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REVIEW

Elevated progesterone during ovarian stimulation for IVF

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Abstract There is an ongoing debate regarding the impact of premature progesterone rise on the IVF outcome. The objective of this review is to assess evidence of poorer ongoing pregnancy rate in IVF cycles with elevated serum progesterone at the end of follicular phase in ovarian stimulation. It also explores the origin of the progesterone rise, potential modifying factors and possible methods to prevent its rise during ovarian stimulation. This review draws on information already published from monitoring progesterone concentrations at the end of follicular phase in ovarian stimulation. The databases of Medline and PubMed were searched to identify relevant publications. Good-quality evidence supports the negative impact on endometrial receptivity of elevated progesterone concentrations at the end of the follicular phase in ovarian stimulation. Future trials should document the cause and origin of premature progesterone in stimulated IVF cycles.

KEYWORDS: IVF, ovarian stimulation, progesterone rise, origin, prevention, pregnancy

Introduction

In the pre-gonadotrophin-releasing hormone (GnRH) analogue (GnRHa) era, late follicular phase elevations of serum progesterone during the course of *in vitro* fertilization (IVF) cycles happened as a consequence of a premature LH elevation and hence was correctly defined as 'premature luteinization'. The introduction of GnRHa in IVF stimulation protocols made feasible the suppression of LH and thus largely prevented this phenomenon. However, it soon become clear that, despite GnRHa administration, premature progesterone rise during ovarian stimulation

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cycles was still possible. It has been suggested that in some patients the pituitary desensitization induced by GnRHa is incomplete (Hofmann et al., 1993). Therefore, increased LH secretion during the late follicular phase could be sufficient to stimulate granulosa cells to produce progesterone, but inadequate to trigger ovulation (Ubaldi et al., 1995). This could be disputed because, since the introduction of the GnRHa protocol, pituitary desensitization has usually been profound and endogenous LH concentrations have usually been very low (Olivennes et al., 2000; Westergaard et al., 2001).

Schoolcraft et al. (1991) reported that in certain patients progesterone concentrations rose above normal follicular-phase concentrations prior to human chorionic gonadotrophin (HCG) administration despite the suppression of endogenous LH by GnRHa. Many researchers continued to describe this rise as premature luteinization (Bosch et al., 2003; Hofmann et al., 1996; Legro et al., 1993; Ubaldi et al., 1996). However, this term suggests that the excess amount of progesterone is produced by granulosa cells that have started the process of luteinization in the presence of LH rise. This apparently is not the case in studies where GnRHa are administered to inhibit an LH surge, and thus the use of this term in the presence of normal LH concentrations is inappropriate (Venetis et al., 2007). However, it should be noted, that despite pituitary down-regulation, luteinization and premature ovulation could occur in GnRH agonist cycles due to the discontinuous manner of administration (missed injections or nasal spray applications). Additionally, premature LH peaks have been reported in antagonist cycles during ovarian stimulation (Albano et al., 2000), as well in modified natural cycles (Pelinck et al., 2007) and natural cycles (Messinis et al., 2010).

The diagnosis of progesterone rise varies between published studies. Most studies use the absolute progesterone concentration on the day of HCG administration as an indicator of progesterone elevation with arbitrarily set cut-off concentrations ranging from 0.8 to 2 ng/ml (Edelstein et al., 1990; Givens et al., 1994; Hofmann et al., 1993; Silverberg et al., 1994; Ubaldi et al., 1996). In recently published studies, using new methods for serum progesterone assessment, this cut-off concentration is usually set at 1.5 ng/ml (Van Vaerenbergh et al., 2011). This cut-off is supported by the presence of a marked difference in endometrial gene expression profile between patients with a progesterone serum concentration above and below the threshold of 1.5 ng/ml on the day of HCG administration (Labarta et al., 2011; Van Vaerenbergh et al., 2011).

Incidence

Although the frequency of elevated serum progesterone concentrations varies, incidences as high as 35% of stimulated cycles in women treated with GnRH agonists (Edelstein et al., 1990; Silverberg et al., 1991) and 38% of cycles in women treated with GnRH antagonists (Bosch et al., 2003; Ubaldi et al., 1996) have been reported. However, in a large retrospective analysis of over 4000 cycles, the incidence of progesterone rise on the day of HCG administration above 1.5 ng/ml was estimated to be 8.4% in agonist and antagonist cycles (Bosch et al., 2010).

This marked variation in the incidence of progesterone elevation could be explained by the method of progesterone assessment. Recently, Coucke et al. (2007) tested this in a number of modern assays and demonstrated broad compliance, but this did not apply to older assays used.

Possible impact of progesterone rise during the follicular phase

The influences of the progesterone rise in the late follicular phase might be exerted both in the ovary and on the endometrium. Progesterone exerts a local paracrine effect in the ovary influencing folliculogenesis. Administration of the progesterone receptor antagonist RU486 in the mid- or late follicular phase causes a dramatic collapse of the dominant follicle and a decline in serum oestradiol. However, these effects of RU486 occurred without any significant changes in serum concentrations of LH and progesterone. This indicates that RU486 exerts a local action within the ovary (Liu et al., 1987).

Since the early 1990s, there has been an ongoing debate regarding the impact of premature progesterone rise on the IVF outcome (Fanchin et al., 1997; Shulman et al., 1996). Several authors have failed to demonstrate any negative impact of this rise on IVF outcome (Bustillo et al., 1995; Edelstein et al., 1990; Givens et al., 1994; Hofmann et al., 1993, 1996; Ubaldi et al., 1995; Urman et al., 1999), while others reported that pregnancy rates were negatively associated with serum progesterone concentrations on the day of HCG administration (Bosch et al., 2003, 2010; Fanchin et al., 1993; Hamori et al., 1987; Silverberg et al., 1991).

In an attempt to resolve this controversy, Venetis et al. (2007) conducted a meta-analysis of published studies. In this meta-analysis, a lower pregnancy rate was present in patients with elevated progesterone on the day of HCG administration; however, the difference did not reach statistical significance. Therefore, it could not be supported that pre-HCG progesterone elevation adversely affects IVF outcome. However, there is evidence of methodological flaws in the late-follicular-phase measurements of progesterone that may have affected the results of an unknown number of studies retained in the meta-analysis by Venetis et al. (2007), which therefore impacted on the conclusions of the meta-analysis itself (De Ziegler et al., 2008).

In a more recent meta-analysis from the same group regarding the impact of progesterone in GnRH antagonist cycles, progesterone elevation on the day of HCG administration was significantly associated with a lower probability of clinical pregnancy (−9%, 95% CI −17 to −2%) (Kolibianakis et al., 2011).

Moreover, Bosch et al. (2010) reported that elevated progesterone concentrations on the day of HCG administration were associated with a decreased probability of an ongoing pregnancy. In particular, serum progesterone concentrations of $>$1.5 ng/ml (4.77 nmol/l) were associated with lower ongoing pregnancy rates following GnRH agonist and antagonist IVF/intracytoplasmic sperm injection embryo-transfer cycles Patients with serum progesterone concentrations $\leq$1.5 ng/ml on the day of final oocyte maturation had significantly higher ongoing pregnancy rates than

those with progesterone concentrations >1.5 ng/ml (31.0% versus 19.1%; $P = 0.00006$) irrespective of the GnRHa used for pituitary down-regulation. Thus, these findings support the concept of a detrimental effect of a progesterone rise in the follicular phase during ovarian stimulation.

Although recent publications have clearly demonstrated a significant negative association between serum progesterone on the day of HCG and the success of IVF, the involved endocrinological mechanism remains unclear. It has been proposed that peripheral progesterone in the late follicular phase is likely to influence endometrial maturation, which may lead to asynchrony between the endometrium and the developing embryo (Achache and Revel, 2006). Kolibianakis et al. (2004) demonstrated that achievement of a pregnancy was not likely if the endometrium at oocyte retrieval showed a discrepancy of ≥3 days between the actual and the expected chronological date as assessed by Noyes criteria. The histological dating results were confirmed at the molecular level in a more recent study (Van Vaerenbergh et al., 2009) in which the endometria with a discrepancy of ≥3 days were shown in a separate molecular cluster profile. Furthermore, Labarta et al. (2011) demonstrated that significant differences are observed at the gene expression level between endometrial samples exposed to low and high concentrations of progesterone on the day of HCG administration. This may explain the impairment of endometrial receptivity in the presence of elevated progesterone reflected in the lower pregnancy rates reported in the literature (Van Vaerenbergh et al., 2011). On the other hand, no negative impact of progesterone rise on oocyte/embryo quality could be found in several studies (Fanchin et al., 1996; Hofmann et al., 1993; Legro et al., 1993; Melo et al., 2006; Polotsky et al., 2009; Silverberg et al., 1994). These data support the notion that progesterone exerts its adverse effect on the IVF outcome by a reduction in endometrial receptivity and not by a negative impact on oocyte/embryo quality.

Whether the above adverse effect of elevated progesterone on the day of HCG administration is due to the effect of elevated progesterone *per se* or simply a reflection of an increased progesterone exposure was investigated by Kyrou et al. (2011b). It was suggested, that a high exposure to progesterone during the end of menstruation and until the day of HCG administration is associated with a decreased probability of ongoing pregnancy.

The origin of progesterone rise during the natural and the stimulated cycle

The second part of this review aims to explore the origin of the progesterone rise, potential modifying factors and ways to prevent its rise during ovarian stimulation.

Ovarian versus adrenal origin

During the menstrual cycle, FSH acts on granulosa cells, promoting cell proliferation and steroid biosynthesis from cholesterol leading to progesterone biosynthesis. Thus, granulosa cells are active manufacturers of progesterone; however, theca cells are also able to produce significant amounts of progesterone (Tsang et al., 1985).

In ovarian folliculogenesis, the initial development from primordial follicle to preantral follicle is independent of gonadotrophins (Visser and Themmen, 2005). Further growth, however, depends on FSH stimulation (Vegetti and Alagna, 2006). As the granulosa cells respond to FSH, proliferation and growth are associated with an increase in FSH receptors (Vegetti and Alagna, 2006). The theca cells are characterized by steroidogenic activity in response to LH, converting pregnenolone into androgens. Aromatization of androgens to oestrogens is a distinct activity within the granulosa cell layer induced by FSH by activation of the P450 aromatase gene (Gore-Langton and Dorrington, 1981). Androgens produced in the theca layer diffuse into the granulosa layer, where they are converted to oestrogens that are released into the follicular fluid and from here into the peripheral circulation. Prior to ovulation, the granulosa cell layer is characterized by aromatization activity and conversion of theca androgens to oestrogens, an FSH-mediated activity. After ovulation the granulosa cell layer secretes progesterone and oestrogens directly into the bloodstream, an LH-mediated activity (Tanaka et al., 1993). The progesterone production rate is a combination of secretion from the adrenal and the ovaries. Including the small contribution from the adrenals, the production rate of progesterone in the preovulatory phase is less than 1 mg/day. During the luteal phase, the production increases to 20–30 mg/day. During the preovulatory phase of natural cycles, in adult females the blood concentrations of progesterone are at the lower limits of immunoassay sensitivity: less than 100 ng/dl. After ovulation, progesterone ranges from 500 to 2000 ng/dl. In congenital adrenal hyperplasia, progesterone blood concentrations can be as high as 50 times above normal.

In the natural cycles, LH concentrations are more or less constant during the follicular phase (Abraham et al., 1972), allowing for a sufficient supply of androgens. This leads to a continuous rise in oestradiol concentrations, determined by the growing number of granulosa cells in the dominant follicle and resultant increase in aromatase activity. Analyses of steroid concentrations in peripheral veins and those draining the active (with dominant follicle) and contralateral ovary revealed the pathways of ovarian steroids (Coutts et al., 1981). Mid-follicular oestradiol concentrations in the periphery as well as the vein draining the contralateral ovary range from 500 to 1000 pmol/l while oestradiol exceeds 6000 pmol/l in the vein draining the active ovary. To attain these values, the oestradiol must cross the basement membrane between granulose cells and theca cells twice. Similarly, mid-follicular progesterone concentration in the periphery as well as the vein draining the contralateral ovary was found to be <6 nmol/l (0.84 ng/l), while the concentration in the vein draining the active ovary was found to exceed 15 nmol/l (2.10 ng/l). Serum progesterone concentration equivalent to that found in the vein draining the active ovary would be indicative of ovulation. These results show that progesterone is the major secretory product of the growing follicle, of which significant quantities reach the general circulation.

However, a stimulated cycle is not comparable to the natural cycle. In stimulated cycles with multiple follicular growth resulting in supraphysiological serum oestradiol concentration during the follicular phase, the progesterone

output to the periphery correlates directly to the number of follicles and the exogenous FSH stimulation. The major components that might contribute to the degree of progesterone secretion from the ovaries would be the number of follicles (granulosa cells), the FSH drive of granulosa cells and the LH drive of theca cells, which might encourage progesterone conversion to androgens and oestrogen (Fleming and Jenkins, 2010). Other factors that are associated with progesterone rise are the prolongation of the follicular phase by delaying HCG administration (Kolibianakis et al., 2005) and the oestradiol concentrations (Al-Azemi et al., 2011). Kolibianakis et al. (2005) reported that if the follicular phase is prolonged by 2 days after the presence of $\geq$3 follicles $\geq$17 mm is confirmed at ultrasound scan in recombinant FSH/GnRH antagonist stimulated cycles, a lower probability of ongoing pregnancy rate can be expected, probably through prolonged exposure of the endometrium to raised concentrations of progesterone. Hence, prolongation of stimulation is an important factor to be considered. Prolongation of follicular phase is related to the rise of oestradiol. Moreover the rise in oestradiol concentration is associated with high risk of premature progesterone rise (Kyrou et al., 2009).

However, besides the ovary, the adrenal gland is another important source of progesterone production. Evidence for an adrenal contribution to the peripheral progesterone concentration is suggested by a number of studies, showing that serum progesterone can be detected in the peripheral blood of oophorectomized women (Strott et al., 1969), that adrenal vein progesterone concentration exceeds that in peripheral blood (Peterson, 1971) and that adrenectomy reduces serum progesterone concentration (Abraham and Chakmakjian, 1973). In addition, adrenocorticotrophic hormone (ACTH), but not HCG, administration increases serum progesterone in dexamethasone-treated menopausal women (Vermeulen, 1976) as well as during the follicular phase in premenopausal women (Strott et al., 1969).

The P450 side-chain cleavage enzyme is required for de-novo progesterone synthesis. In women, this enzyme is present in the corpus luteum, the theca interna layer of the ovarian follicle and in the adrenal gland (Sasano et al., 1989). Therefore, serum progesterone in the follicular phase could be derived from residual activity of the corpus luteum, from the developing follicle or from secretion by the adrenal gland. In an attempt to investigate the relative contribution of the ovary and the adrenal gland to the overall production of progesterone during follicular phase, Judd et al. (1992) compared serum progesterone concentrations in anovulatory women with normal ovulating follicular phase patients. The study concluded that neither the corpus luteum from the previous menstrual cycle nor the developing follicle was responsible for progesterone secretion during the normal follicular phase. The anovulatory women had chronically low concentrations of LH and absent LH pulsatility, which provides the major stimulus to steroidogenesis in theca interna. Despite this, there was no difference in the pulsatility or mean concentrations of progesterone compared with normal women. Moreover, stimulation of the follicle with clomiphene citrate, which restored LH pulsatility and increased serum oestradiol, had no impact on serum progesterone or progesterone pulsatility during the follicular phase. Judd et al. (1992) suggested that the adrenal gland is the major source of pulsatile progesterone secretion during the follicular phase in this setting. The study showed that, in normal women, dexamethasone, through suppression of ACTH, totally suppressed serum progesterone, indicating that ACTH is the stimulus producing pulsatile progesterone secretion. The authors suggested that the dominant source of progesterone during the follicular phase in the patients evaluated seems to be the adrenal gland and the pulsatility of progesterone appears to be determined by ACTH rather than LH (Judd et al., 1992).

Eldar-Geva et al. (1998) evaluated the origin of the serum progesterone rise during ovarian stimulation by comparing its incidence in ovarian stimulation cycles with and without LH suppression and analysing its response to ACTH suppression by dexamethasone administration. It was concluded that the serum follicular-phase progesterone rise in stimulated cycles appears, in part, to be of adrenal origin. High oestrogen concentrations may cause changes in the hypothalamic–pituitary–adrenal axis and in adrenal enzyme activity as a part of cross-talk between the hypothalamic–pituitary–ovarian and the hypothalamic–pituitary–adrenal axes. Furthermore, data from De Geyter et al. (2002) clearly demonstrated that the adrenal is a secretory source of circulating progesterone during early follicular phase. This was demonstrated by the rapid rise of progesterone after administration of ACTH during suppression of endogenous gonadotrophin secretion with triptoreline acetate. ACTH stimulates the conversion of cholesterol to pregnenolone in the adrenal cortex which is rapidly converted to progesterone. Moreover, it seems that the source of progesterone shifts towards the ovaries just prior to the ovulation (De Geyter et al., 2002).

Potential modifying factors for progesterone rise

Progesterone rise during ovarian stimulation appears to originate either from the ovaries and/or the adrenals and there are several factors suggested to contribute to this rise. Some investigators suggested that serum accumulation of HCG from human menopausal gonadotrophin (HMG) would be responsible for progesterone rise in stimulated cycles (Copperman et al., 1995). Assuming this hypothesis, the use of recombinant FSH would avoid the progesterone rise during the GnRHa protocol. However, this was not the case and progesterone rise was even higher in recombinant FSH as compared with HMG ovarian stimulation (Smitz et al., 2007).

Ubaldi et al. (1996) found that high exposure to FSH is associated with progesterone rise and suggested that this might be related to an increased LH sensitivity of the granulosa cells following FSH stimulation. The increased LH receptor sensitivity of granulosa cells might be due to a higher cumulative exposure to oestradiol, which is associated with an increased number of follicles $\geq$17 mm (Bosch et al., 2003; Filicori et al., 2002).

Kyrou et al. (2009) demonstrated that patients with high oestradiol concentrations have significantly higher progesterone concentrations and significantly more oocytes. The association of high oestradiol and progesterone elevation suggests that at least one of the mechanisms that play a role in progesterone rise is linked to the high response of the ovary to ovarian stimulation. An excess number of follicles,

and consequently an excess of proliferating granulose cells, can lead to an increased progesterone production. Recently, Al-Azemi et al. (2011) demonstrated that by measuring the oestradiol concentrations and number of follicles, one could anticipate the risk of premature progesterone rise. Based on the above finding, it seems that an upcoming progesterone rise could be prevented by modification of the protocol and timing of triggering of final oocyte maturation.

Although progesterone rise is often seen in women displaying a good response to ovarian stimulation and is associated with more cumulus–oocyte-complexes retrieved and higher oestradiol concentrations, it can also take place in women whose ovarian responses to ovarian stimulation are weak. In those cases, a per follicle increase in progesterone production is seen (de Ziegler et al., 2003). The nature of this latter phenomenon could be explained by the fact that these patients need longer stimulation and thus a significantly higher total FSH dose, furthermore it could be considered as an indirect sign of ovarian ageing (Fanchin et al., 1997).

The MERIT study compared ongoing pregnancy rates in 731 women undergoing IVF after stimulation with highly purified HMG or recombinant FSH following a long GnRH agonist protocol (Andersen et al., 2006; Smitz et al., 2007). Highly purified HMG contains FSH activity and HCG-driven LH activity whereas recombinant FSH contains only FSH. The cut-off value for defining 'elevated progesterone' was 4 nmol/l (1.26 ng/l) on the last day of stimulation. This study found that serum progesterone concentrations were significantly higher in the recombinant FSH-treated group than in the highly purified HMG-treated group. In addition, elevated progesterone concentrations were associated with high oocyte yield in both treatment groups. Patients with high progesterone values had lower implantation rates compared with those with normal progesterone concentrations. It was also noted that patients in the recombinant FSH group had more echogenic endometrium.

The main confounding factor in that study was that the triggering for final oocyte maturation was administered at a significantly larger follicular size in the recombinant FSH group as compared with the HMG group (Merit study). Nevertheless, the Merit study confirmed the endocrinological mechanism by which high progesterone concentration may affect IVF outcome. Progesterone values in these cases may have exceeded the 'normal' concentrations prior to the day of triggering final oocyte maturation leading to advancement of endometrial maturation and asynchrony with the embryo developmental stage, finally adversely affecting implantation.

The differences in endocrine profile between highly purified hMG and recombinant FSH (Merit study) were attributed to HCG/LH activity. However, if HCG/LH activity would be responsible for this endocrine difference, then one should observe no difference when comparing recombinant FSH with highly purified urinary FSH.

FSH isoforms: a new theory for the endocrine aspect of follicular and oocyte growth

The clinical efficacy of commercially available gonadotrophin preparations has been the subject of an intense debate, which is primarily focused on the origin of FSH activity (urine versus recombinant) and whether the preparation included LH-like activity. FSH isoform composition has received little or no attention and is usually considered to have negligible effect on clinical effectiveness. However, the FSH isoform profile of commercial gonadotrophin preparations is of clinical importance and should be taken into account when evaluating endocrine aspects and efficacy for each preparation (Andersen et al., 2004).

FSH exists as a family of isohormones exhibiting distinct oligosaccharide structures, and the released FSH isoform mixtures change during the follicular phase of the menstrual cycle. The different isoforms cause a number of different and divergent biological effects. Exposure of cumulus–oocyte-complexes to less-acidic FSH isoforms in a pulse-like fashion results in a rapid pattern of cAMP accumulation exceeding that seen with acidic isoforms. It appears that pulsatile and intermittent release of less-acidic/short-living FSH isoforms is sufficient to induce biological responses, while allowing the granulosa cell FSH receptors to regain responsiveness to further FSH stimulation. Together with the interpulse release of more acidic isoforms, overall FSH secretion seems to ensure proper follicular maturation resulting in the release of developmentally competent oocytes. This may explain the elevation of progesterone in recombinant forms (less acidic isoforms) as these isoforms are more potent than their counterparts of urinary origin (Andersen et al., 2004).

Future studies should evaluate the difference in premature progesterone rise in recombinant FSH and urinary FSH cycles.

Proposal for prevention of premature progesterone rise

The risk of premature progesterone rise appears to be associated with the number and the size of follicles and the intensity of FSH stimulation. Elevated progesterone concentrations are likely to lead to embryo/endometrial asynchrony, reducing the probability of implantation. As available data indicate, responses to ovarian stimulation are associated with IVF outcome, necessitating the development of strategies to prevent progesterone rise and increase the probability of pregnancy.

The time to trigger for final oocyte maturation for both GnRH agonist and GnRH antagonist protocols should be defined. Unfortunately, few data are available in the literature evaluating the proper time for triggering in different stimulation protocols. Currently, clinicians depend on the size and number of follicles to administer HCG. Moreover, for that purpose, it might be necessary to take into consideration the patient's response to a certain treatment protocol. It might be preferable, for example, to trigger earlier in high responders than normal and poor responders to avoid premature progesterone rise and consequently poor outcome. Another question, which needs to be answered, is related to the maturity of the oocyte and its relation to the size of the follicle. Jones et al. (1982) investigated the association between follicular fluid volume (follicle size) and oocyte morphology in follicles stimulated by HMG. The authors evaluated this in terms of oocyte maturity,

which is responsible for establishment of pregnancy after single-embryo transfer. Their findings revealed that mature oocytes can be obtained from follicles as small as 11 mm in diameter. This was also reported by Edwards (1980) who reported 69% recovery of mature oocytes from follicles 10—17.5 mm in size. These data suggest that an earlier trigger in high responders in order to avoid premature progesterone elevation is feasible (Kyrou et al., 2011a,b).

Another preventive measure is to adopt mild stimulation protocols. This approach will prevent high oestradiol concentrations, which are associated with progesterone rise in the follicular phase (Kyrou et al., 2009). Along the same line, oestradiol concentrations were found to be predictive for progesterone rise (Al-Azemi et al., 2011) and subsequently by monitoring oestradiol concentration clinicians can trigger once the oestradiol concentration reaches the point of having a risk of premature progesterone rise. Alternatively, the role of dexamethasone should be evaluated in prevention of progesterone rise if the adrenal gland contributes significantly to its production.

Once the progesterone concentration has breached that compatible with successful outcome, then the solution might be vitrification of all embryos and embryo transfer in natural cycle (Fatemi et al., 2010). This approach is supported by Melo et al. (2006), who concluded that progesterone rise does not appear to have a negative impact on ongoing pregnancy rate in oocyte-donation programme. This confirms the negative impact of progesterone rise on the endometrium rather than the oocyte/embryo quality. Furthermore, Polotsky et al. (2009) and Shapiro et al. (2010), demonstrated that in cycles with elevated preovulatory progesterone, 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.

Progesterone should be measured in each cycle using appropriate assay methods and defined threshold values. Furthermore, the design of prospective randomized studies comparing embryo cryopreservation and transfer in a subsequent cycle in one arm and fresh transfer in the other arm, when progesterone concentration is over 1.5 ng/ml, seems to be necessary, in order to draw solid conclusions regarding the effect of progesterone elevation on pregnancy outcomes.

In conclusion, premature progesterone rise in stimulated IVF cycles seems to have a negative impact on the outcome. The aetiology of premature progesterone rise seems to be multifactorial. Moreover, the source of production seems to be the ovary and the adrenal gland. To prevent this rise, future studies should focus on individualizing treatment protocols, proper monitoring of endocrinological profile during stimulation and, subsequently, timing of the trigger according to the patient's response.

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