Ovulation and rate of implantation following unilateral ovariectomy in mice

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ABSTRACT

OBJECTIVES: Removal of an ovary of any side of the body is called the unilateral ovariectomy (ULO). ULO models are used to analyze the existence of functional and physiological asymmetries between the ovaries, including the ovum implantation. **MATERIALS AND METHODS:** ULO was done in mice without any specification on their stages of the estrous cycle. The animals were mated between 3rd and 15th day after ULO to observe the consequence of ULO on ovulation and ovum implantation. **RESULTS:** Ovulation and number of implantation were decreased in proportion to single contralateral ovary if the animals were mated within 36 hrs of ULO. It resulted in increased weight of contralateral ovary due to hypertrophy, and also in compensatory ovulation, formation of corpus luteum (CL) that reached a peak after 14th and 15th day of ULO (sacrificed on 5th day of pregnancy i.e., 19th and 20th day of ULO) compared to animals undergone sham-surgery. Numbers of implantations were also increased progressively on the contralateral ovarian side and get to a peak after similar days of compensation. **DISCUSSION AND CONCLUSION:** Following ULO, the remaining ovary started to compensate within 5th to 6th day of ULO even during pregnancy. Ovarian histology also showed increased formation of CL which is a marker of rate of ovulation.

KEY WORDS: Corpus luteum, follicle stimulating hormone, implantation, ovariectomy, ovulation

INTRODUCTION

Unilateral ovariectomy (ULO) is a time honored procedure which has been useful in elucidating follicular kinetics in species as disparate as pigs, chickens, *Drosophila*.[1] Some of these species normally always ovulates from right ovary, but following its removal the left ovary takes over. These studies permit certain conclusions, firstly the number of oocytes ovulated does not diminish after surgery since the rate of ovulation in the remaining ovary is doubled. For the rat, it has been shown that this compensatory ovulation has occurred by the first estrus after surgery, even if the latter is carried out as late as 1400 hr on the day before estrus.[2] The doubling of the ovulation rate is not a transient phenomenon, it can continue for at least 75 weeks in the mouse[3] and for at least 8 weeks in the hamster.[4] In the rat, it has been reported that compensatory ovulation has ceased by 6 months after surgery.[5] Secondly, the weight of the remaining ovary (or fragment of ovary)[6] progressively increases after surgery (compensatory hypertrophy) and may attain the combined weights of two ovaries in controls. This ovarian weight gain most likely results from the increased number of corpora lutea formed following compensatory ovulation,[7] although preovulatory follicles may also make a contribution.

The effect on mammals can be analyzed in terms of compensatory hypertrophy of the contralateral ovary (i.e., increased weight) representing persistence of increased number of corpora lutea (e.g.,, mice and rats) as well as enhanced follicular activity. The first species in which the immediate effects of ULO on compensatory ovulation established was hamster.[8] Removal of one ovary at morning for first 3 days of the 4-day estrous cycle was followed by doubling in the number of ovulations from the remaining ovary. It was believed that a reduction in atresia of larger follicles between day 3 and 4 spared follicles and resulted in compensatory

ovulation.[8] Unilaterally ovariectomized mice, at random times during the cycle, invariably double the numbers of ova shed within 3 days after the procedure.[7] As there is no report on the effect of ULO on the occurrence of ovulation and implantation irrespective of the stages of the estrous cycle in mice, the present investigation was carried out to study the changes in these parameters subsequent to ULO.

MATERIALS AND METHODS

Adult virgin female mice (7-8 weeks old) of the Park strain weighing 20-25 g were maintained in the Departmental Animal house on Mouse Laboratory Diet and water *ad-libitum*, and at a temperature of 72-78°F and under a light regimen of 14 hrs of light: 10 hrs of darkness. ULO was done between 0800 hrs. and 0900 hrs. randomly in the experimental mice. The ectomized ovaries were fixed in Bouin's solution and processed for routine histology. The ovaries were sectioned by using microtome and stained with Hematoxylin and Eosin stain.

These unilaterally ovariectomized mice were placed with males in the evening on the 3rd, 4th, 5th, 6th, 7th, 14th, and 15th days of ULO and vaginal plugs examined after overnight cohabitation with proven males. The day of observation of plug is considered as 1st day of pregnancy (D1).

On D5, they were anesthetized and pontamine blue dye was injected. The animals were sacrificed by cervical dislocation and laparotomized to see the blue spots in the uterine horn. The blue spots were counted as number of implantation. The remaining ovary of the contralateral uterine horn containing blastocysts was also fixed in Bouin's solution and processed for routine histology. The ovary was sectioned by using microtome and stained with Hematoxylin and Eosin to study the compensatory hypertrophy. The data were statistically analyzed by Student's *t*-test ($P < 0.05$).

RESULTS

Number of implantations along with ovarian weight increased significantly in mice mated on day 3 onward [Table 1]. No mating was observed within 72 hours of ULO. The changes of ovarian weight were consistent from day 5 onward. After day 7 to day 15, the effect of compensatory hypertrophy of the remaining ovary was prominent. The single ovarian weight becomes 14.33 ± 0.33 mg after 20 days of compensation as compared to 5.33 ± 0.33 mg to 7.00 ± 0.12 mg in sham operated animals. Along with the ovarian hypertrophy, number of implantation sites were also be compensated; i.e., 10.5 ± 0.5 implantation sites were observed in sham operated animal whereas 9.00 ± 0.66 number of implantation [Table 1] occurred after 20 days of compensation after ULO The animals were ovariectomized randomly irrespective of their stages considering that it has no effect on the cyclicity. For all these reasons the animals did not mate before day 3 after ULO. Ovarian histology reveals that ovary could compensate its ovulation by D7 because there it is showing at least 7 corpus luteum (CL) [Figure 1].

DISCUSSION

In the present study, the weight of the remaining contralateral ovary increased by compensatory hypertrophy, and by 3 weeks after surgery, it was equivalent to that of two ovaries in controls of the same age [Table 1]; it has been presumed that there is no effect of ULO on their cyclicity and all these changes have been attributed to hormonal alterations in the hypothalamus-pituitary-ovarian (HPO) axis caused by the elimination of a source of ovarian steroids due to ULO.

From the present investigation, it is assumed that the increased output of eggs by the remaining single ovary following surgery could be compensated either by increasing the rate at which follicles leave the pool, or by reducing the number of oocytes lost by atresia, which means compensatory ovarian functions have been explained as resulting from a hormonal alterations in the HPO axis. Following ULO the pituitary increases the release of follicle stimulating hormone (FSH), which in turn enhances the recruitment of small follicles and also decreases follicular

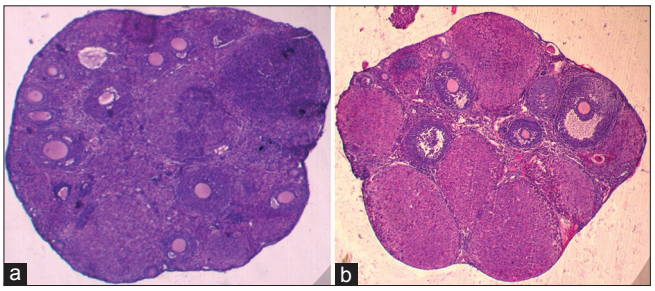

Figure 1: Histological section of (a) control and (b) compensated (of day 7 after ULO) ovary

Table 1: Number of implantation sites and ovarian weights of the mice after ULO showing the compensation

Mating days after ULO	3	4	5	6	7	14	15
Number of implantation sites (IS)	2.33±0.33	6.00±0.42[a]	6.33±0.33[a]	7.00±0.4[a]	7.66±0.33[a]	8.00±0.33[a]	9.00±0.66[ab]
Weight of the single sham operated ovary (mg)	5.33±0.33	5.66±0.33	6.00±0.57	5.66±0.66	6.33±0.88	5.66±0.33	7.00±0.12
Weight of remaining ovary on D5 (mg)	7.00±0.57	8.33±0.33	9.33±0.33	9.00±0.57	10.60±0.33	10.33±0.66	14.33±0.33[cd]
Total day of compensation	8	9	10	11	12	19	20

(Values denote mean±SD of eight samples; superscripts indicate significant difference, [a,b] between number of implantation sites, [c,d] between D5 ovary)

atresia.[9] Aside from the hormonal participation in the compensatory responses observed after ULO, there are evidences suggesting that ovarian innervations arriving to the ovaries through the vagus nerves and the superior ovarian nerve (SON) play a role in compensatory ovarian responses.[9]

The contralateral uterine horn of unilaterally ovariectomized mice contains blastocysts after getting pregnant, having the increase in length during the early stages of implantation. At the same time, the implantation swellings of blastocysts were visible macroscopically before the implantation, after the administration of pontamine blue injection [Figure 2]. It is concluded that growth in length of the uterus occurs during the implantation reaction and this may assist in the accommodation of blastocysts along the length of the uterus.[10] A single horn of the mice uterus is provided for the development of as many as 12 young pups, after 3 weeks[11] and the single remaining ovary in unilaterally ovariectomized animals (assuming the number of corpora lutea to be an index of ovulation) produces as many ova as both ovaries in normal females.

Implantation reduced in proportion to single ovary if the animals were mated within 24 hrs of ULO. There is an increase in ovarian weight along with an increase in implantation number continued in mated mice and reached at peak on 19th-21st day of ULO.[12] After ULO, the contralateral remaining ovary compensated within day 5-day 6 of ULO even during pregnancy. However, in the present study maximum ovulation and pregnancy was observed on D7 which did not increase significantly on D14 [Table 1]. This is to be noted that average number of implantation in this animal colony varies from 6 to10. Compensatory hypertrophy in the present experiment did not occur significantly until D15. But, the normal number of implantation can be corrected within D15. Ovarian histology showed stimulation of small antral follicles in mice mated on day 3 of ULO (sacrificed after 5 days i.e., day 8 of ULO) along with a decrease of large antral follicles and preantral follicles [Figure 1]. Preantral follicles were at peak during day 12–day 14, which may be again due to the increased release of FSH from pituitary.[9] ULO reported to cause a sharp, transient increase in serum levels of FSH with variable and less striking changes in Luteinizing hormone (LH).[13-22] It has also been demonstrated that in rat second surge of FSH which is normally restricted to estrus, is extended to 2nd day of the cycle and appears to be the signal that recruits additional follicles into ovulation range.[23] In mice, ovulation increased by two folds within 3 days after ULO at any

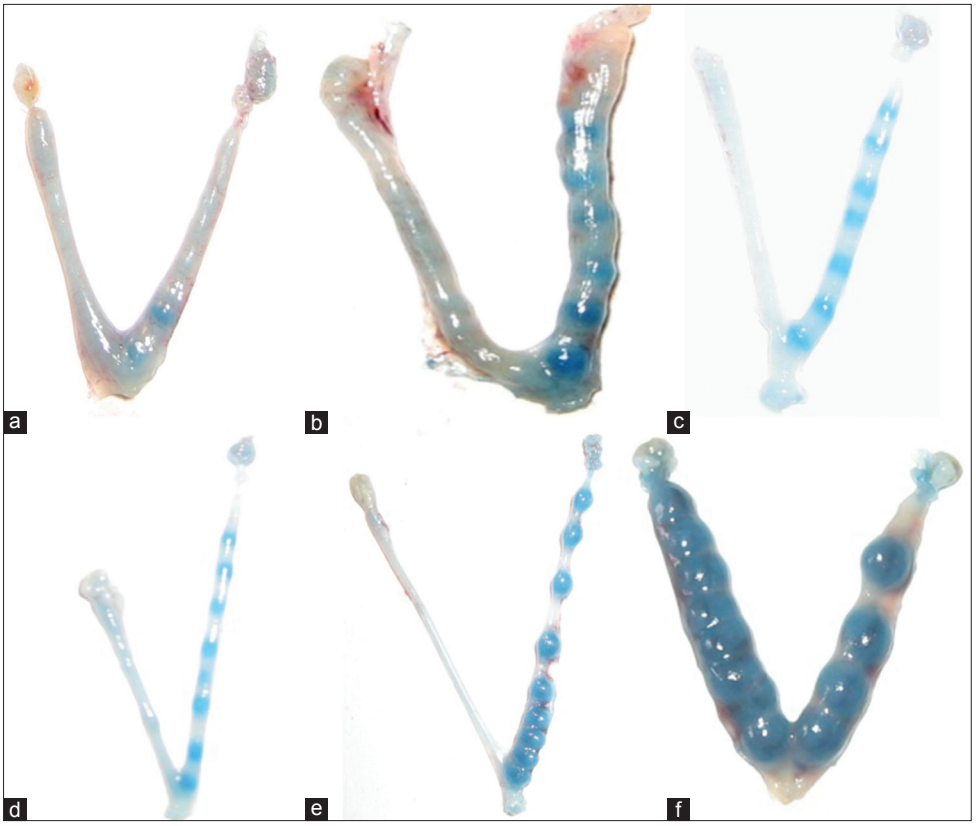

Figure 2: Implantation sites after unilateral ovariectomy (a) showing two implantation sites, 3 days after ULO, (b) showing six implantation sites 4, 5, and 6 days after ULO, (c) showing 6 implantation sites 4, 5, and 6 days after ULO, (d) showing eight implantation sites 14 days after ULO, (e) showing nine implantation sites 15 days after ULO, (f) showing 10 implantation sites of sham operated mice

stage of the cycle.[8] There is no report of implantation just after the ULO or even the short term effect of ULO on the subsequent implantation. During the normal pregnancy in mice, LH peaks on day 4 and day 11 of gestation with no significant changes in FSH; only on the day of parturition there is a sharp increase of both FSH and LH.[23] In ULO animals, implantation occurs when the FSH level is much higher than in normal conditions. Though, FSH level was not measured in the present study, it can be assumed that the occurrence of implantation in high FSH internal milieu.

CONCLUSION

Unilateral ovariectomy does not affect the sexual activities as well as sexual behaviors in female (as in lordosis), it has no impact on reproductive cyclicity too. Number of ova production hampered immediately after ULO but became normal just after few days of compensation. Ovulation and implantation are not affected by ULO due to the compensatory hypertrophy, and thus, it also has no effect on process of fertilization. The fertilized ova do not cross over and implant in the horn of the uterus opposite the side of their origin.

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