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Small animal reproduction: Scientific facts versus dogmas or unverified beliefs

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ABSTRACT

Small Animal Reproduction is a rather recent topic in the field of Animal Reproduction. Although some continuing educational societies aim to improve the level of general knowledge, published scientific facts are still limited, and there are many hypotheses or affirmations that remain unverified or even sometimes dogmatic or empirical. Through examples, this article reviews the main causes that may sometimes challenge a veterinary practitioner faced with a dog or a cat presenting reproductive problems. It could be difficult to orientate the diagnosis or the therapeutic approach, not knowing if what is found in the literature is a scientific proof or just an unverified affirmation. This problem may result from the fact that there are still physiological processes that remain unclear, but also that some common beliefs are based on old studies that have not been repeated or verifiedIn addition, to make it more challenging to seek for the information, there are unverifiedaffirmations,unclear nomenclature, clinical conditions which have not been standardized in the literature, clinical conditions mostly described through case reports, lack of studies on specific topics, areas of small animal reproduction that have been neglected by researchers, contradictory data or even studies that lack objectivity. The growing interest for research in Small Animal Reproduction will probably reduce the gap between unverified beliefs and scientific facts.

1. Introduction

Small Animal Reproduction (SAR) is a topic that has developed recently. Until the eighties, within most veterinary faculties the teaching content in the field of Animal Reproduction was mainly – and sometimes only – based on reproduction in horses and farm animals, that were considered having an economic value. But progressively, following the growing interest in companion animals in modern societies, the scientific and academic interest for SAR expanded. Some precursors such as Professor Patrick W. Concannon in the USA who began publishing some scientific data about canine endocrinology in 1975 [1], found the basic knowledge essential for further research. The first International Symposium on Canine and Feline Reproduction (ISCFR) was organized in Dublin (Ireland) in 1988, bringing together for the first time around 100 specialized academics or practitioners. It was the beginning of an exponential interest for dog and cat reproduction, including both domestic and wild canids and felids, with an international symposium occurring every 4 years since then. In Europe, a species-oriented continuing education society – the European Veterinary Society for Small Animal Reproduction (EVSSAR) - was created in the nineties and held its first annual meeting in 1998 in Barcelona (Spain), and every year since then. In 2001, the first academic book entirely devoted into SAR was written by Shirley Johnston, Margaret Root-Kustritz and Patricia Olson [2]. It extensively reviewed all the published data until that date and is still nowadays considered as a reference book for most students and academics. However, it probably represented a tremendous amount of work to prepare and since 2001, although some dedicated manuals on SAR have been published in different countries, no similar extensive review on published scientific data has been made and the book of Johnston et al. [2] has not been updated. It means that some data found in this book may have changed in some articles published later, but may not be accessible to veterinary students or general practitioners. This probably contributes to the difficulty of differentiating scientific facts and unverified beliefs in the field of SAR.

In 2004, a study based on an open questionnaire was published. The survey was performed in 86 institutions of veterinary education belonging to 32 European countries, 15 within the European Union (EU) and 17 outside the EU, in Central and Eastern Europe and the European Free Trade Association (EFTA). It was aimed to

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provide a view of the general status of education and research in SAR in Europe [3]. It showed that, although more than 60% institutions were providing a well-balanced comparative teaching in animal reproduction, in some places the teaching was more species orientated. The author concluded that, in the absence of comparative data and research used in education and in the development of new technologies in SAR, there was a risk that sound science-based academic education might be constrained. To counteract these negative trends, SAR should be recognized as a very important branch of the department of animal reproduction. This should lead to the development of a high quality research rejecting empiricism and dogmas.

In the present article we aim – using some relevant examples - to demonstrate that even nowadays, our knowledge in SAR is often based on unverified beliefs more than on scientific facts, and that an effort has to be made to catch up the level of excellence that exists in the field of large animal reproduction or even in human gynaecology, andrology or obstetrics. Furthermore, it appears important to develop clinical approaches based on evidence based medicine (EBM).

2. Unclear data in the physiology of reproduction

There are still some unclear physiological pathways that have not been elucidated in SAR. One of the best examples of a common claim that should be more closely looked at is ovulation in the queen. In most books and academic reviews, including the reference book from Johnston et al. [2], the queen is presented as an induced ovulator, and luteinizing hormone (LH) release from the pituitary gland, and subsequent ovulation, is said to be induced by copulation. However, since the early nineties some authors have reported cases of spontaneous ovulation in groups of queens housed together [4,5]. In wild felids, some species such as lions, clouded leopards, leopards, Pallas' cats, fishing cats or margays may show alternatively induced or spontaneous ovulations [6]. Moreover, it is well known that some non-mated queens may develop pyometra during their life, and that this disease – apart from treatment with progestins - is often linked to progesterone impregnation that follows ovulation [7]. Although a study conducted on demand of an insurance company in Sweden [8] showed that the overall incidence rate (IR) of this disease appeared low (17 cats per 10,000), a significant breed effect was observed. The breed with the highest IR (433 cats per 10,000) was the Sphynx, and other breeds with IR over 60 cats per 10,000 were Siberian cat, Ocicat, Korat, Siamese, Ragdoll, Maine coon, and Bengal. Pyometra was more commonly diagnosed in older animals, with a marked increase in cats over 7 years [8]. An objection to the claim that the queen is a strictly induced ovulator is that a so called "spontaneous ovulation", which appears without any coitus, seems especially frequent in breeding catteries. In Domestic Shorthair queens living in groups of females, the level of spontaneous ovulations may reach up to 87% [5]. In a preliminary study conducted in our experimental cattery [9], we aimed to determine the occurrence and frequency of spontaneous ovulations in a group of 11 queens composed of Domestic Shorthair cats (2/11), Thai cats (6/11), and cross-bred cats (3/11). No male cats were housed in the same building. During the study 24 oestruses were detected in a 7 months period and in 6/24 cases (four queens only), a significant increase of progesterone after oestrus was detected (26.26 ± 9.99 ng/mL). Among these four queens that presented this phenomenon, three were Thai cats and one was a cross-bred queen between a Thai and a Domestic Shorthair. Of course, this is not a scientific demonstration but it raises the question of whether the cat should be considered as an alternatively induced or spontaneous ovulatory species, depending on the breed, on the age, and on the housing.

Another point that remained unclear for a long time in cats concerns the steroidogenic capacity of the placenta as a supplemental source of progesterone during pregnancy, and the possibility for a pregnant queen to maintain her pregnancy even after ovariectomy. In 2001, Johnston et al. [2] wrote that the feline corpora lutea are the main source of progesterone in the pregnant queen, and that placental progesterone is of minor or nonexistent importance. Although in 1993, Verstegen et al. [10] had observed a pregnant queen which, after being treated with the dopamine agonist cabergoline, was able to maintain her pregnancy with a plasma progesterone concentration below 1 ng/mL for more than 7 days. These authors suggested – among other possibilities - that placental paracrine progesterone may allow pregnancy to continue. Surprisingly, this very important issue for feline reproduction was not confirmed until 2012, when Siemienuch et al. [11] demonstrated that placental progesterone concentration was low in early pregnant queens, but increased with gestational age. Their results finally confirmed that the feline placenta is an additional source of progesterone in pregnant queens and could be considered as an endocrine organ helping to maintain pregnancy in this species. Of course, further studies have to be conducted to see when, for how long and in which conditions placental progesterone alone could help to maintain pregnancy in an ovariectomised queen, but at least this discovery has helped to solve an unverified belief.

Another frequent statement is that bitches - to the contrary of queens - are non-seasonal breeders, because they may exhibit oestrus at any time of the year and litters are born during each month of the year [2,12]. A study published in 2011 even stated that the bitch is "typically non-seasonal" [13]. Most publications mention that primitive breeds such as the Australian Dingo, the Wolf hybrids (Saarlos, Czeck) or the African Basenji may display oestrus once a year [2]. However, some studies have shown that there may be an influence of the season on reproductive activity in bitches. In a study, Greyhound bitches were mostly in heat in winter, with a highest incidence in February, while the lowest frequency of their cycles was in November [14]. In another study, the data collected during a four year period showed that a seasonal pattern was observed when the cumulative distribution over years was analyzed, with a higher frequency of oestrous cycles observed during winter and summer [15]. An analysis of 319 interoestrous intervals of 36 Beagles, 36 German shepherd and 20 Labrador retrievers claimed that bitches kept under artificial temperature and light conditions are not as influenced by season as are bitches kept outdoors and thus exposed to seasonal variations in climate and day length [16]. An influence of the breed upon the seasonal aspect of cyclicity was also mentioned in these two studies [15,16]. These data are often neglected but could be interesting to develop new approaches in the control of reproduction in the bitch. For example, the prevention of oestrus may be considered differently depending on the breed, or the use of compounds mimicking a change in the photoperiod, such as melatonin, may be considered for the control of oestrus in bitches, as it is the case in the domestic cat.

3. Common beliefs based on old studies

Some dogmas exist in SAR that may not be completely proven by scientific studies. In this respect, the meta-analysis studies made by the group of Beauvais et al. on the effect of neutering on the risk of developing mammary tumours in dogs [17] and also on the risk of developing urinary incontinence in bitches [18] are perfect examples. Concerning the belief that an early neutering lowers significantly the risk for a bitch to develop mammary tumours later in her life, it is based on a study published in 1969 which has not been repeated since then [19]. This may raise some skepticism when we suppose that the way of evaluating the quality of scientific articles

may have changed considerably since the sixties. Indeed in the first study by Beauvais et al. [17], among 13 selected reports in English-language peer-reviewed journals addressing the association between neutering, age at neutering and mammary tumours, nine were judged to have a high risk of bias. The remaining four were classified as having a moderate risk of bias. One study found an association between neutering and a reduced risk of mammary tumours. Two studies found no evidence of association. One reported "some protective effect" of neutering on the risk of mammary tumours, but no numbers were presented. Due to the limited evidence available and the risk of bias in the published results, the authors concluded that evidence that neutering reduces the risk of mammary neoplasia, and the evidence that age at neutering has an effect, are judged to be weak and are not a sound basis for firm recommendations. Even if this belief appears to be probably true for most veterinary practitioners following bitches throughout their lives (very few bitches spayed at an early age develop mammary tumours), it is wrong to present this as a scientific fact.

The association between spaying and the subsequent development of urinary incontinence in bitches is another example. Another study from Beauvais et al. [18] based on a systematic review of seven selected peer-reviewed original English analytic journal articles treating the effect of neutering or age at neutering on the risk of urinary incontinence concluded that four articles were judged to be at high risk of bias. Of the remaining three studies, which were at moderate risk of bias, there was some weak evidence that neutering, particularly before the age of three months, increases the risk of urinary incontinence. For these authors, the evidence overall was neither consistent nor strong enough to allow for strict recommendations on the effect of neutering or age at neutering on the risk of urinary incontinence.

These two studies based on the Cochrane guidelines for systematic reviews of interventions [20] give a good approach on how we should re-analyze, under the light of updated reviewing standards, some old articles or common veterinary protocols that are considered as "true", just because they have been published in peer-reviewed journals.

Some statements considered as true beliefs because that have been put forward by highly considered scientists may have to be reconsidered. Let us take the example of the endocrinology of pregnant cycles in the bitch. Concannon and his colleagues, in their preliminary studies in the eighties, described an increase of blood testosterone and oestrogens during pregnancy, suggesting heightened luteal secretion of these steroids [13,21]. However, in a study in 1994, Hoffmann et al. [22], investigating the hormonal changes around parturition in the dog and the occurrence of pregnancy-specific non conjugated oestrogens, found that no pregnancy specific increase of oestradiol-17 beta could be observed. Oestradiol-17 beta levels decreased prior to parturition concomitant with the decrease of progesterone, suggesting a likewise luteal origin of oestradiol-17 beta in the pregnant and non pregnant dog. No hints in respect to a specific placental oestrogen production were obtained when examining placental tissue. In a review published in 2012, Kowalewski [23] stated that there is no pregnancy- and/or parturition-specific increase in oestrogens in the bitch.

4. Unverified beliefs

One example may be taken on the use of preputial smears to detect an abnormal oestrogenic impregnation in male dogs, often linked with a feminizing syndrome due to a Sertoli-cell testicular tumour. Elevated concentrations of oestrogen in serum are supposed to cause cornification of preputial epithelial cells, which may be evaluated as for a bitch in oestrus [24]. This technique is commonly used in daily practice avoiding measurement of blood oestrogens, which is often complicated to perform (it requires a specific laboratory able to assay oestradiol) and to interpret. To the best of our knowledge, the first study evaluating the sensitivity and specificity of this technique was published only in 2012 [25], on 45 dogs with palpable testicular masses and 30 healthy control dogs. The authors concluded that the preputial cytology has a high sensitivity and specificity for the diagnosis of oestrogen producing testicular tumours in dogs. It means that for many years – preputial smears being an old and common technique – veterinarians were using "empirically" this cytological examination without a real scientific confirmation. To the contrary of preputial smears whose interest has been finally confirmed, there is probably still a huge number of beliefs and practices in the field of SAR that haven't yet had the chance to be confirmed – or infirmed – by scientific works, and are supposed to be useful, although this may not be the case.

Another example is the common belief that a superfoetation (i.e. simultaneous presence in the uterus of foetuses of different gestational ages) may occur in the queen as a consequence of anecdotal oestrous activity during pregnancy and subsequent matings occurring in a pregnant queen [2]. In carnivores, this phenomenon, apart from the cat, has been suspected to occur in the Badger (*Meles meles*), the Geoffroy's cat (*Leopardus geoffroyi*), the Lion (*Panthera leo*); the American mink (*Mustela Neovison*) or the Leopard (*Panthera pardus*) [26]. Personal communication with many cat breeders tend to indicate that most of them have observed queens delivering viable kittens up to several weeks apart. Surprisingly, although this phenomenon seems to exist, there are very few – and only old – case reports that have been published on this topic and still no confirmation of its reality in domestic cats with experimental procedures in which the paternity of the born kittens could for example be tested.

5. Unclear nomenclature

Another factor that may disrupt the establishment of a consensus in the field of SAR is the nomenclature. In other terms, different specialists may use similar terms to designate different problems or diseases. A good example is to be found when speaking about uterine diseases in bitches or queens. A more complete classification of the uterine diseases would be very helpful to ensure that everybody is using the same terminology. For example, there is often a confusion when using the term "metritis". Some authors make a difference between metritis (usually occurring after an oestrus accompanied by mating) and pyometra (occurring during dioestrus) [27]. In 2006, Fieni [28] makes a difference between metritis (putrid vaginal discharge with no enlargement of the uterine lumen), open pyometra (putrid vaginal discharge with enlargement of the uterine lumen) and closed pyometra (enlargement of the uterine lumen without vaginal discharge). Two years later, Verstegen et al., in 2008 [29] only used the term "pyometra".

A similar confusion may exist when using the term "endometritis", as it may designate different pathological conditions, which may be acute or chronic, and may be associated or not with CEH [35]. For some authors [30], it defines a sub-clinical inflammation of the endometrium that does not extend beyond the *stratum spongiosum*, corresponding histologically to a localized breakthrough into the endometrial epithelium, infiltration of inflammatory cells, vascular congestion, stromal oedema and accumulation of lymphocytes and plasma cells in the upper layers of the endometrium. This "endometritis" corresponds more or less to the second stage described by Dow in 1959 [31] from pathological and histological observations. This author described 4 stages of degradation of the uterine endometrium: stage 1 (Increase of the number and irregularity of endometrial glands), stage 2 (plasmocytic infiltration of the endometrium), stage 3 (cystic endometrial hyperplasia (CEH)

with an acute inflammation and even zones of haemorrhage), stage 4 (CEH with chronic endometritis, lympho-plasmocytic infiltration of the endometrium and modifications of the myometrium). This heterogeneity of the nomenclature complicates our understanding of the role of sub-clinical endometritis as creating infertility in the bitch. For example, in a recent study [32] using *in vitro* explants of endometrium taken from bitches suffering from CEH, the authors postulated that these bitches were accurate models of a mating-induced endometritis because the bitches with endometrial hyperplasia had a mild histological inflammatory reaction similar to that noted by De Bosschere et al., in 2001 [33]. However, in 2013 in the study of Mir et al. [34] who analyzed the histological features of surgical biopsies taken on infertile bitches or bitches who suffered from pregnancy arrest, there were several cases of lympho-plasmocytic endometritis or neutrophilic endometritis that were not associated with endometrial hyperplasia.This need to clarify the nomenclature has been put forward by Schlafer and Gifford [36], who defined histologically some different degrees of cystic conditions of the canine and feline uterus, and even introduced the definition of a new condition called “pseudo-placentational endometrial hyperplasia”.

Another unclear statement concerns mammary fibroadenomatosis in the queen, a disease characterized by enlarged mammary glands, often under the influence of luteal progesterone or the use of exogenous progestins [2]. Histologically, two types of conditions have been identified: a lobular hyperplasia – also called intraductal papillar hyperplasia – which appears to be a result of proliferation of the mammary gland duct epithelium, and a fibroepithelial hyperplasia – also called diffuse fibroepithelial hyperplasia which is a general enlargement of the mammary glands [37]. However, in practice two clinical conditions may be found: one is a diffuse mammary hypertrophy mostly diagnosed in young queens with solid – fibrous - content, that is commonly treated with success using the progesterone receptor blocker aglepristone [38], another form is a cystic aspect of the mammary glands, which appear full of liquid with often a blue aspect of the skin. This second form develops often in older queens and doesn’t respond very well to the use of aglepristone. If it is the same disease or if its pathogenicity is exactly the same is not really known. In order to avoid any confusion, the term of “mastosis” (by analogy with a human disease, in which the role of estrogens predominates) has been suggested by some others to describe the cystic clinical condition [39]. But specific veterinary research is still lacking to better characterize this disease in the cat.

6. Clinical conditions without a standardized description

There are also some diseases or abnormalities that are commonly found but do not have a well-described monography. One of them is a perineal fold found in adult bitches and which is supposed to promote the development of vaginitis due to lack of drainage and maceration within the vaginal lumen [40]. This existing condition lacks a consensual description and denomination. Meanwhile the term of “perineal fold” or “occluded vulva” has been proposed [40,41]. Episioplasty, which consists of the surgical ablation of the excess of perineal skin and associated subcutaneous tissue, is of great interest in this case [41].

7. Clinical conditions mostly described through case reports

In the field of SAR, many diseases or clinical findings give rise to the publication of case reports, which may just be anecdotic and not at all representative of the most frequent clinical forms of a disease. In an analysis of all abstracts presented during the successive annual congresses of the EVSSAR and which remained unpublished in peer-reviewed journals afterwards, Robin and Fontbonne [43] discovered that 92/375 abstracts (24.5%) were case reports, which may indicate an over-representation of rare or unusual cases in the field of SAR. This is the case, for example, concerning ovarian diseases. In a recent analysis of the literature, Arlt and Haimerl [42] pointed out that only very little research has been performed on ovarian diseases in the bitch in the last decades, and that our knowledge is mostly based on published clinical reports. In addition, different definitions of diseases and different diagnostic procedures are applied. According to these authors, systematic research results on the clinical and reproductive features of ovarian diseases are not available. Many publications are based on case reports that, in general, provide little evidence. Also, studies with small sample sizes may provide biased impressions about the nature or presentation of a disease, if extreme or rare cases are frequently reported. Regarding cystic ovarian diseases, mostly cases with follicular cysts have been published [42,43]. This might be due to the fact that various amounts of hormones are secreted, either causing overt clinical signs and/or oestrous irregularities. Most published case reports documented large cysts. This suggests that factors such as size, hormonal activities or other rare patterns may cause a publication bias meaning that weird cases are more likely to be published than ordinary ones [43]. This has to be considered when reviewing the literature on ovarian diseases and other rare conditions.

8. Lack of knowledge and lack of studies

The role of infectious diseases in reproduction is a good example. In dogs, with the notable exception of *Brucella canis*, exogenous bacterial pathogens are sporadic causes of reproductive diseases [44]. Most commonly, bacterial infections of the reproductive tract are endogenous in origin; many of the bacteria etiologically involved in reproductive disease are part of the urogenital microflora [44]. Bacterial reproductive diseases are therefore frequently opportunistic, and predisposing factors must be present for disease to develop [44]. The role of *Mycoplasma* and *Ureaplasma* spp. on reproductive problems is still unclear [45]. Certain viruses, such as the minute virus of canines (MVC) or canine Herpesvirus (CaHV), may also play a detrimental role, although the conclusions of different studies are not always clear and are often based on experimental conditions [46]. Faced with pregnancy arrest or abortion in a breeding bitch, veterinarians often do not know what to search for, as the number of proven pathogens towards reproduction is low. Indeed, there is a lack of data on the exact role of many diseases including – but not only - Leptospirosis, Neosporosis, Q fever (*Coxiella burnetii*) or the Blue Tongue virus.

In cats, there are even fewer recent reviews about the role of

infectious agents towards feline infertility [45,46]. Among viral diseases, only retroviruses (FelV, FIV) and the parvovirus have been confirmed as detrimental agents for fertility or pregnancy. The role of Feline Herpesvirus (FHV) or Calicivirus remains unclear and not fully demonstrated in the field, and more studies should be made on this aspect, as these two diseases are of major concern in most catteries. Feline Coronavirus is considered as an uncommon cause of reproductive problems [47]. But all these affirmations are based on very few studies and remain hypothetical. Among bacterial diseases, the main concern is about *Chlamydophila felis*. It has been suggested as a cause of infertility [48], although this has not been fully demonstrated in the field. Other publications report a potential negative role to fertility of *Coxiella burnetii*, *Leptospira* sp. or *Bartonella henselae*. A report indicated also that cats may be sensitive to *Brucella* sp. [49]. There is a special concern about *Toxoplama gondii*. In experimental conditions, *trans*-placental contamination and even abortion may occur [50], but in the field the situation remains unclear.

Another annoying lack of studies concerns the potential promoting effect of recurrent episodes of overt pseudopregnancy with lactation (PSP) in the development of mammary tumours in bitches. According to some authors [51,52] bitches presenting PSP seem to have an increased risk of mammary tumours in comparison with bitches without PSP. They claim that, in these animals, tumours are detected earlier and are more often malignant. The risk seems to be higher if the bitches have developed many successive PSP and when they get older. There seems to be a significant risk after a bitch has undergone three clinical PSP [52]. Due to the very high frequency of PSP diagnosed in veterinary clinics, such studies would be interesting to pursue and of high clinical interest. Unfortunately very few retrospective studies have been presented and they were not conclusive [53].

Sometimes the use of a drug or a procedure is too recent and the results are based on an insufficient number of animals to be able to claim that it brings strong scientific data. For example, the GnRH agonist deslorelin has been commercialized in Europe for about 10 years, and has been used in experimental protocols in dogs and in cats, in-label and off-label. To have data on the reversibility after implanting male cats with the most common 4.7 mg SC implant, there is only one published study based on only 10 male cats housed in experimental conditions [54], although there are some published studies based on the 9.4 mg SC implant. Veterinarians should not consider this study as giving a definitive scientific conclusion. Recent unpublished work in our group tend to show that there are considerable variations depending on the breed and on the environment [55]. Thereforeit is recommended to remain prudent in drawing any conclusion until there is enough data-collected. Another example may be the use of a mechanical drainage in the bitch for the treatment of pyometra. Some authors have described successful treatments using an intra-uterine drainage, followed by subsequent pregnancies [56]. Surgical drainage and intrauterine lavage resulted in further fertility in 100% of eight treated bitches [57]. But there is without any doubt too few studies to draw any conclusion.

9. Contradictory data showing the need of further investigations

In some topics, there may be contradictory data between different authors, which may complicate some decisions in daily veterinary practice. Contradictory results are common in fields of research in which just a few number of patients or cases can be enrolled or in research protocols that do not take into account bias in the proper way. Contradictory results in different researches on the same topic means that no definitive conclusion can be drawn, and therefore there is a need to produce more research in a specific field.

For example, spaying bitches before the first oestrus – with the reserves already mentioned [18] - is often sought by owners to reduce the risk of further mammary tumours. But does it promote the risk of urinary incontinence? A study done in Switzerland on 206 bitches that had been spayed before their first oestrus and for which the owners were questioned on the occurrence of urinary incontinence as a result of spaying in at least the 3 years that followed, the incidence of urinary incontinence was approximately half that of spaying after the first oestrus [58]. A completely different conclusion was drawn by another study producing evidence that neutering before first oestrus increases the risk in comparison to after puberty [59,60]. And as already said, a meta-analysis of all published papers does not permit one to draw conclusions because of the many biases [18].

Many examples of contradictory conclusions exist in the field of SAR. For example, veterinarians often wonder if it is necessary to spay a bitch that has been surgically operated for a mammary carcinoma, and if it will increase her chance of survival. Some studies claim that it is indeed an effective adjunct therapy to tumour removal and that, depending on the timing of spaying, it may influence the survival [61]. Other studies claim the contrary and declare that spaying when mammary tumours are removed does not have a significant effect on the progression of malignant disease and that about one in four bitches with a benign mammary tumour is likely to develop a further tumour in another gland [62].

In the topic of artificial insemination (AI) there are also a lot of contradictory data. In the dog, some authors claim that the addition of autologous prostatic fluid, when using frozen-thawed semen, increases the pregnancy rate and the litter size [63]. Other authors claim that the pregnancy rate, whelping rate and litter size are reduced when frozen-thawed, prostatic fluid-supplemented semen is vaginally deposited [64]. Also when inseminating a bitch with frozen-thawed semen it is a common belief that intra-uterine inseminations give better results than intra-vaginal deposition of semen. Linde-Forsberg et al. [65] obtained a whelping rate of 84.4% with intra-uterine deposition versus 58.4% for intra-vaginal inseminations. Surprisingly, Rota et al., in 2010 [66] claimed that 10/10 pregnant bitches were pregnant after intra-vaginal deposition of semen 4 and 5 days after the estimated LH peak, using 200 millions of sperm per AI. Othaki et al. [67] published successful results of intra-vaginal AIs with frozen semen that were above 85%. One hypothesis may be that nowadays veterinarians detect more carefully the time of ovulation than 10 or 20 years ago and that it may not be necessary anymore to deposit the semen inside the uterine lumen. It could also depend on the fertility of the bitch or the sire, and a distinction has to be made between experimental and clinical trials.

In cats also, there are contradictory data. One of them is the potential detrimental effect that anaesthesia – mandatory in felids due to the difficulty of contention – has on ovulation and on the subsequent pregnancy rate when inseminating with frozen-thawed semen. Howard et al. [68] reported that queens inseminated (laparoscopic intrauterine) after ovulation produced more corpora lutea and embryos and had a higher pregnancy rate than those inseminated before ovulation (50% vs 14.3% pregnancy rate). Because of the concern that anaesthesia may inhibit ovulation, many researchers elect to inseminate after ovulation has occurred (28–40 h). However, others have not found an effect of anaesthesia on ovulation rate and claimed even better pregnancy rate when anaesthetized queens were inseminated before ovulation than after (10/18 (56%) vs 5/24 (21%)) [69]. When freezing cat semen, the addition of the detergent Equex STM-paste is controversial, with some authors claiming that the addition of Equex to the freezing

extender had a significant positive effect on the percentage of intact acrosomes immediately after thawing, but had a negative effect on the longevity of the spermatozoa; the percentages of membrane intact and motile spermatozoa being significantly lower in the presence of Equex than in the controls at 6 h after thawing [70]. Other authors wrote that even if sperm motility and membrane integrity decreased more rapidly in presence of Equex than those in controls, total motility and sperm viability were similar at 3 h and 6 h after thawing [71].

10. Fashionable trends and potentially oriented studies

Some topics are discussed more than others in congresses and appear fashionable at some time. But it is dangerous to claim scientific facts before enough data has been collected and enough studies published. One recent example is the potential increased risk of developing cancer after spaying in the dog. Increased discussion on the influence of neutering on cancer development has been recently prompted with several studies that seem to indicate that incidence of some cancers may be increased with castration or spaying in the canine populations. Indeed these studies tend to show an increased risk of prostatic carcinomas, osteosarcomas, transitional cell carcinomas [72], lymphomas, haemangiosarcomas [73] or mastocytomas [74] in spayed animals, often with a sex-predisposition. But most of them concern only specific breeds and for now it is impossible to say that neutering a dog promotes the development of cancer. Although these data are thought-provoking, we should not extrapolate findings in single dog breeds to the entire species [75].

Another topic that has to be considered with caution is early neutering in dogs and cats. For the past 10–15 years, it has developed following several publications and because it is encouraged by animal welfare societies to limit the number of feral dogs and cats and abandoned animals, but also by dog and especially cat breeders to protect their genetics and avoid others to breed animals bearing their genetics. Sometimes it may appear that publications promoting this approach lack some objectivity. If such a practice seems indeed interesting to limit uncontrolled reproduction of non-Pedigree pets and feral animals, it should not be forgotten that it may also promote potential health problems such as – among others - urinary incontinence [18], diseases of the lower urinary tract, the development of a recessed or infantile vulva [76] or the risk of epiphyseal fractures due to the delay of closure of growth cartilages in long bones [77].

11. Conclusion

At the end of this review based on chosen examples, it appears important to develop clinical approaches using evidence based medicine (EBM), and to build further research on strict and statistically well managed protocols. Following the example of the group of Beauvais et al. [17,18] it would be very valuable to make critical reviews of founding articles that have been published a long time ago. Due to the growing interest in SAR, a high quality of published data should be mandatory, which also implies finding accurate and meticulous reviewers. The growing interest for getting knowledge and learning techniques in the field of SAR will probably reduce the gap between unverified beliefs and scientific facts.

Declaration of competing interest

The authors have no conflict of interest to declare.

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