Ovarian morphometric evolution in two consecutive estrous cycles of female rats treated with steroid-free bovine follicular fluid antiserum

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Abstract

The current study aims to investigate the role of steroid-free follicular fluid antiserum on ovaries morphometric growth and development in virgin female rats at two sequential estrous cycles. Bovine follicular fluid aspired from graafian follicles, steroids were removed by treatment with activated charcoal, in order to obtained steroid-free bovine follicular fluid (S-FBFF) which was used for immunization of male rabbits to prepare S-FBFF antiserum (S-FBFF-ab). Sixty virgin female rats were assigned into control and treatment groups (30 each), intraperitoneal injected with a single dose of distilled water 100µl/rat and S-FBFF-ab 100µl/rat at late metestrus, respectively. At the estrus phase of the first and second estrous cycle, 15 females from each group of each cycle were anesthetized and ovarian samples were obtained for histological examination. In comparison with control, the results of S-FBFF-ab treated female rats revealed a significant increase of relative ovaries and uteri weights at both estrous cycles. Morphometric examination showed progressive ovarian proliferation at the first estrus phase in S-FBFF-ab treated female rats through elevation of the number of primaries, graafian, and total follicles. In conclusion, passive immunization against endogenous inhibin using S-FBFF-ab could augment the reproductive fecundity through increase ovarian growth and development.

***Keywords*:** Inhibin, Passive immunization, Follicular fluid, Ovarian growth; Reproduction

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التطورات المبيضية الشكلية لدورتي شبق متتاليتين في إناث الجرذان المعاملة بمضاد السائل الجريبي منزوع الستيرويدات

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الخلاصة

أجريت الدراسة الحالية للتحري عن تأثير التمنيع ضد السائل الجريبي البقري منزوع الستيرويدات على التطورات الشكلية لمبايض إناث الجرذان في دورتي شبق متتاليتين. تم سحب السائل الجريبي البقري من جريبات كراف وتم التخلص من الستيرويدات عن طريق معاملة السائل مع الفحم النشط للحصول على السائل الجريبي البقري منزوع الستيرويدات الذي أستخدم في تمنيع ذكور الأرانب للحصول على مضاد السائل الجريبي البقري منزوع الستيرويدات. تم تقسيم ٦٠ أنثى جرذ باكر ومنتظمة دورات الشبق على مجموعتي سيطرة ومعاملة (٣٠ لكل مجموعة). حقنت الإناث بجرعة واحدة ١٠٠ مايكرولتر من الماء المقطر ومضاد السائل الجريبي البقري منزوع الستيرويدات في الخلب في طور Metestrus المتأخر، على التوالي. في طور الشبق للدورتين الأولى والثانية، تم تخدير ١٥ أنثى من كل مجموعة باستخدام الكيتامين والزايلازين ٠,٣ و ٠,١ ملليتر/كغم من وزن الجسم، على التوالي. وزنت المبايض والأرحام وأخذت نماذج من المبايض لغرض الفحص النسجي. أظهرت نتائج مجموعة المعاملة زيادة معنوية في الأوزان النسبية للمبايض والأرحام وفي كلتا الدورتين. وأظهر الفحص النسجي تطوراً ملحوظاً في نمو نسيج المبيض في الدورة الأولى مع زيادة أعداد الجريبات الابتدائية وكراف والكلية، بالمقارنة مع السيطرة.

يستنتج من النتائج الحالية أن التمنيع الميسر ضد الأنهبين الداخلي باستخدام مضاد السائل الجريبي البقري منزوع الستيرويدات يزيد خصوبة الإناث عن طريق زيادة نمو وتطور المبايض ونشاطها الوظيفي.

Introduction

The commonly used method for induction superovulation in mice and rats is the treatment with combined equine chorionic gonadotropin (eCG) and human chorionic gonadotropin (hCG), but recently an alternative method was performed for this purpose (1-6). Many studies have used the alternative way in which the induction of superovulation can be attained and overcome the problems that occur in mice and rats. Furthermore, these studies have reported that an active or passive immunization against inhibin has the ability to induce superovulation in both the adult and immature mice and rats (1-11). Inhibin are glycoproteins and members of the transforming growth factor β (TGFβ) family. They are regarded as one of the major internal factors of the ovary that alter the role of the gonadotropins (Gn) as well as considered as one of the regulators of the ovary during the ovarian cycle (12,13). Moreover, inhibin is a potent regulator of FSH secretion by negative feedback mechanism through its endocrine impacts on adenohypophysis (14). Conversely, activins are decisive factors in the ovary, as they act through paracrine and autocrine and paracrine mechanism. Moreover, activins play direct roles in achievement of FSH secretion from the adenohypophysis (15). It has been found that FSH and the steroid in the follicular fluid modulate the expression levels of inhibin or activin subunit genes, their production and secretion (16). Administration of S-FBFF to intact heifers reduces the plasma FSH level and delays the ovulation onset, while termination of S-FBFF treatment re-established FSH secretion, where these effects are concluded to be mediated by inhibin was confirmed (17). New studies were conducted to improve the impacts of antisera against S-FBFF or inhibin-free S-FBFF on hormones profile in cycling female rats that affects reproduction (3,4).

The present study aims to investigate the impact of immunization against S-FBFF on ovarian growth and development, at two consecutive estrous cycles in virgin female rats.

Materials and methods

Preparation of steroid-free bovine follicular fluid antiserum (S-FBFFab)

Follicular fluid was collected from mature follicles ≤15 mm of bovine, centrifuged at 8000 rpm for 15 minutes to remove the debris, then after treated for 1 hour with activated charcoal (10 mg/ ml), and centrifuged again at 14000 rpm for 90 minutes to remove the charcoal with attached steroids. Proteins were detected using Biuret assay and ninhydrin reaction and cholesterol was estimated in the FF (18). Ten adult male rabbits were immunized against S-FBFF. One month after 5 injections (one-week interval), blood was collected, centrifuged and antiserum was obtained and kept at -20 ºC (3).

Experimental animals

The current experiment was accorded by the ethical guidelines and policies of the University of Al-Qadisiyah. Adult virgin cycling female rats of Wistar strain (90 days old and 160-170 g of body weight) were housed at day light of 12L: 12D cycles and controlled temperature at 22-24 °C. Standard laboratory animal food (19% crude protein ratio and 3000 kilocalories energy) and drinking water was accessed *ad libitum*. Only female rats with regular estrous cycles have been used.

Experimental design

Sixty females were divided into two equal groups. The females were injected IP with a single dose of 100 µL of normal saline (control group) and 100 µL of S-FBFF antiserum (treatment group) at late metestrus stage. At the estrus phase of the first and second estrous cycle, 15 females from each group of each cycle were anesthetized by 0.3 ml Ketamine and 0.1 ml of Xylazine/ kg BW. Ovaries and uteri were removed and weighted. Ovarian samples were obtained for histological examination (19).

Statistical analysis

The results were presented as mean ± standard deviation (M±SD). Experimental groups and periods were compared using ANOVA-1 and Newman- Keuls (20). The significant level $P<0.05$ was considered as a significant to compare the differences between the means. All statistical analysis was carried out using the GraphPad Prism-Version 5 (SAS Institute, Inc., USA).

Results

As illustrated in figure 1, significant ($P<0.05$) elevation of relative ovarian weight in S-FBFF-ab injected female rats compared with control was registered at first cycle, whereas second cycle revealed insignificant ($P>0.05$) changes between the studied groups. In comparison between the two cycles for each group, S-FBFF-ab treated group female rats recorded significant ($P<0.05$) elevation of ovarian weight at first cycle in comparison with second cycle, whereas control group demonstrated no significant ($P>0.05$) changes between the cycles.

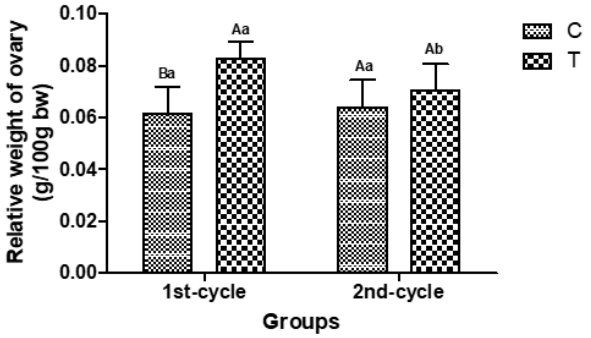

Figure 1: Relative ovary weight (g/100g of body weight) in cyclic female rats treated with steroid-free bovine follicular fluid at late metestrus phase. C: control female rats injected with 100 µl of normal saline *ip*, at late metestrus. T: treated female rats injected with 100µl/rat of steroid-free bovine follicular fluid antiserum *ip*, at late metestrus. Data were presented as Mean ±SD of 15 observations (n=15). Different capital letters denote significant difference (P<0.05) between groups for each cycle. Different small letters denote significant difference (P<0.05) between cycles for each group.

The relative uterine weight of S-FBFF-ab treated group revealed significant (P<0.05) elevation in comparison with control group, at both cycles of the experiment (Figure 2).

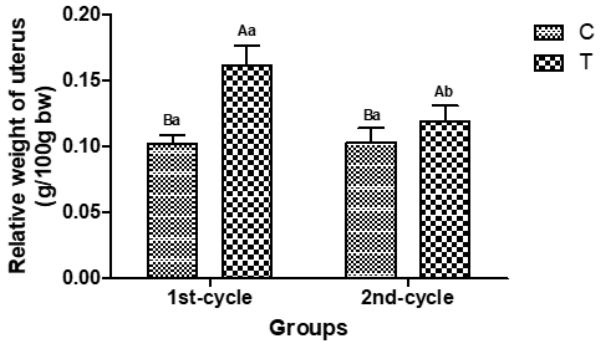

Figure 2: Relative uterus weight (g/100g of body weight) in cyclic female rats treated with steroid-free bovine follicular fluid at late metestrus phase. C: control female rats injected with 100 µl of normal saline IP, at late metestrus. T: treated female rats injected with 100 µl/rat of steroid-free bovine follicular fluid antiserum IP, at late metestrus. Data were presented as Mean ±SD of 15 observations (n=15). Different capital letters denote significant difference (P<0.05) between groups for each cycle. Different small letters denote significant difference (P<0.05) between cycles for each group.

In comparison between periods for each group, S-FBFF-ab treated group recorded significant (P<0.05) elevation of uterine weight at the first cycle in comparison with second cycle, whereas control group showed no significant (P>0.05) difference between the two cycles.

Morphometric appearance (Figure 3T) and histological sections of ovaries from S-FBFF-ab treated females (Figure 4T), the results showed higher number of graafian follicles compared with that of control females (Figures 3C and 4C).

Histological sections from ovaries of treated group (Figure 4T) revealed positive role of S-FBFF-ab injection in ovarian tissue proliferation compared with control (Figure 4C), where it has been found an increment in tissue proliferation especially in number and size of follicles. Furthermore, the sections of treated female rats showed that the predominant type was the graafian follicles that contains oocytes and supporting tissue, as well as multicellular layer of proliferated granulosa cells and antrum filled with fluid (Figure 4T), whereas the sections of control females revealed lower number of graafian follicles and the predominant type of follicles were primary and secondary (Figure 4C).

The results presented in table 1, also confirmed these findings, where the number of primary and graafian follicles of S-FBFF-ab treated females increased significantly (P<0.05) compared with control.

In comparison between first and second estrus cycles for each group, the result of S-FBFF-ab treated group at first cycle (Figure 4T) recorded higher number of primary and graafian follicles and more proliferation than second cycle (Figure 5T), whereas control females showed insignificant differences between the two estrous cycles (Figure 4C and 5C) (Table 1).

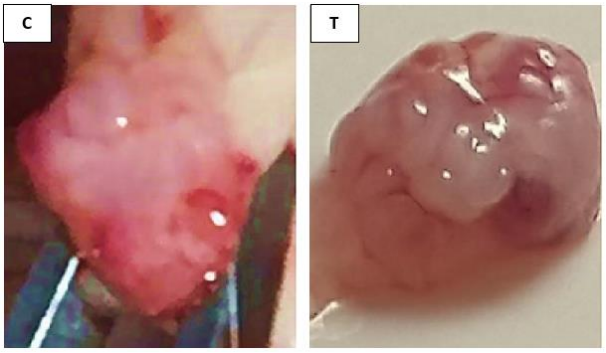

Figure 3: Morphometric appearance of ovary obtained from SFBFF antiserum treated (T) female rats shows more developed and higher number of mature follicles in comparison with that of control (C).

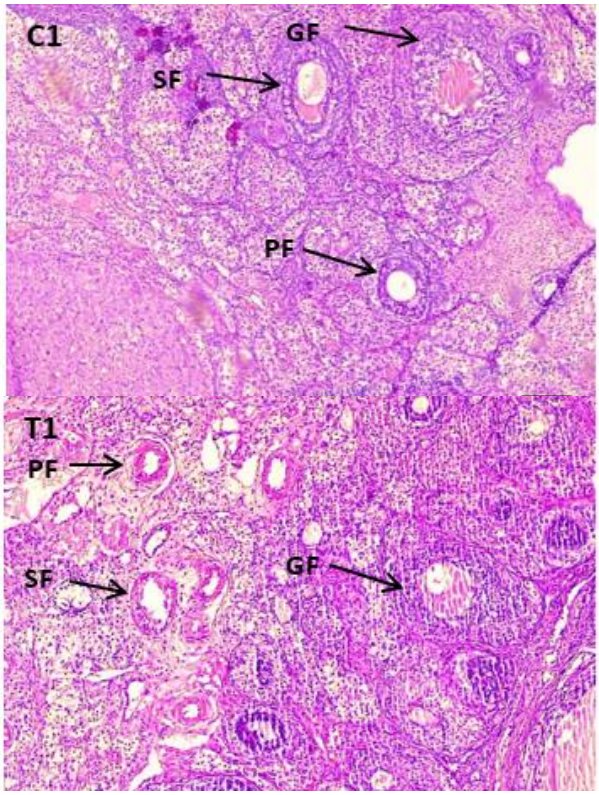

Figure 4: Histological section of ovary obtained S-FBFF-ab treated (T1) female rat at first estrus cycle of the experiment shows obvious follicular growth and development and increased number of primary (PF) and graafian follicles (GF) with decreased number of secondary follicles (SF) in comparison with control (C1) female rats (H&E, x125).

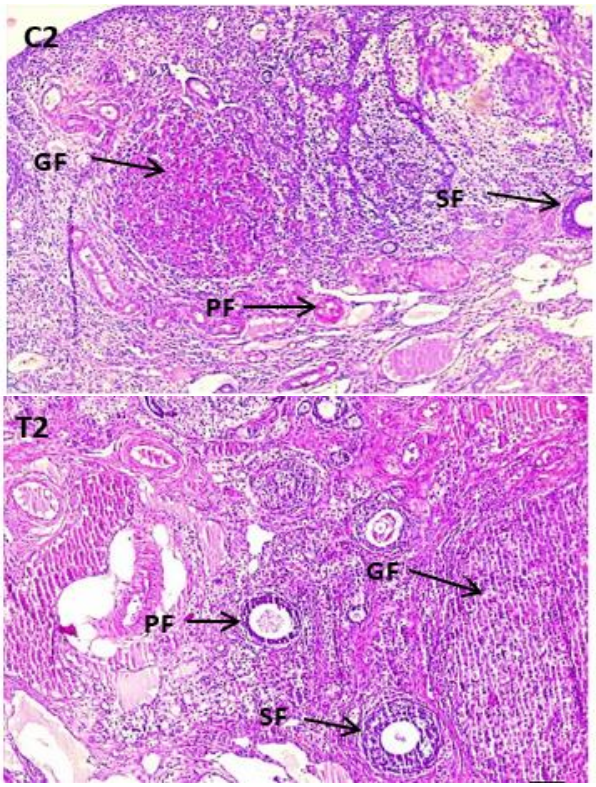

Figure 5: Histological section of ovary obtained from S-FBFF-ab treated female rat at second estrus cycle of the experiment shows normal folliculogenesis and unchanged number of primary (PF), secondary (SF) and graafian follicles (GF) in comparison with control female rat (H&E, x125).

Table 1: Primary, secondary, mature and total ovarian follicles from control and SFBFF antiserum treated female rats

	Groups (Mean ±SD) n=15			
	C_{1st}	T_{1st}	C_{2nd}	T_{2nd}
Primary follicles	10.2±0.78 ^{b}	13.5±0.86 ^{a}	9.98±0.97 ^{b}	10.1±1.02 ^{b}
Secondary follicles	8.65±0.68 ^{a}	6.88±0.84 ^{b}	8.26±0.75 ^{a}	8.44±0.83 ^{a}
Graafian follicles	8.54±0.83 ^{b}	12.7±0.88 ^{a}	9.13±0.92 ^{b}	9.85±0.75 ^{b}
Total follicles	27.3±2.76 ^{b}	33.8±2.16 ^{a}	27.4±3.06 ^{b}	28.3±3.11 ^{b}

C: control female rats injected with 100 µl of normal saline *ip*, at late metestrus. T: treated female rats injected with 100µl/rat of steroid-free bovine follicular fluid antiserum *ip*, at late metestrus. Different capital letters denote significant difference (P<0.05) between groups for each cycle. Different small letters denote significant difference (P<0.05) between cycles for each group.

Discussion

Various experiments confirmed the contribution of inhibin in female reproduction, as a regulator of FSH secretion from the adenohypophysis, is through the growth of dominant follicle during the luteal phase, which is normally associated with the high secretion of estradiol-17β (21). The overall activities of FSH in female reproductive system include ovarian granulosa cell proliferation and differentiation, gametes production, and secretion of inhibin and estrogens (22,23).

The significant increase in the relative weight of the ovaries and uteri of S-FBFF-ab treated group, at the first estrous cycle, could be attributed to the role of passive

immunization against inhibin, which led to an increase in the rate of pituitary FSH secretion, which in turn stimulated the growth of ovarian follicles and the arrival of many of them into the stage of mature follicles (24). On the other hand, in parallel to increased folliculogenesis, estradiol secretion was also increased, since increased proliferation of granulosa cells has been also shown which is already responsible for estradiol secretion (25). It is known that estrogen plays a significant role in building fats and proteins as well as its role in the process of maturation of the ovary and its follicles and increase the level of certain types of proteins such as globulin binding proteins responsible for binding of sex hormones (26). One research demonstrated that progesterone and estrogen interaction increase rat uterine weight (27,28).

Furthermore, the increased level of growth hormone and insulin-like growth factors (3,29) could be the cause of increased ovarian weights, as insulin-like growth factors participates in the growth of follicles by stimulating the bio-synthesis of DNA in granulosa cells and follicle cells (30,31). Pituitary activin (5,6) may also play an indirect role by influencing the secretion of FSH from the pituitary gland, as well as the direct effects of ovarian inhibin and activins on ovarian follicle growth and development (4,32,33).

The significant increase in the uteri weight of S-FBFF antiserum treated group was accompanied by increased growth and development of ovarian follicles, which are responsible for the manufacture of a great amount of estrogens. On the other hand, estradiol is also known to have a stimulatory role in the maturation and development of the endometrium and increasing arterial blood flow (34). The fundamental role of estrogen is in the formation of endometrial tissue to be suitable for implantation of fertilized ova and the development of embryos until the development of placenta and embryonic membranes, which in turn secrete progesterone and estrogen (34). Furthermore insulin-like growth factors in the uterus are controlled by extracted estrogen from the implanted embryos, therefore it has been suggested that insulin-like factors may have a role in implantation (35) as well as to the role of activin to increase the number of estrogen receptors and improves its effectiveness (36).

Moreover, the histological sections from treated female rats revealed high proliferative rates of the ovaries. These coincide changes is due to the passive immunization against endogenous inhibin and consequent rise of FSH and estradiol secretion from the ovaries itself (37-39). In addition, the high level of growth hormone and insulin-like factors (4,41,42) may be the cause of increased ovarian tissue proliferation. Pituitary activin may also play an indirect role by influencing the discharge of FSH through the mechanism of the secretion through the adenohypophysis, as well as the direct effects of ovarian inhibin and activins that have localized effects on ovarian follicle formation (32,41,42).

The increased number of primary, graafian, and total ovarian follicles of treated females, due to the role of passive immunization against inhibin, which increases the concentration of FSH, where this hormone stimulates ovarian folliculogenesis and the production of a large amount of estrogens (25,39). Furthermore, growth hormone and IGFS have important roles in the granulosa cells proliferation, as growth hormone plays a major role in the release of growth promoters by increasing cell proliferation and inhibition of apoptosis (13,43).

Conclusion

It can be concluded that the prepared inhibin antiserum (from S-FBFF) was effective in immunoneutralization of inhibin, increasing the biosynthesis of pituitary FSH and ovarian estrogens production and increases ovarian growth and development, which could be efficient for improvement of female reproduction.

Acknowledgments

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Conflict of interest

We certify that there is no conflict of interest with any organization regarding the materials discussed in the manuscript.

References

1. Ishigma H, Medan MS, Watanabe G, Shi Z, Kishi H, Arai KY. A new alternative method for superovulation using passive immunization against inhibin in adult rats. Biol Reprod. 2004;71:236-243. 10.1095/biolreprod.104.027789
2. Yan L, Li H, Shi Z. Immunization against inhibin improves in vivo and in vitro embryo production laboratory of animal breed improvement and reproduction, institute of animal science, Jiangsu academy of agricultural sciences, Nanjing, China. Anim Reprod Sci. 2015;163:1-9. 10.1016/j.anireprosci.2015.11.001
3. Al-Sa'aidi JA, Majhwol EM. Effect of immunization against steroid-free bovine follicular fluid on reproductive hormones profile in cycling female rats. Asian J Anim Sci. 2017;11:183-188. 10.3923/ajas.2017.183.188
4. Al-Sa'aidi JA, Majhwol EM. Pituitary FSHβ and GH and Ovarian GH-r, IGF-1 and IGF-2 gene expression levels in cycling female rats immunized against steroid-free bovine follicular fluid antisera. Asian J Applied Sci. 2017;5(4):770-775.
5. Kafaji SS, Al-Sa'aidi JA, Khudair KK. Reproductive hormones profile of Iraqi awassi ewes immunized against synthetic inhibin-α subunit or steroid-free bovine follicular fluid. Iraqi J Vet Sci. 2017,31(2):123-128. 10.33899/ijvs.2017.145609
6. Al-Sa'aidi JA, Khudair KK, Khafaji SS. Reproductive fecundity of Iraqi awassi ewes immunized against synthetic inhibin-α subunit or

steroid-free bovine follicular fluid. Asian-Australasian J Anim Sci. 2018;31(8):1169-1175. 10.5713/ajas.17.0660
7. Ishigame H, Medan MS, Wang H, Watanabe G, Kishi H, Arai KY. Induction of superovulation by immunoneutralization of endogenous inhibin in immature rats. J Reprod Dev. 2005;51:559-66. 10.1262/jrd.17020
8. Medan MS, Nambo Y, Nagamine N, Shinbo H, Watanabe G, Groome N, Taya K. Plasma concentrations of ir-inhibin, inhibin A, inhibin pro-alphaC, FSH, and estradiol-17beta during estrous cycle in mares and their relationship with follicular growth. Endocrin Rev. 2004;25(1):7-14. 10.1385/endo:25:1:07
9. Gordon A, Aguilar R, Garrido JC, Guil LS, Sanchez CR, Millan Y, Watanabe G, Taya K, Martin MJ, Sanchez JE. Immunoneutralization of inhibin in cycling rats increases follicle-stimulating hormone secretion, stimulates the ovary and attenuates progesterone receptor-dependent preovulatory luteinizing hormone secretion. Neuroendocrinol. 2010;91:291-301. 10.1159/000299791
10. Du AG, Wu ZH, Zhang CY, Zhang HY, Liu HM, Chen ZY, Zeng XY. The effect of immunization against Xinjiang fine wool sheep inhibin on the reproductive traits of rats. Afr J Agric Res. 2011;6:5040-5046. 10.5897/ajar10.1084
11. Takeo T, Nakagata N. Superovulation using the combined administration of inhibin antiserum and equine chorionic gonadotropin increases the number of ovulated oocytes in C57BL/6 female mice. PLOS ONE. 2015;10(5):1-11. 10.1371/journal.pone.0128330
12. Findlay JK. An update on the roles of inhibin, activin, and follistatin as local regulators of folliculogenesis. Biol Reprod. 1993;48:15-23. 10.1095/biolreprod48.1.15
13. Miyazono K, Kamiya Y, Morikawa M. Bone morphogenetic protein receptors and signal transduction. J Biochem. 2010;147:35-51. 10.1093/jb/mvp148
14. Kumanov P, Kalyana C, Nandipati K, Tomova A, Robeva R, Agarwal A. Significance of inhibin in reproductive pathophysiology and current clinical applications. Reprod BioMed. 2005;10(6):786-796. 10.1016/S1472-6483(10)61124-8
15. Mather JP, Moore A, Li, R. Activins, inhibins, and follistatins: further thoughts on a growing family of regulators. Proc Exp Biol Med. 1997;215:209-222. 10.3181/00379727-215-44130
16. Andreone L, Velasquez EV, Abramovich D, Ambao V, Loreti N, Croxatto HB, Parborell F, Tesone M, Campo S. Regulation of inhibin/activin expression in rat early antral follicles. Mol Cell Endocrinol. 2009;309:48-54. 10.1016/j.mce.2009.05.003
17. Woodruff AK, Krummen LA, Lyon RJ, Stocks DL, Mather JR. Recombinant human inhibin A and recombinant human activin A regulate pituitary and ovarian function in the adult female rat. Endocrinol. 1993;132:2332-73. 10.1210/endo.132.6.8504739
18. Wise T. 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. 1987;64: 1153-1169. 10.2527/jas1987.6441153x
19. Luna LG. Manual of histological staining methods. 3rd ed. Sydney: McGraw Hill Book Company; 1968. 258 p.
20. Schefler WC. Statistics for biological sciences. 2nd ed., Addison-Wesley Publ. co., Reading MA, USA. 230 p.
21. Hillier SG, Yong E, Illingworth PJ, Baird DT, Schwall RH, Mason AJ. Effect of recombinant activin on androgen synthesis in cultured human thecal cells. J Clin Endocrinol Metab. 1991;72:1206-1211. 10.1210/jcem-72-6-1206
22. Rozell TG, Okrainetz RJ. FSH: one hormone with multipule forms, or a family of multiple hormones. In: Chedrese ED (editor). Reproductive endocrinology, A molecular approach. Saskatoon: Springer Science and Business Media; 2009. 144-160 p. 10.1007/978-0-387-88186-7_14
23. Bhardwaj A, Nayan V, Parvati M, Gupta AK. Inhibin: A role for fecundity augmentation in farm animals. Asian J Anim Vet Adv. 2012;7:771-189. 10.3923/ajava.2012.771.789
24. Al-Sa'aidi JA, Al-Jayashi GS. Adenohypophyseal immunohistochemical expression levels of FSHβ in cyclic virgin female rats treated with steroid free bovine follicular fluid antiserum. Online Int Interdicip Res J. 2016;6:18-31. 10.29079/vol17iss2art502
25. Al-Sa'aidi JA, Al-Jayashi GS, Khafaji SS. Ovarian immunohistochemical expression of estradiol 17β in cyclic female rats treated with steroid free bovine follicular fluid antiserum. Al-Qadisiyah J Vet Med Sci. 2018;17(2):35-42. 10.29079/vol17iss2art502
26. Murthy RM. Effect of *Allium cepa* extracts on the estrous cycle and ovarian activity in albino mice. Plant Med. 1997;20(5):117-120. 10.1590/s0036-46652011000300007
27. Bary GA, Saiduddin S, York DA, Swerdloff RS. Effect of estradiol on uterine weight, thyroid function food intake, and pituitary weight of genetically obese (fatty-Zucker) and lean rats. Proc Soc Exp Biol Med. 1976;153(1):88-91. 10.3181/00379727-153-39486
28. Medlock KL, Forrester TM, Sheehan DM. Progesterone and estradiol interaction in the regulation of rat uterine weight and estrogen receptor concentration. Proc Soc Exp Biol Med. 1994;205(2):146-153. 10.3181/00379727-205-43690
29. Al-Sa'aidi JA, AL-Okaily BN, Al-Shwilly HA. Uterine implantations and litter size alternation in anti- inhibin and eCG-hCG treated virgin pregnant female rats. Online Int Interdicip Res J. 2016;6(1):36-46. 10.29350/jops.2018.23.2.732
30. Bley MA, Simon JC, Estevez AG, de Asua LJ, Baranao JL. Effect of follicle stimulating hormone on insulin-like growth factor- I-stimulated rat granulosa cell deoxyribonucleic acid synthesis. Endocrinol. 1992;131:1223-1229. 10.1210/en.131.3.1223
31. Duleba AJ, Spaczynski RZ, Olive DL, Behrman HR. Effects of insulin and insulin-like growth factors on proliferation of rat ovarian theca-interstitial cells. Biol Reprod. 1997;56:891-897. 10.1095/biolreprod56.4.891
32. Findlay JK, Drummond AE, Dyson M, Baillie A, Ethier JF. Production and actions of inhibin and activin during folliculogenesis in the rat. J Mol Cell Endocrin. 2001;180:139-144. 10.1016/s0303-7207(01)00521-4
33. Al-Sa'aidi JA, Baqir SM. Effect of passive immunization against inhibin-a subunit and non-steroid bovine follicular fluid (NSBFF) on mammary gland growth and development in primiparous female Wistar rats. Reprod Dom Anim. 2012;47(4):416-613. 10.1111/j.1439-0531.2012.02119.x
34. Kenny HA, Woodruff TK. Follicle size class contributed to distinct secretion patterns of inhibin isoforms during the rat estrus cycle. J Endocrinol. 2005;10:1210-1242. 10.1210/en.2005-0242
35. Ko Y, Choi I, Green ML, Simmen FA, Simmen RC. Transient expression of the cytochrome P450 aromatase gene in elongating porcine blastocysts is correlated with uterine insulin-like growth factor levels during peri-implantation development. Mol Reprod Develop. 1994;37:1-11. 10.1002/mrd.1080370102
36. Kipp JL, Kilen SM, Bristol GS, Woodruff TK, Mayo KE. Neonatal exposure to estrogens suppresses activin expression and signaling in the mouse ovary. J Endocrinol. 2007;148(5):1968-1976. 10.1210/en.2006-1083
37. Medan MS, Watanabe G, Taya K. Inhibin: Regulation of reproductive function and. practical use in females. Anim Sci J. 2007;78:16-27. 10.1111/j.1740-0929.2006.00399.x
38. Al-Sa'aidi JA, Samir MS. Effect of passive immunization against inhibin alpha subunit on ovarian growth and development in immature female Wister rats. 14th Scientific Congress of Faculty of Medicine; 2010, Assiut, Egypt.
39. Al-Sa'aidi JA, Al-Okaily BN, Al-shwilly HA. Comparative effect of anti-inhibin and eCG-hCG supplementation on reproductive hormonal profile in virgin cycling female rats. Al-Qadisiyah J Pure Sci. 2018;23(2):1-8. 10.29350/jops.2018.23.2.732
40. Al-Sa'aidi JA, Al-Asadi SH. Quantification of mammary Gh-r gene and GH expression in pregnant, delivered and lactating Wister female rats passively immunized at pregnancy against inhibin-α, βa, or βb subunits. Int J Biol Pharm Allied Sci. 2016;5(9):2070-2083. https://ijbpas.com/pdf/2016/September/1472702680MS

41. Al-Sa'aidi JA, ad Thanoon HB. Hypothalamic GHRH and pituitary GH gene expression levels in neonatal inibin immunoneutralized female rats. Aspire. The 5th Congress of the Asia Pacific Initiative on Reproduction; 2014, Brisbane, Australia.
42. Al-Sa'aidi JA, Al-Kalby JM, Al-Saeedi MJ. Effect of passive immunization against inhibin-A, -BA and -BB subunits on serum growth and differentiation hormones profile in pregnant and lactating primiparous Wister rats. Basrah J Vet Res. 2015;14(1):79-96. 10.33762/bvetr.2015.99877
43. Demeestere I, Gervy C, Centner J, Devreker F, Delbaera A. Effect of insulin like growth factor-1 during preantral follicular culture on steroidogenesis, in vitroin oocyte maturation and embryo development in mice. Biol Reprod. 2004;70:1664-1669. 10.1095/biolreprod.103.023317