

## EFFECT OF PROGESTERONE SUPPLEMENTATION ON POST-COITAL UNILATERALLY OVARIECTOMIZED SUPEROVULATED MICE IN RELATION TO IMPLANTATION AND PREGNANCY

TIRTHA BHATTARAI<sup>1</sup>, SOUMITA DATTA<sup>2</sup>, PRASENJIT CHAUDHURI<sup>3</sup>, KOUSHIK BHATTACHARYA<sup>4</sup>, PALLAV SENGUPTA\*<sup>4</sup>

<sup>1</sup> Administrative Chief and HOD, Community Health, Everest College of Nursing, Nepal, <sup>2</sup> Department of Physiology, Vijaygarh Jyotish Ray College, University of Calcutta, Kolkata, West Bengal, <sup>3</sup> Department of Physiology, Sonarpur Mahavidyalaya, University of Calcutta, Kolkata, West Bengal, India, <sup>4</sup> Department of Physiology, Vidyasagar College for Women, University of Calcutta, Kolkata, West Bengal, India. Email: sunny\_pallav\_1984@yahoo.co.in; pallav\_cu@yahoo.com

Received: 7 November 2013, Revised and Accepted: 15 November 2013

### ABSTRACT

Unilateral ovariectomy (ULO) and its consequences with endocrine replacement in pregnant mice are important to examine both follicular dynamics as well as the outcome of implantation and pregnancy. In mice, ovariectomy on fourth day morning (D4), before pre-implantation estrogen secretion induces delayed implantation and embryonic diapyses, *i.e.* a state of suspended animation of embryos. The present study has been undertaken to evaluate the effect of progesterone supplementation on rate of implantation in unilaterally ovariectomized superovulated mice. Our study reveals that progesterone (P<sub>4</sub>) may help to protect the loss of embryo before and after the implantation if ULO is done during pre-implantation period (D4). The present study also shows if ovary is present in one side of the animal, it secretes estrogen (E<sub>2</sub>) in circulation which acts systematically on the uterus rather than locally. The findings of the present study show that progesterone may help to avoid the loss of embryo before and after the implantation, if ULO is done during pre-implantation period (D4) and the serum estrogen (E<sub>2</sub>) acts systematically on the uterus. Thus, it can be concluded that implantation in the uterine horn where ovary is not there.

**Keywords:** estrogen, implantation, progesterone, ovariectomy, ovary, uterus

### INTRODUCTION

Unilateral ovariectomy (ULO) is a time honored procedure which has been useful in elucidating follicular kinetics in species like pigs, chickens, *Drosophila* [1], and the California leaf-nosed bat [2]. The latter species normally ovulates from the right ovary, but following the removal of it, left ovary takes over. The effects of ULO in mammals can be analyzed in terms of compensatory hypertrophy of the contralateral ovary (*i.e.*, increased weight) representing the persistence of increased numbers of corpora lutea as well as enhanced follicular activity. The first experiment involving ULO dates back to an often-quoted study of John Hunter [3] and the first demonstration ovarian hypertrophy and compensation in rat was done by Arai [4]. The first species in which the immediate effects of unilateral ovariectomy (ULO) on compensatory ovulation were established was the hamster. [5] Removal of one ovary in morning for the first 3 days of the 4-day estrous cycle was followed by doubling in the number of ovulations from the remaining ovary. Animal studies carried out in rabbits, cats, mice and pigs on natural cycles have shown on ovarian hypertrophy and compensatory ovulatory rate after unilateral oophorectomy [6]. Mice unilaterally ovariectomized at random times during the cycle, invariably double the number of ova shed within 3 days after the procedure [7]. After ULO, compared to control animals estradiol and progesterone secretion increased, luteal weight increased, but there was no increase in luteal cell numbers. Previously, it had been shown in mice that when one ovary was removed, the other underwent hypertrophy; so that its weight becomes similar to that of two ovaries [8]. Most of the studies in ULO have been undertaken on the folliculogenesis and gonadotropin receptor. Not a good number of publications are available on this subject, pertaining to short term effect of ULO on subsequent pregnancy. During pregnancy the secretion of estradiol and progesterone is increased thoroughly which is secreted from ovary. On Day 4 morning of the pregnancy (D1 when vaginal plug was found after mating). ULO was done on female mice. It will be interesting to check what happens in the outcome of pregnancy after removal of single ovary on D4 morning

(08:00h). In this study the effect of ULO on subsequent implantation and pregnancy in mice has been reported.

### METHODS & MATERIALS

In this study, mature virgin female mice (6 to 8 weeks old) of Park strain weighing 20 to 25g were maintained in Departmental Animal house on Mouse laboratory diet and *ad libitum* water at a temperature of 72°F to 78°F and under a light regimen of 14h and 10h of darkness. Pregnant Mare's Serum Gonadotropin (PMSG) was applied. After 48h of PMSG injection human chorionic gonadotropin (hCG) was injected to get superovulation. Then those PMSG-hCG treated mice were cohabitated with proven males in separated cages for mating immediately. Vaginal plugs were examined after overnight cohabitation to check the pregnancy and that day was considered as D1 of pregnancy. From these 48 pregnant females were selected for the present experiment. On D4 morning (7:45 a.m. to 8:15 a.m.) pregnant females were unilaterally ovariectomized. Females were sham operated (sixteen animals) and taken as control, eight of them was sacrificed on D5 after 15 minutes of pontamine blue dye injection to get normal D5 implantation site. Rest of the 32 pregnant females were divided into four groups, each group contains eight animals. From these four groups two groups of mice (eight animals in each group) were given 2 mg of progesterone from D5 to D9. Two groups (eight animals in each group) (one from progesterone supplemented and one from progesterone non-supplemented) were selected and they were given pontamine blue dye on D5 morning and laparotomized after 15 minutes sacrificed to check their implantation sites. Other two groups (one from progesterone supplemented and one from progesterone non-supplemented) were kept them for later period (*i.e.*, upto D9). At D9 afternoon all the experimental animals (*i.e.*, eight controls, eight progesterone-supplemented and eight progesterone non-supplemented animals) were sacrificed and the numbers of implantation sites were counted in all experimented animals.

## RESULTS &amp; DISCUSSION

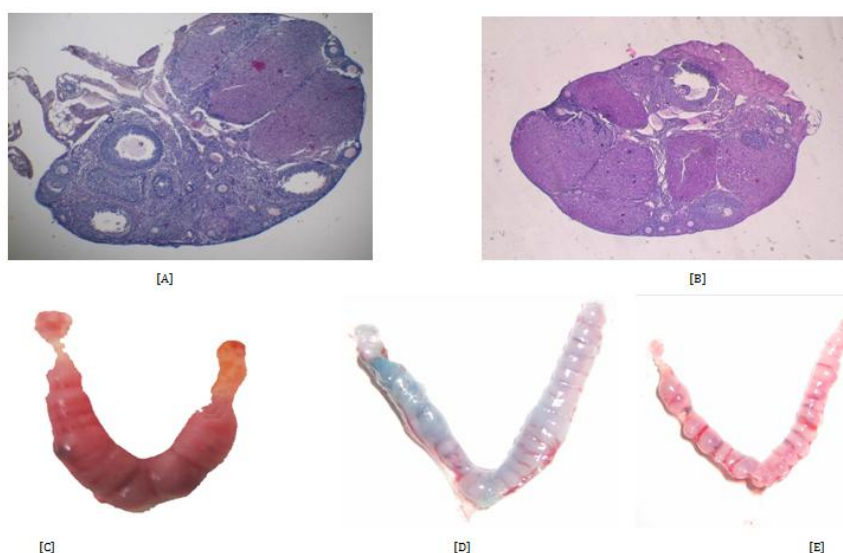
The results of the present experiment are depicted in Table 1, which clearly show that ULO does not have any effect on subsequent implantation. The so called rise of FSH if any cannot affect the exogenous E<sub>2</sub> action to induce implantation in this group. Implantations in both the horns were observed because the ULO was done on D4 morning when the embryos have already reached in the uterine horn. The conjoint injection of progesterone after ULO

showed its effect on D9 when we observed higher number of implantation sites in comparisons to progesterone non-supplemented group (Table 1). We have also checked the occurrence of implantation on D5 in both the progesterone supplemented and non-supplemented groups to confirm the incidence of implantation immediately after ULO. The single ovarian weight becomes 9.5±0.5mg and the numbers of implantation sites are 19.5±0.5 at the day of sacrifice (D9) in control animals.

**Table 1: Changes in ovarian weights and implantaion rates in progesteronesupplemented and non-supplemented groups.**

	Progesteronesupplemented group			Progesterone non-supplemented group		
	Weight of single normal ovary	Ovarian weight on day of sacrifice (D9)	No. of implantation sites observed on day of sacrifice (D9)	Weight of single normal ovary	Ovarian weight on day of sacrifice (D9)	No. of implantation sites observed on day of sacrifice (D9)
Presence of IS checked on D5 and sacrificed on D9	4.00±0.57	5.66±0.33	13.50±0.50	4.20±0.57	7.33±0.66 <sup>b</sup>	17.50±0.50 <sup>b</sup>
Sacrificed on D9	4.66±0.88	11.66±0.88 <sup>a</sup>	14.50±0.50	4.60±0.88	13.00±1.15 <sup>a</sup>	18.50±0.50 <sup>b</sup>

(Values denote mean±SD of 8 samples; <sup>a</sup> between D5 and D9, <sup>b</sup> between progesterone supplemented and non-supplemented groups)



**Fig 1. [A] Histological section of normal ovary, [B] ovary on the day of sacrifice (D9), [C] D9 implantation sites in non-progesterone supplemented mice, [D] D9 implantation sites in progesterone supplemented mice, [E] Normal D9 implantation sites in mice.**

Utero-ovarian relationships have been studied extensively [9-11]. While there is reliable evidence for a local influence on the ovary by the uterine horn of the same side [12, 13], evidence for the reciprocal effect that of the ovary on the ipsilateral uterine horn is much less convincing. The role of ovarian steroids in implantation and pregnancy is well established. Sufficient levels of progesterone and estrogen (E<sub>2</sub>) are necessary for successful implantation. Progesterone is further required for the maintenance of pregnancy. Endocrine control of implantation and delayed implantation in the mouse and the rat was reviewed by Gidley-Baird [14]. Earlier reports have proposed that the oviduct may mediate the local effect of the ovary on the uterus and that this effect may be primarily hormonal.[15] Ovarian steroids secretion is necessary for implantation till the afternoon of D4 when implantation initiates in mice [7]. According to another study [16] a combination of LH and FSH are needed for preimplantation increase in estrogen secretion. It is also suggested that LH is concerned with progesterone production and maintenance of corpus luteum, while FSH is concerned with the production of estrogen required for implantation in mouse. In normal pregnancy in mice there is a report telling that there is a LH surge on D4 but no change in FSH

[17]. In ULO animal's implantation occurs in spite of a higher level of FSH in comparison to normal condition. The pre-coital ULO animals having maximum increased number of implantation due to the compensation of remaining contra lateral ovary[8] with its morphological changes as we saw in male cryptorchid animal also[18]. However, we could not measure FSH in these animals. Highly purified FSH could not induce implantation in lactating rats nursing 6 pups but FSH along with LH can induce implantation. Therefore it is interpreted that combination of LH and FSH is probably required for the secretion of E<sub>2</sub> to support the implantation in lactating rats. The present results show that ovum implantation is possible under the higher levels of FSH [18-21]. From the outcome of our present experiment which shows that removal of one ovary on D4 morning (*i.e.*, 12hour before the prospective implantation) has no inhibitory effect on implantation in both groups (progesterone supplemented and non-supplemented). It is concluded that the amount of estrogen secreted from one ovary on D4 afternoon, is sufficient to result in a successful implantation within 12 hours. Since, there were not enough time intervals for compensatory ovarian hypertrophy (which could be thought of otherwise) between the time of ULO and the E<sub>2</sub> surge required for implantation. On the

other hand the amount of progesterone secreted from one ovary is sufficient to maintain the embryo for implantation. But on D9 when all experimental animals were killed (*i.e.*, normal, progesterone supplemented and non-supplemented) there was an insignificant difference between progesterone supplemented and non-supplemented ULO mice was observed. The number of implantation sites was greater in progesterone supplemented mice than that of progesterone non supplemented mice. It means that the rate of embryo regression is faster in progesterone non supplemented mice than that of progesterone supplemented mice. Hence, progesterone may help in the maintenance of embryo or protect the embryo from loss. Furthermore, the present study may reveal that the occurrence of normal implantations on the contra lateral horn of uterus of the ULO happens by a systemic rise of estrogen or estrogen affects the implantation not by ipsilateral way.

Thus, the present study hereby reveals that progesterone may help to protect the loss of embryo before and after the implantation. It also shows very clearly that if ovary is present in any side in the animal the E<sub>2</sub> secretes in circulation acts systematically on the uterus rather than locally. Thus we found implantation in the uterine horn where ovary is not there.

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