EFFECT OF PROTEIN DEFICIENCY ON RESPONSE OF OVARIES TO EXOGENOUS
GONADOTROPHINS

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Summary: The response of ovaries to injection of PMS and PMS followed by HCG was measured in protein deficient rats, in terms of vaginal cytology, ovarian augmentation, uterine weight gain, histology and ovarian ascorbic acid depletion.

The adult protein deficient rats having anestrus, began to show estrus in vaginal smear after gonadotrophin administration. HCG administration produced same amount of ascorbic acid depletion in ovaries of protein deficient rats as in the control rats. The uterus and ovary weights increased and on histological examination the ovaries showed a follicular and luteal phase; in response to PMS, and PMS and HCG injection respectively.

The immature protein deficient rats had a lower body weight than controls but their ovary and uterus weights were not significantly decreased. Following the injection of exogenous gonadotrophins the weight of these organs, increased and follicular growth was observed both in deficient and control rats. The percentage of ovarian ascorbic acid depletion in response to HCG was significantly higher in deficient rats than in controls.

Key words: protein deficiency ovarian response to gonadotrophins ovarian ascorbic acid depletion

INTRODUCTION

Experimental protein deficiency is known to disrupt estrus cycle in the rat and the menstrual cycles in the monkey (7,3). In these animals the ovarian hormone levels in plasma are decreased (3), the biochemical response of uterus to gonadal hormones is diminished (6) and atrophic changes in the reproductive organs resemble those observed in the hypophysectomised animal (7). These effects are accompanied by changes in the pituitary histology and decreased FSH levels in plasma (2). However, atrophic changes of the ovary in these animals are not accompanied by castration cells in the pituitary which appear after overectomy (12). This suggests that in protein deficiency the ovary is still functioning and secretes some estrogen to provide the feedback, sufficient to prevent the appearance of castration cells. Nevertheless, adequate levels of estrogen and progesterone and normal function of ovary in response to circulating gonadotrophins, perhaps may still be lacking. In this study we have investigated the response of the protein deficient rat ovary to injection of exogenous gonadotrophins.

MATERIALS AND METHODS

Experiments were done on 40 adult rats weighing about 150 g each and having regular estrus cycles; and 20 wear on protein deficient diet were 40 adult deficient diet feeding was 40 dl immature rats were also used with exogenous gonadotrophins to assess the effect of protein deficiency.

Procedure: After the rats were injected with Pregnant Molar chorionic gonadotrophin (PMS) and Human chorionic gonadotrophin (HCG). All injections were same in all rats.

All rats were sacrificed and their ovaries, weight of each group of rats was measured and serially sectioned and histology done. The number of as described by Koering and

Diets: The composition of the diets was isocaloric. The deficient diet contained 20% casein. The rats were

Protein deficient rats showed a marked decrease in body weight of 165 g, the protein deficient rats which had an initial weight of 38.6±10.2 and 22.5±4.0 mg showed a significant weight gain after HCG treatment. The uteri of protein deficient and control rats were almost equal in weight, but the ovaries of protein deficient rats were significantly smaller than those of the controls. The ovary weight of the deficient group was 38.6±10.2 and 22.5±4.0 mg. The ovaries of the deficient group had a mean weight of 38.6±10.2 and 22.5±4.0 mg. The ovaries of the deficient group had a mean weight of 38.6±10.2 and 22.5±4.0 mg.
estrus cycles; and 20 weanling immature rats. Twenty adult and 10 immature rats were fed a protein deficient diet while the rest were fed an iso-caloric control diet. The duration of deficient diet feeding was 40 days for adult rats and 20 days for immature rats. Five adult rats and 5 immature rats were also used to study the state of ovaries during protein deficiency, without treatment with exogenous gonadotrophins. The vaginal smears were taken daily from all adult rats to assess the effect of protein deficiency on estrus cycles.

Procedure: After the deficient or control diet feeding for the specific period, each rat was injected with Pregnant Mare's Serum (PMS) 60 IU and 72 hrs later with 25 IU of Human chorionic gonadotrophin (HCG). Few rats of each group were injected with saline instead of HCG. All injections were given subcutaneously in 0.6 ml of saline and dosage of hormones was same in all rats.

All rats were sacrificed 77 hrs after injection of PMS. The ovaries and uteri were removed, wiped clean of extraneous blood and fluid, and weighed on a Mettlar balance.

Ascorbic acid was estimated, using the technique of Elemendorf and Loraine (13) in the ovaries of each groups of rats which included rats injected with PMS followed by saline, PMS followed by HCG and saline followed by saline. One ovary from two rats of each group was serially sectioned and histologically examined. The follicles were followed through alternate serial sections. The numbers of follicles, their size and inter-stitial tissue activity was assessed as described by Koering (5). The uteri were also sectioned and histologically examined.

Diets: The composition of diets used has been described earlier (3). The two diets used were iso-caloric. The deficient diet contained negligible protein while the control diet contained 20% casein. The rats were fed ad lib and their food intake did not show much difference.

RESULTS

Protein deficient rats: After a schedule of 41 days of protein deficient diet the adult rats showed a marked decrease in body weight. While the control animals attained an average weight of 165 g, the protein deficient rats had an average weight of 100 g only. The immature rats which had an initial average weight of 22 g did not show any weight loss, but they never reached the weight level (42 g) of those immature rats having a normal diet.

All protein deficient adult rats showed disturbed estrus cycles and by the end of 3 weeks almost all went into anestrus. The mean wet weight of uterus and ovaries of these rats was 38.6±10.2 and 22.5±4.0 mg, respectively. On histological examination the ovaries showed very few small follicles. The uterine endometrium showed atrophic changes. The ovary and the uterus of protein deficient and controls of the immature series did not exhibit any differences.

Effect of exogenous gonadotrophins: In adult deficient rats, after PMS injection the vaginal smears of the deficient rats began to show pro-estrous on the second day. Following HCG,
almost all the rats showed numerous cornified cells in their vaginal smear. The mean ovarian and uterine weight of these rats increased to 52.0 ± 6.2 and 149.4 ± 6.8 g respectively (Table I). In response to gonadotrophins the percentage increase in ovarian weight was 5 times and uterine weight 3 times the percentage gain observed in control rats. (Table II). In spite of this the mean ovarian and uterine weight was significantly lower than the mean weight observed in control rats injected with gonadotrophins. The ovaries of deficient rats treated with gonadotrophins showed a number of growing follicles, hyperaemia and blood follicles. The uterus was often thin-walled when compared to the uterus of the control rat injected with gonadotrophins.

**TABLE I: Effect of gonadotrophin administration on wet weight in mg of uterus and ovary (Mean ± SE)**

<table>
<thead>
<tr>
<th></th>
<th>Protein deficient rats</th>
<th>Control rats</th>
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<tr>
<td></td>
<td>Ovary</td>
<td>Uterus</td>
</tr>
<tr>
<td>Before injection</td>
<td>22.5 ± 4.06</td>
<td>38.6 ± 16.9</td>
</tr>
<tr>
<td>After PMS</td>
<td>52.0 ± 6.2</td>
<td>149.4 ± 6.8</td>
</tr>
<tr>
<td>After PMS and HCG</td>
<td>48.9 ± 4.2</td>
<td>171.8 ± 16.9</td>
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**Histology:** The ovaries of the control rats showed a number of growing follicles, hyperaemia and blood follicles. The uterus was often thin-walled when compared to the uterus of the control rat injected with gonadotrophins. The ovaries of the deficient rats treated with gonadotrophins showed a number of growing follicles, hyperaemia and blood follicles. The uterus was often thin-walled when compared to the uterus of the control rat injected with gonadotrophins.

**Fig. 1:** Microphotographs of rats ovary 77 hrs after PMS injection. A-control Rat. A Mature follicle with a large antrum is seen. The ovum with surrounding cells is lying almost free. The thecal layers are thin at a point and suggestive of impending ovulation. B-Protein deficient rat, A maturing follicle with a fairly large antrum, and a well developed theca interna and externa is seen (H & E x 200).

**Fig. 2:** Microphotographs of A-control rat. Note the glandular growth. Compare with B. B-Protein deficient rat. Note the coiling and proliferation (H & E x 200).

**Ovarian ascorbic acid (OAAD)** than the controls. The levels obtained in different levels of treatment and the error that significant OAAD occurs with HCG. The figures underlined by another treatment of HCG, the mean percentage deficient rats.
**Histology:** The ovaries had 4 to 5 follicles, the largest much smaller than that observed in the control ovary (Fig. 1B). The ovaries of rats injected with HCG had a number of old corpora lutea and luteinised follicles. Interstitial tissue was seen in relation with atretic follicles. The uterus of the deficient rat treated with PMS showed much less endometrial proliferation and glandular growth than that observed in control rats. In rats treated with PMS and HCG glandular proliferation and coiling was suggestive of early secretory changes (Fig. 2B).

<table>
<thead>
<tr>
<th>Control rats</th>
<th>Uterus</th>
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<tr>
<td>1 ± 4.5</td>
<td>167.4 ± 8.0</td>
</tr>
<tr>
<td>2 ± 4.5</td>
<td>335.6 ± 5.0</td>
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<tr>
<td>3 ± 3.9</td>
<td>359.6 ± 18.4</td>
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**Fig. 2:** Microphotographs of a transverse section of the uterus of rats after PMS and HCG injections. A-control rat. Note the active proliferation of endometrium, glandular development and coiling of glands. Compare with B. B-Protein deficient Rat. Note the negligible endometrial proliferation and glandular response (H & E x 200).

**Ovarian ascorbic acid depletion (OAAD):** HCG injection in deficient rats produced a higher OAAD than the controls. An analysis of variance was performed on ovarian ascorbic acid levels obtained in different groups of rats to compare the ratio (F) of the mean square as a result of treatment and the error of the mean square due to biological variation (10). Table II shows that significant OAAD occurred in experimental and control rats as a result of treatment with HCG. The figures underlined by the same bar are significantly different (F=4.990) from figures underlined by another bar but not significantly different from each other.

Considering the ovarian ascorbic acid content in rats prior to their treatment with HCG, the mean percentage of ovarian ascorbic acid depletion was 57.5 ± 26.6 in protein deficient rats.
TABLE II: Ovarian ascorbic acid values in different groups of rats.

<table>
<thead>
<tr>
<th></th>
<th>Protein deficient injected PMS</th>
<th>Protein fed injected PMS</th>
<th>Protein deficient injected PMS+LH</th>
<th>Protein fed injected PMS+LH</th>
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<tr>
<td>Mean ovarian ascorbic acid µg/mg ovary in adult rats.</td>
<td>0.989</td>
<td>0.753</td>
<td>0.557</td>
<td>0.504</td>
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<tr>
<td>Critical difference</td>
<td>0.295</td>
<td></td>
<td>0.56</td>
<td>0.72</td>
</tr>
<tr>
<td>Mean ovarian ascorbic acid µg/mg ovary in immature rats.</td>
<td>0.82</td>
<td>1.09</td>
<td>0.56</td>
<td>0.72</td>
</tr>
<tr>
<td>Critical difference</td>
<td>0.0301</td>
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* Values which do not differ significantly have been underlined by a bar.

Control rats: The control rats showed 4-5 day estrus cycles. The mean weight of the ovaries and uterus was 61.4±4.50 and 167.4±8.0 respectively. After PMS injection the mean weight of these organs increased and the uterine weight showed a further rise in rats injected with PMS and HCG (Table II). The percentage gain in weight of these organs in response to gonadotrophins was lower than that of protein deficient rats. The ovaries of control rats that had received gonadotrophins showed 4-8 large follicles of more than 400 µ diameter. The granulosa cells were loosely arranged radially around the ovum, which lay almost detached from the rest of membrane granulosa cells (Fig 1B). The thecal layers surrounding the ovum often showed a thinning out, at a point which was suggestive of impending ovulation. In some ovaries, freshly ovulated follicles with formation of haemorrhagic corpus luteum could be seen. The interstitial issue showed large cells filled with lipid vacuoles. The uterus of these rats was thick walled, distended with fluid and showed intense glandular development and sometimes early secretory changes (Fig 2A). The ovaries of control rats showed a mean depletion of 35.2%±23.6 of ascorbic acid, which was lower than that observed in the protein deficient rats (Table II).

Immature rats: The mean ovarian weight in control and experimental rats treated with PMS was 33.9±1.8 and after treatment with HCG, these increased to 68.2±3.5 and 64.7±7.5 mg respectively. The uterine weight in control rats injected with PMS was 80.3 mg in comparison with 33.4 mg in protein deficient rats, and in both groups, no rise was observed. When the rats were treated with PMS and HCG, the immature rat ovary also showed a depletion of ovarian ascorbic acid in response to PMS and HCG injections. The mean OAAD in the deficient rats was 59.8±14.2% and 31.9±10.7% in control rats. This increase in response of the ovary of the immature protein deficient rat was statistically significant.

Both the experimental and control rat ovaries responded to PMS and HCG by active follicular growth and ovulation.
DISCUSSION

The data presented in this study show that the ovaries of protein deficient rats respond to PMS with follicular growth and gain in weight and show depletion of ovarian ascorbic acid after injection of Human Chorionic gonadotrophin (HCG). The uterine weight gain as well as histological appearance of the endometrium indicates that ovarian hormones are being secreted. The appearance of estrus in protein deficient anaestrous rats is further evidence of estrogen levels in circulation and is suggestive of ovarian secretory function in response to injected gonadotrophins.

Although the mean ovarian weight in protein deficient rats injected with gonadotrophins is lower than that in controls the percentage gain in ovarian weight after treatment with gonadotrophins is 5 times higher.

The histological examination of the ovaries showed numerous follicles in an advanced state of growth. These often appeared to be smaller than those observed in control ovaries and did not show a freshly ovulated state in any ovary. This is probably because the protein deficient rat ovary had been in a more inactive state than the controls, prior to injection of PMS and by the time of sacrifice had probably not reached the pre-ovulatory level.

The depletion of ovarian ascorbic acid has been used as a standard method to estimate luteinising activity in a sample (1, 8), the basis being that with increasing levels of HCG a rise in the ovarian ascorbic acid depletion is observed. Using the same dose of HCG ascorbic acid depletion would increase either, if ovarian ascorbic acid levels prior to injection are high or the ovary has increased sensitivity to the injected dose of gonadotrophin. In the protein deficient rats the ovarian ascorbic acid was higher than that in controls prior to their treatment with HCG. After injection of HCG the deficient rats showed a higher ovarian ascorbic acid depletion than controls. As shown in Table II, the ovarian ascorbic acid in both, experimental and control rats injected PMS was not significantly different, but when compared with the same groups after injection of HCG, significant depletion was seen.

Ovarian response to gonadotrophins in absence of dietary protein, has been studied earlier and compared with that observed in hypophysectomized rats (11). It was shown that the hypophysectomized as well as protein deficient rats showed follicular growth with beginning of antrum formation. The response of ovaries to gonadotrophins appeared to be better in deficient rats than in hypophysectomized control rats. This was probably because the protein deficient animal had in its plasma low levels of gonadotrophins while the hypophysectomized controls had none. Ovarian response has also been measured, using the criteria of uterine growth in protein deficient rats (11).

The proliferative and early secretory changes observed in the uterine endometrium in response to PMS and HCG injections were less in the protein deficient animals than in controls.
in this study. This probably is a poor response of uterus to ovarian hormones rather than a diminished response of the ovary to gonadotrophins.

It has been shown that biochemical changes in the uterus response to ovarian hormones are diminished in protein deficient animals. (6). Experimental protein deficiency disrupts ovarian function resulting in atrophic changes of the reproductive tract (7) and decrease in the plasma progesterone levels during the menstrual cycle (3). The present study shows that these changes are not due to lack of the response of the ovaries to gonadotrophins. The pathological changes in the pituitary of protein deficient animals, and decreased circulating levels of gonadotrophins (2) suggest that disruption may be at the hypophysyal level.

The luteinising hormone releasing factor (LRF) is decreased in the hypothalamus of the underfed rats (9) and the circulating LH levels are also low (4). These observations suggest that hypothalamic-hypophysyal axis is involved and further studies in the neuro-endocrine mechanisms that regulate reproduction during nutritional deficiency need to be investigated.

REFERENCES