

Hormonal requirement for ovum implantation

K. YOSHINAGA

Reproductive Sciences Branch, Center for Population Research, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland 20205, USA

Abstract. Hormonal requirement for ovum implantation varies among the species of animals. The methods attempting to clarify the requirement in each species may be classified as follows: (i) hormonal replacement therapy after removal of the pituitary or/and the ovaries, (ii) hormonal treatment after reduction of specific hormones by its antiserum *in vivo*, (iii) close observation of hormone secretion pattern in early pregnancy, (iv) examination of physiological conditions where implantation is delayed and analyze the hormone levels and the receptivity of the target tissues, and (v) examination of effects on hormone levels and the receptivity of target tissues of drugs which interfere with implantation. The reported results indicate that both progesterone and estrogen are needed for implantation in rats, mice, and Mongolian gerbils; in other species of animals progesterone alone may be sufficient to induce implantation, although synergistic effect of estrogen appears to be seen in some species such as in the rabbit. It remains to be determined whether the blastocysts of those animals that need only progesterone for implantation have greater ability to produce estrogen than the blastocysts of the animals that need both progesterone and estrogen. Control mechanism of secretion of progesterone and estrogen for inducing implantation may be different in various species. It has been suggested that both leutropin and follicle stimulating hormone are needed for pre-implantation estrogen secretion in the rat, whereas only follicle stimulating hormone is needed in the mouse. In the species where the obligatory delay in implantation is observed, neuroendocrine mechanisms are reported to be involved in controlling the pituitary-ovarian function that causes a delay in implantation.

Keywords. Implantation; gonadotropins; progesterone; estrogen.

Introduction

There exists a species variation in the hormonal requirement for ovum implantation. Because of inadequate data on hormonal requirement for ovum implantation for each species of animals, there is a tendency to discuss hormonal requirement in general. In an attempt to delineate the existing species difference, hormonal requirement for ovum implantation will be reviewed separately for each species of animals.

Rats

In the rat both progesterone and estrogen are required to prepare the uterus for implanting blastocysts. Progesterone alone does not induce implantation (Canivenc *et al.*, 1956; Cochrane and Meyer, 1957). Progesterone secretion prior to day 5 of

Abbreviations used: LH, Leutropin or luteinizing hormone; FSH, follicle stimulating hormone; hCG, human chorionic gonadotropin, LHRH, LH-releasing hormone.

pregnancy is controlled by prolactin (Cutuly, 1941; Lyons *et al.*, 1943). It is not clear as to the degree of leutropin (LH) involvement in the luteotropic complex during the preimplantation period. Implantation takes place in the evening of day 5 in the rat (day 1 = sperm positive day). The ovarian steroids necessary for the preparation of the uterus for implanting blastocysts complete their output by 0100 h on day 5. Although gonadotropin secretion for preimplantation increase of estrogen takes place during day 4, it is not clear whether LH alone or both LH and follicle stimulating hormone (FSH) are needed for estrogen secretion. Lactation stimulates prolactin secretion and reduces gonadotropin secretion. Since prolactin is a luteotropic hormone and a new set of corpora lutea is formed at the postpartum ovulation that occurs within 24 h after parturition, progesterone secretion is elevated during lactation (Eto *et al.*, 1962; Tomogane *et al.*, 1969; Yoshinaga *et al.*, 1971). Serum level of progesterone during lactation is proportional to the size of suckling litter. The larger the size of suckling litter, the higher is the prolactin level and thus the higher is the progesterone level. Reduction in estrogen secretion in lactating rats nursing a large litter appears to be due to reduced secretion of gonadotropin(s) since injection of human chorionic gonadotropin (hCG) (Yoshinaga, 1976) or FSH (Raud, 1974) abolished a delay in implantation in lactating pregnant rats.

Although the circulating levels of gonadotropins and prolactin provide important information, the receptor contents in the ovary for these hormones are sometimes crucial for examining the functional state of the ovary. When mated female rats are treated with pharmacologically large doses of LH-releasing hormone (LHRH) or its agonists during preimplantation period, ovum implantation is delayed (Yoshinaga and Fujino, 1979). The delay was found to be due to reduction in the secretion of both estrogen and progesterone. This ovarian dysfunction is not due to reduction in gonadotropin secretion because the level of LH is higher than the control normal pregnant rats and FSH levels do not differ. However, LH/hCG receptor content in the ovary is found to be markedly reduced. Therefore, it is not necessarily true that the higher a hormone level in circulation, the more is the target tissue stimulated. Although the above condition is produced by the pharmacological doses of decapeptide hormones, loss of receptors or unavailability of hormone receptors may be important in the endocrine studies on ovum implantation. Translocation of estrogen-receptor complex from the cytoplasm to the nucleus is observed when estrogen exerts its action on the uterus. In view of the increasing evidence that blastocysts and the uterus can metabolize steroids and that blastocyst estrogen may be playing a role in implantation (Dickmann and Dey, 1974; Dickmann *et al.*, 1976) close examination of steroid actions on the uterus at the site of implantation appears to be important in order to elucidate the endocrine mechanisms involved in implantation. The sequential changes in the uterine sensitivity to blastocyst implantation may well be related to the uterine steroid receptor changes.

Mice and Mongolian gerbils

Steroid hormone requirement for implantation in the mouse, and in the Mongolian gerbils appears to be similar to that in the rat, *i.e.*, both progesterone and estrogen are

necessary (Yoshinaga and Adams, 1966; Norris and Adams, 1971). Endocrine control of implantation and delayed implantation in the mouse and the rat was reviewed by Gidley-Baird (1981). Ovarian steroid secretion is necessary for implantation till the afternoon of day 4 when implantation initiates in the mouse (McLaren 1971). Since implantation takes place in the evening of day 5 in the rat, there is approximately one day difference in the time of implantation between these two species of animals. Pre-implantation estrogen secretion occurs 82 h post coitum (Finn, 1965). Bindon and Lamond (1969) hypophysectomized mated mice at different time intervals from the time of mating and found that implantation was completely prevented when hypophysectomy was performed at 2300 h on day 3 (day 1 = sperm positive day), but implantation took place in the mice whose pituitaries were removed at 0700 h on day 4. Therefore the gonadotropin secretion necessary for implantation is completed before the early morning of day 4. The pattern of preimplantation gonadotropin secretion is not clear. Bindon (1971) suggested that a combination of LH and FSH are needed for preimplantation increase in estrogen secretion. Induction of implantation in hypophysectomized mice was achieved by combinations of FSH and LH, FSH and prolactin, prolactin and LH (Gidley-Baird and Emmens, 1978). Since combination of progesterone and estrogen or progesterone and FSH, but not progesterone and LH, induced implantation in hypophysectomized mice, Gidley-Baird (1981) thinks that FSH is necessary for estrogen secretion.

Hamsters and guinea pigs

Implantation of blastocysts can be induced with progesterone alone in ovariectomized golden hamsters (Orsini and Meyer, 1962). Therefore estrogen does not seem to be necessary for implantation in these species. However when estrogen level is measured during pregnancy, it increases prior to ovum implantation (Joshi and Labsetwar, 1972). Significance of the preimplantation rise of estrogen in the hamster is not clear. Estrogen synthetic capacity of hamster blastocysts has been suggested. Implantation in the hamster begins on the afternoon of day 5 (day 1 = sperm positive day). Implantation is not associated with any sudden increase in plasma or luteal progesterone (Lukaszewska and Greenwald, 1970). Prolactin, FSH and a trace of LH are required for the maintenance of luteal function in the hamster (Greenwald and Rothchild, 1968).

In the guinea pig very small amounts of progesterone are required for implantation, and estrogen is not necessary (Deanesly, 1960). In the guinea pig the corpora lutea require gonadotropin(s) for the first 3–4 days after ovulation and become autonomous thereafter (Aldred *et al.*, 1961). It is tempting to entertain the possibility that the blastocysts of hamster and guinea pig may produce estrogen necessary for implantation. If it is the case, the capacity of estrogen production of these tissues may be greater than that of the tissues in the rat and mouse. Aromatase activity of the uterine tissue must also be examined.

Rabbits

Progesterone alone has been reported to induce implantation and maintain pregnancy in rabbits ovariectomized on day 5 post coitum (Pincus and Werthessen, 1938).

However, in the rabbits ovariectomized at an earlier stage of pregnancy, estrogen is also necessary for ovum implantation (Chambon, 1949).

In hypophysectomized rabbits either LH or estradiol can maintain luteal integrity and progesterone secretion. LH, however, requires the presence of follicles in the ovary, which indicates that LH stimulates estradiol production which, in turn, promotes progesterone secretion (Keyes and Nalbandov, 1976; Keyes and Armstrong, 1968; Eaton and Hilliard, 1971). Therefore estradiol is "the" luteotrofin in the rabbit.

Pigs and sheep

Control of luteal function in these species of animals was reviewed by Nalbandov (1973). The small laboratory animals such as rats, mice and hamsters are incomplete cyclers and the true luteal phase is lacking, thus they have short estrous cycles. Unlike these small laboratory animals, pigs and sheep are complete cyclers and the corpus luteum formed at ovulation secretes progesterone for a period of two or three weeks. In the pig the corpus luteum of the cycle is autonomous and does not require gonadotropin for formation or function. In the sheep, on the other hand, gonadotropins, particularly LH, are needed for maintenance of luteal function (Kaltenbach *et al.*, 1968; Karsch, 1970).

In the pig early embryonic attachment to the uterus and development were maintained by progesterone alone after ovariectomy on day 15, but embryonic mortality was relatively high when examined on day 25. Supplemental treatment with combinations of progesterone and estrogen reduced the high embryonic mortality (Day *et al.*, 1959). This hormonal requirement appears to be similar to that of the rabbit. Pig blastocysts have been known to have steroidogenic capacity. Perry *et al.* (1973) found that day 16 pig embryos could synthesize estrogens, but day 10 embryos could not. It has been suggested that the rise in the level of maternal plasma estrogen sulphate derived from embryos, may be a requirement for initiating blastocyst implantation locally in the uterus. In the sheep and the pig, macromolecules (proteins) produced by blastocysts prevent luteolysis at the end of the cycle (Rowson and Moor, 1967; Longnecker and Day, 1972). Read Dr. Roberts' article in this issue for more detailed account on this very interesting area.

Wild animals with delayed implantation

Refer to Embryonic Diapause in Mammals (1981) for detailed account. It has been known that implantation is delayed in representatives of marsupial and eutherian mammals (Renfree and Calaby, 1981). Most mustelids (western spotted skunk, badgers, marten, wolverine etc.) always exhibit a prolonged period of delayed implantation lasting several months. In these animals luteal cells do not develop fully during the period of delayed implantation and activation of luteal cells occurs a few days before implantation. Attempts to induce implantation by injecting progesterone are not successful, suggesting that other ovarian hormone (s) are necessary for implantation (Mead, 1981). Since, the time of implantation can be hastened or delayed by changing photoperiod, neuroendocrine mechanisms are involved. Increasing the photoperiod

initiates the implantation process in the mink. Administration of a dopamine antagonist, pimozide, resulted in a rapid increase in circulating progesterone and precocious implantation (Murphy, 1983). It has been reported that prolactin increases progesterone secretion in intact mink (Papke *et al.*, 1980, Martinet *et al.*, 1981) and hypophysectomized mink (Murphy *et al.*, 1981), pimozide appears to stimulate prolactin secretion which, in turn, activates progesterone secretion. According to Mead (1981) prolactin may be important in regulating luteal function in mink and ferret but may be less significant in other mustelids such as spotted skunk and European badger. Embryonic diapause in the macropodid marsupials is also associated with undeveloped corpus luteum. Prolactin, in these species, inhibits hyperplasia and hypertrophy of luteal cells. As the prolactin level declines the corpus luteum increases in size, plasma progesterone levels rise and blastocyst resumes its development (Tyndale-Biscoe and Hinds, 1981). Thus the mechanisms involved in the embryonic diapause and resumption of the embryonic growth at the end of the diapause are variable among these species.

Primates

Ovum implantation takes place on the 8th-9th day after fertilization in the rhesus monkey (Wislocki and Streeter, 1938; Atkinson *et al.*, 1975; Enders and Hendrick, 1980); after day 8 in the marmoset (Hearn, 1980), on the 9th-10th day in the chimpanzee (Reyes *et al.*, 1975), and on the 5^½-6th day in the human (Landesman and Saxena, 1976; Ortiz and Croxatto, 1979). In the rhesus monkey, Meyer *et al.* (1969) showed that progesterone treatment of monkeys ovariectomized between the 2nd and 6th day after ovulation was able to induce implantation and maintain pregnancy. Hodgen (1983) transferred preimplantation embryos to the uterus of ovariectomized rhesus monkeys treated with estrogen followed by an estrogen-progesterone combination and obtained implantation of the transferred embryo. It remains to be determined whether estrogen has additional beneficial effect in inducing implantation in this species. This point will be discussed by Dr. Moudgal (1984) in detail elsewhere in this issue.

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