

PATTERN OF CHANGES IN THE PROTEIN METABOLISM OF RAT OVARY DURING IMPLANTATION AND ANTI-IMPLANTATION

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ABSTRACT

Protein metabolism of ovary of Wistar strain albino rats was analysed during implantation and in induced anti-implantation condition. The total protein content of the ovary as well as the levels of DNA and RNA were increased during implantation and in the anti-implantation. These changes are consistent with the development of luteal cells during implantation and the development of follicular cells in the anti-implantation state. Ovarian protease activity was consistently elevated during implantation, and this was attributed to the increased requirement of protease activity for the continual atrophy of the atretic follicles. In the anti-implantation condition the protease activity was inhibited. The free amino acid content of the ovary was elevated both during implantation and in the anti-implantation condition. The soluble and structural protein fractions of the ovary were considerably elevated during implantation as well as in the anti-implantation condition. The α - and β -globulin and γ -globulin levels were significantly decreased in the ovary during implantation and there was a marked elevation in the albumin content. In the anti-implantation condition the α - and β -globulin and γ -globulin levels were significantly elevated, and there was a marked depletion in the albumin content. Thus there was hyperalbuminemia and hypoglobulinemia during implantation, and hypoalbuminemia and hyperglobulinemia in the anti-implantation state.

INTRODUCTION

IMPLANTATION has been considered as a dynamic event involving appropriate responses from uterine endometrium and blastocyst^{1,2}. The decidual cells of the uterus have protein reserves and proteinases which are important for blastocyst implantation³. Changes in endometrial protein synthesis during decidualization have an important role in implantation in rat⁴⁻⁸. During the pre-implantation phase in mouse the endogenous amino acid pool rises because of protein degradation. Free amino acids have been found to be essential for implantation in rodents⁹⁻¹¹.

Gossypol, isolated from *Thespesia populnea*, has been shown to have anti-implantation activity in rats^{12,13}. The possible mechanism of anti-implantation activity of gossypol was suggested to be due to estrogenic action of ovary and blocking of histamine release from the uterus¹².

MATERIALS AND METHODS

Adult female Wistar strain albino rats of age 70 ± 2 days and weight 120 ± 5 g with regular sexual cycles were selected. The rats were examined for

estrus stage by the vaginal smear technique¹⁴ and allowed to mate. The presence of spermatozoa in the vagina was taken to indicate start of pregnancy^{15,16}.

The rats were administered sesame oil by subcutaneous injection. From one group of animals, six animals were sacrificed by cervical dislocation on each of days 1, 3 and 6 of pregnancy, and the ovaries were collected, chilled immediately in ice and used for biochemical analyses. The rats of day 1 were taken as controls and those of day 6 after mating were considered as the implanted group.

Another group of the mated animals received a single subcutaneous injection of 10 mg/kg body weight^{12,13} of gossypol acetic acid (Sigma, USA) in sesame oil on day 6 of pregnancy. From this group, six animals were sacrificed by cervical dislocation on the 7th day of pregnancy and the ovaries were collected for biochemical analyses. This group was considered as the group indicating anti-implantation. Results were compared between day 1 and day 6 of pregnancy for implantation condition and between day 6 of pregnancy and gossypol-treated rats for anti-implantation condition.

The total soluble and structural proteins¹⁷, nucleic acids¹⁸, protease activity¹⁹, free amino acids¹⁹ as described by Colowick and Kaplan²⁰, α - and β -

globulins and γ -globulins, and albumins²¹ were estimated.

RESULTS AND DISCUSSION

The data presented in tables 1 and 2 reveal the pattern of changes in ovarian protein metabolism during implantation and in the anti-implantation condition. The ovarian total protein content was markedly increased on day 3 and day 6 of pregnancy, indicating addition of protein components into the tissue, probably due to activated synthetic activity. The increase in the protein content was higher on day 6 than on day 3, indicating the possibility of

increased addition of protein into the ovary at the time of implantation of blastocyst in the uterus. Since the luteal cells undergo rapid replication in the corpora lutea^{22,23}, the accumulated cells might be responsible for the increased protein content. The increased DNA content of the ovary on day 3 and day 6 also point to cell replication. The RNA content of the ovary was also significantly increased, which also indicates activation of the synthetic phase of protein metabolism during pregnancy. Proteolytic activity was also increased markedly. This observation indicates the possibility of increased tissue protein degradation in pregnancy. All the pre-Graafian follicles undergo atrophy during gestation and lactation periods owing to sufficient FSH

Table 1 Levels of total protein, DNA, RNA, protease activity and free amino acids of ovary during implantation and in the anti-implantation state

	(1) 1st day	(2) 3rd day	% Change between 1 & 2	(3) (Implanta- tion) 6th day	% Change between 1 & 3	(4) (Anti-implanta- tion) Gossypol injected on 6th day of pregnancy	% Change between 3 & 4
Total protein (mg/g wet wt)	113.01 ± 6.42	121.98 ± 5.67	+7.94 <i>P</i> < 0.05	137.19 ± 5.16	+21.39 <i>P</i> < 0.001	150.56 ± 2.02	+9.79 <i>P</i> < 0.005
DNA (mg/g wet wt)	1.64 ± 0.001	2.11 ± 0.002	28.66 <i>P</i> < 0.001	2.45 ± 0.01	+49.39 <i>P</i> < 0.001	4.04 ± 0.14	+64.49 <i>P</i> < 0.001
RNA (mg/g wet wt)	1.08 ± 0.001	1.64 ± 0.01	+51.85 <i>P</i> < 0.001	2.07 ± 0.02	+91.67 <i>P</i> < 0.001	3.26 ± 0.35	+57.49 <i>P</i> < 0.001
Protease activity (μ mol of tyrosine equivalents for- med/mg protein/h)	0.029 ± 0.001	0.034 ± 0.002	+17.24 <i>P</i> < 0.001	0.044 ± 0.002	+51.72 <i>P</i> < 0.001	0.014 ± 0.001	-68.18 <i>P</i> < 0.001
Free amino acids (μ mol/g wet wt)	3.94 ± 0.19	4.56 ± 0.24	+15.74 <i>P</i> < 0.001	5.65 ± 0.29	+43.40 <i>P</i> < 0.001	6.09 ± 0.49	± 7.79 <i>P</i> < 0.05

Each value is mean of six individual observations ± S.D.; '+' and '-' indicate per cent increase and decrease respectively.

Table 2 Levels of soluble proteins, structural proteins, γ -globulins, α - and β -globulins and albumins, and A/G ratios of ovary during implantation and in the anti-implantation state

	(1) 1st day	(2) 3rd day	% Change between 1 & 2	(3) (Implantation) 6th day	% Change between 1 & 3	(4) (Anti-implanta- tion) Gossypol inj- ected on 6th day of pregnancy	% Change between 3 & 4
Soluble proteins (mg/g wet wt)	64.86 ± 5.46	71.45 ± 6.01	+10.16 NS	86.10 ± 6.11	+32.75 <i>P</i> < 0.001	89.69 ± 6.84	+4.17 <i>P</i> < 0.05
Structural proteins (mg/g wet wt)	48.15 ± 3.94	50.53 ± 4.08	+4.96 NS	51.09 ± 4.88	+6.11 NS	60.87 ± 4.45	+19.14 <i>P</i> < 0.001
γ -Globulins (mg/g wet wt)	40.68 ± 3.01	36.44 ± 2.41	-10.42 <i>P</i> < 0.05	32.44 ± 1.22	-20.26 <i>P</i> < 0.001	46.41 ± 3.64	+43.16 <i>P</i> < 0.001
α - and β -globulins (mg/g wet wt)	8.50 ± 0.51	6.95 ± 0.45	-16.23 <i>P</i> < 0.001	6.01 ± 0.39	-29.29 <i>P</i> < 0.001	8.95 ± 0.49	+49.91 <i>P</i> < 0.001
Albumins (mg/g wet wt)	4.59 ± 0.14	6.64 ± 0.31	+22.88 <i>P</i> < 0.001	7.88 ± 0.46	+71.68 <i>P</i> < 0.001	4.82 ± 0.19	-45.72 <i>P</i> < 0.001
A/G ratio	0.093	0.129	+38.71	0.204	+119.4	0.072	-68.73

Each value is mean of six individual observations ± S.D.; '+' and '-' indicate per cent increase and decrease respectively.

circulation^{22, 23}. Hence the observed increase in the protease activity of the ovary might be associated with atresia of the follicles. Consequently, the free amino acid content of the tissue was markedly elevated. Such a condition allows envisaging active mobilization of amino acids into tissue metabolism. The soluble protein fraction of the ovary was markedly increased on day 6 while there was no significant change in the level of structural proteins. Among the soluble proteins, the albumin fraction was conspicuously elevated while the globulin fraction was depleted. This observation indicates the development of hyperalbuminemia and hypoglobulinemia in the ovary during pregnancy. The albumin/globulin (A,G) ratio of the tissue was significantly higher during pregnancy. The observation of non-significant change in the structural protein content of the ovary indicates a possible balance between multiplication of luteal cells on the one hand and atrophy of the atretic follicles on the other. The immunoglobulin content of the blood has been shown to be elevated during early pregnancy^{24, 25}. The decrease in the globulin content of the ovary suggests either their active efflux into the blood or their specific degradation owing to enhanced protease activity.

Albumin functions as an osmotic effector and activator of oxidative enzymes²⁶. The elevation in albumin content is suggestive of development of active transport mechanisms and activation of oxidative metabolism. Thus, the pattern of changes in the protein fractions of the ovary is indicative of dynamic turnover of protein molecules in the tissue, leading to accumulation of the albumin fraction.

In contrast to the conditions during implantation, the anti-implantation condition induced by gossypol administration showed interesting changes in the ovarian protein metabolism, which in some cases were exactly opposite to the changes during implantation. Total protein, DNA and RNA contents were elevated. This observation indicates activation of the biosynthetic machinery probably for the development of follicles. Administration of anti-implantation agents generally terminates the luteal phase and activates follicular growth, shifting the cycle to follicular phase^{12, 13}. Thus, after the administration of gossypol, follicular development was activated. This was evident through anatomical and histological changes. Since follicular development involves the replication of follicular cells, increased DNA content might facilitate cell division. Protease activity was also significantly decreased. Hence, the increased protein content of the tissue was due to

activation of synthetic phase as well as inhibition of proteolysis. The free amino acid content was not very significantly different from that in day 6 ovary. This may be due to active mobilization of amino acids into protein synthesis on the one hand and inhibited proteolysis on the other.

The analysis of protein fractions in anti-implantation ovaries gave interesting results. The structural protein fraction was significantly increased, indicating addition of cells into the ovary owing to follicular development. The globulins were markedly elevated and albumin decreased, a situation just opposite to that in implantation. Thus the anti-implantation state produced hyperglobulinemia and hypoalbuminemia in the ovary. Since the administration of antifertility agents results in the induction of an immune reaction, the increased γ -globulin content is of interest. The decrease in the albumin content of the tissue might induce osmotic imbalance and this may be responsible for the edematous appearance of the ovary with accumulation of water described elsewhere. Since the α - and β -globulin fraction is always associated with mucoproteins and lipoproteins²⁷, the increase in this protein fraction suggests the possible accumulation of organic reserves in the form of mucoproteins and lipoproteins, probably an essential requirement for the initiation of follicular development. The evidence available from the data on protein fractions suggests that the albumin fraction might play an important role in the functional status of the ovary. Hyperalbuminemia was associated with the functional status of the corpora lutea and hypoalbuminemia with the functional status of the follicles. Similarly, hyperglobulinemia seems to be associated with follicular development and hypoglobulinemia with functioning of the corpora lutea. Thus there seems to be a delicate balance between the albumin and globulin fractions of the ovary, and this might regulate the functional status of the organ.

The data also point to the operation of different patterns of protein metabolism in the ovary during implantation and in the anti-implantation state.

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