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An update of luteal phase support in stimulated IVF cycles

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Stimulated IVF cycles are associated with luteal phase defect. In order to overcome this, different doses, durations and types of luteal phase support (LPS) have been evaluated. There is still no agreement regarding the optimal supplementation scheme. The aim of this paper is to assess the past and the current clinical practices of luteal supplementation in IVF. The databases of Medline and PubMed were searched to identify relevant publications. LPS with human chorionic gonadotrophin (hCG) [$n = 262$, odds ratio (OR) 2.72 (95%), confidence interval (CI) 1.56–4.90, $P < 0.05$] or progesterone ($n = 260$, OR 1.57 CI 1.13, 2.17, $P < 0.05$) results in an increased pregnancy rate compared with placebo, however, hCG is associated with increased risk of ovarian hyperstimulation syndrome. Natural micronized progesterone is not efficient if taken orally. The data on oral dydrogesterone are still conflicting. Vaginal and intra muscular progesterone have comparable outcomes. The addition of estradiol (E_2) seems to be beneficial in long GnRH agonist protocol (implantation rate 39.6% with E_2 compared with no E_2; $P < 0.05$) but not in the short GnRH agonist and GnRH antagonist protocol. Despite the early promising results, it is too early to recommend the use of GnRH agonist in LPS. LPS should cease on the day of positive HCG. Since the cause of luteal phase defect in IVF appears to be related to the supraphysiological levels of steroids, milder stimulation protocols should be advocated in order to eventually overcome the luteal phase defect.

Keywords: luteal phase support; IVF; progesterone

Introduction

The luteal phase is defined as the period between ovulation and either the establishment of a pregnancy or the onset of menses two weeks later. (Fatemi *et al*., 2006). Following ovulation, the luteal phase of a natural cycle is characterized by the formation of a corpus luteum, which secretes steroid hormones, including progesterone and estradiol (E_2). 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 fifth gestational week, as calculated by the patients' last menses (Scott *et al*., 1991). Stimulated IVF cycles are associated with a defective luteal phase in almost all patients (Ubaldi *et al*., 1997; Macklon and Fauser, 2000; Kolibianakis *et al*., 2003).

In the context of assisted reproduction techniques, luteal phase support (LPS) is the term used to describe the administration of medication aimed at supporting the implantation process. In an attempt to enhance the probability of pregnancy, different doses, durations and types of treatments for LPS have been evaluated. There is, however, no agreement regarding the optimal supplementation scheme (Fatemi *et al*., 2006).

The aim of this paper is to assess the past and the current clinical practices of luteal supplementation in IVF. Optimal compound, timing and route of administration are ascertained and future prospectives are discussed. Although there is a lack of randomized controlled trails on the issues of LPS and the causes of luteal phase defect, the current clinical approaches will be discussed in the light of the up to date evidence.

Materials and Methods

This update is divided into two sections. The first section, deals with the etiology of the luteal phase defect in stimulated cycles. The second section comprises an update of different possible LPS modalities and lengths. For the purpose of this update, a comprehensive search of the literature was performed using the following search strategy.

Search strategy

A computer-based search was conducted through the bibliographic databases of Medline, Embase and Cochrane Menstrual Disorders

and Subfertility group using the following key words: LPS, luteal phase defect, oral progesterone, vaginal progesterone, intra muscular progesterone, rectal progesterone, progesterone with E_2, hCG and LPS. There was no language restriction.

The cause of the luteal phase defect in stimulated IVF cycles

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 normo-ovulatory patients with primary or secondary infertility was demonstrated to be about 8.1% (Rosenberg *et al*., 1980).

With the advent of IVF, it has been established that the luteal phase of all stimulated IVF cycles is abnormal (Edwards *et al*., 1980). The aetiology 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*., 1992a,b).

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 pituary 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 favour 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; 1 mg/day). However, due to unacceptably low pregnancy rates (PR) (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 can be postulated that one of the main causes of the luteal phase defect in stimulated IVF cycles is related to the supraphysiological 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 role of progesterone in the luteal phase

Csapo *et al*. (1972, 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 7 weeks of gestation led to pregnancy loss (Csapo *et al*., 1972). However, they 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, 2002a,b). 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*. (1998). This study investigated the consequences of uterine contractions (UC) as visualized by ultrasound during embryo transfer. Results indicated that a high frequency of UC 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*., 1998).

The luteal phase support

Progesterone

Currently available formulations of progesterone include oral, vaginal, rectal and intramuscular (IM) (Chakmakjian, 1987;

Penzias, 2002). 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 IM) of progesterone overcomes 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 *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 (POF) who had been treated with oral micronized progesterone when compared with patients treated with IM injections or vaginal micronized 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 micronized progesterone as LPS after IVF. Both DG and progesterone were associated with similar rates of successful pregnancies (24.1 versus 22.8%, respectively; $P = \text{NS}$).

Prior to initiation of a large randomized controlled trial to compare these two treatment schemes for IVF cycles, our group performed a pilot study in patients with POF who were on the waiting list of our oocyte donation programmme (Beck-Peccoz *et al*., 2006). It had been demonstrated in reproductive research that menopausal patients treated for oocyte donation, such as those included in our study, are the best study paradigm for endometrial receptivity (de Ziegler and Fanchin, 2000).

The patients in our study were treated with oral DG and vaginal progesterone in consecutive cycles as progestins in protocols of cyclic steroid replacement after endometrial priming with E_2 (Fatemi *et al*., 2006). The objective was to compare the endometrial histology on day 21 of the artificial cycle in patients with POF treated with oral DG versus vaginal progesterone as progestins in protocols of cyclic steroid replacement. After sufficient estrogen endometrial priming, we found that exogenous administered vaginal micronized progesterone was significantly more effective than oral DG in creating an 'in phase' secretory endometrium ($n = 12$, $P = 0.021$; Fatemi *et al*., 2007). The limitation of this study is clearly the small sample size and the results can not be extrapolated. However, the conclusion corroborates one earlier study (Pellicer *et al*., 1989). Although oral DG might prove to be sufficient for luteal supplementation in IVF cycles, more large randomized controlled trails are needed before a conclusion can be made.

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 (Levin *et al*., 2001). 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 metabolization is absent (de Ziegler *et al*., 1995).

There is recent evidence in the literature that vaginal progesterone is at least as effective as IM progesterone at providing luteal support in induced cycles (Simunic *et al*., 2007). In Europe, there are two different forms of intravaginal progesterone on the market, natural micronized progesterone (Utrogestan® Laboratories Besins International, Paris, France) and Crinone® 8% (Fleet Laboratories Ltd., Watford, UK), a controlled and sustained-release vaginal gel. Utrogestan® 100 mg capsules are administered vaginally three times two capsules daily (600 mg/day) whereas Crinone 8% is administered vaginally once a day, i.e. 90 mg, (Ludwig *et al*., 2002; Simunic *et al*., 2007).

For the vaginal administration of natural micronized progesterone, no dose finding studies have been performed. Most frequently, 300–600 mg of natural micronized progesterone is administered daily, spread over two to three dosages (Tavaniotou *et al*., 2000). However, further prospective randomized trials are essential to define the necessary dose of vaginal micronized progesterone for LPS in IVF.

In a prospective randomized study ($n = 126$), Ludwig *et al*. (2002) compared vaginal Crinone® 8% with vaginal Utrogestan® for LPS. Clinical PR (28.8 versus 18.9%, respectively), clinical abortion rates until 12 weeks of gestation (14.3 versus 10.0%, respectively) and ongoing PR (24.7 versus 17.0%, respectively) were comparable between the two groups (Ludwig *et al*., 2002).

Simunic *et al*. (2007) and Ludwig *et al*. (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 (38/125 with Crinone® 8% gel and 68/132 with Utrogestan®, $P < 0.05$; Simunic *et al*., 2007; Ludwig *et al*., 2002).

Rectal progesterone

A number of publications have evaluated the rectal use of natural progesterone in women undergoing IVF/ICSI (Chakmakijan and Zachariah, 1987; Ioannidis *et al*., 2005). Chakmakijan and Zachariah (1987) studied the bioavailability of micronized progesterone by measuring sequential serum progesterone concentrations after a single bolus of 50–200 mg given sublingually, orally (capsule and tablet), vaginally and rectally (suppositories) during the follicular phase of a group of normally menstruating women. When compared with other modes of administration, rectal application resulted in serum concentration during the first 8 h twice as high as other forms. However, to the best of our knowledge, there are no prospective randomized trails to compare the rectal administration of progesterone with other administration routes for IVF.

IM progesterone

With IM 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 IM progesterone. The doses of

IM progesterone used for LPS vary between 25 and 100 mg/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). In addition to this, injections of progesterone in oil can also cause inflammatory reactions and abscesses (Propst *et al*., 2001).

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

In an open-label trial in 1184 women from 16 US centers, Levine (2000) evaluated the clinical and ongoing PR in IVF cycles involving vaginal and IM progesterone. Vaginal and IM progesterone were found to have comparable clinical (35.1 versus 35.2%, respectively, $P = \text{NS}$) and ongoing PR (30.2 and 33.6%, respectively, $P = \text{NS}$).

A meta-analysis published in 2002 by Pritts and Atwood included five prospective randomized trails comparing IM administration of progesterone with vaginal application (Artini *et al*., 1995; Abate *et al*., 1999; Anserini *et al*., 2001; Guesa *et al*., 2001; Propst *et al*., 2001). A total of 891 cycles were evaluated in those studies. Clinical PR and delivery rate were significantly higher when IM progesterone was used [RR clinical PR/embryo transfer 1.33 (95% CI: 1.02–1.75), delivery rate 2.06 (95% CI: 1.48–2.88)].

Despite the conclusion of Pritts and Atwood's meta-analysis, vaginal administration of progesterone is a viable alternative to the IM injections of progesterone which are associated with a high number of side effects. On the basis of presented evidence, IM progesterone is not recommended as a first choice LPS method in stimulated IVF cycles.

Progesterone with E_2

The two most important hormones produced by the corpus luteum are progesterone and E_2 (Johnson *et al*., 1994). The role of progesterone as luteal support in stimulated cycles is well established (Maslar, 1988). However, it has not yet been clearly demonstrated whether additional supplementation of E_2 in stimulated IVF cycles may be beneficial (Ludwig *et al*., 2001).

In a prospective randomized study, Smitz *et al*. (1993) evaluated the possible benefit of adding E_2 valerate 6 mg per os daily to the vaginal micronized progesterone (600 mg daily) given as luteal supplementation in 378 women treated with a GnRH agonist and human menopausal gonadotrophins (hMG) for IVF. The clinical PR was similar between the two groups (29.2% with the E_2 co-treatment and 29.5% with progesterone-only treatment). Similarly, Lewin *et al*. (1994) in a prospectively randomized study, could not find any advantage in the addition of 2 mg E_2 valerate to progesterone as LPS of long GnRH agonist and hMG-induced IVF embryo transfer cycles in 100 patients (clinical PR 26.5 versus 28% with and without E_2 co-treatment, respectively, $P = \text{NS}$).

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 E_2 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 PR in women undergoing ICSI in agonist cycles ($n = 231$). Significantly higher PR were recorded in those who received low dose E_2 supplementation compared with no E_2 substitution (PR 32.8 versus 23.1%). 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 controlled ovarian hyperstimulation (COH).

Some studies have also indicated that such a beneficial effect of luteal phase E_2 supplementation may depend on the protocol used for IVF 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 E_2 levels of higher than 2500 pg/dl at the day of hCG administration. All patients received progesterone supplementation at a dosage of 150 mg/day starting on the day after the 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 embryo transfer; and those receiving no exogenous E_2 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 E_2 co-treatment, respectively; $P < 0.05$). However, such an effect could not be shown for patients with a short, GnRH agonist protocol.

In a study conducted by our group, which involved patients undergoing IVF stimulation with rec-FSH and a GnRH antagonist, the addition of E_2 to progesterone during the luteal phase did not result in a higher probability of pregnancy ($n = 201$, 26% for progesterone and 29.7% progesterone/E_2 group, $P = \text{NS}$; Fatemi *et al*., 2006).

The difference in results between these two studies cannot be explained by the different forms of stimulation protocols used, since the luteal phase characteristics and dynamics of IVF cycles using GnRH agonist or antagonists have been shown to be similar (Friedler *et al*., 2006). Given the difference in results obtained in these studies, the question arises, why should there be any difference concerning the LPS with these two treatment schemes. Further, prospective randomized trials are clearly needed before any conclusion can be drawn (Table 1).

Co-treatment schemes using additional agents with progesterone for LPS

Progesterone with ascorbic acid.

Ascorbic acid (AA) is a preeminent water-soluble antioxidant (Buettner *et al*., 1993) that has long been associated with fertility (Paeschke *et al*., 1969; Wagner *et al*., 1970 and Millar *et al*.,

Table 1: Summary of studies evaluating the addition of E_2 to progesterone on LPS in different stimulation schemes

COH	Long GnRH agonist				GnRH antagonist
	Smitz *et al*. (1993)	Fahri *et al.* (2000)	Lukaszuk *et al.* (2005)	Lewin *et al.* (1994)	Fatemi *et al.* (2006)
Number of patients	378	271	231	100	201
Dose of E_2 (mg)	6	2	6, 2 and 0	2	4
Implantation rate, % (Progesterone with E_2 versus progesterone)	32.8 versus 35.5	15.2* versus 10.2	29.9* versus 17.8 versus 9.8	_	42.4 versus 37.8
Clinical PR/ET, % (Progesterone with E_2 versus progesterone)	29.2 versus 29.5	39.5* versus 25.6	51.3* versus 32.8 versus 23.1	28 versus 26.5	32.6 versus 28.9

*$P < 0.05$. COH, controlled ovarian hyperstimulation.

1992). Luteal regression is associated with ascorbate depletion and the generation of reactive oxygen species, which inhibit the action of LH and block steroidogenesis (Behrman *et al*., 1989; Margoliny *et al*., 1990). Women with unexplained infertility have a lower total antioxidant status in their peritoneal fluid (Polak *et al*., 2001). Griesinger *et al*. (2002) conducted a prospective, randomized, placebo-controlled study to evaluate the impact of AA of different doses (1, 5 or 10 g/day) as additional support during luteal phase (*n* equals; 620). There was no clinical evidence of any beneficial effect of AA, defined by ongoing PR, in stimulated IVF cycles, regardless of the dose used.

Progesterone with prednisolone

One line of research has investigated whether or not immunosuppression by exogenous corticosteroids as a co-treatment for LPS can be used to improve the rates of embryo implantation and pregnancy in IVF patients (Lee *et al*., 1994). The rationale underlying this approach has been that embryos might be exposed to bacteria or leukocyte infiltration if the protective coating of the zona pellucida is breached. Immunosuppression caused by glucocorticoid administration would decrease the presence of uterine lymphocytes and of peripheral immune cells, particularly of segmented neutrophils, which might invade and destroy the zona-dissected embryos. According to this line of reasoning, glucocorticoids used in conjunction with zona dissection would improve pregnancy and implantation rates in IVF patients (Ubaldi *et al*., 2002).

In a prospective randomized study involving routine ICSI patients, however, Ubaldi *et al*. (2002) did not find any beneficial effect of adding low-dose prednisolone to progesterone during the luteal phase. In this group of patients, pregnancy and implantation rates were unaffected by prednisone administration (Ubaldi *et al*., 2002). This is in accordance with earlier research (Lee *et al*., 1994; Moffitt *et al*., 1995). To date, no large-scale randomized studies have confirmed any increase in PR associated with the use of prednisone.

Progesterone with aspirin

Vane *et al*. (1990) described the mechanism of action of aspirin, showing that it inhibits the enzyme cyclooxygenase, thus avoiding prostaglandin synthesis. Luteal regression is caused by a pulsatile release of prostaglandins from the uterus in the late luteal phase (Okuda *et al*., 2002). Because aspirin has also been shown to increase uterine blood flow (Wada *et al*., 1994), clinicians have postulated that aspirin could improve the receptiveness of the endometrium, thereby increasing implantation and birth rates.

Early studies suggested that low-dose aspirin (100 mg) could increase the implantation and PR in women undergoing IVF (Weckstein *et al*., 1997; Rubinstein *et al*., 1999). However, recent studies have been unable to confirm any improvement in IVF outcomes of patients treated with low-dose aspirin in the luteal phase (Urman *et al*., 2001; Hurst *et al*., 2005). It seems that there is no apparent benefit in the routine use of aspirin during IVF cycles, and this practice should be abandoned.

It should be mentioned that a certain subpopulation of patients may benefit from aspirin and prednisone treatment. Combined treatment of prednisone for immunosuppression and aspirin as an anti-thrombotic agent, administered before ovulation induction, may improve PR in autoantibody sero-positive patients (those with anti-cardiolipin antibodies, anti-nuclear antibodies, anti-double-stranded DNA, rheumatoid factor, and/or lupus anticoagulant) who have had repeated IVF embryo transfer failures (Geva *et al*., 2000).

Human chorionic gonadotropin

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 (Whelan *et al*., 2000). By stimulating the corpora lutea, hCG is an indirect form of luteal support. It is known to generate an increase in E_2 and progesterone concentrations, thus rescuing the failing corpora lutea in stimulated IVF cycles (Hutchinson-Williams *et al*., 1990).

Administration of hCG has also been shown to increase the concentrations of placental protein 14 (Anthony *et al*., 1993), integrin αv (Honda *et al*., 1997) and relaxin (luteal peptide hormone) which has been shown to increase at the time of implantation (Ghosh *et al*., 1997).

In the meta-analysis published by Pritts and Atwood in 2002, hCG was shown to be equally effective as IM and vaginal progesterone ($n = 1756$, RR 0.98; 95% CI 0.68–1.42, RR 0.9; 95% CI 0.72–1.14, respectively) for LPS with respect to clinical PR.

In the latest meta-analysis, conducted by Nosarka *et al*. (2005), hCG has emerged as superior to progesterone ($n = 438$, OR 1.71; 95% CI 1.06–2.76; Table 2). The disadvantage of using hCG for luteal support stems from its potential for increasing rates of ovarian hyperstimulation syndrome (OHSS) when compared with other treatments or no treatment at all. Significant increases in OHSS rates have been confirmed in several studies (Buvat *et al*., 1990; Herman *et al*., 1990; Claman *et al*., 1992; Araujo *et al*., 1994; Mochtar *et al*., 1996).

With regards to OHSS, one should therefore be cautious with the administration of hCG for luteal supplementation in stimulated

Table 2: Comparison of the main differences of two meta-analysis using different LPS schemes

	HCG versus Progesterone		
	HCG versus vaginal progesterone (RR) Pritts and Atwood (2002)	HCG versus IM progesterone (RR) Pritts and Atwood (2002)	HCG versus Progesterone (vaginal and IM) (OR) Nosarka *et al*. (2005)
Number of patients	707	486	438
Clinical PR/ET	0.9 (95% CI 0.72–1.14)	0.98 (95% CI 0.68–1.42)	1.71 (95% CI 1.06–2.76)
Delivery rate	–	1.7 (95% CI 0.52–6.27)	–

RR, relative risk; OR, odds ratio.

IVF cycles (Ludwig and Diedrich, 2001). Luteal support with hCG should be avoided if E_2 levels are $>2500–2700$ pg/ml on the day of hCG administration (Buvat *et al*., 1990; Farhi *et al*., 2000) and if the number of follicles is >10 (Araujo *et al*., 1994).

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 *et al*., 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 PR were enhanced for both protocols, in long GnRH agonist protocol the clinical implantation rate were 29.8 versus 18.2% respectively ($P < 0.05$). Ongoing PR were 46.8 versus 38.0% respectively ($P =$ NS). In patients treated with the GnRH antagonist protocol, clinical implantation rates were 27.1 versus 17.4% respectively ($P < 0.05$) and ongoing PR were 44.8 versus 31.9% respectively ($P < 0.05$).

Luteal-phase GnRH agonist administration additionally increased the luteal-phase serum hCG, E_2 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). In order to establish a potential positive role of GnRH agonist administration in the luteal phase of stimulated IVF cycles, further large prospective trials are needed.

Naloxone, an opiate receptor blockade for LPS?

Short-term opioid antagonism has been shown to increase LH pulsatility during the luteal phase (Rossmanith *et al*., 1998). As a result, it has been postulated that prolonged opioid antagonism might also accelerate LH secretory episodes (Rossmanith *et al*., 1998), which could in turn diminish the luteal phase defect in stimulated IVF cycles.

In clinical trials, however, prolonged opioid blockade did not change LH secretory patterns of the luteal phase in eight normal cycling women, suggesting desensitization of the opiate receptors. (Rossmanith *et al*., 1998). Moreover, naloxone failed to stimulate LH secretion in pregnant women. Given that the same supraphysiological steroid levels are present in pregnancy as in stimulated IVF cycles, this result would seem to confirm that naloxone cannot be used to correct the luteal phase defect by enhancing LH secretions. To this day, there no convincing evidence to support the use of naloxone in the luteal phase of stimulated IVF cycles.

The onset of LPS

Timing of LPS remains the subject of debate. Current clinical practice involves beginning LPS on different days. In one study, delaying LPS until 6 days after OR resulted in a decreased PR of 24% when compared with patients who began luteal support 3 days after OR (Williams *et al*., 2001). No difference has been found when LPS was started at OR compared with starting at embryo transfer (Baruffi *et al*., 2003).

Mochtar *et al*. (2006) compared the effect of three different times of onset of LPS on ongoing PR in patients undergoing treatment with GnRH agonist down-regulated IVF and embryo transfer cycles. A total of 385 patients were randomized into three groups according to the day support was started: the day of hCG administration, the day of OR or the day of embryo transfer (occurring on day 3). LPS was administered until 18 days following OR for all patients. There was no significant difference on ongoing PR in the three groups: 20.8% in the HCG group versus 22.7 and 23.6% in the OR group and embryo transfer group, respectively (Mochtar *et al*., 2006).

Further, studies are needed to establish best timing of onset of the LPS. Referring to the published data, it is evident that the timing of LPS should not be later than day 3 after OR. The HCG administered for final oocyte maturation covers the luteal phase for a maximum of 8 days. However, taking the uterolytic effect of progesterone in account, it is recommended to start treating the patients with progesterone at least as early as the day of embryo transfer (if day 3 following the day of OR).

The duration of LPS

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) were

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 (control group) 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 *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, LPS was administered in the form of 200 mg vaginal progesterone three times daily (600 mg/day) during 14 days from the day embryo transfer 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. A total of 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

LPS with HCG or progesterone after assisted reproduction results in an increased PR (Daya and Gunby, 2006). HCG is associated with a greater risk of OHSS. Luteal support with hCG should be avoided if $E_2 > 2700$ pg/ml (Buvat *et al*., 1990) and if the number of follicles is >10 (Araujo *et al*., 1994). Natural micronized progesterone is not efficient if taken orally (Devroey *et al.*, 1989; Bourgain *et al*., 1990). The oral DG might be sufficient for luteal supplementation in IVF cycles, however, more large randomized controlled trails are needed before a conclusion about oral DG can be drawn (Fatemi *et al*., 2007).

Vaginal and IM progesterone seem to have comparable implantation and clinical PR and delivery rates (Nosarka *et al.*, 2005). The addition of E_2 to the progestin support in long GnRH agonist protocol regimen may have a beneficial effect on pregnancy and implantation rates (Farhi *et al*., 2000). However, no positive effect could be demonstrated in short GnRH agonist protocols. Furthermore, concomitant use of E_2 with progesterone after stimulation with rec-FSH and GnRH antagonist does not enhance the probability of pregnancy (Fatemi *et al*., 2006).

Although there have been attempts to introduce GnRH agonist as a novel LPS in stimulates IVF cycles to improve PR, it is too early to adopt this approach across the board (Lambalk and Homburg, 2006). AA, aspirin, naloxone and prednisolone, all of which have been suggested at some point to be beneficial in IVF cycles, have not been proven useful as co-treatment of luteal phase supplementation. The length of LPS in stimulated IVF cycles should not exceed 14 days from the day of transfer (day 3 post OR) until the day of a positive HCG test (Andersen *et al*., 2002). LPS should begin no later than 5 days following administration of HCG to trigger ovulation.

Reconsidering the cause of the luteal phase defect in stimulated IVF cycles, it now appears that supraphysiological levels of steroids are responsible. This reiterates the need to revise approaches to ovarian stimulation of IVF patients. With a growing tendency towards the transfer of a reduced number of embryos (Fauser and Devroey, 2003), and with an increasing number of European investigators advocating single embryo transfer (Strandell *et al*., 2000; Nygren and Andersen, 2001; Papanikolaou *et al*., 2006), attitudes should shift towards milder stimulation protocols. In the coming years, IVF stimulation may evolve into a more physiological process—a milder stimulation—with the significant fringe benefit of reducing or eliminating the current luteal phase defect.

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