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The binding of progesterone in different parts of the rabbit uterus during implantation*

RAJ K. PURI and S. K. ROY

Division of Endocrinology, Central Drug Research Institute, Lucknow 226 001,

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Abstract. Progesterone receptors, both nuclear and cytosolic, were determined in the embryonic and inter-embryonic segments of the rabbit uterus at 6, 7 and 8 day *post-coitum.* At day *6 post-coitum* a higher concentration of nuclear receptor in the embryonic segment was observed compared with that in the inter-embryonic segment. A reverse situation was observed in the case of cytoplasmic receptors. On the 7th day *post-coitum,* no significant alteration in the concentration of either kind of the receptors was observed. However, on day 8, a higher concentration of both nuclear and cytosolic receptors at the embryonic site was observed compared to that in inter-embryonic segment. Since receptors are influenced only in the immediate vicinity of the blastocyst, it can be suggested that the blastocyst plays a role in the induction of its own implantation. Further, at day 8 increase in receptor concentration at the embryonic site may be related to the presence of decidual tissue at this site.

Keywords. Rabbit; uterus; progesterone; receptors.

Introduction

It is well-known that attachment of the embryo to the maternal uterus is an important aspect of pregnancy. It is also established that hormonal requirements and various morphological and biochemical events occurring in the process of implantation vary considerably among the different mammalian species. In the rabbit, attachment of the embryo to the uterus occurs day 7 *post-coitum* (Enders and Schlappe, 1971) and this process is not dependent on the ovarian oestrogen content (Hafez and Pincus, 1956). However, it has been postulated that oestrogen from the blastocyst is essential for implantation (Dickman *et al,* 1976; Paychoyos, 1976; Dickman, 1979). The presence of significant amounts of oestrogen and progesterone in the preimplanting blastocyst of rabbit has been established (Seamark and Lutwak-Mann, 1972; Fuchs and Beling, 1974; Dickman *et al.,* 1975) suggesting that these steroids may have a role in the attachment of the embryo to the uterus. Hormonal action is mediated through its binding to receptors in the cystosol and subsequent translocation to the nucleus (Jensen *et al.,* 1968) which can be correlated to subsequent biological changes (Jensen, 1964) . The role of these hormones from the blastocyst at the implantation

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site can be delineated by studying hormone receptors in the embryonic and inter-embryonic segments of the uterus during implantation. Variations of estradiol binding in the cytosol at these sites in the rat uterus at 6th day *post-coitum* have been reported (Ward *et al,* 1978). In a preliminary report that variations of progesterone receptors at day 6 only were examined (Puri and Roy, 1979).

The present report, describes such receptors at the embryonic and inter-embryonic sites of the rabbit uterus prior to, during and after implantation.

Materials and methods

Steroids and buffer

[l∝, 2∝ – ^{3}H]-progesterone (sp. act, 49 Ci/mmol) was obtained from Radiochemical Centre, Amersham, England. Its purity was checked by thin-layer chromatography on silica gel in benzene: ethyl-acetate (7:3, v/v) system.

In most experiments 10 mM Tris-HCl buffer, pH 7.4, containing 1 mM EDTA and 10% (v/v) glycerol was used.

Animals

Adult (virgin) female albino rabbits (2.0-2.5 kg) of our Institute colony were used in this study. The animals were divided into three groups consisting of 6 animals in each group. Animals from all the groups were mated with proven bucks and mating was confirmed by the presence of spermatozoa in vaginal smears. Rabbits were killed at day 6, 7 and 8 of pregnancy (day 0=day of mating).

Preparation of nuclear and cystosol fractions

After sacrifice, uterine horns were excised and kept in ice-cold buffer. The uteri were divided into embryonic and inter-embryonic segments by carefully opening the uterine cornua from the mesometrial side to expose implanting blastocysts. The uterine tissue surrounding the area of each blastocyst was separated as the embryonic site and rest as the inter-embryonic site. Pregnancies with at least three implantation sites were included in the study.

The embryonic and inter-embryonic segments of each group were pooled from 2 animals and weighed separately. Each type of tissue was homogenized with 10 vol. (w/v) buffer in an all glass homogenizer at 0-4°C. The nuclear fraction was obtained by centrifuging the homogenate at 800 *g* for 10 min. The supernatant was centrifuged at 105,000 *g* for 60 min to get the cytosol fraction. The nuclear pellet obtained was washed thrice with cold buffer (1.0 ml each time) and then suspended in cold buffer at a concentration of 100 mg tissue/ml. The cytosol was mixed with dextran-coated charcoal suspension (0.05% dextran-70 from Pharmacia and 0.5% Norit A; prepared with 10 mM Tris-HCl, 1 mM EDTA) in the ratio of 1:1 (v/v). The suspension was agitated for 60 min in cold (0-2°C) and centrifuged at 800 g for 10 min. Supernatants thus obtained were immediately used for assay of progesterone binding.

Measurement of the concentration of pregesterone receptor

Cytosol: Cytosol receptor assay was done according to the method of Vu Hai and Milgrom (1978a). The aliquots (0.2 ml of cytosol) were mixed with [^{3}H]-progesterone

(25 nM at the final concentration) solution in Tris EDTA buffer (set A) or [^{3}H]-progesterone at the same concentration with 250×progesterone (set B). Unlabelled Cortisol at a concentration of 1 μM was used to minimize the interaction of progesterone with corticosteroid binding globulin like protein. Mixtures were incubated for 1 h at 0°C. The free hormone was removed by the addition of 0.3 ml of dextran-coated charcoal suspension, followed by a 10 min incubation at 0°C and centrifugation (800 *g* for 10 min). Supernatants were decanted into vials and mixed with 12 ml of scintillation cocktail and the radioactivity was measured.

Preliminary experiments demonstrated that the concentration of [^{3}H]-progesterone (25 nM) used was sufficient for saturating the cytosol receptors. This equlibrium is reached within 60 min at 0°C (data not shown).

Nuclei: The concentration of nuclear progesterone receptor was measured by the method of Vu Hai and Milgrom (1978b). The crude nuclear suspensions (0.2 ml) were incubated for 3 h at 0-4°C with 0.1 ml of [^{3}H]-progesterone solution (set A) or [^{3}H]-progesterone at the same concentration with 250×progesterone (set A). After subsequent centrifugation, the pellets were washed three times with the homogenizing buffer and extracted twice with 1.0 ml of ethanol each time. The extracts were mixed with 8.0 ml of scintillation cocktail and counted for radioactivity. Preliminary experiments demonstrated that the concentration of [^{3}H]-progesterone (25 nM) utilized was saturating and the equilibrium obtained at 3 h of incubation, which was stable for the next 3 h at this temperature (data not shown).

To calculate the specific [^{3}H]-progesterone binding by the progesterone receptors radioactivity measured in the supernatants of set B (non-saturable binding) was subtracted from that of the set A (total binding-saturable+non-saturable).

Radioactivity and other measurements

Radioactivity determination was carried out in a Packard model 3330 Liquid Scintillation Spectrometer; the vials contained 0-0.6 ml of the sample in aqueous phase plus scintillation cocktail (3.25 g PPO; 65 g POPOP, 52 g naphthalene, 300 ml toluene, 300 ml dioxane and 150 ml methanol). The efficiency of the counting was approximately 52%. Quenching corrections were done using the internal standard technique.

DNA content was measured by the diphenylamine method (Burton, 1951) using highly polymerised DNA (Sigma Chemical Co., St. Louis, Missouri, USA) as standard.

Statistical analysis

The results were subjected to analysis of variance. The difference between the mean value of two groups was calculated by the method of least significant difference. A 'P' value of 0.05 or less was considered to be significant.

Results

It was observed (table 1) that at day 6 of pregnancy the concentration of progesterone nuclear receptors was significantly higher in the embryonic portion compared to that in the inter-embryonic segment of the uterus. At day 7 after coitus no such difference was observed. However, on 8th day *post-coitum,* a significantly higher (P<0.02) concentration was obtained at the embryonic site as compared with the inter-embryonic portion.

Table 1. Progesterone nuclear receptors at embryonic and inter-embryonic segments of the rabbit uterus prior to and after implantation. (Results are expressed as fmol/µg DNA).

Days *post-coitum*	Progesterone bound to the embryonic segment	Progesterone bound to the inter-embryonic segment
6	12.94±2.87	3.24±0.77 [a]
7	11.16±1.77	6.68±1.70 [b]
8	10.78±0.59	6.24±0.85 [c]

Values are mean ±S.E. of 3 pooled samples from 2 animals in each pool.

[a] $P < 0.05$
[b] $P < 0.02$
} Significantly different from embryonic segment

[c] Not significant ($P > 0.05$) as compared to embryonic segment.

Table 2 shows that cytosol receptors were significantly higher ($P<0.01$) at the inter-embryonic segment as compared to the embryonic segment of the uterus. At day 7, after mating no such difference was observed. However, at day 8 *post-coitum,* a significantly higher concentration ($P<0.02$) at the embryonic segment was observed when compared to the inter-embryonic segment.

Table 2. Progesterone cytosol receptors at the embryonic and inter-embryonic segments of the rabbit uterus prior to and after implantation. (Results are expressed as fmol/µg DNA).

Days *post-coitum*	Progesterone bound to the embryonic segment	Progesterone bound to the inter-embryonic segment
6	10.10± 0.47	13.33±0.50 [a]
7	26.71± 3.00	28.18±0.81 [b]
8	94.23±10.23	49.12±2.54 [c]

Values are mean ±S.E. of 3 pooled samples from 2 animals in each pool.

[a] $P< 0.01$
[b] $P< 0.02$
} Significantly different from embryonic segment.

[c] Not significant as compared to embryonic segment ($P >0.05$).

Discussion

The present study demonstrated higher concentration of nuclear receptors at the embryonic site than at the inter-embryonic site of the uterus just before the implantation day (i.e. at day 6 *post-coitum).* The cytosol receptors, however, showed the opposite trend as compared to nuclear receptors. This situation of receptors at the embryonic site of the uterus can be ascribed to the translocation of cytosol progesterone receptors towards the nucleus under the influence of higher concentration of progesterone and estradiol at this site. Progesterone receptors are under dual control of both estrogen and progesterone; estradiol increases cytosolic progesterone receptors, while progesterone promotes its transfer into the nucleus

and also decreases its concentration (Milgrom *et al.,* 1973). Our results suggest that a similar mechanism operates during the changes in the nuclear and cytosolic progesterone receptor content at the implantation site of the uterus caused by progesterone and estradiol. The hormones causing such change at the embryonic site could be produced locally because delivery to the uterus via the general circulation would affect the entire uterus. The obvious local source is the blastocyst. The presence of estrogen and progesterone in the pre-implanting blastocyst has been demonstrated in the rabbit (Singh and Booth, 1978; Angle and Mead, 1979) but their synthesis in the blastocyst has not been shown.

An inflammatory-like reaction (e.g., increase in capillary permeability) has to precede implantation at the prospective site of attachment of the blastocyst (Psychoyos, 1967). Further, it has also been shown that progesterone can act as an antiinflammatory agent. Thus it may be postulated that while progesterone dominance is obligatory for the uterus as a whole it is inhibitory for the local inflammatory-like reaction. Hence, it is necessary to nullify or sufficiently reduce the local progesterone dominance to permit a local inflammatory-like response. Estradiol, which is known to counteract progesterone locally could thus be secreted by the blastocyst.

The above results can be correlated with the hypothesis of Dickman *et al.* (1975, 1976) that mammalian blastocysts secrete steroid hormones, which diffuse out into the adjacent endometrium and induce localized effects in the process of implantation. The verification of diffusion of estrogen from the pre-implanting blastocyst have been indirectly inferred by studying cytosolic estradiol receptors at embryonic site in the rat uterus (Ward *et αï.,* 1978). The above possibility has also been confirmed by the increase of various lysosomal (Abraham *etαl.,*1970; Murdoch, 1972; Moulton *et al.,* 1978) and macroscopic reactions (Dickman *et al.,* 1977) at the embryonic site at 6 day *post-coitum* in the rabbit uterus. Irrespective of the origin of steroids it seem s likely from these findings that estrogen and progesterone secreted could be the stimulus from the blastocyst to the mother for implantation.

However, as implantation progressed (i.e at day 7 *post-coitum*) there was no significant difference in the concentrations of nuclear and cytosolic receptors at either site. This phenomenon may be correlated with no localized influence or secretion of any of these hormones by the blastocyst at this time and needs further clarification. At day 8 of pregnancy, when trophoblastic invasion of this blastocyst is widely spread, increased levels of both cytoplasmic and nuclear receptors at the embryonic site may directly be attributed to the presence of decidual tissue at this time (De Feo, 1967), which is shown to be influenced by progesterone (Wiest, 1970).

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