J. Biosci., Vol. 6, *Supplement* 2, July 1984, pp. 121–126.

Trends in blastocyst research with relevance to development of contraceptives: General discussion.

Coordinator: M. R. N. PRASAD

The Indo-US symposium on Blastocyst Research concluded with a round table discussion on trends in research with particular relevance to the development of contraceptives. A number of events crucial to the initiation and maintenance of implantation were identified and possibilities of developing a contraceptive modality based on interference with these events in implantation were discussed.

There is a great demand from women and national family planning programmes for simple, effective and safe methods of fertility regulation which can be self-administered either post-coitally or for use when menses are delayed by a few days. The availability of such contraceptive technology would limit exposure to fertility regulating agents only to such occasions when coitus takes place or when there is a probability of pregnancy. Moreover, if fertilization had indeed occurred, interruption would take place at the earliest stage of pregnancy thereby reducing the excessive bleeding encountered with later termination of pregnancy. Methods of post-coital contraception used so far have been reserved primarily for emergency situations to protect women from unwanted pregnancy resulting from rape or acts of unprotected coitus or failed barrier methods.

Probabilities that an act of coitus will lead to conception, with reference to the estimated time of ovulation, is highest during the fertile period (9·6+ 2·6 days). The objective of any form of contraception related to coitus would be to interfere with implantation irrespective of the day when the drug is taken. A drug effective in inhibiting implantation during the most fertile period is likely to be effective in other periods of the menstrual cycle also.

The areas selected for discussion were:

Uterine sensitivity

—What are the factors that regulate uterine sensitivity and maternal recognition of the blastocyst?

—What are the factors involved in the development of uterine refractoriness? Can refractoriness be induced?

—Are there specific uterine and blastocyst proteins involved in implantation?

—Is induction of a short luteal phase a possible approach to modify uterine environment and render it hostile for implantation?

Blastocyst

—What is the role of estrogens (ovarian or produced by the blastocyst) in implantation? Can this be interfered with by antiestrogens administered systemically? What is the role of catechol estrogens in implantation?

Luteolysis

Research needs, animal models, status of R and D with methods to inhibit implantation and disruption of established implantation.

Uterine sensitivity:

Initiation of implantation is dependent on a coordinated series of events in the uterus triggered by the sequential action of estrogens and progesterone secreted by the ovary during the pre, peri and post-ovulatory period. Uterine sensitivity and receptivity to implantation is variable in different species and is perhaps less than two days during the fertile period in the human. At other times in the menstrual cycle, the uterine environment is hostile to the implantation of the blastocyst. Refractoriness is a condition when the process of decidualization does not occur in rodents. The biochemical basis for the development of uterine sensitivity/refractoriness is not fully understood in non-human primates and human. The generation, storage and release of vasodilatory mediators, catechol amines (endometrial monoamine oxidase and catechol-*O*-methyl transferase involved in catechol amine deactivation), prostaglandins, cathepsins, phospholipase A etc., appear to be involved in the modulation of uterine vascular functions and uterine sensitivity. The crucial question is the identification of factor/s involved in the onset of uterine refractoriness and whether such a condition can be induced. Recent studies in baboons have led to the isolation of a low molecular weight substance (400–500 daltons) from the refractory uterus which appears to be toxic. Further studies are required to confirm and extend these observations in other non-human primates and human. Biochemical marker/s for the recognition of the refractory period need to be identified.

The uterine epithelium and subepithelial stroma destined to decidualize show a differential temporal response to the action of estrogen and progesterone. A number of proteins synthesized by the luminal epithelial cells in response to estrogens to achieve sensitivity have been identified but the specificity of any of these to implantation is not established. Likewise, a number of proteins secreted by the unimplanted blastocyst have been isolated, but the role of none of these, other than human chorionic gonadotropin (hCG), has been clarified. It is unlikely that any of the presently known uterine/blastocyst proteins can be used to develop an immunological approach to interfere with implantation. hCG subunits or smaller peptides of hCG constitute the basis for the only immunological approach to interfere with implantation which is under development. There is need for the development of an *in-vitro* model to culture the endometrium in different phases of the menstrual cycle with the blastocyst to elucidate the biochemical entities involved in the interaction between the two. Such a model would also permit analysis of the sequential events and concurrent biochemical

changes associated with implantation, *viz*, apposition, adhesion and penetration.

It is desirable to modify uterine sensitivity to implantation by simulating naturally occurring condition of 'short luteal phase' which is generally associated with infertile cycles. Such a condition has been induced in the rhesus monkey by reduction of folical stimulating hormone (FSH) in the follicular phase. This leads to disruption of the luteal phase and reduction in levels of progesterone. This is an area in which further work is needed.

One of the methods to modify uterine sensitivity is to interfere with the action of progesterone on the endometrium. A progesterone antagonist or antiprogestin, taken orally during very early pregnancy or after missed menses could, by binding to endometrial progesterone receptors, interfere with the maintenance of the secretory endometrium essential for the continuation of pregnancy. One of these compounds, RU-38486 has received considerable attention recently and is being evaluated clinically. An antiprogesterone monoclonal antibody has been shown to inhibit implantation in mice possibly due to the reduction by the progesterone antibody of readily available progesterone in circulation for maintaining uterine sensitivity for implantation. The acceptability/safety of a passive immunization method and its efficacy in other species remains to be established.

Blastocyst

Interaction between the blastocyst and uterus is essential for the initiation and maintenance of implantation. The role of estrogen produced by the ovary (which appears in the form of a second peak during the week following ovulation in women) to the initiation of implantation is not clear. Estrogen of embryonic/endometrial origin has been implicated in the process of maternal recognition in several species including the rat, mouse, rabbit, pig and hamster but remains to be demonstrated in the non-human primates and human. Implantation in the hamster is considered to be solely dependent on progesterone but it has recently been shown that the number of implantation sites increase with estrogen supplementation. The steroidogenic activity of the embryo and the endometrium shows wide qualitative and quantitative species variations. The role of these local estrogens at the time of implantation is not clear. The blastocyst secretes several proteins besides estrogens, *viz*, proteins (hCG is the most well defined), catechol estrogens and several enzymes. A specific ovine trophoblastic protein besides prolonging the function of the corpus luteum, interacts with the endometrium by altering the pattern of protein synthesis by endometrial explants cultured *in vitro*. The presence and role of similar proteins in non-human primates remain to be established.

Steroidal and non-steroidal antiestrogens administered systemically post-coitally inhibit implantation in the rat but are ineffective in the hamsters and primates. However, antiestrogens administered intraluminally into the uterine lumen post-coitally interfere with implantation in the hamster, a species which is not dependent on ovarian estrogen for implantation. This has been interpreted as evidence for the role of estrogen secreted by the blastocyst in triggering implantation in the hamster. The failure of antiestrogens to inhibit implantation in non-human primates may be due to the insufficient dose and/or time of administration of the compounds. Zuclomiphene, a

potent antiestrogen, administered to rhesus monkeys between days 5–11 of the cycle at doses which do not affect hypothalamic function, inteferes with fertility of the treated females. It is necessary to evaluate the effects of antiestrogens administered at different stages in the menstrual cycle of non-human primates specially during the week following ovulation and mating. Catechol estrogens which represent metabolites of estrogens, are secreted by the blastocyst (rabbit) and may mediate in the stimulatory effects of estrogens on prostaglandin synthesis in the embryo and/or uterus and thus participate in the establishment of implantation. Catechol estrogens are formed uniquely in the embryo and it should therefore be possible to selectively interfere with the blastocyst by inhibitors/antagonists of catechol estrogens. However, such compounds need to be synthesized and evaluated.

Luteolysis:

The role of prostaglandins in implantation is not fully established. Earlier studies indicate a luteolytic role for prostaglandins (PGs) in a few species of animals other than non-human primates. The role of PGs in capillary permeability has been studied. If implantation is considered to involve inflammatory and immunosuppressant actions, perhaps PG-E2 can do both, but in reality this class of PGs does not interfere with implantation. The cyclooxygenase system has been studied in relation to implantation recently. Leucotrienes have been identified in the blastocyst and these are known to have chemotactic action. Are these involved in the initiation of attachment of the blastocyst? This is an interesting area for further studies.

Monoclocal antibodies to LHRH agonist have been reported to terminate pregnancy in baboons when administered around the periimplantation period. It is suggested that this effect may be due to the direct effects on the trophoblast. Considerable work is needed to confirm this effect and establish the safety of a passive immunization approach for fertility regulation.

Luteinizing hormone receptor binding inhibitors (LHRBI) have been isolated from the follicular fluid and corpora lutea. The identity of these compounds, purification and demonstration of their effects *in vivo* need many years of basic research before they can be considered for use in regulation of fertility.

Deglycosylated derivatives of hCG have been shown to antagonize the action of hCG and interfere with established pregnancy in rats. Studies on the stability of the derivatives and their effects on the termination of pregnancy in non-human primates are in progress. Appropriate methods of delivery, possible immunogenicity and alternate methods of production of hCG need to be studied before evaluation of the derivatives for clinical use.

Research needs

There are a number of gaps in our knowledge of the processes regulating implantation which need to be investigated. Some of these are indicated in the preceeding discussion. Other areas are:

—changes in the concentration of steroid receptors during the peri- and post-implantation period.

—are there any enzymes secreted by the blastocyst which are unique and crucial to the initiation of implantation?

—whether the functional life of the corpus luteum can be prolonged by hCG administration, if so, for how long? Such a model would be useful in screening luteolytic agents.

—There are at present no biochemical markers for the prediction of implantation. It is necessary to determine if some of the proteins secreted by the preimplantation blastocyst enter the saliva/urine and can be detected by simple tests. hCG has been detected in the preimplanting blastocyst but a diagnostic and sensitive test to determine its presence in saliva/urine as a method to predict implantation remains to be developed.

—develop more potent luteolytic agents. Deglycosylated hCG needs to be evaluated in non-human primates.

—Progesterone receptor blockers: recent reports on the development of a potent progesterone receptor blocker interfering with early pregnancy has evoked considerable interest in these compounds. There is need for the initiation of a programme of synthesis and evaluation of new classes of antiprogestins (steroidal or non-steroidal) based on an understanding of the structure of the progesterone receptor.

Animal models

It is most unlikely that any of the rodent or non-human primate species will prove ideal as a model for all aspects of implantation in the human. It may be necessary to carry out some studies in the human after obtaining clearance from the appropriate Ethics Committees of the institutions where such studies are carried out and without compromising safety. Non-human primates are preferred for the reason that they are close to the human but their widespread use depends on a number of factors such as availability, cost, ease of management and captive breeding performance. It is necessary to establish international collaboration between countries/institutions where primate facilities exist to optimize their use in research on regulation of fertility. Likewise, there is need for standardization of methods and models and exchange of scientists and information to achieve the objectives outlined earlier.

Status of research and development with post-coitals and menses inducers

Methods of post-coital contraception used so far include IUD’s inserted post-coitally, estrogens used alone or in combination with estrogens. These are reserved primarily for emergency situations to protect women from unwanted pregnancy. Levonorgestrel which has shown satisfactory contraceptive efficacy is being further evaluated clinically. Centchroman, a non-steroidal drug has been evaluated clinically as a once-a-week pill but its future as a fertility regulating agent depends on re-evaluation of its toxicology and determination of a suitable dose to avoid undesirable side effects.

Menstrual regulation could be achieved by a number of approaches: (a) block progesterone receptors and interfere with the preparation of the uterus for implan-

tation, (b) luteolysis leading to decreased progesterone levels and interruption of pregnancy and (c) termination of early pregnancy by prostaglandin analogues. A number of progesterone antagonists have been evaluated clinically. One of these compounds, RU-38486 has received considerable attention recently and is being evaluated clinically for termination of early pregnancy. Preliminary results seem to indicate that the drug, even in high doses, may not result in complete evacuation of the product of conception. A number of prostaglandin analogues are being evaluated clinically for termination of early pregnancy specially under conditions when they are self-administered at home.

It is perhaps reasonable to expect that a post-coital pill or a menstrual regulating agent based on ongoing clinical studies may be available for general use before the end of the decade. However, any method based on new leads which may emerge from basic research studies is unlikely to be available for clinical use in regulation of fertility before the turn of the century.