Adenohypophysial Expression Level of *PRL* Gene and Lactotrophs in Pregnant Wister Rats passively Immunized Against Inhibin-α Subunit

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Abstract

Passive immunization against inhibin raised follicle stimulating hormone secretion during the estrous cycle with high estradiol secretion (follicular phase). Multiple ovulations have been induced successfully by immunoneutralization of endogenous inhibin in several species. The present study has been conducted to examine the role of passive immunization against endogenous inhibin-α subunit on pituitary expression level of *PRL* gene and immunohistochemical expression of lactotrophs during pregnancy and lactation in primiparous female Wister rats. Seventy two pregnant females were assigned to 2 groups (36 per each). On the 5^{th} and 10^{th} day of gestation, the control (C) was injected with saline (100µl, *i.p.*) and the treated group (Anti-Inha) was injected with inhibin-α antiserum (1µg in 100µl of saline, *i.p.*). Each group was allocated to 3 equal subgroups: pregnancy, delivery, and lactation, and sacrificed at day 16 of gestation, parturition, and 11^{th} day of lactation, respectively. At the end of each subgroup period, females were anesthetized, dissected and adenohypophysis was removed and kept at -70 Cº for molecular evaluation and others kept in formaline (10%) for immunohistochemistry. Serum PRL concentration showed no significant difference between groups during pregnancy and lactation, whereas delivery period registered significant elevation in Anti-Inhibin group. In comparison between periods, both groups showed higher levels at delivery followed by lactation and pregnancy. Pituitary *PRL* gene expression levels in Anti-Inha group increased significantly compared with control at delivery. Expression of pituitary lactotrophs showed higher number of positive stained cells and intensity of staining in Anti-Inha group at delivery and lactation. In comparison between periods, both groups showed higher score at delivery. In conclusion, passive immunization against inhibin-α subunit at the 5^{th} and 10^{th} day of pregnancy, has a role in PRL secretion in primiparous female rats.

Key words: passive immunization, inhibin, activin, Prolactin, pituitary gland.

Introduction:

Immunization against inhibin has been used to study the physiological role of inhibin. Subsequently, immunoneutralization against endogenous inhibin has been performed to enhance gamete production and fertility such as induction of superovulation in adult cycling rats (Rivier and Vale, 1989; Ishigame et al., 2004) and mice (Medan et al., 2004), increase sperm production in many live stock species including rams (Voge and Wheaton, 2007) and bulls (Martin et al., 1991), enhance oocyte development (Ishigame et al., 2005), and accelerate puberty in immature female rats (Al-Sa'aidi and Samir, 2010).

One of the characteristic features that distinguishes mammals from all other animals accomplishes its unique task of producing and delivering adequate amount of milk from the mammary gland to provide nourishment for newborn after birth. The mammary gland is one of a few organs able to undergo repeated phases of growth, differentiation and regression. At the onset of puberty in the female, the increase in ovarian steroids induces elongation and side-branching of the rudimentary mammary gland ductal system. Some differentiation of the ductal system occurs at this stage, resulting in a compact glandular structure. The gland, then remains relatively inactive until pregnancy (Sternlicht, 2006). Structural differentiation of the mammary gland is directed by hormonal balances and major steps of this differentiation include, sequentially, formation of a lobulo-alveolar structure, appearance of specific secretory activity, hypertrophy of epithelial cells of mammary gland characterized by an intense synthesis and secretion of milk (Delouis *et al.*, 1980). Studies on the hormonal control of mammary gland differentiation have been extensive. Stricker and Grueter (1928) demonstrated the lactogenic potency of rat pituitary extracts. As purified hormones became available, many endocrinologists worked on animals deprived of endocrine glands, but recipient of specific hormonal therapies. Results of these experiments demonstrated the role of the pituitary, gonads, adrenals, and thyroid on mammary gland function (Cowie, 1970; Denamur, 1971). Milk yield generally considered to be limited by mammary size (number of secretory cells) and activity per cell. Considerable research effort has been directed toward enhancing mammary function and augmenting established lactation. In addition, the possibility exists that augmenting mammary gland development (either at puberty or during pregnancy) could increase lactational performance. Because of the exponential nature of mammary gland development observed in most species, relatively small changes in the growth rate of the mammary gland during pregnancy potentially can result in large changes in ultimate mammary size and lactational performance (Sheffield and Anderson, 1985).

The present study aimed to determine the role of passive immunization against endogenous inhibin-α subunit on pituitary expression level of *PRL* gene and immunohistochemical expression of lactotrophs during pregnancy and lactation in primiparous female Wister rats.

Materials and methods

Preparation of Inhibin-α subunit antiserum 1%: Inhibin-α antiserum (1µg/100µl) was prepared according to the manufacture instructions (ABO, Switzerland).

Experimental animals: Sixty five days old mature primiparous female Wister rats, born at the animal house of the College of Veterinary Medicine, Al-Qadisiya University, and reared under controlled conditions (12 L:12 D cycles and ambient temperature at 22 ± 2 °C) and fed on standard laboratory food (19% protein ratio and 3000 kilocalories energy) and drinking water *ad libitum*. Female rats 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. Seventy two pregnant females were randomly assigned to 2 groups (36 per each). On the 5^{th} and 10^{th} day of gestation, control group (C) was injected with saline (100µl, *i.p.*) and the treated group (Anti-Inha) was injected with inhibin-α antiserum (1µg in 100µl of saline, *i.p.*). Each group was allocated to 3 equal subgroups (12 females each): pregnancy, delivery, and lactation, and sacrificed on day 16 of gestation, at parturition, and 11^{th} day of lactation, respectively. 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 anterior pituitary was removed and kept at -70 C° for evaluation of mRNA expression level of *GAPDH* gene as Housekeeping gene and *PRL* gene using quantitative reverse transcriptase Real-Time PCR technique based on Syber Green dye. Other pituitaries have been reserved in the formalin (10%) for immunohistochemical study.

Hormonal assays in blood serum by ELISA technique: According to the manufacturer instructions (ABO Switzerland), PRL concentrations (ng/ml) have been assessed.

Quantitative Reverse Transcriptase Real-Time PCR: qRT-PCR technique was used for quantification of *PRL* gene expression levels relative to Housekeeping gene *GAPDH* gene in the pituitary. This technique was done according to method described by Wang and Hardy (2004).

Immunohistochemistry-Paraffin protocol: According to Luna (1968), histological sections have been prepared from pituitaries. According to the manufacture instructions (Abcam, UK; **www.abcam.com/technical)**, immunohistochemistry has been performed for demonstrating the presence and location of PRL in lactotrpic cells of the pituitaries in tissue sections.

Statistical Analysis: Mean and standard error of the variables included in the present study has been calculated for each group. Student's *t*-test has been performed to test the effect of treatment in each period and one way analysis of variance (ANOVA1) and newman- keuls has been performed to test the effect of periods. Differences were considered to be significant at the level of $P<0.05$. All statistical analysis were carried out using the GraphPad Prism-5.

Results

Serum prolactin concentration: There is no siginificant difference ($p>0.05$) between experimental groups during pregnancy and lactation periods. At delivery, the concentration of Anti-Inha group registered siginificant increase ($p<0.05$) compared with control. In comparison between periods, the concentration in the two experimental groups showed same picture as the significant ($p<0.05$) higher levels have been recorded at delivery period followed by lactation period, whereas the lowest ($p<0.05$) levels recorded at pregnancy period (figure 1).

Molecular analysis:

Quantitative Reverse Transcriptase Real-Time PCR: Data analysis of SYBR®green based reverse transcriptase RT-PCR assay were divided into primer efficiency estimation and relative quantification of *PRL* gene expression normalized by housekeeping gene expression (*GAPDH*).

Primer efficiency estimation: Threshold cycle numbers (Ct) were calculated from amplification plot of real-time PCR detection system, during exponential phase of fluorescent signals of SYBR® green primer of different genes that react with cDNA of rat pituitary mRNA, where, the amount of PCR product (DNA copy numbers) in master mix reaction is approximately doubles in each PCR cycle. First, series dilution of pituitary cDNA of control group was prepared, this concentrations was used with the primer of *PRL* gene to form the amplification plot of each gene and then from this amplification plot threshold cycle (Ct) was used to calculate a linear regression based on the data points, and inferring the efficiency of the primer from the slope of the line.

Relative quantification of target gene expression: The $2^{\wedge-\Delta\Delta Ct}$ livak and Schmittgen method was used by normalize gene expression of target gene with expression of housekeeping gene (*GAPDH*) as reference gene. 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 was normalized to that of reference gene in treatment group and calibrator. Second, the ΔCt of treatment group 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 pituitary *PRL* gene expression: Figure (2) demonstrates the levels of *PRL* gene expression in the pituitary during pregnancy, delivery, and lactation. The expression level during pregnancy and lactation showed no difference ($p>0.05$) between groups. At delivery, Anti-Inha group recorded significant ($p<0.05$) elevation of pituitary *PRL* gene expression levels.

Immunohistochemical analysis: The lactotrophic cells have been detected in control and passive immunized pregnant rats anterior pituitary using IHC staining technique, at 16 day of pregnancy (figure 4), parturition (figure 5), and mid lactation (figure 6). Quantitative scoring results of pituitary lactotrophs in treated group at 16 day of pregnancy (table 1), parturition (table 2), and mid lactation (table 3) recorded significant increase ($p<0.05$) compared with control (figure 3).

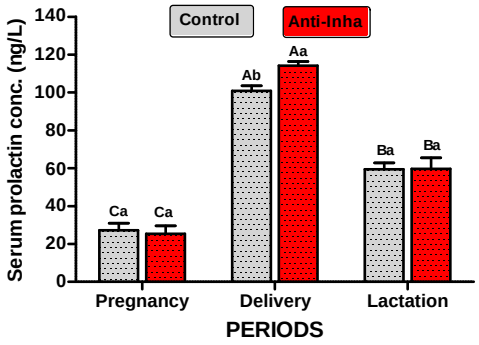

Figure (1): Effect of passive immunization against inhibin-α subunit on serum prolactin concentration (ng/L) during pregnancy, delivery, and lactation in pregnant rats.

Values represents mean±standard error. 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. Anti-Inha: pregnant rats injected with inhibin-α antiserum (1µg, *ip*) on 5th and 10th day of pregnancy.

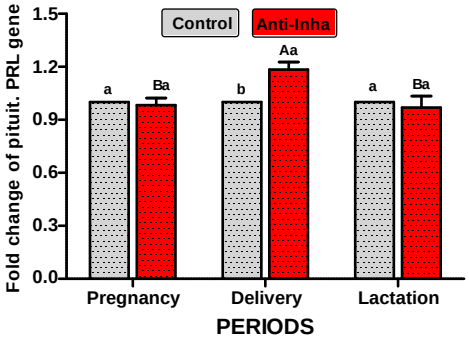

Figure (2): Effect of passive immunization against inhibin-α subunit on pituitary *PRL* gene expression level (fold changes) during pregnancy, delivery, and lactation in pregnant rats.

Values represents mean±standard error. 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. Anti-Inha: pregnant rats injected with inhibin-α antiserum (1µg, *ip*) on 5th and 10th day of pregnancy.

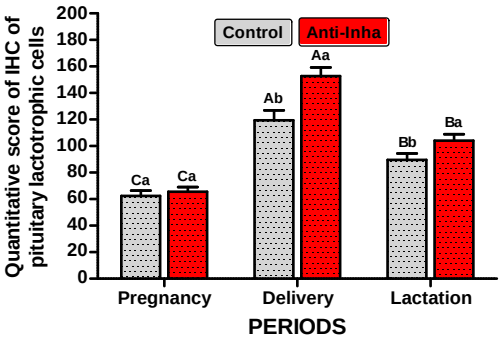

Figure (3): Effect of passive immunization against inhibin-α subunit on quantitative score of IHC of pituitary lactotrophic cells during pregnancy, delivery, and lactation in pregnant female rats.

Values represents mean±standard error. 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. Anti-Inha: pregnant rats injected with inhibin-α antiserum (1µg, *ip*) on 5th and 10th day of pregnancy.

Table (1): Qualitative scoring of IHC of pituitary lactotrophic cells at the 16th day of pregnancy.

Score		0	1+	2+	3+	Q = P*I
Positive Cells (P)		<10%	10-25%	25-50%	50-75%	
Score		1	2	3		
Staining Intensity (I)		weak	Moderate	strong		
C-1	P			35		35*2=70
	I		2			
C-2	P		24			24*2=48
	I		2			
C-3	P			29		29*2=58
	I		2			
C-4	P			38		38*2=76
	I		2			
C-5	P			30		30*2=60
	I		2			
Mean ± S.E.						62.4±5.45 a
Anti-Inha-1	P			30		30*2=60
	I		2			
Anti-Inha-2	P		21			21*3=63
	I			3		
Anti-Inha-3	P			35		35*2=70
	I		2			
Anti-Inha-4	P			29		29*2=58
	I		2			
Anti-Inha-5	P			38		38*2=76
	I		2			
Mean ± S.E.						65.4±3.74 a

Values represents mean±standard error.
Different small letters represents significancy ($p<0.05$) in comparison between groups.
C: pregnant rats injected with normal saline (100 µl, *ip*) on 5th and 10th day of pregnancy.
Anti-Inha: pregnant rats injected with inhibin-α antiserum (1µg, *ip*) on 5th and 10th day of pregnancy.

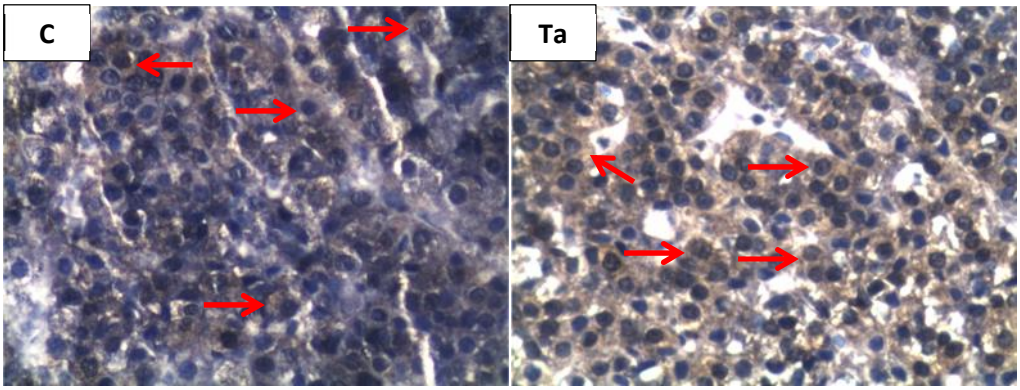

Figure (4) Pituitary from control (C) and inhibin-α (Anti-Inha) antisera injected female rats at the 16th day of pregnancy reveals actively staining of lactotrophic cells with immunohistochemistry (red arrows). Immunohistochemistry, 500X.

Table (2): Qualitative scoring of IHC of pituitary lactotrophic cells at the 1st day after parturition.

Score		0	1+	2+	3+	Q = P*I
Positive Cells (P)		<10%	10-25%	25-50%	50-75%	
Score		1	2	3		
Staining Intensity (I)		weak	Moderate	strong		
C-1	P				55	55*2=110
	I		2			
C-2	P				58	58*2=116
	I		2			
C-3	P				55	55*2=110
	I		2			
C-4	P				51	51*2=102
	I		2			
C-5	P				53	53*3=159
	I			3		
Mean ± S.E.						119.4±10.26 b
Anti-Inha-1	P			48		48*3=144
	I			3		
Anti-Inha-2	P				60	60*3=180
	I			3		
Anti-Inha-3	P				55	55*3=165
	I			3		
Anti-Inha-4	P				70	70*2=140
	I		2			
Anti-Inha-5	P				65	65*2=130
	I		2			
Mean ± S.E.						151.8±9.51 a

Values represents mean±standard error.
Different small letters represents significancy ($p<0.05$) in comparison between groups.
C: pregnant rats injected with normal saline (100 µl, *ip*) on 5th and 10th day of pregnancy.
Anti-Inha: pregnant rats injected with inhibin-α antiserum (1µg, *ip*) on 5th and 10th day of pregnancy.

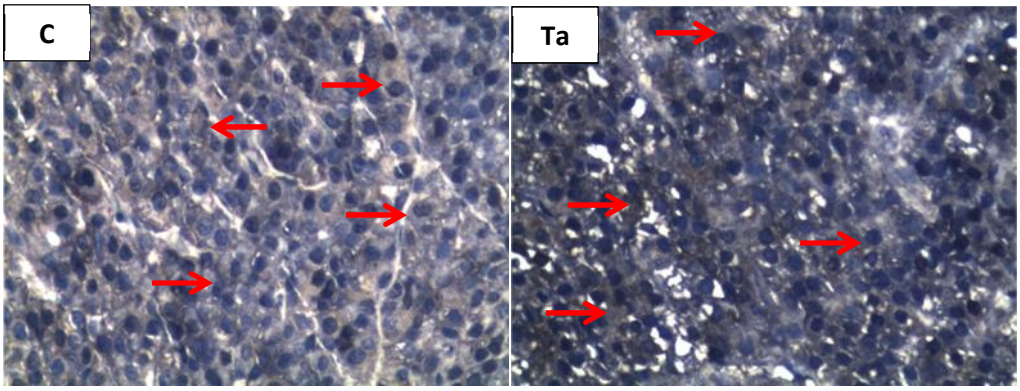

Figure (5) Pituitary from control (C) and inhibin-α (Anti-Inha) antisera injected female rats at the 1st day after parturition reveals actively staining of lactotrophic cells with immunohistochemistry (red arrows). Immunohistochemistry, 500X.

Table (3): Qualitative scoring of IHC of pituitary lactotrophic cells at the 11th day of lactation.

Score		0	1+	2+	3+	Q = P*I
Positive Cells (P)		<10%	10-25%	25-50%	50-75%	
Score		1	2	3		
Staining Intensity (I)		weak	Moderate	strong		
C-1	P				51	51*2=102
	I		2			
C-2	P			43		43*2=86
	I		2			
C-3	P				50	50*2=100
	I		2			
C-4	P			35		35*2=70
	I		2			
C-5	P			40		40*2=80
	I		2			
Mean ± S.E.						87.6±6.01 b
Anti-Inha-1	P				65	65*2=130
	I		2			
Anti-Inha-2	P			45		45*2=90
	I		2			
Anti-Inha-3	P			35		35*3=105
	I			3		
Anti-Inha-4	P			45		45*2=90
	I		2			
Anti-Inha-5	P				58	58*2=116
	I		2			
Mean ± S.E.						106.2±7.83 a

Values represents mean±standard error.
Different small letters represents significancy ($p<0.05$) in comparison between groups.
C: pregnant rats injected with normal saline (100 µl, *ip*) on 5th and 10th day of pregnancy.
Anti-Inha: pregnant rats injected with inhibin-α antiserum (1µg, *ip*) on 5th and 10th day of pregnancy.

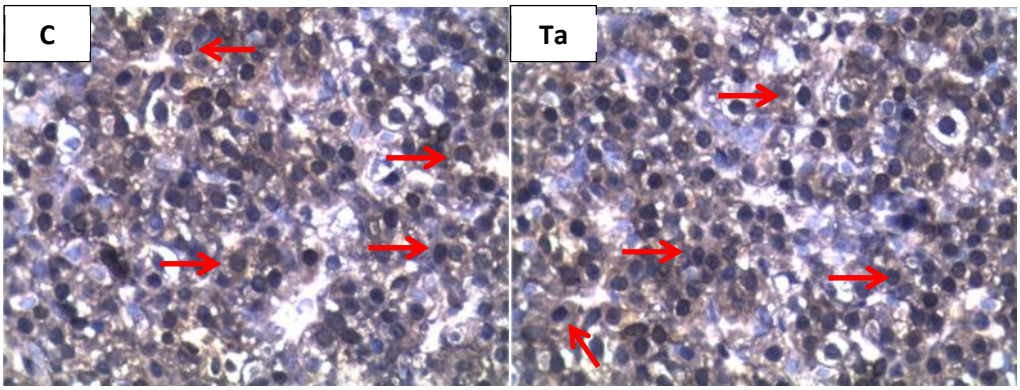

Figure (6) Pituitary from control (C) and inhibin-α (Anti-Inha) antisera injected female rats at the 11th day of lactation reveals actively staining of lactotrophic cells with immunohistochemistry (red arrows). Immunohistochemistry, 500X.

Discussion

During pregnancy, the level of inhibins could decrease in immunized group compared with control due to the immunoneutralization of inhibin-α subunit, as it is cosidered as the unique constituent of inhibins (Campbell and Baird, 2001). The significant high expression level of *PRL* gene, recorded in Anti-Inha group compared with control, could be attributed to the decline of inhibins and elevation of activins and estrogen, as it has been reported by Thanoon (2013) that passive immunization of neonate female rats against inhibin-α subunit increased activins and estrogen levels. Immunization against α subunit could neutralize both types of inhibin (A and B).

At delivery, both groups recorded significant elevation of the expression level of *PRL* gene compared with their corresponding levels at pregnancy, with the appearance of difference in Anti-Inha group compared with control. During this period, the elevation of PRL may be attributed to the decrease of serum progesterone and the increase of estrogen secretion at the end of gestation. So, the elevation of serum prolactin concentration at delivery could be attributed to the sharp increase of estradiol and the low level of progesteron, as it has been mentioned that estradiol promotes prolactin secretion from pituitary gland by inhibiting dopamine (Norman and Litwach, 1997). Our result was in agreement with that of other studies which illustrates that, in the rats, the fall in the circulating progesterone is followed by an increase in serum prolactin (Bussmann and Deis, 1979). Also, as the ovarian hormone, estrogen, is critical regulator of pubertal mammary development and is responsible for the tremendous surge in growth occurring during this period that generates a functional mammary gland. For a long time, it was unclear whether hormones such as estrogen had direct effects on mammary gland development or whether, instead, they function indirectly to stimulate the release of hormones such as PRL from the pituitary (Lieberman *et al.*, 1978).

It has been reported, at delivery and mid lactation in the present study, that immunoneutralization against inhibin-α subunit increased the functional activity of the pituitary gland in protein synthesis which was over that recorded in control. This increase during pregnancy could be attributed to the increased activin-B level which is required for branching and alveologenesis (Schneyer *et al.*, 2003), as activin enhance aromataes activity and estradiol production (Xiao *et al.*, 1990; Xiao and Findlay, 1991; Miro *et al.*, 1991), so it enhance side branching in the presence of estrogen and progesterone. At delivery and mid lactation, the increment could not be related to activin-A secretion, because of expulsion of placenta, which is the main source of activin-A during pregnancy, but instead may be attributed to the mitotic activity of the different cells, which were increased in number as well as a response to the functional status in regards to the preparation for lactation at delivery and promotion of lactation at mid lactation period. These findings may explain the increment of RNA concentrations (Reeves, 1987).

Some studies demonstrated that extracts of the pituitary gland regulate mammary gland function, with researchers observing enhanced mammogenesis and lactogenesis upon its administration (Trott et al., 2008). The identified hormones that are responsible for these effects: growth hormone and prolactin. Other studies showed 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 (Ruan and Kleinberg, 1999).

The high level of pituitary *PRL* gene expression in Anti-Inha group in this study could be attributed to the decrease of inhibin-B and/or increase of activin-A, as it has been postulated that activin-A inhibit GH synthesis (Billestrup *et al.*, 1990), and GH seems to be inversely correlated with serum prolactin concentration (Jahn *et al.*, 1993). While the expression returned to the basal levels at mid lactation. The expression level of pituitary *PRL* gene at lactation period could not related to the activin-A concentration, but instead affected neuroendocrinologically mainly through suckling than the immunoneutralization one, this finding is supported by the non siginificant difference observed in serum PRL concentration during lactation peroid in the present study.

Lactotrophic cells have been detected in the anterior pituitary glands of the experimental groups, using immunohistochemical staining technique, to qualify the quantitative scoring (number of positive stained cells and intensity of staining) differences between normal and inhibin-α immunoneutralized pregnant rats. The present findings, in regards to the significant elevation of quantitative scoring of lactotrophic cells in Anti-Inha group, could be attributed to the decrease in the concentrations of inhibins (namely inhibin-B) together with the increase of activins (namely activin-A) during pregnancy and at delivery. These event was in orchestration with the result of pituitary *PRL* gene expression levels, reported in the present study. The quantitative scoring of lactotrophic cells increased significantly at delivery and in turn decreased at mid lactaion. These changes were accompanied by similar changes in serum prolactin concentrations and pituitary *PRL* gene expression levels. It is well known that prolactin concentration sharply increases at delivery and during lactogenesis. These observations may build to the idea that the hormonal and physiological state of female rat is the main effector of quantitative scoring of lactotrophic cells than the effect of immunoneutralization against inhibin subunits. The higher score of lactotrophic cells at delivery is attributed to the sharp droppness of progesterone level, the main prolactin antagonist hormone, after delivery, while low scoring degree at pregnancy peroid is attributed to the progesterone level which is essential for secretory initiation in mid and late pregnancy. The prolactin concentration rose after 48 hours as a consequence of the fall in serum progesteron concentration (Jahn *et al.*, 1993).

References

Al-Saaidi JAA, 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. Vet. Med., Assiut Univ., Egypt.

Billestrup N, Gonzales-Manchin C, Potter E, Vale W, (1990): Inhibition of somatotroph growth and growth hormone biosynthesis by activin in vitro. Molecular Endocrinology, 4: 356-362.

Bussmann LE, Deis RP, (1979): Studies concerning the hormonal induction of lactogenesis by prostaglandin F2a in pregnant rats. J. Steroid Biochemistry, 11: 1485-1489.

Campbell BK, Baird DT, (2001): Inhibin A is a follicle stimulating hormone responsive marker of granulosa cell differentiation, which has both autocrineand paracrine actions in sheep. J. Endocrinol., 169: 333–345.

Cowie AT, (1970). Influence of hormones on mammary growth and milk secretion. In: Lactation. I.R. Falconer (ed.). Butterworths. London. P:123.

Delouis C, Djiane J, Houdebine LM, Terqui M, (1980). Relation between hormones and mammary gland function. *J. Dairy Sci.*, 63: 1492.

Denamur R, (1971). Reviews of the progress of dairy science. Section A. physiology. Hormonal control of lactogenesis. *J. Dairy Res.*, 38: 237.

Ishigame H, Medan MS, Wang H, Watanabe G, Kishi H, Arai KY, (2005): Induction of superovulation by immunoneutralization of endogenous inhibin in immature rats. *J. Reprod. Dev.*, 51: 559-66.

Ishigame H, Medan MS, Watanabe G, Shi Z, Kishi H, Arai KY, Taya K, (2004). A new alternative method for superovulation using passive immunization against inhibin in adult rats. *Biol. Reprod.*, 71: 236-243.

Jahn G, Rastrilla A, Deis R, (1993): Correlation of growth hormone secretion during pregnancy with circulating prolactin in rats. J. Reprod. Furt., 89: 327-333.

Lieberman ME, Maurer RA, Gorski J, (1978): Estrogen controlof prolactin synthesis in vitro. Proc. Natl. Acad. Sci., USA, 75: 5946–5949.

Luna LG, (1968). Manual of histological staining methods of the armed forces institute of pathology. 3rd edition. Mc Graw. Hill book. Co. London.

Martin TL, Williams GL, Lunstra DD, Ireland JJ, (1991). Immunoneutralization of inhibin modified hormone secretion and sperm production in bulls. *Biol. Reprod.*, 45: 73-77.

Medan MS, Akagi S, Kaneko H, Watanabe G, Tsonis CG, (2004). Effects of re-immunization of heifers against inhibin on hormonal profiles and ovulation rate. *Reproduction*, 128: 475-482.

Miro F, Smyth CD, Hillier SG, (1991): Development-related effects of recombinant activinon steroid synthesis in rat granulosa cells. Endocrinology, 129: 3388-3394.

Norman A, Litwack G, (1997): Hormones of pregnancy and lactation In: Hormones 2nd. Ed. San. Diego California Academic Press**,** 387-411.

Reeves JJ, (1987): Endocrinology of reproduction. In: Reproduction in farm animals. (5^{th} edition). By Hafez, E. S. E., Lee, and Febiger. PP: 85-106.

Rivier C, Vale W, (1989). Immunoneutralization of endogenous inhibin modifies hormone secretion and ovulation rate in rat. *Endocrinol.*, 125: 152-157.

Ruan W, Kleinberg DL, (1999): IGF-1 is essential for terminal end bud formation and ductal morphogenesis during mammary development. Endocrinology, 140: 5075–5081.

Schneyer A, Schoen A, Quigg A, Sidis Y, (2003): Differential binding and neutralization of activin A and activin B by follistatin and follistatin like-3. Endocrinology, 144(5): 1671-4.

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

Sheffield LG, Anderson RR, (1985). Interspecies variation in mammary gland growth rate: relationship to gestation length. *J. Dairy Sci.*, 68: 2571.

Sternlicht MD, (2006). Key stages in mammary gland development: the cues that regulate ductal branching morphogenesis. *Breast Cancer Res.*, 8: 201.

Stricker P, Gruetter F, (1928). *C.R. Soc. Biol.*, 99: 1978. Cited in: Multiple hormone interaction in the developmental biology of the mammary gland. By: Topper YT, and Freeman CS, *Physiol. Rev.*, 60: 1049. (1980).

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-Qadesiya Universitu, IRAQ.

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

Voge JL, Wheaton JE, (2007). Effects of immunization against two inhibin antigens on daily sperm production and hormone concentrations in ram lambs. *J. Anim. Sci.*, 1-24.

Wang G, Hardy MP, (2004). Development of leydig cells in the insulin-like growth factor-I (igf-I) knockout mouse: effects of igf-I replacement and gonadotropic stimulation. Biol. Reprod., 70: 632–639.

Xiao S, Findlay JK, (1991): Interactions between activin and FSH suppressing protein and their mechanisms of action on cultured rat granulosa cells. Mol. Cell Endocrinol., 79: 99-107.

Xiao S, Findlay JK, Robertson DM, (1990): The effect of bovine activin and follicle-stimulating hormone (FSH) suppressing protein/follistatin on FSH-induced differentiation of rat granulosa cells in vitro. Mol. Cell Endocrinol., 69: 1-8.

مستوى تعبير جين هرمون البرولاكتين والخلايا المحرضة اللبنية في الغدة النخامية لإناث جرذان الوستر الحوامل الممنعة ضد وحدة الانهبين ألفا

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الخلاصة

يعمل التمنيع الميسر على زيادة إفراز الهرمون محفز الجريب مع زيادة ملحوظة في زيادة إفراز الاستروجين أثناء دورة الشبق (الطور الجريبي). كما أن فرط الإباضة تم إستحداثه بنجاح باستخدام التعادل المناعي لوحدة الانهبين ألفا في العديد من أجناس الحيوانات. أجريت الدراسة الحالية لاختبار دور التمنيع الميسر ضد وحدات الانهبين ألفا في مستوى تعبير جين البرولاكتين والتعبير المناعي النسجي الكيميائي للخلايا المحرضة اللبنية في الغدة النخامية لإناث جرذان الوستر الأباكير أثناء مرحلتي الحمل والرضاعة. تم تقسيم 72 إنثى حامل على مجموعتين (36 لكل مجموعة)، وفي اليومين الخامس والعاشر من مدة الحمل، حقنت الاولى (السيطرة) بالمحلول الفسلجي (100 مايكرولتر في البريتون)، وحقنت الثانية بالمصل المضاد للانهبين (1 مايكروغرام مذابا في 100 مايكرولتر من المحلول الفسلجي، في البريتون). وزعت كل مجموعة على ثلاث مجموعات ثانوية متساوية العدد تمت التضحية بها في اليوم السادس عشر من الحمل (مجموعة الحمل) واليوم الأول بعد الولادة (مجموعة الولادة) واليوم الحادي عشر من الرضاعة (مجموعة الرضاعة). تم تخدير الإناث وتشريحها وإزالة الغدد النخامية وحفظها بدرجة -70 مئوية لغرض التقييم الجزيئي وأخرى حفظت في الفورمالين (10%) لغرض التقييم المناعي النسجي الكيميائي. أشارت النتائج الى عدم وجود فروق معنوية في تركيز برولاكتين مصل الدم بين إناث المجموعتين أثناء مرحلتي الحمل ودر اللبن، بينما سجلت مرحلة الولادة زيادة معنوية في التركيز في المجموعة الممنعة. وعند المقارنة بين المراحل، أظهرت كلتا المجموعتين أعلى التراكيز أثناء الولادة تلتها مرحلة در اللبن ومن ثم مرحلة الحمل. كما أدى التمنيع ضد الانهبين ألفا الى زيادة مستوى تعبير جين البرولاكتين في النخامية بالمقارنة مع السيطرة أثناء مرحلة الولادة. أظهر الفحص المناعي التسجي الكيميائي زيادة في نسبة الخلايا الموجبة للصبغة وشدة الصبغة في نماذج المجموعة الممنعة أثناء الولادة ودر اللبن. وعند المقارنة بين مدد الدراسة، أظهرت المجموعتان درجة عالية للتلوين أثناء الولادة بينما أظهرت مرحلة الرضاعة أقل درجة للتلوين. يستنتج أن للتمنيع الميسر ضد وحدة الانهبين ألفا في اليوم الخامس والعاشر من مدة الحمل دورا في مستوى افراز البرولاكتين من نخامية إناث جرذان الوستر الأباكير أثناء مرحلتي الحمل والرضاعة.

الكلمات المفتاح: التمنيع الميسر، الانهبين، الاكتفين، هرمون البرولاكتين، الغدة النخامية.