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Progesterone Administration after Ovarian Stimulation: Effects on Ovary Mast Cell Count and Histamine Level

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Abstract

Introduction: Induction of ovulation results in changes in ovary including the tissue immune cells. Administration of progesterone following induction of ovulation may ameliorate some of these changes. The present study was conducted to examine the effect of this administration of progesterone on mast cell count and tissue histamine level in mice ovary at pre-implantation time.

Materials and Methods: An experimental study on 15 NMRI mice was carried out. The groups of study were control group, induction of ovulation group and a group for administration of progesterone after induction of ovulation. Mast cells count was through toluidine blue staining and tissue histamine level measure was through spectrophotometry. Analysis of variance (ANOVA) was used for data analysis.

Results: The mean of mast cell count was significantly different between the groups (P <.001). Pairwise comparisons showed that induction group had significantly higher mast cells in comparison with control group (P =.039), indicating the effect of ovulation induction on mast cell count. The count was higher in progesterone group in comparison with induction group (P =.020). Mean of histamine level was significantly different between the groups (P <.001). Induction group had significantly higher histamine level in comparison with control group (P <.001). However, histamine level was not significantly different between induction and histamine groups (P =.998).

Conclusion: Ovulation induction raises ovary mast cell population and histamine level. However, progesterone could not improve this change. Further studies should be conducted to find further roles of mast cells and histamine in ovary.

Keywords: Mast cells, Histamine, Ovulation induction, Progesterone.

1. Introduction

Female infertility has a number of reasons which can be attributed to ovary or uterus[1]. From the ovary related reasons, unsuccessful ovulation can be pointed out. To resolve this problem, induction of ovulation is used with some medications such as anti-estrogens like clomiphene citrate [2]. Induction of ovulation is not complication-free. Therefore, progesterone may be used in order to reduce these complications and improve the outcome. Of the probable positive outcomes of using progesterone is improvement of angiogenesis[3]. Immune cells are affected by both ovulation induction and progesterone treatment. One group of the affected immune cells are mastocytes (mast cells). Mast cells are abundantly present in reproductive tract of males and females[4, 5]. It has been observed in male reproductive system

that mast cells and histamine improve tissue remodeling[6]. In general, histamine has a lot of effects on immune system consisting of increase in vessel permeability, change in T lymphocyte subtype equilibrium and some non-inflammatory regulations. The histamine effects are also dependent on their receptors[7]. About the role of histamine in fertility and pregnancy, some studies have been performed in the previous century [8-10].

All over the world, there are different reported statistics for prevalence of infertility based on different definitions [11]. According to a population based study in Iran (2015), about 17.3% of the Iranian couples face primary infertility during their life time [12]. In a meta-analysis, the world prevalence of infertility was calculated to be about 10% [13].According to the global importance of the issue, assisted reproduction technics (ART) such as *in vitro* fertilization (IVF) are commonplace. Nevertheless, these technics are mostly unsuccessful although advances have been made in this regard over the recent years [14].

As it was mentioned, mast cells are one of the groups of immune cells, playing an important role in fertility. Progesterone is one of the factors influencing mast cell population and histamine release. It has been observed that progesterone reduces histamine release from the mast cells [15]. However, some other studies show otherwise. It seems that sex hormones have influence on mast cell function. The reason behind this is expression hormone receptors on the surface of mast cells. Because of histamine release and having hormone receptors, mast cells are called as single cell glands [16]. A cell culture study has shown that progesterone results in reduction of mast cell proliferation [17].

So far, many studies have investigated the role of mast cells in women fertility and pregnancy. It has been determined that the mast cells of reproductive system have hormone receptors and act under the influence of hormonal changes. In spite of the existence of such evidence, some points are not clear. For instance, it is not clear what the role of progesterone is in mast cell population and their histamine release, and also it is not clear whether increase in mast cell population and their activity is beneficial or harmful for female reproductive system.

Given the doubts on the role of mast cells and histamine in female fertility, we decided to investigate the effects of progesterone administration followed by induction of ovulation on mast cell count and tissue histamine level in mice ovary at pre-implantation time.

2. Materials and Methods

An experimental study was conducted on fifteen 6-8 week NMRI mice. Under the standard condition, the mice were kept for two estrus cycles. Ethical guidelines of working with laboratory animals were regarded and the protocol of this project was approved in the ethics committee of Lorestan University of Medical Sciences with registration number IR.LUMS.REC.1400.067.

The animals were divided into three groups (n=5, for each) consisting of control group (control), induction of ovulation group (induction) and a group for administration of progesterone after induction of ovulation (progesterone). For the control group, the process of pseudo pregnancy was done by vaginal swabbing and after 3.5 days the animals were euthanized with cervical dislocation after general anesthesia. For induction group, induction of ovulation with a single dose of 10 IU human menopausal gonadotropin (hMG) hormone was injected intraperitoneal (IP) followed by a single dose of 10 IU human chorionic gonadotropin (hCG) hormone IP injection after 48 hours. After ovulation induction, pseudo pregnancy was done and after 3.5 days the animals were euthanized with cervical dislocation after general anesthesia. For progesterone group, in addition to the process of induction group, daily administration of 1 mg subcutaneous (SC) progesterone was performed up to 3.5 days. The mentioned protocols have been previously used by Narimani et al. [3].

As for tissue study, the right ovaries were separated and fixed in diluted formalin solution for about three days. After tissue processing, the samples were embedded in paraffin. Then 5 µm serial sections were prepared for staining. Hematoxylin and eosin (H&E) staining was used for morphological study and toluidine blue staining (Figure 1) was used for mast cell count.

To study tissue histamine level, the left ovaries were separated and after clearing fat and surrounding tissues, they were put at -70 ºC freezer until the time of histamine analysis. The samples were thawed and homogenized. Briefly, the samples were added to their four folds of their volume sodium chloride .85% in homogenizer. After that, the samples were passed through a filter. Then, the samples were centrifuged at 12000 rpm for 10 minutes. We used a cold reagent

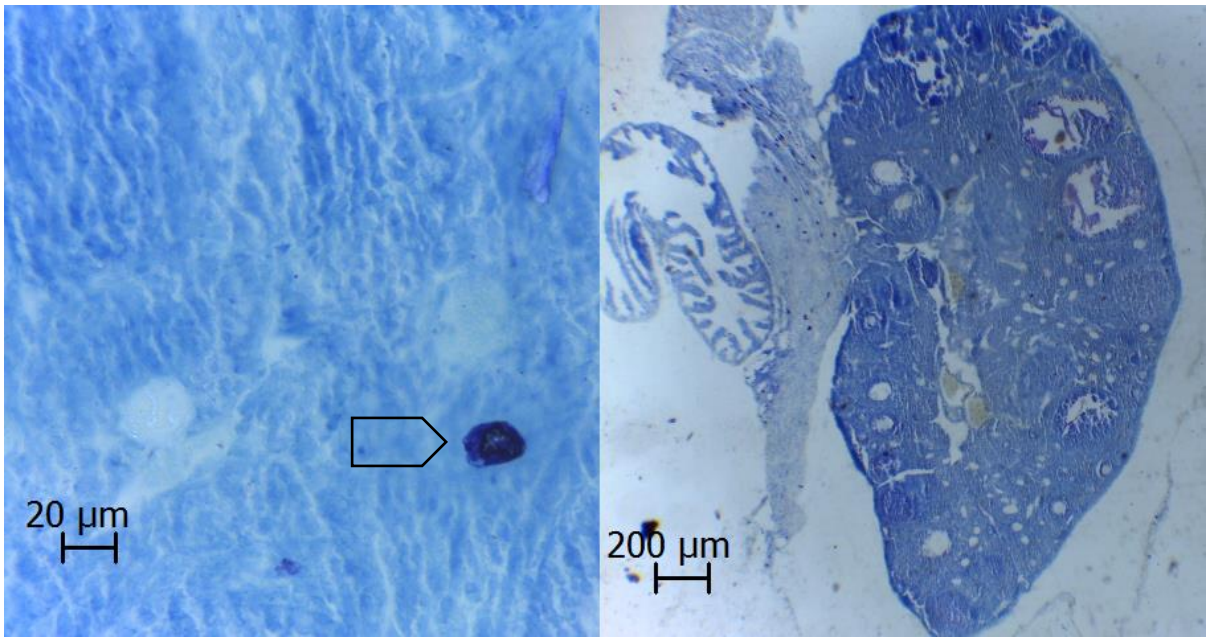

Figure 1. Toluidine blue staining of the ovary samples (a mast cell is shown at left by the magnification of 1000)

consisting of 1.5 ml of sulfanilic acid (.9% w/v) in 4% hydrochloric acid plus 1.5 ml of sodium nitrite 5% w/v poured into a 50 ml volumetric container and kept in an ice bath for 5 minutes. Six ml of 5% sodium nitrite was added and then cooled to 25 ml with cooled distilled water and was kept in an ice bath for 15 minutes. To measure the histamine level, 5 ml of sodium carbonate 1.1% was slowly mixed with 2 ml of the cold reagent and then 1 ml of the homogenized sample was added to it, and then after 5 minutes of absorption at 496 wavelength, it was read by spectrophotometer. Distilled water was used as a blank. As the standard, histamine was prepared from 0-100 µg / ml and measured. Finally, histamine level was reported with unit µg/g-tissue.

After collection of the data, one way analysis of variance (ANOVA) with Tukey post hoc test was used to compare the results between the groups at the significance level of .05 in SPSS 24 software (IBM, US).

3. Results

After qualitative and quantitative approval of the samples and the modeling of ovulation induction by H&E staining based on comparison of the number of antral follicles and corpora lutea (P <.05, for comparison between control and induction group), the mast cells were counted in toluidine blue slides at each transection of an ovary. Accordingly, the mean of mast cell count in the groups control, induction and progesterone were significantly different based on ANOVA (P <.001). Post hoc wise, induction group had significantly higher mast cells in comparison to control group (P =.039), showing the effect of ovulation induction on mast cell count. The count was higher in progesterone group in comparison with induction group (P =.020) (Table 1, Figure 2).

Table 1. Mast cell count in each group and the results of Tukey post hoc analysis

Group	Mean	Standard deviation	Compared with the group	P value
Control	9.00	1.58	Induction Progesterone	0.039 <0.001
Induction	11.46	1.02	Progesterone Control	0.020 0.039
Progesterone	14.25	2.19	Control Induction	<0.001 0.020

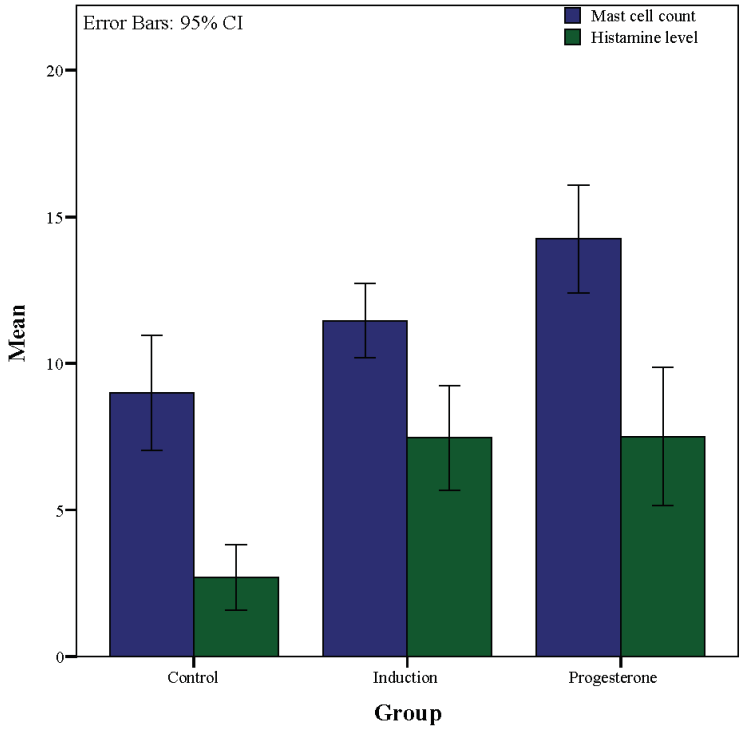

Figure 2. Comparison of mast cell count and tissue histamine level between the groups.

Table 2. Tissue histamine level in each group and the results of Tukey post hoc analysis.

Group	Mean	Standard deviation	Compared with the group	P value
Control	2.69	0.90	Induction	<0.001
			Progesterone	<0.001
Induction	7.46	1.44	Progesterone	0.998
			Control	<0.001
Progesterone	7.51	1.89	Control	<0.001
			Induction	0.998

Tissue histamine level was compared between the groups. The mean of histamine level in the groups control, induction and progesterone were significantly different based on ANOVA (P <.001). Post hoc wise, induction group had significantly higher histamine level in comparison with control group (P <.001), showing the effect of ovulation induction on tissue histamine level. However, histamine level was not significantly different between induction and histamine groups (P =.998) (Table 2, Figure 2).

4. Discussion

The present study was conducted to demonstrate what changes induction of ovulation brought about considering mast cell count and tissue histamine level of mice ovary, and then to find whether progesterone could improve these changes. For both outcomes (i.e. mast cell count and tissue histamine level), significant increase in induction group compared with the control group indicated both the effects of ovulation induction and successful modeling of the animals. In spite of increase in mast cell count in progesterone group in comparison with induction group, no significant difference was observed for histamine level regarding this comparison.

Investigation of histamine role in pregnancy has a rather old background. For instance, in 1962, Kameswaran et al. declared that placental histamine level in rat increased during mid-pregnancy resulting in better blood supply [8]. Nevertheless, some studies indicated negative roles for mast cells and histamine. For instance, Derbala et al. (2019) by studying 46 women with recurrent abortion showed that mast cells mediators were up-regulated [18]. Gensen et al. (2010) believed that mast cells had receptors for estradiol and progesterone, stimulation of which resulted in mast cell distraction and histamine release, and release of other factors such as vascular endothelial growth

factor (VEGF), protease and metalloproteinase. The protease and metalloproteinase destroy extra cellular matrix, and the VEGF modulates neovascularization [19].

In spite of a good history on the role of mast cells and histamine release in female reproduction and finding some beneficial and harmful mechanisms [20], there was no study directly investigating the mast cell population and histamine level in ovary tissue after induction of ovulation by hMG and hCG. Hence, conducting an animal study was necessary to complete this evidence gap according to the importance of induction ovulation in assisted reproduction. In the present study, we showed that induction of ovulation might affect mast cell population. However, as the limitations of our study, we could not show whether such increase in mast cell population was beneficial or harmful. In addition, we could not show any modulatory effect for progesterone administration.

5. Conclusion

Induction of ovulation results in increase of ovary mast cell population and histamine level. Progesterone administration increased mast population without increasing tissue histamine level. Briefly, progesterone could not ameliorate the change of mast cell population and histamine level. Further studies should be undertaken to find further roles of mast cells and histamine (beneficial or harmful) in ovary.

Ethical Considerations

Compliance with ethical guidelines

Ethical guidelines of working with laboratory animals were regarded and the protocol of this project was approved in the ethics committee of Lorestan University of Medical Sciences with registration number IR.LUMS.REC.1400.067.

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Author's contributions

NA: Data collection, interpretation and drafting. NBB: Data interpretation and critical revision. LN: Data collection and drafting. MBB: design and conceptualization, interpretation and critical revision. All the authors approved the final manuscript.

Conflict of interest

There is no conflict of interest.

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