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Study of compensatory ovarian hypertrophy and cyclicity in ovariectomized female laboratory rat

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Abstract- In this experiment an investigation was made to analyze the effect of ovariectomy on the cyclicity and compensatory ovarian hypertrophy in female laboratory rats. Various parameters were used to study this phenomenon such as Unilateral Ovariectomy (ULO) and Bilateral Ovariectomy (BLO) and daily estrous cycle was observed till 4th week. Body weight, weight of remaining ovary (in unilateral Ovariectomy), adrenal weight, uterus weight and total protein content in uterus was observed. Control group were showing 4-day estrous cycle, bilateral Ovariectomy group completely lost the estrous cycle and underwent permanent diestrus stage whereas ULO group had irregular estrous cycle but within 21 ± 2 days they gained their estrous cycle and showed 4-day estrous cycle. Significant body weight gain was observed in BLO group as compared to ULO group ($p<0.001$). The ovarian weight in ULO group showed significant increase as compared to control group ($p<0.001$). Total protein was significantly reduced in bilateral ovariectomy group as compared to control group and unilateral ovariectomy group ($p<0.001$). These results supported that in ULO group during compensation of remaining ovary the cyclicity was irregular due to removal of one ovary (source of estrogen decreases) but after compensation estrous cycle become regular.

Keywords: Ovariectomy, Estrous cycle, Cyclicity, Hypertrophy, Diestrus

INTRODUCTION

Ovariectomy (surgical removal of ovary) in rats and mice is used as a surgical menopausal model to investigate the effects of permanent reduction in the plasma level of steroid hormones.[1] Similar to the cyclic menstrual cycle in human female, rodents have estrous cycle. The estrous cycle consists of four stages: proestrus, estrus, metestrus and diestrus and lasts for approximately 4-5 days. The proestrous phase is homologous to the human follicular stage associated with a rise in circulating estradiol level leading to a rise in Luitenizing hormone and Follicle Stimulating Hormone release. Estrous phase corresponds to ovulation with decline in estradiol level, whereas metestrus and diestrus corresponds to early and late secretory stages of human reproductive cycle, respectively, along with high level of progesterone.[2] Because rats are continuously polyestrous (i.e., cycle constantly throughout the year) diestrus is immediately followed by the proestrus phase of the next cycle. Anestrus, a period of reproductive quiescence between estrous cycles, is thus not usually observed in healthy, cycling female rats. Estrous cyclicity only ceases during pseudo pregnancy, pregnancy, and lactation, although a fertile postpartum estrus does occur within 24 hours after birth.[3] Ovariectomy can be unilateral (partial) or bilateral (complete) when one ovary or both ovaries are removed, respectively.

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As paired organs have a complementary and common function, the total loss of one such organ may have little immediate overall effect. Impairment of or loss of the second organ of the pair commonly results in a major increase in disability ULO results in weight increase compensatory ovarian hypertrophy (COH) and in a compensatory ovulation (CO). Unilateral oophorectomy has been reported to cause an increase in mass of the contralateral ovary and reduction of follicular atresia in different experimental animal models.[4] Compensatory ovarian functions have been explained as resulting from a hormonal imbalance of the hypothalamus-pituitary-ovarian axis caused by the elimination of one source of steroids (i.e., ULO). Asymmetries between paired endocrine organs (ovaries, adrenals, thyroid, testis) are related to the regulation exerted by the pituitary trophic hormones. For the bilateral Ovariectomy, there should be a substantial decrease in the weight of the uterine when compared to both the control and unilateral surgery as the hormones from the anterior pituitary gland will not be able to act without ovaries. So, in this study an effort has been made to investigate the effect of unilateral and bilateral ovariectomy on estrous cycle and histological architecture of ovary and uterus in female rats.

MATERIALS & METHODS

Animal collection and acclimatization:

The female rats were acclimatized for 2 weeks in ambient lab conditions and were fed with commercial feed and red gram at a scheduled time every day. Water was provided *ad libitum* throughout the study period.

Experimental groups:

15 mature female rats weighing 90-110 g were assorted and divided into 3 groups and marking was done. Each group contained 5 animals.

- Control group (CON) for 30 days study
- Unilateral Ovariectomy (UNI OVX) group for 30 days study
- Bilateral Ovariectomy (BI OVX) group for 30 days study

Surgical procedure of Ovariectomy:

Rats were anesthetized using ether at the time of operation. Before operation all the instruments were sterilized with 70% alcohol. Re-etherizer was kept ready. Rats were placed on dissecting tray dorsally. Longitudinal incision was performed 2 cm below the last rib; affecting skin, muscle, and peritoneum. Before incision hairs of this area were shaved off and were cleaned with 70% alcohol. Body wall was cut and ovary was exposed. Ovary was located in fat pads just beneath the muscles. Using forceps, the peri ovarian fat will be made around the fallopian tube to avoid spillage of blood. Ovary was excised and placed in a watch glass containing normal saline. In this manner all animals were made bilaterally and unilaterally OVEX and kept for recovery. All these animals were weighted before operation.

Autopsy procedures:

The animals were sacrificed by decapitation.

Histology:

The tissues of ovary and uterus from different rat groups were fixed by immersion in Bouin's solution. All these tissues were processed to form paraffin blocks and sections (6 μm thick) were cut using microtome. The sections of ovary and uterus were stained with H & E.

Estrous cycle:

In the morning hr. (9:00-9:30am) vaginal smear of each marked rat were taken separately with normal saline by inserting a blunt dropper inside the vagina but not deeply. Vaginal fluid was placed on glass slide. Slides were allowed to air dry. These slides were stained with methylene blue for about 5-7 min and then washed in normal water; stained slides were observed under a light microscope. 3 types of cells were characterized:

1. Round and nucleated cells are epithelial cells
2. Irregular shaped cells without nucleus are cornified cells
3. Small round cells are leukocytes

The proportion among them was used for determination of estrous cycle. This procedure was continued for 30 days at the same time.

Estimation of Total protein:

Content of protein in uterus was estimated by using Lowry Method.

Statistical analysis:

Data on estrous cycle, body weight, ovaries weight, uterus weight, adrenal weight, total protein content in uterus was statistically analyzed using one way ANOVA method.

RESULTS

Effect of ovariectomy on body weight and weight of uterus and adrenal gland:

The present study showed that there was significant increase after 4th week study, in body weight of bilateral group in comparison to unilateral and control group (p<0.001) (Fig 1.a.). Uterine weight was significantly decreased in bilateral OVX group as compared to control and unilateral OVX group (p<0.001) (Fig 1.b.). In this study there was no significant gain observed in weight of adrenal in bilateral OVX group as compare to control group and unilateral OVX group (Fig 1.c.).

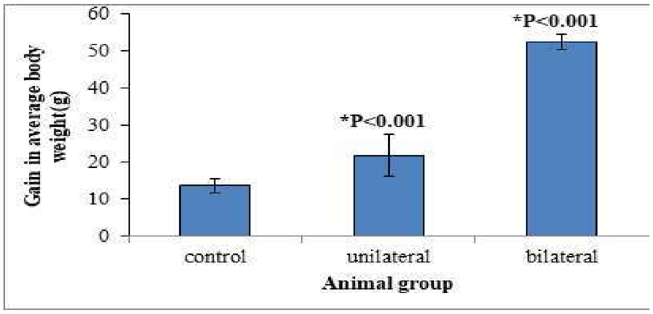


Fig 1.a. Graph showing weight gain in different groups. Error bar represents the mean values±SEM. Data was analyzed by on way ANOVA.

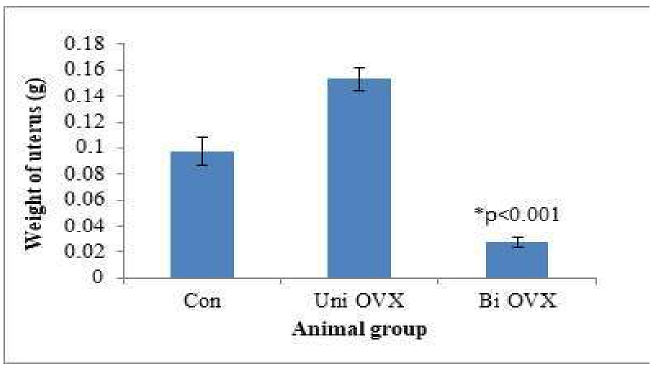


Fig 1.b. Graph showing the effect of ovariectomy on the weight of uterus. Error bar represents the mean value±SEM. Data was analyzed by one way ANOVA.

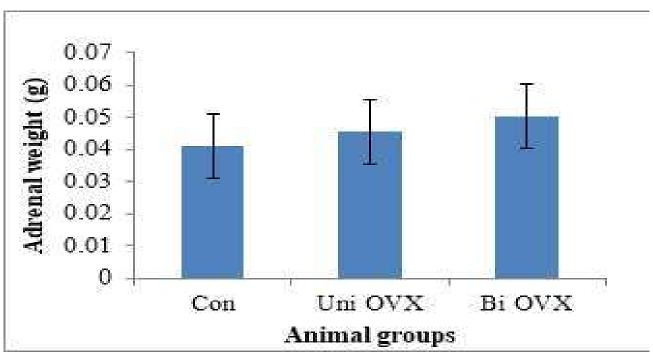


Fig 1.c: Graph showing the effect of ovariectomy on the weight of adrenal gland. Error bar represents mean value±SEM. Data was analyzed by one way ANOVA.

Effect of ovariectomy on total protein content in uterus:

Total protein was significantly reduced in bilateral ovariectomy group as compared to control group and unilateral ovariectomy group (p<0.001) (Fig 2).

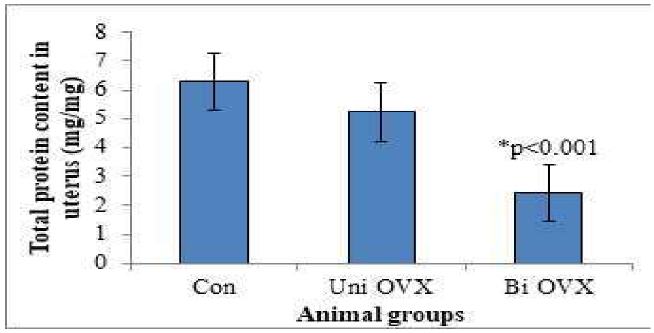


Fig 2: Graph showing protein content in uterus of control and experimental groups. Error bar represents the mean values ±SEM.

Effect of ovariectomy on estrous cycle length and vaginal cytology:

Estrous cycle was completely lost in bilateral group (Fig 3.a.) whereas in control group normal estrous cycle was exhibited (Fig 3.b.). Unilateral group showed disturbed estrous cycle after ovariectomy but after few days they got back to normal estrous cycle (Fig 3.c.). Length of cycle has been shown in table-1. In the control group there was 4-day estrous cycle. In BLO group there was loss of estrous cycle and they underwent complete diestrus stage but a constant estrous stage repeatedly interrupted by diestrus type was observed after unilateral Ovariectomy. Approximately 22±2.1 days after surgery, ULO group rats led to a functional compensation that was confirmed by occurrence of 4 days estrous cycle (Fig 3.a.- 3.c.). The cellular representation of different stages of estrous cycle in control and experimental groups have been presented in fig 4 (a-c).

Table-1: Length of estrous cycle in different groups.

Group	Estrous cycle length (days)
Normal Control	4±0.4
Unilateral OVX	4±0.2
Bilateral OVX	--

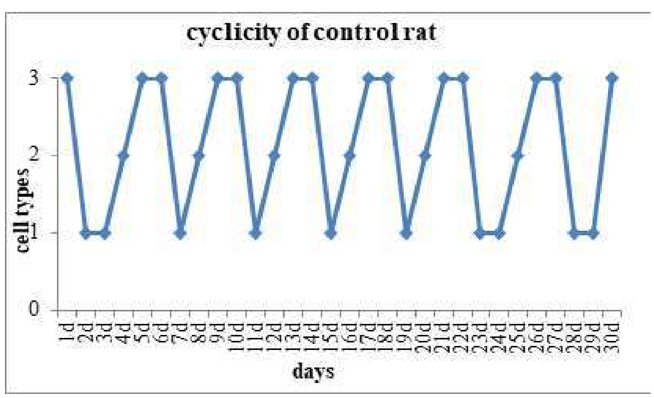


Fig 3.a. Graph showing cyclicity of estrous cycle in control group rat. 1=leukocytes, 2=epithelial cells, 3=cornified cells

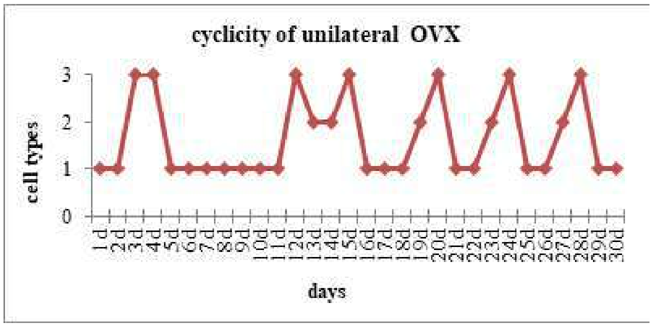


Fig 3.b. Graph showing cyclicity of estrous cycle following unilateral ovariectomy. 1=leukocytes, 2=epithelial cells, 3=cornified cells

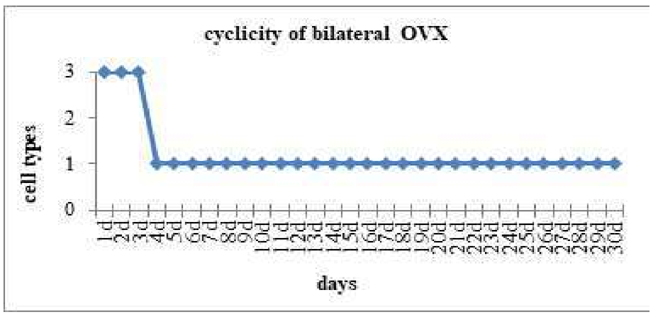


Fig 3.c. Graph showing cyclicity of estrous cycle following bilateral ovariectomy. 1=leukocytes, 2=epithelial cells, 3=cornified cells

Estrous cycle of control group:-

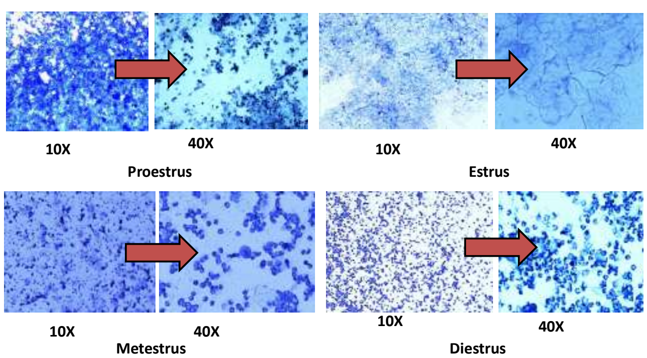


Fig 4.a. Cellular representation of different stages of estrous cycle in control group rat.

Estrous cycle in Unilateral OVX group

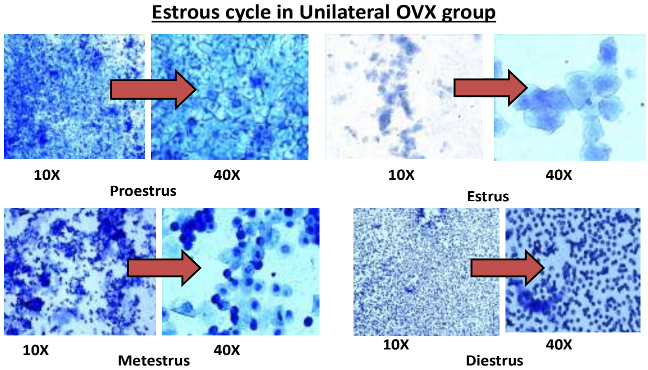


Fig 4.b. Cellular representation of different stages of estrous cycle following unilateral ovariectomy.

Estrous cycle lost in BILATERAL OVX group

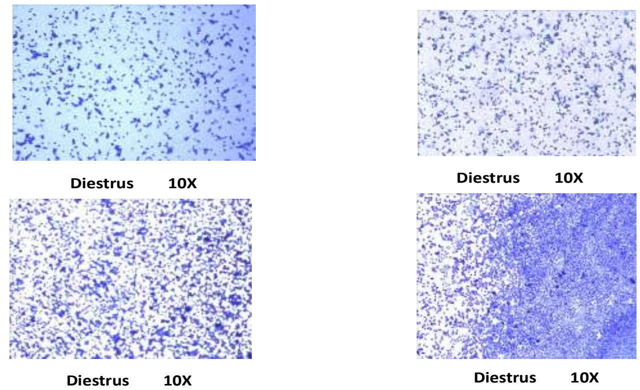
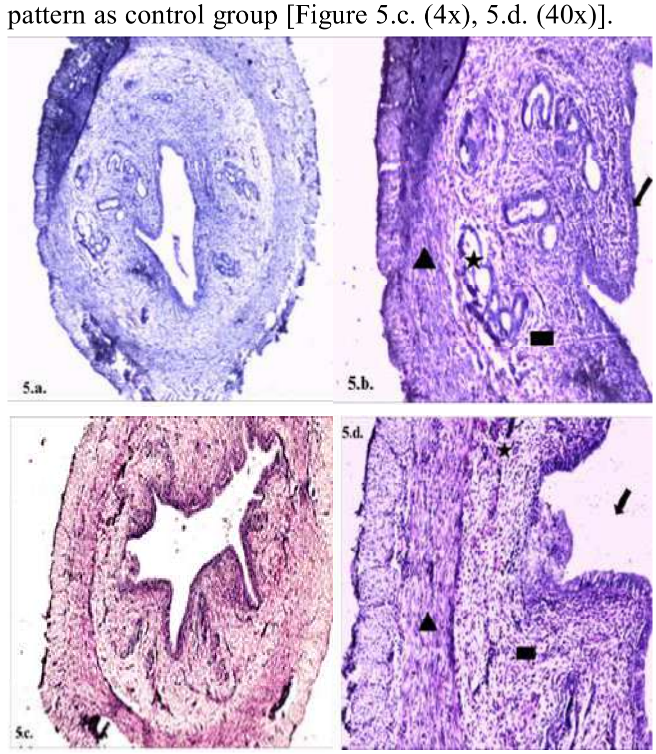


Fig 4.c. Cellular representation of different stages of estrous cycle following bilateral ovariectomy.

Effect of ovariectomy in histology of uterus:

Appearance of uterine epithelium and stroma was normal in control group. Its covering epithelial cells were a mixture of ciliated and secretary simple columnar cells and the connective tissue of the lamina propria were rich in fibroblasts and contained abundant amorphous ground substance. Connective tissue fibers were mostly reticular (Figure 5.a. (4x), 5.b. (40x). The length of the epithelium and the number of the uterine glands decreased, moreover the appearance of stromal tissue was looser in bilaterally ovariectomy group in compare to control and unilateral ovariectomy group [Figure 5.e. (4x), 5.f. (40x)] in compare to the unilateral ovariectomy group, which having similar pattern as control group [Figure 5.c. (4x), 5.d. (40x)].



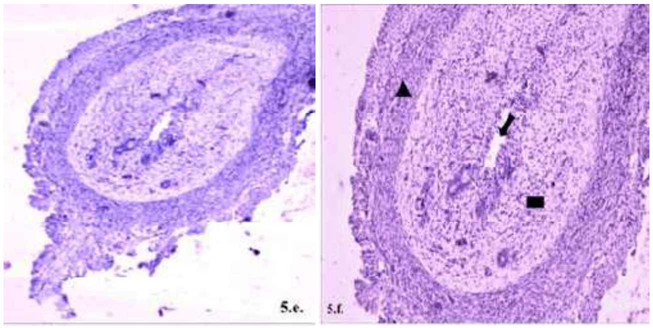


Fig 5: Histology of uterus in control group rat (Fig 5.a. & 5.b.), unilateral OVX group (Fig 5.c. & 5.d.) and bilateral OVX group rat (Fig 5.e. & 5.f.). (➔lumen, ★endometrium glands, ▲myometrium & ■endometrium).

Effect of ovariectomy in histology of ovary:

In this experiment the ovary of Control group contained large number of antral follicles as well as preovulatory follicles and less corpus luteum (Figure 6.a., 6.b. at 10x) as compared to unilateral ovariectomy group (Figure 6.c., 6.d. at 10x). Antral follicles were very few and large number active corpus luteum were abundant in unilateral ovariectomy group.

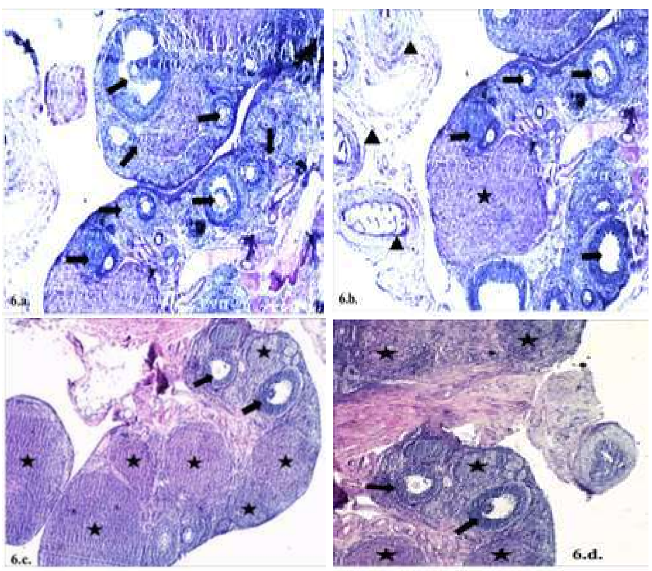


Fig 6: Histology of ovary in control group (6.a. & 6.b.) and Unilateral ovariectomy group (6.c. & 6.d.) rats. ➔Antral follicles, ▲Regressed corpus luteum,★Active corpus luteum.

DISCUSSION

Rodent ovariectomy is an experimental tool to remove the main source of female sex steroids, estrogen.[5,6] Significantly lower level of estrogen was documented in bilaterally ovariectomized rats.[7] In females, estrogen (source=ovary) promotes the development of secondary sexual characters, participates in the maintenance of reproductive system (involved in the thickening of the endometrial layer, regulating the menstrual cycle) and also known as body weight inhibiting agent. Similar to the present finding, ovariectomy in rats and mice caused a marked decline in uterine weight. This loss in uterine weight can be associated with the diestrus stage.[5] In this experiment, all animals underwent diestrus stage following bilateral ovariectomy but in unilateral ovariectomy group constant vaginal smear (estrus) of animals was repeatedly interrupted by diestrus stage and after 21±1 days normal 4-days estrous cycle was restored (Fig 3). Unilateral ovariectomy doubled the rate of ovulation and shortened the expected cycle length.[8] The fact that alteration in cycle is caused due to insufficient estrogen and restoration of cycle clearly indicates unilateral Ovariectomy leads to a functional compensation of remaining ovary; unilateral surgeries had an ovary which underwent hypertrophy to compensate for the other missing ovary but due to removal of both ovaries cause complete disruption of estrous cycle. In addition, there was no change in uterine weight for unilateral ovariectomy rats.[9] This was expected since estrogen levels would balance out as the compensating ovary-maintained estrogen levels to preserve sex characteristics and reproductive organs whereas in bilateral Ovariectomy group all animals showed diestrus stage because there is no estrogen source.[10]

Body weight significantly increased in bilateral group in comparison to control and unilateral group. Ovariectomy induced weight gain has been reported by Burch *et al.*, (2022)[11] also. They further stated that the estrogen treatment can be helpful in overcoming ovariectomy induced weight gain or post- menopausal obesity in females.[11] It supports that estrogen regulates body weight in females. Ovariectomy induced body weight gain and increased food intake was reported by Souza *et al.*, (2019)[12]. Compensatory ovarian functions have been explained as resulting from a hormonal imbalance of the hypothalamus-pituitary-ovarian axis caused by the elimination of one source of steroids (i.e., ULO). Following ULO treatment, the pituitary increases the release of follicle stimulating hormone (FSH) which in turn increases the recruitment of small follicles and a decrease in follicular atresia. Uterus structure of all these 3 groups varies. Data for the bilateral treatment also met expectations. The uterus had deteriorated, in most cases, by almost 75%. Removing both ovaries prevented estrogen production which prevented perpetuation of the sex characteristics. This in turn led to the degeneration of the uterine horn, lumen is slit like and the epithelial lining of lumen is very poor, endometrial layer is devoid of glands and total protein content is low whereas in unilateral group lumen of uterus is wide, columnar epithelial lining, endometrial glands are abundant (same as control) but in case of total protein content in uterus of control group (normal condition) high quantity of protein in compare to both groups (experimental condition).[13] These results supported that the maintenance of uterus is under the control of estrogen. Many studies in non-human animal models have demonstrated that unilateral ovariectomy leads to Compensatory ovarian hypertrophy in the remaining ovary.[14-17]

CONCLUSION

The results of present study showed that the ovariectomy caused significant alterations in functioning and maintenance of female reproductive system. As ovary is a prime source of production of estrogen, the ovariectomy can be used as an effective tool to study the effects of estrogen on the female reproductive health. Bilateral ovariectomy led to complete removal of source of estrogen, whereas in unilateral ovariectomy, compensatory response was shown by the intact ovary. The decrease in estrogen led to a severe deterioration of the uterine horn in bilateral ovariectomy animal and hypertrophy of the remaining ovary in unilateral ovariectomy animals. By showing how hormone levels in the female reproductive system change based on the experimental variables, this information will prove critical in helping patients with hormone level problems and missing parts. Hormone treatment is becoming increasingly important and this experiment provides an important insight into the body's control over them.

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