

Original Article

Iodine in Excess : Impact on Ovarian and Uterine Histology in Adult Rats

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Abstract

Iodine is a key component of thyroid hormones. Iodine deficiency as well as iodine excess causes thyroid disorders. Universal salt iodization has led to exposure to iodine above the recommended levels in environmentally iodine sufficient regions disrupting thyroid physiology and in turn affecting reproductive physiology. Effects of excess iodine on male reproductive organ is well studied but studies on ovarian and uterine structural changes are scanty. Hence the present study investigates the histological changes in the ovary and uterus after prolonged exposure to iodine in excess. The study clearly indicates changes in ovarian follicular and corpus luteum number and structure. This was also accompanied by altered luminal structure and number of secretory glands in the uterus. The probable cause was ovarian iodine accumulation and altered thyroid physiology. Thus prolonged exposure of iodine in excess altered the normal histological structure of ovary and uterus, posing detrimental to female reproductive system.

Introduction

Iodine is an essential microelement needed for the maintenance of the thyroid physiology. It is a major component of the thyroid hormones (THs) (1, 2). As such deficiency of iodine causes severe disruption

of thyroid function (3). As thyroid gland gets affected, another important system to bear the brunt is the reproductive system. The reproductive system is affected at various degrees depending on the extent of iodine deficiency but the most important effect has been loss of fertility (4). The iodine deficiency disorders (IDDs) was proposed to be curbed by universal salt iodization programme. However, this lead to the consumption of iodine equally in both environmentally iodine deficient as well as environmentally iodine sufficient areas (5). Prolonged consumption of excess iodine (EI) in environmentally iodine sufficient regions further lead to thyroid dysfunctions like iodine induced hypothyroidism, hyperthyroidism, autoimmune disorders and even thyroid cancer (6,7). Under such circumstances it

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might be stated that disturbed thyroid physiology would probably also alter the physiology of the reproductive system. And as such few recent studies have shown that excess iodine altered the structure and function of the male reproductive system through ROS generation and hypothyroidism that led to severe morphological changes in the testis (8) and accessory organs (9). It may here be stated that thyroid physiology has also been known to alter ovarian function as well as female fertility (10). However, studies on the effects of prolonged exposure to excess iodine in environmentally iodine sufficient regions on ovary are scanty.

It is known that iodine is incorporated into the thyroid follicular cells through the sodium-iodide symporter (NIS) present on the basolateral membrane of the thyroid follicles (2). Recent evidences have also shown the presence of NIS in the mammalian ovary and uterus whose expression varies through the ovarian cycle (11, 12, 13). The iodine accumulation is higher in the ovary than all other iodine accumulating organs except thyroid gland (14). TSH has been shown to up-regulate NIS gene expression whereas estradiol has been found to increase proliferation (15).

Thus under the present scenario it is evident that not only there is presence of NIS in the ovary but also accumulation of iodine and as such excess iodine accumulation is also known to affect thyroid gland. However, as stated earlier, available reports on the effects of excess iodine exposure to ovary are scanty. Thus the present study was undertaken to investigate the structural alterations in the ovary and uterus due to prolonged exposure to iodine in excess. Excess iodine was administered in the form of potassium iodide (KI) dissolved in distilled water, at two different doses for a period of 60 days. One dose was 100 times excess of the normal required level of iodine (100EI) and was tolerable to the thyroid gland (13). The other was 500 times excess of the normal required level of iodine, that affects the thyroid physiology (13, 16).

Material and Method

Reagents

Potassium Iodide (KI) was obtained from Merck, Mumbai, India. All other reagents were procured from Sisco Research Laboratories (SRL), Mumbai, India.

Animal Maintenance

A total of 120 healthy adult (90±5 days) female albino rats (*Rattus norvegicus*) of Wistar strain weighing 100±10 gm were taken for the study. The animals were divided into three groups (I, II, III), each consisting of 40 animals. The animals were maintained according to the protocol approved by the Institutional Animal Ethics Committee (IAEC), Department of Physiology, University of Calcutta with assigned study approval reference number IAEC/IV/Proposal/AC-02/2014 dated 11.08.2014.

The animals were housed in clean polypropylene cages and maintained in a temperature of 22±2°C and relative humidity of 40-60% in an air-conditioned animal house. A constant 12:12 light:dark cycle was maintained throughout the period of study. The animals were fed on standardized normal diet consisting of 70% wheat, 20% Bengal gram, 5% fish meal powder, 5% dry yeast powder, 0.75% refined til oil, 0.25% shark liver oil, 4% non-iodized salt and water *ad libitum* (17). Besides this, KI at a dose of 0.007mg/100g body weight (BW) were provided to all the animals along with the balanced normal diet (18). All efforts were made to reduce suffering of the animals.

Experimental Design

The total number of 120 healthy adult female albino rats (*Rattus norvegicus*) of Wistar strain weighing 100±10 gm were divided into 3 groups (Group I, Group II and Group III), each consisting of 40 animals. Group I was designated as the Control (CON) group. Groups II and III were the excessive iodine treated groups at two different doses respectively. All the

animals were treated for 60 days. Prior to treatment the estrous cycle of all the animals were studied for at least two consecutive complete cycles, to confirm normal cyclicity of all the experimental animals. The normal duration of the estrous cycle corresponds to 5 ± 1 days (19).

Group I – Group I was the control group, consisting of 40 animals and fed with a normal diet, as mentioned earlier and an adequate iodine of $7\ \mu\text{g}/100\ \text{g}$ body weight per animal per day (16).

Group II – Excess iodine (100EI) administered group. This group consisted of 40 animals and were administered excessive KI at a dose of $0.7\ \text{mg KI}/100\ \text{g BW}$ dissolved in sterile water. This dose corresponded to 100 times the physiological daily required dose of iodine (16) and henceforth would be referred to as 100 EI (100 times excessive iodine). This dose is known not to cause any significant alteration in the thyroid physiology and hence the serum THs levels and thus considered as physiologically tolerable dose (20).

Group III - Excessive iodine (500EI) administered group. This group consisted of 40 animals and were administered excessive KI at a dose of $3.5\ \text{mg KI}/100\ \text{g BW}$ dissolved in sterile water. This dose corresponded to 500 times the physiological daily required dose of iodine (16) and henceforth would be referred to as 500 EI (500 times excessive iodine).

Treatment duration for all the three groups was set at 60 days so as to provide enough time for the animals to undergo a number of estrous cycles that also correspond to an equivalent number of ovarian cycles. The animals were sacrificed 24 hours after the last treatment. Animals were grouped and sacrificed according to the stage of estrous cycle (proestrous, estrous, metestrous and diestrous), all animals having in the same phase being sacrificed together. This was done to study the changes in the ovary throughout the different stages of estrous cycle.

Both the doses of excessive iodine as used in the form of KI (100 EI and 500 EI) were non-toxic as evident from the serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic

transaminase (SGPT) levels (13). The animals were sacrificed by cervical dislocation following protocol and ethical procedures. Fresh ovary and uterine tissue samples were collected and processed for histology.

Histological study of Ovary and Uterus

Immediately after the sacrifice, the ovary and uterus of experimental animals (of all groups- I, II and III) from all stages of estrous cycle were collected and were fixed in Bouin's fluid followed by embedding in paraffin. Five ($5\ \mu\text{m}$) thick paraffin sections of each of these organs were taken and subsequently stained with hematoxylin and eosin. Each slide was examined under a Bright Field Microscope (Model: EVOS XL Core Imaging System, Life Technologies, Cat.No. AMEX 100) at different magnifications of 80X and 200X for observable histological changes. It may be mentioned here that in group III, that is, 500EI administered group of animals, metestrous stage of estrous cycle was absent. Hence in the present study, histology of both ovary and uterus in this stage could not be represented.

Statistical Analysis

The data was expressed as Mean \pm standard deviation (SD). One way Analysis of Variance (ANOVA) was done with multiple comparisons using Tukey's post hoc test for statistical evaluation of the data. In all experimental data sets, a value of $P<0.05$ was considered as statistically significant. Statistical analyses were performed using GraphPad Prism 6 for Windows (GraphPad Software Inc., USA) and MS-Office Excel 2010 (Microsoft Corporation, Washington, USA).

Results

The histological changes in the ovary and uterus are represented in the Figures 1 (A-K) and 2 (A-K) respectively.

The ovary in the diestrous stage (magnification 80X) of the control group (Figure 1A) shows the presence of numerous corpora lutea (CL) in various stages of development and a few follicles in early stages of development. The diestrous stage of the 100 EI

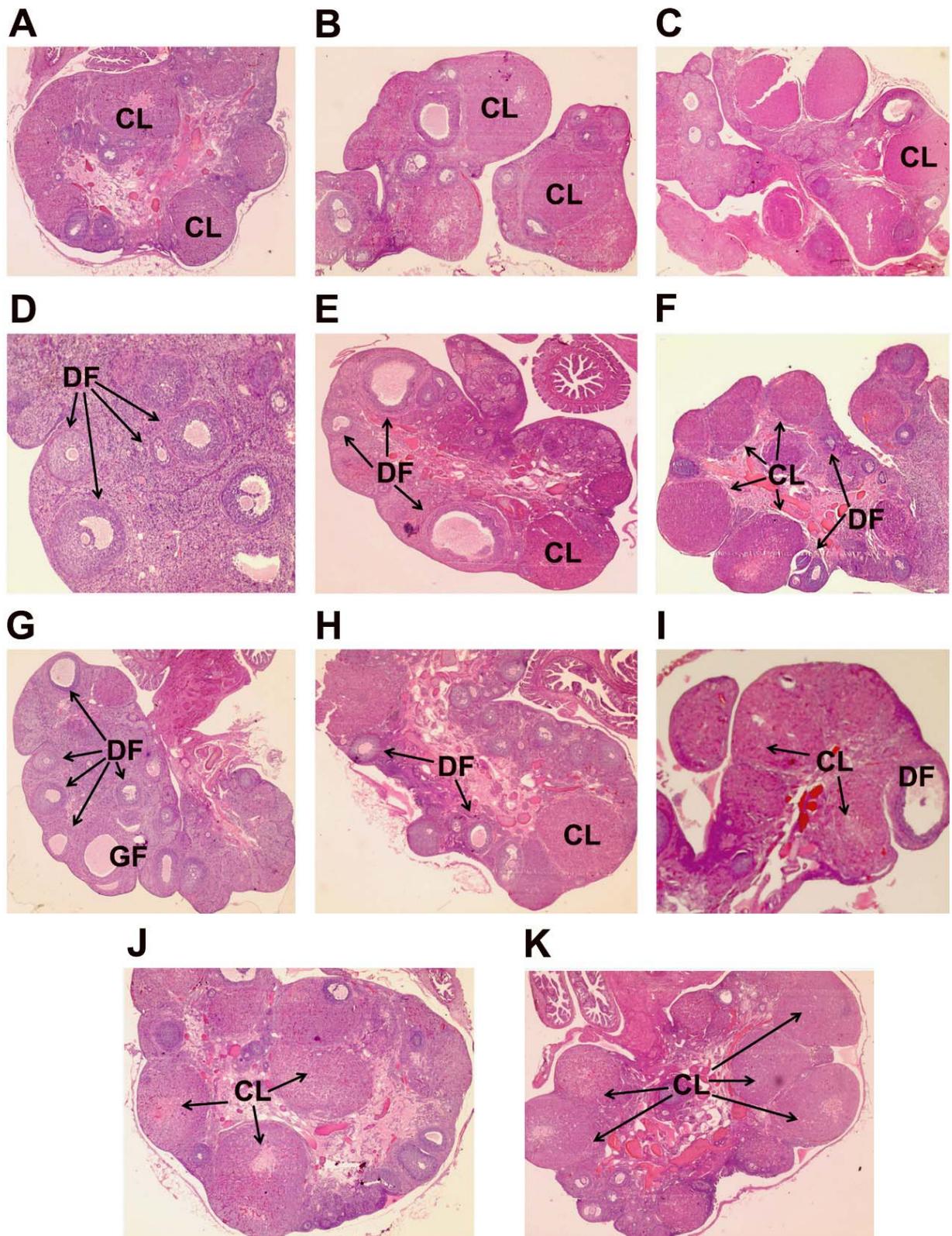


Fig. 1 : Photomicrographs of H & E stained ovary sections showing the structural changes in the ovary (magnification 80X) in diestrous, proestrous, estrous and metestrous stages of control (Fig. 1A, 1D, 1G and 1J), 100 EI (KI at a dose of 0.7 mg KI/100g BW) (Fig. 1B, 1E, 1H and 1K) and 500 EI (KI at a dose of 3.5 mg KI/100g BW) (Fig. 1C, 1F and 1I). In the micrographs, CL represents corpus luteum and DF developing follicles.

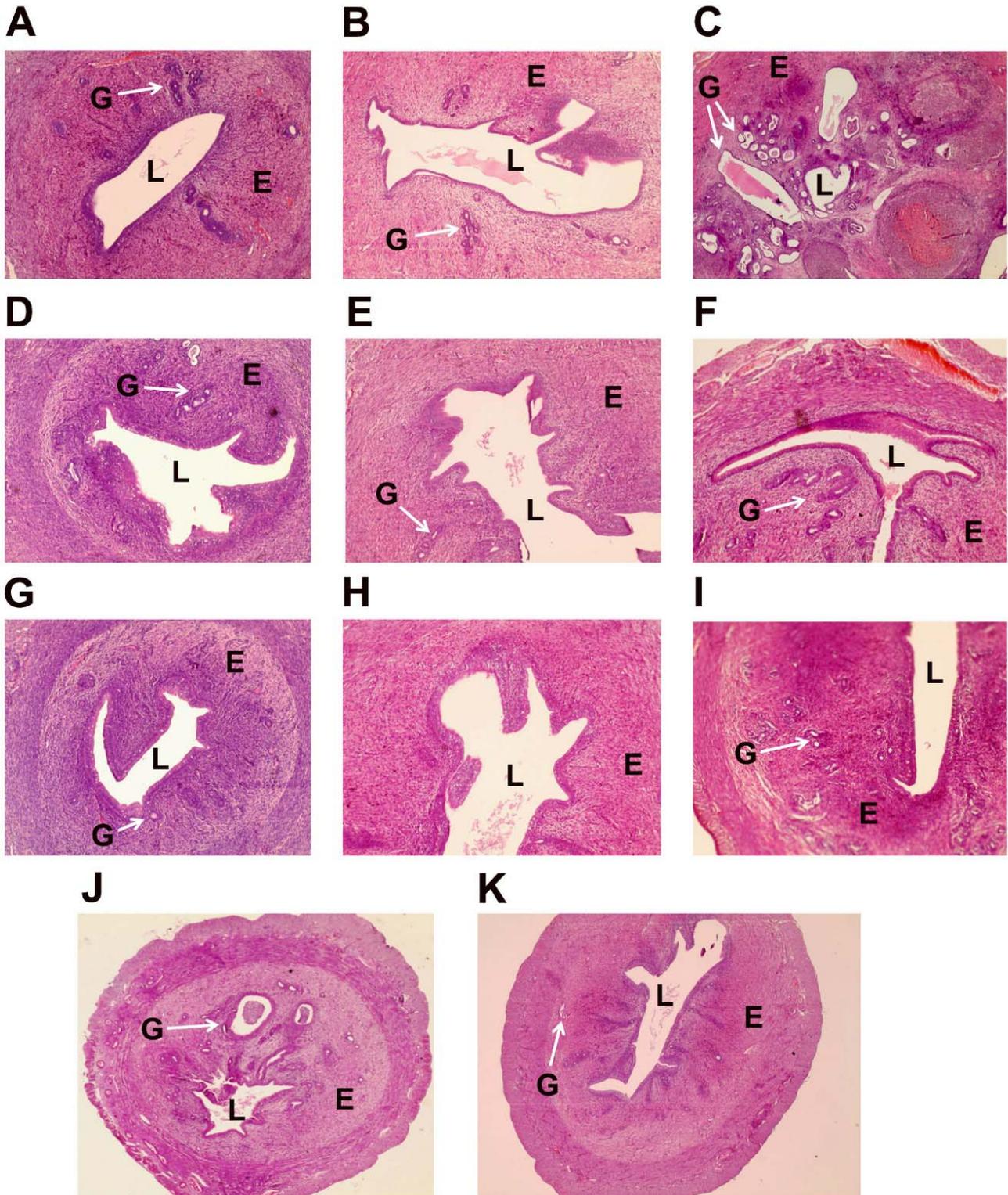


Fig. 2 : Photomicrographs of H & E stained uterine sections showing the structural changes in the uterus (magnification 200X) in diestrous, proestrous, and stages of control (Fig. 2A, 2D and 2G), 100 EI (KI at a dose of 0.7 mg KI/100g BW) (Fig. 2B, 2E, and 2H) and 500 EI (KI at a dose of 3.5 mg KI/100g BW) (Fig. 2C, 2F and 2I) and in metestrous stage (magnification 80X) of control (Fig. 2J) and 100 EI (KI at a dose of 0.7 mg KI/100g BW) (Fig. 2K). In the micrographs, E represents endometrium, G and white arrow points to uterine secretory glands and L represents lumen of the uterus.

administered animals (Figure 1B) show the presence of developing follicles (DF) in different stages of development which is not the characteristic of normal diestrous stage, one or two corpora lutea and slightly regressed ovary. The ovary of the 500 EI administered animals (Figure 1C) in the diestrous stage shows hypertrophied ovary with many corpora lutea like bodies and very few follicles.

The ovary in the proestrous stage (magnification 80X) of the control group (Figure 1D) shows developing follicles (DF) in various stages, primordial to secondary and normal stroma. The ovary in the proestrous stage of the 100 EI administered group (Figure 1E) shows regressed ovary with few follicles but also the presence of corpora lutea (CL) like bodies. The ovary in the proestrous stage of the 500 EI administered group (Figure 1F) show an increased number of corpora lutea with very few developing follicles.

The ovary in the estrous stage (magnification 80X) of the control group (Figure 1G) shows the presence of normal developing follicles (DF) in the various stages of development. The ovary in the estrous stage of the 100 EI administered group (Figure 1H) shows the presence of few follicles besides corpora lutea (CL) like bodies. However the ovary in the estrous stage of 500 EI administered group (Figure 1I) shows the presence of many corpora lutea (CL) like bodies and very limited number of follicles.

The ovary in the metestrous stage (magnification 80X) of the control group (Figure 1J) shows the presence of few follicles and newly formed corpora lutea (CL). The ovary in the metestrous stage of the 100 EI administered group (Fig 1K) shows corpora lutea and few developing follicles. However, as stated earlier, in the 500 EI administered group of animals the metestrous stage was found to be absent.

The uterus in the diestrous stage (magnification 200X) of the control group (Figure 2A) shows a well arranged structure showing the three layers of the uterus, viz., perimetrium, myometrium and endometrium. The endometrium (E) has few secretory glands (G), with a uterine lumen (L) lined by endothelial cells. The uterus in the diestrous stage of the 100 EI

administered animals (Figure 2B) show enlarged uterine lumen (L) with luminal lining being discontinuous and detached from endometrium at certain areas. There are hypertrophic changes in the uterus. But the uterus of the 500 EI administered animals (Figure 2C) in the diestrous stage gives a dismantled appearance of the endometrium with more than one lumen-like structures, few glandular structures and also some large secretory tissues (G) with compact cellular arrangement and high blood supply. The luminal lining is slightly indistinct. There is hypertrophic changes in the uterus.

The proestrous stage (magnification 200X) of the control animals shows (Figure 2D) well-arranged structure of the uterus showing myometrium, endometrium (E) and uterine lumen (L) with well distinguished luminal endothelial cell lining and few secretory glands (G). The uterus of the 100 EI administered animals in the proestrous stage (Figure 2E) shows an enlarged lumen with less distinct luminal lining and very few secretory glands. However, the uterus of the 500 EI administered animals (Figure 2F) in the proestrous stage shows a hypertrophied state with distorted uterine lumen showing disrupted and deformed luminal lining with an altered shape of the lumen.

The estrous stage (magnification 200X) of the control group shows (Figure 2G) normal arrangement of all the layers with a proper lumen (L) and a couple of secretory glands (G). The uterus of the 100 EI administered animals (Figure 2H) in the estrous stage shows enlarged uterine lumen (L) with the endothelial lining jutting out into the lumen with almost no secretory glands. The uterus of the 500 EI administered animals (Figure 2I) in the estrous stage shows a hypertrophied uterus having an elongated, narrow lumen and few secretory glands.

The metestrous stage (magnification 80X) of the control group shows (Figure 2J) almost a star shaped lumen (L) surrounded by numerous secretory glands (G) of various sizes in the endometrium (E) and surrounded by myometrium and perimetrium. The uterus in the metestrous stage of the 100 EI administered animals (Figure 2K) show an enlarged lumen with discontinuous and disoriented luminal lining at some areas and regressed endometrium.

Discussion

The ovary is the principal organ in females producing the steroidal hormones estrogens (estradiol and estriol) and progesterone. The synthesis and degradation of these hormones are said to be affected by a number of both external as well as internal factors (21). Of the internal factors thyroid hormones is of tremendous importance (22). Any alteration in the serum thyroid hormonal (THs) levels and thyroid function induce changes in the functional status of the ovary and also cause a respective alterations in the activity of the ovarian steroidogenic enzymes, serum estrogen levels and so on (4). The altered functional status is also represented by the altered histology of the ovary as well as uterus. Similarly external factors that cause compromised ovarian function also lead to an altered ovarian histology (23).

Iodine is an important trace element required for the synthesis of THs. Under euthyroid conditions, iodine availability is normal, thus the THs are maintained within their physiological range. However, prolonged consumption of excess iodine (EI) beyond 100 times the required level altered the thyroid function causing changes in the serum THs levels (5, 16). Excess iodine under such conditions poses as an external factor which also seemed to cause changes in the serum estradiol levels by upregulating the expression of estrogen metabolising enzymes as well as by down regulating estrogen responsive genes (24, 25).

As observed in the present study, the micrographs of both the ovary as well as the uterus, revealed significant alterations in the histological features in both the 100EI administered group of animals as well as the 500EI administered groups of animals as compared to that of the control group of animals in the respective stages of estrous cycle. There was also a significant difference in the altered histology between the 100 EI and 500EI treated groups.

As stated earlier, excess iodine at 100 times the normal required level, does not alter the thyroid functional status significantly (5). There is an ovarian

accumulation of iodine taking place under normal iodine exposure as shown in previous studies (13, 14). Thus, there is an increase in the iodine accumulation in ovary with excess iodine consumption as reported in a recent study (13). Iodine accumulation is also found in case of testes and thyroid (8). Previous studies have revealed that this iodine accumulation is further affected by altered estrogen levels as well as altered thyroid function (23). In the present investigation, the micrographic images of ovarian sections of the 100 EI administered group of animals (Fig. 1B, 1E, 1H and 1K) show a somewhat slightly regressed ovary with the presence of developing follicles (DF) and also corpus luteum irrespective of the stage of estrous cycle as compared to the micrograph images of ovarian sections of normal group of animals (Fig. 1A, 1D, 1G and 1J). Thus it may be stated that the observed changes in the ovarian histology in the 100 EI administered group of animals may be attributed to the excess iodide accumulation in the ovary as excess iodine is well known to bring about changes in the ovarian steroid hormonal profile which is known to be instrumental in bringing about further alterations in the ovarian structure (24, 25).

The photomicrograph images of the ovarian sections of the 500 EI administered group of animals (Fig. 1C, 1F, and 1I) depicted several other changes in the follicular structure and arrangement. Several studies have reported that altered THs levels cause alterations in the ovarian structure and function (10, 13). Previous studies have shown that excess iodine above 100 times the normal required level affects thyroid function (5, 10, 13). Besides, this evidences show that altered thyroid physiology leads to elevated serum E_2 levels along with low serum LH and high serum FSH levels that altogether lead to altered ovarian physiological changes (13, 27). Thus the observed changes in the present study in the 500 EI administered group of animals may be attributed to the summated effects of both excess iodide (13, 17, 18) as well as altered THs (4, 13, 22). The absence of a distinct metestrous stage in the 500 EI administered group of animals may also be attributed to the altered steroidogenesis, serum hormonal status of estrogens, progesterone and gonadotrophins as reported earlier (13).

The micrograph images of uterine sections of the control group of animals show normal characteristics of uterus as also previously reported (26) throughout the entire period of estrous cycle. However, the images of uterine sections of both the 100 EI as well as the 500 EI administered group of animals show a remarkable alteration in the lumen size, luminal lining as well as the uterine glands. Most prominent changes were observed in the diestrous stage of 500 EI administered group of animals. Profuse vasculature, numerous uterine glands and no characteristic lumen as observed were probably due to the hormonal alterations that resulted from altered ovarian structure and THs (13, 26). Uterine structure in the proestrous stage in the control shows dilation of the lumen as also seen in earlier studies of normal uterine morphology (26). The 100 EI administered groups of animals also showed similar structure. However, the 500 EI administered group shows a somewhat slit-like appearance. As reported by earlier studies, under normal hormonal profile in control and/or normal animals, the uterine sections show normal features (26). But excess iodine (24, 25) as well as altered thyroid function (4, 22) is well known to bring about changes in the steroid hormonal profiles (estrogen and progesterone), they cause altered ovarian morphology (as also evidenced) and reported earlier (13). This altered ovarian structure and the modified hormonal profile (13) further causes the observed changes in the uterus. Thus the observed changes may be attributed to the changes in the progesterone and estrogen profiles of these

animals, as these hormones act synergistically to bring about the changes in the uterine morphology (13, 26).

Thus from the present investigation it may be stated that prolonged exposure to excess iodine over and above the normal required dosage in environmentally iodine sufficient regions may be responsible for significant alterations in the ovarian and uterine histology even at dosages where thyroid function is unaffected. This may further lead to altered functional status of the female reproductive system of which ovary is the main functional organ. Hence in the present scenario the evident altered ovarian and uterine structure may pose to be a novel finding as a consequence of the exposure to excess iodine through various sources in environmentally iodine sufficient regions. And this excess iodine may thus further be considered to be detrimental to the female reproductive system besides the thyroid gland as previously established. However, further investigation is needed to assess other changes associated with this structural change and the probable reason behind the present observations.

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