Effect of passive immunization of pregnant rats against inhibin α, βA, and βB subunits on litters weight at delivery and lactation

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Abstract:

Transforming growth factor β (TGFβ) superfamily members are closely associated with reproductive processes. The present study has been designed to test the role of immunoneutralization of endogenous inhibin-α, βA, and βB subunits on mammary gland growth and development in primiparous female Wister rats, by evaluating cumulative dam body weight during pregnancy, and litter weigh at delivery and during lactation. Fifty six pregnant rats were randomly divided into four groups (14 per each). On 5^{th} and 10^{th} day of gestation, control was injected with saline (100µl, *i.p.*), Ta, Tba, and Tbb groups were injected with inhibin-α, βA, and βB antiserum (1µg in 100µl of saline, *i.p.*), respectively. Body weights of females have been monitored during pregnancy. Litters weight at parturition and daily weight gain until the 11^{th} day of lactation have been recorded. The results demonstrate significant increase in cumulative dam weight in Tba group during pregnancy period starting from 8^{th} of gestation compared with control, Ta, and Tbb groups. Litters of Ta group at parturition, revealed siginificant higher weight compared with Tba group, and no siginificant difference compared with control and Tbb groups. Litter of Ta group at the 11^{th} day of lactation recorded a highest siginificant weight gain among experimental groups. In conclusion, passive immunization against inhibin-α elevate litter weight gain at delivery, whereas immunization against inhibin-βA elevate cumulative dam weight gain and litter weight gain at 11^{th} day of lactation in primiparous female Wister rats.

Key words: passive immunization, inhibin α, inhibin βA, inhibin βB, litter weight gain.

Introduction

During pregnancy, a surge of hormones result in major structural changes in the mammary gland (1). Exposure to progesterone and prolactin results in extensive epithelial proliferation, increased side branching of both secondary and tertiary ducts and differentiation of milk-filled alveolar lobules that uniformly fill the interductal space by late pregnancy (2). Lobule and ducts grow to fill the fat pad. Present of progesterone receptor (PRs) is critical for mediating ductal outgrowth and lobuloalveolar differentiation (2). During the late pregnancy, the alveolar cells fill with lipids and luminal cells fill with basophilic sab stance. The number of blood vessels increase to provide nutrients to the cells of the rapidly growing gland (3,4).

At parturition, alveolar cells filled with lipid and lactose-containing secretary vesicles. Crescent shaped cytoplasm enclosed secretory vesicles. Cresent shaped cytoplasm enclosed milk fat globules containing lactose and milk protein are expelled into the alveolar lumen. Following parturition, the secretory lobuloalveolar structures become more apparent as the luminal space expand,and the epithlial cells layer become more promenant against the adipocytes the large lipid droplet, which are present at day 18 of pregnancy, are not present, having been replaced by small droplet at the apical surface of the epithelial cells (5). Processes of myopithlial cells cells contract and push the contents of the lumen (milk) into draning ducts (4). Nearing parturition alveolar tight junction close and milk and colostrum protein move into the alveolar lumen in preparation for active milk secretion (6).

During early lactation, mammary growth in rodents, may double the umber of mammary cells present at parturition (7,8). In it the second stage of alveolar morphogenesis (Lactogenesis II) occur around milk secretion (9,10) lactation is the process and milk secretion which can occur for up to 3 weeks. It is stimulated by suckling pups which cause the release of oxytocin (11). The initiation of lactation appear to be induce by decrease in estrogen and progesterone. About 20% of total mammary growth occur during the first 14 days of lactation. Mammary epithelial proliferation continuse in to early lactation (12). The peak of mammary differentiation occur approximately 1 day prior parturition and culminates with formation of alveoli and a fully gland (13).

Various stimuli induce massive changes that convert the mammary gland into milk factory. The development of functional alveoli, alveolar morphogenesis is a combined process of proliferation and differentiation. All phases of functional differentiation occur during pregnancy which start by proliferative phase (ductal and alveolar proliferation) in early

pregnancy, secretory activation in mid pregnancy and part of secretory activation phase at around parturition and contine during lactation (14). Multiple hormonal and growth factor input as well as the tremendous change in gene expression, signaling and metabolizing involves in morphological and functional change in the mammary gland (9).

Inhibins were originally characterized as proteins produced by the gonads that act in an endocrine manner to negatively regulate FSH synthesis and secretion from the anterior pituitary. As such, inhibins are essential for normal reproductive and endocrine function (15). Inhibins are disulfide-linked heterodimers comprising an α-subunit and either a βA or βB subunit to form inhibin A and inhibin B, respectively. Activins are structurally related proteins involved in the control of cell proliferation, differentiation, apoptosis, metabolism, homeostasis, differentiation, immune response, and endocrine function (16). Activins are produced in the gonads, pituitary gland, placenta, and other organs. Activins enhance follicle-stimulating hormone (FSH) biosynthesis and secretion, and participates in the regulation of the menstrual cycle. Activins are secreted as homodimers or heterodimers of inhibin β-subunits. Dimers composed of βA/βA (activin A), βB/βB (activin B), and βA/βB (activin AB) subunits have been shown to be biologically active (15). A participation of locally produced inhibin in alveolar development has been implied by the observation that treatment of rats with chorionic gonadotropin induce alveolar development (17), which is accompanied by an increase and shift of immunoreacting for inhibin from the stroma to the alveolar cells (18). In addition, mammary epithelial cell lines have been shown to contain activin receptor I, II, and IIB and thus have the potential to respond to activin signal (19,20,).

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: Sixty five days old mature primiparous female Wister rats, born at the animal house of the College of Veterinary Medicine, Basrah 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. Fifty six pregnant females were randomly divided into 4 groups (14 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.*). Body weights of all pregnant rats have been monitored throughout the experimental period. At parturition, litter weight has been recorded, and litters number was modulated as 9 per each dam (21). Daily litter weight gain was recorded until the 11^{th} day of lactation.

Results

Cumulative dam weight: Figure (1) reveals significant increase of cumulative body weight in Tba group during pregnancy period starting from the 8^{th} day of gestation and continued throughout the remaining gestational period, whereas the Tbb group registreted siginificant elevation in their body weight starting on the 16^{th} day of pregnancy and continued to last day of gestation.

Cumulative litter weigh: Rsults of cumulative litter weight (g.) reveals siginificant increase in cumulative litter weight of Tba group litter starting from 9^{th} day and continue to 11^{th} day of lactation figure(2).

Litter weight at the delivery: A higher siginificant increase in body weight of litters born from Ta group has been recorded by result clarified in figure(3).

Litter weight gain at the 11th day of lactation: Tba group recorded the significantly highest litter weight gain at the 11th day of lactation among the experimental groups by result clarified in figure (4).

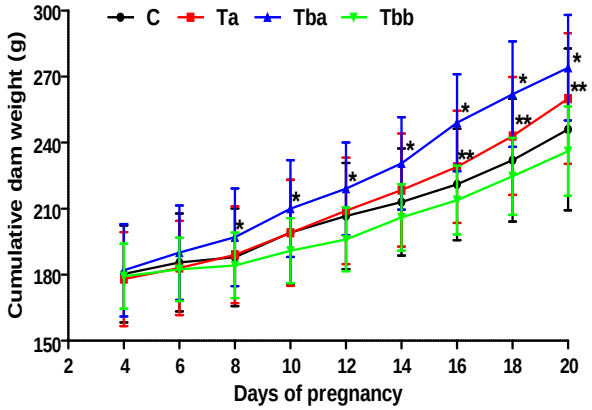

Figure (1): Effect of passive immunization against inhibin-α, -βa, and –βb subunit on cumulative body weight (g) in pregnant female rats.

Values represents mean±standard error.
Single and double stars represent significancy (p<0.05) compared with control.
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.

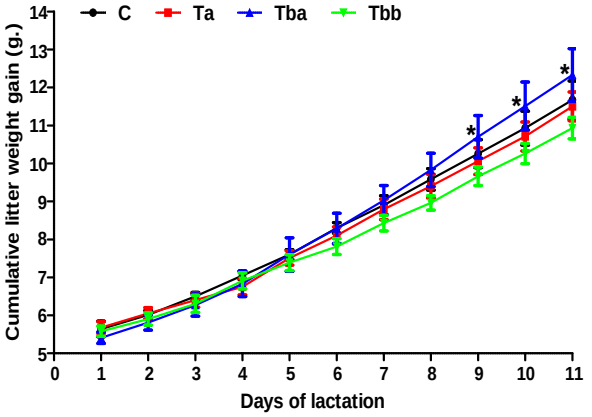

Figure (2): Effect of passive immunization against inhibin-α, -βa, and –βb subunit on cumulative litter weight gain (g) in pregnant female rats.

Values represents mean±standard error.
Stars represent significancy (p<0.05) compared with control.
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.

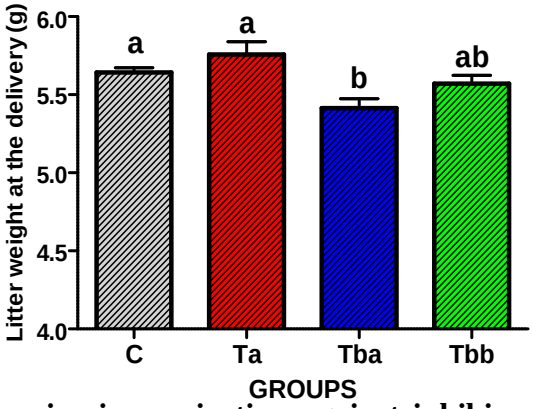

Figure (3): Effect of passive immunization against inhibin-α, -βa, and –βb subunit in pregnant female rats on litter weight (g) at the 1st day after parturition.

Values represents mean±standard error.
Different 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.
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.

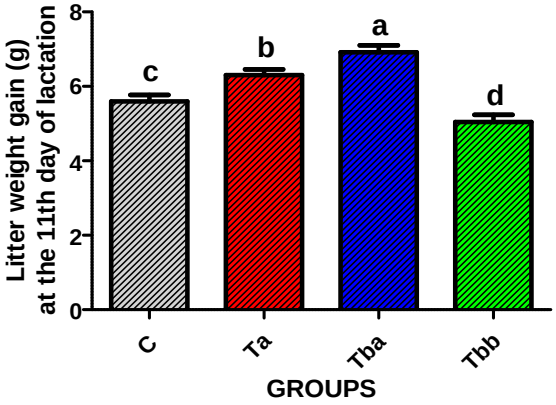

Figure (4-4): Effect of passive immunization against inhibin-α, -βa, and –βb subunit in pregnant female rats on litter weight (g) at the 11th day of lactation.

Values represents mean±standard error.
Different 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.
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.

Discussion

Cumulative dam weight

The significant increase of cumulative dam body weight in Tba group during pregnancy, which started early on the 8thday of gestation and continued to the end of gestation, may attributed to the decrease of activin-A and activin-AB level and increase of activin-B due to the passive immunization against βA subunit, as it has been observed that activin-A perform its action as an antiproliferative and proapoptotic effect in many different cell types (22). Also the decrease level of activin-A, which expressed during mid pregnancy, could causes inhibition of adipocyte development and block the JAK/STAT- induce cyclin D1 expression which responsible for activation of the G1 phase of mitotic cell division to induce cell proliferation (23,24). Since the immunization against inhibin- βA subunit enhance cells number and growth, this could increase cumulative dam weight.

The present study demonstrated decreased body weight in the inhibin-α immunized pregnant rats, as it has been shown that expression of inhibin-α subunit has been described, again in glandular epithelium, but to a lesser degree than activin-β subunits, indicating that activin dimers are prefer entially produced. Indeed, isolated epithelial cells in culture secrete activin A at 1000-fold higher concentrations than either inhibin A or B; similarly, activin A is secreted from epithelial glands in vivo into uterine fluid (25). The present significant increase in body weight of pregnant females in Ta group started at 16th day of gestation and continued until delivery, because passively immunized females against inhibin-α subunit resulted in the elevation activins and FSH. Therefore activins may enhance aromatase activity and estrogen production, and therefore cell proliferation. These results can be supported by the increase of GH concentration in Ta and Tba groups.

Litter weight at delivery and cumulative litter weight during lactation

In comparison with control, the present findings refered to no significant increase in litter weight at delivery of Ta group, whereas those of Tba and Tbb groups refered to no significant decrease and significant decrease, respectivly. The changes in Ta group could attributed to decrement of inhibins (inhibin A and B) with the increment of activins (A, B, and AB) and GH, as it has been reported by Thanoon (26) who found that passive immunization against inhibin-α subunit increase the expression level of pituitary GH and hypothalamic GHRH as well as the increment of serum activin A. Whereas in spite of the increase in GH, Tba and Tbb groups recorded decrease in activins (activin A and AB in Tba group and activin B and AB in Tbb group). From these results it appears that GH require high level of activins to perform its proliferative action. On the other hand, it has been found that activin A promotes embryo development, by increasing blastocyte cells number, reaching time taking to reach blastocyte stage rate (27). In fact, inhibin subunit mRNA (α, βa, and βb) were localized in placental trophoblast (28).

Cumulative litter weights of the experimental groups recorded no significant differences during the first eight days of lactation. At the 9thday of lactation, litters of Tba group recorded significant increase among the experimental groups and continued in the increase to the 11th day of lactation. These finding may attributed to the lactation itself (quantity and quality of milk), as it has been reported by Tucker (21) that litter weight gain during lactation reflect the galactopoiesis status in rats and other laboratory animals. Same auther mentioned that the 11th day of lactation represent the peak of lactation in female rats. The suggested increase of lactation could attributed to the increase of lactogenic factors particularly prolactin and GH. On the other hand, decreases of activins during this period enhances the action of prolactin, since activins act as a negative regulator of prolactin expression and secretion in mammary gland (29), as it has been mentioned that prolactin hormone responsible for maintainance of milk production and stimulation of the transcription of Wnt4 (30), Rankl (23) and cyclin D1 via induction of IGF-1 (23,24).

At the 11th day of lactation, the results obviously refered to significant increase of Ta and Tba groups litter compared to control, whereas Tbb group litters recorded the lowest weight among the experimental groups.This improvement could attributed to the early mammogenesis from the proliferative actions of activins before delivery and/or the high level of prolactin and GH and the number of their receptors in the secretory cells of the mammary acini after delivery. As it has been mentioned that activins have an important role in development of mammary gland during pregnancy and in preparation for lactation (31). Also it has been reported that activin B is required for succesful mammogenesis at the last period of pregnancy leading to lactation (32). At late pregnancy,activin inhibits adipocyte development (33). In a study, transgenic mice with activin subunit βB were generated to test the effect of activin in the metabolism (34), the results suggested the role of activin in restriction of adipocyte even in higher caloric diet (35). Activin, however, promotes insulin synthesis and secretion and enhance glucose stimulate insulin secretion (36).

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تأثير تمنيع الجرذان الحوامل الميسر ضد وحدات الانهبين ألفا وبيتا أي وبيتا بي على أوزان المواليد أثناء الولادة ودر اللبن

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الخلاصة

لأعضاء عائلة عوامل نمو بيتا الانتقالية TGF-β علاقة وثيقة في التكاثر. هدفت الدراسة الحالية لاختبار دور التمنيع ضد وحدات الانهبين (ألفا و بيتا أي وبيتا بي) في اليوم الخامس و العاشر من مدة الحمل في مستوى نمو وتطور الغدد اللبنية في إناث الجرذان عن طريق دراسة التغيرات الوزنية للأمهات أثناء الحمل وأوزان المواليد أثناء الولادة ومعدل الكسب الوزني للمواليد أثناء مدة در اللبن. تم تقسيم 56 من إناث الجرذان الحاملة على أربع مجموعات (14 لكل مجموعة). في اليوم الخامس والعاشر من مدة الحمل، تم حقن إناث السيطرة بالمحلول الفسلجي (100 مايكرولتر في البريتون)، وحقنت مجموعات التمنيع (Ta و Tba و Tbb) بالمصل المضاد للانهبين أي وبيتا أي وبيتا بي (1 مايكروغرام مذابة في 100 مايكرولتر من المحلول الفسلجي، في البريتون)، على التوالي. سجلت أوزان الأمهات أثناء مدة الحمل وأوزان المواليد أثناء الولادة وأثناء مدة در اللبن ولغاية اليوم الحادي عشر. أظهرت النتائج زيادة معنوية في أوزان أمهات مجموعة Tba ابتداءً من اليوم الثامن من الحمل بالمقارنة مع المجاميع الأخرى. مواليد مجموعة Ta سجلت أعلى الأوزان أثناء الولادة. سجلت مواليد مجموعة Ta أعلى معدل للكسب الوزني في اليوم الحادي عشر من الرضاعة من بين المجاميع الأخرى. يستنتج أن التمنيع ضد وحدات الانهبين (الفا وبيتا أي) في اليوم الخامس والعاشر من الحمل له دور بتحسين نمو وتطور الغدد اللبنية.

الكلمات المفتاح: التمنيع الميسر، الانهبين ألفا، الانهبين بيتا أي، الانهبين بيتا بي، الزيادة الوزنية للمواليد.