IJBPAS, September, 2016, 5(9): 2070-2083

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

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

JABBARA.A.AL-SA'AIDI[1*] AND AL-ASADI, S.H. JARI[2]

1: Prof. Dr., Dept. Physiology and Pharmacology, College of Pharmacy, Al-Qadisiya University, Iraq

2: Head, Dept. Physiology and Pharmacology, College of Vet. Med., Al-Qadisiya University, Iraq

***Corresponding Author: E Mail: jbr20042002@yahoo.com. Mobile: +964-780-1156473**

Received 28th May 2016: Revised 20th June 2016: Accepted 30th July 2016: Available online 1st Sept. 2016

ABSTRACT

The present study has been designed to examine the effect of inhibin- α, βa, or βb isotypes immune-neutralization at pregnancy on the expression levels of mammary *GH-r* gene and GH at pregnancy, delivery and lactation in female Wistar rats. Eighty four pregnant rats were randomly assigned to four equal groups (21 each). On 5th and 10th day of gestation, the females were injected with saline (100µl, *i.p.*) (Control group), inhibin-α antiserum (1µg in 100µl of saline, *i.p.*) (Ta group), inhibin-βa antiserum (1µg in 100µl of saline, *i.p.*) (Tba group), inhibin-βb antiserum (1µg in 100µl of saline, *i.p.*) (Tbb group). Each group was allocated to equal pregnancy, delivery, and lactation subgroups. Females were sacrificed on the 16th day of gestation, the 1st day after parturition, and the 11th day of lactation, respectively. Samples from inguinal mammary gland from each female were obtained for the evaluation of expression levels of *GH-r* gene and GH using real time polymerase change reaction and immunohistochemistry, respectively. Result of mammary GH expression during pregnancy and delivery increased significantly in Ta than Tba and Tbb groups, whereas Tba was significantly higher than Tbb group. *GH-r* gene expression during pregnancy and delivery registered significant elevation in Ta group. At lactation, no significant difference has been shown in treated groups. In conclusion, the upregulation of GH and/or GH-r due to immunoneutralization of inhibins subunits, during pregnancy, have potent role as an augmented factor for lactation.

Keywords: Pituitary, mammary gland, GH, GH-r

INTRODUCTION

Ovarian follicular advancement and relapse relies on upon various endocrine, paracrine, and autocrine signals (Tesone et al., 2009). Follicle stimulating hormone (FSH) synthesized by pituitary gonadotrophs have basic direct action in the female reproductive system, including growth, division, and development of granulosa cells, creation of female gametes, and in addition generation of hormones (estradiol and inhibin) that impact in the control of FSH secretion from the pituitary (Rozell and Okrainetz, 2009).

The transforming growth factor-β (TGF-β) family include TGF-β1, TGF-β2, TGF-β3, activins (A, B, and AB), inhibins (A and B), and anti-mullerian hormone (AMH). Inhibins are an important regulator from which, both in male and female reproduction. In females, inhibins are delivered fundamentally in ovarian granulosa cells and follows up on oocyte theca cells for direction of cell proliferation (Rozell and Okrainetz, 2009). Inhibins join in the control of estrus cycle by controlling pituitary FSH discharge. This control is prepared by a feedback mechanism, since FSH induces the development of ovarian follicles, where granulosa cells produce inhibins (de Kretser et al., 2002).

Growth hormone, GH is a polypeptide hormone, synthesized by somatotrophs in the anterior pituitary gland. GH is a member of family, which includes prolactin and placental lactogen, both of which have impacts in female reproduction (Neville et al., 2002).The import role of GH in enhancing postnatal growth and lactogenesis is well studied by many researchers in different species. However, the more important thing is the requirement of GH and PRL in stimulating fetal growth and mammogenesis during pregnancy (Antony et al., 1998).Numerous studies pointed out on the role for GH in the production of viable gametes, since GH participates, in the early ovarianfolliculogenesis in the absence of gonadotrophin action as well as its role at the late folliculogenesisin the presence of gonadotrophin action, by increasing cell proliferation and inhibiting atresia, and therefor influencing fertility (Miyazono*et al.*,2010).Therefore pituitary somatotrophsand GHRH number and maturation, as well as the level of GH secretion are the main determinants responsible for the efficient output of an adult mammalian pituitary secretion and thus function of gonads and extra-gonad organs concerned with female reproduction (Griswold and McLean, 2006), sinceGH attributes in gonadal steroidogenesis, gametogenesis and ovulation. However, as GH

is also produced in gonadal, placental and mammary tissues, it has been suggested that GH has a paracrine or autocrine action in the regulation of local processes that are strategically regulated by pituitary GH (Hull and Harvey, 2001).Pituitary GH induces the production of hepatic IGF-I, and both pituitary GH and hepatic IGF-I act to regulate ovarian function. Therefore, pituitary GH and hepatic IGF-I may be involved in strategic maintenance of ovarian function, whereas ovarian GH may be involved in emergency modulation of ovarian function (Hull and Harvey, 2001). Due to these direct endocrine actions, of pituitary GH, or its mediation through induction of hepatic or local IGF-I production, the present study has been conducted to investigate the impact of inhibin-α, βa, or βb isotypesimmunoneutralization at pregnancy on the expression levels of mammary *GH* and *GH-r* genes at pregnancy, delivery and lactation as well as its role in mammary gland growth and development in female Wistar rats

MATERIALS AND METHODS

Preparation of Inhibin Subunits Antiserum 1%: Inhibin-α, βA, and βB antiserum (1µg/100µl) were prepared according to the manufacture instructions (ABO, Switzerland).

Experimental Animals: Primiparous female Wistar rats (average weight was 146±5.8g. and age was 75 days), were allowed one week to acclimatize to the animal house environment before beginning of experiment. The animals were housed under laboratory conditions (12L:12D cycles at 20-22 C°) and fed on standard laboratory food (19% protein ratio and 3000 kilocalories energy) and drinking water *ad libitum*.

Experimental Design: Eighty four female were allowed to mate with experienced males (1 male with 2 females). The appearance of vaginal plug was considered as the first day of pregnancy. The females were randomly divided into 4 groups (21 females per each). On 5^{th} and 10^{th} days of gestation, control (C) females were injected with physiological saline (100µl, *i.p.*), antiinhibin-α group (Ta) females were injected with inhibin-α antiserum (100µl of physiological saline containing 1µg of antiserum, *i.p.*), antiinhibin-βA group (Tba) females were injected with inhibin-βA antiserum (100µl of physiological saline containing 1µg of antiserum, *i.p.*), and antiinhibin-βB group (Tbb) females were injected with inhibin-βB antiserum (100µl of physiological saline containing 1µg of antiserum, *i.p.*). Each group was allocated to three subgroups (7 females per each): subgroup1 (pregnancy) females were sacrificed on the 16^{th} day of gestation, subgroup2 (Delivery) females were sacrificed

on the 1st day after parturition and in subgroup3 (lactation) litters number was modulated as 9 per each dam (Tucker, 1987) and femaleswere sacrificed on the 11th day of lactation. At the end of each treatment and control subgroups period, female rats were anesthetized (by injection of 0.3 ml ketamine + 0.1 ml xylazine/kg body weight, *i.p.*) (Sharpe and LaRegina, 1998), dissected and two samples from inguinal mammary glands (from each female) were obtained. First sample was quickly kept at -80 ºC for the evaluation of mRNA expression levels of GAPDH, as housekeeping gene, and GH receptor (GH-r) gene using quantitative reverse transcriptase-real time PCR based on Syber Green dye, and second samplewas kept in formalin 10% and processed in paraffin blocks for evaluation of GH expression using immunohistochemical technique.

Quantitative Reverse Transcriptase Real-Time PCR: According to the method described by Wang and Hardy (2004), qRT-PCR technique was used for quantification of*GH-r* gene expression levels relative to Housekeeping gene *GAPDH* gene in the mammary gland.

Immunohistochemistry-Paraffin Protocol: According to Luna (1968), histological sections have been prepared from mammary glands. According to the manufacture instructions (Abcam, UK; **www.abcam.com/technical)**, immunohistochemistry has been performed for demonstrating the presence and location of GH in the glandular tissues of inguinal mammary gland sections.

Statistical Analysis: Results were expressed as mean ± standard deviation. Comparisons between groups and periods values were performed using one way analysis of variance (ANOVA1) and newman- keuls. Differences were considered to be significant at the level of $P<0.05$. Statistical analysis was carried out using the GraphPad Prism (SAS Institute, Inc., USA).

RESULTS

RNA Concentration in the Mammary Gland: mammary glands RNA concentration (ng/µl) of experimental groups during pregnancy registered insignificant difference ($p>0.05$) between control and Ta groups but they were siginificantly ($p<0.05$) higher than that recorded in Tba and Tbb groups. At delivery and lactation periods, the concentration of Ta group was the highest among experimental groups ($p<0.05$), where Tba group was significantly ($p<0.05$) higher than Control and Tbb groups which showed no significant difference ($p>0.05$) when compared with each other.

Relative Quantification of Target Gene Expression: The gene expression in control was expressed as (calibrator) or control in both target gene and reference gene (*GapdH*), at first, the threshold cycle number of target gene normalized to that of reference gene in all treatment groups and calibrator. Second, the ΔCt of treatment groups normalized to the ΔCt of calibrator, and finally the expression ratio (fold change) was calculated. In all periods, fold changes were normalized according to control (which is equal to 1).

Relative Quantification of Mammary *GH-r*Gene Expression: Figure (2) illustrates the result of *GH-r* gene expression levels in mammary gland tissues during pregnancy, delivery, and lactation periods. At pregnancy, the results registered siginificant ($p<0.05$) elevation of *GH-r* gene expression levels in Ta group compared with Tba and Tbb groups. At delivery, the highest ($p<0.05$) level of *GH-r* gene expression has been registered in Ta compared with Tba and Tbb groups, where Tbagroup was significantly ($p<0.05$) higher than that of Tbb group. At lactation, statistical analysis showed insiginificant difference ($p>0.05$) between female rats of treated groups.

Results of Mammary GH Immunohistochemical Examination: GH has been detected in normal pregnant rats mammary gland (control group) and passive immunized pregnant rats against inhibin-α, -βA, and βB subunits (Ta, Tba, and Tbb groups, respectively) using immunohistochemical staining technique. At the 16th day of pregnancy (figure 4), the 1st day after parturition (figure 5), and the 11th day of lactation (figure 6). Quantitative scoring results of mammary GH at the 16th day of pregnancy showed significant ($p<0.05$) higher score (number of positive stained cells and intensity of staining) in Ta group among the experimental groups, whereas Tba and Tbb groups, which showed insignificant ($p>0.05$) difference between each other, registered significantly ($p<0.05$) higher scores compared with control. At the 1st day after parturition, the score of the Ta group increased siginificantly ($p<0.05$) when compared with Tbbgroup,which also showed significant ($p<0.05$) difference when compared with control and Tba groups At the 11th day of lactation, Ta and Tbb groups registered insiginificant ($p>0.05$) difference in comparison between them, whereas they recorded siginificant ($p<0.05$) higher score than that of control and Tba groups. In comparison between periods of the experiment, in all experimental groups, higher scores of staining have been shown at the 1st

day after parturition, whereas the lowest scores recorded at the 11th day of lactation (figure 3).

DISCUSSION

During pregnancy, the significant high level of GH expression, recorded in Ta, Tba, and Tbb groups compared with control, could attributed to the decrease in inhibins, as it has been reported by Thanoon (2013) that passive immunization against inhibin-α subunit increases the expression levels of hypothalamic GHRH and pituitary GH genes. The significantly higher expression of GH in Ta group compared with Tba and Tbb groups, could be a result of immunoneutralization of both types of inhibins (A and B), whereas immunization against βA (Tba group) and βB (Tbb group) subunits neutralized either inhibin-A or inhibin-B, respectively. At delivery, all groups recorded significant elevation in GH expression levels compared with their corresponding levels at pregnancy, with the remaining of differences in Ta and Tbb groups, whereas the concentration in Tba group recorded no significant difference compared with control. It seems that Inhibin-A protein is more important in regulating GH secretion than inhibin-B. Also the immunization against βB subunit could decrease the levels of activin-B and activin-AB but increase the level of activin-A. Therefore, activin-A decreases the secretion of GH, as it has been registered that activin-A being able to inhibit basal GH secretion from pituitary gland (Vale *et al.*, 1986; Plotsky*et al.*, 1991; Bilezikjian*et al.*, 1991). During this period, the elevated GH may attributes to the increase in serum estradiol secretion at the end of gestation (Armario*et al.*, 1984; Simard*et al.*, 1986). At mid lactation, the levels of GH expression decreased sharply in all groups, which accompanied by significant increase in inhibin-B, activin-A, and activin-AB, and may also attributes to the decrease of estradiol (Shaikh, 1971; Thanoon, 2013). Ta groups, at all experimental period, revealed higher levels of GH expression levels among experimental groups which may attribute to the increase of activins and estradiol levels after immunoneutralization against inhibin-α subunit, since subsequent stages of postnatal development (pubertal growth, pregnancy, lactation, and involution) occur under the regulation of hormones, as branching morphogenesis requires GH and estrogen, as well as insulin-like growth factor 1 (IGF1), whereas upon pregnancy, the combined actions of progesterone and prolactin required for alveogenesis and therefor milk secretion during lactation. Pregnancy is considered as a period of allometric growth, keeping up with overall body development, until puberty when expansive proliferation occurs, filling the fat

pad under the influence of hormones and growth factors (Macias and Hinck, 2012). It has been reported that pituitary extracts regulate mammary gland function by enhancing mammogenesis and lactogenesis upon its administration (Trott*et al.*, 2008). These effects could be attributed to the effects of GH and prolactin. Other studies demonstrated that mammary pubertal development was disrupted in mice lacking *Gh*, insulin-like growth factor 1 (*Igf1*) or estrogen receptor (α) (*Esr1*), genes that mediate pathways regulating ductal outgrowth and morphogenesis. In contrast, development of the adolescent gland occurs normally in mice lacking *Prl*or progesterone receptor (*Pgr*), genes that mediate signaling pathways regulating alveologenesis (Zhou *et al.*, 1997; Gallego*et al.*, 2001).

The present findings confirmed the indirect role of GH as an important regulator of mammary gland growth and development, which could be mediated through IGF1, as *Ghr*-depleted mice display a dramatic reduction in serum IGF1 levels (Zhou *et al.*, 1997) and delayed mammary gland development (Gallego*et al.*, 2001). Although circulating levels of IGFs are primarily determined as a result of hepatic secretion under the control of the GH (LeRoith*et al*.,2001), they also are synthesized locally in various tissues including ovaries (Tesone et al., 2009), where IGF-I acts as a co-gonadotropin with FSH to stimulate granulosa cells to produce estrogen and progesterone , and with LH to stimulate thecal androgen production. On the other hand, the locally produced IGF-I in ovary has other actions including enhancement of cell proliferation, aromatase activity, and progesterone biosynthesis (Adashi*et al.*, 1985). Supplementation of bovine embryo culture with IGF1 has been reported to increase rate of blastocyst development, embryo survival as well as reducing the effects of heat shock on embryos (Bonilla *et al.*, 2011).

From the present observations, it appears that increased GH expressions has been augmented by the high level of activins to perform its proliferative action, as it has been reported that activin A promotes embryo development, by increasing blastocyte number, reaching time taking to reach blastocyte stage rate (Orimo *et al.*, 1996; Yoshioka *et al.*, 1998; Mtango*et al.*, 2003). Previous studies demonstrate that inhibin immunonutralization could improve litter sizes (Medan *et al.*, 2005; Padilla *et al.*, 2007). It has been mentioned that activins have an important role in development of mammary gland during pregnancy and in preparation for lactation (Bloise*et al.*, 2010), where at late pregnancy, activin inhibits adipocyte

development (Zamani and Browm, 2010). Also the ovarian actions of GH are partly mediated by changes in ovarian steroidogenesis, where GH enhances aromatase and 3-β-hydroxysteroid dehydrogenase activity (Tapanainen*et al.* 1992). GH may induce steroidogenesis by potentiating gonadotrophin action, since GH only stimulates oestradiol production from follicles (Van der Kraak*et al.* 1990). Other studies indicate that GH and gonadotrophins act synergistically to increase oestradiol synthesis in granulosa cells (Carlsson*et al.* 1992, Lanzone*et al.* 1992). This synergy may reflect upregulation of gonadotrophin receptors by GH or the upregulation of GHRs by gonadotrophin induced cAMP (Adashi*et al.* 1994).

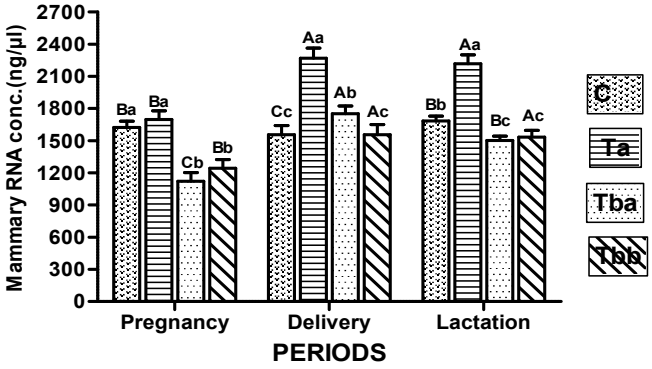

Figure (1): Effect inhibin-α, -βa, and –βb subunits immunoneutralization on Mammary RNA conc. (ng/µl) during pregnancy, delivery, and lactation in pregnant rats.

Values representsmean±standard deviation. Different small letters represents significancy (p<0.05) in comparison between groups. Different capital letters represents significancy (p<0.05) in comparison between periods. C: pregnant rats injected with normal saline (100 µl, *ip*) on 5th and 10th day of pregnancy. Ta: pregnant rats injected with inhibin-α antiserum (1µg, *ip*) on 5th and 10th day of pregnancy. Tba: treated rats injected with inhibin-βa subunit antiserum (1µg ,*ip*) on 5th and 10th day of pregnancy. Tbb: treated rats injected with inhibin-βb subunit antiserum (1µg, *ip*) on 5th and 10th day of pregnancy.

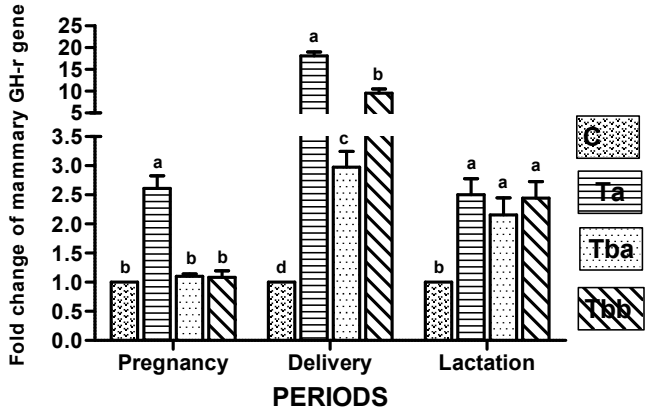

Figure (2): Effect of inhibin-α, -βa, and –βb subunits immunoneutralization on mammary *GH-r* gene expression level during pregnancy, delivery, and lactation in pregnant rats (fold changes).

Values representsmean±standard deviation. Different small letters represents significancy (p<0.05) in comparison between groups. Different capital letters represents significancy (p<0.05) in comparison between periods. C: pregnant rats injected with normal saline (100 µl, *ip*) on 5th and 10th day of pregnancy. Ta: pregnant rats injected with inhibin-α antiserum (1µg, *ip*) on 5th and 10th day of pregnancy. Tba: treated rats injected with inhibin-βa subunit antiserum (1µg ,*ip*) on 5th and 10th day of pregnancy. Tbb: treated rats injected with inhibin-βb subunit antiserum (1µg, *ip*) on 5th and 10th day of pregnancy.

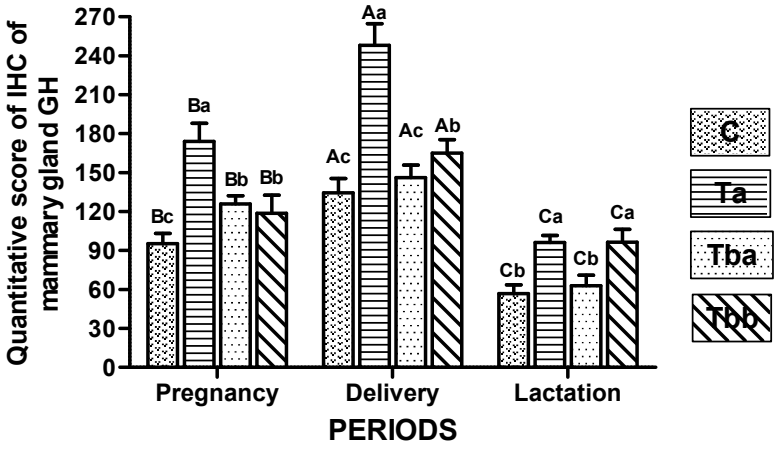

Figure (3): Effect of inhibin-α, -βa, and –βb subunits immunoneutralization on quantitative score of IHC of mammary gland GH during pregnancy, delivery, and lactation in pregnant female rats.
Values represents mean±standard deviation. Different small letters represents significancy ($p<0.05$) in comparison between groups. Different capital letters represents significancy ($p<0.05$) in comparison between periods. C: pregnant rats injected with normal saline (100 µl, *ip*) on 5th and 10th day of pregnancy. Ta: pregnant rats injected with inhibin-α antiserum (1µg, *ip*) on 5th and 10th day of pregnancy. Tba: treated rats injected with inhibin-βa subunit antiserum (1µg ,*ip*) on 5th and 10th day of pregnancy. Tbb: treated rats injected with inhibin-βb subunit antiserum (1µg, *ip*) on 5th and 10th day of pregnancy.

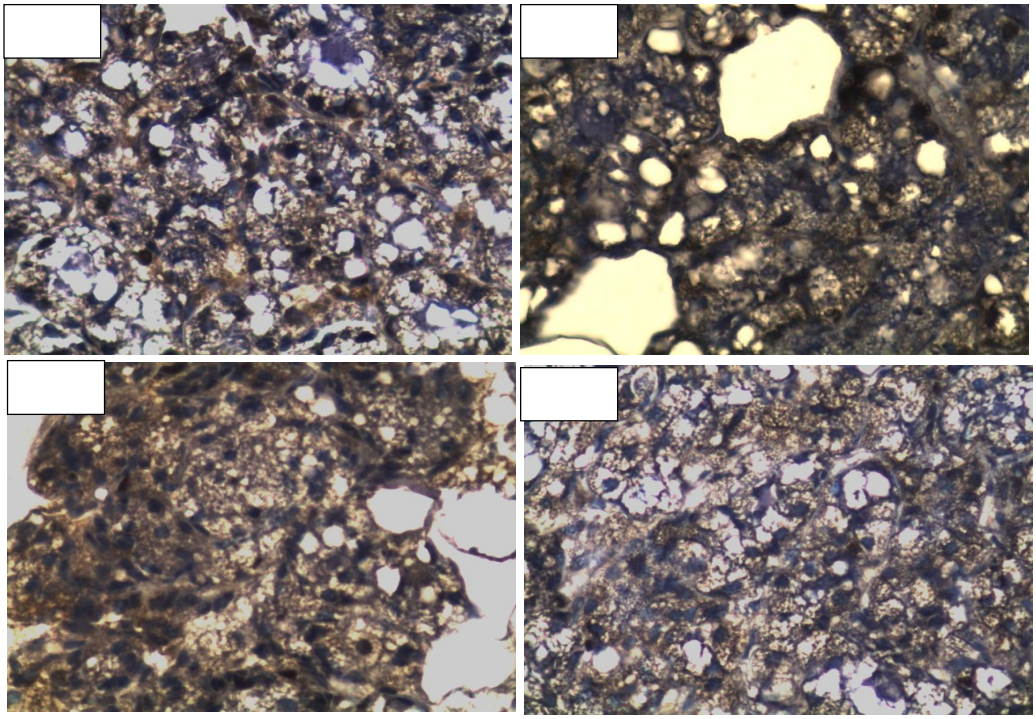

Figure (4) Mammary gland from control (C) and inhibin-α (Ta), inhibin-βA (Tba), and inhibin βB (Tbb) antisera injected female rats, at 16th day of pregnancy reveals the density of actively staining GH with immunohistochemistry. Immunohistochemistry, 500X.

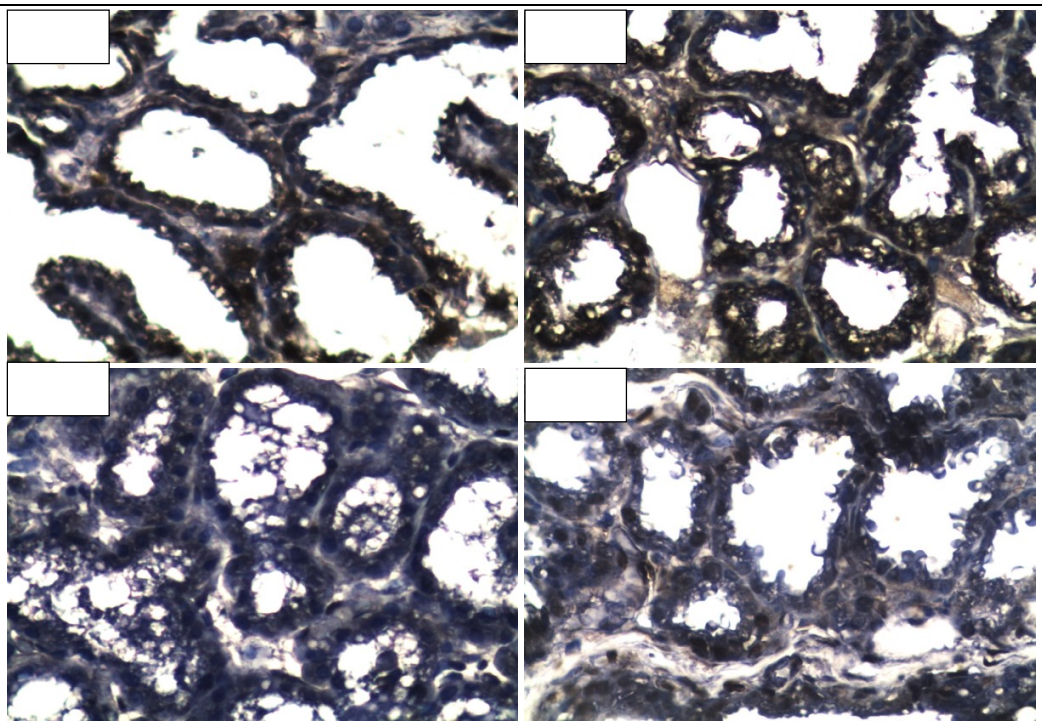

Figure (5) Mammary gland from control (C) and inhibin-α (Ta), inhibin-βA (Tba), and inhibin βB (Tbb) antisera injected female rats, at 1st day after parturition reveals the density of actively staining GH with immunohistochemistry. Immunohistochemistry, 500X.

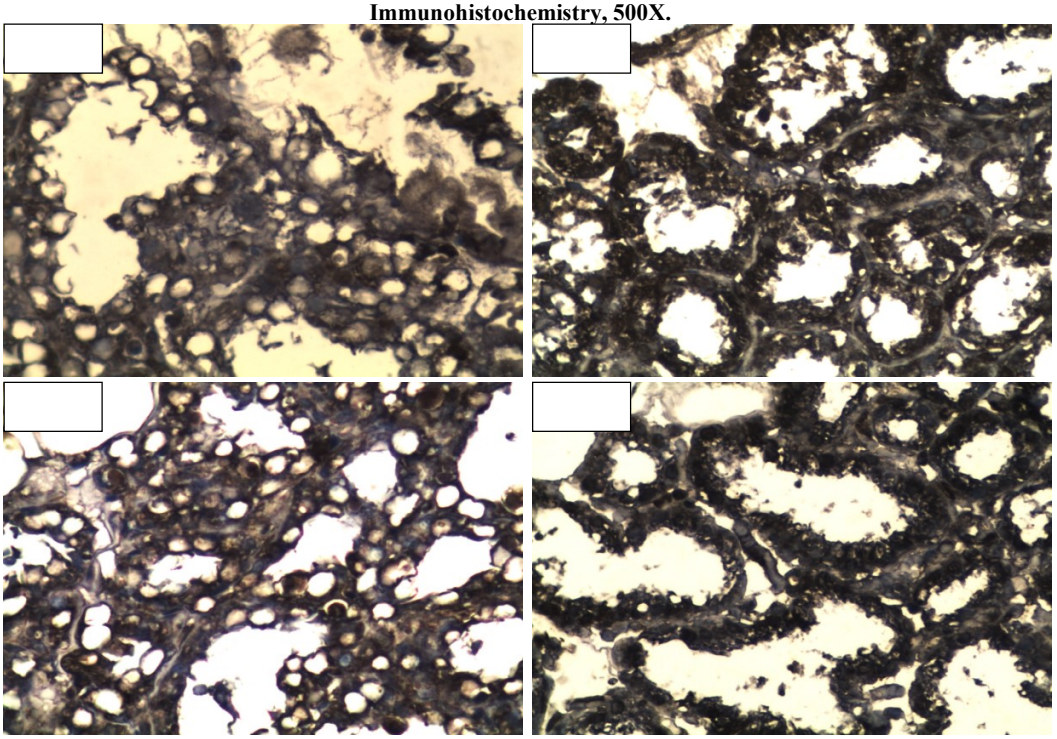

Figure (6) Mammary gland from control (C) and inhibin-α (Ta), inhibin-βA (Tba), and inhibin βB (Tbb) antisera injected female rats, at 11th day of lactation reveals the density of actively staining of GH with immunohistochemistry (red arrows). Immunohistochemistry, 500X.

REFERENCES

[1] Adashi EY, Thorner MO, and Jaffe RB (1994).The somatotrophic axis and the reproductive process in health and disease [editorial]. Fertility and Sterility, 61: 1014–1015.

[2] Adashi EY, Resnick CE, D'Ercole J, Svoboda ME, and Van Wyk JJ. (1985). Insulin-like growth factors as intraovarian regulators of granulosa cell growth and function. Endocrine Reviews, 6: 400-420.

[3] Anthony RV, Limesand SW, Fanning MD, and Liang R. (1998).Placental Lactogen and Growth Hormone: Regulation and Action, Chapter 16, Endocrinology of Pregnancy, Volume 9 of the series Contemporary Endocrinology. Humana Press, Springer Science and Business Media, New York, pp 461-490.

[4] Armario A, Castellanos JM, and Balash J. (1984).Dissociation between corticosterone and growth hormone adaptation to chronic stress in the rat. Hormone and Metabolic Res., 16: 142-145.

[5] Bilezikjian LM, Blount AL, Campen CA, Gonzalez-Manchon C, and Vale W. (1991). Activin A inhibits proopiomelanocortinmessengersRNA accumulation and adrenocorticotropin secretion of AtT20 cells. Mol. Endocrinol., 51: 1389-1395.

[6] Bloise E, Cassali GD, Ferreira MC, Ciarmela P, Petraglia F, and Reis FM. (2010).Activin-related proteins in bovine mammary gland: localizationand differential expression during gestational development anddifferentiation. J. Dairy Sci., 93(10):4592–601.

[7] Bonilla AQ, Ozawa M, and Hansen PJ. (2011). Timing and dependence upon mitogen-activated protein kinase signaling for pro-developmental actions of insulin-like growth factor 1 on the preimplantation bovine embryo. Growth Horm. IGF Res., 21: 107–111.

[8] Carlsson B, Bergh C, Bentham J, Olsson JH, Norman MR, Billig H, Roos P, and Hillensjo T. (1992). Expression of functional growth hormone receptors in human granulosa cells. Human Reprod., 7: 1205–1209.

[9] deKretser DM, Hedger MP, Loveland KL, and Philips DJ. (2002). Inhibins, activins and follistatin in reproduction. Hum. Reprod. Update, 8: 529-541.

[10] Neville MC, McFadden TB, and Forsyth I. (2002).Hormonal

regulation of mammary differentiation and milk secretion. J. Mammary Gland Biol. Neoplasia, 7(1): 49-66.

[11] Gallego MI, Binart N, Robinson GW, Okagaki R, Coschigano KT, Perry J, Kopchick JJ, Oka T, Kelly PA, and Hennighausen L. (2001). Prolactin, growth hormone, and epidermal growth factor activate Stat5 indifferent compartments of mammary tissue and exert different and overlapping developmental effects. Dev. Biol., 229:163–175.

[12] Griswold MD, and McLean D. (2006). The Sertoli cells. In: Knobil and Neill's Physiology of Reproduction. 3rded, by: Jimmy D. Neill (ed.), Elsevier, New York. P: 949-976.

[13] Hull KL, and Harvey S. (2001). Growth hormone: roles in female reproduction K L Hull and S Harvey; review. J. Endocrinol., 168: 1-23.

[14] Lanzone A, Di Simone N, Castellani R, Fulghesu AM, Caruso A, and Mancuso S. (1992). Human growth hormone enhances progesterone production by human luteal cells *in vivo*: evidence of a synergistic effect with human chorionic gonadotropin. Fertility and Strelity, 57: 92-96.

[15] LeRoith D, Bondy C, Yakar S, Liu JL, Butler A. (2001). The somatomedin hypothesis: Endocr. Rev., 22: 53-74.

[16] Luna LG. (1968). Manual of histological staining method .3rd ed. Mc. Graw Hill.Book.Company, Sydney. Pp:258.

[17] Macias H, and Hinck L. (2012). Mammary gland development. Wiley Interdiscip Rev. Dev. Biol., 4: 533-557.

[18] Medan MS, Watanabe G, Sasaki K, Groome N, Sharawy S, and Taya K. (2005). Follicular and hormonal dynamics during the estrous cycle ingoats. J. Reprod. Dev., 51: 455-463.

[19] Miyazono K, Kamiya Y, Morikawa M. (2010). Bone morphogenetic protein receptors and signal transduction. J. Biochem., 147: 35-51.

[20] Mtango NR, Varisanga MD, Dong YJ, Rajamahendran R, and Suzuki T. (2003). Growth factors and growth hormone enhance in vitro embryo production and post-thaw survival of vitrified bovine blastocysts. Theriogenology, 59: 1393–1402.

[21] Orimo T, Taga M, Matsui H, and Minaguchi H. (1996).The effect of activin-A on the development of mouse preimplantation embryos in vitro. J. Assi. Reprod. Genet., 13: 669–674.

[22] Padilla MA, Shriner D, Vaughan LK, and Tiwari HK.(2007). Problems with genome-wide association studies. Science, 316(5833): 1840–1842.

[23] Plotsky PM, Kjaer A, Sutton SW, Sawchenko PE, and Vale W. (1991). Central activin administration modulates corticotropin-releasing hormone and adrenocorticotropin secretion. Endocrinology, 128:2520-2525.

[24] RozellTG, and Okrainetz RJ. (2009). FSH: One Hormone withMultiple Forms, or a Family of Multiple Hormones (Chapter 14). In Reproductive Endocrinology: A Molecular Approach. By Chedrese, PJ (Editor), Springer, New York, USA.

[25] Shaikh A. (1971). Estrone and estradiol levels in ovarian venous blood from ratsduring the estrous cycle and pregnancy. Biol. Reprod., 5: 297-307.

[26] Sharpe PE, and LaRegina MC. (1998). The laboratory rat.CRC Press. London, New York. PP:115.

[27] Simard J, Hubert J, Hosseinzadeh M, and Labrie F. (1986).Stimulation of growth hormone release and synthesis by estrogens in rat anterior pituitary cells in culture. Endocrinology, 119: 2004-2011.

[28] Tapanainen J, Martikainen H, Voutilainen R, Orava M, Ruokonen A, and Ronnberg L. (1992). Effect of growth hormone administration on human ovarian function and steroidogenic gene expression in granulosa-luteal cells. Fertility and Sterility, 58: 726–732.

[29] Tesone M, Abramovich D, Irusta G, and Parborell F. (2009).Autocrine and Paracrine Regulation of the Ovary (Chapter 21). In: Reproductive Endocrinology: A Molecular Approach. By Chedrese, PJ (Editor), Springer, New York, USA.

[30] Thanoon HB. (2013). Hypothalamic GHRH and pituitary GH genes expression levels in sequential neonatal inhibin immunineutralized female rats. MSc Thesis, Collage of Vet. Med., Al-QadesiyaUniversitu, IRAQ.

[31] Trott JF, Vonderhaar BK, and Hovey RC.(2008). Historical perspectives of prolactin and growth hormone as mammogenslactogens and galactagogues for the future. J. Mammary Gland Biol. Neoplasia, 13:3–11.

[32] Tucker HA. (1987). Quantitative estimation of mammary growth during various physiological states: a review. J. Diary Sci.,70:1958.

[33] Vale W, Rivier J, Vaughan J, McClintock R, Corrigan A, and Woo W. (1986). Purification and characterization of an FSH releasing protein from porcine ovarian follicular fluid. Nature, 321: 776-779.

[34] Van der Kraak G, Rosenblum PM, and Peter RE.(1990). Growth hormone-dependent potentiation of gonadotropin-stimulated steroid production by ovarian follicles of the goldfish. Gen. Comp. Endocrinol., 79: 232–239.

[35] Yoshioka K, Suzuki C, and Iwamura S. (1998).Activin A and follistatin regulate developmental competence of in vitro-produced bovine embryos. Biol. Reprod., 59:1017–1022.

[36] Zamani N, and Brown CW. (2010). Emerging roles for the transforming growth factor-beta superfamily in regulating adiposity and energy expenditure. Endocr.Rev., 2010.

[37] Zhou Y, Xu BC, Maheshwari HG, He L, Reed M, Lozykowski M, Okada S, Cataldo L, Coschigamo K, and Wagner TE. (1997). A mammalian model for Laron syndrome produced by targeted disruption of the mouse growth hormone receptor/binding protein gene (the Laron mouse). Proc. Natl. Acad. Sci. USA, 94: 13215–13220.