Effect of Experimentally Induced Hypothyroidism and its Treatment by Thyroxine on the Number of Follicles in an Ovary of Wistar Rats.

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Abstract

**Background:** To observe the effects of hypothyroidism on histological structure of ovaries and also to evaluate the effect of treatment of hypothyroidism on the morphological structure of ovary.

**Methods:** In this experimental study forty five Wistar Albino rats were monitored for 10 days to rule out any diseased or medically unfit animals by keeping them in an experimental room. They were kept on balanced diet ad-libitum and normal tap water. The constant weight gain by an animal was taken as a healthy parameter. Forty five animals were divided into 3 groups, comprising of fifteen animals in each group. The control group only received tap water and normal balance diet. The hypothyroid group received carbimazole at a dose of 0.02% per ml in normal drinking water through feeding tube for six weeks. The thyroxine treated group initially received carbimazole at a similar dose for four weeks and then received intra-peritoneal injections of thyroxine at a dose of 5µg daily as a single dose for following two weeks. All the animals were sacrificed at the end of six weeks by deep ether anesthesia. Both ovaries of rats were isolated, fixed in Bouin’s fixative overnight and about 5µm thick sections were prepared and stained with hematoxylin and eosin (H&E) for assessing the follicular development.

**Results:** The total number of follicles was markedly reduced in hypothyroid group on both the right and left sides and an improvement in the number of follicles were observed in the thyroxine treated group. There was no significant difference in the size and number of follicles between the two sides of an animal.

**Conclusion:** Hypothyroidism produces significant changes in the morphology of ovarian structure. This change in the morphology can revert back to approximately normal by treatment with thyroxine.

**Key Words:** Hypothyroidism, Infertility, carbimazole, thyroxine and ovarian follicles

**Introduction**

Thyroid gland is a butterfly shaped endocrine gland responsible for the secretion of Triiodothyronine (T3) and Thyroxine (T4). These are the only amine containing hormones in vertebrates which contain iodine. They are the basic hormones considered to be important regulators of growth, differentiation, proliferation and metabolism of most tissues and organs. Literature shows that there is a strong influence of thyroid hormones in the regulation of ovarian and endometrial function. It is therefore important that thyroid gland should function normally to maintain healthy reproduction. The increase or decrease in the levels of thyroid hormones leads to impaired reproductive functions. Hypothyroidism is found to be the most important and leading cause of female infertility as it leads to dysfunction of ovaries. An important cause of infertility is found to be a decrease in number of follicles in the women of reproductive age group which will lead to a condition called premature ovarian failure.

Ovaries are highly dynamic endocrine and exocrine glands which lead to the production of ova and lead to the continuity of species. Hypothyroidism is found to be the most important and leading cause of female infertility as it leads to dysfunction of ovaries. An important cause of infertility is found to be a decrease in number of follicles in the women of reproductive age group. The journey of eggs begins as oocytes in primordial follicles. The primordial follicles are present in the ovary either during embryonic development in most mammals as in primates or they are present immediately after birth as rodents (mice, rats). It is known that in females the life time endowment of oocytes is established prenatally and diminishes progressively thereafter. Even the number of growing follicles changes each day throughout life.
This number of growing follicles is dependent on the number of follicles in a non-growing pool which if larger leads to the growth of more number of follicles.10 There are certain other important age related and developmental factors which are responsible for the growth and development of small follicles of each species.10 If the supply of primordial follicles from non-growing pool is reduced or stopped, the natural process of growth and development of follicles will stop permanently and the women will enter the menopause whereas the continuous non reproductive phase will start in the rodents.7 The effect of developmental factors on the number of follicles leading to its depletion is still not understood.7 The number of follicles can be determined by directly examining the ovaries. Previous data indicates that the counting of follicles present in 1% of serial sections of ovaries are sufficient to reveal the difference in between the control and treated group.11,12 The ovaries are one of the most important targets for xenobiotics that affect female fertility.13 The proper functioning of the ovary is influenced by the other endocrine glands like the thyroid gland.7

**Materials and Methods**

This experimental study was based on animal model and especially designed to produce hypothyroidism through Neomercazole and then treatment of hypothyroidal symptoms related to ovaries. For this purpose, Eltroxin was used in one of the group of animals to assess changes produced and to compare with different groups. For the selection of animals, it was made sure that all the animals were adult female, fertile rats of wistar strain and albino species. They were monitored for 10 days to rule out any diseased or medically unfit animals by keeping them in an experimental room, 10 days prior to the start of an experiment. Any animal observed to be lethargic, inactive or undergoing constant decline in weight was considered unfit for the experiment and was replaced. They were kept on balanced diet ad-libitum and normal tap water. The constant weight gain by an animal was taken as a healthy parameter. After the end of initial 10 days period, 45 animals were selected for the project and were divided into 3 groups. Each group comprised of 15 animals which were labeled as control, hypothyroid group and thyroxine treated group. During experimentation the control group was maintained on tap water and normal balanced diet.

Stock solution of Carbimazole was prepared by adding 1 tablet (5mg) of Carbimazole to 250 ml of distilled water. A concentration of 0.02% was obtained. This stock was kept in the fridge for use. The strength of the drug was measured constantly during the experiment by spectrophotometer. It was made sure that the dose of drug is same every day. Whenever the strength of drug was changed the stock was discarded and the new solution was prepared. The stock of thyroxine was also prepared by dissolving 50 µg tablet in 0.01M NaOH sol and 0.9% normal saline.

Animals were made hypothyroid by giving 0.02% per ml Carbimazole orally for 6 weeks daily through an oral feeding tube.4 The thyroxine treated group at first received an anti-thyroid drug Carbimazole at a dose of 0.02% per ml through feeding tube for 4 weeks and then Thyroxine at a dose of 5µg intra-peritoneally daily as a single dose for following two weeks.13,14 All the surgical procedures on animals were done under general anesthesia and the samples from both the right and left ovaries were isolated. All the external features of ovaries like the color, consistency, shape, vascularity, mottling, areas of hemorrhage and necrosis were observed and noted. The organ was then fixed in Bouin’s solution, embedded in paraffin and the sectioning of ovaries was done on rotary microtomes producing 5µm thick serial sections. The counting was done at the 10X objective lens of the microscope and 10X ocular of the microscope. The slides were thoroughly examined for microscopic and morphometric changes. The cortex and the medulla of ovaries were identified. The gross and histological changes were observed and compared in the ovaries of different groups.

**Results**

The mean total number of follicles ± SD counted in right ovaries of control group was 54.28 ± 30.64 and in hypothyroid group was 29.40 ± 11.67. The mean total number of follicles ± SD counted in left ovaries of control group was 54.42 ± 27.12 and in hypothyroid group was 29.40 ± 11.67. The mean total number of follicles ± SD counted in right ovaries of control group was 54.28 ± 30.64 and in hypothyroid groups (p value > 0.05) and in hypothyroid groups (p value > 0.05). The mean total number of follicles ± SD counted in right ovaries of hypothyroid group was 29.40 ± 11.67 and in thyroxine treated group was 34.69 ± 11.29. The mean total number of follicles ± SD counted in left ovaries of hypothyroid group was 26.06 ± 6.68 and in thyroxine treated group was 36.61 ± 6.61.
When the comparison of these two groups was done, no significant change in the total number of follicles was observed on right side (p value > 0.05) and left side (p value > 0.05) of ovaries. When the right and left sides of the same groups were compared, no significant result was seen in between the hypothyroid (p value > 0.05) and in thyroxine treated groups (p value > 0.05). The mean total number of follicles ± SD counted in right ovaries of control group was 54.28 ± 30.64 and in thyroxine treated group were 34.69 ± 11.29. The mean total number of follicles ± SD counted in left ovaries of control group was 54.42 ± 27.12 and in thyroxine treated group was 36.61 ± 15.19. When the comparison of these two groups was done, no significant change in the total number of follicles were observed on right side (p value > 0.05) and left side (p value > 0.05) of ovaries. When the right and left sides of the same groups were compared, no significant result was seen in between the control (p value > 0.05) and in thyroxine treated groups (p value > 0.05) (Table 1-3; Figures 1-3).

**Table 1:** Comparison of total number of follicles found in left and right side of ovaries

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total no of follicles in right ovary (µm); Mean ± S.D</th>
<th>Total no of follicles in left ovary (µm); Mean ± S.D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>54.28 ± 30.64</td>
<td>54.42 ± 27.12</td>
</tr>
<tr>
<td>Hypothyroid</td>
<td>29.40 ± 11.67</td>
<td>26.06 ± 6.68</td>
</tr>
<tr>
<td>Thyroxine treated</td>
<td>34.69 ± 11.29</td>
<td>36.61 ± 6.61</td>
</tr>
</tbody>
</table>

S.D is the Standard Deviation

**Table 2:** Statistical comparison of total number of ovarian follicles among all groups of rats

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Right ovary (p value)</th>
<th>Left ovary (p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control with hypothyroid</td>
<td>0.017*</td>
<td>0.00*</td>
</tr>
<tr>
<td>Hypothyroid with thyroxine treated</td>
<td>0.509</td>
<td>0.089</td>
</tr>
<tr>
<td>Control with thyroxine treated</td>
<td>0.237</td>
<td>0.141</td>
</tr>
</tbody>
</table>

*p-value < 0.05 is significant

**Table 3:** Statistical comparison of total number of ovarian follicles in right and left side in similar groups of rats

<table>
<thead>
<tr>
<th>Comparison</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control with control</td>
<td>0.623</td>
</tr>
<tr>
<td>Hypothyroid with hypothyroid</td>
<td>0.252</td>
</tr>
<tr>
<td>Thyroxine treated with thyroxine treated</td>
<td>0.141</td>
</tr>
</tbody>
</table>

*p-value < 0.05 is significant

**Discussion**

Hypothyroidism is the leading cause of various reproductive disorders in both sexes in human as well as most mammals. The hypothyroidism can be due to many different functional and structural...
abnormalities which can lead to impaired production of thyroid hormones. This altered state of thyroid hormones in turn leads to many physiological disorders. The hypothalamic-pituitary ovarian axis (HPO) and hypothalamic pituitary thyroid axis (HPT) are related to each other. They act together as a single system in different pathological conditions. Different studies have shown that in majority of cases, the reason for infertility in females is due to an abnormality in the ovaries. Thus the female fertility depends on the proper development of ovaries and the maturation of oocytes and follicles. Even the growth and differentiation of surrounding somatic cells are playing a major role in infertility.

It is also reported that even mild hypothyroidism can increase the risk of miscarriages and fetal deaths and can also impair the development of cognitive function in an offspring, but the relationship between them is still not yet clear. Therefore, in present study, an attempt has been made to investigate and discover the morphological effects of hypothyroid rats and compare this model with the control and thyroxine treated hypothyroid rats. The current study is also an attempt to fulfill the needs on histological and morphometric knowledge of ovaries by correlating them with the changes observed in different groups of rats.

When the number of follicles was observed in three different groups, a significant difference was observed in between the groups. It was found that there was a significant decrease in number of follicles in hypothyroid group in comparison to control group. This number was increased in the thyroxine treated group not to the extent as in control group but no significant difference was observed in between the number of follicles of control and thyroxine treated group. In the present study, the significant difference in the number of ovarian follicles observed among different groups of rats is in conformity with previous studies regarding reduced number of ovarian follicles in hypothyroid animals. Saha SK et al reported to have difficulty in identifying the different types of follicles in hypothyroid animal as there was difficulty in identifying the different stages of maturation. This finding regarding the number of follicles correlates with the present study, which also showed a marked reduction in the number of follicles.

Previous studies did not report any significant difference in the mean number of graafian, primary and developing follicles of control and PTU treated group but some researchers, reported marked difference in the number of follicles in hypothyroid ovary. This study agrees with the findings of the previous studies which also showed marked difference in the number of follicles in hypothyroid group. Thus the decrease in number of follicles in hypothyroid group found in the present study clearly indicates that the difficulty in conceiving in females is due to the ovarian structural abnormality. This morphological abnormality leads to infertility.

When the control group was compared with the thyroxine treated group, no significant finding was seen in the number of primary, secondary and graafian follicles. It was noted that the two groups were not of the same statistics but the number of follicles and the morphological state of ovary was better in thyroxine treated group. This finding is also in agreement to studies done by different researchers, who also found in their study that T4 alone or together with Equine chorionic gonadotropin markedly increased the number of healthy small or larger antral follicles respectively. A study of one of the researchers suggested that the developments of follicles are markedly reduced in hypothyroidism but the thyroxine treatment can improve the development of follicles.

**Conclusion**

1. Hypothyroidism produces significant changes in the morphology of ovarian structure which leads to infertility in females.
2. Even the short term treatment by thyroxine is beneficial in changing the morphology, as the changes produced by hypothyroidism in the morphology of ovaries can revert back to approximately normal.

**References**

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