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## LUTEAL PHASE DEFICIT AND PROGESTERONE SUPPLEMENTATION IN ART

**Abstract:** Luteal phase deficit (LPD) is defined as a defective corpus luteum functioning, which can lead to infertility and pregnancy loss. It is estimated that this deficit is present in 6-33% of infertile women. LPD in the natural cycle is diagnosed by serum progesterone level in mid-luteal phase of less than 10ng/ml or delayed more than two days in the development of endometrial histology in relation to the expected for that day of the cycle. The main causes of the LPD at the unstimulated cycles are considered poor follicle production from the ovary, premature exhaustion of the corpus luteum, and weakness of the inner layers of the uterus to respond on the normal concentrations of progesterone. Assisted reproduction techniques (ART) and stimulated IVF cycles (with gonadotrophin releasing hormone (GnRH) agonists or antagonists) are associated with luteal phase defect. Therefore, almost all IVF centers use luteal phase supplementation after controlled ovarian stimulation. Elements of luteal support are administration of progesterone, estradiol and human chorionic gonadotrophin (HCG). Application of HCG and progesterone is efficient and significantly improves pregnancy rates in ART cycles. However HCG is associated with significant increased risk of ovarian hyperstimulation syndrome when used with GnRH agonists, especially in patients at high risk. Application of progesterone per orally is less efficient in comparison to intramuscular or transvaginal route of administration, both although showed similar clinical outcomes, carry

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a significant difference in the safety profile, given that i.m. is associated with the occurrence of serious adverse effects and supraphysiological levels of progesterone in serum. Vaginal administration route proved to be most acceptable. Optimal duration of supplementation is still open, but proposal of majority of authors is up to 12th week of pregnancy.

**Key words:** corpus luteum, luteal phase deficit, progesterone, artificial reproductive techniques, IVF, luteal phase support

Luteal phase deficit (LPD) represents one of the most controversial topics in reproductive medicine. It is defined as a condition of defective corpus luteum function resulting in infertility and pregnancy loss [1]. The incidence of LPD in the fertile women is assessed on up to 4% while it is found in 6-33% of the infertile women [1, 2]. In this paper we should address the function of the hypothalamo-pituitary-ovarian axis, role of gonadotrophins and steroids in the ovary and during the menstrual cycle, possible etiopathogenetical causes leading to luteal phase deficiency, and the therapeutical possibilities.

### ***Hypothalamo-pituitary-ovarian axis and the steroidogenesis in the reproductive women***

Gonadotropin-releasing hormone (GnRH) is a hypothalamic factor that is secreted into pituitary portal blood flow in the form of periodic pulses. This is leading to the synthesis and secretion of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) from the pituitary [3].

According to the "Two cell – two gonadotropin theory", both FSH and LH are necessary for ovarian follicular maturation and the synthesis of ovarian steroid hormones [4]. LH promotes the production of androgens (dehydroepiandrosterone, androstenedione, and testosterone) from cholesterol and pregnenolone, by stimulating  $17\alpha$ -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 [5]. 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 while at higher levels of estradiol (200pg/ml) for more than 48 hours, estrogen enhances the LH release [6].

The local estrogen-FSH interaction in the dominant follicle induces LH receptors on the granulosa cells resulting in their luteinization, secretion of progesterone and initiation of ovulation. Ovulation 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 [7]. The mid-cycle LH surge is responsible for a dramatic increase in local concentrations of

prostaglandins and proteolytic enzymes in the follicular wall [8]. Due to these substances, the follicular wall consisting of collagens, is progressively weakened and is perforated with a slow extrusion of the oocyte through this opening [9].

The functional relationship between GnRH neurons from the pituitary gland is established at the end of the first trimester of pregnancy. In this way all the components gonadal system including the hypothalamus, pituitary and ovary, are present even before birth. However, high concentrations of estradiol and progesterone originating from the placenta are suppressing hormone production in the fetus. After the birth and cessation of the placental steroid activity, the level of gonadotropins increases. The increase in FSH was associated with activation of the ovary, which is observed by ultrasound examination and increased levels of inhibin B and estradiol. FSH level was higher in girls than in boys. In the period from 12 to 20 months, the reproductive axis is again suppressed and a period of relative inactivity lasts until puberty. At the beginning of puberty, pulsatile secretion of GnRH induces the production of pituitary gonadotropins, initially registered only during sleep, and later during the day and night [10].

### ***Ovulation and the luteal phase function***

Follicle rupture during ovulation is associated with the creation of a rich capillary network induced angiogenic factors such as vascular endothelial growth factor (VEGF) from granulosa cells [11]. Luteinized granulosa cells expresses genes that are involved in the synthesis of progesterone. Luteal cells produces 17-hydroxyprogesterone, which is a substrate for aromatization by the luteinized granulosa cells. Because LH and human chorionic gonadotropin (hCG) bind to a common receptor, the role of LH in support of the corpus luteum can be replaced with hCG in the first 10 weeks after conception [12].

The luteal phase is defined as the period between ovulation and either the clinical establishment of a pregnancy or the onset of menses two weeks later [13].

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. 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 vascular endothelial growth factor [14] and the granulosa becomes vascularized. This angiogenic response allows large amounts of luteal hormones to enter the systemic circulation. 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 [15]. If conception and im-

plantation 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 [16]. The estimated onset of placental steroidogenesis (the luteo-placental shift) occurs during the 5th gestational week, as calculated by the patients' last menses [17].

Earlier, the premature onset of menses was recognized as a luteal phase deficiency of progesterone production, which was shown to be correctable by exogenous progesterone administration [18]. 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 [19]. The three main causes of luteal phase defect in unstimulated cycles include poor follicle production in the ovary, premature demise of the corpus luteum, and failure of the uterine lining to respond to normal levels of progesterone.

Although LPD has been clearly described in research settings, the clinical diagnosis remains controversial. A defective luteal phase in natural cycle has been defined when the serum mid-luteal progesterone levels are less than 10ng/ml [20]. However, mid-luteal progesterone levels do not always reflect the endometrial maturation [21]. Therefore, it was suggested that 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 [22].

### ***Luteal phase support in ART***

Normal corpus luteum function is essential to prepare the uterus for implantation and stabilization of the endometrium for pregnancy. Normal luteal phase is characterized by the appropriate hormonal milieu, including adequate levels of progesterone secretion by the corpus luteum and the secretory transformation of endometrium [23]. Corpus luteum function, among other things depends on the support of pituitary gonadotropins during the luteal phase in ovulatory cycles. Without LH signal, corpus luteum may be dysfunctional, and consequently the secretion of estradiol and progesterone may be abnormal.

Implantation involves a specific interaction between blastocyst and maternal endometrium. Ovarian steroids induce specific differentiation of the endometrium. Implantation window is defined as the period when uterus is receptive, 8 – 10 days after ovulation [24]. Without proper stimulation by progesterone or estradiol, endometrial receptivity may be compromised, leading to reduced implantation and pregnancy rates.

After conception and implantation, the blastocyst in development secretes hCG, which maintains the corpus luteum and its secretion. The shift from ovarian to placental production of gonadal steroids occurs in the period that lasts for weeks. Placental progesterone can be early detected on about the 50th day of gestation [17]. Premature LH peak occurs in 20 – 25% of IVF cycles. Exposure to high levels of LH results in premature luteinizing and induction of maturation of oocytes, having as a consequence increased rates of excluded cycles and reduced pregnancy rate after IVF [25, 26].

Administration of GnRH analogues completely suppresses the release of pituitary gonadotropins and reduces rates of excluded cycles. Edwards and Steptoe [27] first suggested that inadequate luteal phase due to ovarian stimulation can lead to failure of IVF. Other authors then showed that the supplementation of luteal phase after the application of a long protocol of controlled ovarian stimulation with Gn-RH agonists is useful in occurrence of pregnancy after IVF [28-31].

Aspiration endometrial biopsy on the day of puncture and aspiration of preovulatory follicles in IVF cycles with administration of Gn-RH agonists revealed a rapid maturation of the endometrium in 90% of patients. If this acceleration exceeds three days, in these cycles, pregnancy was not found [32, 33].

Pinopodes occur during the early luteal phase in women undergoing controlled ovarian stimulation [34]. Endometrial biopsies during the mid-luteal phase indicated a delay of 2 – 4 days in stimulated cycles [30]. In cases of luteal phase supplementation with progesterone or hCG, histology of the endometrium during mid and late luteal phase is normal [35].

One group of authors believe that luteal phase defect found in cycles with administration of Gn-RH analogues for ovulation induction, can not happen by accident, but rather depends on the preovulatory profile of physiological peak of hormones involved in the process of ovulation [36].

Luteal phase of ART cycles may be defect due to one or more of the following factors:

- Suppression of endogenous LH secretion during the luteal phase as a result of persistent pituitary suppression by Gn-RH agonists,
- Supraphysiological levels of estradiol and progesterone in the early luteal phase may lead to faster development of the endometrium. Hence, asynchrony between embryo and endometrium, and reduction of pregnancy rates in iVF cycles. Receptive endometrium facilitates timed dialogue between embryo and endometrium. Altered hormonal environment, either with a high level of progesterone or changed estradiol – progesterone ratio, may affect the development of endometrial and at the end of the implantation of the embryo[37-40].

- Duration of ovarian steroid production is shorter in stimulated cycles compared with spontaneous. Early maturation can prevent successful implantation because of sudden decrease in concentration of estradiol and progesterone in ART cycles compared with normal.
- Aspiration of granulosa cells surrounding the oocyte may affect the production of progesterone.

Similar observations on luteal phase defect were observed with administration of Gn-RH antagonists during controlled ovarian stimulation. It was found that the levels of serum LH in the early and middle luteal phase of ART cycles treated with gonadotrophin – Gn-RH antagonists, are also low, despite the regimen used for the maturation of oocytes [41, 42].

In one study on 55 patients stimulated by the scheme: r-FSH + Gn-RH + HCG, endometrial biopsy done on the day of oocyte aspiration, showed that there were no pregnancies if there was histological deviation in endometrial maturation of more than three days [43]. In the cycles without luteal phase supplementation, its length is shortened and leads to early bleeding [44].

Elements of luteal support the administration of progesterone, estradiol and HCG.

**Progesterone supplementation.** Progesterone is the basis of luteal phase. It is so crucial in the preparation of endometrium and pregnancy support that many clinicians see it as the only important hormone in luteal physiology. It should be exogenously supplied in all programmed cycles in most of the stimulated cycles in order to achieve adequate pregnancy rates.

**Estradiol supplementation.** Conventional protocols of luteal phase supplementation in ART consisted of administration of progesterone only, although the estradiol and progesterone are secreted together and both early fall in most cases after a cycle of controlled ovarian stimulation. Whereas estradiol does not directly affects luteinization, a certain amount of estradiol is still necessary to stimulate increase of the number of progesterone receptors, in order progesterone to act. As a support of a finding that luteal estradiol may be used, is the result of meta-analysis which found that the administration of HCG (which stimulates the production of estradiol and progesterone) is superior form of luteal support in comparison to progesterone itself, whereas progesterone itself, in contrast, is better than if there is no luteal support at all [30, 45]. Recent tests of HCG administration when estradiol levels in mid-luteal phase were low in the IVF cycles also showed increased rates of pregnancy with this method of application [46]. However, since there is an increased risk of ovarian hyperstimulation syndrome, caused by HCG administration, the use of HCG to get a combined estradiol and progesterone secretion is not widely accepted.

**Ways to administer supplementation.** Possible ways of administration of progesterone include oral, parenteral (i.m.), transvaginal and rectal.

Peroral administration. The development of the process of micronization of progesterone enabled its better absorption by oral route of administration. However, levels of progesterone in the circulation are very low after oral administration, in order to provide adequate support for the endometrium. First pass through the liver after oral intake of progesterone leads to its significant metabolic degradation, so in the best case, only 10% of administered dose enters circulation as the active progesterone [47]. Any increase in dosage orally administered in order to achieve the necessary serum levels of progesterone cause a degree of somnolence unacceptable for most patients. Recent clinical trials of oral supplementation of IVF cycles confirmed the inadequacy of this method of application. It was noted that patients taking only oral progesterone in their IVF cycles had lower rates of implantation and pregnancies and higher rates of spontaneous abortion.

Intramuscular administration. Parenteral administration releases progesterone in relatively high concentrations without metabolic degradation, which is connected with oral administration. However, it sometimes causes serious side effects, where recovery from them can take several weeks, because half-life of oil solution in the muscle is long. The fact is that this protocol leads to the highest levels of progesterone in serum, which may be supraphysiological levels. Endometrial architecture has generally proved adequate "in phase" development and rates of miscarriage and pregnancy seem to be normal [13, 48].

Transvaginal administration. There are several important advantages:

- Suitable and acceptable for the patient,
- It is not painful and does not require any special equipment, and exercises for application,
- Rarely causes allergic reactions.

Initial tests done to compare different ways of application of progesterone in ART cycles supported the benefits of transvaginal approach. Devroy et al. [49-51] have shown that transvaginal use is as good (if not better) as i.m. and clearly better than oral administration. Based on these considerations, the following protocols are used with different effectiveness in the preparation of the endometrium and the pregnancy support:

- Progesterone in oil 50mg i.m. once a day or depo progesterone 250mg i.m. on the second day,
- Utrogestan 200mg, 3 – 4 times a day,
- Crinone Gel 8% 90 mg, 1 – 2 times a day.

### ***Supplementation in stimulated ART cycles***

Almost all IVF centers apply luteal phase supplementation after controlled ovarian stimulation, at least in the second half of the cycle (Luteal Phase). It is usual

to start on the day of oocyte aspiration or immediately after. There are following possibilities:

- The administration of progesterone with or without estradiol on the day of oocyte aspiration or three days later and continued until a pregnancy test around the 14th day of aspiration,
- HCG every 3 – 5 days during the luteal phase in cycles with low risk of OHSS.

### ***Supplementation in recipient cycles***

In cycles of egg donation, the recipient must be synchronized with the donor, to ensure the receptivity of the endometrium at the appropriate time. ‘‘Follicular’’ phase of these cycles can vary from short, even 7 days, to long, even 35 days, and not being related to any disorder [52, 53]. Most of the center is trying to start supplementation with estradiol several days prior to donor begins with the stimulation. Start of progesterone administration varies from the day when the donor receives her HCG to the day of oocyte aspiration.

### ***Gn-RH agonists: new support of luteal phase?***

Gn-RH agonists have recently been proposed as a new support of luteal phase, which can act on the pituitary gonadotrophic cells, endometrium and the embryo itself [54]. It is assumed that Gn-RH agonists may support the corpus luteum by stimulating the secretion of LH by the pituitary gonadotrophic cells, or by acting directly on the endometrium by local expression of Gn-RH receptors [55].

In a prospective randomised trial Tesarik et al. [54] evaluated the effect of administration of Gn-RH agonist (0,1 mg triptorelin) in the luteal phase on the outcome of the protocol with Gn-RH agonists and antagonists in controlled ovarian stimulation (n=300, n=300). Randomly were given an injection of Gn-RH agonist (study group) or placebo (control group) on 6-th day after ICSI. Pregnancy rates were increased in both protocols, in the long protocol rates were 29.8% vs. 18.2%. Rates of pregnancies in progress were 46.8% vs. 38%. From the patients treated with protocol Gn-RH antagonists implantation rates were 27.1% vs. 17, %, and during pregnancy 44.8% vs. 31.9%. Despite these initial encouraging results, it is too early to accept general administration of Gn-RH agonist in the luteal phase supplementation. With respect to safety there is a great concern about possible adverse effects on oocytes and, more importantly, the embryos [56]. To prove the possible role of administration Gn-RH agonist in the luteal phase of stimulated IVF cycles large prospective study are needed.



After all above mentioned, it can be concluded that progesterone and estradiol have a crucial role in maintain early pregnancy. Until luteo-placental shift occurs around 7<sup>th</sup> week of gestation, ovarian production of these hormones is critical. After 7 weeks of gestation, the placenta normally produces adequate levels of these hormones. The administration of HCG and progesterone is efficient and significantly improves pregnancy rates in ART cycles. However HCG is associated with significant increased risk of OHSS when used with Gn-RH agonists, especially in high risk patients.

Progesterone administration per orally was clearly less efficient way of application in relation to intramuscular or transvaginal time of application and is associated with increased rates of adverse effects. However, intramuscular administration route may also cause serious side effects, like severe inflammatory reactions, sterile abscess and inconvenience, thus alternatives are necessary. Vaginal administration route of progesterone has multiple advantages, which is supported by clinical studies.

Optimal duration of luteal phase supplementation is still open, most authors propose up to 12 weeks of gestation. However, this also remains subject for further research in order to avoid compromised rate of successful pregnancies.

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