EXPERT REVIEWS

The role of progesterone and the progesterone receptor in human reproduction and cancer

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Insufficient progesterone, effect possibly more on immune factors rather than adequate endometrial development, can be an easy remedial cause of infertility by simply supplementing the luteal phase with either vaginal or intramuscular or oral (dydrogesterone) progesterone. Progesterone will also help to reduce miscarriage rates when follicle maturing drugs are used for those with regular menses but follicular maturation defects, or women with recurrent miscarriages. One mechanism of action seems to be related to production of an immunomodulatory protein, the progesterone-induced blocking factor either in the cytoplasm or in the circulation. PIBF inhibits cytotoxicity of natural killer cells. Cancer cells may 'borrow' the same mechanism to escape NK cell immunosurveillance.

KEYWORDS: cancer • growth factors • immunosuppression • infertility • miscarriage • preterm delivery • progesterone • progesterone receptor • progesterone receptor antagonist • progesterone-induced blocking factor

Infertility related to luteal phase deficiency: diagnosis

The suggestion that a deficiency of progesterone (P) can cause a miscarriage probably can be attributed to a publication as early as 1929 [1]. A lack of progesterone as a cause of infertility was probably first published in 1949 by Georgianna Jones who coined the term 'luteal phase deficiency' [2].

There is little question that if one blocks P effect with even one day of 600 mg of the progesterone receptor (PR) antagonist, mifepristone, that one will most likely cause a miscarriage [3]. However, how important is insufficient P as a cause of infertility and/or miscarriage and how effective is P therapy in obviating these problems is still a highly debated subject.

The basic question is even if the corpus luteum of some women makes less P than others is a small secretion of P able to induce adequate changes in the endometrium to allow implantation? To answer that question the treating physician must first find a method to diagnose a luteal phase deficiency. Early studies evaluated the serum P level in the mid-luteal phase and concluded that the threshold value must be over 1 ng/ml but probably less than 10 ng/ml. Of course, this was the minimal level to establish the appropriate secretory changes in the endometrium, which at that time were criteria established by Noyes *et al.* in 1950 [4]. Unfortunately, there have been many subsequent studies questioning the criteria of the endometrial biopsy established by Noyes *et al.* as a valid test for detecting subfertility.

Certainly, if the biopsy is taken in the late luteal phase and shows early secretory changes, there appears to be a definite problem. Thus, a far out-of-phase endometrium would probably be detected by the less invasive low mid-luteal phase serum P level. However, what is needed is a sensitive assay that can predict a P deficiency as a cause of infertility in a woman who has an adequate mid-luteal phase serum P. One of the problems with the study by Noyes *et al.* is that they did not biopsy infertile women, but instead made the assumption that if a woman has regular menses and has tubal factor then they probably have normal luteal phase function. This does not seem to be a valid assumption since subsequent studies

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found that tubal disease including hydrosalpinges or tubal phimosis can make the endometrium resistant to progesterone as evidenced by finding lower levels of biomarkers that are normally found during the 'window of implantation' and the restoration of these putative biomarkers following salpingectomy [5–7]. Furthermore, there is still no answer as to whether the proper timing for the endometrial biopsy is mid-luteal phase during the window of implantation or late luteal phase to allow an accumulation of the progesterone effect on the endometrium. Also, there was debate about whether an abnormality was 1 or 2 or more days out-of-phase to be considered abnormal. Finally, there was a high rate of discordance in the interpretation of the exact day of secretory advancement by experienced pathologists using the criteria of Noyes *et al.* All of these issues have led to the endometrial biopsy as a diagnostic tool being abandoned by many infertility centers.

Research began around 1988 hoping to find certain molecular products that are the result of progesterone interacting with the PR in the endometrium that are responsible for the endometrial side of embryo receptivity [8]. Many of these studies evaluated luminal epithelium, which is a layer of specialized epithelial cells separate from the stroma and glandular epithelium of the endometrium and is considered the primary barrier to embryo attachment and invasion [9].

MUC1 is one of these luminal biomarkers and is a glycoprotein extending from the luminal surface [10]. MUC1 is considered a barrier to implantation but disappears at the time of implantation [11,12]. Other luminal biomarkers studied include L-selectin ligand [13,14] and trophinin [15].

Many studies have also been conducted concerning α v β3 integrins [16]. A review in 2006 by Achache and Revel seemed to provide encouragement that this could be a good biomarker of uterine receptivity [17].

Unfortunately, despite years of investigation not one of these biomarkers has evolved as an accurate determination of a lack of progesterone of an endometrium not primed for implantation. Even α v β3 integrin has been challenged as a molecular biomarker of inadequate progesterone effect as far back as 1998 [18]. Most recently, a study from one of the most successful *in vitro* fertilization (IVF) centers in the world failed to find any lowering of success rates following IVF-embryo transfer (ET) in women found to be negative for α v β3 integrin compared with those where its presence was detected [19]. Since these women were supplemented in the luteal phase with progesterone and estrogen, it is possible that this supplementation overcame the α v β3 integrin defect. In other words, possibly the pregnancy rates would have been lower in those with negative α v β3 integrins compared with those where it was detected if luteal phase support was not given.

At one time based on the studies of electron microscopy of the endometrium, microscopic projections known as pinopodes were found only during the window of implantation in the rat uterus [20]. However, unfortunately this phenomenon of development and disappearance of pinopodes turned out to be specific to the rat. In the human, pinopodes develop shortly after ovulation and persist right into the first trimester of pregnancy [21].

Several possible conclusions could be reached from a relatively recent study finding that a relatively low level of serum P at 5 ng/ml was not only associated with normal secretory changes upon classical histological changes but so were other markers of endometrial receptivity including endometrial integrins and quantitative reverse transcription–polymerase chain reaction analysis for nine putative biochemical endometrial functional markers [22]. What suggested hypotheses may be derived from this latter study: i) perhaps endometrial development is so well organized that even if the corpus luteum is secreting much less progesterone than the average fertile woman, except for an extreme low value, the endometrium will be prepared for implantation on a molecular basis; ii) perhaps there is no such entity as a luteal phase deficiency where some women who appear to ovulate and can generate at least a serum progesterone level of 5 ng/ml will still have an endometrium that will not allow implantation as the cause of infertility; iii) possibly all women who secrete progesterone sufficiently to surpass 2 ng/ml at mid-luteal phase but <5 ng/ml may have a severe follicular maturation defect and should be treated with a follicle maturing drug, for example, clomiphene citrate or gonadotropins as if they were completely anovulatory; iv) those who still consider that a luteal phase deficiency can be a cause of infertility unrelated to a major follicular maturation defect could argue that though these molecular events are needed for implantation, we are still not measuring the main defective factor. New areas of research involve detecting differences in gene expression during the window of implantation, for example, HOXA-10 expression [23–25]. One group of investigators could not find any histologic or biomarker abnormalities with a serum progesterone in the mid-luteal phase of 3 pg/ml but did find gene expression altered [26]. Progesterone interacting with its receptor has multiple effects on the immune status and the establishment of immune tolerance to the fetal semi-allograft. This will be discussed in the next section; v) the possibility exists that whereas a relatively low level of progesterone is sufficient to develop an endometrium structurally sufficient for embryo implantation it is not prepared to inhibit immune rejection of the fetus because of the need for more progesterone to interact with the P receptors and produce an immunomodulatory protein such as the progesterone-induced blocking factor (PIBF). This will be discussed in the next section.

Proteonomics: a new growing field of investigation

There have been many attempts to try to see if diminished PRs in the endometrium may be the cause of infertility [27–29]. This has been evaluated and there have not been any studies concluding that diminished PRs are the cause of the majority of women with 'documented' tests, especially histology where luteal phase deficiency is detected (although the accuracy of these 'standard' tests are themselves under scrutiny). However, the PR regulates implantation, glandular development and

decidualization through a complex signaling network. There has been an explosion of new information in this area especially using the mouse model. This very complex relationship of the PR initiating paracrine signaling within the uterine microenvironment during the pre-implantation period, its regulation of post-implantation support for the developing embryo and the role of progesterone interaction with its receptor for glandular development has been superbly summarized by Wetendorf and DeMayo [30]. Possibly a defect in paracrine signaling of the Indian Hedgehog gene and the Hedgehog signaling pathway of the three growth factor ligands (sonic hedgehog, desert hedgehog and Indian hedgehog) may be found to play a significant role in achieving a successful pregnancy. Other paracrine factors may include COUP-TF11 (nuclear receptor subfamily 2, group F, number 2), the transcription factor H and 2 which may be the critical mediator between active progesterone signaling and inhibition of estrogen-induced proliferation of the endometrium [30]. Other growth factors may include bone marrow morphogenetic protein (Bmps) which are activated by progesterone and is important in the post-implantation of the developing embryo. Bmps are growth factors that are part of the transforming growth factor-beta superfamily [30]. There are just a few of potential new targets for investigation of molecular markers of progesterone insufficiency [30].

Role of P in allowing immune tolerance to the fetal semi-allograft: the PIBF

The PIBF is a protein which when detected in serum measures 34 kDa and is a splice variant of the parent compound which measures 90 kDa. The parent compound resides in the nucleus at a centrosomal position [31]. The protein seems to be unique in that it shows no significant amino acid sequence homology with any known protein [32].

The full-length protein consists of 757 amino acid residues and is encoded by PIBF1 cDNA. The 48 kDa N-terminal part of PIBF is biologically active [32]. Over 25 years ago, data were presented suggesting that the sensitivity of the immunosuppressive effects of progesterone on natural killer (NK) cell activity were markedly enhanced in the pregnancy state by demonstrating the need to increase the progesterone concentration 100-fold to obtain the same suppressive effect on NK cell activity by non-pregnant versus pregnant lymphocytes [33,34].

The use of the PR modulator (mifepristone) abrogated the immune suppression by a factor secreted by gamma/delta TCR^+ and/or $CD8^+$ lymphocytes. This suggested that progesterone was needed to interact with a PR on these lymphocytes to activate them. Thus, activation by progesterone results in the secretion by these gamma/delta T cells of some immunosuppressive factor [35–38]. The term coined for this immunosuppressive factor is the PIBF and it has now been purified and synthesized by recombinant DNA technology [39,40].

Initially, both *in vitro* and *in vivo* studies suggested that the allogeneic stimulus of the fetal placental unit may be responsible for a hormone independent upregulation of P receptors in gamma/delta T cells [35,41]. The concept was further strengthened by the demonstration that the allogeneic stimulus of lymphocyte immunotherapy can increase PRs on lymphocytes and can increase PIBF expression [42,43].

Early studies of PIBF predominantly used a less sensitive immunocytochemistry technique for its detection because the PIBF antigen had not been purified and only polyclonal antibodies to PIBF could be made. Nevertheless, the PIBF protein was detected in the luteal phase shortly after presumed implantation [44,45]. With the development of a sensitive ELISA assay with the advent of purification of the PIBF protein, PIBF is detected in most women even in the follicular phase, but abruptly rises in the luteal phase shortly after ET [46]. Furthermore, very preliminary studies suggest a correlation with higher levels of PIBF and successful pregnancy [46]. Subsequent studies even found that very high levels of PIBF can be achieved just by exposure to progesterone without an allogeneic stimulus (even in males) [47,48].

One of the main functions of PIBF is to stabilize perforin granules, that is, inhibit their release from large storage granules in NK cells, thus abrogating their cytotoxicity [39,49]. However, the PIBF gene mediates several immunological effects of progesterone. PIBF has been shown to activate STAT 6 by binding to a novel IL-4 receptor [50]. This PIBF effect helps to switch a thymic helper (TH) 1 dominant environment to a TH2 dominant environment, which results in increased production of IL-3, -4 and -10 [51]. Studies from over 20 years ago established that a TH1 dominant environment as opposed to a TH2 dominant environment was associated with poor fetal outcome [52,53]. Subsequent prospective studies confirmed that a synthetic oral progesterone, dydrogesterone, changes a TH1 cytokine environment in women with unexplained recurrent miscarriage or preterm labor to a TH2 type of cytokine dominance [54,55]. Previous studies by the same group had found that the cytokine production by maternal lymphocytes from women with unexplained recurrent miscarriage were mostly TH1 cytokines ones in contrast to normal pregnancy where TH2 dominated [56].

Now that a sensitive ELISA assay has been developed it is hoped that certain threshold serum levels will be found at certain critical parts in the luteal phase or first trimester below which an increased risk of miscarriage or non-conception is detected. This could determine if adding progesterone or increasing the dosage can raise the PIBF level over the minimum threshold, and see if this correlates with successful conception or not. The great advantage of a serum test with a potential rapid assay is that the test can be safely performed in a conception cycle without jeopardizing the pregnancy as with endometrial sampling.

As will be discussed subsequently in the oncology section, it may be the presence of a 34–36 kDa PIBF protein in the cytoplasm of the cancer cells that may prove to be even more important for immune protection. Obviously, this would not lend itself to testing of a live pregnancy but could possibly be performed on an abortus.

This section emphasized PIBF because of the authors' personal experience. It could turn out that for conception the

main role of progesterone interacting with endometrial receptors is to influence the HOX-A-10 gene, which helps promote proliferation of uterine stromal cells [57]. Progesterone may play a critical role in the secretion of certain chemokines, for example, CXC28 and CXCL10 which helps recruit the appropriate type of NK cells (high-density CD56 known as CD56 bright but without CD16 (in contrast to peripheral NK cells)), which may play a critical role in trophoblast invasion [58]. Progesterone may also help regulate the galactin-1 gene, which may be involved in immune tolerance to the fetal semi-allograft and increase the proliferation of IL-10 secreting Treg [59].

The possibility exists that there is a need for higher progesterone secretion in some individuals to achieve the desired immunological effect for successful pregnancy than to achieve the ideal molecular structure of the endometrium. We are only in the early stage of hopefully finding accurate methods to determine if there is inadequacy of immune suppression. This fact will be taken into account when treatment paradigms for unexplained infertility or recurrent miscarriage are discussed in a subsequent section.

There are recent data demonstrating that progesterone can act rapidly by extranuclear (non-genomic) interaction with membrane receptors, for example, PR membrane 1 [60]. The membrane receptors are directly coupled to G proteins which lead to downregulation of adenyl cyclase activity. This interaction can also lead to rapid activation of protein kinases (MAPK, PI3K AKt and c-Src) particularly by a ligand-induced interaction between C-krc kinase and the PR [61]. There are some data to support the concept that progesterone interacting with PR membrane 1 may suppress in an epigenetic manner T cell rejection of the fetal semi-allograft [62].

Role of progesterone in inhibiting immune response against cancer cells

It seems logical to begin the oncology section with a continuation of the discussion of a possible role of progesterone effecting PIBF secretion in allowing cancer cells to avoid immune surveillance. There are at least two forms of PIBF. One is a 90 kDa molecule that has a nuclear location in the centrosome [31]. This is the dominant form present in most rapidly growing cells as evidenced by western blot analysis using PIBF-specific antibodies [31]. There has been identification of the exon 1–5 + 17–18 transcript encoding for a 35 kDa protein [31]. The deletion observed in this transcript preserves the open reading frame for the full-length PIBF protein [31]. Translation of the transcript results in a 35 kDa isoform of PIBF containing the N-terminal 223 and C-terminal 75 amino acids [31]. The PIBF gene has been identified on chromosome 13 in the vicinity of BRCA1 and BRCA2 mutations which are associated with increased breast and ovarian cancers [63]. Variations in other centrosome proteins, for example, p53, are also associated with increased risk of cancer [64]. RNA expression analysis has shown that centrosomal PIBF is overexpressed in rapidly proliferating cells irrespective of whether they have been found to be positive or not for PRs [31].

One study showed that all 29 human leukemia cell lines tested were found to express a considerable amount of mRNA for PIBF [65]. Furthermore, 4 of 10 leukemia cells lines tested by immunocytochemistry were found to express the PIBF protein [65]. Interestingly, adding progesterone to the media upregulated PIBF expression whereas adding the PR antagonist mifepristone downregulated PIBF expression [65]. The question arises as to whether only 4 of 10 human leukemia cell lines did express the PIBF protein or was the immunocytochemistry technique using a polyclonal antibody to PIBF too insensitive to detect the protein secretion by the other 6 cell lines?

Immunofluorescence microassay demonstrated a 35 kDa form of PIBF localized to the cytoplasm of tumor cells [31]. As previously mentioned, until very recently it was believed that the allogeneic stimulus of the fetal semi-allograft was needed to induce P receptors in gamma/delta T cells to allow PIBF expression after exposure to P [37,43]. This led to the hypothesis that the allogeneic stimulus of certain tumor antigens may induce PRs in gamma/delta T cells in the tumor microenvironment leading to PIBF expression and subsequent suppression of NK cytolytic activity and a shift of TH1 to TH2 cytokines similar to the pregnancy state [66]. Through the demonstration of marked palliative effect of PR antagonists for both murine and human cancers (which will be discussed in a later section), data have provided support for the hypothesis that similar to the pregnancy state, PIBF may play a role in allowing cancer cells to escape immune surveillance [67]. A case of acute leukemia and possible lung cancer dramatically responding to mifepristone (a PR antagonist) but without increased levels of PIBF, suggests that it may be the intracytoplasmic location of PIBF that confers immune protection [68]. This intracytoplasmic presence of PIBF and thus potential immunoprotection may be present in all rapidly growing cells, even in tumors in which present techniques have not detected the presence of PRs [31,67,68].

Cancers with known progesterone receptors

As mentioned previously, if it is true that all rapidly growing cells, for example, tumor cells, have nuclear PIBF then it serves to reason that all cancer cells must have PRs [31]. Some tumors may depend on PRs for continued growth and evasion of immune surveillance but these proteins are below the limit which present day antibody techniques can detect. Nevertheless, mechanisms exist which can make these PRs more sensitive, yet they may be non-detected because of downregulation by ubiquitination or decreased by rapid protein loss by proteasome-mediated turnover of activated receptors [69]. In some instances, growth factors can cause a reversible decrease in mRNA expression [70]. Nevertheless, it may be that for cancers in which the PRs are easily detected, these PRs could play an even more pivotal role in the continued growth of the cancer.

The biological activity of progesterone is mediated by genomic pathways through nuclear PRs or by non-genomic pathways using membrane receptors [60]. There are three isoforms of the nuclear receptors: PR-A, PR-B and PR-C. The PRs are ligand activated transcription factor members of the steroid hormone

receptor subfamily of nuclear receptors. PR B is the full-length receptor and PR-A is the N-terminal portion of PR-B minus the first 164 amino acids. It is important in mammary gland development and tumorigenesis [71]. Protein C is an N-terminal truncated form of PR-A and it plays a role in the onset of labor by the inhibition of PR function [72,73]. PRs function not only as critical regulators of transcription but also activate signal transduction pathways.

Breast cancer

One of the malignancies most associated with the PR is breast cancer. Yet the role of the PRs has definitely 'taken a backseat' to studying the estrogen receptor (ER) and determining the mechanism of how the ER eventually escapes from endocrine therapy.

An excellent expert review has already been published in this journal in 2011 concerning PR action and its role in breast cancer [74]. It would be redundant to elaborate in this review on the complex potential mechanisms by which the PR may be involved in cancer cell proliferation since it has been expertly reviewed by Daniel *et al.* [74]. The authors will present only the summary of the review by Daniel *et al.* For more additional information and other references the reader may want to read the excellent review by Daniel *et al.* [74].

The authors review that the PR gene is differentially regulated by two independent promoters: homodimers of A:A or B:B exist along with heterodimers A:B. These dimers can bind DNA at progesterone response elements and/or by tethering to other transcription factors, for example, STATS (signal transducers and activation of transcription), SPI (specificity protein I) and activator protein I. Both PRA and PRB exhibit both ligand-dependent and ligand-independent activity. They make note that these PR functions are greatly influenced by cross-talk and input from peptide growth factor-initiated signal transduction pathways [74].

The authors' emphasis concerns mechanisms of how the PR may be involved in breast cancer cell proliferation rather than the role it may play in avoidance of immune surveillance. More studies are needed to determine the relative importance of these two areas in allowing breast cancer progression.

The authors refer to recent studies suggesting that one of the main mechanisms in involvement of PR in breast cancer cell growth may be an epigenetic extra nuclear action especially with the rapid activation of protein kinases (MAPK, PI3K/Akt and c-Src) in part by a ligand-induced interaction between PR and c-Src kinase. The authors make note that this interaction also involves the estrogen receptor alpha (ERα) and that treatment with anti-estrogens blocked progesterone-induced MAPK activation [74].

The authors emphasize that similar to other steroid receptors, the PRs are significantly post-translationally modified by phosphorylation, acetylation, sumoylation and ubiquitination. An example of these complex interactions and modifications is seen when studying BRCA1. BRCA1 encodes a mainly nuclear protein with two highly conserved domains: a Ring domain at N-terminus and two BRCT motifs at C-terminus. Several cancer predisposing mutations have been found within these regions indicating that BRCA1 is critical in suppressing tumor formation [75]. Progesterone has a stimulating role in breast cancer and BRCA1 was found to inhibit the activity of the ER [76,77]. BRCA1 was also shown to inhibit the activity of the PR [78].

The RING domain of BRCA1 shows E2 ubiquitin ligase activity which is markedly enhanced by heterodimerization with BARD1 [79,80]. Thus, BRCA1 counteracts progesterone action by ubiquitination leading to PR degradation and epigenetic silencing of target promoters [81]. Therefore, mutation of BRCA1 leads to breast cancer, at least partly related to downregulating the PR. Whether this leads to abnormal cell proliferation by excess production of growth factors, for example, kinases, or related to higher intracytoplasmic levels of PIBF thus inhibiting immune destruction remains to be determined with further studies. Interestingly, there is also evidence that ubiquitination may be involved in BRCA1 inhibiting the function of ERα [82]. Thus, where there is mutation of the normally protective BRCA1, steroid receptor cells will respond excessively to estrogen and progesterone (and possibly androgens) which would increase proliferation in surrounding negative cells besides exposing these cells to the effects of lack of functional BRCA1. Also more PIBF could theoretically be produced and thus help to avoid immune surveillance especially but not limited to NK cells. The receptor negative stem cells would then be primed for initiating tumorigenesis.

ER and PR are important prognostic and predicative biomarkers in women with breast cancer [83–85]. More than 70% of breast cancers express ERs and PRs. Patients with hormone receptor negative disease have a higher risk of mortality compared with those with hormone receptor positive disease [83–85]. Even women with very early breast cancer were found to have a greater risk of local recurrence if they were ER+ PR- versus ER+ PR+ [86]. Some data suggest that an early event in breast cancer development is the finding of a change of the normal 1:1 ratio of PR-A to PR-B ratio with a decrease in PR-B [87,88]. For an interesting insight into the significance of this loss of PR-B activity, see the review by Daniel *et al.* [74].

Expert commentary

Treatment with progesterone for infertility

There are three main stages of ovulation: developing a mature follicle, releasing the oocyte from the follicle and steroidogenesis by the resulting corpus luteum including progesterone and estradiol. In an unpublished study of 200 women aged <35 who were not trying to conceive and were previously fertile, the authors found that over 90% attained an 18–24 mm average sized diameter for the dominant follicle and the serum estradiol level was >200 pg/ml.

In 1962, Jones and Poumand published an uncontrolled series of 555 private patients and found exclusive use of progesterone in the luteal phase to be associated with achieving pregnancies [89]. However, possibly because natural progesterone was not commercially produced but had to be compounded by the pharmacies, the use of the commercially produced follicle

maturing drug, clomiphene citrate, became the popular treatment choice for luteal phase deficiencies as diagnosed by either low serum progesterone levels or out-of-phase endometrial biopsies [90–91].

In searching the literature the only prospective randomized controlled study using exclusive progesterone in the luteal phase for infertility was published by the authors' group in 1988 [92]. The method of randomization would not be considered ideal by today's standards for a number of reasons including the lack of a placebo control.

Nevertheless, the study compared the use of clomiphene citrate (or human menopausal gonadotropins (hMG) if the post-coital test was poor related to the anti-estrogen effect of clomiphene citrate) versus the exclusive use of progesterone vaginal suppositories in the luteal phase. The study group consisted of women with a minimum of 1 year of infertility with a male partner with normal semen parameters, bilateral tubal patency and a normal post-coital test. After enrollment, if they showed evidence of an unruptured follicle in their initial evaluation of follicular maturation, they were not included. Only women with endometrial biopsies performed in the late luteal phase which dated 2 or more days out-of-phase were included.

One hundred consecutive women were stratified into two groups based on their initial observation cycle: those who seemed to make a mature dominant follicle (using the aforementioned definition described earlier in this section), and those who showed follicle collapse and secretion of progesterone in the luteal phase but did not seem to attain a mature follicle based on serial transvaginal sonography and serum estradiol levels.

Fifty-eight women making a mature follicle were randomized into treatment with clomiphene citrate or low dosage hMG (n = 27) or just with vaginal progesterone (n = 31). Only 3 of 27 conceived with follicle maturing drugs and 2 of 3 miscarried during the first 6 months of therapy. Thus, the live delivery rate was only 3.7%. By contrast, 24 of 31 women conceived with luteal phase progesterone supplementation with only 1 miscarriage. The live delivery rate was 74.2% [93]. Interestingly, 25 women who failed to conceive during the 6-month study with follicle maturing drugs during the first 6 months were switched to just progesterone in the luteal phase, and 16 of 25 conceived within 6 months with only 1 miscarriage [93].

By contrast, with a three-way randomization in 42 women who did not attain a mature follicle, 7 of 10 conceived with follicle maturing drugs but there were 4 miscarriages. Combining follicle maturing drugs in the follicular phase and progesterone in the luteal phase, the same percent achieved a pregnancy (14 of 20, 70%) but there was only 1 miscarriage. There were only 3 of 12 conceiving with just progesterone supplementation alone but no miscarriages [93].

If one did not separate the group according to follicle maturation, overall 43.8% achieved a clinical pregnancy with follicle maturing drugs versus 60.4% with exclusive use of progesterone. The authors are not aware of any subsequent study that refutes these data. Nevertheless, even to the present day, the authors have evaluated thousands of infertility couples and have found that a high percentage had been previously treated empirically with follicle maturing drugs by other infertility specialists or gynecologists.

As previously mentioned, the endometrial biopsy as performed in the aforementioned study has been criticized as to its accuracy in diagnosing luteal phase deficiency. This has led to a treatment philosophy in the infertility practice to empirically treat women with infertility with regular menses who seem to make mature follicles, have male partners with normal semen parameters, normal post-coital tests and bilateral tubal patency, with progesterone in the luteal phase. This is especially important in women aged 30 or above or even younger women with symptoms or signs of endometriosis.

Though, as mentioned, there have been no studies refuting the aforementioned study published about 30 years ago, there had been no studies corroborating it either. The authors decided to attempt to corroborate their previous study. However, with no remuneration, it would be difficult to convince women to be treated with a placebo for a period of time or give women follicle maturing drugs despite the previous negative data when using these drugs in the presence of mature follicles. Thus, the authors decided to perform a prospective observational series of exclusive use of progesterone in the luteal phase without the use of an endometrial biopsy in women with a minimum of 1 year of infertility [93].

For 32 women aged <39 with an average length of infertility of 2.3 years 23 (71.7%) achieved a live pregnancy past the first trimester within 6 months of progesterone therapy [93]. Also, of great importance, 26 of the 32 women had failed to have a successful pregnancy despite being previously treated for at least 3 cycles of follicle stimulation drugs. One may question how to reconcile these data with previous publications finding that superovulation with intrauterine insemination (IUI) results in higher pregnancy rates than IUI alone [94]. This can be explained by the fact that the aforementioned study of 100 women with regular menses found 42% to release the oocyte before the follicle was mature and 70% conceived within 6 months after using follicle maturing drugs [92]. In the study by Guzick *et al.*, no luteal phase progesterone was given [94]. They may not have reached the same conclusions had the IUI only group been given supplemental progesterone.

Clomiphene citrate and/or letrozole may cause vasomotor side effects, depression, thin endometria, ovarian cysts, hostile cervical mucus and multiple follicles and thus multiple births. Gonadotropins, though not causing vasomotor symptoms, hostile mucus or thin endometria, have an even greater likelihood of causing multiple births or persistent ovarian cysts (from unruptured follicles), but worst of all, they are extremely expensive. Based on these data, the authors would recommend empirical luteal phase progesterone therapy for infertility in women with 'unexplained infertility' rather than empirical use of follicle-stimulating drugs, or worse, going to the most expensive of all therapies, IVF. These data suggest that luteal phase deficiency is common but there is no good method at present

to detect it. If the diagnosis is wrong, the treatment is without risk and relatively inexpensive.

There has been a recent randomized controlled study showing that luteal phase support can increase live birth rates in natural cycles. Bjuresten *et al.* prospectively randomized women having frozen ET in natural cycles to receive luteal phase progesterone (400 mg twice daily vaginal micronized progesterone from the time of the ET) [95]. The controls did not receive any progesterone. The live birth rate was 30% (65 of 219) for those receiving progesterone versus 20% (44 of 216) for those not receiving progesterone ($p = 0.0272$). There was a non-significant trend for a higher clinical pregnancy rate in those taking progesterone (32 vs 25%, $p = 0.1614$). The article did not seem to mention if there were any criteria of follicular maturation to allow them to perform a natural frozen ET versus hormonal replaced cycle. The study by Bjuresten *et al.* would be even more impressive if it was clear that all women were required to produce mature follicles for inclusion. Also, it is not clear whether progesterone was continued in those achieving a pregnancy who were taking it during the first trimester or whether those not taking progesterone were started on it once a positive pregnancy test was achieved [95].

A recent 'integrated view on the luteal phase: diagnosis and treatment in subfertility' in non-assisted reproduction cycle was published [96]. The manuscript was a literature review of the subject, but not a meta-analysis. In this review by Sonntag and Ludwig, they stated "Despite the existing recommendation for rational work-up in subfertility, luteal phase evaluation and progesterone therapy alone is still common in daily practice". Thus their view, in contrast to the authors' suggestion, is that treatment with progesterone in the luteal phase is not rational. According to the second part of this statement, perhaps luteal phase support as sole therapy is common in Germany but not in the USA. Of course, it is possible that the authors' experience is biased since maybe the women previously treated exclusively with progesterone have had a high pregnancy rates and they are just seeing the follicle maturing drug failures.

The statement by Sonntag and Ludwig seems to imply that empirical use of progesterone is being used possibly inappropriately based on 'old fashioned endometrial biopsies' [96]. Their conclusion from evaluating the literature is to 'use ovarian stimulation as the first-line therapeutic option in different subsets of patients with sub-fertility including luteal phase deficiency' [96]. Obviously, based on the authors' positive experience with progesterone, they do not agree with their conclusions, though they do agree that the minority of women who have luteal phase deficiency but release an oocyte before the follicle is fully mature should take follicle maturing drugs (even a small boost of follicle-stimulating hormone (FSH) from mid- to late follicular phase). One caveat is to recall that in the authors' small series though the pregnancy rate was 70% for those conceiving with follicle maturing drugs in the group not developing a mature follicle but 4 of 7 miscarried compared with only 1 of 14 had a miscarriage who also took supplemental progesterone [93]. Thus, in the authors' opinion progesterone supplementation should be used whenever follicle maturing drugs are given.

The 2012 article by Sonntag and Ludwig is the latest review the authors could find on the subject. It did not include their aforementioned study or the one by Bjuresten *et al.* Thus, the authors feel justified in summarizing the data from these studies in the present review [96].

The use of progesterone in the luteal phase for IVF-ET cycles is almost universal. From discussions with various colleagues, and from attending lectures, the authors' impression is that the majority of healthcare providers in the IVF industry think that the reason for luteal phase deficiency may be related to depletion of granulosa-theca cells following follicle aspiration or the use of gonadotropin-releasing hormone (GnRH) agonist suppressing luteinizing hormone (LH) with a slow recovery of LH in the luteal phase. However, it should be noted that Kerin *et al.* in 1981 showed that women having natural cycle IVF-ET did not have associated luteal phase defects [97]. Yet a year prior, Edwards *et al.* found a high frequency of luteal phase defects when follicle stimulation drugs were used to create more follicles [98]. Edwards *et al.* did not use GnRH agonists or antagonists. Thus, it seems likely that the main cause of luteal phase deficiency in IVF-ET is the controlled ovarian hyperstimulation (COH) regimen itself with the change in LH and FSH ratios during the follicular phase and the higher levels of estradiol generated both in the follicular and the luteal phase.

One could argue that perhaps it is the extreme COH used for IVF-ET that leads to luteal phase deficiency not the smaller dosages used merely to induce ovulation in anovulatory women or correct follicular maturation defects. In a quasi-randomized study, the authors found that 28 of 100 (28%) anovulatory women treated with hMG had a miscarriage versus 21 of 130 (16%) treated by progesterone vaginal suppositories [99]. This article was published in 1985, and in those days much smaller dosages of progesterone supplementation (50 mg/day) were used than at present [99]. In another study of 100 anovulatory women that included clomiphene citrate or hMG-treated women (60% took clomiphene), there was a 6% miscarriage rate in those treated with 50 mg/day of vaginal progesterone in the luteal phase versus 28% in the untreated controls [100].

There has been many studies supporting the use of progesterone in the luteal phase for IVF-ET cycles. A recent meta-analysis of these IVF-ET studies have concluded that in 875 women (eight studies), there was an increased live birth rate and clinical birth rate (seven studies) in favor of progesterone versus placebo or no treatment [101]. There were 15 studies and 2117 women comparing luteal phase progesterone versus hCG injection. No difference in pregnancy rates were found but higher rates of ovarian hyperstimulation syndrome was found with hCG [101].

The largest part of the meta-analysis evaluated 9839 women (32 studies) comparing types of progesterone administration. The main results of this comparison did not indicate a difference of effect except in some subgroup analogs. One subgroup demonstrated that synthetic progesterone (dydrogesterone)

showed a significant benefit over oral micronized progesterone [101]. Oral micronized progesterone comes in 100 and 200 mg tablets but it is rendered mostly inactive by rapid first pass in the liver and thus despite good serum levels does not advance the secretory change of the endometrium effectively when compared with the intramuscular or vaginal route [102,103]. Also the metabolites of oral progesterone can cause significant side effects, for example, light headedness, vertigo, drowsiness and gastric discomfort. The use of vaginal and/or intramuscular progesterone after artificial endometrial development by estrogen administration has resulted in high live delivered pregnancy rates following transfer of embryos derived from donated oocytes or frozen thawed embryos.

As mentioned, endometriosis has been associated with progesterone resistance [19,23,25]. However as far back as 1987, the authors had shown that infertility or miscarriage associated with endometriosis can be corrected by luteal phase progesterone supplementation [104]. Surgical treatment of endometriosis is sometimes associated with improved pregnancy rates but it can also damage oocyte supply and thus further impair fertility [105]. Hence, in young women with a clinical history suggesting endometriosis, as long as follicular maturation is achieved and there is the absence of any other infertility factor, they are empirically treated with vaginal progesterone [106].

Progesterone to prevent miscarriage

It has been demonstrated that surgical removal of the ovary with the corpus luteum of pregnancy prior to 8 weeks without progesterone supplementation will lead to miscarriage [107]. Taking a PR antagonist, for example, mifepristone, even 1 day during early pregnancy can terminate a live fetus [108]. Thus, it seems logical that some miscarriages may be related to insufficient progesterone.

Of course, the aforementioned examples were those of the extreme absence of progesterone. The question is whether there is some minimum critical level of progesterone that is needed to maintain a live pregnancy. A study by Yeko *et al.* found that 17 of 18 had a miscarriage if the serum progesterone level was less than 15 ng/ml [109]. Is the low progesterone the cause of the miscarriage or is it merely a reflection of a failing early placenta? If the low progesterone level is the cause of the problem, some of these pregnancies should be salvaged by aggressive treatment with progesterone. Indeed, the authors found that if aggressive progesterone therapy was initiated in the first trimester when the progesterone was <15 ng/ml 70% did not have a miscarriage [110]. The authors even found that 60% could be salvaged with aggressive progesterone therapy with a serum progesterone level <8 pg/ml [111]. Thus, the authors are under the impression that at least some miscarriages may be caused by a corpus luteum of pregnancy not making enough progesterone and intervention with supplemental progesterone can allow a majority to have successful pregnancies. Some of the 30–40% pregnancy losses could have been from an associated chromosome abnormality, and some may have indeed been a reflection of a failing early placenta, and thus a pregnancy that was inevitably doomed.

However, in some instances the low progesterone may have been the cause of the miscarriage but the eventual loss is related to irreparable damage from cellular immune responses not being initially suppressed by an adequate amount of progesterone. Thus, it makes sense for women with a past history of frequent miscarriages not to watch serum progesterone levels carefully and intervene if the level drops to a certain point, but instead to prophylactically treat with progesterone from the early to mid-luteal phase.

Empirical use of progesterone also makes sense also because in some instances the progesterone levels may be normal but there may be resistance to progesterone. If one considers the mounting evidence that endometriosis may be associated with infertility related to progesterone resistance (and it is hoped that supplementing more progesterone can overcome this resistance), it makes sense to start progesterone in the luteal phase. Studies of a baboon model of endometriosis suggest that the progesterone resistance may be associated with epigenetic modification of progesterone-related genes and also its chaperone immunophilins [112]. Fazleabas and collaborators proposed, based on his studies, that both ERs (α and β) were reduced in stromal cells and PRs were reduced in glandular epithelial cells, and that PRs in stromal cells were less responsive to ligand stimulation [113]. Thus, Fazleabas proposes that the reduction of PR-A in the glandular epithelium may be due to dysregulation of ERα and β in the stromal cells that alters the normal paracrine signaling between the two cell types [112]. Of course, there may be some individuals whose progesterone resistance is so extreme that even the use of extra progesterone will not allow the proper endometrial environment for successful implantation or will be inadequate to suppress the immune attack against the fetus.

A recent Cochrane meta-analysis including 4 studies and 421 participants found a reduction in the rate of threatened abortion by the use of progesterone compared with placebo or no treatment (risk rate: 0.53; 95% CI: 0.35–0.79) [114].

Preterm labor

There are numerous published studies demonstrating a benefit of progesterone in having a tocolytic effect and in the reduction of preterm birth as discussed in the excellent summary article by Di Renzo *et al.* [115]. The authors believe that institution of progesterone with bleeding or cramping in the first, second or third trimester can extend the length of gestation. However, in individuals at risk from a previous history of preterm delivery the progesterone should be started in the luteal phase prior to establishment of pregnancy [116]. It is the authors' policy that when giving progesterone in the luteal phase for infertility or risk of miscarriage, they generally taper the dosage to zero after 12 weeks, but continue it even to 36 weeks if cramping or bleeding occurs with the slowly decreasing progesterone dosages.

Progesterone receptor antagonists for cancer treatment

As previously mentioned, P and the PR seem to have a significant effect on cell growth and production of molecules that affect the immune system. The authors previously demonstrated that not only did all 29 human leukemia cell lines produce a

large amount of mRNA for the PIBF protein but a few cell lines that were tested and actively secreted the PIBF protein showed upregulated PIBF expression when progesterone was added to the media and downregulated PIBF expression when the PR antagonist mifepristone was added [65]. It will be recalled, among other things, PIBF inhibits NK cell cytolytic activity.

The structure and function of PRs in breast cancer was described by Horwitz in 1987, but he along with McGuire hypothesized potential endocrine therapy related to inhibiting progesterone in 1975 [117,118]. Mifepristone was given to advanced stage tamoxifen-resistant women with breast cancer. Tumor regression was found in 18% in one study, and only 1 of 11 in another study but this study did show 6 others to exhibit stabilization of the metastatic lesions [119,120]. However, another subsequent study seemed to put a quash in the interest in PR antagonists since PR+ women with metastatic breast cancer only showed a 'partial response' in about 10% of the women and most reported side effects to 200 mg/day of mifepristone (the authors find this interesting since they find 200 mg mifepristone to be tolerated extremely well). However, another study using another antiprogesterone onapristone found in 19 patients that two-third showed clinical evidence of tumor regression, 56% showed partial response and 11% had stable disease [121].

Based on the authors' observation with human leukemia cell lines and PIBF and the work by Lachmann *et al.* finding PIBF in all highly proliferating cells, it was decided to first treat spontaneous murine cancers with mifepristone to test the hypothesis that was published in 2001 that PIBF may be involved in establishing immune tolerance to cancer cells similar to the conceptus by producing PIBF [31,65,66]. The authors gavaged mice with spontaneous leukemia, lung cancer, testicular cancer and prostate cancer, and found improvement in length and quality of survival compared with controls [122–124]. The authors subsequently published some anecdotal reports showing significant palliative effects in patients with very advanced and highly metastatic cancers, all of which were resistant to standard therapies including colon cancer, thymic cell epithelial cell cancer, transitional cell carcinoma of the renal pelvis, leiomyosarcoma, pancreatic cancer, malignant fibrous histiocytoma and acute leukemia [125–127]. Recently, mifepristone was shown to cross the blood–brain barrier by demonstrating an objective clinical improvement in a male with end-stage glioblastoma multiforme [128]. In some of these aforementioned studies, there was demonstration of significant improvement on predicted length of life and marked clinical improvement [125–127]. These aforementioned cancers are not known to be PR+.

The reader should be aware that this section is reserved for the authors' expert opinion which will obviously be biased based on the authors' research experience. To be fair it is important to mention other possible ways that progesterone can promote cancer growth and why mifepristone may thwart progression of cancer cells. As mentioned earlier, when discussing escape of the fetal semi-allograft from immune surveillance that progesterone may interact in an extranuclear (epigenetic or non-genomic manner) manner to suppress T-cell rejection of the fetal semi-allograft [62,129,130]. Thus similarly, progesterone acting on extranuclear membrane PRs could inhibit T-cell rejection of cancer cells. It should be recalled that mifepristone is a selective PR modulator. Similar to selective ER modulators in some ways, it may act as an antagonist to progesterone and in some ways as an agonist. Chien *et al.* showed that mifepristone was antagonistic to the membrane PR non-genomic response [131]. However, it can act as an agonist to progesterone by enhancing the inhibition of phytohemagglutinin-stimulated T-cell proliferation by progesterone [131]. Thus, the beneficial palliative effects of mifepristone may possibly not only promote NK cell 'attack' on tumor cells by a PIBF mechanism, but could suppress T-cell response by its interaction with non-classical membrane PRs.

One way that progesterone can promote immune tolerance is by suppressing human lymphocyte proliferation (as seen with a shift of TH1 to TH2 cytokines). Glucocorticoids similarly suppress T-cell proliferation. Mifepristone also has weaker suppression of the glucocorticoid steroid receptor and thus inhibits the suppression of PHA-lymphocyte proliferation by cortisol [132]. Interestingly, however, mifepristone fails to negate the suppression of PHA-induced lymphocyte proliferation by progesterone but was in fact synergistic [132]. Thus, mifepristone may act through other mechanisms than suppression of PIBF to help inhibit cancer growth.

Five-year view

It would make sense for those pharmaceutical companies manufacturing progesterone to try to immensely expand their market by convincing the obstetrician generalist to empirically treat with progesterone in the luteal phase for infertile women with circumstances likely to be associated with luteal phase deficiency (e.g., advancing age, pelvic pain, premenstrual syndrome, or premenstrual spotting, or short time interval from cervical mucus to menses) rather than empirical use of clomiphene citrate (as so many physicians are presently doing now). Hopefully, recent presentations and publications re-establishing high success rate of correcting infertility with this methodology, and making physicians aware of the potential adverse effects of follicle maturing drugs including compounding the infertility problem, will lead to renewed interest in exclusive progesterone managements for infertility, especially in women who appear to make mature follicles.

The empirical use of progesterone for infertility can be extended to those with frequent and/or recurrent habitual miscarriage. Unfortunately, the authors do not think that reproductive endocrinologists will be influenced greatly by innocuous but effective treatment measures since it seems that the majority of them are more enthralled with assisted reproductive technology.

The practicing endocrinologist has seen over the years a dwindling of their patient load because a great percentage of these endocrine problems, for example, thyroid disease, diabetes and hypertension have been usurped by family physicians, internal medicine specialists or other medical subspecialists. The medical endocrinologist is better suited to understand the complexities of reproductive endocrinology and would be more patient in using non-invasive technology. Hopefully

manuscripts, for example, the present one, will evoke an interest in this area for medical endocrinologists and enable them to increase their clinical population by treating both medical endocrinology and infertility (non-assisted reproductive techniques). This could lead in the next 5 years to more teams of medical and reproductive endocrinologists banding together with the former treating the medical aspects of infertility and the latter the surgical and IVF aspects.

There will be more studies in the next 5 years trying to determine abnormal endometrial gene sequencing to diagnose luteal phase deficiency. Just recently, transcriptomics of the human endometrium has led to the definition of a genomic signature of human endometrial receptivity [133]. An endometrial receptivity array (ERA) has been designed to identify endometrial receptivity by comparing the genetic profile of a test sample with LH+ 7 controls in a natural cycle, or day 5 of progesterone administration after estradiol priming in a hormonal replacement cycle (as for donor oocyte recipients or frozen ETs) [134]. It consists of a customized array containing 238 genes that are differentially expressed between these profiles, which is coupled to a computational predictor that can diagnose the personalized endometrial window of implantation of a given patient regardless of their endometrial histology [134]. The accuracy and reproducibility of the ERA has been demonstrated [135]. Thus, there is now established a method for detection of the expression of a cluster of endometrial biomarker genes to assess endometrial receptivity [134–136]. The next 5 years should see the ERA used to evaluate endometrial receptivity during the window of implantation to detect cases of low receptivity despite normal mid-luteal phase P levels in natural cycles, which may determine when the dosage of P supplementation is inadequate in natural cycles, those with mild stimulation with follicle maturing drugs, and when used for COH in IVF-ET cycles by detecting an abnormal gene signature.

The ERA could possibly be used to answer other questions, for example, is there an abnormal gene signature during the window of implantation if the peak serum estradiol prior to the hCG injection is very high? There are some case reports that vividly demonstrate that in some instances the COH regimen results in implantation failure [137–139]. Of course if the ERA does show an abnormal gene signature during the window of implantation following COH and correctable by mild stimulation, one would still not be sure if the etiologic factor was the high estradiol or abnormal FSH pattern during the follicular phase [140].

The ERA could possibly help to ascertain if the use of estrogen supplementation in the luteal phase in addition to progesterone is more likely to result in a normal genetic signature during the window of implantation than the use of progesterone alone. Some IVF-ET studies using GnRH agonists have found a mild beneficial effect of adding estradiol to progesterone supplementation in the luteal phase [141,142]. However, an IVF-ET study using GnRH antagonists did not find that adding E2 to P was beneficial for improving pregnancy rates [143]. A prospective randomized study added estradiol valerate 6 mg to progesterone vaginal suppositories (600 mg/day) in women who superovulated but were not undergoing IVF-ET and found no difference in pregnancy outcome [144]. Nevertheless, there may be a minority of women where just adding progesterone in the luteal phase is insufficient to correct endometrial receptivity and possibly this could be determined by ERA (or maybe by insufficient generation of PIBF). Hopefully, this will prove more beneficial than detection of putative biomarkers.

The authors will be launching shortly a very large study to hopefully find a critical value for PIBF below which either a woman does not conceive or a minimal level associated with greater risk for miscarriage. If so, one can determine if extra progesterone treatment in the luteal phase can raise the PIBF level over this discriminatory level. If not, one could determine if other therapies could improve levels, for example, salpingectomy for hydrosalpinx, surgical removal of endometriosis or other therapies including lymphocyte immunotherapy. In a quasi-prospective randomized study, it was found that the addition of lymphocyte immunotherapy plus progesterone resulted in less miscarriages than progesterone alone in women with recurrent miscarriage [145]. If some women fail to increase PIBF above the threshold level below which there is an association with miscarriage (assuming this is found) despite progesterone therapy, and if lymphocytic immunotherapy will be found similar to the previous study to raise serum PIBF (but now using a more accurate and sensitive assay), possibly the medical governing agencies will re-allow clinical trials with lymphocyte immunotherapy in properly selected cases.

As mentioned, most reproductive endocrinologists are enamored with assisted reproductive technology. In the authors' opinion, the trend will be for reproductive endocrinolgists to recommend women with frequent or habitual miscarriages to have IVF, grow the embryos to day 5 blastocysts, perform a trophectoderm biopsy, cryopreserve the blastocysts with vitrification and transfer back the frozen-thawed embryo in a subsequent cycle but only those embryos that have normal chromosomes according to a 'comprehensive chromosome screening' (evaluation of all 24 chromosomes). The type of cycle for frozen ET would most likely not be a natural one but one of graduated estradiol followed by progesterone supplementation.

Recently, the Colorado Center for Reproductive Medicine sent out an advertisement to reproductive endocrinologists showing a miscarriage rate of only <3% for women up to 40 and about 6% in women >41 using the comprehensive chromosome screening technique. Although these are highly selected patients, the very low miscarriage rates emphasize that the majority of women will be able to deliver a live baby if a chromosomally normal embryo implants. However, it is not clear how much the supplemental progesterone contributed to this low miscarriage rate.

There is no evidence that recurrent aneuploidy is a cause of recurrent miscarriage [146]. However, the possibility exists that there are some exceptions and it will be interesting to see what happens to those women choosing to have IVF-ET with 24 chromosome analysis to see if there are some women who are much more prone to meiosis errors. Of course, these types of data will

only determine if some women are just more prone to meiosis errors under the conditions of COH. There are data suggesting that the increase in early follicular serum FSH by COH in women with diminished oocyte reserve can lead to meiosis errors [147].

There will be many women with recurrent pregnancy loss who cannot afford IVF-ET and comprehensive chromosome screening. Again, this is an area, that is, recurrent miscarriages, where the medical endocrinologist can increase the patient volume by also indicating an interest in treating recurrent or frequent miscarriage. Progesterone and establishing a mature follicle would be taken over by the medical endocrinologist.

Lack of side effects and convenience will influence physician's prescribing tendencies as long as efficacy is established. A progesterone vaginal ring which is replaced weekly was found to be clinically efficacious to progesterone vaginal gel for IVF-ET cycles and has less local side effects [148]. It will soon be available for commercial use on the US market. Oral dydrogesterone has been found to be well tolerated and equally efficacious to vaginal progesterone gel and should find increased usage in the next 5 years in countries where this drug has been approved [149].

From the cancer treatment standpoint, the authors think there will be an increase in interest to use PR antagonists for breast cancer but possibly other cancers as well [150]. Probably, one of the things thwarting the use of mifepristone for cancer therapy has been its use for performing therapeutic abortions. Of course, many drugs used to treat cancer can terminate a live fetus. However, there are many new PR antagonists either recently approved for clinical use or are soon to come onto the market including lonaprisan (which is more PR specific and thus has less antiglucocorticoid side effects) and patients with breast cancer are being recruited for a Phase II clinical trial [151]. There are other selective PR modulators with low glucocorticoid affinity including asoprisnil which act as agonists on the ovary but antagonists in the breast and the endometrium. Thus, this compound would not only markedly produce less side effects of adrenal insufficiency but also eliminate the risk of endometrial hyperplasia that is present with mifepristone [152].

Should a positive benefit be found in breast cancer trial for lonaprisan this could lead to clinical trials with other cancers perhaps in the next 10 years. In the meantime, hopefully recent articles and new antiprogestins will spark interest in treating on an individual basis which hopefully will lead to the publication of more positive case reports. The authors are aware of two clinical trials recruiting patients for early stage breast cancer: one for mifepristone and one for lonaprisan [150]. Some of these newer PR antagonists will not block progesterone effect at the endometrial level and thus create less risk of endometrial hyperplasia compared with mifepristone.

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Key issues

- Endometrial biopsy, measurement of progesterone receptors (PRs) and molecular markers have proven disappointing in detecting luteal phase deficiency.
- Progesterone alone is able to interact with PRs on gamma/delta T cells to express a unique 34 kDa protein which suppresses natural killer (NK) cell cytolytic activity and causes a shift of thymic helper 1 (TH1) to thymic helper 2 (TH2) cytokines.
- Purification of the progesterone-induced blocking factor (PIBF) protein and the development of a monoclonal antibody has led to the development of an ELISA assay. Thus, PIBF may be the next target to evaluate even if progesterone secretion is adequate for successful conception.
- Follicular maturation studies should be used to determine if women with infertility and regular menses but suspected luteal phase deficiency should be treated exclusively with progesterone in the luteal phase or have the addition of follicle maturing drugs in the follicular phase.
- Progesterone treatment is the main method by which a physician can help prevent miscarriage or preterm labor other than *in vitro* fertilization and transfer of embryos that are chromosomally normal using 24 chromosome analysis and trophectoderm biopsy.
- The PIBF 90 kDa molecule has a centrosomal location near BRCA1 and BRCA2 but a split variant of 34–36 kDa is present in the cytoplasm of most rapidly proliferating cells. Intracytoplasmic PIBF may be most important in providing immune protection for cancer cells.
- There is a complex relationship and cross-talk involving the estrogen receptor and PR and isoforms of the PR which through both genomic and non-genomic mechanisms control cell growth.
- Mutations, for example, BRCA1, where there may be inadequate degradation of the PR by ubiquitination may lead to uncontrolled cell growth or the production of an excess of factors, for example, PIBF preventing immune destruction.
- PR antagonists, for example, mifepristone, has been found to prolong life and diminish suffering not only in animal and human models where tumors are known to be PR+, but also in ones not known to have PRs.
- New more specific PR antagonists that have little antiglucocorticoid receptor antagonists and ones that will not block progesterone effect at the endometrial level are in the pharmaceutical pipeline clinical trials for cancer are forthcoming.

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