OVARIAN AND UTERINE WEIGHT CHANGES IN ASSOCIATION WITH ESTROUS PHASES OF CYCLIC FEMALE WISTAR RATS TREATED WITH ANTI-SERA AGAINST BOVINE FOLLICULAR FLUID (STEROID-FREE)

Salim Hussein Jari

Department of physiology, Biochemistry and pharmacology, College of Veterinary Medicine, University of Al-Qadisiyah, Iraq.

(Received January 2016, Accepted December 2017)

Corresponding Author: salim.jari@qu.edu.iq

Key words: Estrus Cycle, Sexual Performance, Ovary, Uterus.

ABSTRACT

This is an investigative study to explore the changes in the duration of different estrus cycle stages of cyclic female Wistar rats treated with anti-sera against bovine follicular fluid-steroid-free (ANBFF-SF), and to examine the changes of ovarian and uterine weight in association with these different stages of the cycle. The current study recruited 160 cyclic rat females, which were assigned equally into two groups. At proestrus phase, the control group received 100µl/female normal saline intraperitoneally, and the treatment group received 100µl/female ANBFF-SF. The duration of the four estrous phases were determined for two consecutive cycles. For vaginal smears, scarification of 20 females/phase/group was conducted, and ovaries and uteri were removed and weighted. The findings reported potential elongation of proestrus and diestrus durations and potential shortening of diestrus duration in treatment groups compared with control, whereas metestrus and total duration showed insignificant changes. The mean weight of both ovaries and uteri weight increased significantly at estrus phase in treatment group compared with control, whereas other phases showed insignificant changes between groups. In conclusion, SFBFF-AS administration has a potent role in enhancing sexual performance of cyclic female Wistar rats.

INTRODUCTION

Together, the female reproductive system organs are responsible for ovulation and transportation of ova, oocyte implantation, embryonic development, birth, postpartum care and nutrition, and the generation of female hormones. Ovaries, oviducts, uterus, vagina, and the mammary glands are the key components of the reproductive system for rat females (1).

Structurally, the ovary is composed of cortex and medulla. In the cortex, different follicular sizes were found Inside each follicle, ovum with number of surrounding layers of granulosa and theca cells are present, depending on the maturation state of the ovum. Earlier stages of follicle development are independent on reproductive hormones secretion, whereas late stages are dependent on reproductive hormones secretion by the anterior pituitary. During folliculogeneis, the growing follicle releases estradiol and inhibin from the granulosa cells (2). There are two phases belong to the ovarian cycle: the follicular phase, during which an ovum is formed, and the luteal phase, during which the corpus luteum grows and regresses through the development of follicular phases (3).

It has been reported that the 4-5 days of estrous cycle of the rat has four phases of proestrus, estrus, metestrus, and diestrus (4, 5). In cyclic rat, FSH level was still high during the first period of estrus phase (6), but it down-regulated when serum levels of inhibin increased (7). After the beginning of the proestrus, estrogen levels surged, resulting in enhanced peaking of LH and FSH. Circulating peak of estradiol induces the ovulation, which takes place at estrus (5). Throughout the pro- and estrus phases, various structural changes will be occurring, due to the increased estradiol levels, in the female genitalia, such as dry vaginal wall, swollen vulva, edematous of the oviduct and uterus (8). At the end of proestrus and beginning of estrus only, a rat female allows a male for mating (9).

The activins and inhibins of the transforming growth factor ß superfamily have significant impacts on reproduction. The combination of reproductive activities relies heavily on the antagonistic connection between activins and inhibins. In a negative feedback process, inhibin of gonadal origin blocks the paracrine activities of activin, preventing it from promoting follicle-stimulating hormone (FSH) release from the adenohypophysis. The structural similarities between them account for their operational antagonistic effect (10).

As a new paradigm, neutralization of endogenous inhibin has been examined to increase FSH surge and induction of high level of folliculogenesis and superovulation (11-14). Immunoneutralization of inhibin has been conducted using antibodies against synthetic inhibin alpha subunit and against partially purified inhibin from the steroid free follicular fluid, as a rich source of inhibin (15). Inhibin, a proteinaceous compound generated by granulosa cells, was reported by Henderson and Franchimont (16) from follicular fluid of bovine ovary. This fluid inhibits FSH release in sheep (17). FSH release regulation is maintained by the inhibin that may have a function in the regulation of folliculogenesis. Miller et al. (17) observed that injection of bovine follicular fluid resulted in a BFF-based effect on the ovary-pituitary gland axis that delayed the occurrence of estrous and ovulation.

The present study aimed to investigate the changes in the duration of different estrus cycle stages of cyclic female Wistar rats treated with anti-sera against bovine follicular fluid-steroid-free (ANBFF-SF), and to examine the alterations of ovarian and uterine weight in association with the stages of the cycle.

MATERIALS AND METHODS

Follicular fluid was aspirated from follicles of bovine ovaries (215mm in diameter), spun at 8000rpm for 15mins at 4°C to eliminate cell wreckage, and then processed with activated charcoal (10mg/ ml) for 60mins at 4°C to create BFF-SF. In order to get rid of the charcoal, they centrifuged it at 14000rpm for 90mins at 4°C. FF that had been processed with charcoal was chilled to -20 degrees centigrade before being used. The proteins in the FF have been detected using the Biuret test and the ninhydrin reaction. Calculations for cholesterol were made.(18)

Rabbits have been inoculated with BFF-SF to acquire ASBFF-SF. Five adult male rabbits were administered five times with S/C-1ml of BFF-SF (one-week interval). Antiserum was acquired through blood collection and centrifugation 30 days after the final injection and kept at -20 C° till usage. The use of rats in this research was done in accordance with AL-Qadisiyah University's regulations and standards for animal experimentation in Iraq. In this experiment, 60-day-old, 1565.13-gm-weighted female Wistar rats were employed. They were provided with typical laboratory diet (a 19% protein ratio, with 3000kcal of energy), water, and lighting conditions (a 12Light: 12Dark cycle). Only rodents with at a minimum two separate 4-5 day cycles were employed, and vaginal smears were tested daily.

Experimental Design: One hundred sixty females were randomly divided into two groups. Each female received an intraperitoneal (ip) injection of 100µl of either normal saline (control) or ASBFF-SF (treatment) during proestrus. Over two cycles in a row, the length of each of the four estrus stages was measured. Twenty animals were sacrificed at each phase, and their ovaries and uteri were excised and weighed in accordance with the vaginal smear.

Statistical Analysis: All data were presented as means +/- standard errors based on statistical assessment. The student t test was used to compare the length of each phase of the estrous cycle and the overall length of the cycle among groups, while the two-way ANOVA and Newman-Keuls test were used to compare the ovarian and uterine weights across groups. Based on P less than 0.05, changes were determined to be statistically significant. GraphPad Prism was used for all statistical analyses.

RESULTS

Figure (1) displays significant increase ($p<0.05$) of proestrus and estrus phases and significant decrease of diestrus phase in the rodents treated with ASBFF-SF compared with control. Total duration of the estrus cycle showed insignificant difference ($p>0.05$) among groups (figure 2). When use group comparisons, ovarian and uterine weights of treated group reported significant increase ($p<0.05$) at estrus phase than control, whereas other phases showed insignificant differences ($p>0.05$) between experimental groups (figure 3 and 4, respectively). The figures also documented significant ($p<0.05$) elevation of ovarian and uterine weights of both groups at estrus, whereas other phases reported insignificant differences ($p>0.05$) between groups.

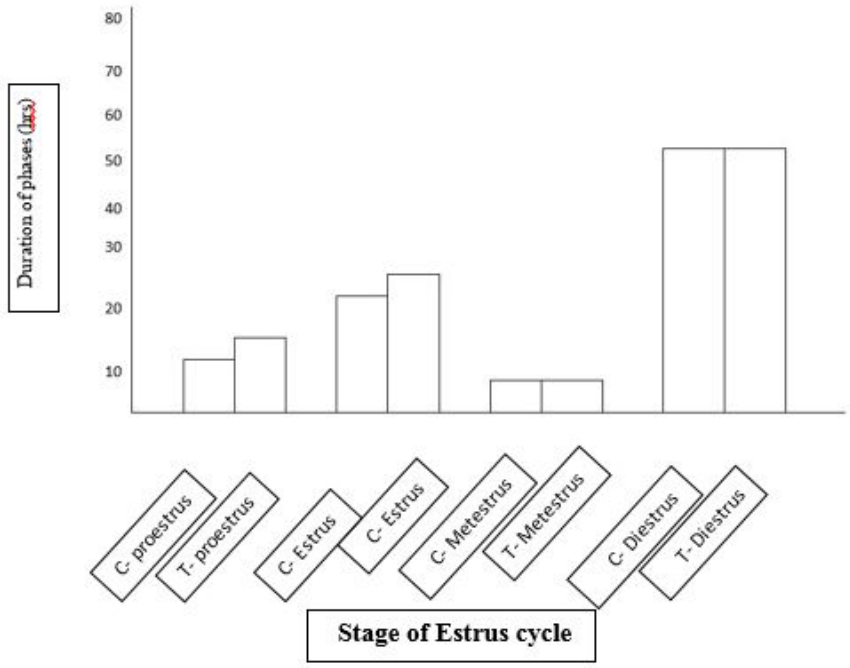

Figure (1): Effect of SFBFF antiserum in the duration of phases of estrus cycle in cyclic virgin female rats. The results presented as mean standard error of the mean. Star denote significant difference ($p<0.05$) between groups for each phase of the estrus cycle. C(control) cyclic virgin female rats injected with 100 µl of normal saline (ip), at early proestrus T (created) cystic virgin female rats injected with 100 l of steroid free BFF antiserum (ip), at early proestrus.

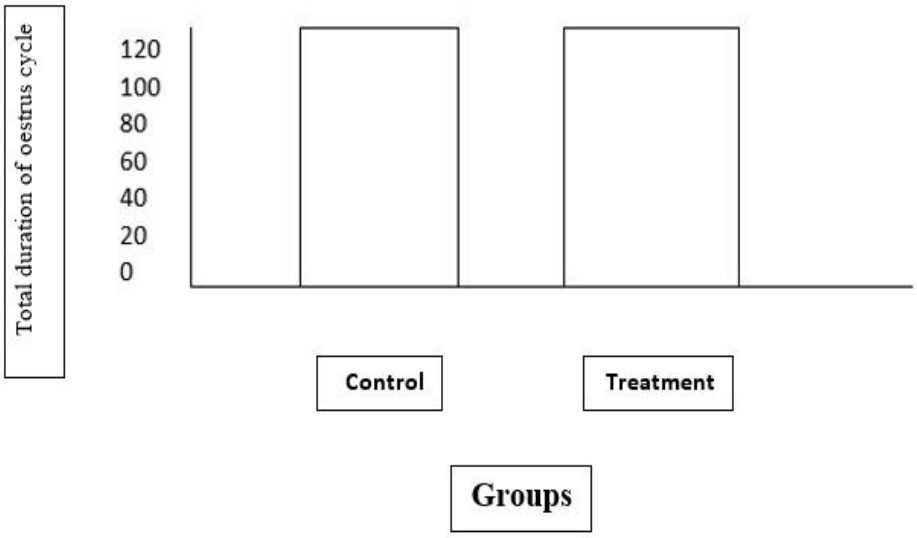

Figure (2): Effect of SFBFF antiserum on total duration of the estrus cycle in cyclic virgin female rats. The presented as mean standard error of the mean. C) cyclic virgin female rats injected with 100 µl of normal saline (p), at early proestrus. Toad cyclic virgin female rats injected with 100 µl of steroid-free BFF antiserum (ip), at early proestrus.

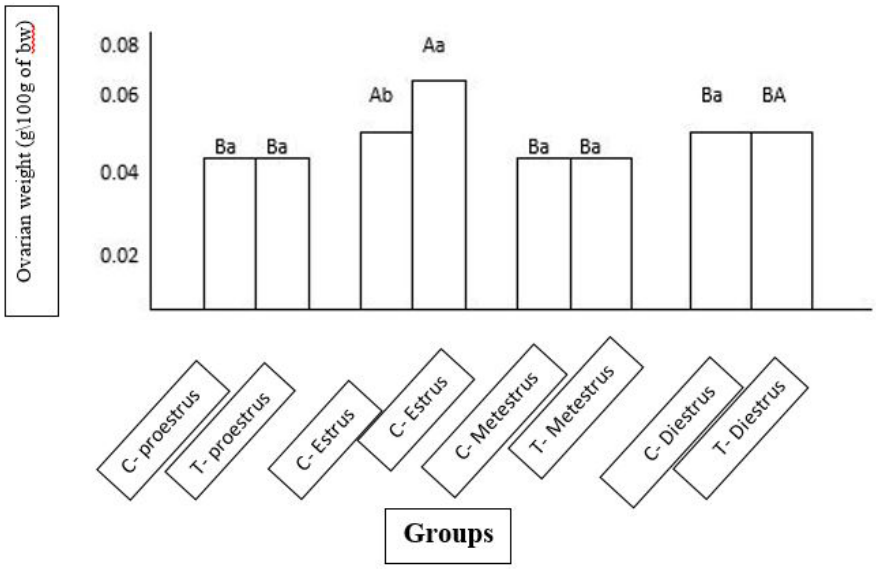

Figure (3): Effect of SFBFF antiserum on ovarian weight (g) of cyclic virgin female rats. The results presented as mean standard error of the mean, Different small letters denote significant difference ($p<0.05$) between groups for each phase of the estrus cycle. Different capital letters denote significant difference ($p<0.05$) between phases of the estrus cycle for each group. C (control) cyclic virgin female rats injected with 100 µl of normal saline (ip), at early proestrus. T (treated) cyclic virgin female rats injected with 100 µl of steroid-free BFF antiserum (ip), at early proestrus.

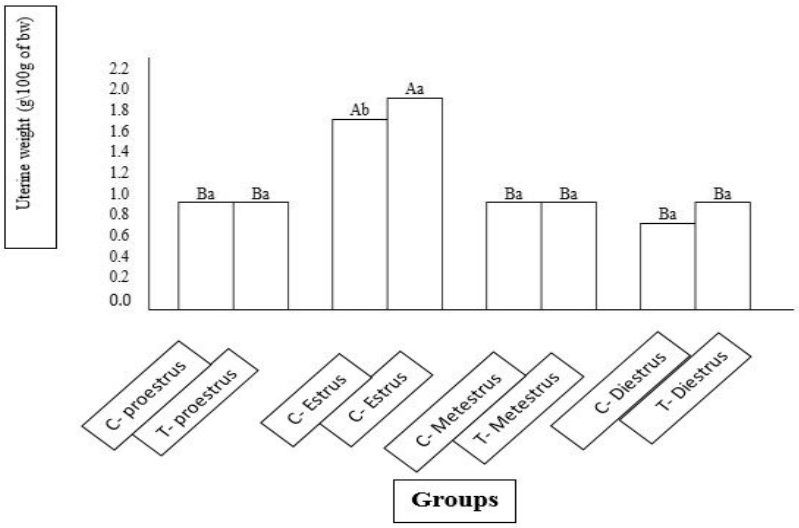

Figure (4): Effect of SFBFF antiserum on uterine weight (g) of cyclic virgin female rats. The results presented as mean standard error of the mean Different small letters denote significant difference ($p<0.05$) between groups for each phase of the estrus cycle. Different capital letters denote significant difference ($p<0.05$) between phases of the estrus cycle for each group. C (control cyclic virgin female rats injected with 100 µl of normal saline (ip), at early proestrus. T (treated) cyclic virgin female rats injected with 100 µl of steroid-free BFF antiserum (ip), at early proestrus.

DISCUSSION

As ovarian Graffian's follicles have been recognized as a promising supply for these peptides, they were used to produce the anti-inhibin in the current work (19), have been tested to clarify its role estrus cycle duration and weights of ovaries and uteri, as a result of reproductive hormonal changes due to inhibin immunoneutralization. Various studies reported that passive or actively immunizing against endogenous inhibin alpha subunit result in elevated FSH secretion from anterior pituitary (20-22) and enhancement of ovulation rate (23,14)

Adult male rabbits were injected with ASBFF-SF five times at weekly intervals to generate inhibin-neutralizing antibodies, allowing pituitary-released activin to stimulate FSH release from the pituitary. Consequently, by exploring the result of their impacts on reproductive organs and the occurrences of the ovarian cycle, the present research may provide beneficial data concerning the in vivo functions of inhibins and activins throughout ovarian follicles, due to its autocrine and paracrine activities, and in pituitaries, owing to its endocrine intervention. Where it has been well reported that dysfunction of these factors may result in infertility (24,25).

From the current findings, exposing the crucial functions, ovarian inhibin and activin might give significant understanding about the ovarian function. However, the fact that these variables may play a regulatory function in ovarian cell proliferation and maturation and steroidogenesis suggests there may be novel ways to improve conceiving and reproduction success.

In treated females, increased proestrus and estrus duration with decreased diestrus duration could be due to the hormonal changes, namely pituitary surge of FSH and then ovarian estradiol production. It has been evidenced that inhibin immunoneutralization increased FSH secretion from adenohypophyses in various animals (11-14). The high level of folliculogenesis which followed by high estradiol secretion from granulosa cells (14,26). It can be hypothesized that a rise in endogenous FSH release causes a stream of ovarian folliculogenesis and leads to manufacturing of a substantial quantity of estradiol release, which in turn causes an elevation in the weight of ovary and uterus, thus explaining the observed changes in proestrus and estrus stages after the use of anti-inhibin serum. Estradiol plays an important role in mammalian biology due to its biological effects in mammalian target cells, which include stimulating signals in the reproductive organs, mammary cells, and pituitary cells. Estradiol is essential for the maturation of the uterus during gestation by stimulating the production of contractile proteins (30).

It can be concluded that steroid free BFF antiserum have a role in stimulating pituitary FSH secretion and therefore in ovarian folliculogenesis, where FSH is recognized as the primary hormone responsible for stimulating the ovarian follicle formation and maturation, in association with ovarian and uteri growth, which could be a result of high secretion of estradiol-178.

التغيرات في أوزان المبايض والأرحام وعلاقتها بأطوار دورة الشبق لإناث الجرذان المعاملة بمضاد السائل الجريبي البقري منزوع الستيرويدات

سالم حسين جاري

قسم الفسلجة، الكيمياء الحياتية والادوية، كلية الطب البيطري، جامعة القادسية، العراق.

الخلاصة

أجريت دراسة التحري الحالية لتحديد فترات أطوار دورة الشبق الأربعة والتغيرات الحاصلة في أوزان المبايض والأرحام أثناء تلك الأطوار في إناث الجرذان المعاملة بمضاد السائل الجريبي البقري منزوع الستيرويدات (SFBFF). تضمنت التجربة توزيع 160 إنثى ناضجة عشوائيا على مجموعتين متساويتين. حقنت الأولى (السيطرة) 10 مايكرولتر من المحلول الفسلجي في البريتون وحقنت الثانية (المعاملة) 100 مايكرولتر من بمضاد السائل الجريبي البقري منزوع الستيرويدات. تم حساب مدة أطوار دورة الشبق الأربعة(proestrusو estrus و metestrus و diestrus) لدورتين متعاقبتين باستخدم طريقة المسحة المهبلية. بضوء نتائج المسحات المهبلية، تمت التضحية بـ20 إنثى من كل مجموعة في كل طور وازيلت المبايض والأرحام وسجلت أوزانها بينت النتائج حصول ارتفاع معنوي في مدة طوري proestrus و estrus وانخفاض معنوي في مدة طور diestrus وعدم تغير في مدة طوري diestrus في إناث المعاملة بالمقارنة مع السيطرة. كما حصلت زيادة معنوية في أوزان مبايض وأرحام إناث المعاملة أثناء

طور الشبق في حين لم تظهر تغيرات معنوية خلال الأطوار الثلاثة الأخرى. يمكن الاستنتاج أن حقن معضاد السائل الجريبي البقري منزوع الستيرويدات يساعد بشكل فاعل في رفع الكفاءة التناسلية لإناث الجرذان.

الكلمات المفتاحية : دورة الشيق, الكفاءة التناسلية، المبيض، الرحم.

REFERENCES

1- **Simmons, K. (2005)**, Rat Reproductive System February 21. Retrieved December 17, 2008, from http://o.uwinnipeg.ca-simmons/16labman05/1b8pg8.htm.

2- **Beshay, VE, and Carr, BR. (2013).** Hypothalamic-Pituitary-Ovarian Axis and Control of the Menstrual Cycle. T. Falcone and W.W. Hurd (eds.), Clinical Reproductive Medicine and Surgery: A Practical Guide, Springer Science and Business Media, New York. http://www.springer.com/978-1-4614-6836-3.

3- **Dadmarzi, M, Yadi, J, and Eila, N. (2015)**. Comparison of some metabolites in blood and ovarian follicular fluid in different sizes in Holstein dairy cows. Res. Opin. Anim. Vet. Sci., 5(6): 256-258

4- **Fata, JE, Chaudhary, V, and Khokha, R. (2001)** Cellular turnover in the mammary gland in correlated with systemic levels of progesterone and not 17-tradiol during the estrous cycle, Biol. Reprod., 65: 680-688

5- **Wood, GA, and Fata, JE (2006)** Circulating hormones and estrous stage predict cellular and stromal remodeling in murine uterus 2007 Society for Reproduction and Fertility.

6- **Freeman**, **ME. (1988).** The ovarian cycle of the rat. In E. Knobil & 7. Neil (eds), Physiology of reproduction. Raven Press Lad, New York, pp. 1893-1928

7- **Tebar, M. Uilenbroek, JTHJ, Kramer, P, van Schaik, RHN, Wienkx, CDI, Ruiz, A, de Jong, FH, Sánchez-Criado, JE (1997)** Effects of progesterone on the secondary surge of follicle-stimulating hormone in the rat. Biol. Reprod., 57: 77-84.

8- **Baker, DEJ. (1979)**. Reproduction and breeding. In HJ. Baker, JR. Lindsey, and SH. Weisbroth. The Laboratory Rat, volume 1. (pp. 153-168). New York Academic Press.

9- **Marcondes**, **FK, Bianchi, FJ, and Tanno, AP. (2002)** Determination of the estrous cycle phases of rats: some helpful considerations Braz. J. Biol, 62: 609-614.

10- **Channing, H, Okada, T, Kawara, S., Oka, M. Taya, K. Watanabe, G, and Sasamoto S, (1985)**. Effects of passive immunization against estradiol-17b and inhibin on the secretion of gonadotropin in the cyclic golden hamster (Mesocricetus auratus) Reprod Fernt Dev. 9447-453

11- **Kishi, H, Okada, T, Kawara, S., Oka, M. Taya, K. Watanabe, G, and Sasamoto S, (1997)**. Effects of passive immunization against estradiol-17b and inhibin on the secretion of gonadotropin in the cyclic golden hamster (Mesocricetus auratus) Reprod Fernt Dev. 9447-453

12- **Wang, HC, Herath, GX, Watanabe, G, and Taya, K. (2001)** Superovulation, fertilization and in vitro embryo development in mice after administration of an inhibin neutralizing antiserum Reproduction 122, 809-816.

13- **Ishigame, H. Medan, MS, Wang, H. Watanabe, G, Kishi, H, and Arai, KY, (2005)**, Indoction of superovulation by immunoneutralization of endogenous ibi in immature rats. J Reprod Dev. 51, 559-566.

14- **Al-Saaidi**, **JAA, and Samir, MS, (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.

15- **Leversha, LJ, Robertson, DM, deVos, FL. Morgan, Fl, Heam, MTW, Wettenhall, REH Findlay, JK, Burger, HG, and de Kreber, DM. (1987)** Isolation of inhibin from evine follicular fluid J. Endocrinol 113: 213-221.

16- **Henderson**, **KM, and Franchimont, P. (1983)**: Inhibin production by bovine ovarian tissues an vitro and its regulation by androgens. J. Reprod. Fertil. 67. 291-298

17- **Miller**, **KF, Critzer, JK, and Ginther, OJ. (1982)**. Inhibin and subsequent rebound of FSH secretion following treatment with bovine follicular fluid in the ewe. Theriogenol. 184552

18- **Wise**, T. **(1987)** Biochemical analysis of bovine follicular fluid: Albumin, total protein, lysosomal enzymes, ions, steroids and ascorbic acid content in relation to follicular size, rank, atresia classification and day of estrous cycle. J. Anim. Sci. 64: 1153-1169.

19- **Nashimoto**, **H, Hamano, S, Hill, GA, Miyamoto, A, Tetka, M. (2009)** Classification of bovine follicles based on the concentrations of seroids, glucose and lactate in follicular fluid and te status of accompanying follicles. J Reprod. Dev., 53(2): 219-224.

20- **Culler, MD, Negro-Vilar, A. (1988):** Passive immunoneutralization of endogenousinhibin: sex related differences in the role of inhibin during development. Mol. Cell Endocrinol, 58: 263- 273.

21- **Abdull**a, WS. (2013): Sequential postnatal Evolution of mRNA expression level of pituitary. gonadal and extragonadal GnRH-r, IGF-1, IGF-2 genes in inhibin-passive immunized female rats. MSc Thesis, Vet. Med., Al-Qadesiya University, IRAQ.

22- **Thanoon**, **HB. (2013)** Hypothalamic GHRH and pituitary GH genes expression levels in sequential neonatal inhibin immunoneutralized female rats. MSc Thesis, Collage of Vet. Med. Al-Qadesiya University, IRAQ

23- **Rivier, C, and Vale, W. (1989)**. Immunization of endogenous inhibin modifies hormone secretion and ovulation rate in the rat. Endocrinology, 125, 152-157.

24- **Shelling**. **AN, Burton, KA, Chand, AL, van Ee, CC, France, IT, Farquhar, CM. Milsom, ER Love, DR. Gersak, K, Aittonaki, K, et al. (2000).** Inhibin a candidate gene for premature ovarian failure. Hum Reprod., 15: 2644-2649.

25- **Marozzi, A. Porta, C. Vegeni, W, Crosignani, PG, Tibiletti, MG, Dalpra, L, Ginell, E. (2002)** Mutation analysis of the inhibin alpha gene in a cohort of Italian women affected by ovarian failure. Hum Reprod., 17: 1741-1745.

26- **Al-Sa'aidi, JAA, Al-Okaily, BN, Al-Shwilly, HAJ. (2016).** Uterine implantations and litter size alteration in anti-inhibin and eCG-hCG treated virgin pregnant female rats. Online Intern. Interdiscip. Res. J., VI(I): 36-46.

27- **Taya**, **K. (1993)**: Role of inhibin in regulation of FSH secretion and folliculogenesis in mammalians. Curr. Trends Exp. Endocrinol. 1:97-116.

28- **Taya**, **K, and Watanabe, G. (1999)**: Inhibin as a key hormone in determining species-specific ovulation rates in mammal In: Kwon HB, Joss JMP, Ishi S. (eds.), Recent Progress in Molecular and Comparative Endocrinology. Kwangju, Korea: Hormone Research Center, Chonman National University, 134-143.

29- **Helmy**, **SA, Emarah, HA, and Abdelrarek, HMA (2014**) Estrogenic effect of way phytoestrogens on the uterus of ovariectomized female rats. Clin Pharmacol. Biopharmaceut $2: 1-7.001. doi:10.4172/2167-065X.82-001.

30- **Borekci**, **B, Ingec, M. Kumtepe, Y, Karaca, M. Koc, F. Salman, S, Gulaboglu, M, and Suleyman, H. (2009)**. Effect of estrogen, progesterone, LH, and FSH on oxidant and antioxidant parameters in rat uterine tissue. Int. J. Fert. Steril., 3(3): 119-128.