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*REVIEW ARTICLE*

# HORMONAL REQUIREMENT FOR BLASTOCYST IMPLANTATION AND A NEW APPROACH FOR ANTI-IMPLANTATION STRATEGY

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**Abstract:** There is a growing awareness of a need for developing novel methods of contraceptive technology which should not only be effective in providing protection against conception but also take into consideration the reproductive health issues confronting men and women. This paper considers the process of embryo implantation as one such potential target. The hormonal basis of embryo implanation in primates has been discussed to indicate that progesterone, and not estrogen, from ovarian source is the primary determinant of embryo-endometrial maturation and their synchronization for implantation. Thus, low dose administration of the anti-progesterone, mifepristone, during early luteal phase has been shown to be an effective anti-implantation approach for fertility control. Furthermore, the dissociation of endometrial-hormonal synchrony at the time of blastocyst implanation following the post-ovulatory mifepristone administration has been shown to be the physiological basis of its anti-implantation effect with undisturbed circulatory hormone profiles and ovarian functions. Further studies are required to appreciate the full potential and to mollify the limitations of this approach.

**Key word :** blastocyst, fertility, regulation, implanation, estrogen, mifepristone, primates, progesterone

**A simple question having manifold projections**

Why do birds sit on eggs? This simple question has several aspects (1). First, why birds sit only on *eggs* or things which look like eggs. Second, why birds *sit* on eggs during the breeding period instead of eating them. These questions are related to immediate or proximal causation of the issue. There could be other questions as well. For example, why only *birds,* and not cats or monkeys sit on eggs! And lastly, *why* at all do birds sit on eggs. These questions deal with ultimate causation of the phenomenon. Indeed, all these four questions are interlinked. Still, the first two questions and other related issues of immediate or proximal causation are the ones often considered important in biomedical research.

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In the same way, the question : *why uterine endometrium allows blastocyst to implant*, can be reframed. We are particularly interested in the knowledge which may give us a lead to regulate blastocyst implanation in the human. While the process of implantation appears highly intricate and non-cartesian in nature (2), the requirement for hormonal priming and support for blastocyst implantation and maintenance of pregnancy appears obligatory. Thus, the module of hormonal requirement can be approached in terms of its influence on implanation either way, to inhibit or to facilitate it.

Because of practical and ethical constraints, these studies cannot be performed with human samples. Although the phenomenon of blastocyst implantation is seen in a variety of eutherian mammals, the mode, hormonal regulation and physiological details of implantation are diverse among species. Different animal models have been used over the last one hundred year. It now appears that no single model is completely satisfactory for human use. Old world monkeys and apes appear to be the models closest to the human. In our laboratory, we use the rhesus monkey as the experimental animal model to investigate different aspect of the mechanism of blastocyst implantation.

In the present review, our aim is (i) to evaluate the issue of hormonal requirement for implantation in the rhesus monkey and other primates, and (ii) to attempt a tentative extrapolation towards the possible manner of controlling implantation in the human.

***Ovarian steroid hormones are primary determinants of endometrial receptivity***

Prior to implantation, a viable mammalian blastocyst sheds its zona. Zona free blastocysts are generally sticky in nature and innately tend to adhere to any polarised substratum. However, uterine endometrium needs to be properly hormone-primed to support implantation of blastocyst and establishment of pregnancy. Unprimed endometrium is in fact hostile to the growth of any tissue, even of tumour explants (3). Also, inappropriate hormonal priming is inadequte to support implantation (4). Thus, if we are aware of minimum hormonal requirements for inducing endometrial receptivity to blastocyst implantation, and then if we can manipulate hormonal action and milieu at the uterine level, it is possible to render it hostile to blastocyst implantation.

It appears that in several species including many non-human primates and human ovarian steroid hormones, namely progesterone and estrogen are important hormonal agents in mediating this process (5). Failure of pregnancy due to ovarian hormone insufficiency can be successfully treated with the appropriate combination of these two hormones in the human (6). However, the ratio and the sequence of exposure to these hormones for uterine priming are important for achieving successful implanation, as has been observed in human *in vitro* fertilization and embryo transfer (IVF-ET) laboratories (7, 8).

***Circulatory profile of estrogen and progesterone during implantation reveals little about hormonal requirement***

In the human and in many other menstruating primate species, concentrations of estrogen and progesterone in the peripheral circulation rise during the midluteal phase when blastocyst implantation ensues (9, 10). In the rhesus monkey, there is only midluteal rise of progesterone concentration in serum, and serum concentration of estradiol is

maintained throughout the luteal phase (11, 12). Although, midluteal period of women and rhesus monkeys in conception cycles may exhibit slightly higher plasma concentrations of these hormones compared with that in normal ovulatory, non-conception cycles (12, 13), such changes are not considered important because high degree of individual variations exist in these species. Interestingly, in baboons and pans, the levels of both ovarian steroid hormones in a conception cycle are distinctly higher even during preimplantation period as compared to normal midluteal period of a non-conception cycle (10).

While this information is important, it is not possible to deduce the hormonal requirement for blastocyst implantation from such circulatory profiles of hormones in natural pregnancies in non-human primates and in women, for several reasons. Firstly, it does not provide any indication about the minimum hormonal requirement. Generally, in biological processes, a certain degree of redundancy, synergism and permissiveness operate as safety factors (14). It is likely that such factors are present in an essential but vulnerable process like blastocyst implantation. Secondly, circulatory hormonal data fail to provide us with any precise idea about their concentrations at the endometrial level. In fact, studies in women and in monkeys have shown that there is little parallelism between concentrations of steroid hormones in serum and target tissues such as endometrium during luteal phase (15, 16). Local factors like tissue vascularity, permeability, concentrations of high capacity binders and metabolism at the target tissue bed can modulate the local hormonal milieu (17). Thirdly, concentration of high affinity specific binders of receptors in target cells is a function of hormone receptivity of target tissue. Several experimental models have been used in order to narrow this gap and to obtain an objective understanding about the hormonal requirement for endometrial receptivity to blastocyst implantation.

***Progesterone is essential but hormone replacement studies in monkeys yield contradictory results about the need of ovarian estrogen***

Progesterone is essential for the endometrial preparation at implantation and for pregnancy maintenance. Luteal phase insufficiency along with progesterone deficiency leads to implantation failure (18). Furthermore, successful blockade of progesterone action at endometrial level by the application of antiprogestin can inhibit blastocyst implantation in women and monkeys (19).

Although it is believed that midluteal estrogen is also necessary for blastocyst implantation, there is no robust supportive evidence to this effect. In rhesus monkeys, luteal phase progesterone alone could support blastocyst implantation and pregnancy maintenance (20). In this study, Meyer and his coworkers performed complete bilateral ovariectomy to eleven successfully mated and ovulated monkeys during days 2 to 6 after ovulation and these monkeys were treated with daily intramuscular progesterone injection; nine monkeys became pregnant. Unfortunately, blood levels of steroids were not measured in this study. Bosu and Johansson (21) however observed that only progesterone supplementation in form of subdermal silastic capsule failed to support the establishment of pregnancy in mated rhesus monkeys subjected to bilateral oophorectomy during days 4 to 6 after ovulation; administration of estrogen with progesterone was found to be highly effective. However, progesterone alone could support

implantation in those mated monkeys which were subjected to only bilateral luteectomy. They monitored the levels of estradiol and progesterone in the peripheral circulation, which were found to be within physiologic ranges (21). Bosu and Johansson (21) concluded that (i) along with progesterone some amount of extraluteal estrogen is essential for implantation in the rhesus monkey, and (ii) Meyer's group had in fact administered supraphysiologic amount of progesterone. It however remained unsolved how supraphysiologic amount of progesterone might negate the necessity of estrogen. Furthermore, it is notable that one out of six mated oophorectomized monkeys treated with only progesterone could establish pregnancy in the study of Bosu and Johansson (21).

***Luteal phase ovarian estrogen is not essential for establishment of pregnancy***

It now appears that ovarian luteal phase estrogen is not essential for implanation in primates including women. Although there is no known marker for uterine receptivity, it has been suggested that an endometrium undergoing typical secretory maturation during midluteal phase is sufficient to support implantation, provided the developmental ability of preimplantation stage blastocyst is not compromised (22). It has been shown in IVF-ET laboratories that progesterone to estrogen ratio in circulation at this time is critical, and higher level of estrogen may inhibit implantation (7,8). On the other hand, the absence or antagonism of luteal phase estrogen failed to inhibit the secretory maturation of human endometrium and such endometrial maturation appears to be adequate to support successful implantation (23-25). This possibility was proved to be correct from the report of a study performed in our laboratory. We have shown that overiectomized, hormone primed monkeys treated with progesterone alone could induce sufficient secretory maturation, and could support embryo implantation and live birth of babies following embryo transfer (26). Afterwards, it has been shown in a human study that luteal phase estrogen was not required for the establishment of pregnancy (27).

While these results clearly show that luteal phase estrogen from ovaries is not obligatory, provided sufficient progesterone is available, for successful implantation and establishment of pregnancy, the possibility that estrogen may be locally available at the site of implantation cannot be ruled out (28). This possibility is being supported from several lines of evidence. Blastocysts of different mammalian species including human IVF blastocyst have been shown to possess capacity to handle steroids and to aromatize testosterone to estrogen *in vitro* (29, 30). Furthermore, human endometrial stromal cells exhibit high degree of aromatase activity under progesterone dominance in culture (31). We have also observed that endometrial tissue obtained from overiectomized monkeys receiving only progesterone and subjected to traumatization had detectable amount of immunoreactive estradiol (32). Thus, the possibility of locally available estrogen during blastocyst implatation needs to be closely studied.

***Estrogen antagonism appears to be an inadequate target for anti-implantation approach***

It has been suggested that high level of periovulatory estrogen plays a significant role in the process of endometrial priming for subsequent endometrial maturation at implantation. Thus, administration of zuclomiphene citrate, an estrogen antagonist, blocked pregnancy in 4 out of 5 mated female rhesus monkeys (33). However, this

suggestion is being debated now, especially based on reports from IVF-ET laboratories. It has been shown that a constant dose of estradiol priming may lead to implantation without periovulatory type estrogen surge (34,35). It now appears that preovulatory and periovulatory estrogen levels in circulation actually reflect the state and quality of oocyte maturation, and has little correlation with endometrial preparation *per se* (36).

When multiple doses of tamoxifen, an antiestrogen , was given during the midluteal period to mated bonnet monkeys, a high percentage of monkeys failed to be pregnant (37). Also, treatment with antiestrogen antibody inhibited establishment of pregnancy in bonnet monkeys (38). However, a single dose of tamoxifen given on day 14 of cycle in *Macaca fasicularis* failed to inhibit pregnancy as compared to control monkeys, and in fact concentration of tamoxifen in circulation of treated monkeys was non-detectable (39). In a pilot study, we also observed only a marginal protection against pregnancy with tamoxifen treatment in rhesus monkeys (unpublished data). Thus, we conclude that the approach of estrogen antagonism in the midluteal phase appears to be an inadequate approach for inhibiting implantation.

***Dissociation of endometrial synchrony with antiprogestin appears to be a promising anti-implantation approach***

Generally, synchrony between mother and embryo is considered critical for successful implantation and establishment of pregnancy (40). This synchrony is held in the developmental conditions in the dimensions of time and space between three interacting entities : ovary-uterus-embryo. Despite apparently inseparable functional synchrony between endocrine duty of corpus luteum, differentiation of endometrium, environment of uterine luminal space, and embryonic development, it is possible to dissociate these constituents of synchrony. For example, activities of several endometrial hydrolytic enzymes, concentration of sex steroid hormone receptors in endometrial samples and endometrial morphology on day 6 after ovulation in a conception cycle display a leftward shift on 24-48 h as compared to a non-conception ovulatory cycle, without any significant change in the circulating concentration of ovarian steroid hormones (12, 41-43). This strongly supports the feasibility of a dissociation between these three factors-corpus luteum, endometrium and embryo.

It is possible to desynchronize endometrial maturation by timed application of antiprogestin which blocks progesterone action at the receptor level in the target cells, without any significant effect on ovarian hormonal profiles (44-47). Thus, administration of antiprogestin like mifepristone (RU486) during post-ovulatory early luteal phase (days 2-5 after ovulation) could inhibit endometrial receptivity and implantation in monkeys and women (48-51). We believe that this approach of affecting endometrial synchronization with antiprogestin is a meaningful lead in the area of anti-implantation strategy for fertility regulation.

***Limitations perceived***

First there is an essential need of monitoring the occurrence of ovulation to apply mifepristone in timed manner. When this chemical agent is given before ovulation, ovulation may be delayed (52, 53) giving rise to a chance of unprotected pregnancy, or a change in the menstrual cycle patterns. When this chemical agent is given during mid- to late luteal periods, the anti-implantation

efficacy is relatively low (54). It may also change the cycle pattern characterized by extension of cycle length (53, 54). Also, there is a chance of two episodes of vaginal bleeding : one from the direct effect of RU486 on endometrium unrelated to the hormone levels, and second being the normal menstrual bleeding from natural luteal demise (54). Thus, further studies are needed in a directed manner to overcome these limitations of the proposed approach, and thereby to achieve an anti-implantation method of birth spacing with minimal medication, least physical and emotional trauma, maintenance of normal menstrual patterns, unchanged circulatory hormonal profiles and undisturbed ovulation.

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