Iranian Journal of Reproductive Medicine Vol.7. No.2. pp: 91-95, Spring 2009

Short communication

Effect of oxytocin injection on folliculogenesis, ovulation and endometrial growth in mice

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Received: 3 September 2008; accepted: 27 May 2009

Abstract

Background: Induction of ovulation in ART is necessary for superovulation and the side effects of superovulatory drugs are debated. Oxytocin as a natural hormone, have receptors and is synthesized by several reproductive organs. Preovulatory presence of oxytocin receptor mRNAs in granulosa cells indicating a role for oxytocin in follicular development.

Objective: The aim of the present study was to investigate the effect of exogenous oxytocin injection on folliculogenesis, ovulation and endometrial growth in mice.

Materials and Methods: Forty adult female mice were divided into two groups as control and experimental. The mice at their sterous cycle received 1 IU/gr oxytocin, in experimental, and the same volume of solvent in control groups. Half of the mice in each group are sacrificed at 24 hours post injection and the other half, 48 hours after the injection. Ovarian samples fixed in 10% formalin, embedded in paraffin and sections were stained with H and E and studied using stereological techniques. The data were analyzed with Man – Whitney test.

Results: Microscopic examination revealed that the number and morphological features of follicles at different stages were similar at 24 and 48 hours post injection in both groups. The volumes of the ovaries were similar in both groups at 24 hour. However, at 48 hour, the volume of ovaries, corpora lutea and endometrial thickness, in experimental group, were significantly higher than those in control group ($p< 0.05$).

Conclusion: According to the increased volume of corpus luteum in the experimental group, it is concluded that oxytocin injection has a stimulatory effect on induction of ovulation.

Key words: *Oxytocin, Folliculogenesis, Ovulation, Endometrium, Mouse.*

Introduction

Ovulation induction and acceleration of endometrial maturation are important factors in assisted reproductive technologies (ART). Using synthetic drugs for superovulation could result in hyperstimulation syndrome, production of atretic follicles and impairment of endometrial maturation which would result in imbalanced synchronization between endometrial maturation and blastocyst development and consequently implantation failure (1- 3). Therefore, a substance lacking such side effects would highly be preferred. It appears that oxytocin, a peptide hormone, mainly produced by neurohypophysis is involved in reproduction biology (4-7).

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Recent studies suggest that oxytocin is also synthesized in corpus luteum and endometrial epithelial cells. Oxytocin mRNA increases more than 150 times during gestation and at term (8). It is shown that this hormone has receptors on granulosa cells, cumulus cells, endometrial and luteal cells (8-11). Presence of oxytocin receptor mRNA in granulosa cells, before ovulation, indicate that oxytocin may involved in follicular development (12).

There are also evidences that oxytocin has a role on regulation of GnRH secretion, synthesis and release of LH (8, 13-16) and progesterone secretion (15,17). On the other hand, administration of oxytocin antagonist resulted in decreasing of LH concentration (17, 18). In spite of the above evidences that oxytocin is involved in reproduction, the role of this hormone on endometrial growth and maturation is not exactly known. Furthermore, its effect on follicular development and ovulation is controversial (19, 20). That is, continuous injection of oxytocin to heifers, for 12 days (1.9 mg/d), did not change the number of growing follicles and had no effect on the size of the ovulating follicles (19). On the other hand, slow-releasing injection of 10 IU oxytocin to Marino ewes showed that after 10 days of oxytocin treatment, the ovulation rate was higher in oxytocin –treated group (20). It is obvious that finding of a role for oxytocin in these basic steps of pregnancy would provide a chance for its application in ART. The aim of the present study was to investigate the effect of exogenous oxytocin injection on folliculogenesis, ovulation and endometrial growth.

Materials and methods

In this study, 40 female balb/c mice, 2.5-3 months old with 25-30 gr weight were used. The mice were obtained from Tabriz University of Medical Sciences' animal-house and kept in an air conditioned room with a 12h light/12h dark cycle. All animals' procedures complied with an approved Tabriz University of Medical Sciences' animal care and use committee.

The mice were cocycled and used at proestrus phase, which was determined based on cytological morphology and proportion in vaginal smear (19). Then, they were divided into two groups of control and experimental. The mice in experimental group were received 10 mlU/gr oxytocin as IP injection according to Robinson and Evans (13). Oxytocin was purchased from Menoro Pharmaceutical Co. (Tehran, Iran) as 1ml vial and diluted in 1.5 ml distilled water. The mice, in control group, received the same volume of solvent. Ten out of 20 mice, in experimental group, were sacrificed 24 hours post-injection and the rest at 48 hours after the injection. According to estrous cycle in mice, the 24 and 48 hours was regarded as equivalent to follicular and luteal phases respectively.

A similar number of control mice were sacrificed accordingly. The mice were anesthetized with chloroform, and 1-1.5 ml blood was obtained from the heart for biochemical analysis. Ovaries and uteri were removed and fixed in 10% formalin, and embedded in paraffin. Ovarian specimens were sectioned serially with 50µm intervals and 5µm thick sections of ovary and uterine were stained with H and E and studied with light microscope. The 50µm interval was chosen based on the size of corpora lutea and graffian follicles. The follicular and luteal phases were reconfirmed according to uterine endometrial morphology.

Stereological studies

Ovarian volume from control and experimental groups were calculated as mm3 using point grid counting in serial sections according to $v=T\times a/f \times \sum p$. Where, v=volume, T=distance between sections, a/f =area/field and $\sum p$=the number of points hitting the image. In the sections, from follicular phase, the number of ovarian follicles was counted in 10 sections from each specimen and the total number of follicles in an ovary was calculated according to the following equation:

$$N(v) = \frac{\sum Q}{a/f \times h}$$

Where, $\sum Q$ = number of follicles, h=distance between selected sections, and a/f=area/field. For counting of follicular number, in Motic software, the photographs of sections were transferred to monitor and counting was carried out on the monitor.

In the sections, from luteal phase, the volume of ovaries and volume of corpora lutea were calculated in a similar manner. For determination of endometrial thickness the least and maximum thickness of endometrium was measured in each specimen and the mean values was calculated for each group. In the collected blood, the sera were separated by centrifugation and concentration of LH and estradiol was determined by radioimmunoassay technique. The collected data were compared between control and experimental groups.

Statistical analysis

The data were analyzed using Mann Whitney test and $p<0.05$ level was considered significant.

Results

Histological study of ovarian sections from both control and experimental groups revealed that the quality of follicles, including; oocytes, granulosa cells, and thecal layers were similar. Based on morphometric studies, the number of follicles at different stages of development is summarized in table I. As it is shown in the table, there are no differences between control and experimental groups regarding the number of primordial, primary, growing, secondary, and antral (including graffian follicles) and the volume of ovary on follicular phase. However, on luteal phase, as indicated in table II, the volume of corpora lutea and ovaries and endometrial thickness were increased significantly ($p<0.05$) in experimental group in comparison to control group. Histological studies are shown in figures 1 and 2. As it is shown in the figures, the ovary in experimental group (Figure 1) is larger than the ovary in control group (Figure 2). Furthermore, the corpora lutea is also very extensive in experimental group compared with the control group. Morphometric determination of endometrial thickness revealed that endometrium, in experimental group was significantly thicker than the endometrium in the control group ($p<0.03$). Concentrations of estradiol and LH are shown in tables III at 24 and 48 hours after oxytocin injection. As it is indicated in table III, the concentration of estradiol, in control group, from 24 hour to 48 hour decreased ($p<0.04$). On the contrary , in experimental group , its concentration at 48 hour in relevance to 24 hour increased significantly ($p<0.04$). However, the changes of LH from 24 hour to 48 hours were similar in both groups and did not differ significantly.

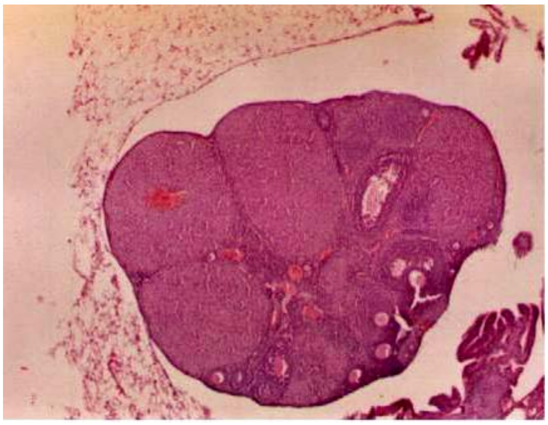

Figure 1. Ovarian section from a control mouse. Note several corpora lutea in the section. H & E staining, 50X.

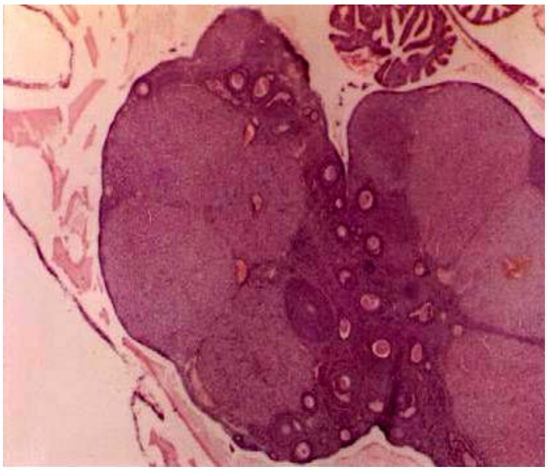

Figure 2. Ovarian section from an experimental mouse. Note size of the ovary and extensive corpora lutea . H & E staining, 50X.

Table I. Number of different follicles and volume of ovary (mm^3) in control and experimental groups at follicular phase.

Follicular phase	Experimental	Control	p-value
Primordial follicle	65.31 ± 5.3	78.12± 4.68	0.66
Primary follicle	166.66 ± 20.83	144.53±30.07	0.41
Growing primary follicle	1453.57 ± 206.78	1890.62±281.06	0.47
Secondary follicle	366.007±57.74	365.62±51.05	0.96
Antral follicle	98.75±22.46	112.81±34.81	0.23
Ovarian volume	83.55±1161(mm^3)	91.04±101.95(mm^3)	0.81

$p< 0.05$ is significant .

Table II. Volume of ovaries and corpora lutea (mm^3) and endometrial thickness (μm) in control and experimental group at luteal phase.

Organ	Experimental	Control	p-value
Endometrial thickness	1085.43±27.73	438.41±22.83	0.03
Volume of ovary	162.54±3.21	84.37±1.06	0.001
Volume of corpus luteum	53.32±1.01	28.25±0.84	0.001

$p<0.05$ is significant.

Table III. Blood serum concentrations of LH and estradiol (IU/L) in experimental and control groups at 24 and 48 hours after the injection of oxytocin.

Parameters (group)	24 hours post injection	48 hours post injection	p-value
LH (Experimental)	6.61±2.82	8.25±4.5	NS
Estradiol (Experimental)	5.7±0.23	7.54±0.65	0.04
LH (Control)	8.74±1. 47	9.76±3.07	NS
Estradiol (Control)	6.84±0.81	6.01±0.68	0.04

p<0.05 = significant NS = not significant

Discussion

The aim of the present study was to investigate the effect of oxytocin on folliculogenesis, ovulation and endometrial thickness. Our results showed that at follicular phase, the number of follicles at different stages of development did not differ between control and experimental groups. This finding indicates that oxytocin injection has not a significant effect on the number of follicules. In agreement with our findings, it is shown that injection of oxytocin in rat (22) and calf (19) have no effect on folliculogenesis or size of the follicules.

However, unlike superovulatory drugs, that could affect oocyte maturation and consequently pregnancy rate (23, 24), oxytocin has no adverse effect on oocyte maturation (25). The fact that, at follicular phase, ovarian volume in experimental group in comparison to control group, did not change, is another confirmation for the above mentioned results (Table I). On the other hand, in luteal phase, after oxytocin injection, the volume of ovaries and corpora lutea increased significantly ($p<0.05$).

It means that oxytocin did stimulate ovulation and resulted in increasing of corpora lutea and ovarian volume. In support of our findings, there are several reports suggesting that oxytocin may have a role on ovulation (26-29).

Our findings in luteal phase, together with the results from follicular phase, means that stimulatory effect of oxytocin on ovulation is a gradual effect rather than a sudden effect. In agreement with our hypothesis, Tallam and Walton (2000) have shown that continuous infusion of oxytocin, in heifers, delays luteal regression without inhibiting follicular development (19). Increasing of endometrial thickness in oxytocin injected animals was another finding of the present study.

This finding, well correlates with stimulatory effect of oxytocin on ovulation, i.e. oxytocin injection increased ovulation and consequently the formation of corpora lutea is increased and resulted in an elevation of progesterone secretion. The luteinizing effect of progesterone on endometrium is well known (11). The above mentioned morphological changes well

correlated with hormonal changes in our experiment. Hormonal assay in the present study showed that in control group, estradiol concentration decreases from 24 hour to 48 hour post injection, which is an indication of stop of folliculogenesis. While, in experimental group the concentration of estradiol increased at the same time interval, meaning that follicular growth continued or the period of folliculogenesis is prolonged.

The effect of oxytocin on steroidogenesis has already been shown. Progesterone secretion is stimulated by oxytocin in cultured granulosa cells isolated from preovulatory follicles (30). Furthermore, it is shown that oxytocin regulates progesterone release in human granulosa-lutein cells in culture medium (31). However, the changes of LH concentration, in control and experimental groups, were similar. Since the commonly used superovulatory drugs cause a sudden increase in folliculogenesis and formation of numerous graffian follicles, it increases the chance for hyperstimulation syndrome (1). The main point of our finding is that, oxytocin, by acting in a gradual manner would not result in hyperstimulation syndrome.

Conclusion

Taking together, the stimulatory effects of oxytocin on esteroidogenesis, ovulation and endometrial luteinization, it is concluded that oxytocin promotes several different mechanisms on reproductive system which go together and keep pace. In this sense, it is not similar to superovulatory drugs that act isolately on a single event.

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