

Ovarian Proliferation in mature female rats passively immunized against inhibin alpha subunit

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Abstract

Inhibin is a non-steroidal glycoprotein hormone of gonadal origin with major action as negative feedback control of the production of Follicle Stimulating Hormone by anterior pituitary gland which in turn modulates female reproductive functions. The present study has been undertaken on mature female rats to examine the potent of passive immunization against inhibin- α subunit in induction and progress in proliferation of ovarian follicles. Sixteen mature female rats (aged 50 day and weighted 190 ± 5 g) were randomly assigned into two equal groups: control and treated groups were intraperitoneally injected with 100 μ l of normal saline and 1 μ g of inhibin antiserum (dissolved in 100 μ l of normal saline), respectively, on the 55th, 56th, 57th, 58th, and 63rd days of age. At the second proestrus, female rats were anesthetized, dissected and ovaries were obtained, weighted and fixed in formalin 10% for histophysiological study. Ovarian of treated group registered significant weight compared with control. Histological findings showed significant increment of primary, Graffian and total numbers of follicle in treated group, whereas secondary follicles number recorded insignificant differences between the two groups. Ovarian sections of treated female rats revealed higher level of proliferation in the follicular tissue and predominance of Graffian follicles compared with control ovaries that revealed lower proliferation and predominance of primary and secondary follicles. It is concluded that passive immunization against inhibin alpha has potent role in induction of follicular growth and development in mature female rats.

Key words: Passive immunization, Inhibin, Ovarian proliferation.

Introduction

Inhibin is a member of the transforming growth factor β (TGF β) superfamily, a group of structurally similar but functionally diverse growth factors, and it shares several structural features with this family. Researches suggests that inhibin is a main regulator of follicle stimulating hormone (FSH) synthesis and that it exerts paracrine and autocrine effects on gonadal and extragonadal tissues (Kumanov *et al.*, 2005).

The most common method for induction of superovulation is treatment with a combination of equine chorionic gonadotropin (eCG) and human chorionic gonadotropin (hCG). However, several studies have reported high rates of pre- and post- implantation losses in animals

superovulated with eCG (Leveille and Armstrong, 1989). And there are other problems related with inducing superovulation by using these hormones like: early developmental failure, which is thought to be a result of excessive follicular stimulation because of the inherent long biological half- life of eCG. High doses of eCG may have a detrimental effect on oocyte quality, such as the ability to be fertilized (Kandiel *et al.*, 2008).

Therefore, many researchers tried to find an alternative method for producing large numbers of viable oocytes and zygotes in adult rats and they succeeded to induce superovulation in many species without occurrence of any of previous problems (Ishigame *et al.*, 2004).

Immunization against inhibin has originally been used to study the physiological role of inhibin. Subsequently, immunoneutralization against endogenous inhibin is considered as a new paradigm to enhance gamete production and fertility. It has been used by many researchers for induction of superovulation as a substitution for eCG-hCG method in adult cycling rats (Rivier and Vale, 1989; Ishigame *et al.*, 2004) and mice (Medan *et al.*, 2004). Passive immunization against α -inhibin antigens has also been used to increase sperm production in many live stock species including rams (Voge and Wheaton, 2007) and bulls (Martin *et al.*, 1991). Immunoneutralization against inhibin α -subunit has been shown to enhance oocyte development (Ishigame *et al.*, 2005) and puberty in immature female rats (Al-Sa'aidi and Samir, 2010; Samir, 2010). The present study aimed to enhance superovulation using passive immunization against inhibin alpha subunit as alternative method instead of hormonal treatment.

Materials and Methods

Preparation of inhibin antiserum: Inhibin antiserum has been prepared according to the manufacture instruction (ABO, Switzerland) by dissolving 1 μ g of lyophilized inhibin antiserum in 100 μ l of normal saline.

Vaginal Smear: The purpose of making vaginal smear was to determine the rats that have different histological changes during 4 sequential days, and only those rats were included in the experiments.

Preparation and staining of histological sections: has been done according to Edne and Lestie (1991).

Experimental animals: Fifty days old female Wister rats (weight was 190 ± 5 g.), were reared under controlled conditions (12L:12D cycles at 22-24 C°) and fed standard laboratory food (19% protein ratio and 3000 kilocalories energy) and drinking water *ad libitum*. On the 55th day of age the animals have been treated as follow:

- 1) **Control (C):** Eight mature female rats were injected with normal saline (100 μ l *ip*) on the 55th, 56th, 57th, 58th and 63rd days of age.
- 2) **Treatment (T):** Eight mature female rats were injected with 1 μ g of inhibin- α antiserum (*ip*) (dissolved in 100 μ l of normal saline) on the 55th, 56th, 57th, 58th and 63rd days of age.

After the last injection, animals were monitored for estrus synchronization (Al-Sa'aidi, unpublished data), daily vaginal smears for females were performed for 4-5 consecutive days to detect the phases of estrus cycle. At second proestrus, female rats were anesthetized (by injection of 0.4 ml of thiopental sod./ animal), dissected and ovaries were obtained, weighed and kept in formalin 10% for histophysiological study.

Statistical Analysis: All data were expressed as mean $\pm$ SE.. Analysis of data was statistically performed by using the student *t* test to determine the statistical differences between mean values. P-value (0.01) was considered significantly different (Scheffler, 1980).

Results and discussion

Ovarian weight

The results clarified in Figure (1) showed that passive immunization against inhibin α of mature female rats has been caused significant ($P < 0.01$) increase in ovarian weight (0.0484 ± 0.005 g/100g bw) compared with that of control female rats (0.0395 ± 0.004 g/100g bw).

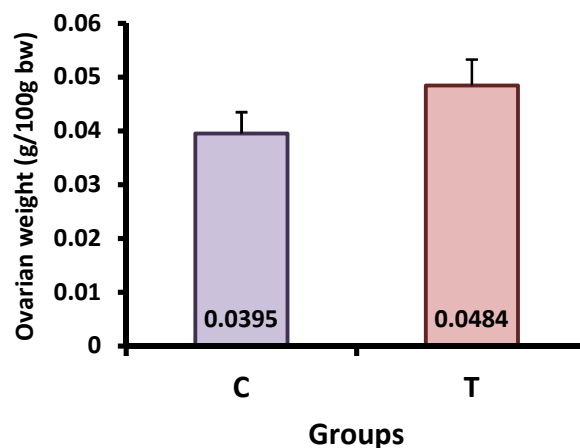


Fig. (1): Effect of passive immunization against inhibin- α on ovarian weight (mg/ 100g BW) in mature female rats.

Number of ovarian follicles

Results revealed significant differences ($P < 0.01$) in the mean number of primary and Graffian follicles in the ovaries of mature female rats between experimental groups. No significant difference was observed in the mean number of secondary follicles (Figure 2).

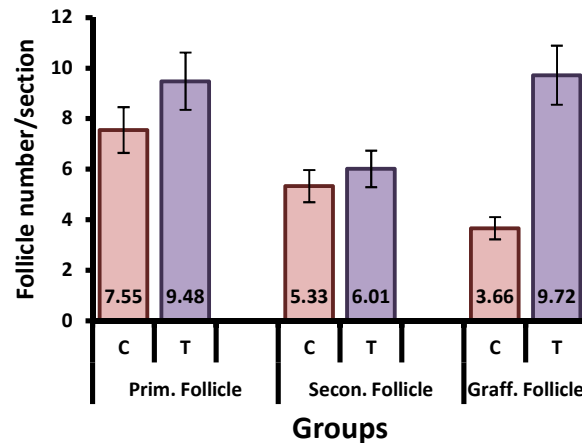


Fig. (2): Effect of passive immunization against inhibin- α on the number of primary, secondary, and Graafian follicles in the ovaries of mature female rats.

Histological Observations

Histological sections obtained from ovaries of treated female rats (figure 3B) revealed progressed ovarian tissue proliferation compared with control (figure 3A) particularly in number and size of follicles. Other sections from treated female rats showed that the predominant type was the Graafian follicles that contain oocytes and supporting tissue, as well as multi-cellular layer of proliferated granulosa cells and antrum filled with fluid, while ovaries from control animals revealed lower number of Graafian follicles. In spite, the predominant types of follicles were primary and secondary. The secondary or growing follicles showed little proliferative cells in their lining.

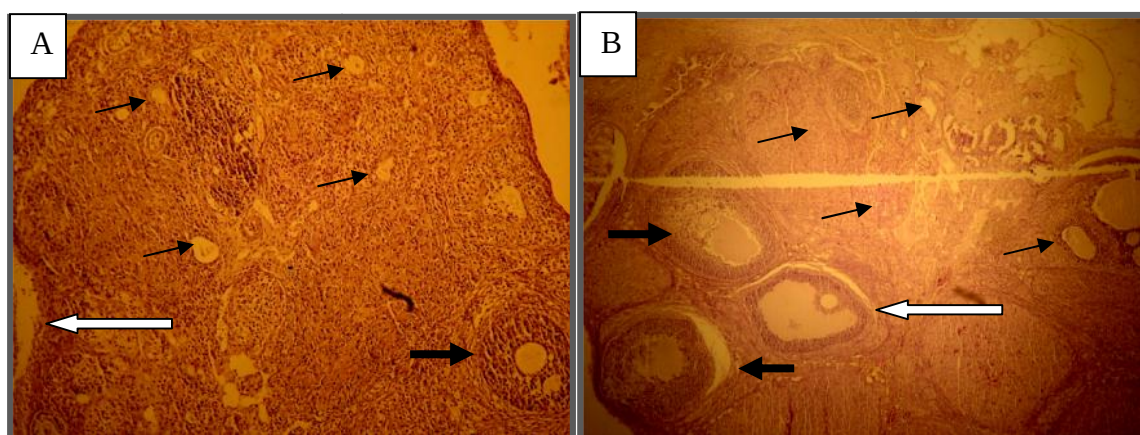


Fig. (3): sections from ovaries of treated (B) and control (A) mature female rats showed the proliferative difference. Primary follicle (light black arrow), secondary follicle (thick black arrow), and mature follicle (white arrow). H&E 20x.

Discussion

Results of the present study demonstrated significantly higher mean value of ovarian weight in mature female Wistar rats passively immunized against inhibin α subunit which might be attributed to the increment of follicular proliferation by gonadotropic hormones released from anterior pituitary gland, especially FSH, as the number of follicles that develop to ovulatory size depends on both the amount of FSH and the time of exposure to FSH (Campbell *et al.*, 1990; McNeilly *et al.*, 1991). The hypersecretion of endogenous FSH following immunoneutralization of inhibin α stimulated the development of large numbers of follicles (Medan *et al.*, 2006). This finding might be supported by other results of the present study concerning with the increased number of growing follicles. During folliculogenesis, the surrounding granulosa cells proliferate to become several layers thickness, and secrete a lot mass of glycoprotein substances that form a layer called the zona pellucida between themselves and the oocytes. Cells of stroma condense on the membrane propria to form the theca cells layer, which is vascularized. FSH and LH receptors are also developed (Quirk *et al.*, 2004). This agrees with the result of ovarian weight in the present study which accompanied by a significant increase in serum concentration of FSH as well as the striking changes in ovarian follicular populations. The present result was in agreement with that recorded on immature female rats by Al-Sa'aidi and Samir (2010).

Since immunoneutralization of endogenous inhibin, in the present study, led to neutralize biologically active inhibin in rats, serum concentration of FSH will increase, as mentioned by other investigatnrs (Medan *et al.*, 2007). In addition, FSH increment due to passive immunization against inhibin α could remains at high level throughout the experimental period, and this elevation induced folliculogenesis in the ovaries. As previously mentioned that, on long term basis, inhibin immunization may be stimulates ovarian function by interfering with the inhibin– dependent modulation of follicular development at the ovarian level (Campbell *et al.*, 1995). These results clearly indicate that high follicular proliferation due to passive immunization against inhibin α subunit, could enhances biosynthesis of estrogens from ovarian follicles. In female ovulatory cycle, FSH is responsible for inducing follicular growth, while also increasing estrogen production. It means that increased estrogen level strongly support the indirect role of inhibin as a selective suppressor of FSH. The increment of reproductive hormones over control animals through passive immunization against inhibin α subunit mainly attribute to the immunoneutralization of endogenous inhibin in mature female rats which in turn, primarily increased serum concentration of FSH. These findings suggest that high level of endogenous FSH stimulate the wave of follicular

development and results in production of large amount of estrogens, which induces the LH surge by positive feedback effect to the hypothalamus and pituitary axis and this surge may leads to induction of superovulation.

The present study clearly proved that the endocrine function of inhibin act as a major suppressor of FSH secretion and ovarian follicular development. Passive immunization of mature Wistar rats on days 55, 56, 57, 58, and 63 resulted in a striking changes in ovarian follicular populations.

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