Sequential alterations of reproductive hormones in prepubertal female rats by inhibin alpha immunoneutralization

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Abstract

To investigate the sequential changes of female hormones related to reproduction, the present study has been conducted by induction of inhibin immunoneutralization during rats female life early as 15-20 days. Forty eight neonate *Wistar* female rats (weighted 24.3±1.7 g., aged 15 days) were randomly assigned to two equal groups; treated and control, injected (ip) with inhibin-α antiserum (1µg dissolved in 100µl of normal saline) and normal saline (100µl), respectively, in the 15th, 16th ,17th , and 20th days of age. Eight females from each group were sacrificed in the 23rd, 30th and 45th days. Blood samples were obtained for assessment of FSH, inhibin-B, LH, activin-A and estrogen concentrations. In all of the experimental periods (23d, 30d and 45d), female rats of treated group showed significant elevation of serum FSH, activin-A and estrogen concentrations, and significant decline of inhibin-B concentration, whereas LH concentration unchanged during 23rd and 30th days but slightly decreased from control level during the 45th day. In conclusion, passive immunization against endogenous circulating inhibin during neonatal age of female rats can perform an important role in sexual maturity, pituitary function and gonadal activity after puberty.

***Key words*: Inhibin, passive immunization, immunoneutralization, ovary.**

Introduction

Inhibins and activins are members of the TGF-β superfamily of extracellular signaling molecules (1). These members have been implicated as autocrine and paracrine regulators of ovarian follicle development and survival. Of these members, inhibins and activins are a physiologically relevant pair that are functional antagonists of each other. Activin stimulates whereas inhibin blocks follicle-stimulating hormone biosynthesis and secretion from pituitary gonadotrope cells, and together, inhibin and activin control the pituitary gonadal axis essential for normal ovarian function (2,3).

Immunoneutralization of endogenous inhibin was thought to result in diminished negative feedback on the anterior pituitary resulting in increased

secretion of FSH, greater follicular development and increased ovulation rate (4,5) and puberty in immature female rats (6,7). Passive immunization against α-inhibin subunit has also been used to increase sperm production in many live stock species including rams (8) and bulls (9). Early onset of puberty (time of first ovulation) was also observed after immunization early with an inhibin-enriched bovine follicular fluid preparation (10).

The present study aims to investigate the role of passive immunization against endogenous circulating inhibin as early in the neonatal ages of female rats on reproductive hormones secretion during prepubertal stage until development of puberty.

Materials and Methods

Animals and experimental design :

Fifteen days old female Wister rats (average weight was 24.3 ±1.7 g), were randomly assigned to two equal groups (24 female each); antiserum and control, daily injected (*ip*) with inhibin alpha antiserum (1µg dissolved in 100µl of normal saline) and normal saline (100µl), respectively during rats female life early as 15-18 days. Eight female rats from each group were sequentially sacrificed at 23d , 30d , and 45d of age. At the end of each treatment and control period, blood samples were obtained from abdominal vein for assessment of reproductive hormones (FSH, LH, estrogen, inhibin B, and activin A) concentrations in serum.

Hormonal assays in blood serum by using ELISA technique:

Serum FSH, LH, Estrogen, Inhibin B, and Activin A concentrations were assessed described by the manufacture company (ABO, Switzerland).

Statistical Analysis

Mean and standard deviation of the variables included in the present study has been calculated for each group. Student's *t*-test and F-test has been performed to test the effect of treatment in each period and between periods, respectively. 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

Results shown in figure (1A) clarified the concentrations of FSH in serum of the experimental female rats. It has been found that treated females revealed significantly ($P<0.05$) higher levels of FSH concentrations at first

(23d) and second (30d) periods when compared with control females, but they registered values near each other in the third (45d) period. Figure (1B), showed insignificant differences ($P>0.05$) in serum LH levels of female rats between experimental groups throughout the three experimental periods (23d, 30d, and 45d). Results shown in figure (1C) demonstrates the serum concentration of estrogen of the experimental female rats. It has been found that female rats injected with Inhibin antiserum registered higher significant ($P<0.05$) level in all of the experimental periods (23d, 30d, and 50d) compared with control. Figure (1D) illustrates the effect of passive immunization against inhibin alpha subunit on inhibin-B concentration in serum. Treated female rats registered lowest significant ($P<0.05$) level of inhibin-B throughout the three experimental periods (23d, 30d, and 45d) compared with control group. Figure (1E) clarified serum activin A concentrations of female rats. Treated group registered the significant ($P<0.05$) highest levels compared with that of control throughout the three experimental periods (23d, 30d, and 45d).

Discussion

The present study pointed out that passive immunization against inhibin alpha subunit, as early in the neonatal stage of female life, has potential effect on reproductive hormone concentrations during pre- and post-pubertal stages. From the present results, the decrement of inhibin-B concentration was due to the immunoneutralization caused by infusion of inhibin alpha antiserum , and that lead to significant increased in activin A serum concentration because inhibins and activins are functionally antagonistic members of the evolutionarily conserved TGFb family of extracellular signaling molecules (1). Circulating concentrations of FSH was increased due to the decrement of inhibin-B, since inhibins blocks FSH biosynthesis and secretion from adenohypophysis (2,3). High level of endogenous FSH stimulates follicular development especially granulose cell proliferation and its secretions of estrogen. This might simply explain the significant increased of serum estrogen concentration in our study. Recent studies revealed that inhibin regulates FSH secretion by reducing the amount of activin available at the binding site and also by reducing activin binding with activin type II receptors(11). Activin binding to its receptors has been shown to increase FSH secretion (12). Thus supposed our result where serum FSH concentration

was significantly increased in the three studied period, where as serum LH concentration was unchanged. GnRH is released in pulses, and the nature of these pulses (both amplitude and frequency) affects relative synthesis and secretion of FSH and LH (13,14), Changes in pulse frequency might explain situations in which LH and FSH are differentially secreted.

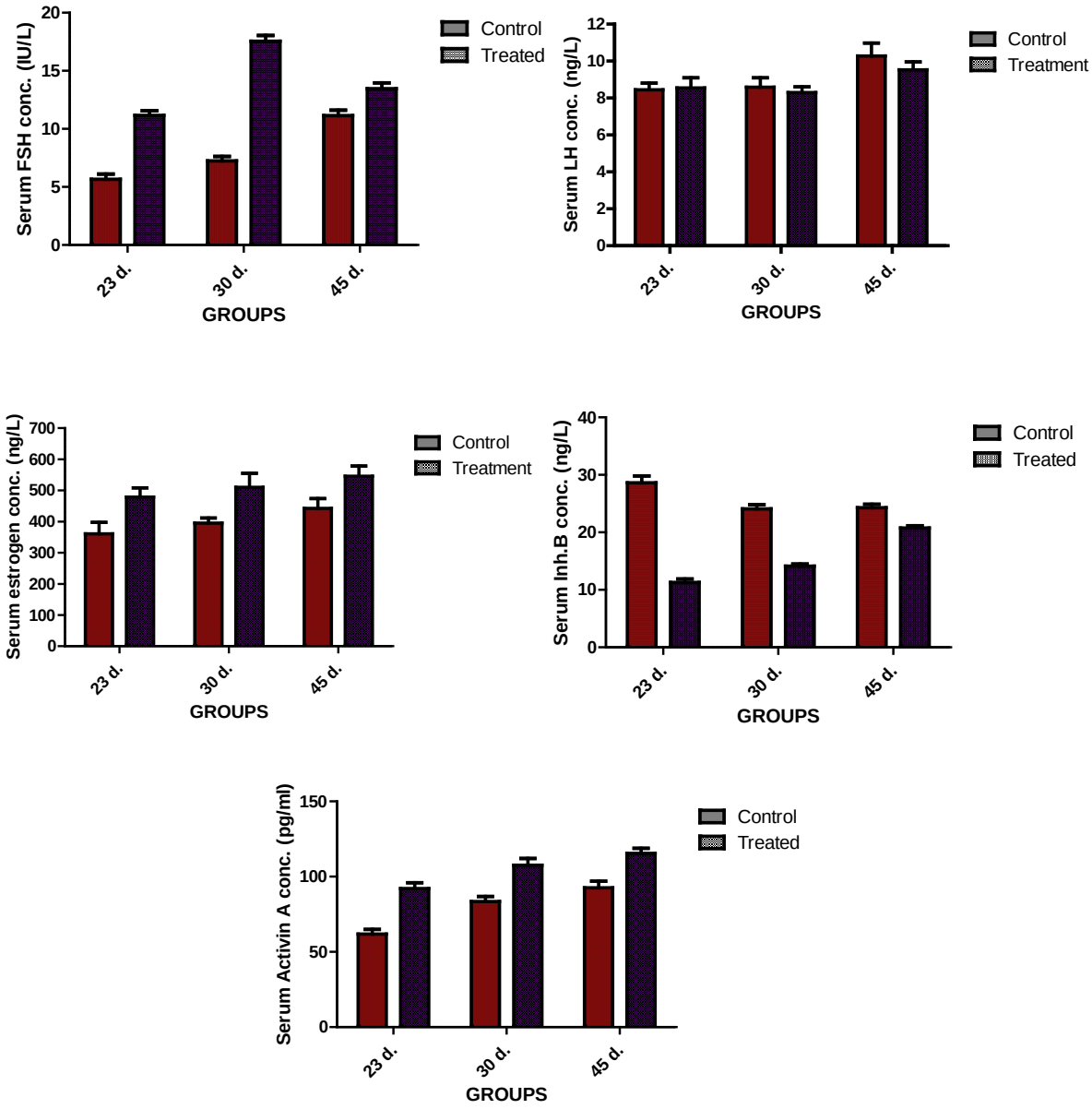

Figure (1): effect of passive immunization against inhibin-*α* subunit on serum FSH (IU/ L), LH (ng/L), estrogen (ng/L), inhibin-B (ng/L), and activin-A (pg/ml) concentrations of 23d, 30d, and 45d old femal rats.

- **Values represents M±SE.**
- **C: control female rats injected with normal saline (100 µl, *ip*).**
- **T: treated female rats injected with inhibin alpha subunit antiserum (1 µg, *ip*).**

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التغيرات المتعاقبة للهرمونات التكاثرية في اناث الجرذان غير البالغة جراء التعادل للمناعي للانهبين

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الخلاصة
للتحري عن التغيرات الهرمونية ذات العلاقة بالتكاثر في اناث الجرذان، تم اجراء الدراسة الحالية باستحداث التعادل المناعي للانهبين في عمر مبكر (٢٠-١٥ يوم) . تم توزيع ٤٨ من اناث الجرذان، بعمر ١٥ يوم ومعدل وزن ٢٤,٣ ± ١,٧ غم، عشوائيا على مجموعتين متساويتين (السيطرة والمعالجة) حقنت بمضاد الانهبين (١ غم مذاب في ١٠٠ مايكرولتر من المحلول الفسلجي) والمحلول الفسلجي (١٠٠ مايكرولتر) على التوالي في عمر ١٥ و ١٦ و ١٧ و ٢٠ يوم. تمت التضحية بثمانية جرذان من كل مجموعة في الأعمار ٢٣ و ٣٠ و ٤٥ يوم وأخذت منها عينات دم لغرض تقدير تركيز الهرمون محفز الجريب والهرمون اللوتيني والاستروجين والانهبين- بي والاكتفين – أي. في جميع مراحل الدراسة، أظهرت اناث مجموعة المعالجة ارتفاعا معنويا في تركيز كل من الهرمون محفز الجريب والاكتفين – أي والاستروجين وانخفاضا معنويا في تركيز الانهبين – بي بينما لم يتغير مستوى الهرمون اللوتيني في المرحلتين ٢٣ و ٣٠ يوما الا أنه انخفض قليلا في مرحلة ٤٥ يوما. يستنتج من الدراسة الحالية أن التمنيع ضد الانهبين ألفا خلال مرحلة قبل البلوغ يؤدي دورا مهما في البلوغ الجنسي ووظيفة الغدة النخامية وفعالية المبايض بعد البلوغ.