Assessment of the luteal phase in stimulated and substituted cycles

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The endocrine profile of the natural cycle

Central to the modern concept of reproduction in all mammalian is the brain, from which springs the function of all the rest. It is therefore appropriate to start this part of the physiology of the reproductive system with the role of the brain.

The hypothalamus

It has long been surmised that the reproductive processes, such as the menstrual cycle or ovulation, must in some way be under nervous control, since many reproductive phenomena arise in consequence of environmental changes. For instance amenorrhoea in a woman can result from psychological stress (Bomba *et al*., 2007).

Within the brain, there are two major sites of action that are important for the regulation of the reproductive function: the hypothalamus and the pituitary gland (Speroff *et al*., 1994). The pituitary gland is divided into three regions or lobes: anterior, intermediate, and posterior. The anterior pituitary (adenohypophysis) is quite different structurally from the posterior neural pituitary (neurohypophysis), which is a direct physical extension of the hypothalamus. The adenohypophysis is derived embryologically from epidermal ectoderm from an infolding of Rathke's pouch. Therefore, it is not composed of neural tissue, as is the posterior pituary, and does not have direct neural connections to the hypothalamus (Berek, 2002).

The elevation of the pituitary at the beginning of the 20th century put physiologists in a dilemma. No nervous connection between the brain and the anterior pituitary could be revealed. The mystery was solved by G. Harris (1970) who showed that while there was no nervous connection between the brain and the anterior pituitary, there was a direct *vascular* channel between the hypothalamus above and the pituitary below, which serves as a mean to convey a biological signal (neurohormones) from the nervous system to the gland.

The specific secretory cells of the anterior pituitary have been classified based on their hematoxylin- and eosin-staining pattern. The gonadotropins, LH and FSH, are secreted by basophilic cells. Acidophilic-staining cells primarily secrete GH and prolactin and, to a variable degree, ACTH (Duello and Halmi, 1979).

The neurohormone that controls gonadotrophins is called gonadotrophin-releasing hormone (GnRH) also called luteinizing hormone - releasing hormone (LHRH) (Blackwell *et al*., 1973). The biochemical structure of GnRH was first described by Andrew Schally and Roger Guillemin in 1971, an accomplishment, for which the authors received the Nobel Prize.

It is a decapeptide produced by neurons with cell bodies primarily in the nucleus arcuatus of the hypothalamus with a half life of 2-4 minutes (Krey *et al*., 1975; Plant *et al*., 1978; Amoss *et al*., 1971). The short half-life of GnRH is the result of rapid proteolytic cleavage (Soules *et al*., 1985; Filicori *et al*., 1986).

GnRH is unique among releasing hormones in that it simultaneously regulates the secretion of two hormones- FSH and LH. It also is unique among the body's hormones because it must be secreted in a

Table 1. — Menstrual cycle variation in LH pulse Frequency and Amplitude.

Cycle Phase	Mean frequency (minutes)	Mean Amplitude (mIU/mL)
Early follicular	90	6.5
Mid-follicular	50	5
Late-follicular	60-70	7
Early luteal	100	15
Mid-luteal	150	12
Late luteal	200	8

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pulsatile fashion to be effective, and the pulsatile release of GnRH influences the release of the two gonadotropins (Dierschke *et al*., 1970; Knobil E, 1980; Belchetz *et al*., 1978).

GnRH is released into portal blood and regulates LH and follicle-stimulating hormone (FSH) release from the pituitary gonadotropes by binding to its specific receptors located on these cells. GnRH receptors are upregulated by pulsatile GnRH, while they are submitted to down regulation when LHRH or its analogues are administered in a non-pulsatile fashion (Melcangi, 2002).

The pulsatile secretion of GnRH varies in both frequency and amplitude throughout the menstrual cycle and is tightly regulated (Table 1, Soules *et al*., 1985; Filicori *et al*., 1986).

Among many factors that integrate the activity of the GnRH neuronal system, estrogens play the most important role. Estrogens exhibit a negative feedback action on LH secretion. However, in addition to the negative feedback, E_2 also exhibits a positive feedback influence upon the activity and output of GnRH neurones to generate the preovulatory LH surge and subsequent ovulation (Herbison AE, 1998). Despite the evidence supporting the essential role of estradiol in triggering the preovulatory surge of gonadotropins, there is substantial evidence that indicates an important role of progesterone (P) in inducing or in facilitating this surge (Hotchkiss *et al*., 1982). P appears to act at several levels, since it may exert direct regulatory effects on pituitary cells, it is also able to act at the hypothalamic level, via the modulation of GnRH synthesis and of its pulsatile release (Ramirez *et al*., 1985). Moreover, P appears to be required for a full pituitary responsiveness to GnRH. In fact, after ovariectomy plus adrenalectomy, E_2 alone is not able to induce a preovulatory LH surge (Mahesh and Brann, 1998).

Numerous neuroactive substances have also been implicated as neurotransmitters and neuromodulators controlling GnRH release (Barraclough *et al*., 1984; Kalra, 1986; Terasawa, 1995). Among them NPY (Neuropeptide Y neurons), Norepinephrine (NE), GABA, glutamate, and Nitric oxide are contributors controlling pulsatile GnRH release (Terasawa, 1998). The main modulators dopamine, serotonin, opioid (mainly β-endorfin and dynorphin) decrease GnRH release from the hypothalamus (Andersen, 1987; Genazzani and Petraglia, 1989). Moreover, ovarian sex steroids can increase the secretion of central endorphins, further depressing gonadotropins (Reid *et al*., 1981). Endorphin levels vary significantly throughout the menstrual cycle, with peak levels in the luteal phase and a nadir during menses (Gindorff and Ferin, 1987). This inherent variability, although helping to regulate gonadotropin levels, may contribute to cycle-specific symptoms experienced by ovulatory women (Halbreich and Endicott, 1981).

Gonadotropins

The gonadotropins FSH and LH are produced by the anterior pituitary gonadotroph cells and are responsible for ovarian follicular stimulation. Structurally, there is a great similarity between FSH and LH. They are both glycoproteins that share an identical α-subunit and differ only in the structure of their β-subunit, which confers receptor specificity (Fiddes, and Talmadge, 1984). The synthesis of the β-subunit is the rate regulating step in gonadotropin biosynthesis (Lalloz *et al*., 1988). The α-subunit consists of 92 aminoacids stabilized by 5 disulfide bonds, while the β-subunit contains 118 amino acids and 5 sialic acid residues. Neither subunit has any intrinsic biologic activity by itself. The variation of the sialic acid component is responsible for the different half life of these hormones. Sialic acid prevents the hepatic clearance; thus, the greater the sialic acid component, the longer the half life (Morell *et al*., 1971). HCG, for example, with 20 sialic acid residues, has the longest half life (about 24 hours), whereas LH (1 to 2 sialic acid residues) has a very short half life (20-30 minutes) (Morell *et al*., 1971).

Two cells - two gonadotropins

The fundamental principle of follicular development is the two cells - two gonadotropins theory (Erickson, 1986). This theory states that there is a subdivision and compartmentalization of steroid hormone synthesis activity in the developing follicle.

According to the "Two cell two gonadotropin theory" (Kobayashi *et al*., 1990), both FSH and LH are necessary for ovarian follicular maturation and the synthesis of ovarian steroid hormones. LH promotes the production of androgens (dehydroepiandrosterone, androstenedione, and testosterone) from cholesterol and pregnenolone, by stimulating 17α-hydroxylase activity in the thecal cells. The androgens then diffuse to the granulosa cells where FSH stimulates the expression of the cytochrome P450 aromatase, which converts the androgens to estrogens (Erickson *et al*., 1985).

Rising estrogen levels have a negative feedback effect on FSH secretion. Conversely, LH undergoes biphasic regulation by circulating estrogens. At lower concentrations, estrogens inhibit LH secretion. At higher levels of estrogen (200pg/ml) for more than 48 hours, estrogen enhances the LH release (Young and Jaffe, 1976).

The local estrogen-FSH interaction in the dominant follicle induces LH receptors on the granulosa cells that results in luteinisation of the granulosa cells, production of progesterone and initiation of ovulation, that will occur in the single mature follicle 10-12 hours after the LH peak or 34-36 hours after the initial rise in mid-cycle LH (Pauerstein *et al*., 1978).

The mid-cycle LH surge is responsible for a dramatic increase in local concentrations of prostaglandins and proteolytic enzymes in the follicular wall (Yoshimura *et al*., 1987). Due to these substances the follicular wall is progressively weakened and is perforated with a slow extrusion of the oocyte through this opening (Yoshimura and Wallach., 1987).

The luteal Phase:

The luteal phase is defined as the period between ovulation and either the establishment of a pregnancy or the onset of menses 2 weeks later (Fatemi *et al*., 2007).

When the ovum is discharged at ovulation, it takes with it a covering of granulosa cells. The remaining granulosa cells staying behind are attached to the wall of the collapsed follicle. The exit hole of the ovum is sealed by a fibrinoid plug. From the endocrine point of view the most significant event in the early development of the corpus luteum is the fact that the capillaries of the theca interna penetrate the basal membrane in response to secretion of angiogenic factors such as the vascular endothelial growth factor (VEGF) (Anasti *et al*., 1998) and the granulose layer becomes vascularized. This angiogenic response allows large amounts of luteal hormones to enter the systemic circulation. The granulosa cells remaining in the follicle begin to uptake lipids causing the characteristic yellow lutein pigment. These cells are active secretory structures that produce progesterone, estrogen and inhibin A.

In women and other primates, steroid hormone production by corpora lutea depends on the presence of continued LH production (Devoto *et al*., 2000).

If conception and implantation occur, the developing blastocyst secretes human chorionic gonadotrophin (hCG). The role of hCG produced by the embryo is to maintain the corpus luteum and its secretions (Penzias, 2002). The estimated onset of placental steroidogenesis (the luteoplacental shift) occurs during the 5th gestational week, as calculated by the patients' last menses (Scott *et al*., 1991).

Early History of the Corpus Luteum

Coiter (1573) described the presence of cavities filled with a yellow solid in the ovary, but it was de Graaf (1943) who gave the first definitive description of these structures. Malpighi (1689) provided an accurate microscopic description of these structures and was the first to apply the name corpus luteum, literally the yellow body. Beard (1897) postulated that corpora lutea were responsible for the suppression of ovulation and estrus during pregnancy, and about that time, Prenant (1898) suggested that the corpus luteum might be a gland of internal secretion directly benefiting the egg with which it appeared to be associated. It was, however, Fraenkel (1903) who demonstrated that corpora lutea were necessary for implantation and the subsequent maintenance of pregnancy in the rabbit. Corner and Allen (1929) and Allen and Corner (1930) prepared a relatively pure alcoholic extract of corpora lutea from sows and showed that this extract maintained pregnancy in ovariectomized rabbits. A few years later, the isolation of the pure crystalline hormone was reported simultaneously by four groups (Butenandt *et al*., 1934; Hartmann and Wettstein, 1934; Slotta *et al*., 1934; Wintersteiner and Allen, 1934). Slotta *et al*. (1934) named the compound progesterone and suggested a structural formula, and in the same year, the compound was synthesized by Butenandt and Westphal (1934).

The endometrium

The endometrium is the mucosal lining of the uterine cavity. Its basic function is the creation of a suitable

environment for embryo nidation. Though implantation could occur in any human tissue, the endometrium is the only tissue, which is not receptive to embryo implantation except during a restricted frame of time called the 'implantation window' (Minas *et al*., 2005).

The endometrium can morphologically be divided into an upper two third 'functionalis" layer and a lower one third "basalis" layer. The purpose of the functionalis layer is to prepare for implantation of the blastocyst and therefore it is the site of proliferation, secretion and degeneration. The purpose of the basalis layer is to provide the regenerative endometrium following menstrual loss of the functionalis (Speroff *et al*., 1994).

As the major target of sex steroid hormones, the endometrium will undergo characteristic cycles of proliferation, secretory changes and tissue shedding in response to ovarian steroid hormones (Bourgaine C., 2001/2002). The endometrial cycle is a reflection of the ovarian cycle, corresponding with two phases of cellular development, separated by ovulation.

The primary control over endometrial maturation is considered to be exerted by P and E_2. Studies on pregnancy outcome suggest that an optimal balance of the two hormones is necessary for a normal progression of pregnancy (Lejeune *et al*., 1986).

The endometrium proliferates due to the stimulation of E_2 produced by the granulosa cells in the follicular phase. The highest response is in the glands. There is first an increase in mitotic activity and secondly there is formation of a loose capillary network in the spiral vessels (Tavanioutu, 2006).

After the ovulation, there is a secretory transformation of the endometrium due to the progesterone produced by the corpus luteum.

Under the action of progesterone, endometrial proliferation ceases and glandular secretion initiates. The endometrial glands become tortoise and spiral vessels coiled. In the glandular epithelium subnuclear intracytoplasmic glycogen vacuoles appear that start to move towards the glandular lumen, followed by an active secretion of glycoproteins and peptides in the endometrial cavity. During the secretory phase, a short specific period of uterine receptivity toward embryonic implantation is designated as the ''implantation window'' (Harper, 1992). The peak of the secretory endometrial activity is around the 5-7th post-ovulatory day, coinciding with the time of the embryo implantation.

Luteal Phase defect

As early as 1949, the premature onset of menses was recognized as indicative of a luteal phase deficiency of progesterone production, which was shown to be correctable by exogenous progesterone administration (Jones, 1979). The prevalence of a luteal phase defect in natural cycles in normoovulatory patients with primary or secondary infertility was demonstrated to be about 8.1% (Rosenberg *et al*., 1980).

Pathophysiologic alterations of the complex reproductive process that lead to delayed endometrial maturation characteristic of LPD include disordered folliculogenesis, defective corpus luteum function, and abnormal luteal rescue by the early pregnancy. A variety of clinical conditions, such as hyperprolactinemia, hyperandrogenic states, weight loss, stress, and athletic training may result not in oligo- or anovulation, but rather may be manifest as LPD (Ginsburg, 1992).

The three main causes of luteal phase defect in unstimulated cycles include poor follicle production, premature demise of the corpus luteum, and failure of the uterine lining to respond to normal levels of progesterone.

Luteolysis

Basal levels of LH in the human appear to be essential to maintain the secretory function of the corpus luteum (Van de Wiele *et al*., 1970). In the rhesus monkey, bilateral lesions in the arcuate nucleus of the hypothalamus caused a cessation of ovarian ovulatory activity that could be restored by chronic circhoral infusions of GnRH (Knobil *et al*., 1980). If plasma levels of LH were reduced to undetectable levels during the midluteal phase by halting GnRH infusions in these lesioned monkeys, plasma progesterone fell to undetectable levels. However, when LH levels were restored 3 days later by resuming circhoral GnRH infusions, the corpus luteum resumed a normal pattern of progesterone secretion but regressed at the expected time (Hutchinson and Zeleznik, 1985). These studies suggest that LH acts to promote progesterone synthesis by the corpus luteum but that other factors are responsible for the loss of function and structural integrity of the primate corpus luteum during luteolysis.

It has long been considered that luteolysis might be an intraovarian event. Early studies suggested that estrogen produced by corpus luteum mediated luteolysis (Knobil, 1973). Subsequent work indicated that estrogen may act by increasing PGF_2 levels in the ovary. This view was based on the finding that exogenous estrogen increased the concentration of PGF_2 in ovarian venous blood (Auletta *et al*., 1973) and that indomethacin blocked estrogen-induced luteolysis in the rhesus monkey (Auletta *et al*., 1976). However, it was found that high levels of estrogen (10 μg/ml) inhibited progesterone synthesis by

human luteal cells, both in the presence and absence of indomethacin (Thibier *et al*., 1980). Later studies suggested that the luteolytic effect of exogenous estrogen in the primate may be due to its suppression of pituitary gonadotropin secretion rather than a direct effect on the ovary (Schatz *et al*., 1985). Moreover, estrogen receptors are absent in all cell types of the primate corpus luteum (Hild-Petito *et al*., 1997). The finding that administration of either an aromatase inhibitor (Ellinwood *et al*., 1983) or an estrogen antagonist (Albrecht *et al*., 1981) does not prolong the life span of the corpus luteum in monkeys indicates that estrogen may not be a direct mediator of luteolysis in primates. However, it is possible that estrogen may have indirect actions in the ovary or the corpus luteum other than via estrogen receptors. However, the exact mechanism of luteolysis is not known and in future studies it will be required to resolve this question.

How to define a luteal phase defect?

Although LPD has been clearly described in research settings, the diagnosis remains controversial (Jordan *et al*., 1994). A defective luteal phase in the natural cycle was defined if the serum mid-luteal progesterone levels are less than 10 ng/ml (Jordan *et al*., 1994). However, mid-luteal P levels do not always reflect the endometrial maturation (Batista *et al*., 1994). Therefore, in the literature the most reasonable consensus of a defective luteal phase is a lag of more than two days in endometrial histological development compared to the expected day of the cycle (Jones, 1991, Dawood, 1994).

Ovarian stimulation and luteal phase defect

However, with the advent of ovarian stimulation for IVF, it has been established that the luteal phase of all stimulated IVF cycles are abnormal (Edwards *et al*., 1980). The etiology of luteal phase defect in stimulated IVF cycles has been debated for more than two decades. Initially, it was thought that the removal of large quantities of granulosa cells during the oocyte retrieval (OR) might diminish the most important source of progesterone synthesis by the corpora lutea, leading to a defect of the luteal phase. However, this hypothesis was disproved when it was established that the aspiration of a preovulatory oocyte in a natural cycle neither diminished the luteal phase steroid secretion nor shortened the luteal phase (Kerin *et al*., 1981).

Another proposal suggested that the prolonged pituitary recovery that followed the GnRH agonist co-treatment, designed to prevent spontaneous LH rise in stimulated cycles resulting in lack of support of the corpus luteum, would cause a luteal phase defect (Smitz *et al*., 1992). It was also suggested that the hCG administered for the final oocyte maturation in stimulated IVF cycles could potentially cause a luteal phase defect by suppressing the LH production via a short-loop feedback mechanism (Miyake *et al*., 1979).

However, the administration of hCG did not down-regulate the LH secretion in the luteal phase of normal, unstimulated cycles in normo-ovulatory women (Tavaniotou and Devroey, 2003).

The introduction of GnRH antagonists in IVF raised speculations that a rapid recovery of the pituitary function (Albano *et al*., 1996) would obviate the need for luteal phase supplementation (Elter and Nelson, 2001).

Preliminary observations in intrauterine insemination (IUI) cycles seemed to favor this contention. Ragni *et al*. (2001) explored the luteal phase hormone profiles in gonadotrophin stimulated cycles both with and without GnRH antagonist therapy for IUI. No deleterious effects of GnRH antagonist administration could be noted on either the luteal progesterone concentration or the duration of the luteal phase in that study.

However, various studies of GnRH antagonist co-treatment in IVF have since found different results. Luteolysis is also initiated prematurely in antagonist co-treated IVF cycles, resulting in a significant reduction in the luteal phase length and compromising the chances for pregnancy (Albano *et al*., 1998; Beckers *et al*., 2003).

Beckers *et al*. (2003), evaluated the non-supplemented luteal phase characteristics in patients undergoing ovarian stimulation with recombinant FSH combined with a GnRH antagonist (antide; 1mg/day). However, due to unacceptably low pregnancy rates (overall 7.5%), the decision was therefore made to cancel this study after 40 patients were included. Luteolysis also started prematurely with the administration of GnRH antagonist.

Despite the rapid recovery of the pituitary function in GnRH antagonist protocols (Dal Prato and Borini, 2005), luteal phase supplementation remains mandatory (Tarlatzis *et al*., 2006).

It appears that the main cause of the LPD, observed in stimulated IVF cycles, is related to the multifollicular development achieved during ovarian stimulation, which alter completely the hormonal environment. It can be postulated that one of the main causes of the luteal phase defect in stimulated IVF cycles is supra-physiological levels of steroids secreted by a high number of corpora lutea during the early luteal phase, which directly inhibit the LH release via negative feedback actions at the hypothalamic-pituary axis level (Fauser and Devroey, 2003).

Studies in human and primates have demonstrated that the corpus luteum requires a consistent LH stimulus in order to perform its physiological function (Jones, 1991). LH support during the luteal phase is entirely responsible for the maintenance and the normal steroidogenic activity of the corpus luteum (Casper and Yen, 1979). As a result, withdrawal of LH, unnecessary causes premature luteolysis (Duffy *et al*., 1999)

The HCG administered for final oocyte maturation covers the luteal phase for a maximum of 8 days (Fatemi *et al*., 2007). In normal circumstances, thereafter LH would stimulate the corpora lutea, but due to the suppressed LH levels in IVF cycles, there is no stimulus of the corpora lutea.

The luteal phase support

Progesterone

Csapo *et al*. (1972 and 1973) demonstrated the importance of progesterone during the first weeks of a pregnancy. In their initial study, the removal of the corpus luteum prior to seven weeks of gestation led to pregnancy loss (Csapo *et al*., 1972). However, the authors found that pregnancy could be maintained even after removal of the corpus luteum by external administration of progesterone (Csapo *et al*., 1973).

Progesterone induces a secretory transformation of the endometrium in the luteal phase (Bourgain *et al*.,1990). By inducing this change after adequate estrogen priming, progesterone improves endometrial receptivity (Kolibianakis and Devroey, 2002). Endometrial receptivity is a self-limited period in which the endometrial epithelium acquires a functional and transient ovarian steroid-dependent status that allows blastocyst adhesion (Martin *et al*., 2002). Decreased endometrial receptivity is considered largely responsible for the low implantation rates in IVF (Paulson *et al*., 1990).

Progesterone also promotes local vasodilatation and uterine musculature quiescence by inducing nitric oxide synthesis in the decidua (Bulletti and de Ziegler, 2005). Inadequate uterine contractility may lead to ectopic pregnancies, miscarriages, retrograde bleeding with dysmenorrhea and endometriosis (Bulletti and de Ziegler, 2005).

The uterine-relaxing properties of progesterone were supported by a study of IVF embryo transfer outcomes by Fanchin *et al*. (2001). This study investigated the consequences of uterine contractions (UC) as visualized by ultrasound (US) during embryo transfer. Results indicated that a high frequency of uterine contractions on the day of embryo transfer hindered transfer outcome, possibly by expelling embryos out of the uterine cavity. A negative correlation between UC frequency and progesterone concentrations was detected underlining the benefits of progesterone in IVF (Fanchin *et al*., 2001).

Currently available formulations of progesterone include oral, vaginal, rectal and intramuscular (i.m.) (Penzias, 2002; Chakmakjian, 1987). Progesterone administered orally is subjected to first-pass prehepatic and hepatic metabolism. This metabolic activity results in progesterone degradation to its 5α- and 5β-reduced metabolites (Penzias, 2002). Parenteral administration (vaginal, rectal and i.m.) of progesterone surpasses the metabolic consequences of orally administered progesterone (De Ziegler *et al*., 1995).

Oral progesterone

Oral micronized progesterone was used for luteal support in IVF with poor results until the end of 1980s (Buvat J, *et al*., 1990). Devroey *et al*. (1989) and Bourgain *et al*. (1990) reported an absence of the secretory transformation of the endometrium in patients with premature ovarian failure who had been treated with oral micronised progesterone when compared to patients treated with intramuscular injections or vaginal micronised progesterone. This finding suggested that oral administration reduced the hormone's bioavailibility.

To overcome this problem, dydrogesterone (DG) was introduced to support the luteal phase of stimulated IVF cycles (Belaisch-Allart *et al*., 1987). DG, a retroprogesterone with good oral bioavailability, is a biologically active metabolite of progesterone and has an anti-estrogenic effect on the endometrium, achieving the desired secretory transformation (Whitehead, 1980; Chakravarty *et al*., 2005).

Recently, Chakravarty *et al*. (2005) undertook a prospective, randomized study (n = 430) that compared the efficacy, safety and tolerability of oral DG with vaginal micronised progesterone as luteal phase support after in-vitro fertilization (IVF). Both DG and P were associated with similar rates of successful pregnancies (24.1% vs. 22.8%, respectively; P = NS).

However, it has been demonstrated clearly that after sufficient estrogen endometrial priming, exogenous administered vaginal micronised progesterone is significantly more effective than oral dydrogesterone in creating an 'in phase' secretory endometrium. (Fig. 1, Fig. 2, Fatemi *et al*., 2007).

The oral DG might be sufficient for luteal supplementation in IVF cycles; however more large randomized controlled trails are needed, before a conclusion can be made.

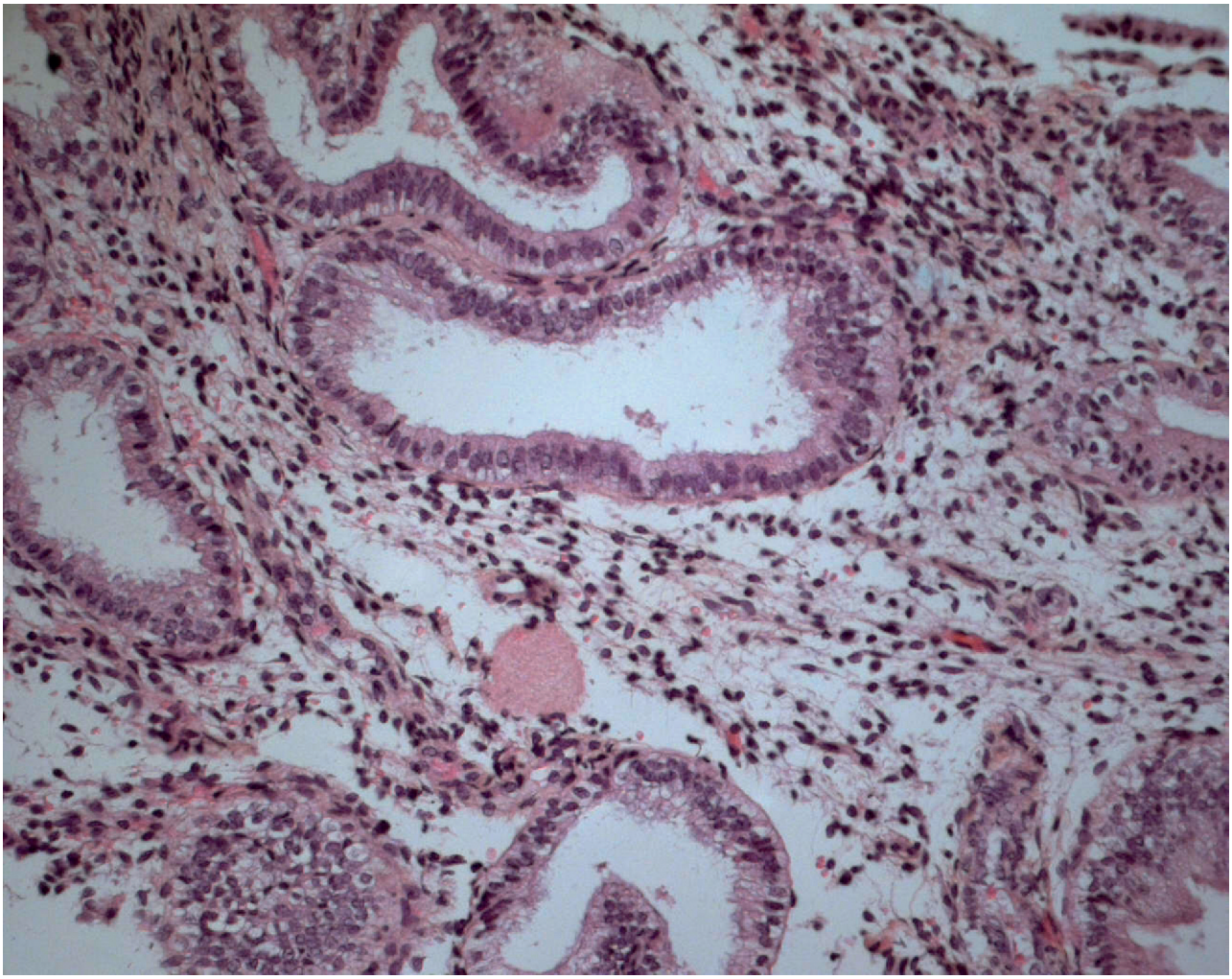

Fig. 1. — Endometrial biopsy after micronized progesterone. Coiled glands with active secretion and minimal residual vacuoles. Stromal edema. Absence of mitotic activity. The maturation corresponds to day 6 of the luteal phase (HES, 200×).

Rectal progesterone

A number of publications have evaluated the rectal use of natural progesterone in women undergoing IVF/ICSI (Chakmakijan *et al*., 1987; Ioannidis *et al*., 2005). Chakmakijan *et al*. (1987) studied the bioavailability of micronized progesterone (P) by measuring sequential serum P concentrations after a single bolus of 50-200 mg P given sublingually, orally (capsule and tablet), vaginally and rectally (suppositories) during the follicular phase of a group of normally menstruating women. When compared to other routes of P administration, rectal application resulted in serum concentrations during the first eight hours twice as high as other forms. However, to the best of our knowledge, there are no prospective randomized trials to compare the rectal administration of progesterone with other administration routes for IVF.

Vaginal progesterone

The intravaginal route of progesterone supplementation in IVF has gained wide application as a first choice luteal support regimen, mainly due to patient comfort and effectiveness (Levine *et al*., 2000). Following intravaginal administration of progesterone, high uterine progesterone concentrations with low peripheral serum values are observed, due to counter-current exchange in progesterone transport between anatomically close blood vessels (Cicinelli *et al*., 2000) and due to the uterine first pass effect, where liver metabolisation is absent (De Ziegler *et al*., 1995).

There is increasing evidence in the literature that vaginal P is at least as effective as i.m. P at providing luteal support in induced cycles (Simunic *et al*., 2007). In the latest meta-analysis by Nosarka *et al*. (2005), vaginal and intramuscular progesterone had

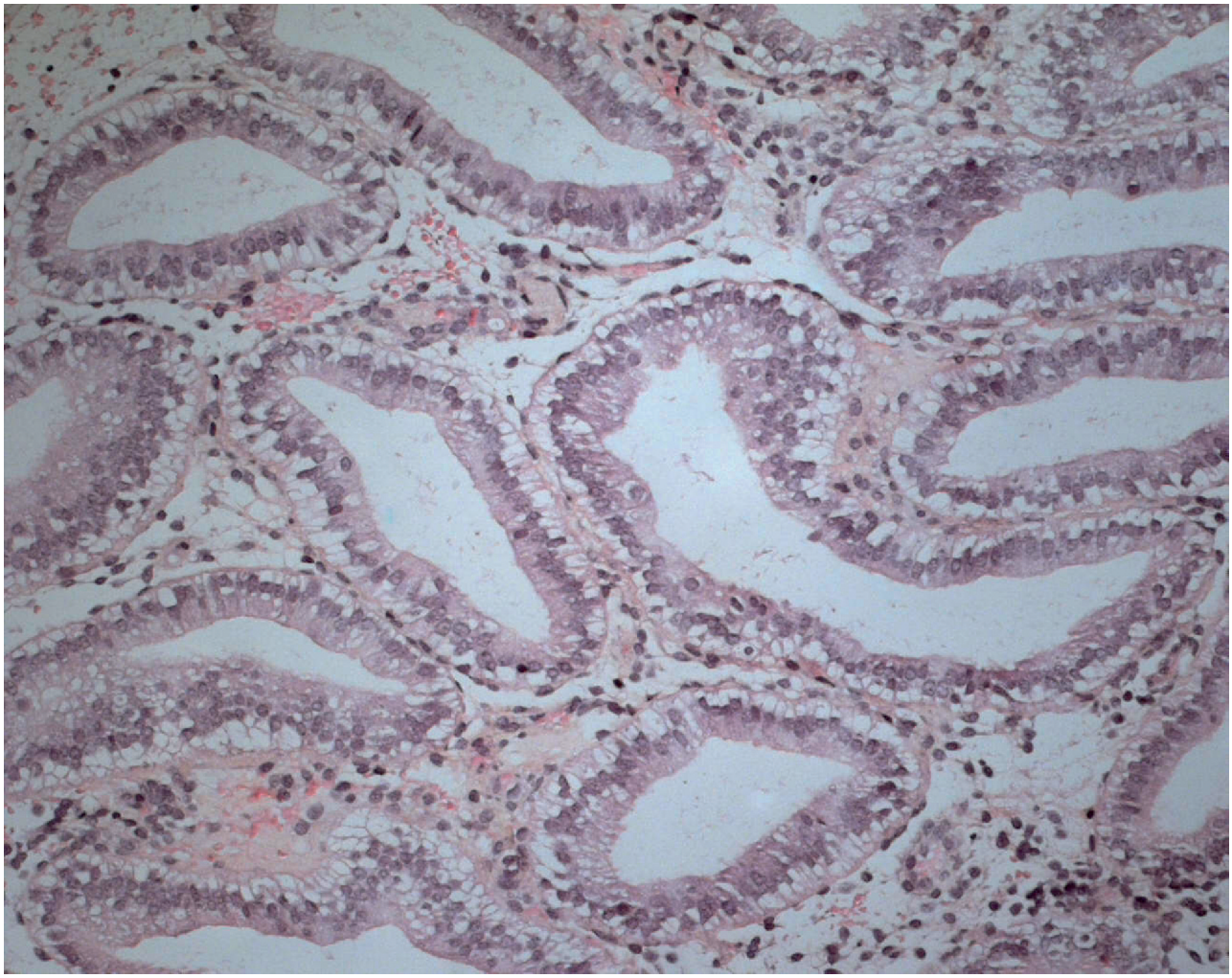

Fig. 2. — Endometrial biopsy after Dydrogesterone. Small glands with minimal coiling and persistant homogeneous subnuclear vacuoles and pseudostratified nuclei. No stromal edema. Focal mitotic activity. The maturation corresponds to day 2-3 of the luteal phase (HES, 200×).

comparable implantation and clinical pregnancy rates. In Europe, there are two different forms of intravaginal progesterone on the market, natural micronised progesterone (Utrogestan® Laboratories Besins International, Paris, France) and Crinone ®8% (Fleet Laboratories Ltd., Watford, United Kingdom), a controlled and sustained-release vaginal gel. Utrogestan ® 100 mg capsules are administered vaginally three times two capsules daily (600 mg/d) whereas Crinone 8% is administered vaginally once a day, i.e. 90 mg, (Simunic *et al*., 2007; Ludwig *et al*., 2002).

To establish the minimal effective dose of vaginal micronized progesterone, Chanson *et al*. (1996) conducted a small (n = 40) prospective randomized study comparing two different dose regimens (400 mg versus 600 mg each day). No differences in clinical pregnancy rates were noted. However, further prospective randomized trials are essential to define the necessary dose of vaginal micronized progesterone for luteal phase support in IVF.

In a prospective, randomized study Ludwig *et al*. (2002) compared vaginal Crinone 8% with vaginal Utrogestan® for luteal phase support. Clinical pregnancy rates, clinical abortion rates until 12 weeks of gestation and ongoing pregnancy rates were comparable between the two groups (Ludwig *et al*., 2002).

Simunic (2007) and Ludwig (2002) evaluated the tolerability and acceptability of both preparations from patients' point of view. Crinone® 8% gel proved more tolerable than Utrogestan® vaginal capsules because of a lower number of side effects (Simunic *et al*., 2007; Ludwig *et al*., 2002).

Intra muscular (i.m.) Progesterone

I.M.. progesterone supplementation is given as an injection of natural progesterone-in-oil (Costabile *et al*., 2001).

In 1985, Leeton *et al*. first demonstrated the extension of the luteal phase of stimulated IVF cycles

treated with 50 mg i.m. progesterone. The doses of i.m. progesterone used for luteal phase support vary between 25 and 100mg per day without any significant difference concerning the outcome (Pritts and Atwood, 2002).

This route of administration is often associated with a number of side effects, including painful injections and a rash (Lightman *et al*., 1999), causing a lack of enthusiasm for this treatment modality (Costabile *et al*., 2001). Injections of Progesterone in oil can also lead to inflammatory reactions and abscess formation (Propst *et al*., 2001).

In addition, several case reports have been published in which patients receiving i.m. progesterone for luteal supplementation have developed acute eosinophilic pneumonia (Bouckaert *et al*., 2004; Veysman *et al*., 2006). This drug-induced disease shows that the use of i.m. progesterone can also be associated with a severe morbidity in otherwise healthy young patients (Bouckaert *et al*., 2004).

In an open-label trial in 1184 women from 16 U.S. American centers Levine evaluated the clinical and ongoing pregnancy rates in IVF cycles involving vaginal and i.m. progesterone. Vaginal and i.m. progesterone were found to have comparable clinical (35.05% V.S. 35.2%, respectively) and ongoing pregnancy rates (30.2% and 33.64%, respectively) (Levine, 2000).

A meta-analysis published in 2002 by Pritts and Atwood included five prospective randomized trails comparing i.m. administration of progesterone with vaginal. A total of 891 cycles were evaluated in those studies. Clinical pregnancy rate and delivery rate were significantly higher when i.m. progesterone was used (RR clinical pregnancy rate/ET 1.33 (95% CI:1.02-1.75, Delivery rate 2.06 (95% CI:1.48-2.88)).

Progesterone plus estradiol

The two most important hormones produced by the corpus luteum are progesterone and estradiol (Fatemi *et al*., 2007). The role of progesterone for luteal support in stimulated cycles is well established (Fatemi *et al*., 2007). However, it has not yet been clearly demonstrated whether additional supplementation of E_2 in stimulated IVF cycles may be beneficial (Fatemi *et al*., 2007).

In a prospective randomized study, Smitz *et al*. evaluated the possible benefit of adding estradiol valerate 6 mg per os daily to the vaginal micronised progesterone (600 mg daily) given as luteal supplementation in 378 women treated with a gonadotrophin releasing-hormone agonist and human menopausal gonadotrophins for in IVF (Smitz *et al*., 1993). The clinical pregnancy rate was similar between the two groups (29.2% with the estradiol co-treatment and 29.5% with progesterone only treatment). Also Lewin *et al*., (1994) in a prospectively randomized study, could not find any advantage in the addition of 2 mg estradiol valerate to Progesterone as luteal phase support of long GnRH agonist and hMG-induced IVF-ET cycles in one hundred patients (clinical pregnancy rate 26.5% versus 28% with and without estradiol co-treatment, respectively)

A meta-analysis by Pritts and Atwood (2002) suggested that addition of estrogen to progesterone might improve the implantation rates. However, the authors referred to only one study confirming the beneficial effect of estradiol in the luteal phase (Farhi *et al*., 2000).

Any beneficial effect of adding E_2 to progesterone might depend upon its dosage. Lukaszuk *et al* (2005), in a prospective, randomized study, recently evaluated the effect of different E_2 supplementation doses (0, 2, or 6 mg) during the luteal phase on implantation and pregnancy rates in women undergoing intracytoplasmic sperm injection (ICSI) in agonist cycles (n = 231). Significantly higher pregnancy rates (PR) were recorded in those who received low dose E_2 supplementation compared with no estradiol substitution (PR 23.1% vs. 32.8%). The best pregnancy results were found in the group with high dose E_2 supplementation (PR 51.3%). It was shown that the addition of a high dose of E_2 to daily progesterone supplementation significantly improved the probability of pregnancy in women treated with a long GnRH analogue protocol for COH.

Farhi *et al*. (2000), in a prospective, randomized study, evaluated the effect of adding E_2 to progestin supplementation during the luteal phase in 271 patients undergoing IVF who had E2 levels of higher than 2500 pg/dL at the day of hCG administration. All patients received progesterone supplementation at a dosage of 150 mg/d starting on the day after the oocyte retrieval (OR). Patients were randomized into two groups: those receiving 2 mg of E_2 (Estrophem; Novo Nordisk, Bagsvaerd, Denmark), given orally, starting on day 7 after ET; and those receiving no exogenous E2 supplementation during the luteal phase. It was shown that for those patients who had been treated with the long GnRH agonist protocol for COH, the addition of E_2 to the progestin support regimen had a beneficial effect on pregnancy and implantation rates (39.6%, and 25.6% with and without estradiol co-treatment respectively; $P < .0.05$). However, such an effect could not be shown for patients with a short, GnRH agonist protocol.

Different studies were conducted to examine whether the probability of pregnancy is increased by adding estrogen to progesterone for luteal phase

support in patients treated by IVF. However, the currently available evidence as published in meta-analysis by Kolibianakis *et al*., 2008 suggests that the addition of estrogen to progesterone for luteal phase support does not increase the probability of pregnancy in IVF in both GnRH agonist and antagonist cycles.

Human Chorionic Gonadotropin (hCG)

Since it was found that the corpus luteum can be rescued by the administration of hCG, this treatment has become the standard care for luteal support since the late 1980s (52). By stimulating the corpora lutea, hCG is an indirect form of luteal support. It is known to generate an increase in estradiol and progesterone concentrations thus rescuing the failing corpora lutea in stimulated IVF cycles (Fatemi *et al*., 2007).

Administration of hCG has also been shown to increase the concentrations of placental protein 14, integrin and relaxin (luteal peptide hormone) which has been shown to increase at the time of implantation (Fatemi *et al*., 2007).

In the meta-analysis published by Pritts and Atwood in 2002, hCG was shown to be equally effective as progesterone for luteal phase support with respect to pregnancy rates.

The disadvantage of using hCG for luteal support stems from its potential for increasing hyperstimulation rates when compared with other treatments or no treatment at all. Significant increases in hyperstimulation rates have been confirmed in several studies (Fatemi *et al*., 2007).

With regard to ovarian hyperstimulation syndrome (OHSS), one should therefore be cautious with the administration of hCG for luteal supplementation in stimulated IVF cycles (Fatemi *et al*., 2007). Luteal support with hCG should be avoided if estradiol levels are above 2500-2700 pg/ml on the day of hCG administration (Fatemi *et al*., 2007) and if the number of follicles is above 10 (Fatemi *et al*., 2007).

GnRH agonist: a novel luteal-phase support?

GnRH agonist was recently suggested as a novel luteal-phase support that may act upon pituitary gonadotrophs, the endometrium and the embryo itself (Tesarik, 2006).

It has been hypothesized that GnRH agonist may support the corpus luteum by stimulating the secretion of LH by pituitary gonadotroph cells or by acting directly on the endometrium through the locally expressed GnRH receptors (Pirard *et al*., 2005).

In a prospective randomized study, Tesarik *et al*. (2006) evaluated the effect of GnRH agonist (0.1 mg triptorelin) administration in the luteal phase on outcomes in both GnRH agonist (n = 300) and GnRH antagonist (n = 300) ovarian stimulation protocols. They were randomly assigned to receive a single injection of GnRH agonist (study group) or placebo (control group) 6 days after ICSI.

The pregnancy rates were enhanced for both protocols, in long GnRH agonist protocol the clinical implantation rate were 29.8% (97/325) vs. 18.2% (60/330) respectively ($P < 0.05$). Ongoing pregnancy rates were 46.8% (66/141) vs. 38.0% (54/142) respectively (P = NS).

In patients treated with the GnRH antagonist protocol, clinical implantation rates were 27.1% (86/317) vs. 17.4% (57/328) respectively ($P < 0.05$) and ongoing pregnancy rates were 44.8% (65/145) vs. 31.9% (46/144) respectively ($P < 0.05$).

Luteal-phase GnRH agonist administration additionally increased the luteal-phase serum HCG, estradiol and progesterone concentrations in both ovarian stimulation regimens. It was postulated that the beneficial effect may have resulted from a combination of effects on the embryo and on the corpus luteum.

Despite these initial encouraging results, it is too early to adopt this treatment wholesale.

With regard to safety, great concern exists about possible adverse effects on oocytes and, more importantly, on embryos (Lambalk and Homburg, 2006).

To establish a potential positive role of GnRH agonist administration in the luteal phase of stimulated IVF cycles, further large prospective trials are needed.

The duration of luteal phase support

Until recently, there were no studies to either support or contest the generally accepted practice of prolonging progesterone supplementation during early pregnancy.

Schmidt *et al*. (2001) was the first to publish a retrospective study to compare the delivery rate with IVF or ICSI in women who received progesterone supplementation with those who did not during the first weeks of pregnancy. For three weeks following a positive hCG test, 200 pregnant women received progesterone and 200 pregnant women received none (study group). The results showed no difference in the delivery rate. Of the 200 pregnancies in the study group, 126 (63%) ended in live birth, 46 (23%) were biochemical, 5 (2.5%) were ectopic and 23 (11.5%) ended in abortion. In the control group, 128 pregnancies (64%) ended in a live birth, 35 (18%) were biochemical, 7 (3.5%) were ectopic, and 30 (15%) ended in abortion.

Subsequently, a prospective randomized controlled trial was conducted. Nyboe Andersen *et al*.,

(2002) evaluated whether the prolongation of luteal support during early pregnancy had any influences on the delivery rate after IVF. In this study, luteal phase support was administered in the form of 200 mg vaginal progesterone three times daily (600 mg/d) during 14 days from the day ET until the day of a positive HCG test. The study group (n = 150) withdrew vaginal progesterone from the day of positive HCG. The control group (n = 153) continued administration of vaginal progesterone during the next 3 weeks of pregnancy. 118 (78.7%) patients delivered in the study group given no progesterone versus 126 (82.4%) in the control group who continued with progesterone. The difference was not significant. Results indicated that prolongation of progesterone supplementation in early pregnancy had no influence on the miscarriage rate, and thus no effect on the delivery rate.

It would appear that the increase in endogenous HCG level during early pregnancy makes up for any possible lack of endogenous LH that has been caused by stimulated IVF cycles.

First trimester progesterone supplementation in IVF may support early pregnancy through 7 weeks by delaying a miscarriage but it does not improve live birth rates (Proctor *et al*., 2006).

Conclusions

The cause of luteal phase defect in stimulated IVF cycles seems to be related to the supra- physiologic levels of steroids.

Luteal phase support with HCG or progesterone after assisted reproduction results in an increased pregnancy rate (Fatemi, *et al*., 2007).

HCG is associated with a greater risk of OHSS. Luteal support with hCG should be avoided if E_2 >2700pg/ml (Fatemi, *et al*., 2007) and if the number of follicles is >10 (Fatemi, *et al*., 2007).

Natural micronised progesterone is not efficient if taken orally (Fatemi, *et al*., 2007). Vaginal and intra muscular progesterone seem to have comparable implantation and clinical pregnancy rates and delivery rates (Fatemi, *et al*., 2007).

The addition of oral E_2 to the progestin for luteal phase support still seems not to be beneficial (Kolibianakis *et al*., 2008).

The length of luteal phase support in stimulated IVF cycles does not need to exceed 14 days from the day of transfer (day 3 post OR) until the day of a positive HCG test (Nyboe Anderson *et al*., 2002). In the coming years, IVF stimulation may evolve into a more physiologic process – a milder stimulation – with the significant fringe benefit of reducing or eliminating the current luteal phase defect.

Future prospects

It appears that the cause of luteal phase defect in IVF is related to the supraphysiological levels of steroids, it would be interesting to find out which is the threshold, where the luteal phase defect initiates.

Further more it should be more specified whether it is the progesterone, E_2 or both causing the luteal phase defect in stimulated cycles. Therefore a progesterone antagonist could be administered in oocyte donors and the luteal endocrine profile of those patients should be evaluated. Also the combined use of an anti-estrogen, i.e. an aromatase inhibitor and a progesterone antagonist in oocyte donors should be further evaluated.

CC occupies the hypothalamic estrogen receptors for several weeks (Dickey *et al*., 1996). The long term receptor occupancy might lead to higher luteal LH concentrations, correcting the luteal phase defect observed in stimulated IVF cycles (Van Steirteghem *et al*., 1988). It would be interesting to evaluate, whether there is a luteal phase defect in cycles stimulated with clomiphene citrate/ recombinant FSH and gonadotropin-releasing hormone antagonist, despite the significantly higher LH levels measured in the luteal phase of these cycles (Tavaniotou *et al*., 2002).

Furthermore the administration of very low dose of HCG for luteal phase support in stimulated IVF cycles without the co-administration of P and E_2 should be evaluated.

Last but not least, further genetic research of endometrium should be performed, to find out why anno 2009 still we have such a low ongoing pregnancy rates after IVF/ICSI.

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