocytes and plasma cells. These are prominent in the interstitial and periductal connective tissue, and lymphoid nodules form. Fibrosis is not a feature. Degeneration and loss of acinar and ductular epithelium that is present in advanced lesions may be a consequence of inflammation and not a direct virus effect.

The lentivirus of **caprine arthritis-encephalitis** is closely related to maedi-visna virus (MVV), and the pathogenesis is similar. Mastitis, although similar to the ovine disease, is not a prominent feature.

Mammary injury is reported in lactating goats that ingest persin, the toxic principle of the **avocado,** *Persea americana*. The gland becomes swollen, firm, and red, especially in dorsal parts, and there may be subcutaneous edema affecting the gland and adjacent body wall. Histologically, there is widespread degeneration and necrosis of secretory epithelium, especially at the centers of the lobules. Ductal epithelium is similarly affected. Cellular inflammatory response is minimal.

Mastitis in dogs and cats

Mastitis is uncommon in dogs and cats. It occurs in lactating or pseudopregnant animals and occasionally in those with mammary neoplasia. Staphylococci, streptococci, and coliforms are usually responsible. Staphylococcal mastitis is more common. Necrosis can occur (Fig. 4-129), and affected animals are systemically ill; abscesses form. The severely affected glands are large, firm, and edematous, and the overlying skin becomes taut and shiny. Only a small amount of gray secretion can be expressed; it may be blood tinged or contain pus. Mastitis is often superimposed on cystic dilation of mammary ducts, with or without mammary neoplasia.

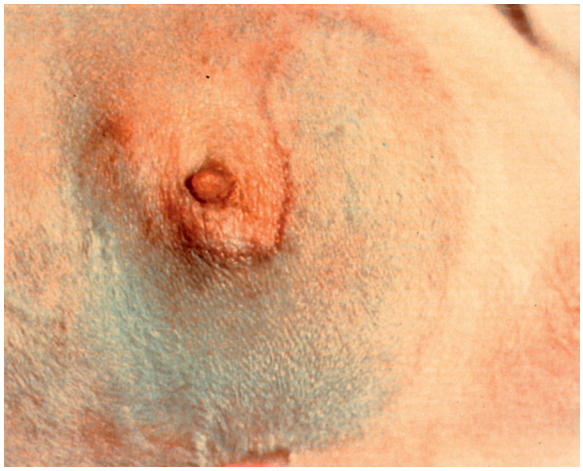

Figure 4-129 Severe mastitis in a cat with a distinct region of mammary necrosis.

Mastitis in camelids

Surveys of the milk from camels, llamas, and alpacas suggest that infection of the mammary gland is very common; most cases are subclinical. Clinical disease appears to be very uncommon. Based on culture, *Staphylococcus* spp. and *Streptococcus* spp. represent the vast majority of bacteria isolated. There is the same range of clinical disease as in other species, and the pathophysiology is assumed to be the same. Severe mastitis with sepsis, suppurative mastitis, and chronic fibrosing mastitis all occur. Poor cria growth rate is often an indication of mastitis.

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MAMMARY MASSES INCLUDING NEOPLASIA

Neoplasia of mammae is extremely important from an individual or comparative perspective. Not every mass in a mamma is neoplastic, but neoplasia is usually the top of the list of diagnostic hypotheses. Neoplasia is very common in the dog, less so in the cat, and rare in other species. The prognosis for neoplasia in the mammary glands is most important. It is usually poor for all species except the dog. Diagnosis of mammary neoplasia is best achieved by excisional biopsy; cytology can be of use in identifying inflammation and malignant stromal neoplasms in dogs, and is very accurate in all other species, especially in cats.

This section will cover non-neoplastic diseases first; neoplastic disease will follow.

Non-neoplastic mammary enlargement and masses

Mammary tissue undergoes physiologic change at the time of puberty when ducts proliferate under hormonal influences of estrogen, progesterone, growth hormone, and prolactin, to name a few. With pregnancy, the gland undergoes further development and enlargement, especially with lactation. Involution of the gland follows, and acinar tissue disappears in most species. The common non-neoplastic lesions of the mammary gland are cystic dilation of mammary ducts and mammary hypertrophy and hyperplasia.

Cystic dilation of mammary ducts (Fig. 4-130) is very common, especially in dogs and cats. It is usually secondary to obstruction of a duct or the papillary canal by periductal fibrosis, localized hyperplasia, or neoplasia. Continued secretion and buildup of content dilates the duct, and the epithelium accommodates the larger diameter by a lining that is attenuated or normal in appearance.

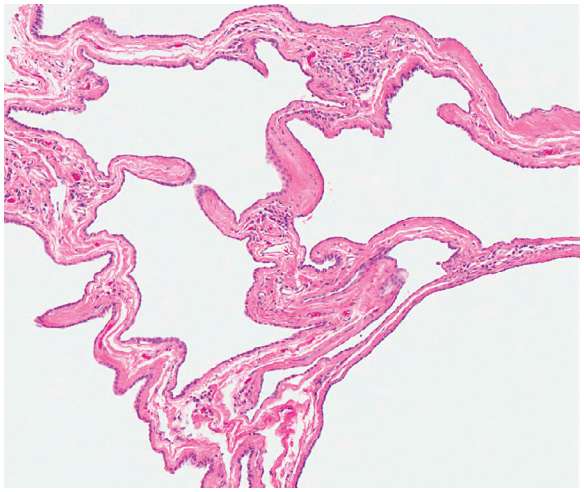

Figure 4-130 Cystic dilation of mammary ducts secondary to obstruction of secretion outflow in a cat.

Mammary hypertrophy is when a whole mamma or mammae become larger than normal for the physiologic state. It is common in dogs because of their propensity to lactate during pseudopregnancy, as an adaptation to enhance survival of pups in a pack situation. Precocious mammary development and lactation occurs with exposure to exogenous hormones, such as transcutaneous human hormonal replacement therapy. Some high-producing lines of dairy cattle and goats have mammary hypertrophy and lactation, including males. Some dogs spayed while in diestrus will develop hypertrophy and lactation because of a prolactin surge in response to a sudden reduction in progesterone concentration. The histologic change is generalized hyperplasia of all mammary tissue.

Localized **mammary hyperplasia** is recognized when the gland should be involuted (Fig. 4-131). Hyperplasia within a gland is either of the epithelium of a duct or of the lobule of a gland. *Papillary hyperplasia* within a duct is often an incidental finding or causes cystic ductal dilation. *Lobular hyperplasia* (Fig. 4-132) or subsequent dysplasia occurs at any stage of mammary physiologic events and results in enlargement of one or more lobules, a whole mammary gland, and or mamma and mammae. Lobular hyperplasia has 3 main manifestations. The first is hyperplasia of the epithelium of the glandular acini, often with luminal dilation from secretion. Second is

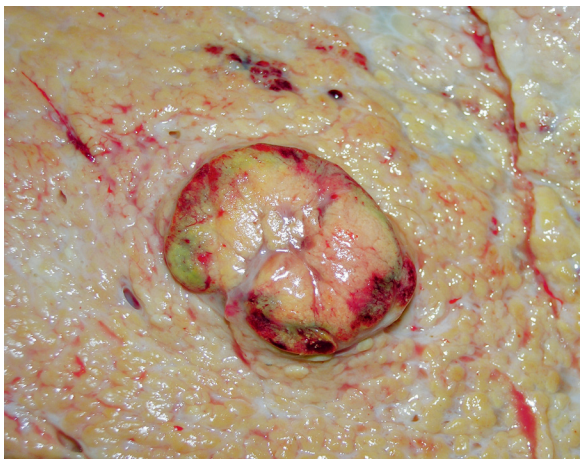

Figure 4-131 Focal hyperplasia of the mammary gland in a cow.

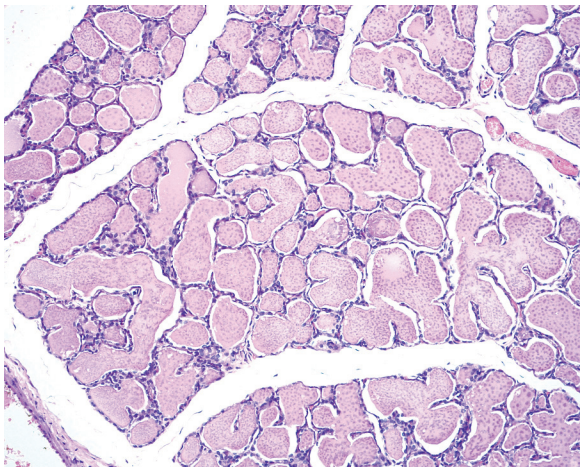

Figure 4-132 Mammary lobular hyperplasia with lactation in a dog.

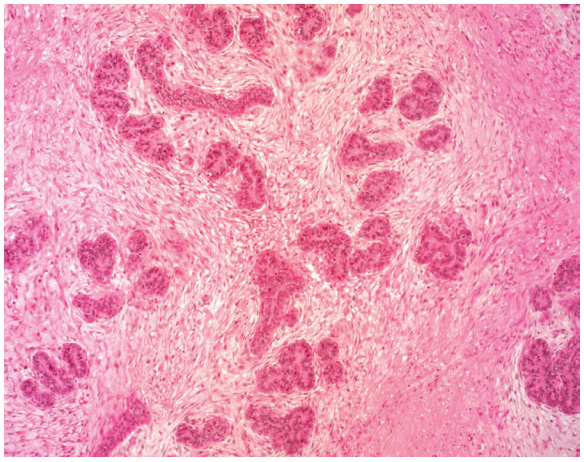

Figure 4-133 Proliferation of lactiferous ducts and surrounding stroma in **fibroepithelial mammary hyperplasia** in a young cat.

adenosis (also called epitheliosis), when the epithelium undergoes hyperplasia and the lumen is filled with basiloid epithelial cells. The third is fibroadenomatous hyperplasia.

Fibroadenomatous hyperplasia is common in cats, especially intact female cats <2 years of age. This spontaneous condition occurs in the luteal phase of estrus, early in pregnancy or after progestin therapy, and accompanies a high serum concentration of progesterone.

Progesterone mediates hyperplasia through growth hormone and insulin-like growth factor 1 in the ductal buds. In young queens, there may be an exaggerated tissue response to prolactin. Resolution is spontaneous; antiprogestin therapy or ovariohysterectomy are effective. Megestrol acetate or other progestins will induce this in old neutered males and females, and drug withdrawal and mastectomy is curative. Whole lobules, glands, or mammae are affected. Clinical enlargement is dramatic, and the affected mamma or mammae are hot and painful, mimicking mastitis. The histologic change is dramatic proliferation of lactiferous ducts that are widely separated by loosely arranged and often concentrically arranged fibrous stroma (Fig. 4-133). Hemorrhage and/or coagulative necrosis of affected glands may occur.

Galactostasis is when a lactating mammary gland is filled with milk, but there is no let-down of milk because of inhibition of release of oxytocin, often from stress. It also occurs after weaning or in pseudopregnancy. It is common in dairy animals of all species. The mammae become engorged, hot, and painful from milk retention; there is no systemic illness or other indicators of mastitis. **Agalactia** is the other extreme, where no mammary enlargement or lactation occurs after parturition. This is rare and the cause is unknown. The udders are hard (hence the colloquial name of "hard udder"), but no milk is produced. In caprine arthritis encephalitis virus (CAEV) in goats and maedi-visna virus (MVV) infection in sheep, there is usually microscopic lymphocytic interstitial inflammation.

Mammary neoplasia in dogs

There is much research in canine mammary neoplasia because it is common and may be a model for breast cancer in women. Little is known of its cause or pathogenesis. Risk factors are well known, and age at neutering has a major effect on its development. Overall prevalence of mammary neoplasia is 2-4%. Study populations vary considerably as spaying bitches at a young age is very common in some regions and not in others. The window of susceptibility of developing mammary neoplasia is up to 2 years of age. Ovariectomy before the first estrus reduces the prevalence to about 0.05%, after the first estrus and before the second about 0.8%, and after the second estrus increases it to about 26%. Late ovariectomy has some protective effect. A high-protein diet decreases susceptibility; medroxyprogesterone acetate treatment and being a purebred increases it. Although the initiating event is unknown, most accept progression from mammary hyperplasia to dysplasia to benign neoplasia and to malignant neoplasia. The majority of publications examine aspects of pathogenesis, particularly molecules, receptors, and genes that are then related to one or more of the classified phenotypes.

Prognosis and grading of mammary neoplasia

The basis of prognosis of neoplasia is determining phenotype and relating this to patient outcome, often with determining a grade. More than 35 phenotypes are described for mammary neoplasia; 7 are benign and the rest are considered histologically malignant (Table 4-2). The large number of types and subtypes is because of wide heterogeneity of mammary tumors in dogs. Surprisingly, studies of prognosis based on patient outcome are few and often have considerable methodologic bias. Results vary greatly, so it is difficult to provide accurate survival data.

The majority of publications concern canine mammary carcinoma. Little is written about mammary sarcomas or those with malignant epithelial and stromal elements (carcinosarcomas) (see later). Some specific types of mammary neoplasia have a poor prognosis. Mammary osteosarcoma, anaplastic carcinoma, lipid-rich carcinoma, micropapillary invasive carcinoma, inflammatory carcinoma, comedocarcinoma, and squamous cell carcinoma are aggressive and usually have short survival times after diagnosis. These are the minority of cases.

Benign mammary neoplasms

Mammary neoplasms with an epithelial and mixed epithelial and myoepithelial component are the most common type in dogs. Benign neoplasms represent more than half of mammary masses. Affected dogs may have one clinically detectable tumor, but they usually have multiple microscopic neoplasms. It is common to see different types within the same mamma. The masses are usually well circumscribed and solid (Fig. 4-134). They have a histologically benign appearance, including lack of invasion, minimal anisokaryosis/pleomorphism, and a low mitotic index (usually <10 per 10 high-power fields). These are divided into adenomas (simple, complex, and basaloid) (Fig. 4-135), fibroadenomas (low and high cellularity), benign mixed tumors (those complex tumors containing bone), and ductal papillomas (Fig. 4-136).

Mammary carcinomas

Although less common than their benign counterparts, mammary carcinomas are usually the main focus of studies of pathogenesis and prognosis. They are very heterogeneous, and classification schemes are becoming increasingly complex. There are special types of carcinoma with a poor prognosis and short survival times; for the others there are prognostic factors. Few studies have an end point of death from neoplastic disease. Many studies use the end point of carcinoma

Table • 4-2

Histologic classification of canine mammary tumors

Benign mammary tumor	Adenoma, simple Intraductal papillary adenoma Ductal adenoma (subtypes include basaloid adenoma and adenoma with squamous differentiation) Fibroadenoma Myoepithelioma Complex adenoma Benign mixed tumor
Mammary carcinomas	Carcinoma, in situ Carcinoma, simple (subtypes are tubular, tubulopapillary, cystic-papillary, cribriform) Carcinoma, solid Carcinoma, anaplastic Carcinoma, micropapillary invasive Comedocarcinoma Carcinoma arising in a complex adenoma/mixed tumor Carcinoma, complex type Carcinoma and malignant myoepithelioma Carcinoma, mixed type Ductal carcinoma Intraductal papillary carcinoma
Mammary carcinomas, special types	Squamous cell carcinoma Adenosquamous carcinoma Mucinous carcinoma Lipid-rich (secretory) carcinoma Spindle cell carcinomas Malignant myoepithelioma Squamous cell carcinoma, spindle cell variant Carcinoma, spindle cell variant Inflammatory carcinoma
Mammary sarcomas	Osteosarcoma Chondrosarcoma Fibrosarcoma Hemangiosarcoma Other sarcomas
Malignant mixed mammary tumor	Carcinosarcoma, malignant mixed mammary tumor

classification, lymphatic invasion, or regional lymph node metastasis as a proxy instead. Publications that use death from neoplasia as an end point report that 20-50% of those diagnosed as carcinoma on histologic criteria result in death from neoplasia. The metastatic rate is variable in reports and is as low as 10%. Most types of mammary carcinoma do not have a sufficient number of cases with 2-year follow-up to have meaningful results. Mammary carcinomas are more prevalent in the older age groups that have a shorter life expectancy anyway. This adds to the complexity and difficulty in providing a valid and accurate prognosis.

Simple carcinomas, solid carcinomas, and complex carcinomas are the more common types. There is considerable

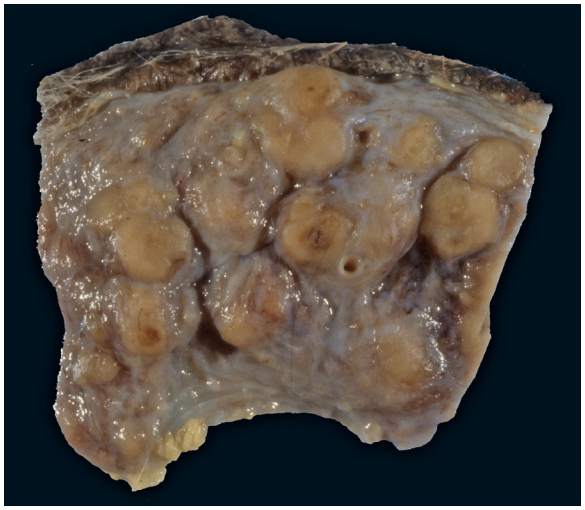

Figure 4-134 Multiple mammary adenomas in a dog.

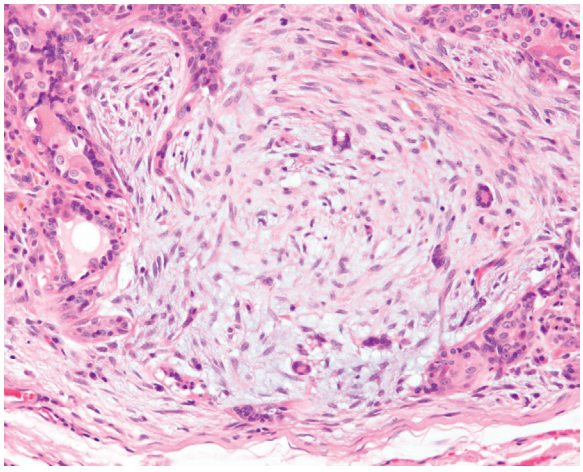

Figure 4-135 Complex mammary adenoma with epithelial and myoepithelial components. Myoepithelial proliferation predominates in this example in this dog.

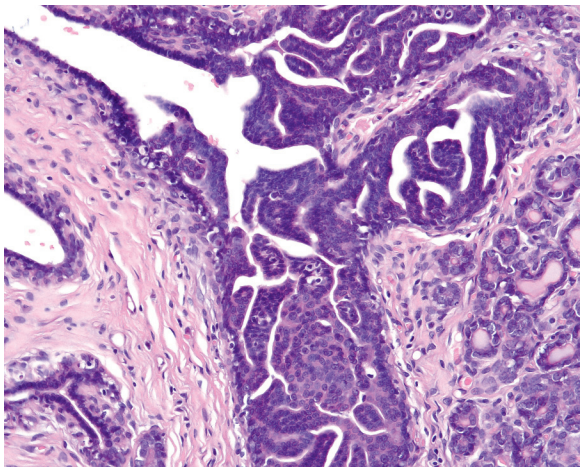

Figure 4-136 Intraductal papilloma in a dog that resulted in cystic ductular dilation.

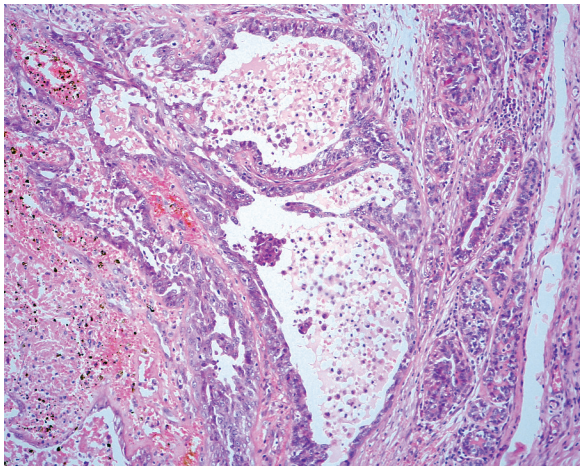

Figure 4-137 A **well-circumscribed mammary carcinoma** in a dog showing compressed fibrous tissue at the periphery. The prognosis is much better than a similar carcinoma that has peripheral invasion.

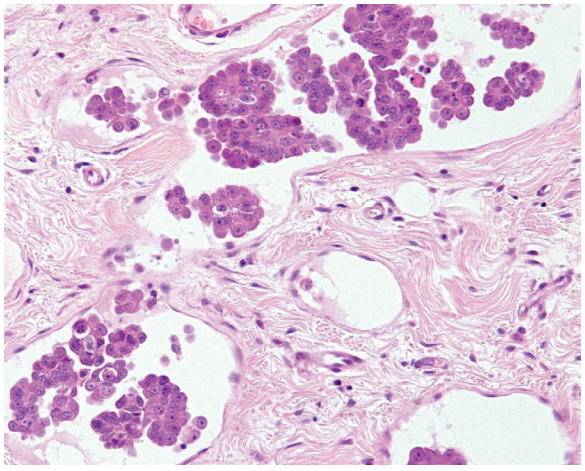

Figure 4-139 **Lymphatic invasion** of a canine mammary carcinoma indicates a very poor prognosis.

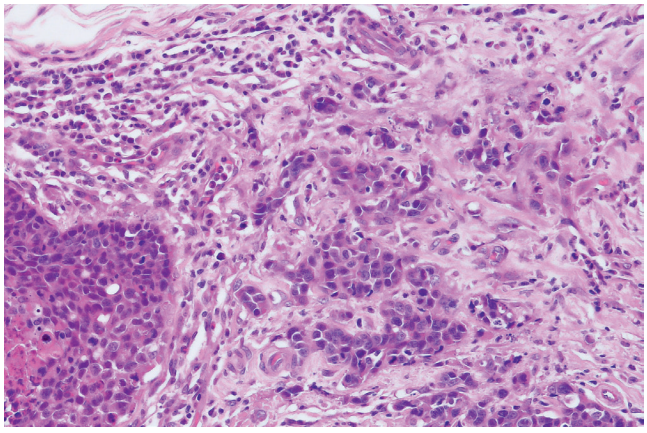

Figure 4-138 A **poorly circumscribed canine mammary carcinoma** with peripheral invasion has a poor prognosis.

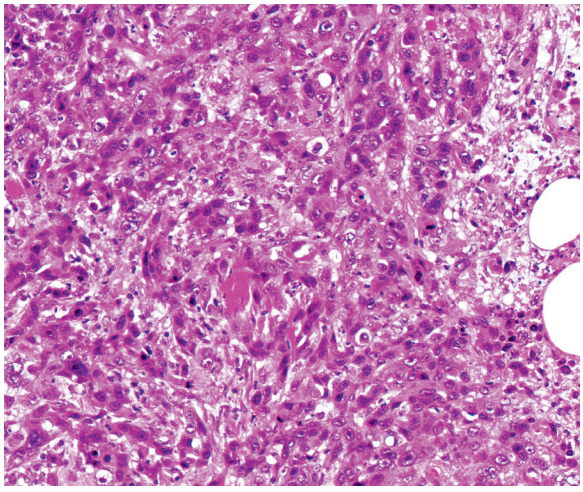

Figure 4-140 **Anaplastic canine mammary carcinoma** has poor differentiation and invasion; both are poor prognostic indicators.

variation in the survival times reported, but only low numbers of these metastasize. Cytology and metastatic potential do not correlate well. In general, the prognosis of mammary carcinomas is based on:

- Age of dog at diagnosis
- Size of neoplasm
- Peripheral infiltrative growth pattern (poorly circumscribed tumors)
- Lymphatic invasion
- Lymph node metastasis
- Poor differentiation and special types
- Grade

A mammary carcinoma >3 cm in diameter has a poorer prognosis, with a significantly shorter disease-free interval and time until death.

Infiltration of a carcinoma into surrounding tissue, variously called peripheral invasion, poorly circumscribed, or infiltrative pattern (Figs. 4-137, 4-138), indicates a poor prognosis, with markedly reduced survival after diagnosis. Despite this, not all carcinomas with this behavior metastasize. Poorly circumscribed or invasive types have a much shorter survival time; for example, 26% of invasive solid carcinomas have a 12-month survival versus 73% for well-circumscribed examples.

Lymphatic invasion (Fig. 4-139) or lymph node metastasis (clinical stage) in mammary neoplasms carries a poor prognosis with short survival intervals after diagnosis. This is particularly the case in the subtype of mammary carcinoma known as *inflammatory carcinoma*. Inflammatory carcinoma is named for its clinical signs of swelling and heat. Histologically, there are neoplastic emboli in dermal lymphatics and no histologic inflammatory reaction.

Carcinomas with decreasing degrees of differentiation are more likely to metastasize; thus simple, solid, and anaplastic carcinomas have, in turn, a greater chance of metastasis and a shorter survival time. Thus classification of the tumor is prognostic (Fig. 4-140). Anaplastic carcinoma, lipid-rich carcinoma, micropapillary invasive carcinoma, comedocarcinoma, and squamous cell carcinoma are aggressive and usually have short survival times after diagnosis.

Grading schemes for carcinomas, using modification of the Elston and Ellis/Nottingham method for human breast carcinoma, are available. There is variability in how the criteria are applied, and the number of cases with good follow-up is

Table • 4-3

Grading scheme for mammary carcinomas

Characteristic	Feature	Points
Tubule formation	Formation of tubules in >75%	1
	Formation of tubules in 10-75% (moderate formation of tubular arrangements admixed with areas of solid growth)	2
	Formation of tubules in (<10%) (minimal or no tubule formation)	3
Nuclear pleomorphism	Uniform or regular small nucleus and occasional nucleoli	1
	Moderate degree of variation in nuclear size and shape, hyperchromatic nucleus, presence of nucleoli (some of which can be prominent)	2
	Marked variation in nuclear size, hyperchromatic nucleus, often with >1 prominent nucleoli	3
Mitoses per 10 HPF	0-9 mitoses/10 HPF	1
	10-19 mitoses/10 HPF	2
	≥20 mitoses/10 HPF	3
Grade	3-5 total points	**I** (low, well differentiated)
	6-7 total points	**II** (intermediate, moderately differentiated)
	8-9 total points	**III** (high, poorly differentiated)

HPF, High-power fields.

relatively low. The schemes are based on degree of tubule formation (in mixed tumors, this is best done in the epithelial-predominant part), nuclear pleomorphism, and particularly, mitotic index (in the region with the highest number of mitoses) (Table 4-3). Those with a grade of III have a worse prognosis.

Mammary sarcomas

Mammary sarcomas represent about 5% of mammary tumors. These are located within mammary tissue and are separate from those that arise in the skin and subcutis. They are identical to their soft tissue counterparts in their appearance and histologic criteria (see Neoplasms of skin and soft tissue). The most well-known is the *mammary osteosarcoma* (Fig. 4-141), a unique type of soft tissue osteosarcoma that has an especially poor prognosis and a very short survival time. The overall prognosis for mammary sarcoma is very poor, and the 6-, 12-, and 24-month survival is 50, 30 and 10%, respectively.

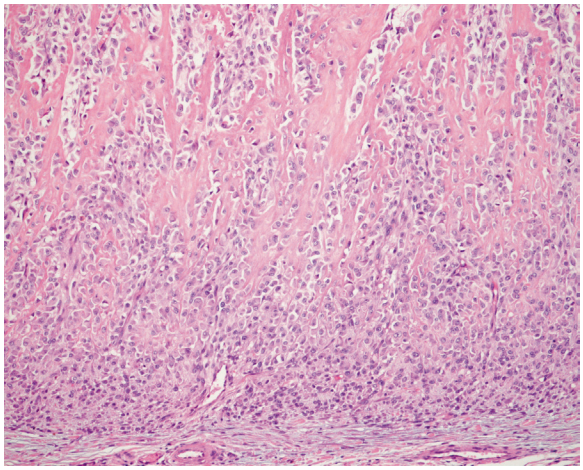

Figure 4-141 Mammary osteosarcomas in dogs are highly metastatic and have a very short survival time after diagnosis.

Mammary neoplasia in cats

Mammary neoplasms are much less common in cats than in dogs, at 20-31 per 100,000 females. Neoplasia in males also occurs. About 75-90% are carcinomas, and most of these are eventually metastatic. The primary tumor is usually adjacent to the nipple. It metastasizes to lymph nodes, lungs, and other mammae. The Siamese and other short-haired pure breeds are particularly affected. There is a 7-9 year-old risk plateau. Intact animals are at a slightly greater risk. Cats spayed before 1 year of age have much less risk of developing mammary carcinoma.

Mammary carcinomas in cats are much less heterogeneous than in dogs, and a myoepithelial component is rare, as are unique or special types such as inflammatory carcinoma. Most are tubulopapillary, solid, or cribriform carcinomas (Figs. 4-142, 4-143). There is no convincing difference in outcome, so most pathologists do not subclassify feline mammary carcinomas. The majority are estrogen receptor, progesterone receptor, and human epidermal growth factor receptor-2 negative on immunohistochemistry too.

The prognosis of feline mammary carcinomas is based on

- Age at diagnosis
- Volume or diameter of the tumor
- Aggressiveness of surgery
- Presence of lymph node involvement (clinical stage)
- Grade

There is a general lack of good long-term follow-up of mammary neoplasia; most studies have only low numbers and are not based on thorough postmortem and histologic evaluation. It does appear that older cats diagnosed with mammary carcinoma have a shorter survival time, but why is not known. Carcinomas usually occur in older cats, however. Mammary carcinomas >3-cm diameter have the worst

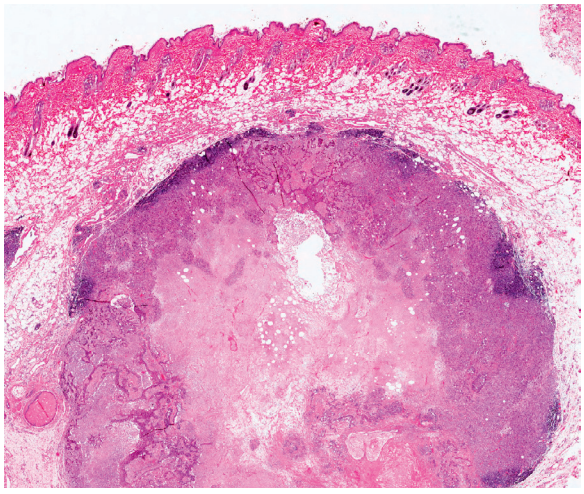

Figure 4-142 Feline mammary carcinoma that is well circumscribed with a necrotic center. Most are metastatic.

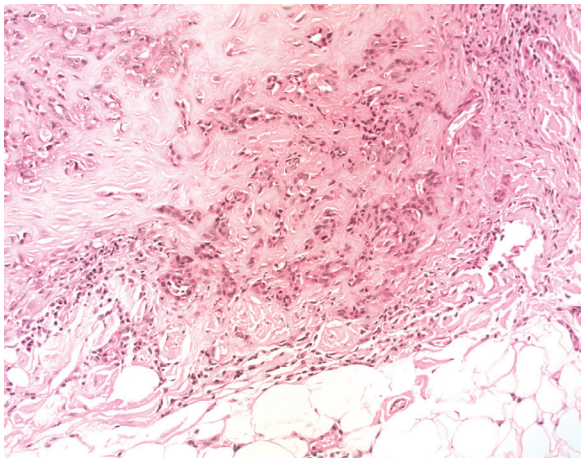

Figure 4-143 Photomicrograph of **tubule formation** in a feline mammary carcinoma.

prognosis, and a survival time of 6 months is anticipated. Those with a tumor diameter of <2 cm have a median survival of >3 years, and those between 2 and 3 cm, 2 years. Mammary carcinomas treated with complete mastectomy appear to have a longer survival compared to lumpectomy. Clinical stage as determined by lymphatic invasion and metastasis to the superficial inguinal or axillary lymph nodes is a poor prognostic indicator.

Grade is an independent prognostic factor. The human mammary carcinoma grading scheme of Elston and Ellis/Nottingham scheme (see Table 4-2) is applied to feline mammary carcinomas, and there is a significant difference in survival time between grades. Those with grade II and III tumors seldom survive beyond 48 and 12 months, respectively. Most mammary carcinomas are grade II or III. Mitotic index is especially important in this scheme.

Mammary neoplasia in other species

Mammary neoplasia is reported in every domestic species, but apart from the dog and cat, is very rare. Mammary adenomas are especially rare and would be undetected unless they cause enlargement of the whole mamma. Reported neoplasms are carcinomas, and most have metastases. Nothing is known of risk factors or of pathogenesis.

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For more information, please visit the companion site: PathologyofDomesticAnimals.com

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