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Review

The Impact of Serum Progesterone Levels on the Results of *In Vitro* Fertilization Treatments: A Literature Review

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ABSTRACT

The aim of this review is to analyze the relationship between preovulatory progesterone (P) rise and in vitro fertilization (IVF) pregnancy outcomes. It also investigates the sources and effects of rises in progesterone levels, including the underlying mechanisms and potential strategies in preventing its elevation during ovarian stimulation. Progesterone is produced in the early follicular phase in the adrenal gland, which shifts toward the ovaries prior to ovulation. Several factors contribute to the etiology of P level increase including the number of multiple follicles, the overdose of gonadotropins and poor ovarian response. Nowadays, the influence of the preovulatory P rise on IVF outcome remains controversial. Several authors have failed to demonstrate any negative impact, while others reported a detrimental effect associated with the rise of P. It seems that P rise (1.5 ng/ml or 4.77 nmol/l) may have deleterious effects on endometrial receptivity, namely, accelerating the endometrial maturation process that subsequently narrows the period for implantation and thus decreases pregnancy rates. Recent studies have proposed different cutoffs according to the ovarian response, which may be a little high in patients with high response in relation to those of normal response or low response. To prevent a P rise, it might be preferable to use milder stimulation protocols, earlier trigger of ovulation, cryopreservation of all embryos and transfer in the natural cycle.

Keywords: Fertilization *in vitro*, Ovulation induction, Pregnancy rate, Progesterone (P), Embryo implantation, Luteinization

INTRODUCTION

In the physiologic cycle during the follicular phase, luteinizing hormone (LH) exerts a stimulatory effect on the P450c17 (17-R- hydroxylase/17,20-lyase cytochrome P450) enzyme, which catalyzes 17alfa-hydroxylation and 17,20 bond scission that promote the conversion of progesterone (P) to 17-hydroxy-P, leading to a decrease in P secretion (Lindeberg *et al*., 2007). It was clearly demonstrated that the adrenal gland is a secretory source of P during the early follicular phase. However, the source of P shifts toward the ovaries during the late follicular phase, when both theca and granulosa cells express the enzymes converting cholesterol to pregnenolone (P450 side-chain cleavage enzyme, P450scc) and pregnenolone to progesterone (3-beta-hydroxysteroid dehydrogenase, 3betaHSD) (De Geyter *et al.*, 2002; Payne & Hales, 2004, Miller, 2008). Serum progesterone level rise is often observed during the late follicular phase at the end of ovarian stimulation on the day of human chorionic gonadotropin (hCG) administration in women undergoing in vitro fertilization (IVF), before the introduction of gonadotropin-releasing hormone (GnRH) analogues. This occurs because of an uncontrolled LH surge and hence correctly named as ''premature luteinization''. It was believed that after the introduction of GnRH analogues in IVF cycles, P levels would decrease because LH elevation would be effectively inhibited. However, despite the wide use of GnRH analogues, this unexpected phenomenon of subtle increases in serum P levels is still observed in the GnRH agonist-treated group. The lower serum P levels following the GnRH antagonist protocol are mainly explained by lower granulosa cell steroidogenic activity (Hugues *et al*., 2011). The consequences of serum P increase on the clinical impact have been highly controversial for many years. Some studies could not find any association between P levels and pregnancy rates (Andersen *et al*., 2011; Venetis *et al*., 2007), whereas others have reported a negative impact on cycle outcome when serum P levels are increased (Al-Zemi *et al*., 2012; Huang *et al*., 2012; Kiliçdagc *et al*., 2010). These divergent conclusions could be explained by the lack of consensus on the threshold values and by the huge variability among methods of P measurement. Earlier studies used the absolute P concentration on the day of hCG as an indicator of P elevation with arbitrary set cut-off levels ranging from 0.8 to 2.0ng/ml (Silverberg *et al*., 1994; Ubaldi *et al*., 1996a; 1996b). In recently published studies that use new methods for serum P measurement, this cut-off concentration is usually set at 1.5 ng/ml or 4.77 nmol/l. This cut-off is supported by the presence of a marked difference in the endometrial gene expression profile between patients with a P serum concentration above and below the threshold of 1.5ng/ml (Van Vaerenbergh *et al*., 2011; Labarta *et al*.,2011). In addition to the variables mentioned by various studies, the total dose and duration of FSH ovarian stimulation in GnRH analogue regimens as well as the number of follicles may act as confounding factors (Coucke *et al*., 2007; Andersen *et al*., 2006).

Incidence

The frequency of elevated serum P levels varies according to the stimulation regimen. The incidence has been reported between 5% and 35% of stimulated cycles in women treated with GnRH agonists (Silverberg *et al*., 1991), and between 20% and 38% of cycles when the GnRH antagonist protocol was used (Ubaldi *et al*., 1996a; 1996b). These marked variations in the frequency of progesterone elevation could be explained by the method of P assessment (Andersen *et al.*, 2011).

Why does Progesterone Increase?

Because this subtle increase in P occurs in the presence of GnRH analogues and is associated with low serum LH concentrations, this serum P rise widely differs from the so-called premature luteinization. Only a few studies on the role of the endogenous LH environment similarly concluded that preovulatory P level rise is not related to serum LH levels (Al-Azemi *et al*., 2012; Bosch *et al*., 2003). Nevertheless, in a recently published study, a weak but significantly positive correlation with serum LH/hCG levels was found in patients treated with the GnRH agonist regimen, suggesting that while LH may not play a pivotal role, it may

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still contribute to the serum P rise (Hugues *et al*., 2011). In the etiology of preovulatory P rise, several possible mechanisms were documented, that is, the number and size of multiple follicles, the overdose of exogenous gonadotropin and poor ovarian response with increased LH sensitivity, proliferating granulosa cells as well as increased activity of FSH-stimulated granulosa cells and LH-stimulated theca cells (Fleming & Jenkins, 2010). It was found that the patients with high estradiol concentrations have significantly higher P concentrations and more oocytes (Kyrou *et al*., 2009). Prolongation of the follicular phase by delaying hCG by 2 days after the presence of 3 follicles of 17 mm in recombinant-FSH/GnRH antagonist cycles resulted in raised concentrations of P. Hence, prolongation of ovarian stimulation is an important factor to be considered (Kolibianakis *et al*., 2004). By investigating the relationship between premature P rise and serum estradiol levels and the number of follicles in GnRH antagonist/r-FSH stimulated cycles, a significant impact is demonstrated on P rise by estradiol and the number of follicles on the day of hCG. Patients with a P >1.5 ng/ml had significantly higher concentrations of estradiol and increased number of follicles compared to those with P < 1.5 ng/ml. However, patients with a P >1.5 ng/ml showed lower pregnancy rates than those with a P < 1.5 ng/ml (17.8% versus 32.7%, respectively). It was found that the increased LH receptor sensitivity in the granulosa cells is due to a higher cumulative exposure to estradiol, which in conjunction with FSH, could be one of the mechanisms responsible for the premature elevations in serum P concentrations (Ubaldi *et al*., 1996a; 1996b). Serum P increase is usually seen in patients displaying a good response to ovarian stimulation, which is associated with more cumulus-oocyte complexes retrieved and higher estradiol levels (Fanchin *et al*., 1997a). However, P elevation can also take place in women whose ovarian response is weak, but this could be explained by the fact that these patients need longer stimulation and thus a significantly higher total FSH dose (Fanchin *et al.*, 1997a; 1997b). In addition to the ovary, the adrenal gland seems to be another source of P biosynthesis during ovarian stimulation in the follicular phase of menotropin-stimulated cycles. High estrogen concentrations may cause changes to the hypothalamic–pituitary–adrenal axis and in adrenal enzyme activity as a part of the complex ''cross- talk'' between the hypothalamic–pituitary–ovarian and the hypo- thalamic–pituitary–adrenal axes (Eldar-Geva *et al*., 1998).

Concerning the issue of predisposing factors to elevated progesterone, several of them have already been described - which are proportional to it and summarized as: total dose of gonadotropins (Bosch *et al*., 2010; Andersen *et al*., 2006), duration of ovarian stimulation (Bosch *et al*., 2003; Kolibianakis *et al*., 2004; Filicori *et al*., 2002), and number of oocytes retrieved, as well as estradiol level on the day of HCG (Bosch *et al*., 2010). Regarding the type of pituitary suppression, it has been reported that there is no difference between the use of agonist analogues vs. antagonists, (Bosch *et al*., 2010), because even the long cycles of agonists are not exempt from premature elevation of progesterone (Ubaldi *et al.*, 1996a; 1996b; Bosch *et al*., 2003; Hofmann *et al*., 1993). Whilst paradoxically, what has been seen as a risk factor is the use of FSH alone without LH due to results from studies which compared cycles with rFSH vs. HMG (Andersen *et al*., 2006; Filicori *et al*., 2002).

The Consequences of Increased P

Since the early 1990s, there has been an ongoing debate regarding the impact of preovulatory P on IVF outcome. Its clinical influence has been highly controversial for many years, with some studies reporting a negative impact on cycle outcome when serum P levels are increased on the day of hCG (Venetis *et al*., 2007; Huang *et al*., 2012; Kiliçdagc *et al*., 2010; Bosch *et al*., 2003; Kolibianakis *et al*., 2004; Bosch *et al*., 2010; Schoolcraft *et al*., 1991; Kolibianakis *et al*., 2012), whereas others could not find any association between P levels and pregnancy rates (Andersen *et al*., 2011; Silverberg *et al*., 1994; Fanchin *et al*., 1997a; 1997b). In the first report in 1991, the authors stressed that the premature P rise was associated with a lower pregnancy rate during pituitary suppression with GnRH agonists. P concentrations greater than 0.5 ng/ml were associated with a significantly lower rate of pregnancy (20%) compared with less than 0.5ng/ml (54%). The results suggested that ovarian stimulation might cause excessive luteinization and an adverse cycle outcome even in the presence of low-LH levels (Schoolcraft *et al*., 1991). Venetis *et al.* (2007) published the first systematic review and meta-analysis and found a lower pregnancy rate in patients with elevated P, but the difference did not reach statistical significance. Therefore, the association between P elevation and the likelihood of clinical pregnancy in women undergoing ovarian stimulation with GnRH analogues and gonadotropins for IVF could not be supported and they denied the negative effect of premature P elevation. However, that paper was criticized for the heterogeneity of the studies included, and methodological flaws in the late follicular-phase measurements of P, that may have affected the results of an indeterminate number of studies retained in the meta-analysis. Taking into account the limitations of the meta-analysis when considering the study's conclusions, the clinical consequence of pre-hCG P elevation should be analyzed within the context of the ovarian response to stimulation in which it happens (de Ziegler *et al*., 2008). However, in a more recent analysis from the same group of women undergoing ovarian stimulation for IVF using GnRH antagonists and gonadotropins, P elevation on the day of hCG is significantly associated with a lower probability of clinical pregnancy (Kolibianakis *et al*., 2012). In an analysis on the outcomes of 2,566 patients undergoing their first IVF/ICSI cycles treated with long or short protocols of GnRH agonists it was demonstrated that a premature P rise negatively correlated with live birth rate in fresh embryo transfer (ET) cycles. However, live birth rates in frozen ET cycles have no significant difference between groups with or without P rise (29.31% versus 25.35% in long protocol; 24.84% versus 24.22% in short protocol), implying that P rise may have deleterious effects on endometrial receptivity (Huang *et al*., 2012). In women with good ovarian response treated with long GnRH agonist and hMG/FSH for IVF, elevated serum P levels were associated with diminished implantation rates and live birth rates regardless of ovarian reserve (Kiliçdagc *et al.*, 2010). Assessing the effects of altering the timing of hCG administration on ongoing pregnancy rates in patients stimulated with r-FSH and GnRH antagonists for IVF, it was found that prolongation of the follicular phase does not affect oocyte or embryo quality, but it is associated with a significantly reduced ongoing pregnancy rate (Kolibianakis *et al.*, 2004). An earlier study by Bosch *et al.* (2003) demonstrated that elevated P concentrations were associated with a negative IVF outcome during GnRH antagonist cycles, with a pregnancy rate (25.8% versus 54.0%) significantly lower in the P-elevated group. In a larger analysis of over 4,000 cycles by the same group, it was confirmed that ongoing pregnancy rates following IVF/ICSI cycles were inversely associated with serum P levels on the day of hCG, irrespective of the GnRH analogue used. In particular, patients with serum P levels < 1.5ng/ml or 4.77 nmol/l had significantly higher ongoing pregnancy rates than those with P levels >1.5ng/ml (31%versus 19.1%), supporting the concept of a detrimental effect of P rise in the follicular phase during ovarian stimulation. It seems that high-serum P levels is a

frequent event in GnRH analogue cycles and its occurrence seems to be directly related to the total FSH dose used during ovarian stimulation and the number of oocytes obtained. The results suggest that the negative association between P elevation on the day of hCG and the likelihood of pregnancy could be used to optimize the treatment of patients undergoing IVF/ICSI–ET (Bosch *et al*., 2010).

Although many studies have described an adverse relationship between elevated P concentrations and IVF pregnancy outcomes, the precise endocrinological mechanism is unclear. It has been proposed that peripheral P, in the late follicular phase, is likely to influence endometrial maturation, which may lead to an asynchrony between the endometrium and the developing embryo (Achache & Revel, 2006). In the first analysis of endometrial biopsies using Noyes' criteria, it was found that follicular phase prolongation by delaying hCG administration for 2 days as soon as at least 3 follicles > 17 mm was present on ultrasound results in a higher incidence of endometrial advancement on the day of oocyte retrieval in GnRH antagonist cycles. It can be hypothesized that the delaying of hCG during ovarian stimulation intensifies the receptor changes induced by the significantly higher exposure of endometrium to supra-physiological estradiol levels in the late hCG group (Kolibianakis *et al*., 2005).

Although improved embryo quality has been found in the presence of high-estradiol levels, no subsequent increase in rate of pregnancy was noticed. This observation suggests that elevated estradiol concentrations could have a deleterious effect on endometrial receptivity. An increase in P during the follicular phase, a reflection of heightened ovarian response, can also have a negative impact on the endometrium (Kyrou *et al*., 2009). It was reported that endometrial advancement exceeding 3 days between the histological dating and the cycle day never resulted in an ongoing clinical pregnancy (Kyrou *et al.*, 2009). In addition, significantly higher P levels on day hCG+1 were seen in the late hCG group as compared to the early hCG group (median level 9.1 versus 4.8ng/ml, respectively). P values > 6ng/ml on day hCG+1 have been associated with an earlier downregulation of P receptor expression, as well as with accelerated glandular development and pinopode expression within the implantation window. Thus, advanced endometrial maturation induced by high-P levels on day hCG+1 may be associated with an earlier closure of the implantation window and decreased pregnancy rates in stimulated cycles (Develioglu *et al*., 1999). Moreover, it has been shown that the timing of steroid receptor downregulation in the endometrial epithelium marks the establishment of endometrial receptivity (Lessey *et al*., 1996). The histological dating results by Noyes' criteria in GnRH analogue IVF cycles were confirmed recently at a molecular level in a study in which the advanced endometrial maturation exceeding 3d was shown in a separate molecular cluster profile (Van Vaerenbergh *et al*., 2009). In a recent functional genomics analysis using microarray technology to compare endometrial gene expression profiles at the window of implantation according to the levels of circulating P, it was revealed that elevated P levels on the day of r-hCG can induce significant alterations in the gene expression profile of the endometrium (Labarta *et al*., 2011). A similar study recently confirmed that P rises in GnRH antagonist/rFSH stimulated cycles affects endometrial gene expression on the day of oocyte retrieval (Van Vaerenbergh *et al*., 2011).

On the other hand, several authors failed to demonstrate any negative effect of P rise on IVF outcome (Andersen *et al*., 2011; Silverberg *et al*., 1994; Fanchin *et al*., 1997a; 1997b; Martinez et al., 2004). Using a previously described breakpoint in serum P concentration of 0.9 ng/ml (2.86 nmol/l) in an earlier study, it was found that an elevated serum P level on the day of hCG does not adversely affect the quality of oocytes or resulting embryos. The results suggest that the pregnancy rate in the elevated serum P group is at least equal to the observed rate in the low P group (Silverberg *et al*., 1994). A later investigation demonstrated that in the presence of an adequate response to ovarian stimulation P levels > 0.9 ng/ml were not associated with lower pregnancy rates, indicating that good embryo quality may compensate for the adverse endometrial effects of P. However, when the response to ovarian stimulation was weak, premature P elevation led to drastically reduced pregnancy rates (Fanchin *et al*., 1997a; 1997b). To further assess the potential impact of ''high'' concentrations of circulating P on pregnancy rates and outcomes, the threshold value (0.9 ng/ml) was applied to discriminate between women with ''high'' and ''normal'' P. No significant differences were found with respect to pregnancy and miscarriage rates between these two groups. It was concluded that serum P concentrations could not predict pregnancy in IVF cycles using GnRH agonists and gonadotropins (Martinez *et al*., 2004). The recently published study demonstrated that there is no association between late follicular serum P concentration on the day of hCG and the biochemical and clinical pregnancy rates obtained after ovarian stimulation for IVF/ICSI using a long GnRH agonist protocol and stimulation with r-FSH and r-LH. Instead, a strong significant association was found between the number of follicles/oocytes and serum P concentration, suggesting that each individual follicle contributes to the collective concentration observed in the circulation. Paradoxically, the highest pregnancy rate in the study was found in the group of patients who had the highest late follicular P concentrations (i.e.47nmol/l) and thus developed many follicles. (Andersen *et al*., 2011).

Some authors submit that the progesterone cutoff value that negatively impact the results should be considered according to the ovarian response (Xu *et al*., 2012; Griesinger *et al*., 2013); which can be "normally higher" (about 1.75 ng / ml) in the cycles with ovarian hyperresponsiveness. It is worth to notice that these two studies included patients with only one type of GnRH analogue for pituitary suppression: one with agonists and the other with antagonists.

The group of the "Instituto Valenciano de Infertilidad" (IVI) has also sought to elucidate the effects of premature elevated progesterone on endometrial luteinization, showing through studies involving cycles of frozen-thawed oocyte donation and frozen-thawed embryos that the effect of this alteration is more harmful to endometrial receptivity than to the oocyte and/or embryo (Bosch *et al*., 2010; Bosch *et al*., 2004; Melo *et al*., 2006).

How to Prevent Premature P Elevation

As responses to ovarian stimulation are associated with IVF outcome, a few preventive measures and strategies should be recommended to avoid the adverse consequences of premature P rise to achieve a successful pregnancy outcome (Al-Zemi *et al*., 2012).

The proper time of triggering hCG in different ovarian stimulation protocols currently depends on the size and number of follicles. The criteria for hCG administration usually include estradiol levels and the confirmed presence of >3 follicles of >17 mm diameter by means of sonography (Kolibianakis *et al*., 2004). To avoid the late follicular phase P elevation and consequently its detrimental effect on IVF outcome, it may be reasonable to trigger hCG in advance in high responders than in normal and poor responders (Al-Zemi *et al*., 2012). An earlier trigger of hCG in patients stimulated with r-FSH/GnRH antagonists for IVF resulted in significant differences between the early hCG and the late hCG group regarding estradiol (1388 versus

2040pg/ml, respectively) and P (0.8 versus 1.1ng/ml, respectively) levels on the day of hCG. No significant differences were observed between the early hCG and the late hCG group regarding positive hCG (46.2% versus 50%, respectively) and ongoing pregnancy rates (34.6% versus 40.7%, respectively) (Kyrou *et al*., 2011). In addition, investigating the association between follicular size and oocyte morphology in follicles stimulated by hMG, it was found that oocytes could be obtained even from follicles as small as 11mm in diameter. Therefore, triggering hCG in advance would not compromise the overall quality of the oocyte cohort when most of the trailing follicles are still small (Papanikolaou *et al*., 2009). These data suggest that an earlier trigger of hCG in high responders to prevent a P rise is feasible and useful as a preventive measure.

Another preventive strategy is the use of mild stimulation protocols usually characterized with lower estradiol levels, which would enable P elevation in the late follicular phase (Kolibianakis *et al*., 2004). Because the level of serum estradiol and the number of follicles affect the level of P on the day of hCG, an increase in P during the follicular phase can be anticipated and prevented by modifications to the protocol and timing of triggering final oocyte maturation (Kyrou *et al*., 2012).

In a recent study, it was found that P elevation on the day of hCG was associated with impaired pregnancy outcomes in day 3 single-ETs, while it had no effect on day 5 single-blastocyst transfers. It was proposed that high-follicular P concentrations greatly advance the endometrium, and therefore, the placement of a day 3 embryo in an asynchronous endometrium results in the failure to establish an embryo-endometrium cross-dialogue and thus a failed implantation. On the other hand, the negative impact of P rise on pregnancy rates with blastocyst transfers suggests that the endometrium has already significantly recovered from the violation induced by the supra-physiological steroid concentrations on the fifth luteal day (Papanikolaou *et al.*, 2009).

Because P elevation or high-P levels accompanying high-estradiol concentrations on the day of hCG negatively correlate with live birth rates in fresh ET cycles, the most appropriate choice to avoid the negative effects on pregnancy rate is to transfer frozen embryos rather than fresh embryos (Huang *et al*., 2012; Wu *et al*., 2012). The results in the ongoing pregnancy rate after transferring frozen-thawed embryos in natural cycles with spontaneous LH-P rise compared with natural cycles controlled by hCG for final oocyte maturation and ovulation confirmed the superiority of cryopreserved-thawed human ET (Shapiro *et al*., 2010). It seems that embryo cryopreservation rescues cycles with premature luteinization. In cycles with elevated preovulatory P, the probabilities of implantation and ongoing pregnancy are increased if all 2-pronuclear oocytes are cryopreserved and subsequently thawed and cultured to the blastocyst stage before transfer (Lee *et al*., 2012). In a comparative study about the impact of fresh variants on the success of frozen-thawed ET cycles using 2-pronuclear sibling embryos in women with/without polycystic ovary syndrome, the success of fresh ET was the most important predictor of pregnancy in the frozen-thawed ET cycles in the non-polycystic group. However, the relationship between serum P on hCG day in the fresh cycle and the outcome of subsequent frozen-thawed ET would benefit further evaluation in polycystic ovary syndrome group (Lee *et al*., 2012).

From these publications and those mentioned in the previous paragraphs, we can say that there are several steps to prevent premature elevation of progesterone such as: to use the lowest possible dose of gonadotropins especially in patients with risk of ovarian hyperstimulation, and although it sounds a little strange, to evaluate the option of using FSH combined with LH in patients with a history of premature luteinization in previous cycles. Regarding the monitoring cycle, it is advisable to measure progesterone serially, especially in patients at risk, and also take into account the number of growing follicles in order to try to take measures to prevent premature P elevation, it is like making the decision to cryopreserve either embryos or oocytes when their values exceed the cutoff that each center uses on the day of HCG. The "Instituto Valenciano de Infertilidad" proposed for this value: 1.5 ng/ml (Bosch *et al.*, 2010; Remohí *et al*., 2012)

CONCLUSIONS

The success of an IVF treatment depends not only on oocyte and embryo quality, but also on the endometrial receptivity that allows adequate dialogue between the embryo and the uterus so that a correct implantation occurs. Elevated levels of estradiol and progesterone in a cycle of IVF can affect endometrial receptivity. Thus, the elevation of preovulatory progesterone during IVF cycles appears to have a detrimental effect on pregnancy outcome.

When exogenous FSH is used, it induces expression of LH receptors in granulosa cells, which leads to an excessive sensitivity to the same LH, which in turn could lead to a production of progesterone prematurely, even with low concentrations of serum LH (Bosch *et al*., 2003; Filicori *et al*., 2002; Glamoclija *et al*., 2005). It appears that high progesterone values do not affect embryo quality (Silverberg *et al*., 1994), and that it real deleterious effects occur to the endometrium (Glamoclija *et al*., 2005). It has been proposed that increased P produces asynchrony between the endometrium and the embryo. The source of P production in the early follicular phase is the adrenal, which shifts toward the ovaries just prior to the ovulation.

Premature luteinization (LP) refers to the increase in serum progesterone levels at the end of ovarian stimulation. Its importance lies in the damaging effects it has on IVF cycle outcomes. There is no consensus on the cutoff to define the increased concentration of P, and there are differences in the methods of measuring it, which can cause considerable variation in the relatively low concentrations of the follicular phase. However, different cutoff points have been established in the literature to establish the diagnosis of premature luteinization (LP), ranging between 0.5 and 2 ng / ml (Legro *et al*., 1993; Edelstein *et al*., 1990; Ubaldi *et al*., 1996a; 1996b; Bosch *et al*., 2003; Silverberg *et al*., 1991; Hofmann *et al*., 1993). The major risk factors for progesterone elevation are ovarian hyperresponsiveness, the use of high dose of gonadotropins and the length of ovarian stimulation. LP can appear in either cycles with GnRH agonists, with a reported incidence, which varies between 5 and 35% (Edelstein *et al*., 1990; Fanchin *et al*., 1993; Ubaldi *et al*., 1995; Silverberg *et al*., 1991; Venetis *et al*., 2007), or with antagonists, in 20-38% of cases. (Bosch *et al*., 2003; Ubaldi *et al*., 1995).

There have already been many papers published on this topic; however, some of them do not show a relationship between progesterone levels and pregnancy rates (Check, 1994; Bustillo *et al*., 1995; Levy *et al*., 1995; Miller *et al*., 1996; Venetis *et al.*, 2007; Edelstein *et al*., 1990; Ubaldi *et al.*, 1995; Silverberg *et al*., 1991). The latter work is a meta-analysis, which has some points of controversy like the lack of uniformity regarding the type of GnRH analogue used as well as the cutoff to define elevated serum progesterone. The vast majority of studies offered 0.9 ng/ml as a cutoff value, taken arbitrarily without previous analysis to assess the correlation between the progesterone level and pregnancy rate.

On the other hand, other studies have shown an inverse relationship between the value of progesterone on the day of HCG and pregnancy rates. (Venetis *et al*., 2013;

Bosch *et al*., 2003; Fanchin *et al*., 1997a; 1997b; Bosch et al., 2010). Some authors submit that the progesterone cutoff level that negatively impacts the outcomes should be considered according to the ovarian response (Xu *et al*., 2012; Griesinger *et al.*, 2013); which can be "normally higher" (about 1.75 ng/ml) in the cycles with ovarian hyperresponsiveness. It is worth to note that these two studies included patients with only one type of GnRH analogue for pituitary suppression: one with agonists and the other with antagonists.

Bosch *et al.* (2010) established 1.5 ng/ml as a cutoff level, to define significant effects on pregnancy rates; which was independent of ovarian response and the type of analogue used. Based on this background, it is believed that there is a clear tendency to assert that high values of progesterone on the day of HCG affect pregnancy rates, supported by studies of high methodological quality. Some studies suggest higher cutoffs than others do while others consider that this cutoff depends on the ovarian response. It seems that the type of analogue used does not interfere in it.

Could the premature luteinization pathophysiology be different from ovarian hyperresponsiveness? More studies seeking to establish the relationship between different cutoffs in the value of progesterone on the day of HCG and pregnancy outcomes, dividing the population into several groups according to the ovarian response and the type of GnRH analogue used, are probably needed.

Among the most important measures and modifiable factors that exists to avoid premature elevation of progesterone, we have: mild stimulation protocols, serial measurement of serum progesterone, early HCG injection, association of LH activity in cases of history of stimulation with FSH alone and LP. In oocyte donors, there is no reason to cancel unless progesterone levels suggest premature ovulation. Finally, in IVF cycles with high serum progesterone, one should consider embryo/oocyte vitrification, and otherwise cancel it if levels suggest premature ovulation.

CONFLICT OF INTERESTS

No conflict of interest have been declared.

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