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Research Article

BIOCHEMICAL CHANGES IN ASCORBATE AND PEROXIDASE ACTIVITY IN THE FALLOPIAN TUBE DURING PREGNANCY IN RABBIT

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ABSTRACT

An inverse relation between peroxidase activity and ascorbate content suggests the involvement of ascorbate-peroxidase system in the increased secretion of progesterone resulting from LH action in rabbit. Also the same system renders some evidence for the involvement of this enzyme along with other factors in the process of implantation.

Keywords: Peroxidase-Ascorbate system, Fallopian Tube Pregnancy, Implantation.

INTRODUCTION

It has been reported that the LH in various species of animals stimulates progesterone biosynthesis or secretion by the luteal tissue or the luteinized ovary (Savard *et al.*, 1965; Amstrong, 1968). Injection of LH to rabbits increased progesterone, 20α-OH-progesterone and estrogen in ovarian venous plasma (Eaton and Hilliard, 1971; Moudgal *et al.*, 1974). LH has been shown to maintain the CL in hypophysectomized rabbit (Kilpatrick *et al.*, 1964). Progesterone is a key hormone for the maintenance of pregnancy in the rabbit (Ryan, 1973). Progesterone influences gestation by action in the uterus, where it induces endometrial proliferation and inhibits myometrial activity, and by its action on the hypothalamo-hypophyseal system, where it regulates release of gonadotropins (Heap *et al.*, 1973). On the other hand, recent studies have shown that LH can increase progesterone synthesis in the leutinizedrat ovarian tissue by the induction of peroxidase (Agrawal and Harper, 1983) and the well known depletion of ascorpic acid in the leutinized rat ovary in response to LH may be related to this steroidogenic action (Agrawal and Laloraya, 1977). The action of LH in inducing peroxidase activity and associated depletion of ascorbate in the CL of rats can be blocked by treatment with antiserum to LH (Agrawal and Laloraya, 1980a) and that the extended luteotropic function of ovary during estrous and pregnancy is shown to be regulated by a similar mechanism (Agrawal and Laloraya, 1979; Agrawal and Harper, 1983). However, peroxidase can be demonstrated histochemically in the CL, but not in growing follicles of rabbit ovaries, while Δ^5 -3β- Hydroxysteroid dehydrogenase was found in the theca interna of follicles, CL and interstitial cells (Agrawal and Laloraya, 1979, 1980b). The rabbit being an induced ovulator, it was of interest in that mature animals are at estrous and corpora lutea are formed only after coitus. It appeared likely that extended luteotropic function of ovary during pregnancy in rabbits also is regulated by a similar mechanism. The present study describes the changes in the activity of peroxidase and associated depletion of ascorbate during different days of pregnancy i.e. from mating through implantation and early gestation period.

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MATERIAL AND METHODS

Colony bred mature female white rabbits of our departmental colony were caged individually in a controlled environment with a light-dark cycle of 14:10 hours. Water and food were supplied *ad libitum*. They were mated twice with different bucks of proven fertility followed by i.v. injection of 100 i.u. of human chorionic gonadotropin (CG-5, Sigma Chemicals Co., USA) to induce ovulation and were designated as pregnant rabbits. The pregnant females were anesthesized by i.v. injection of Sodium pentabarbitone at various stages of pregnancy. The dissected tissue (Fallopian tube) was stored at -20^{0}C and then subjected to the following biochemical studies.

Total Proteins: A total protein was estimated by the method of Lowry *et al.* (1951) after proceeding for calibration of caesin.

Ascorbic Acid: Ascorbate was determined by the colorimetric method fo Mindlin and Butler (1938) by following the decolorization of 2-6, dichlorophenolindophenol in metaphosphoric acid.

Peroxidase: Total peroxidase activity was measured using guaiacol as donor by the method of Maehly and Chance (1954).

RESULTS

Figure1 shows the changes in the peroxidase activity and ascorbic acid content in the fallopian tube while the blastocyst is inside it. During the first two days of pregnancy, the ascorbate content as well as the peroxidase activity increases from its value of estrous. Peroxidase activity and ascorbate levels decline on day 3. However, on day 4 while ascorbate content continues to decline, peroxidase acivity exhibits a marked rise showing a peak. It declines sharply on day 5 and thereafter the level of peroxidase activity decreases until day 7. Ascorbate keeps low steady level during this period. A very high peroxidase activity is seen in the fallopian tube and blastocyst which correlates very well with our histochemical observations.

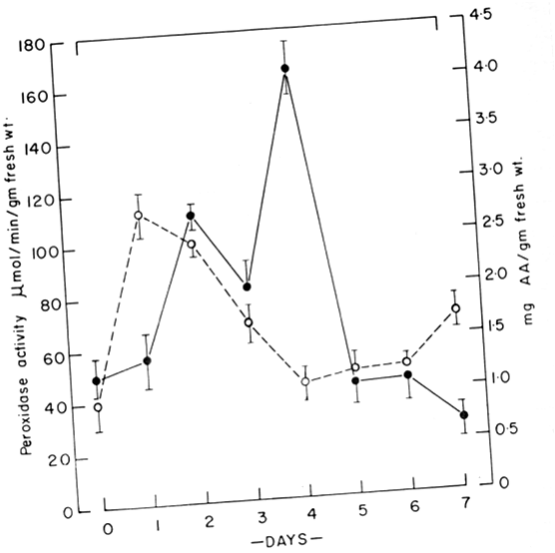

Figure 1: Changes in ascorbate and peroxidase activity in the fallopian tube while blastocyst is inside it in rabbit (Smooth line: for Peroxidase and Dotted Line: for Ascorbate).

DISCUSSION

The presence of a high ovarian peroxidase activity in the rabbit ovary, blastocyst and in the endometrium of the fallopian tube from 1st to 5th day of pregnancy, while the blastocyst is still in the fallopian tube. The results presented are in agreement with that of luteal activity of the ovary and progesterone secretion during the early period of pregnancy in rats characterized by the presence of high peroxidase activity and depletion of ascorbate (Agrawal and Laloraya, 1979). The presence of high peroxidase activity in the fallopian tube containing the blastocyst and in the uterus after day 5, when the blastocyst is about/ready to implant, suggests that peroxidase may also be involved in regulating the adhesiveness of the deciduoma for the attachment of the blasotcyst as suggested earlier (Agrawal and Laloraya, 1979). Peroxidase was suggested to have a role in regulating the stickiness of the uterine endometrium and blastocyst or of both, which is essential for implantation (Agrawal and Laloraya,1979).

LH is a luteotropic hormone secreted during the early phases of pregnancy (Savard *et al.*, 1965; Eaton and Hilliard, 1971; Moudgal *et al.*, 1974). LH is shown to promote cholesterol ester hydrolysis by activating cholesterol esterase (Behrman and Armstrong, 1969, 1970: Behrman *et al.*, 1979), this stimulates the synthesis of the enzyme.

Δ^5-3β-hydroxysteroid dehydrogenase which plays a key role in the early biosynthetic pathway of all the biologically active steroid hormones (Wiest *et al.,* 1968). However it has been shown that increased formation of progesterone in the CL is brought about by the induction of peroxidase system (Agrawal and Laloraya, 1977) and conversion of pregnenolone to progesterone by the peroxidase of the rabbit CL at day 6 of gestation has also been shown (Agrawal and Harper, 1982). The finding of a high ovarian peroxidase during the first 5 days of pregnancy while the blasocyst is in the fallopian tube, and the increase in uterine peroxidase activity as the blastocyst descends to it, suggest that increased luteotropic action of LH on the ovary during the early pregnancy period i.e., from mating through implantation and early gestation may be regulated by the peroxidase system as suggested earlier for rat and mice (Agrawal and Laloraya, 1979).

Involvement of histamine, PGs and cyclic AMP have been shown in implantation reactions of the blastocysts (Dey *et al.,* 1978 and1979). Furthermore, alkalinity of the mucolemma layer has also been attributed a function in implantation reactions (Boving, 1963). Simmer (1968) showed that the enzyme alkaline phosphatase appeared in the endometrial stroma around an implanting blastocyst.

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