Ovarian follicular growth in mature female rats immunized against inhibin

S. H. Jari
College of Veterinary Medicine\ Al-Qadisiya University

Abstract

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 growth 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 intraperitonally injected with 100 µl of normal saline and 1µg of inhibin antiserum (dissolved in 100 µl of normal saline), respectively, on the 55th, 56th, 57th, 58th and 63rd days of age. At the second proestrus, dissected and ovaries were removed , weighted and fixed in formalin 10% for histopathological ovaries of treated group showed significant increased in weight as compared with control group. Histological findings showed significant increment of primary, Graffian and total numbers of follicle in treated group, whereas secondary follicles numbers were 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 as compared with control ovaries that revealed lower proliferation and predominance of primary and secondary follicles.It was concluded that passive immunization against inhibin alpha hasapotentrole in induction of follicular growth and development in mature female rats.

النمو الجريبي لمبايض إناث الجرذان الممنعة ضد الانهبين ألفا

سالم حسين جاري
كلية الطب البيطري/ جامعة القادسية

الخلاصة

أجريت الدراسة الحالية على إناث الجرذان الناضجة بهدف تقييم فعالية التمنيع الميسر ضد الانهبين الفا في حث ونمو وتطور الجريبات المبيضية. تم تقسيم 16 أنثى عشوائياً على مجموعتين متساويتين (السيطرة والمعاملة) وسجلت أوزانها الابتدائية. حقنت إناث السيطرة بالمحلول الملحي الفسلجي (100 مايكروليتر في البريتون) وحقنت إناث المعاملة بالمصل المضاد للانهبين (Inhibin antiserum) (بجرعة 1 مايكروغرام مذاب في 100 مايكرولتير من المحلول الملحي الفسلجي في البريتون) في الأيام 55، 56، 57، 58، 63 من عمر حيوانات التجربة. بعد الحقن مباشرة، تم وضع الحيوانات تحت المشاهدة لتوحيد الشبق، تم جمع وفحص المسحات المهبلية لتحديد مراحل الشبق ولمدة أربعة أيام متتالية. في مرحلة ما قبل الشبق للدورة التالية، تم تخدير الإناث وشرحت وأخذت منها نماذج المبايض وسجلت أوزانها وأخذت منها نماذج حفظت في الفورمالين 10% لغرض إجراء الدراسة النسيجية عليها. أظهرت النتائج تقارباً في معدلات أوزان الجسم ومعدل الكسب الوزني بين مجموعتي الدراسة. أما أوزان المبايض فقد سجلت ارتفاعاً معنوياً في إناث مجموعة المعاملة عند مقارنتها مع السيطرة. من جهة أخرى أظهرت نتائج الدراسة النسيجية وجود زيادة معنوية في إعداد الجريبات الابتدائية وجريبات كراف وجريبات الكلية جراء حقن المصل المضاد للانهبين الفا في إناث المعاملة مقارنه مع السيطرة في حين لم تسجل أعداد الجريبات الثانوية أي فرق إحصائي بين المجموعتي كما بينت المقاطع

النسيجية المأخوذة من مبايض إناث المعاملة نمو أعلى في الأنسجة الجريبية مع زيادة إعداد جريبات كراف بالمقارنة مع المقاطع المأخوذة من إناث السيطرة التي أظهرت إعدادا اقل من جريبات كراف مع زيادة الجربيات الابتدائية والثانوية في المقاطع. يستنتج من نتائج الدراسة الحالية أن للتمنيع الميسر باستخدام المصل المضاد للانهبين تأثيراً فعالاً في تحفيز ونمو وتطور الجريبات المبيضية عند حقنه في الأيام34، 55، 58 من عمر جرذان الوستر الناضجة.

Introduction

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. Inhibin is a member of the transforming growth factor β (TGFß) superfamily, a group of structurally similar but functionally diverse growth factors, and it shares several structural features with this family. It has been suggested by many researcharsinhibin is a main regulator of follicle stimulating hormone (FSH) synthesis and that it exerts paracrine andautocrine effects on gonadal and extragonadal tissues (1). 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 (2). 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 (3). 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 problems (4). 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 (4,5) and mice (6). Passive immunization against α-inhibin antigens has also been used to increase sperm production in many live stock species including rams (7) and bulls (8). Immunoneutralization against inhibin α-subunit has been shown to enhance oocyte development (9) and puberty in immature female rats (10, 11). 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 1µg of lyophilized inhibin antiserum in 100µl 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 Edna, B. and Lestie, H. (12).

- **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 µl*ip*) on the 55th, 56th, 57th, 58th and 63rd days of age.
2) **Treatment (T)**: Eight mature female rats were injected with 1 µg of inhibin- α antiserum (*ip*) (dissolved in 100 µl 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, weighted and kept in formalin 10% for histophysiological study.

Statistical Analysis: All data were expressed as mean ± SE. Analysis of data wasstatistically performed by using the student *t* test to determine the statistical differences between mean values. P-value (0.01) was considered significantly different (13).

Results

- **Ovarian weight:** The results clarified in Fig. (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) as compared with that of control female rats (0.0395 ± 0.004 g/100g bw).

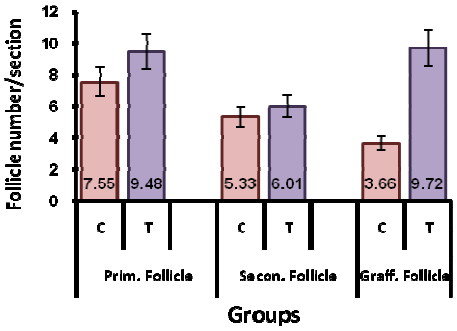

Fig. (1) Effect of passive immunization against inhibin- α on ovarian weight (mg/ 100g BW) in mature female rats

- **Number of ovarian follicles:** Results revealed significant increase (P<0.01) in the mean number of primaryandGraffian follicles in the ovaries of mature female rats Passively immunized aganestinhibin α. Compared with control. No significant difference was observed in the mean number of secondary follicles (Fig. 2).

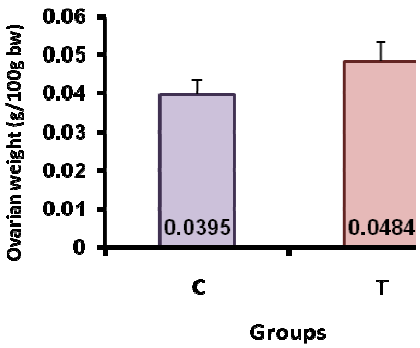

Fig. (2) Effect of passive immunization against inhibin- α on the number of primary, secondary, and Graffian follicles in the ovaries of mature female rats.

Histological Observations

Histological sections obtained from ovaries of treated female rats (Fig. 4) revealed progressed ovarian tissue proliferation compared with control (Fig. 3) particularly in number and size of follicles. Other sections from treated female rats showed that the predominant type was the Graffian follicles that contain oocytes and supporting tissue, as well as multi-cellular layer of proliferated granulosa cells and antrum filled with fluid, while predominant types of follicles were primary and secondary. The secondary or growing follicles showed little proliferative cells in their lining.

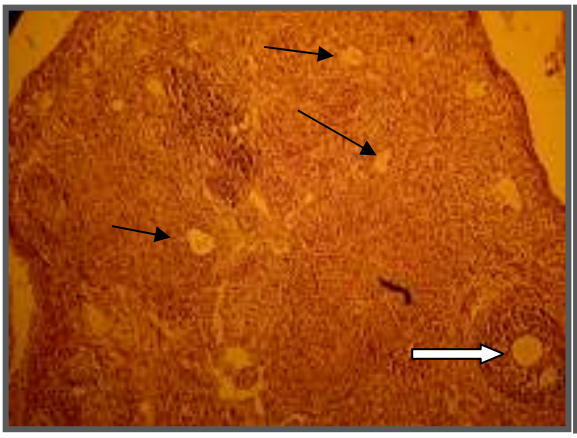

Fig. (3) Ovary. As control group There is several immature follicles: primary follicles (thin arrows) and secondary follicles (thick arrows)

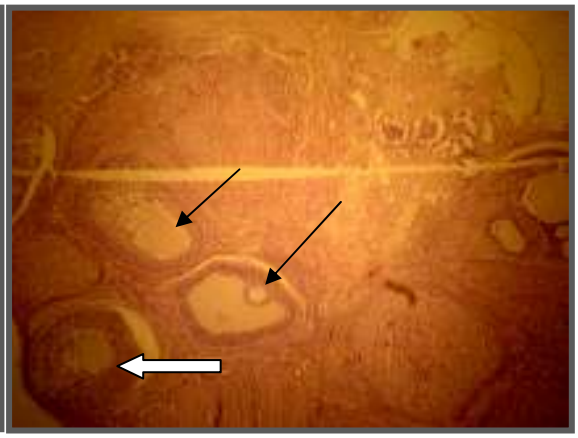

Fig. (4) Ovary. Treated group. There is several immature follicles: primary follicles and secondary follicles (thick arrow) and presence of mature graffian follicles (thin arrows)

Fig. (5) Ovary. One mature (vesicular) follicle which characterized by secondary oocyte (head of arrow), corona radiate (thick arrow), and theca interna (arrow) and theca externa (double arrow). 200× H&E

Fig. (6) Uterus. There is normal columnar epithelium with much, large (arrows) and hyperplasic uterine glands (double arrow). 20× H&E

Discussion

Results of the present study Showed significantly higher mean value of ovarian weight in mature female Wistar rats passively immunized against inhibin α subunitThis might be attributed to the increment of follicular growth 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 (14,15). The hypersecretion of endogenous FSH following immunoneutralization of inhibin α stimulated the development of large numbers of follicles (16). This finding might be supported by other results concerning with the increased number of growing

follicles. During folliculogensis, the surrounding granulosa cells proliferate to a several layers thickness, and secrete a lot of glycoprotein substances that form a layer called the zonapellucida 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 (17). This agrees with the result of ovarian weight which accompanied by a significant increase in serum concentration of FSH as well as the striking changes in ovarian follicular populations.Our result was in agreement with that recorded on immature female rats by Al-Sa'aidi and Samir (10). Since immunoneutralization of endogenous inhibin, in, led to neutralize biologically active inhibin in rats, serum concentration of FSH will increase, as mentioned by other investigatnrs (18). In addition, FSH increment due to passive immunization against inhibinα couldremains at high level throughout the experimental period, and this elevation induced folliculogenesis in the ovaries (15). 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 (19). These results clearly indicate that high follicular growth due to passive immunization against inhibinα subunit, could enhances biosynthesis of estrogens from ovarian follicles (16). In female ovulatory cycle, FSH is responsible for inducing follicular growth, while also (16). 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 inhibinα subunit mainly attribute to the immunoneutralization of endogenous inhibin in mature female rats which in turn, primarily increased serum concentration of FSH (15). 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 (9). It was concluded from this study 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.

References

1. Kumanov, P.; Kalyana, C.; Tomova, A.; Robeva, R. & Agarwal, A. 2005. Significance of inhibin in reproductive pathophysiology and current clinical applications. Rev.; RBM Online- Vol. 10. No. 6. 786-796. Reproductive BioMedicine Online: www.rbmonline.com/Article/1704
2. Leveille, M. & Armstrong, D. 1989. Pre-implantation embryo development and serum steroid levels in immature rats induced to ovulate or superovulate with pregnant mares serum gonadotropins injection or follicle stimulating hormone infusions. Gamete Res., 23: 127-138.
3. Kandiel, M. M. M.; Watanabe, G.; Li, J. Y.; Manabe, N.; El Azab, A. E. I. & Taya, K. 2008. Physiological roles of inhibin in regulation of FSH secretion and follicular development during early pregnancy in goats. Dom. Anim. Endocrinol., 35: 157-163.
4. Ishigame, H.; Medan, M. S.; Watanabe, G.; Shi, Z.; Kishi, H.; Arai, K. Y. & Taya, K. 2004. A new alternative method for superovulation using passive immunization against inhibin in adult rats. Biol. Reprod., 71: 236-243.

5. Rivier, C. & Vale, W. 1989. Immunoneutralization of endogenous inhibin modifies hormone secretion and ovulation rate in rat. Endocrinol., 125: 152-157.
6. Medan, M. S.; Wang, H.; Watanabe, G.; Suzuki, A. K. & Taya, K. 2004. Immunization against inhibin increase normal Oocyte/ embryo production in adult mice. Endocrinol. 24: 115-119.
7. Voge, J. L. & Wheaton, J. E. 2007 Effects of immunization against two inhibin antigens on daily sperm production and hormone concentrations in ram lambs. J. Anim. Sci., 1-24.
8. Martin, T. L.; Williams, G. L.; Lunstra, D. D. & Ireland, J. J. 1991. Immunoneutralization of inhibin modifies hormone secretion and sperm production in bulls. Biol. Reprod., 45: 73-77.
9. Ishigma, H.; Medan, M. S.; Wang, H.; Watanab, G.; Kishi, H. & Arai, K. Y. 2005. Induction of superovulation by immunoneutralization of endogenous inhibin in immature rats. J. Reprod. Dev., 51: 559-566.
10. Al-Sa'aidi, J. A. A. & Samir, M. S. 2010. Effect of passive immunization against inhibin alpha subunit on ovarian growth and development in immature female Wister rats. 14th Scientific Congress of Fac. Med., Assiut Univ., Egypt.
11. Samir, M. S. 2010. Effect of passive immunization against inhibin-alpha subunit on puberty of immature female Wister rats. M.Sc. thesis, College of Vet. Med., Al-Qadisiya Univ., Iraq.
12. Edna, B. & Lestie, H. 1991 Laboratory Methods in Histotechnology. Washington, D. C. American Registry of Pathology.
13. Schefler, W. C. 1980. Statistics for the Biological Sciences. 2nd ed., Addison-Wesley.
14. Campbell, B. K.; Mann, G. E.; McNeilly, A. S. & Baird, D. T. 1990. The pattern of ovarian inhibin estradiol and androstenedione secretion during the estrous cycle in the ewe. Endocrinol., 127:227-235.
15. McNeilly, A. S.; Crow, W. & Campbell, B. K. 1991. Effect of follicular fluid and inhibinimmunoneutralization on FSH-induced preovulatory follicle growth in the ewe. J. Endocrinol., 1991: 31: 401-409.
16. Medan, M. S.; Takedomi, T.; Aoyagi, Y.; Konishi, M.; Yazawa, S.; Watanabe, G. & Taya, K. 2006. the effect of Active immunization against inhibin on gonadotropins secretions and follicular dynamics during the estrous cycle in cows. J. Reprod. Dev., 52: 107-113.
17. Quirk, S. M.; Cowan, R. G.; Harman, C. L. & Porter, D. A. 2004. Ovarian follicular growth and atresia: the relationship between cell proliferation and survival. J. Anim. Sci., 82 (E.suppl.): E40-E52.
18. Medan, M. S.; Arai, K. Y.; Watanabe, G. & Taya, K. 2007. Inhibin, Regulation of reproductive function and practical use in animals. J. Anim. Sci., 78:16-27.
19. Campbell, B. K.; Gordon, B. M.; Tsonis, C. G. & Scaramuzzi, R. J. 1995. The effect of acute immuno-neutralisation of inhibin in ewes during the early luteal phase of the oestrous cycle on ovarian hormone secretion and follicular development. J. Endocrinol., 145: 479-490