Editorial

Progesterone therapy versus follicle maturing drugs - possible opposite effects on embryo implantation

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Summary

Progesterone (P) is an essential hormone to allow the establishment of a pregnancy and to prevent spontaneous abortion during the first trimester. One way to treat P deficiency is simply to supplement extra P during the luteal phase and during the first trimester. Another way to increase serum P is to use follicle maturing drugs. However, these latter drugs may also cause an increase in contra-P hormones so that luteal phase deficiency is common when anovulatory women are treated with follicle maturing drugs. Improved pregnancy and especially reduced miscarriage rates, will be found when P is supplemented in the luteal phase in women requiring follicle maturing drugs. When women have fairly regular cycles and appear to be ovulating they may still have endometrial biopsies that are out of phase and be classified as having luteal phase defects (LPD). A slight majority of women with LPD make mature follicles and respond much better to supplemental P than to follicle maturing drugs. In some cases the use of follicle maturing drugs creates a hostile uterine environment possibly related to premature trophoblast invasion.

Key words: Progesterone therapy; Follicle maturing drugs; Embryo implantation.

Introduction

Progesterone (P) is an essential hormone for early implantation and maintenance of pregnancy until the placenta begins to function. Surgical removal of the ovary with the corpus luteum of pregnancy prior to eight weeks will lead to spontaneous abortion (SAB) [1]. A deficiency of P in the second half of the menstrual cycle, known as a luteal phase defect, has been considered a cause of infertility for over 50 years [2]. The preferred method for diagnosing a luteal phase defect has been endometrial biopsy [3] and most clinicians use even today the criteria of Noyes *el al.*, which was published more than 50 years ago [4]. Probably even in the modern infertility era with sophisticated techniques of assisted reproductive technology, endometrial biopsy remains as the gold standard for most physicians for diagnosing luteal phase defects. However, there is still controversy as to whether it should be performed late in the luteal phase to gain more accumulative effect of P [5-7] or mid-luteal phase when implantation occurs [8-10] or whether an abnormality exists if it is ≤2 days out-of-phase [11, 12] or >2 days out-of-phase [13, 14].

Follicle maturing drugs vs supplemental progesterone

With the advent of follicular maturation drugs, e.g., clomiphene citrate or gonadotropins, it was clear that these drugs raised serum P levels to supraphysiological levels. Thus it was not surprising that these drugs were considered by some as the treatment of choice for luteal phase defects [15, 16]. However, it does not seem intuitively clear that follicle maturing drugs should correct luteal phase defects since some studies have found that when these drugs are used for ovulation induction, 30-50% or more of these cycles are associated with luteal phase defects [17, 18].

Luteal phase defect with mature follicles

In an unpublished study we found that almost all women who achieved a pregnancy attained a dominant follicle of average diameter of 18-24 mm with a serum estradiol (E2) level ≥ 200pg/ml. Using these crite-

ria as a definition of follicular maturity, Check *at al.*, found that 58 of 100 women with luteal phase defects by endometrial biopsy appeared to attain a mature follicle [19]. These 58 women with luteal phase defects and mature follicles were randomized by the last digit of their social security number to receive treatment by P vaginal suppositories in the luteal phase (n = 31) versus a follicle maturation drug (n = 27) (initially clomiphene citrate but it was switched to human menopausal gonadotropins (hMG) if the post coital test was poor) [20]. The six-month pregnancy rate (PR) was 77.4% in the group taking luteal phase P versus only 11.1% in the group receiving follicle maturation drugs without P supplementation. The SAB rate in those taking P supplementation was only 4.2% vs 67% in those taking follicle maturing drugs.

Luteal phase defects without mature follicles

The 42 patients releasing eggs before the follicle was mature had a different response to therapy. The PR was only 25% for those taking only supplemental P compared to 70% for those taking follicle maturing drugs [19]. There were no aborters amongst those taking supplemental P exclusively. However, the miscarriage rate for those taking follicle maturing drugs exclusively was 57.1% vs only 7.1% for those also taking P vaginal suppositories in the luteal phase [19].

Difference in the two types of luteal phase defects

These results suggest that there may well be two types of luteal phase defects: one related to release of an oocyte before a follicle is mature which responds better to treatment with follicle maturing drugs for purposes of conception, but still does not fully correct the problem so miscarriages are more likely unless supplemental progesterone is also given [21]. Then there is a second type where the follicle is mature, in which follicle maturing drugs are ineffective and the proper treatment is just supplemental P. There is the possibility that the type of luteal phase defect resulting from the release of an egg from an immature follicle is more likely to be associated with lower mid-luteal phase serum P levels, and those with mature follicles may more likely require endometrial biopsy to establish the diagnosis [22].

Follicle maturing drugs and toxic uterine environment

There are some data supporting the concept that controlled ovarian hyperstimulation (COH) may adversely affect implantation [23]. One of the first studies matched patients having in vitro fertilization (IVF) to oocyte recipients using donor oocytes with regards to age and previous conception [24]. Despite the transfer of similar numbers of embryos and the finding of no difference in embryo morphology between standard IVF patients and recipients, the clinical and ongoing PRs and implantation rates were significantly higher in recipients [24].

In another study using a shared oocyte program for donors and recipients, the PRs following fresh embryo transfer (ET) were twice as high in the older recipients than the younger donors [25]. However, no significant differences were seen in the PRs between donors and recipients for frozen ETs (though there was a small trend for higher PRs in recipients following frozen ET) [25]. This trend, even with frozen ET, was subsequently found to be related to a lower implantation rate because of hydrosalpinges [26, 27]. In this subsequent study, which also corroborated higher implantation rates in recipients vs donors in fresh ET cycles, the trend for higher PRs in recipients, even with frozen ET, was abrogated once the policy to perform salpingectomies for hydrosalpinges was invoked [28]. A very vivid example of the potential adverse affect of the COH regimen in certain individuals was provided by a case report of a 38-years-old woman with ten years of infertility related to polycystic ovarian syndrome who failed to conceive after six years of ovulation induction and even ten cycles of IVF-ET where 92 embryos had been transferred; however, she conceived on her first frozen ET on an estrogen-P therapy cycle [29]. Interestingly, following delivery, and after cessation of nursing, for the first time in this patient's life her menses became regular. However, she failed to conceive despite nine regular cycles. Yet the first time that P was supplemented in the luteal phase she conceived and she delivered a healthy baby [30].

Mechanism for causing toxic uterine environment

The assumption has been made that the COH regimen somehow inhibits implantation. However, another possibility is that implantation is not impaired but there is premature trophoblast invasion. A study was conduc-

ted to determine if premature trophoblast invasion could be an explanation for poor fecundity in some women following COH and IVF-ET by evaluating whether the appearance of some early pregnancy factor, possibly detected at an earlier time than normal, and which would require trophoblast invasion, might correlate with poor pregnancy outcome [31]. The factor measured for this study was progesterone-induced blocking factor (PIBF), a protein whose production by gamma/delta T-cells requires the induction of P receptors which is initiated by the allogeneic stimulus of the invading trophoblast [32-36]. The earliest that PIBF has been previously detected is in the late luteal phase, and its presence has been associated with a positive pregnancy outcome [37]. For this study, the attempt was made to measure PIBF at the peri-implantation time to see if detection might be associated with a negative pregnancy outcome [31]. Progesterone-induced blocking factor was positive in 14 of 67 (20.7%) women undergoing IVF-ET. Clinical PRs were 7.1% for those positive for PIBF versus 43.4% for those negative for PIBF [31].

Progesterone and the immune system

Normal lymphocytes in non-pregnant women do not demonstrate P receptors [38]. However normal human lymphocytes express P receptors after in vitro allogeneic or mitogenic stimulation [34]. Furthermore, P receptors were also demonstrated in peripheral lymphocytes of liver transplanted and transfused patients [39]. In the peripheral blood of pregnant women there is an increased ratio of gamma/delta T-cell receptor positive lymphocytes and more then 90% of these cells are activated and express P receptors [36, 40]. The exact nature of the paternal antigen that stimulates the P receptor is not known but some data suggests that the antigens may be class I or class I-like molecules [41-43]. In the presence of P, P-receptor positive lymphocytes synthesize the immunomodulatory protein PIBF.

During pregnancy, the maternal immune response is modulated; there is a decrease in cellular immune response and an increase in humoral immunity [44]. The change in immune parameters may be mediated by the secretion of cytokines by T-helper cells (TH cells) [45]. In normal pregnancies, there is a shift in the decidua from the production of TH1 to TH2 cytokines leading to a decrease in cell mediated responses and an increase in immune globulin synthesis [46]. Progesterone-induced blocking factor may exert immunomodulation through its effects on cytokines, especially influencing a shift to TH2 cytokines, which inhibit NK cell activity [32, 35]. Progesterone-induced blocking factor has been found to increase IL-10 (TH2) cytokine production by murine spleen cells and to decrease IL-12 (TH1 cytokine) production [47].

There are data demonstrating that PIBF expression of maternal T-lymphocytes is more likely to be present in the luteal phase in those who eventually have positive pregnancy test [37]. Furthermore, it was found that replacing embryos in the uterine cavity did not result in higher PIBF expression by lymphocytes than non-IVF cases when the pregnancy test was negative suggesting that implantation rather than mere fertilization may be the main factor correlated with PIBF expression [48]. Other studies confirm that P alone without a corpus luteum can cause PIBF expression [49].

Progesterone's role in preventing miscarriage may be through immune modulation

Szekeres-Bartho *et al.*, by using an enzyme-linked immunosorbent assay, measured PIBF levels in normal pregnancies, at the termination of pregnancy (at onset of labor, at time of SAB, and at time of preterm deliveries) and in the 16^{tf} week of women who subsequently spontaneously aborted [50]. All women at pregnancy termination had sera PIBF levels lower then those of healthy pregnant women [50]. They also found that using a cut-off value of 197.5 ug/ml for PIBF, that 52 of 87 women who would eventually abort either immediately or up to 12 weeks later had low PIBF levels [50].

By using a similar immunocytochemistry method as the one presented in this manuscript, Szekeres-Bartho *et al.* compared PIBF expression in pregnant women between the 9th and 40th week of gestation (only seven were tested in the first trimester) [51]. They found that the percentage of PIBF-expressing lymphocytes in the peripheral blood of 96 healthy pregnant women was 67±2.99% vs. only 6.5±1% in 62 women with pathological pregnancies [51].

The data from Szekeres-Bartho *et al.* found that over 90% of pathological pregnancies had PIBF levels below the established cut-off of normal for these later pregnancies (13%) [51]. Interestingly, 90% of these same blood samples showed NK cell activity > 40%, a level considered to be high [51].

Progesterone-induced blocking factor expression requires the induction of P receptors in gamma/delta T

cells by allogeneic stimulation and requires high concentrations of P. The patients used in the studies of PIBF by Szekere-Bartho *et al.* were not treated with supplemental P [50, 51].

Check *et al.* evaluated PIBF in aborters vs non-aborters in women aggressively supported with P so that serum P levels were maintained > 40ng/ml. PIBF expression was observed in 78.4% of the 292 women with ongoing pregnancies compared to 79.1% of 91 women with failed pregnancies in the first trimester [52].

Lymphocyte immunotherapy has been demonstrated to increase PIBF expression [53]. Theoretically, lymphocyte immunotherapy would be helpful for couples not generating sufficient P receptors in gamma/delta T cells so that insufficient PIBF is generated even if the exposure to P is sufficient. In fact, for primary aborters with three or more miscarriages, lymphocyte immunotherapy has been shown to improve outcome when combined with P therapy [54]. However, based on the aforementioned study [52], a deficiency in P seems to be a much more likely mechanism for immune rejection rather than fortuitous sharing of certain transplantation antigens leadingo to inadequate P receptor induction.

Conclusions

Progesterone supplementation in the luteal phase and through the first trimester seems to reverse subfertility and decrease miscarriage rates. Though endometrial biopsy remains the gold standard for diagnosis of luteal phase defects, the future will probably evaluate the effect of P on immunomodulatory proteins, cytokines, and effects on cellular immunity.

Follicle maturing drugs may contribute to subfertility by increasing the risk of premature luteinization [55-58] and the luteinized unruptured follicle syndrome [59-63], or create hostile cervical mucus [64]. However follicle maturing drugs may also decrease fecundity by creating a hostile uterine environment [23-26, 29, 30], which actually may be more related to premature trophoblast invasion rather than implantation failure [31]. Future studies are needed to determine if testing for markers for premature trophoblast invasion may determine if in a given woman follicle maturing drugs are creating an environment not conducive for successful pregnancy outcome.

It is important to perform follicle maturation studies and to treat with follicle maturing drugs if the peak mid-cycle serum E2 is low plus supplemental P in the luteal phase to maximize the conception rate and decrease the miscarriage rate. However, when the follicle appears mature, the therapy should be relegated to exclusive use of P in the luteal phase and through the first trimester.

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