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ARTICLE

Oestrogen and progesterone action on endometrium: A translational approach to understanding endometrial receptivity

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Abstract Embryo attachment and implantation is critical to successful reproduction of all eutherian mammals, including humans; a better understanding of these processes could lead to improved infertility treatments and novel contraceptive methods. Experience with assisted reproduction, especially oocyte donation cycles, has established that despite the diverse set of hormones produced by the ovary in a cycle-dependent fashion, the sequential actions of only two of them, oestrogen and progesterone, are sufficient to prepare a highly receptive endometrium in humans. Further investigation on the endometrial actions of these two hormones is currently providing significant insight into the implantation process in women, strongly suggesting that an abnormal response to progesterone underlies infertility in some patients.

KEYWORDS: embryo implantation, endometrium, oestradiol, progesterone

Introduction

A thorough understanding of the processes governing human embryo implantation would be of significant benefit for the treatment of infertility and the development of novel contraceptives. However, implantation processes remain poorly understood, largely due to differences between humans and experimental animals and appropriate ethical, moral and legal barriers to direct examination of implanting human embryos. Despite these barriers, significant knowledge has been gained through experience with assisted reproduction coupled with application of improving analytic techniques applied to human tissues and non-human primate models.

Experience with donor oocyte IVF cycles has allowed profound clinical insights into the regulation of human endometrial receptivity. Donor oocyte cycles achieve the highest implantation rates of all assisted reproduction approaches

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(Sunderam et al., 2009), suggesting that the hormonal preparation of the endometrium has been well optimized (van der Linden et al., 2011). In donor oocyte cycles, the endometrium of the recipient is prepared by sequential treatment with oestrogen and progesterone, using protocols that prevent ovulation and corpus luteum formation. Notably, these protocols work just as well in a woman without ovaries. Thus, these two hormones, without any other ovarian or corpus luteum products, are sufficient for excellent preparation of human endometrium to accept an implanting embryo. Their primacy is further supported by the requirement of both hormones for pregnancy initiation and early survival in all eutherian mammals, despite major species-specific differences in ovarian and uterine anatomy and physiology. Given the critical and fundamental role that oestrogen and progesterone play in establishment of receptivity, a deep understanding of the action of these steroid hormones on the human endometrium will allow clear insight into the mechanisms determining endometrial receptivity. This review will attempt to summarize the current, albeit limited, understanding of oestrogen and progesterone action in determination of endometrial receptivity.

Molecular biology of oestrogen and progesterone action

Both oestrogen and progesterone act through specific, high-affinity, low-capacity nuclear receptors that function as ligand-activated transcription factors and chromatin modifiers to directly regulate expression of a large number of genes (Cheung and Kraus, 2010; Huang et al., 2010). The products of steroid receptor-regulated genes can also act in a downstream, autocrine, paracrine or endocrine fashion to regulate expression of additional genes. It is important to recognize that some non-steroidal ligands can also bind the steroid receptors. Examples of non-steroidal ligands which act through oestrogen receptors include endogenous lipoxin A4 (LXA4), an eicosanoid produced in the endometrium (Russell et al., 2011), bisphenol A, an environmental compound (Li et al., 2012), and clomiphene citrate, a pharmaceutical agent. Thus, nuclear steroid receptors are responsible for the so-called 'classical' actions of oestrogen and progesterone (**Figure 1**).

It is important to point out some significant simplifications made to improve readability in **Figure 1**. For example, oestrogen receptors and progesterone receptors are bound to chaperone proteins and are released from them after ligand binding. Chaperone binding may regulate steroid receptor availability and access to the nucleus, and therefore function. Another key feature of the classical actions of oestrogen and progesterone, not included in **Figure 1**, is that there are multiple oestrogen receptor and progesterone receptor isoforms, each having distinct actions on the genome. Differential expression of these isoforms in different cell types and physiological states results in differential effects of the steroids.

There are two nuclear oestrogen receptors — oestrogen receptor α and oestrogen receptor β — each derived from a distinct gene (*ESR1* and *ESR2*, respectively). These genes have high sequence homology, likely resulting from an ancient gene duplication event, since homologous genes

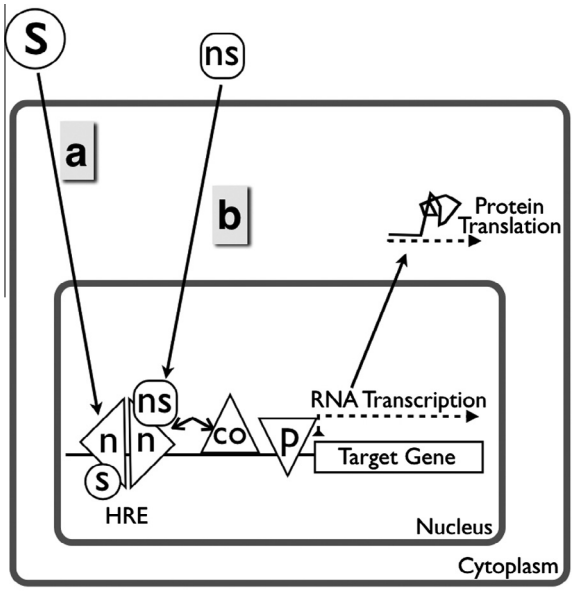

Figure 1 Classical actions of nuclear oestrogen and progesterone receptors. (a) Steroid receptors bind steroid and then bind cognate DNA sequences. (b) Non-steroidal ligands can also act through nuclear steroid receptors. co = co-regulator; HRE = hormone response element; n = nuclear steroid receptor monomer; ns = non-steroid; p = RNA polymerase; s = steroid.

are seen in fish and amphibians as well as mammals (Katsu et al., 2008). Although similar in structure, oestrogen receptors α and β have distinct effects in experimental model organisms and distinct patterns of expression in human disease (Hewitt and Korach, 2003). For example, overexpression of oestrogen receptor β is observed in endometrioma lesions due to hypomethylation of the promoter leading to a molecular cascade resulting in inflammation and other pathophysiological changes (Bulun et al., 2010).

The progesterone receptors have at least two isoforms — progesterone receptor A and progesterone receptor B. Unlike oestrogen receptors, the progesterone receptor isoforms are derived from alternate transcription and translation start sites in a single gene (*PGR*; Jacobsen and Horwitz, 2012; Ogle, 2002). Progesterone receptor A and B are identical in structure except that the progesterone receptor B isoform contains a 164-amino acid N-terminal sequence, which is lacking in the progesterone receptor A isoform. The presence or absence of the N-terminal extension appears to be responsible for the distinct differences in progesterone receptors A and B actions. Truncated isoforms — progesterone receptor C and progesterone receptor M — that retain the progesterone-binding domain but lose the DNA-binding domain have been described as a possible suppressor of progesterone receptors A and B action, but their relevance *in vivo* is controversial (Samalecos and Gellersen, 2008; Taylor et al., 2009; Wei et al., 1990).

A further level of complexity is seen in the interaction between steroid receptors and co-activators and co-repressors. These co-activators and repressors mediate the effects of the nuclear receptors on gene transcription (**Figure 1**). The expression and activity of the co-activators and co-repressors can be determined both developmentally

and dynamically in the adult, providing a further basis for the pleiotropic effects of steroid hormones. In this regard, it is important to note that there are distinct mechanistic differences between mammalian species in steroid hormone and co-activator expression. For example, oestrogen receptor β appears to be significantly more expressed in human endometrium as opposed to the mouse. A more extreme example is the progesterone receptor B specific co-activator, MAGEA-11, which is only present in primates and appears to play an important role in the human endometrial response to progesterone (Su et al., 2012).

The effects of progesterone via its receptor also depend on other signals and transcription factors. An indisputably critical action of progesterone on endometrial stroma is decidualization. However, full decidualization requires signalling by both progesterone receptor and cAMP (Kajihara et al., 2013). Interestingly, cAMP induces expression of many transcription factors, including FOXO1, C/EBPb (CCAAT/enhancer-binding protein b), STAT5 (signal transducers and activators of transcription 5) and HOXA11, all of which directly interact with and modulate progesterone receptor (Kajihara et al., 2013). These factors, including progesterone receptor, form multimeric complexes at promoters for genes critical to a decidualized phenotype. Without this synergistic interaction between other cellular signals and transcription factors, progesterone would not exert this important effect on endometrial stroma. Emerging data suggesting that progesterone-driven decidualization may act as a biosensor of embryo quality during early implantation is reviewed by Lucas in this issue (Lucas, 2013).

Another simplification in **Figure 1** is that steroid receptors dynamically interact with chromatin in a manner regulated by chromatin remodelling, chaperones, the proteasome and binding of other transcription factors (Grontved and Hager, 2012). Oestrogen receptor and progesterone receptor isoforms can only bind DNA if the chromatin structure is open enough to allow access. The areas of open and closed chromatin in a particular cell type in a particular physiological environment are yet another mechanism for tissue-specific actions of oestrogen and progesterone.

In this context, it is important to note that epigenetic mechanisms and microRNA expression may be important modifiers of progesterone action. Initial studies in humans have shown epigenetic changes with cycle phase, including alterations in DNA methyltransferase and histone-modifying enzyme expression (Guo, 2012). Initial studies have also shown significant cycle-regulated changes in microRNA through the cycle (Altmae et al., 2013; Sha et al., 2011). The role of microRNA in both normal endometrium and in endometriosis are discussed in the review by Hull and Nisenblat (2013, in this issue).

In addition to their direct, genomic effects, both oestrogen and progesterone also exert rapid, 'non-classical' effects on the cell via action at the plasma membrane, via nuclear receptors interacting with other transcription factors or via less well-understood effects on mRNA stability (**Figure 2**). Oestrogen can act through both membrane-associated oestrogen receptor α and a structurally unrelated, integral membrane, G-protein coupled oestrogen receptor, GPR30, to stimulate one or more cytoplasmic signalling cascades in response to oestrogen. The effects of signalling via GPR30 in the endometrium are unclear, but there is a profound cyclic regulation of this receptor (Plante et al., 2012).

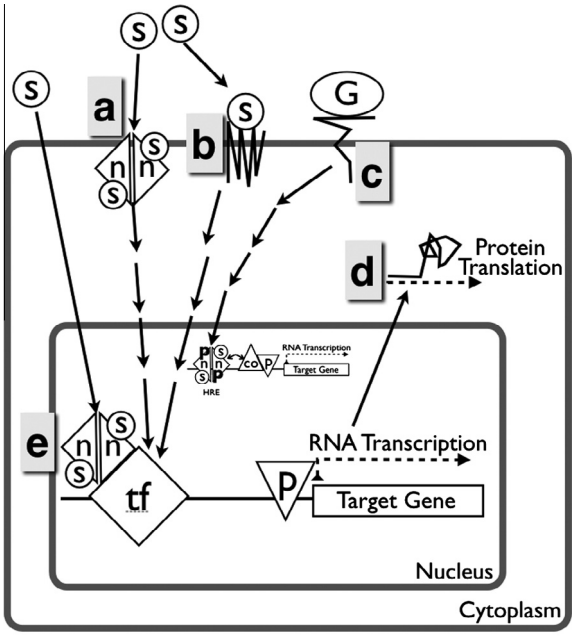

Figure 2 Non-classical actions of nuclear oestrogen and progesterone receptors. (a) Membrane-associated steroid receptors, either isoforms of classical receptors, or (b) unrelated transmembrane receptors recognize steroid hormones and initiate a cytoplasmic signalling cascade. (c) Growth factors signalling can act by causing post-translational modifications of nuclear steroid receptors. (d) Additionally, oestrogen and progesterone can modulate expression by altering mRNA turnover and translation. (e) Alternatively, steroids can bind classical nuclear receptors, which act by binding other proteins rather than DNA. co = co-regulator; G = growth factor; HRE = hormone response element; n = nuclear steroid receptor monomer; ns = non-steroid; p = RNA polymerase; s = steroid; TF = transcription factor.

The non-classical actions of progesterone are less-well understood, but no less complex. As mentioned above, alternative transcription start sites in PGR may result in production of progesterone receptor M or progesterone receptor C, although conflicting evidence exists regarding their relevance *in vivo*. A separate family of membrane progesterone receptors, mPRα (PAQR VII), mPRβ (PAQR VIII) and mPRγ (PAQR V) that are structurally unrelated to the PGR gene, can also bind progesterone and are thought to activate G-protein coupled signalling pathways (Dressing et al., 2011; Zhu et al., 2003). Significant controversy exists regarding the structure and function of this molecular family. For example, the predicted structure of PAQR family members shows eight transmembrane domains rather than the seven seen in the G-protein coupled receptor family and there is no significant sequence similarity to known G-protein coupled receptors (Moussatche and Lyons, 2012). Furthermore, the PAQR family shows sequence motifs more closely related to alkaline ceramidases and may have similar enzymic activity (Moussatche and Lyons, 2012). Thus, the function of the PAQR family receptors remains to be firmly

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established and although expression of the mRP family has been shown in the human endometrium, their role in endometrial function remains unclear (Fernandes et al., 2005). Finally, a newly described membrane channel/receptor on human spermatozoa, CatsPer, is capable of binding progesterone (and other compounds released by the cumulus–oocyte complex) and causing calcium influx (Brenker et al., 2012; Lishko et al., 2011). However, CatsPer expression appears to be sperm specific and is, therefore, unlikely to play a role in the endometrium.

Endometrial receptivity to embryo implantation exists for a brief period of time and this timing is driven by time of progesterone exposure, only after sufficient exposure to oestrogen. Given this temporally specific process, it is not surprising that expression and localization of steroid receptors and their co-regulators vary markedly in different menstrual cycle phases (**Table 1**). In all eutherian mammals studied, oestrogen receptor disappears from the endometrial epithelium at the time of embryo implantation (Donaghay and Lessey, 2007). In the human endometrial epithelium, both oestrogen receptor and progesterone receptor immunohistochemical staining diminish markedly during the midsecretory implantation window (Lessey et al., 1988; Young and Lessey, 2010). Further analysis of the mid- and late proliferative phases shows that progesterone receptors A and B are easily detected in both epithelial and stromal compartments of the human endometrium (Mote et al., 2000; Wang et al., 1998). In the secretory-phase epithelium, progesterone receptor A expression is virtually absent during the mid- and late secretory phases, while progesterone receptor B expression is maintained at low concentrations through the mid-secretory phase and falls to even lower concentrations by the late secretory phase. In the stroma, progesterone receptor A expression is significantly higher than progesterone receptor B throughout the cycle, although present in low abundance in the late secretory phase. Given the absence or paucity of oestrogen receptor and progesterone receptors A and B in the mid- to late secretory endometrial epithelium, it is likely that epithelial effects of oestrogen and progesterone during these cycle phases results from oestrogen- or progesterone-induced paracrine factors, produced in the stroma and acting on the epithelium, termed oestromedins and progestomedins. Potential human endometrial oestromedins and progestomedins include insulin-like growth factor 1 (Giudice et al., 1993), heparin-binding epidermal growth factor (Leach et al., 1999; Young et al., 2002) and fibroblast growth factor 7 (Koji et al., 1994).

Role of oestrogen in embryo implantation

While molecular studies of oestrogen and progesterone receptors provide the mechanistic framework for understanding endometrial function, it is the physiological and clinical studies that provide the most practical insight into implantation mechanisms. Oestrogen is essential for endometrial proliferation, as repeatedly demonstrated in humans and experimental animals lacking ovaries and those in whom oestrogen production or action has been prevented.

The role for oestrogen in the secretory phase and in implantation is less clear. In mice, oestrogen appears to be critical to support implantation and early pregnancy (Dey et al., 2004). Interestingly, the decidualized mouse endometrium appears to produce its own oestradiol and does not require corpus luteum-derived oestrogens (Das et al., 2009). As far as is known, there is no substantive data to support this pathway in human decidua.

There are, of course, many differences between human 28-day menstrual cycle and the mouse 4-day oestrus cycle, including circulating oestradiol concentrations. Mouse peak serum oestradiol concentrations in pro-oestrus are equal to or lower than typical perimenstrual nadir concentrations in the human and 10–20 times lower than peak preovulatory concentrations. However, oestrogen action in the human midsecretory phase could possibly occur through other, non-steroidal oestrogen receptor agonists. An eicosanoid, LXA4, was recently shown to bind oestrogen receptor α and act as an agonist, and the biosynthetic pathway for LXA4 appears to be present in the human endometrium (Russell et al., 2011). Further work is needed, however, to determine any role that LXA4 might play in the human endometrium.

Studies in women without functional ovaries demonstrate that luteal oestrogen is not necessary for normal day-25 morphology or normal changes in oestrogen receptor and progesterone receptor immunolocalization (de Ziegler et al., 1992). Surprisingly no vaginal spotting was noted in

Table 1 Cyclic steroid receptor expression in the human endometrium.

Compartment	*Phase*			
	Proliferative	*Early secretory*	*Mid-secretory*	*Late secretory*
Epithelium				
Oestrogen receptor α	++++	++	–	–
Oestrogen receptor β	++	++	++	++
Progesterone receptor A	+++	++	–	–
Progesterone receptor B	+++	++	+	–
Stroma				
Oestrogen receptor α	+++	++	– or +	–
Oestrogen receptor β	++	+	+	+
Progesterone receptor A	++	++	++	++
Progesterone receptor B	++	++	+	–

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the subjects during the 10 days of progesterone treatment without any oestrogen given. In another study employing oestrogen receptor antagonism with clomiphene begun 2 days after LH surge in a spontaneous cycle and continued until biopsy on day 13 resulted in consistently delayed histological maturation (Fritz et al., 1987). The clomiphene antagonism study findings are echoed by experiments in the bonnet macaque; in these studies, peri-implantation administration of aromatase inhibitor (fadrozole) or oestrogen antagonist (tamoxifen) markedly decreased, but did not eliminate, conception. In another primate study, this time in oophorectomized rhesus macaques, provision of progesterone alone was able to support endometrial receptivity, early post-implantation embryo development and normal pregnancy (Ghosh et al., 1994).

In order to better understand these apparently conflicting data, this study group analysed gonadotrophin-releasing hormone downregulated cycles followed by oestrogen (at varying doses) and progesterone replacement (Groll et al., 2009). Effects on endometrial histology and immunohistochemical staining for integrin subunit β3, osteopontin, oestrogen receptor α and progesterone receptors A and B were examined. These studies demonstrated no difference in between groups not receiving oestradiol and those receiving physiological or supraphysiological oestradiol.

It is striking that the oestrogen receptor inhibitor studies demonstrate a necessity for luteal-phase oestrogen, while progesterone (with or without oestrogen) replacement studies show no luteal-phase requirement. A possible explanation is that in studies where exogenous progesterone is given, there is sufficient extra-ovarian conversion of progesterone to oestrogen (via testosterone) to maintain endometrial function. The oestradiol antagonism and aromatase inhibition studies might provide a more profound impact by blocking oestrogen action (even that derived in the endometrium). The data in the ovariectomized rhesus macaque, however, remains remarkable, because systemic oestradiol concentrations were measured and shown to be very low, even with administration of progesterone. Taken together, the data suggest that the (human or non-human) primate endometrium appears to function normally with very low concentrations of oestradiol.

Clinical data are also mixed. It is well known that use of gonadotrophin-releasing hormone agonists or antagonists in non-donor IVF cycles results in a shortened luteal phase and possibly other qualitative luteal defects. Thus, luteal support with progesterone and sometimes oestrogen is given. Clinical outcomes are mixed demonstrating a benefit of luteal oestrogen supplementation in IVF (Farhi et al., 2000; Lukaszuk et al., 2005) or no benefit (Fatemi et al., 2007; Lewin et al., 1994; Smitz et al., 1993). The most recent systematic review suggests no overall benefit (Fatemi et al., 2007). Given the experimental results in women and monkeys with absent luteal function and the mixed evidence in clinical trials, any possible clinical benefit of luteal oestrogen support in IVF must accrue only to a small subset of patients.

Role of progesterone in embryo implantation

Progesterone is absolutely required for successful embryo implantation and pregnancy maintenance. In fact, progesterone was discovered because of its effects on the endometrium and early pregnancy survival (Allen and Corner, 1929; Allen and Doisey, 1923). The effects of progesterone on the endometrium were confirmed in non-human primates (Zuckerman 1937), leading Georgeanna Seeger Jones to characterize patients with possible progesterone deficiency leading to infertility (Jones, 1949, 1973). The concept that progesterone insufficiency will cause infertility is logically irrefutable. Progesterone is necessary for implantation and pregnancy survival and thus, at some lower threshold, there will be insufficient progesterone for these functions. However, the methods of diagnosing progesterone insufficiency (or sufficiency) and therefore its role in patients have been controversial.

There are three major contributors to the uncertainty regarding the role of luteal-phase defect in infertility. The first is that the corpus luteum releases progesterone in pulses, which are rapidly cleared from the body, resulting in marked fluctuations of progesterone serum concentrations (Filicori et al., 1984), changing as much as 6-fold within a few hours. The rapidly fluctuating concentrations preclude using individual serum progesterone measurements as a measurement of progesterone sufficiency. Secondly, there is no 'gold standard' marker of endometrial receptivity to embryo implantation that would allow evaluation of endometrial function outside of a conception cycle. Current progress in the identification of markers of the receptive endometrium is discussed by Salamonsen et al. (2013, in this issue). Thirdly, there are clear differences between species in the mechanisms regulating embryo implantation, but profound ethical issues prevent systematic study of human embryo and endometrial interactions *in vivo*.

To avoid the aforementioned barriers to understanding progesterone sufficiency in endometrial function, this study group has utilized a modelled cycle, in which progesterone concentrations are experimentally determined (**Figure 3**). The controlled cycles are highly similar to endometrial preparation for an oocyte donor IVF cycle, and thus should result in a highly receptive endometrium, if physiological progesterone is provided. The protocol begins with lupron downregulation, followed by transdermal oestrogen replacement at physiological concentrations, followed by oestrogen plus daily i.m. progesterone at physiological and subphysiological concentrations, and subsequent biopsy on day 10 of progesterone treatment. Using this model, endo-

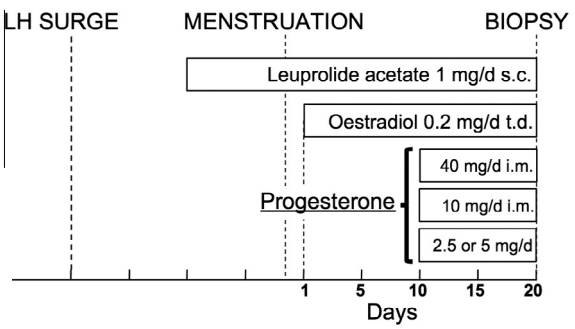

Figure 3 Protocol for modelled cycles (adapted from Usadi et al., 2008).

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metria from healthy women exposed to physiological concentrations of progesterone (40 mg dose, steady-state concentration about 15–25 ng/ml) were compared with those exposed to subphysiological (10 mg dose, steady-state concentration about 4–6 ng/ml) and assessed histological dating of endometria, immunohistochemistry for endometrial integrins and quantitative real-time PCR analysis for nine putative functional markers (Usadi et al., 2008). However, despite a 4-fold difference in progesterone, none of the assessed markers of endometrial structure and function showed a significant difference between groups. Given the critical importance of progesterone action in the endometrium and the expectation of a dose-dependent response, a further reduction in dose will certainly have effects on both histology and gene expression. However, the data to date clearly demonstrate that progesterone concentrations in the low end of what is seen in ovulatory women do not cause profound changes in human endometrial structure or function. Thus, it would appear that, in normal women, a progesterone dose threshold can be defined, below which consistent alterations in gene expression and in histological maturation can be seen. Since this threshold concentration is below the lowest serum concentrations encountered clinically, the data strongly suggest the following two conclusions: (i) isolated progesterone deficiency is very unlikely to be a cause of infertility in couples; and (ii) normal secretory-phase endometrial structure and function in young healthy women can be achieved across a wide range of progesterone concentrations. It must be noted that these experiments were performed on young healthy women without any evidence of endometriosis or infertility.

In all of the above studies, it must also be recognized that local effects of sex steroids can be strongly influenced by local metabolism. For example, a recent study examined oestrogen metabolizing enzyme concentrations in human endometrial tissue as well as serum and tissue oestradiol and oestrone concentrations (Huhtinen et al., 2012). These studies showed marked differences between serum and tissue oestradiol/oestrone ratios, which depended on cycle phase and correlated with the type of 17β-hydroxysteroid dehydrogenase expressed.

Progesterone and endometriosis

Abnormalities in endometrial oestrogen and progesterone action

It has been postulated that women with endometriosis-related infertility may be partially resistant to progesterone actions on the endometrium (Bulun et al., 2010; Burney et al., 2007; Fazleabas, 2010). Strikingly, the baboon model demonstrates that simply inducing peritoneal lesions can result in changes in progesterone action, consistent with progesterone resistance (Fazleabas, 2010). It is presumed that local inflammation is involved in the observed alterations in progesterone action, although the mechanism for this remains unclear. This hypothesis could explain why some women have persistently delayed histological maturation or persistently abnormal expression of progesterone-regulated genes. If progesterone resistance is truly present in some women, then, depending on the mechanism conferring resistance, such women might achieve normal secretory-phase structure and function with a higher progesterone dose or with treatments targeted at abnormal inflammation.

Given the known mechanisms of progesterone action, resistance might occur through a variety of means. Abnormal expression of specific progesterone receptors is one possible mechanism and women with endometriosis often show failure of mid-secretory downregulation of epithelial progesterone receptor (Lessey et al., 1988) and evidence for specific suppression of progesterone receptor B, but not progesterone receptor A, at multiple cycle phases (Attia et al., 2000). Another possible mechanism of resistance is an alteration of expression or function of progesterone receptor chaperones and co-chaperones. Overexpression of co-chaperone FKBP51 (Hubler et al., 2003) or lack of co-chaperone FKBP52 (Tranguch et al., 2005, 2006, 2007) causes progesterone resistance in experimental models. Interestingly, high FKBP51 expression appears to be responsible for the relative progesterone resistance seen in normal squirrel monkeys (Hubler et al., 2003); however it also leads to glucocorticoid and androgen resistance, which has not been described in women with endometriosis. FKBP52 gene knockout in mice leads to progesterone resistance and embryo implantation failure, which can be overcome with supplemental progesterone (Tranguch et al., 2007).

Co-regulators, which bind steroid receptors and modify their nuclear effects, are also potential modifiers of progesterone resistance. One co-activator, Hic-5, has recently been shown to be deficient in the stroma of proliferative and late-secretory endometria of women with endometriosis (Aghajanova et al., 2009), and null mutations in the progesterone receptor co-activator, steroid receptor co-activator 2 (SRC-2) cause mice to have severe defects in endometrial receptivity. KLF9 is another progesterone receptor co-regulator, whose absence in the mouse results in partial progesterone resistance, subfertility and reduced HOXA10 expression (Simmen and Simmen, 2002; Simmen et al., 2002; Zhang et al., 2003). KLF9 was recently shown to be reduced in a mouse model of endometriosis (Lee et al., 2009) and in infertile women with endometriosis (Pabona et al., 2012). Whether these findings are a root cause or an effect of endometriosis remains to be evaluated, but they lend further credence to the concept of progesterone resistance.

Summary and conclusions

To summarize, although a plethora of hormones are produced by the corpus luteum, the sequential actions of oestrogen and progesterone, without any other corpus luteum hormones, are sufficient to drive a highly receptive endometrium in humans. The mechanisms by which oestrogen and progesterone act are highly complex and involve multiple nuclear receptors as well as recently described membrane receptors. Cell-type specific effects of oestrogen and progesterone depend on differential expression of receptors, chaperones and co-regulators as well as chromatin structure. The role of oestrogen in endometrial proliferation and the importance of that proliferation in embryo implantation are clear. It is also likely that a small amount

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of oestrogen is necessary for normal luteal-phase endometrium in humans, but the sources of oestrogenic activity and dose requirements remain unclear and the possibility remains that oestrogen or oestrogen-like substances are made locally within the endometrium.

Progesterone is absolutely necessary, during the secretory phase, to allow the endometrium to be receptive to the implanting embryo. However, evidence in normal women suggests that only a very small amount of progesterone is necessary, a concentration achieved by the vast majority or perhaps all ovulatory women. Thus, in women with otherwise normal endometrial function, only small amounts of oestrogen and progesterone appear to be required in the luteal phase for full reproductive function. There is also evidence that some women, especially those with endometriosis-related infertility, may be somewhat resistant to the actions of progesterone and it seems that some of these defects are likely to be overcome with higher concentrations of progesterone, but that hypothesis remains to be proven.

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