Editorial Articles

Luteal phase support for in vitro fertilization-embryo transfer – present and future methods to improve successful implantation

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Summary

Purpose: To present reasons for luteal phase deficiency when taking controlled ovarian hyperstimulation (COH) for purposes of inducing multiple oocytes for in vitro fertilization (IVF), and to suggest strategies to overcome the defect. *Methods:* Treatment options presented include luteal phase support with human chorionic gonadotropin (hCG) injection, progesterone, estradiol, gonadotropin releasing hormone agonists, cytokines, e.g., granulocyte colony stimulating factor, and lymphocyte immunotherapy. *Results:* hCG and progesterone produce the best results and are comparable or at best a slight edge to hCG but the latter is associated with too high a risk for ovarian hyperstimulation syndrome. Vaginal progesterone is the most efficacious with the least side-effects. *Conclusions:* Better methods are needed to adequately assess full correction of the luteal phase defect. In some cases the luteal phase defect associated with COH is not correctable and FSH stimulation should be reduced or all embryos frozen and defer transfer to an artificial estrogen progesterone or natural cycle.

Key words: Progesterone; Controlled ovarian hyperstimulation; Human chorionic gonadotropin; Estradiol; Gonadotropin releasing hormone agonist.

Etiology of luteal phase defects with in vitro fertilization-embryo transfer (IVF-ET)

In 1980 Edwards, Steptoe and Purdy suggested that the luteal phase of all stimulated IVF cycles is abnormal [1]. In 1985 and 1989 publications by Check *et al*., showed lower miscarriage rates in women using clomiphene citrate or human menopausal gonadotropins, not in high dosages as used for IVF but in lower dosages just to achieve ovulation when the luteal phase was supplemented with progesterone [2, 3]. Thus the studies by Check *et al*. suggested that the luteal phase defect (LPD) reported by Edwards *et al*. was not related to the process of IVF itself combined with high-dose gonadotropin stimulation but something disruptive about the stimulating drugs.

Further support for this concept was provided by Kerin *et al*. in 1981 who showed that aspiration of a preovulatory Graafian follicle following spontaneous ovulation did not cause an apparent luteal phase defect [4]. Thus the study by Kerin *et al*. showed that the removal of large quantities of granulosa cells during oocyte retrieval is not the reason for LPD in stimulated IVF-ET cycles [4].

One study suggested that the injection of human chorionic gonadotropins (hCG) to enable advancement of meiosis to the metaphase II stage would suppress endogenous luteinizing hormone (LH) production by a short loop feedback mechanism and thus causes LPD [5]. However, this potential etiologic factor for LPD in women taking COH for IVF-ET was shown not to be a likely explanation because taking hCG in natural cycles did not down-regulate LH secretion in the luteal phase [6].

The demonstration of LPD from controlled ovarian hyperstimulation (COH) for IVF-ET occurred before the common practice of using gonadotropin-releasing hormone (GnRH) agonist to prevent premature luteinization. Nevertheless, a popular theory arose that LPD was created by the delay in pituitary recovery from suppression by the GnRH agonist [7]. However, if this was the case then the problem would be obviated by the replacement of using a GnRH agonist with a GnRH antagonist protocol. However, Albano *et al*. published the first of several subsequent studies showing that despite the rapid recovery of pituitary function LPD using GnRH antagonists still persists and pregnancy rates suffer greatly unless supplemented progesterone or hCG injections are given [8]. Thus these studies using GnRH antagonists obviate the popular concept that LPD was related to pituitary suppression by the GnRH agonist.

The prevalent theory today for the etiology of LPD for COH for IVF-ET is related to the supra-physiological concentration of steroids secreted by multiple corpora lutea during the early luteal phase which directly inhibit LH release by negative feedback to the pituitary and the hypothalamus.

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Main Methods of Treating LPD from COH for IVF-ET

Human chorionic gonadotropins

Human chorionic gonadotropin has been found to rescue the corpus luteum in IVF-ET cycles since as early as 1990 [9]. Besides increasing serum estradiol (E2) and progesterone (P) concentrations hCG may also work by increasing integrin alpha-5, placental protein 14, and relaxin [10-12].

Perhaps by increasing these other luteal peptide hormones besides E2 and P, hCG may be somewhat superior to exclusive P supplementation [13]. Unfortunately luteal support with hCG has been associated with a significant increased risk of ovarian hyperstimulation syndrome [14]. Because of this risk probably P support should be the treatment of choice for most IVF-ET cycles involving the usual COH despite a possible mild superiority in achieving pregnancy with hCG vs P [15]. Human chorionic gonadotropin supplementation could be considered in mild stimulation protocols especially in women with diminished oocyte reserve, women who fail to have sufficient length to the luteal phase or who fail to attain evidence of adequate endometrial P, e.g., failing to attain a homogeneous hyperechogenic endometrial echo pattern by mid-luteal phase despite P therapy, or women who cannot tolerate vaginal or intramuscular P taking standard COH as long as the serum E2 is not too high or there are too many follicles [16, 17].

Progesterone therapy

The two most effective forms of luteal phase P therapy is either intramuscular (IM) or vaginal. The first method of P used for IVF-ET was IM [18]. A large prospective multi-center open label study found no significant differences in pregnancy rates with IM vs vaginal P [19]. Similarly a recent meta-analysis reached the same conclusions [20].

From a side-effect standpoint vaginal P is much more tolerated than IM P which can cause severe pain at the injection sites, severe inflammatory reactions with erythema and swelling, and even abscess formation [21]. There are even reports of a rare but very serous complication of eosenophilic pneumonia [22, 23]. For this reason vaginal P is favored over IM administration.

Compounded P vaginal suppositories have been used for over 20 years [24-27]. The first two commercial products of P put into use for IVF-ET cycles were a controlled and sustained release vaginal micronized P gel (Utrogestan - taken as 2 100 mg capsule 3x day) (a product not available in the United States) and a controlled and sustained release vaginal gel known as Crinone 8% which is either administered once daily or twice. One study showed a non-significant trend for higher pregnancy rates with Crinone vs Utrogestan [28]. Another study found fewer side-effects with Crinone vs Utrogestan [29].

A vaginal tablet was introduced to the market – in contrast to the oral micronized P tablet which had been used vaginally by some IVF centers – which was designed to absorb the vaginal secretions and disintegrate into an adhesive powder that adheres to the vaginal epithelium, thus facilitating sustained absorption and also causing less perineal irritation [30]. It is known commercially as Endometrin (vaginal tablets) [31]. We participated in a multi-center randomized prospective trial and found that the dosage of 100 mg 3x/day produced comparable ongoing live delivery rates compared to Crinone 90 mg (8%) once daily [32]. We also participated in a multi-center P trial evaluating a silastic ring with slow release of P (change weekly) and found that it produced comparable pregnancy rates to Crinone. This product will soon be released to the U.S. market.

Oral micronized P (Prometrium) is subjected to first-pass prehepatic and hepatic metabolism and is thus degraded to its 5 alpha and 5 beta reduced metabolites [33]. These reduced metabolites create frequent side-effects of nausea and light headedness and abdominal discomfort. Though the oral Prometrium is able to induce menses in an estrogen replete woman with amenorrhea, studies have shown very little evidence of P secretory effect following endometrial biopsies when given to women with ovarian failure or when using estrogen followed by P [34].

In contrast there is an oral P (not available in the United States) that has good oral bioavailability known as dydrogesterone [35]. Appropriate endometrial secretory transformation has been demonstrated with dydrogesterone [36]. A randomized study of IVF-ET cycles found dydrogesterone to produce comparable pregnancy rates compared to micronized P administered vaginally [37]. However other studies found that dydrogesterone is not as effective as vaginal P in establishing in phase endometrium in women with amenorrhea and estrogen deficiency when treated first with estrogen followed by P [38, 39].

There does not appear to be any difference in live delivered pregnancy rates in IVF-ET cycles if P is started the day of hCG injection or the day after, the day of the oocyte retrieval or even at day 3 ET [40]. One study compared the live delivered pregnancy rates in 150 women who conceived with IVF-ET and P was stopped on the day of a positive serum beta-hCG level (78.7%) vs those continuing for three weeks beyond the positive beta-hCG (82.4%) [41]. Obviously, the study did not have enough power to determine if the five extra women who delivered with P extended to three weeks was real or fortuitous but I would rather error on the conservative side and extend the therapy. In fact, it is possible that even an higher successful live delivery rate could be achieved if P was extended to the end of the first trimester as in the practice of this author.

Possible other ancillary therapy for an inadequate corpus luteum

Estrogen supplementation

E2 is secreted in significant amounts by the corpus luteum and reaches a second peak by the mid-luteal phase [42]. During the follicular phase E2 plays a major role in inducing P receptors in the endometrium. Its role in the luteal phase is less clear but it definitely is needed for pregnancies to be maintained. For example, adequate pregnancy rates would not be achieved by embryo transfers using donor oocytes in a graduated estrogen replacement cycle by merely adding P without continuing the E2.

With COH generally there are higher levels of E2 made by the corpus luteum but there are also higher levels of P and yet P supplementation is needed for ideal pregnancy rates following IVF-ET or at least hCG injections to stimulate more P production by the corpus luteum. The question is once there has been adequate priming of the endometrium by follicular phase E2 production with induction of P receptors in the luteal phase is E2 necessary to maintain the progesterone-induced changes in the endometrium? There is a possibility that too much estrogen in the earlier luteal phase may be luteolytic normally by increasing nitric oxide production which causes apoptosis of the luteal cells leading to decreased P production [43-45].

A prospective randomized study added E2 valerate 6 mg to P vaginal suppositories (total 600 mg per day) in patients superovulated but not undergoing IVF-ET and found no difference in outcome [46]. No difference in pregnancy outcome was found with addition of E2 to P vs P alone in a progesterone IVF-ET study using luteal phase GnRH agonist [47]. Some other IVF-ET studies using GnRH agonists have found a mild beneficial effect of adding E2 to P [48, 49]. An IVF-ET study with GnRH antagonists did not find that adding E2 to P was beneficial for improving pregnancy rates in women taking a GnRH antagonist protocol [50].

Luteal phase GnRH agonist

I personally think that adding a GnRH agonist has the most potential to improve live delivered pregnancy rates following COH and IVF-ET since the discovery years ago of using hCG supplementation or P. One of the most fascinating studies was by Tesarik *et al*. who found that a time mid-luteal nasal spray of buseriline significantly improved live delivered birth rates in estrogen/progesterone primed women receiving donated oocytes [51]. This study showed that it can be beneficial even when there is no corpus luteum [51]. The GnRH agonist could have a direct effect on the endometrium because GnRH receptors in the endometrium have been found [52].

However, the data from Tesarik *et al*. could also suggest a direct effect of GnRH on the embryo itself [51]. A previous study found that women with singleton pregnancies have higher serum beta-hCG levels when they have had luteal treatment with GnRH agonists compared to pregnant women not receiving a GnRH agonist at the same time post-ovulation [53]. Some data support the concept that the GnRH acts on a GnRH receptor in the placenta and augments hCG output by the early placenta [54].

A subsequent study by Tesarik *et al*. found that 0.1 mg of triptorelin for six days after oocyte retrieval resulted in increased live delivered pregnancy rates in both GnRH agonist and antagonist IVF-ET cycles versus placebo in which both groups were also supplemented by vaginal P and oral estrogen [55]. We will be presenting data at the 2012 annual meeting of the American Society of Reproductive Medicine showing a 30% in pregnancy rates following embryo transfer but no increase in beta-hCG levels suggesting a direct effect on endometrial GnRH receptors to explain its mechanism of action.

Cytokines

Besides E2 and P which are the major products of the corpus luteum, there are a plethora of cytokines secreted by the corpus luteum. However, based on very high pregnancy rates found in donor oocyte cycles or frozen embryo transfer cycles which are devoid of corpora lutea, it is clear that the secretion of these cytokines is not important for successful pregnancy in most women receiving embryos. However, one cytokine in particular may prove useful for improving the receptivity of the endometrium for successful embryo implantation, and that is granulocyte colony stimulating factor (G-CSF) which is expressed and produced by decidual cells [56-58]. A randomized controlled trial of women with recurrent miscarriage found a significantly improved live delivery rate in women treated with 1 μg/kg/day G-CSF subcutaneously from the mid-luteal phase to the end of the 9th week of gestation [59]. Recently, an intrauterine infusion prior to ovulation of G-CSF was found to significantly increase endometrial thickness in women with persistently thin endometria leading to successful pregnancies in a series of anecdotal cases, and this group is now conducting prospective randomized studies to see if this method could improve embryo implantation following COH and IVF-ET [60].

Methods to determine if luteal support is adequate in IVF-ET cycles

The oldest method to determine adequacy of the luteal phase at the endometrial level is the endometrial biopsy [61]. However, there are great difficulties in the interpretation and many IVF-ET centers no longer use this technique [62]. There are data suggesting that failure to attain a homogeneous hyperechogenic (HH) endometrial echo pattern by mid-luteal phase is associated with much lower pregnancy rates [63]. Increasing the dosage of P from mid-luteal phase in those women not attaining an HH pattern has resulted in improved pregnancy rates [64].

There is evidence that one way P may help implantation is by interacting with P receptors that are induced de novo on gamma/delta T cells by the allogeneic stimulus of the fetal semi allograft causing the expression of the immunomodulatory protein known as the P induced blocking factor (PIBF) [65, 66]. This protein has been found to suppress natural killer cell activity especially by stabilizing perforin granules and causes a shift from thymic helper (TH) cytokines (which evoke cellular immune responses) to TH2 cytokines (which evoke a humoral response which may be immunoprotective) [67, 68].

Recently an ELISA method has been developed that can rapidly measure PIBF [69]. We will be presenting research at the 2012 American Society of Reproductive Medicine meeting showing that PIBF is not only present in high amounts 3 days after embryo transfer but it actually begins to rise 1 hour after embryo transfer. Our ELISA method differs slightly from that of Hudic *et al*. [69]. If this can be determined it could prompt raising the dosage of P, since we have determined that most of the time low PIBF levels are related to inadequate P exposure [70].

The two main factors involved in secreting PIBF from gamma/delta T cells are the development of P receptors in the gamma/delta T cells and exposure to P at the maternal fetal interface. In a minority of cases the relatively low immunogenicity of the fetus is sometimes for some reason insufficient to properly induce sufficient P receptors in the gamma/delta T cells leading to poor PIBF expression despite adequate P exposure. In this case, injection of the far more immunogenic lymphocytes (i.e., lymphocyte immunotherapy) can increase PIBF expression [71]. Through a highly debatable subject, one study found a 51% ongoing/live delivered pregnancy rate following lymphocyte immunotherapy in women undergoing another IVF-ET cycle (despite failing to achieve a live pregnancy even with average of 4.3 previous embryo transfers) vs only 16% in the controls [72]. Progesterone-induced blocking factor secretion does not require a corpus luteum [73]. However, there are various cytokines and interleukins involved in PIBF secretion but this suggests they are not corpus luteum derived. Thus if a certain level of PIBF after a certain time after implantation is found to be associated with a positive outcome, there is potential for a treatment paradigm of first using P to increase PIBF and if ineffective try lymphocyte immunotherapy.

Other strategies taking into consideration that present techniques fail to completely identify full correction of the luteal phase defect by COH

Sometimes a single case report can accurately present the crux of a problem. A case was reported of a woman with polycystic ovarian syndrome and amenorrhea who failed to conceive despite six years of ovulation induction with luteal phase P support with no other infertility factors identified. Subsequently she had ten IVF-ET cycles with 92 embryos transferred in three of the world's foremost IVF centers but still failed to conceive. A decision was made to perform another IVF cycle but to purposely cryopreserve all the embryos and defer ET in case the COH caused a hostile uterine environment that was not correctable by P supplementation. She was successful with a live delivery following her first and only frozen ET at age 38 [74].

Interestingly three months following her delivery she menstruated and had nine regular menstrual cycles for the first time in her life. She returned for a consult to have another frozen ET, and she was in the early luteal phase. She had not taken P in her previous cycles. She was placed on P this cycle and we prepared for frozen ET the next cycle but she conceived in this natural cycle with just intercourse [75]. This case shows that luteal phase defects exist even without COH especially in women of advanced reproductive age, and P therapy in the luteal phase may successfully improve chance of a live pregnancy [76]. It should be remembered that she failed to conceive after six years of ovulation induction and P supplementation showing that follicle maturing drugs even when used in mild dosages can create an endometrial defect not correctable by P or hCG [74, 75].

Though many IVF centers find very low pregnancy rates following COH and IVF-ET in women with diminished ovarian oocyte reserve and elevated day 3 serum FSH, it has been clearly demonstrated that this observation is not related to defective oocytes prone to meiosis errors but more related to the adverse effect of high FSH dosage [77, 78]. This adverse effect can be negated to a large degree by using mild ovarian stimulation and avoids even low dosages of FSH when the serum FSH level is elevated, and in some instances lowering the FSH by ethinyl estradiol or other means [79-81].

These data underscore the importance of a good cryopreservation program to maximize the chance of conception from a given COH oocyte retrieval cycle and to consider that failure to conceive following the transfer of a normal appearing embryo could be from the COH itself [82, 83].

High-dose COH and cryopreservation may not be the ideal answer for women with diminished oocyte reserve since the adverse effect may be in the embryo itself interfering with some implantation factor present on the embryo [84].

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