



## EFFECT OF THIOUREA ON OVARY OF FEMALE MICE

### KEYWORDS

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### Introduction

The female reproductive system consists of a pair of ovaries attached to the dorsal wall of the abdomen behind the kidneys of the mesentery called mesovarium. Closely associated with each ovary but not in contact with it lies the funnel shaped end of the oviduct called fimbriated funnel. Each funnel leads into a narrow thin walled slightly convoluted fallopian tube which is lined internally by ciliated epithelium. Posteriorly each fallopian tube is continued into a thick walled, muscular, richly vascular and highly distensible uterus. The uteri open separately into a single median tube known as the vagina. The lower end of the vagina also meets the urethra from the urinary bladder so that there is a single wide but short urinogenital canal or vestibule which is dorsal to the pubic symphysis. The vestibule opens to the exterior of the vulva.

### OVERY:

The ovary lies within the peritoneal cavity attached by a fold of peritoneum. It has no intrinsic duct or tubules like the testis and consequently lacks a glandular appearance even though it has endocrine features.

Each ovary is made up of fibrous and vascular connective tissue called stroma. The ova are situated in the stroma in their various stages of maturation. Groups of germinal cells found in stroma are known as follicles. One primary follicle develops into a mature follicle. A fluid filled cavity, the antrum, appears in the follicle which separates an outer layer of cells, the membrane granulosa form the cells of discus proligerous. The follicular cells form layer around the ovum called zona pellucida. Such a mature follicle is known as a Graffian follicle. The Graffian follicle migrates towards the surface of the ovary and ruptures to release the ovum under the influence of LH. After ovulation all that is left is a mass of follicular cells surrounding a blood clot. Gradually, these follicular cells proliferate and the blood clot transforms into a yellow coloured corpus luteum, which produces progesterone.

The uterus, a thick walled hollow, pear shaped organ with anterior flattening, lies about the center of the pelvis. The uterus is freely mobile and is capable of growth during pregnancy. The uterus is made up of three major connective tissue layers (1) a thin outer perimetrium (2) a massive myometrium of smooth muscle and connective tissue, and (3) an inner most endometrium. From the physiological point, only two layers are recognised, the endometrium and the myometrium. The endometrium varies in thickness from 1-6 mm depending on the stage of the menstrual cycle as estrous cycle. It also varies with changes in the ovarian hormones and pregnancy. Progesterone causes the stromal cells to enlarge, become pale and filled with lipid and glycogen. The normal endometrium responds to the fluctuating levels of ovarian steroids, this reflecting the stages of the ovarian cycles. The myometrium is arranged in intricate layers of interlacing bundles of smooth muscle cells which respond to oxytocin.

It is very clear from the review of literature that thyroid hormone within a narrow range is required for proper functioning of somatic and gonadal tissues; otherwise

hypothyroidic or hyperthyroidic condition develops which requires proper attention. Incidence of both hyperthyroidism, especially during pregnancy and hypothyroidism are comparatively higher in females than males. Detailed changes in the reproductive function of females during hypothyroidism is not clearly understood. Hence the present investigation was undertaken. We preferred the use of antithyroid agent (thiourea) over surgery because of the presence of cells producing thyrocalcitonin required for calcium homeostasis, and risk due to high regenerating capacity of thyroid cells. Mice were selected for experimental studies due to short estrous cycle and prolific breeding capability.

### Materials and methods

#### ANIMALS:

Healthy, adult female albino mice (*Mus musculus*) of Swiss strain, weighing about 30-40 grams were used in the present experiments. The animals were housed in an air conditioned animal house at  $26 \pm 2^\circ\text{C}$  and maintained on 12 hours of day light. The control as well as experimental animals were given standard chow and water ad-libitum. Animals of different groups of treatments were caged separately with a maximum number of 6 to 8 animals per cage.

#### PREPARATION OF THIOUREA:

Thiourea (A.R.) ( $\text{NH}_2\text{CS.NH}_2$ ) (mol wt 76.12) was obtained from S.D. Fine Chemicals Pvt. Ltd., Boisar-401501.

Required concentration of thiourea was dissolved in normal saline (0.9% sodium chloride in glass distilled water) prior to treatment.

#### DOSAGE, DURATION AND MODE OF TREATMENT:

##### Group-I: Control:

Animal of this group received intraperitoneal injections of 0.2 ml normal saline daily for 15 days.

##### Group-II: Low Does:

Animal of this group were injected with thiourea at a dose of 0.2 mg/0.2 ml saline/mouse/day intraperitoneally for 15 days (10 mg/kg body weight).

##### Group-III: High Does:

Animal of this group were injected with thiourea 0.4 mg/0.2 ml saline/mouse/day intraperitoneally for 15 days (20 mg/kg body weight).

The various groups of animals, pretreatment, number of animals, duration of treatment and day of autopsy are summarized in the following table

Groups	Treatment	Duration of treatment (days)	Autopsy day
I Control	0.2 ml saline/mouse/day	15	16th day
II Low Dose	0.2 mg thiourea/0.5 ml/mouse day	15	16th day
III High does	0.4 mg thiourea/0.5 ml/mouse day	15	16th day

At the end of treatment, the animals were weighed and sacrificed by cervical dislocation. Required organs were isolated, blotted free of blood and weighed.

#### ESTROUS CYCLE OF FEMALE MICE:

The estrous cycle or reproductive cycle in female mice is completed in 4-5 days. The vaginal epithelium undergoes changes controlled by the changing hormonal milieu and these changes are correlated to changes in the uterine wall.

The estrous cycle occurs in 4 stages during which characteristic changes occur in the vaginal smear. The estrous cycle is therefore studied by making stained preparations of the vaginal smear.

#### Procedure:

A medicinal dropper containing 1-2 drops of normal saline was gently inserted into the vagina of mouse. After aspirating once or twice, the vaginal fluid collected in the dropper was smeared on a clean microscopic glass slide and allowed to semi-dry. Vaginal smears, stained with Ehrlich's Haematoxyline for 4-5 minutes were observed under the microscope.

#### Results:

##### Estrous cycle:

Estrous cycle in control mice was of 4-5 days. Treatment of mice with low and high dose of thiourea drastically changed the estrus cycle. In treated animals cyclicity was normal upto 11-12 day of treatments; thereafter it was abruptly stopped and animals remained continuously in one stage (Table-3).

Table-3 Showing changes in estrous cycle of control and hypothyroidic mice

Groups	Days of treatments														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Control	P	E	M	D	E-P	P	E	M	D	L-D	P	E	M	D	P
Low dose	P	E	M	D	L-D	E-P	P	E	M	D	P	E	E	E	E
High dose	P	E	M	D	P	E	M	D	P	E	E-M	M	M	M	M

P = Proestrous; E = Estrous; M = Metaestrous; D = Diestrous; E-P = Early proestrous; L-D = Late diestrous; E-E = Early estrous. n = 10.

#### DISCUSSION

The results revealed that the body weight did not alter significantly in all the treated groups of mice as compared to that of control. Non-significant increase in body weight might be due to the subcutaneous depositions of fat and/or other metabolic alterations. Absolute and relative organ weights did not significantly change in all treated groups of animals as compared to control.

Results shown in Table-3 revealed anovulatory and irregular cycles in thiourea treated mice. The estrous cycle is shorter (4 or 5 days vs. 21 days) in rat/mice (Butcher *et al.*, 1974; Smith *et al.*, 1975) since the corpora lutea of estrous cycle are only partially activated steroidogenically (Butcher *et al.*, 1974; Smith *et al.*, 1975). Stages of estrous cycle and their inter-conversions are mainly governed by the female sex hormones, estrogen and progesterone, mainly synthesized in the ovary. The serum level of these hormones are controlled by the secretion of pituitary gonadotrophins and hypothalamic releasing hormones (Lerner, 1969). Furthermore through feedback mechanism, the level of gonadotrophins are controlled by the estrogens and progesterone. Progesterone is known to maintain the luteal phase in cyclic rats (Nalbandov, 1970).

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