J. Biosci., Vol. 6, *Supplement* 2, July 1984, pp, 93–96.

Luteal function during the periimplantation period and requirement for estrogen for implantation and pregnancy maintenance in the non-human primate

N. R. MOUDGAL

Center for Advanced Research in Reproductive Biology (ICMR), Laboratory of Endocrine Biochemistry, Department of Biochemistry, Indian Institute of Science, Bangalore 560 012, India

Abstract. An attempt has been made in this paper to review our present understanding of luteal function during the periimplantation period and in particular hormonal requirement for implantation and maintenance of early pregnancy in the non-human primate.

In a fertile cycle the *corpus luteum* is apparently rescued from luteolysis by chorionic gonadotropin secreted by the implanted blastocyst, In the bonnet monkey the serum progesterone titers during the luteal phase of a fertile cycle seems higher compared to that of nonmated cycling monkeys. This suggested that the *corpus luteum* is receiving some stimulatory signal from the blastocyst even prior to implantation. The recent demonstration that human blastocyst in culture secretes into the medium human chorionic gonadotropin essentially support the above assumption. However, attempts to extend the luteal phase of cycling unmated monkeys with exogenous human chorionic gonadotropin injection has hitherto not met with complete success suggesting that there could be other than chorionic gonadotropin, additional luteal stimulatory factors the unimplanted blastocyst is secreting.

Corpus luteum is the principle source of both progesterone and estrogen produced during the periimplantation period and dysruption of luteal function, brought about by either lutectomy or ovariectomy or luteinizing hormone antiserum treatment, followed by progesterone supplementation leads to maintenance of pregnancy. This has lead to questioning the need for estrogen in the maintenance of early pregnancy. Recent work using Zuclomiphene, an antiestrogen during days 5–11 of cycle in rhesus monkeys mated between day 9–14, has however, suggested that estrogen may be required for implantation. Further work is needed to arrive at an unequivocal decision regarding the need for estrogen in maintenance of early pregnancy in the primate.

Keywords. Estrogen; implantation; *corpus luteum*; non-human primate.

It is presently well accepted that the *corpus luteum* (CL) is essential in the primate for establishment of pregnancy. The CL need apparently ceases once implantation occurs and the placenta takes over the function of producing steroids (Booher *et al.*, 1981). The period between fertilization and implantation in the non-human primate is around 9 days (Hendrickx and Enders, 1980) and limited information is known about the events occurring during the intervening period. The CL of the fertile mated monkey secretes both progesterone and estrogen. Particularly, that the latter is of CL in origin is shown by lutectomizing monkeys. Such monkeys in addition to showing drastic and immediate reduction in both progesterone and estrogen levels do not respond to exogenous luteinizing hormone (LH)/chorionic gonadotropin (CG) treatment (Walsh *et al.*, 1979; Wilks and Noble, 1983; Sheela Rani, C. S., Ravindranath, N., Kotagi, S. G.

Abbreviations used: CL, *Corpus luteum;* LH, luteinizing hormone; hCG, human chorionic gonadotropin.

and Moudgal, N. R., unpublished observations). A comparison of serum progesterone and estrogen levels of cycling fertile and non-fertile bonnet monkeys between days 19–23 of cycle shows that the level of both these steroid hormones is significantly high in the former group (Rao and Moudgal, 1984). Similar observations have been made for other primates, notably the rhesus (Atkinson *et al.* 1975; Hendrickx and Enders, 1980). The increase in progesterone levels of cycling fertile bonnet monkeys even prior to implantation according to Mukku and Moudgal (1979) is perhaps due to the CL receiving some type of signal from the as yet unimplanted blastocyst. The first report that the unimplanted blastocyst could be secreting chorionic gonadotropin was made by Saxena *et al.* (1974) and recently Fishel *et al.* (1984) have obtained confirmatory evidence for this by measuring human chorionic gonadotropin (hCG) in culture medium of human blastocysts. Attempts to extend the luteal phase of cycling unmated monkeys by giving exogenous hCG have, however, generally not met with complete success. hCG given daily either in increasing doses (to simulate pregnancy) or sustaining small doses from day 18–20 of cycle has lead to increase in progesterone titers only for a 3–4 day period, the levels falling rapidly thereafter. Continued hCG injection, however, seems to maintain estrogen at high levels, the cycle length itself being extended by a maximum of 5–7 days. These results using both the rhesus (Wilks and Noble, 1983) and the bonnet (Sheela Rani, C. S., Ravindranath, N., Kotagi, S. G. and Moudgal, N. R., unpublished observations) suggest that in addition to hCG, perhaps the CL during the preimplantation period is receiving some other stimulus from the blastocyst to keep producing high titers of progesterone. The nature of this stimulus is yet to be ascertained.

Based essentially on morphological/histological criteria it has been concluded that implantation in most of the non-human primates examined thus far occurs approximately 9 days after fertilization (Hearn, 1980). This day could coincide with the day of CL rescue as determined in non-fertile cycling monkeys (Atkinson *et al*., 1975). In the bonnet monkeys, based on the *in vitro* sensitivity of CL, removed on days 19,23, 25 and 28 of cycle to exogenous LH it was concluded that the CL rescue must be occurring on or about day 23, 9–10 days after fertilization (Mukku and Moudgal, 1979). At present other than measurement of elevation in hormone levels (progesterone and CG for example) there is no other way, except by morphological examination, to fix the time of implantation. With the advent of newer protein separation techniques which will permit isolation of specific protein in small quantities (see paper by Roberts in this issue) coupled to radioimmunoassay we can hope that a specific marker protein will be isolated from blastocysts in culture which will permit us to arrive at the time of implantation little more accurately.

Paralleling the increase in CG levels, first detectable in the serum of bonnet monkeys by day 28 of cycle there is a significant increase in both estradiol and testosterone levels (Rao and Moudgal, 1984). While the need for progesterone in implantation and maintenance of pregnancy is fairly well established that of estrogen in these events is questioned. Meyer *et al.* (1969) thus observed using rhesus monkeys ovariectomized on day 6/7 post fertilization that it is possible to obtain implantation and pregnancy maintenance by supplementing with progesterone alone. Bosu and Johansson (1975), however, report that induction of implantation and/or maintenance of pregnancy was poor in ovariectomized monkeys maintained on progesterone alone. Dosing of mated

cycling bonnet monkeys with antisera to oLH or its β subunit (*a/s*) from days 18–20 post fertilization has been shown to reduce drastically serum progesterone and estrogen levels, this leading ultimately to the termination of pregnancy (Prahalada *et al*., 1975; Moudgal *et al.*, 1978). However, supplementing *a/s* treated monkeys with varying doses of progesterone permits continuation of normal pregnancy suggesting that in the bonnet monkey progesterone alone is adequate to maintain pregnancy during the periimplantation period (Sheela Rani, C. S., Ravindranath, N., Kotagi, S. G. and Moudgal, N. R., unpublished observations).

In the rodents, particularly in rats and mice the requirement for estrogen in the implantation process is well established. Implantation in the hamster, though believed till recently to be solely progesterone dependent has been shown to improve (by increase in the number of implantation sites) with estrogen supplementation (Sengupta *et al.*, 1981). Recent studies have shown that the unimplanted blastocyst (Sengupta *et al*.,1984) as well as the uterine endometrium have the ability to synthesize estrogen. In the light of this, perhaps some of the experiments done in ovariectomized or *a/s* treated monkeys supplemented with progesterone need to be re-examined. The use of medroxyprogesterone acetate, a non metabolizable progestational compound instead of progesterone in the above studies might provide more definitive result. Alternately a higher titer estrogen antibody or a specific aromatase inhibitor or a chemical analogue having potent antiestrogenic activity will have to be used to establish beyond any doubt the need for estrogen.

Recent work of Adiga *et al.* (1983) provides supporting evidence for the need for estrogen to support pregnancy. They have established that estrogen induces the synthesis of a series of vitamin carrier proteins in both the pregnant rodent and primate (Adiga, P. R., personal communication). Using a pregnant rat as the experimental model they have shown that there is a critical need for these estrogen induced proteins in transporting vitamins across the placenta to the growing foetus, neutralization of these proteins with specific antibodies resulting in the termination of pregnancy. It is, however, not yet clear from their studies, if this 'critical' requirement extends to the primate and if so whether this need is felt during the preimplantation period itself.

The use of a variety of antiestrogens to study the role of estrogens in implantation *per se* in the primate has generally not been successful (Prasad and Sankaran, 1975). This failure has been attributed by some workers to the doses used and in particular to the time of administration of the drug. Assuming that the estrogen surge that occurs at mid cycle may be adequate to sensitize the uterus for subsequent progesterone action, Zuclomiphene, a potent antiestrogen has been administered to rhesus monkeys between days 5–11 of cycle in doses (2 mg/kg/day) that presumably did not effect estrogen function at the hypothalmo-pituitary end. Such a treatment has been shown to render these monkeys infertile (Sankaran M. S., Prahalada S. and Hendrickx A. G., personal communication). Though it is suggested that clomiphene could be acting here as an anti-implantation agent by interfering with the sensitization of uterus to progesterone, the possibility that it could also be acting by bringing about premature expulsion of fertilized ova from the follopian tube cannot be excluded.

In summary, though the need for a functional CL to support pregnancy during the periimplantation period is well established there is as yet no clear-cut evidence to suggest that estrogen is needed for either implantation or immediate post-implantation

survival of the blastocyst. The work of Sankaran and his co-workers (Sankaran, M. S., Prahalada, S. and Hendrickx, A. G., personal communication) is a pointer in this direction, but further work needs to be done to establish estrogen requirement beyond any doubt. A more accurate biochemical marker for implantation as well as mapping of steroid receptor levels during the periimplantation period in the primate is also required and may together help in better understanding the role of steroid hormones in implantation.

Acknowledgements

The author wishes to thank the Indian Council of Medical Research, New Delhi and the World Health Organization, Geneva for financial assistance.

References

Adiga, P. R. and Ramana Murthy, C. V. (1983) *Vitamin carrier proteins during embryonic development in birds and mammals in Molecular Biology of egg maturation,* Ciba Foundation Symposium No. 98 (London: Pitman) p. 111.

Atkinson, L. E., Hotchkiss, J., Fritz, G. R., Surve, A. H., Neill, J. D. and Knobil, E. (1975) *Biol. Reprod*., **12,** 335.

Booher, G., Enders, A. C, Hendrickx, A. G. and Hess, D. L. (1981) *Am. J. Anatomy*, **160,** 17.

Bosu, W. T. K. and Johansson, E. D. B. (1975) *Acta Endocrinol.,* **79,** 598.

Fishel, S. B., Edwards, R. G. and Avans, G. J. (1984) *Science*, **223,** 816.

Hearn, J. P. (1980) in *Immunological aspects of Reproduction and Fertility Control,* ed. J. P. Hearn (London: MTP Press) p. 229.

Hendrickx, A. G. and Enders, A. C. (1980) in *Non human primate models for study of human reproduction*, ed. T. C. Anand Kumar (Basel: S. Karger) p. 109.

Meyer, R. K., Wolf, R. C. and Arslan, M. (1969) in *HOFER Recent Advances in Primatology* (Basel: S. Karger) **2,** 30.

Moudgal, N. R., Mukku, Y. R., Prahalada, S., Murty, G. S. R. C. and Li, C. H. (1978) *Fertil. Steril.* **30,** 223.

Mukku, V. R. and Moudgal, N. R. (1979) in *Recent Advances in Reproduction and Regulation of Fertility*, ed. G. P. Talwar. (Elsevier/North Holland) p. 135.

Prahalada, S., Mukku, V. R., Rao, A. J. and Moudgal, N. R. (1975) *Contraception*, **12,** 137.

Prasad, M. R. N. and Sankaran, M. S. (1975) *J. Sci. Ind. Res.* (*India*), **34,** 336.

Rao, A. J. and Moudgal, N. R. (1984) *Gonadal hormonal profiles during the periimplantation period in the bonnet monkeys,* Proceedings of the Indo-US Symposium on Ovum Implantation, ICMR, New Delhi, p. 92.

Saxena, B. B., Hasan, S. H., Haour, F. and Schmidt-Gollwitzer, M. (1974) *Science*, **184,** 793.

Sengupta, J., Paria, B. C. and Manchanda, S. K. (1981) *J. Reprod. Fertil*. **62,** 437.

Sengupta, J., Roy S. K., Paria, B. C. and Manchanda, S. K. (1984) *Role of embryonic estrogen during early gestation and implantation,* Proceedings of the Indo-US Symposium on Ovum Implantation, ICMR, New Delhi, p. 54.

Walsh, S. W., Wolf, R. G, Meyer, R. K. and Robinson, J. A. (1979) *Biol. Reprod*., **20,** 606.

Wilks, J. W. and Noble, A. S. (1983) *Endocrinology* **112,** 1256.