Quantification of *Inha, Inhba, and Inhbb* genes expression levels in Wister rat testis during Pre- and Post-Pubertal Stages

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Abstract:

The present study has been designed to quantitate the mRNA expression level of testicular *Inha, Inhba, and Inhbb* genes at pre- and post-pubertal stages of male Wister rats. Fifty male Wister rats (25 immature and 25 mature) were used in the present study. At pre-pubertal stage, 5 male rats of 25, 30, 35, 40, and 45 days old, and at post-pubertal stage, 5 male rats of 55, 60, 65, 70, and 75 days old were anasthetized and testis were obtained for molecular study to investigate the expression levels of *Inha, Inhba,* and *Inhbb* genes using qRT- PCR. The expression level of *Inha* gene in testis decreased as the age progress until 40 day period, and then slightly increased at 45 day period. At 55 day period, the expression significantly increased. At 60 and 65 periods, the levels recorded no increase, but 70 and 75 day periods recorded significant increase. The expression level of *Inhba* gene increased significantly as the age progress at the pre-pubertal stage, where the highest level was recorded at 45 day. At 55, 60, and 65 day periods, the highest expression level has been recorded, thenafter, the levels decreased at 70 and 75 day periods. The expression level of *Inhbb* gene increased significantly at 30, 35, 40, and 45 day periods of the pre-pubertal stage. At 55 and 60 day periods, no significant difference was recorded compared with 45 day period. At 65 day period, the highest level was recorded, thenafter, the levels decreased at 70 and 75 day periods. It can be concluded that fold changes of *Inha, Inhba,* and *Inhbb* genes in testis, correlated with the alterations of inhibins and activins concentrations at different periods of pre- and post-pubertal stages of male Wister rats life.

Key words: Testis, *Inha* gene, *Inhba* gene, *Inhbb* gene, Inhibin, Activin.

Introduction:

Inhibins and activins are dimeric proteins of the transforming growth factor-β superfamily, a class of peptides that can regulate the growth and differentiation of a variety of cell types. They are structurally related, sharing common β subunits. Activins are dimeric proteins composed of βA and βB subunits (activin-A [βAβA], activin-AB [βAβB], or activin-B [βBβB]), which are linked by a single covalent disulfide bond, while inhibins are heterodimers of αβ subunit and the structurally related α subunit (inhibin-A [αβA] and inhibin-B [αβB]) (1). Activins and inhibins are thought to be regulators of reproductive functions through their influence on both the pituitary and the gonads (2). The proteins and messenger RNAs (mRNAs) of the three subunits of inhibin/activin have been localized in the immature and adult testis but little is known about the pattern of expression and function of these peptides in the fetal and neonatal gonads (3). it is noted that expression of testicular inhibin RNA subunits decreases during sexual maturation (4).

The source of inhibin is predominantly the testis, the source of activin is ubiquitous, being synthesised by a large number of tissues (3). The autocrine or paracrine action of activin was apparent from the studies that successfully localized the expression of inhibin/activin α- and β-subunit mRNAs and proteins in the pituitary cells. Inhibin α-subunit mRNA was expressed in follicle cells of adult ovary more abundantly than in adult testis (5). The activation of inhibin –α gene during testis development correlated with the histological maturation of the testis and the acquisition of fertility in male mice (6). Inhibin α mRNA is primarily expressed in the granulose cells of the ovary (3), the sertoli cells of the testis (7) and the adrenal cortex (8).

Also inhibin is a gonadally derived protein that plays an important role in feedback hormone regulation between the gonads and the pituitary gland. The regulation of inhibin-B secretion appears to be dependent on Sertoli cell proliferation, maintenance, and spermatogenesis in males, the increase in inhibin-B levels during the neonatal period and during puberty occurs at times in which Sertoli cell number increases. FSH administration increases inhibin-B in prepubertal males in whom Sertoli cells can still divide (9). Regulation of inhibin βA- and βB-subunit expression is controlled in an independent manner during maturation of the rat testis (10). The α, βA, βB and βC subunit proteins were detected in Sertoli and Leydig cells of developing and adult mouse testes (11).

Inhibin is secreted mainly in the testis by the Sertoli and Leydig cells (12). Inhibin B is a direct product of the seminiferous tubules, reflecting the total testicular tissue (13). Inhibin B is an important marker for the functioning of seminiferous tubules (14). Inhibin B is the

afferent arm of the feedback loop from the testis that regulates FSH secretion, and investigation of inhibin B levels during reproductive development and in a variety of physiological and pathological states (15). Immunocytochemistry studies have shown that the Leydig cells as well as the Sertoli cells can produce both subunits of the inhibin B dimer in the pre- and early postnatal testis in humans and rats (16). Inhibin can bind specifically to testicular interstitial cells throughout development and may be an important regulator of Leydig cell testosterone production or interstitial cell function (17).

This study aims to quantitate the mRNA expression levels of testicular *Inha, Inhba, and Inhbb* genes at pre- and post-pubertal stages of male Wister rats to try gain insight in to its functions in the male and roles of these proteins in normal testicular development.

Materials and Methods

Animals:

Fifty male Wistar rats, were breed at the animal house of the college of veterinary medicine, Al-Qadisiya University. Animals were reared under controlled conditions (12L:12D cycles at 22±2 C°) and fed on standard laboratory food (19% protein ratio and 3000 kilocalories energy) and drinking water *ad libitum*. The study has been performed in two stages: at pre-pubertal stage, 5 males of 25 days old (weighted 34±0.89g), 30 days old (weighted 46±0.93g), 35 days old (weighted 58±0.91g), 40 days old (weighted 71±1.15g), and 45 days old (weighted 89±1.10g), and at post-pubrtal stage, 5 males of 55 days old (weighted 102±9.84g), 60 days old (weighted 111±1.22g), 65 days old (weighted 127±1.74g),70 days old (weighted 138±1.55g) and 75 days old (weighted 150±1.64g) were sacrificed after general anesthesia by combination of Xylazine and Ketamine (10mg and 90mg/kg, *ip*, respectively). Testis were obtained for molecular study to investigate the expression levels of *Inha*, *Inhba*, and *Inhbb* genes using qRT- PCR.

Molecular analysis:

RNA isolation from rat testis:

RNA was isolated from rat testis according to the protocol described by the TRIzol® reagent manufacturer with some modification: testis tissues were homogenized by grinding in liquid nitrogen, and the tissue powder was transferred into DEPC – treated epindorf tube contains 1 ml of TRIzol® reagent. The tubes were shaken vigorously for 30 seconds. Chloroform (200 µl) was added to each epindorf tube, vortexed for 15 sec and kept at room temperature for 10 min. Then were spined at 11,000 rpm, 4 C° for 10 min. Supernatant was transferred to a new epindorf tube, and isopropanol (500 µl) was added, vortexed for 15 sec. and kept at room temperature for 10 min. Then spined at 11,000 rpm, 4 C° for 10 min. and

supernatant was discarded. One ml of ethanol (75%) was added, vorttexed again, and spined at 8,000 rpm, 4 C° for 5 min. Supernatant was discarded and the pellet dried. RNase free water (50µl) was added to the sample with vortexing until dissolving.

Assessing RNA yield and qualit

There are three quality controls were performed on isolated RNA. The first one is to determine the quantity of RNA (ng/µL) that has been isolated using Nanodrop UV/VIS spectrophotometer (OPTIZEN POP. MECASYS Korea), the second is the purity of RNA by reading the absorbance in spectrophotometer at 260 and 280 nm in the same Nanodrop machine, and the third is the integrity of the RNA by prepared gel electrophoresis, as follows: After opening up the Nanodrop software, the appropriate application was used (Nucleic acid, RNA). A dry Chem-wipe was taken and cleaned the measurement pedestals several times. Then, 1µl of ddH2O was carefully withdrawn onto the surface of the lower measurement pedestal. The sampling arm was lowered and clicked OK to initialize the Nanodrop, then cleaning off the pedestals and 1µl of the appropriateblanking solution was added as black solution which is same elution buffer of RNA samples. After that, the pedestals were cleaned and 1µl of RNA sample was carefully withdrawn for measurement.

The purity of RNA also determined by reading the absorbance in Nanodrop spectrophotometer at 260 nm and 280 nm, so the RNA has its absorption maximum at 260 nm and the ratio of absorbance at 260 nm and 280 nm is used to assess the purity of DNA and RNA. A ratio of ~1.8 is generally accepted as “pure” for DNA; a ratio of ~2.0 is generally accepted as “pure” for RNA. If the ratio is appreciably lower in either case, it may indicate the presence of protein, phenol or other contaminants that absorb strongly at or near 280 nm.

After that, the integrity of the RNA was determined by prepared gel electrophoresis as following: 1% agarose gel was prepared in 0.5X TBE buffer and heated by using (hot magnetic stirrer) for 2 minutes until disappear all crystals in agarose solution. After cooling, (3 µL) of Ethidium bromide was added to the solution, then the gel was poured in the tray and left until solidifying. Then, it was transferred into electrophoresis machine which contains the same 0.5X TBE buffer. The RNA samples were prepared by mixing 5µl of RNA sample with 1µl of loading dye. Then, all amount was transferred into agarose gel wells, then running the electrophoresis power at 100 Volt for 1 hours, then the RNA bands were seen by U.V light.

DNase inactivation (DNase I) Treatment

The extracted total RNA samples were treated with DNase I enzyme to remove the trace amounts of genomic DNA from the eluted total RNA by using samples (DNase I enzyme) and done according to method described by promega company, USA instructions as follows:

RNA (1µl) and 10 × reaction buffer with Mgcl2 (1µl) and DNase I, RNase-free (1µl) and DEPC treated water (7µl) were added to Eppendorff tube. The mixture was incubated at 37C° for 30 minutes. 1µl EDTA was added and incubated at 65C° for 10 minutes. A volume of DNase inactivation reagent equal to 20% of RNA sample was added to each RNA sample. The tubes vortexed to mix the DNase Inactivation Reagent with RNA sample. All RNA samples left at room temperature for 2 minutes with flicking the tubes once or twice during this period to resuspend the DNase inactivation regent. The tubes were centrifuged at (12,000 rpm) for 1 minute to allow the DNase inactivation reagent separated from RNA sample solution, then, the RNA solutions transferred to new eppendorff tube.

cDNA synthesis

DNase-I treatment total RNA samples were used in cDNA synthesis step using AccuPower® RocktScript RT PreMix kit that provided from Bioneer company, Korea and done according to company instructions as follows: All DNase-I treatment total RNA samples were adjusted at the same concentrations that measured by nanodrop by DEPC water. RNA was converted to cDNA by preparation of reverse transcription PreMix reaction. 20 µL of RT PreMix was added into AccuPower RocketScript RT PreMix tubes that contain lyophilized reverse transcription enzyme. The lyophilized pellet were dissolved completely by vortex and briefly spinning down. The RNA was converted into cDNA under the following thermo cycler conditions. Finally, the samples were stored at -20 C° until performed qRT-PCR.

Step	Temperature	Time
cDNA synthesis	50°C	1 hour
Heat inactivation	95°C	5 minutes

qRT-PCR based SYBER Green I Dye Detection

qRTPCR was performed using AccuPower® Greenstar™ qPCR PreMix reagent kit (Bioneer, Korea) and Exicycler™ 96 Real-Time Quantitative Thermal Block (Bioneer, Korea). According to a method described by Cheon *et al.* (18). The Syber Green based qRT-PCR PreMix reagent kit is designed for PCR amplification of cDNA for target genes (*Inha*, *Inhba*, *Inhbb* genes) by using it's primers and (*GapdH*) Housekeeping gene, as well as for quantification of PCR amplification copy numbers comparatively to copy numbers of Genomic DNA qRT-PCR standard curve. The Syber Green dye that was used in this kit is DNA binding dye which reacted with new copies of amplification specific segment in target and housekeeping gene, then the fluorescent signals were recorded in Real Time PCR

thermocycler. A genomic DNA standard curve was generated from *GapdH* gene of *Rattus norvegicus* (27.9Mbp) were taken from NCBI-Gene Bank information was approximately (~1×107) copies, and serial dilution representing (1×107, 1×106 and 1×105) gDNA copies were used as genomic DNA standard curve.

Experimental design of qRT- PCR

For quantification of gene expression in treatment and control samples at duplicate, internal control gene as house keeping gene (GAPDH) was used to normalization of gene expression levels, therefore, qRTPCR master mixes were prepared for gDNA standard curve, target genes, and GAPDH housekeeping gene as following tables:

A) -qRT-PCR Master Mix for target genes

qPCR PreMix		**Volume**
cDNA template		10 µL
Primers	Target gene –F	2 µL
	Target gene –R	2 µL
DEPC water		6 µL
Total		20 µL

B) - qRT-PCR Master Mix for GAPDH housekeeping gene

qPCR PreMix		**Volume**
cDNA template		10 µL
Primers	GAPDH-F	2 µL
	GAPDH-R	2 µL
DEPC water		6 µL
Total		20 µL

After that, qPCR PreMix were added into *AccuPower* GreenStar qPCR PreMix tube. Then, real-time PCR tubes sealed by the optical adhesive film and mixed by vigorous vortexing for resuspension of PreMix pellet. The tubes centrifuge at 3,000 rpm, for 2 min, then Exicycler™ 96 Real-Time Quantitative Thermal Block instrument was started and loaded the following program according to the kit instruction. After reaction is completed, data analysis was performed.

Step	**Temp.**	**Time**	**Cycle**
Pre-Denaturation	95 °C	5 min	1
Denaturation	95 °C	20 sec	45
Annealing/Extension	60 °C	45 sec	
Detection(Scan)			
Melting	-		1

Data analysis of qRT-PCR

The housekeeping gene (*GapdH*) was represented as a normalize gene that can be used for calculation of the relative gene expression or fold change in target genes (Inha ,Inhba ,Inhbb genes). Therefore, the quantities (Ct) of target gene were normalized with quantities (Ct) of house keeping gene (*GapdH*) by the relative quantification gene expression levels (fold change) using Livak method (19). The relative quantification method, quantities obtained from q RT-PCR experiment must be normalized in such a way that the data become biologically meaningful. In this method, one of the experimental samples is the calibrator such as (Control samples) each of the normalized target values (CT values) is divided by the calibrator normalized target value to generate the relative expression levels. After that, the ΔCT Method with a Reference Gene was used as following equations:

ΔCT (calibrator) = CT (target, calibrator) – CT (ref, calibrator)

Ratio (target / reference) = $2^{\text{CT (reference) – CT (target)}}$

Table (3.7): CT values required for relative quantification with reference gene as the normalizer.

	Test	Calibrator (cal)
Target gene	CT(target, test)	CT(target, cal)
Reference gene	CT(ref, test)	CT(ref, cal)

First, normalize the CT of the reference (ref) gene to that of the target gene, for calibrator sample. Second, normalize the CT of the reference (ref) gene to that of the target gene, for the test sample:

ΔCT (Test) = CT (target, test) – CT (ref, test)

ΔΔCT= ΔCT (test) – ΔCT (calibrator)

Fold change = $2^{-\Delta\Delta CT}$

Ratio (reference/target) = $2^{\text{CT (reference) – CT (target)}}$

So, the relative expression was divided by the expression value of a chosen calibrator for each expression ratio of test sample.

Statistical analysis:

Results were expressed as mean ± standard error. Comparisons were performed using one way analysis of variance (ANOVA1) and newman- keuls to test all groups unpaired values. Differences were considered to be significant at the level of $P<0.05$. All statistical analysis were carried out using the GraphPad Prism (SAS Institute, Inc., USA).

Results:

Molecular analysis:

The Concentrations and Purity of Total RNA

Total RNA concentrations (ng/µl) and purity were assessed in the testis of male Wistar rats using Nanodrop spectrophotometer in absorbance readings (260/280 nm). All tissue samples that used in the present study during the pre-pubertal period (25 day, 30 day, 35 day, 40 day, and 45 day periods) and post-pubertal period (55 day, 60 day, 65 day, 70 day, and 75 day periods) gave high concentrations of total RNA and appeared quantitatively enough to proceed in quantitative reverse transcriptase real-time PCR.

Results illustrated in figure (1) revealed that the higher RNA concentrations in the testis (ng/µl) have been recorded at 25 day period of the pre-pubertal stage and 55, 60, and 75 day periods of the post-pubertal stage, whereas the lowest concentrations recorded at 35 and 40 day periods of the pre-pubertal stage and at 65 and 70 day periods of the post-pubertal stage.

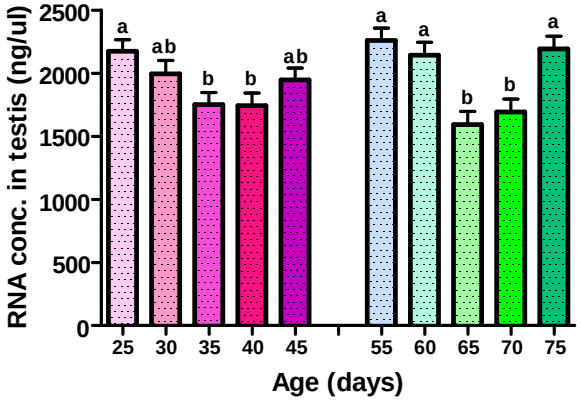


Figure (1): RNA concentration in the testis of male Wistar rats during pre- and post-pubertal stage.
Different letters represents significancy ($p<0.05$) in comparison between groups.
25, 30, 35, 40, and 45 days represent pre-pubertal stage.
55, 60, 65, 70, and 75 days represent post-pubertal stage.

Quantitative Reverse Transcriptase Real- Time PCR:

Data analysis of SYBR®green based reverse transcriptase real-time PCR for *Inha*, *Inhba*, and *Inhbb* genes expression levels were presented as a relative quantification normalized by housekeeping gene expression (GAPDH). The data of 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 *Inha*, *Inhba*, and *Inhbb* genes that react with complementary DNA (cDNA) of rat testis mRNA, where, the amount of PCR product (DNA copy numbers) in master mix reaction is approximately doubles and more in

each PCR cycle. First prepared series dilution of testis cDNA was used with the primer of different genes separately to draw the amplification plot of each gene, and then from this plot, threshold cycle was used to calculate a linear regression based on the data points, and inferring the efficiency of each primer from the slope (figures 2).

Relative quantification of target genes expression:

To calculate the relative expression of target gene (*Inha*, *Inhba*, and *Inhbb*) in rat testis. The $2^{\wedge-\Delta\Delta Ct}$ (livak) method used by normalize gene expression of target gene with expression of housekeeping gene (GAPDH) as reference gene. The gene expression in 25 day period was expressed as calibrator or control in both target genes (*Inha*, *Inhba*, and *Inhbb*) and reference gene (GAPDH), at first, the threshold cycle number of target gene normalized to that of reference gene in all ages and calibrator (control groups). Second, the ΔCt of ages normalized to the ΔCt of calibrator, and finally the expression ratio (fold change) was calculated. In all periods, fold changes were normalized according to 25 day period (which is equal to 1).

Relative quantification of *Inha, Inhba, and Inhbb* genes expression in the testis:

Figure (3) demonstrates the result of *Inha*, *Inhba*, *and Inhbb* genes expression levels in the testis obtained from male Wistar rats at pre-pubertal stage (25, 30, 35, 40, and 45 day periods) and post-pubertal stage (55, 60, 65, 70, and 75 day periods). The expression level of *Inha* gene decreased as the age progressed until 40 day period, and then slightly increased at 45 day period. At 55 day period of the post-pubertal stage, the expression level significantly increased ($p<0.05$) compared with control (25 day period). At 60 and 65 periods of the same stage, the expression levels recorded no significant increase ($p>0.05$), but 70 and 75 day periods recorded significant increase ($p<0.05$) (figure 3A). The expression level of *Inhba* gene increased significantly ($p<0.05$) as the age progressed at the pre-pubertal stage, where the highest level has been recorded at 45 day period. At 55, 60, and 65 day periods, the significant ($p<0.05$) highest expression level has been recorded, thenafter, the levels decreased significantly ($p<0.05$) at 70 and 75 day periods compared with the previous periods of the same stage, but their levels still significantly ($p<0.05$) higher than that of control (25 day period) (figure 3B). The expression level of *Inhbb* gene increased significantly ($p<0.05$) at 30 and 35 day periods of the pre-pubertal stage. Further significant increase ($p<0.05$) has been recorded at 40 day period, whereas the highest level has been recorded at 45 day period of this stage. At 55 and 60 day periods of the post-pubertal stage, there is no significant difference ($p>0.05$) was recorded compared with that at 45 day period of the previous stage . At 65 day period, the highest significant ($p<0.05$) level has been recorded, thenafter, the levels decreased significantly ($p<0.05$) at 70 and 75 day periods (figure 3C).

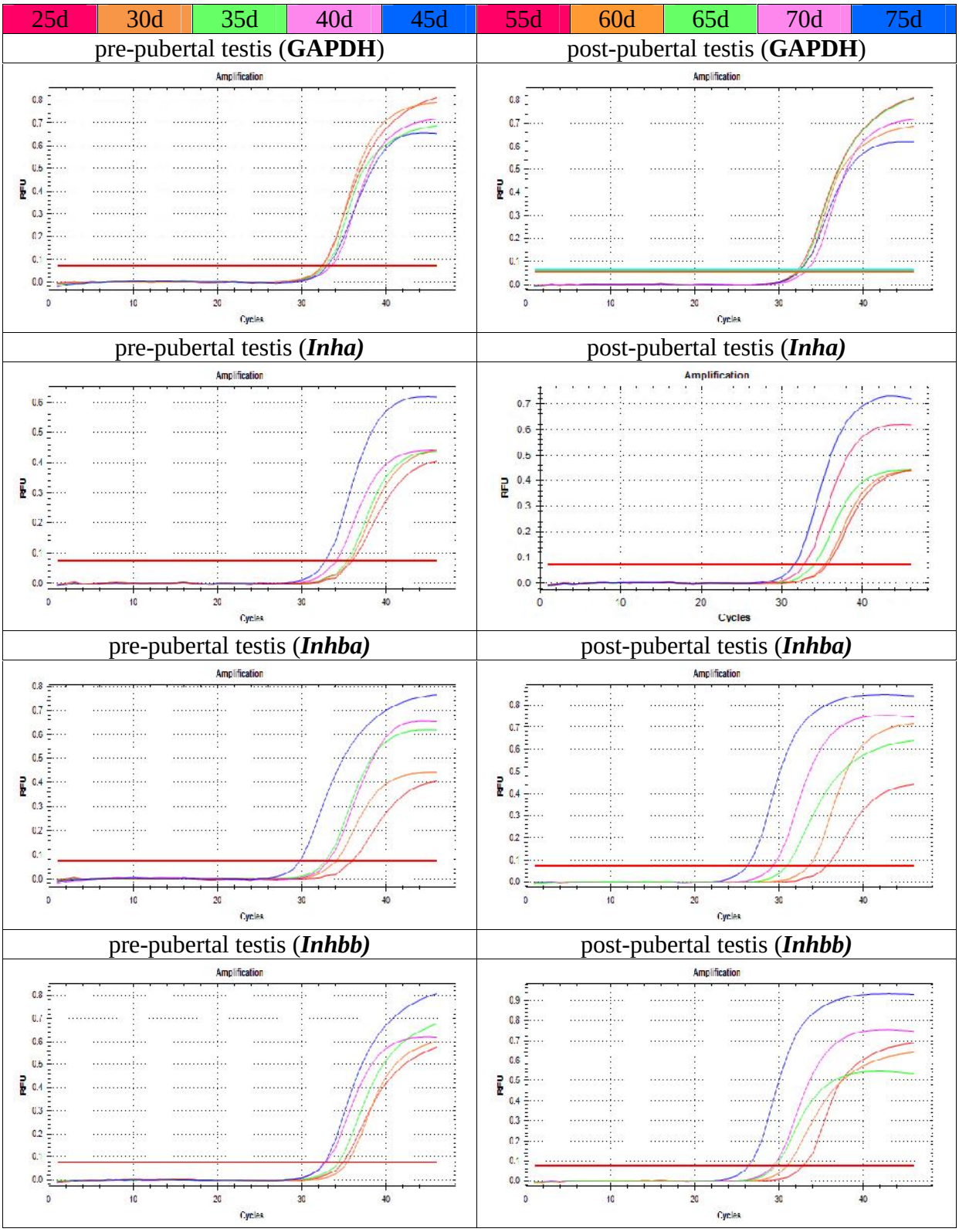
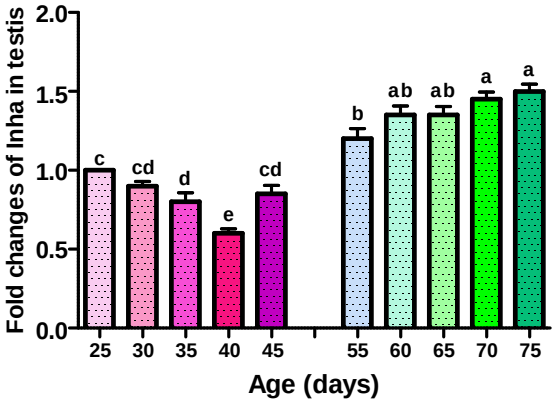
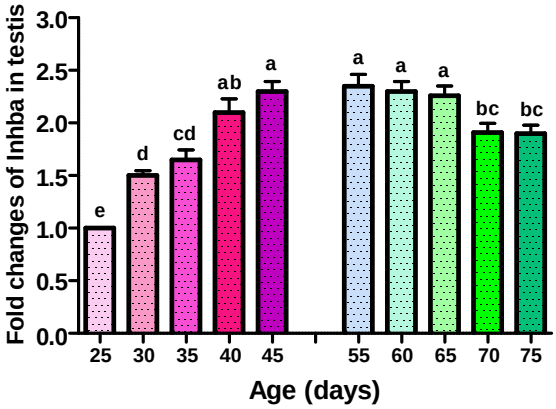


Figure (2): Fold changes of GAPDH, *Inha*, *Inhba* and *Inhbb* in the testis of male Wistar rats at pre-pubertal stage (25, 30, 35, 40, and 45 day periods) and post-pubertal stage (55, 60, 65, 70 and 75 day periods).

A-Inha



B-Inhba



C-Inhbb

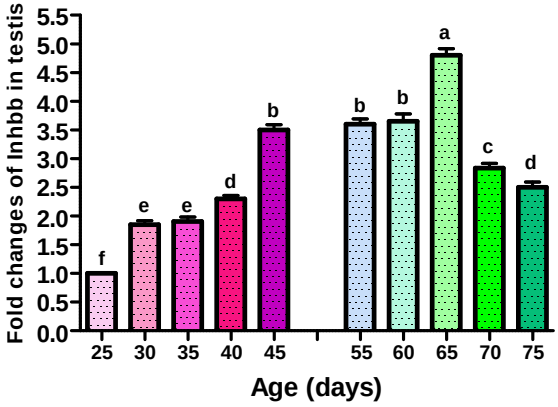


Figure (3): Fold changes of *Inha, Inhba* and *Inhbb* in the testis of male Wistar rats at pre-pubertal stage (25, 30, 35, 40, and 45 day periods) and post-pubertal stage (55, 60, 65, 70 and 75 day periods).

Discussion

In the present study, It has been found high concentration of total RNA in studied tissues. In testis, RNA concentration flactuated as it was high at 25 day priod and slightly decreased at 30 and 35 day periods and then recorded further increase at 40 and 45 day periods. During post-pubertal periods, RNA concentration sharply increased at 55 day period, then slightly decreased at 60 and 65 day periods, then sharply increased again at 70 and 75 day periods.

These changes may resulted in accordance with the level of protein synthesis and the proliferative status inside the seminiferous tubules, which may reflect the testicular function in steroidogenesis and spermatogenesis. This finding may relates to the circulating inhibin levels (20), where Sertoli cells produce higher levels of inhibin subunits in the early stage of testicular maturation and eventually reach very low levels of production in the mature animal (3). In the rat, studies have indicated that immunodetectable levels of the inhibin/activin subunits as well as their mRNA levels in the testis vary greatly with age. Specific measurement of the levels of inhibin in circulation have usually been based on assays that appear not to distinguish between dimeric (active) inhibin and free α-subunit (7;21).

Controlled studies in the nonhuman primate revealed that the number of Sertoli cells increases between the neonatal and the juvenile period, with a further increase during puberty (22). Both the neonatal and the post-pubertal increase in Sertoli cell number are paralleled by a rise in circulating levels of inhibin-B (23;24), and in normal adult rhesus monkeys, inhibin-B is strongly positively correlated with the number of Sertoli cells (25).

On the other hand, it has been found that the testis is not the only source of inhibins and activins in the male reproductive tract, and there are several reports of inhibin/activin subunits mRNA and proteins expression in the prostate, seminal vesicles, and epididymis (26). These changes related to the testicular activities after puberty until reaching the maturity under the activity of testosterone, as the concentration of testosterone increased gradually during the post-pubertal stage. On the other hand mRNA expression levels of *Inha*, *Inhba*, and *Inhbb* genes during this stage were in accordance with the increment status reported for testosterone. Meaningwhile that the expression levels of *Inha*, *Inhba*, and *Inhbb* genes may negatively related to the activity of epididymis, prostate, and seminal vesicle after puberty until the maturity period or in other hand, the level of testis function may reflected in an indirect way on reproductive accesory organs functions through its steroid secretions and other gonadal factors. It has reported that *Inha*, *Inhba*, and *Inhbb* levels in the testis were 4-10 fold higher in 8-15 day old animals when compared to mature (90 day old) animals. Although the relative abundance of all three inhibin subunit RNAs decreased with age, it is clear that the ba-RNA levels declined most rapidly (3). Parallel with this sexual maturation, inhibin–α subunit protein synthesis increased, whereas the synthesis of the activin βA and activin βB followed with a delayed time course (6). In rats inhibin–α gene expression have been reported high levels of inhibin–α mRNA from 8-25 days after birth (3), also Ito *et al*. (27) refered to a gradual increased in inhibin α- subunit gene expression after birth.

References

1. **Chen**, Y.G.; Lui, H.M.; Lin, S.L.; Lee, J.M. & Ying, S.Y. (2002). Regulation of cell proliferation, apoptosis, and carcinogenesis by activin. *Exp. Biol. Med. (Maywood)* 227:75–87.
2. **Vassalli**, A.; Matzuk, M.M.; Gardner, H.A.; Lee, K.F. & Jaenisch, R. (1994). Activin/inhibin beta B subunit gene disruption leads to defects in eyelid development and female reproduction. *Genes and Development*. 8: 414–427.
3. **Meunier**, H.; Rivier, C.; Evans, R.M. & Vale, W. (1988). Gonadal and extragonadal expression of inhibin alpha, beta A, and beta B subunits in various tissues predicts diverse functions. *Proc. Natl. Acad. Sci. USA.*85:247-251.
4. **Rabinovici**, J.; Goldsmith, P.C.; Roberts, V.J.; Vaughan, J.; Vale, W.W. & Jaffe, R.B. (1991). Localization and secretion of inhibin/activin subunits in the human and subhuman primate fetal gonads. *J. Clin. Endocrinol. Metab*. 73:1141–1149.
5. **Tone**, S.; Katoh, Y.; Fujimoto, H.; Togashi, S.; Yanazawa, M.; Yoshihiro, K.Y. & Higashinakagawa, T. (1990). Expression of inhibin α-subunit gene during mouse *gametogenesis.* 44(1): 62–68.
6. **Seok**, O.S.; Ahn, J.M.; Mayo, K.E. & Cho, B.N. (2004). Developmental changes in inhibin –α Gene expression in the mouse testis.*mol.cells.* 17(1):67-72.
7. **Roberts**, V.; Meunier, H.; Sawchenko, P. & Vale, W. (1989). Differential production and regulation of inhibin subunits in rat testicular cell types. *Endocrinology.* 125:2350–2359.
8. **Crawford**, R.J.; Hammond, V.E.; Evans, B.A.; Coghan, J.P.; Haralambidis, J.; Hudson, B.; Penschow, J.; Richaras, R. & Treageear, G. (1987). α-inhibin gene expression occurs in the ovine adrenal cortex, and is regulated by adrenocorticotropin .*Mol.Endocrcrinol*., 1: 699-706.
9. **Raivio**, T.; Toppari, J.; Perheentupa, A.; McNeilly, A.S. & Dunkel, L. (1997). Treatment of prepubertal gonadotrophin-deficient boys with recombinant human follicle-stimulating hormone. *Lancet*. 350:263–264.
10. **Esch**, F.S.; Shimasaki, S.; Cooskey, K.; Mercado, M.; Mason, A.J.; Ying, S.Y.; Ueno, N. & Ling, N. (1987). Complementary deoxyribonucleic acid (cDNA) and DNA sequence analysis of rat ovarian inhibins. *Mol. Endocrinol*; 1:388–396.
11. **Barakat**, B.; O'Connor, A.E.; Gold, E.; de Kretser, D.M. & Loveland, K.L. (2008). Inhibin, activin, follistatin and FSH serum levels and testicular production are highly modulated during the first spermatogenic wave in mice. *Reproduction* .136: 345–359.

12. **Nistal**, M.; Gonzalez-Peramato, P. & DeMiguel, M.P. (2010).Immunodetection of inhibin in the human testis and epididymis during normal development and in non-tumoural testicular lesions. *Reprod. Fertil. Dev.* 22(3):558-63.
13. **Hu**, Y.A. & Huang, Y.F. (2002). A serum marker of spermatogenesis inhibin B. *Zhonghua Nan. Ke. Xue.*8(1):57-60.
14. **Pierik**, F.H.; JAN, T.M.; Vreeburg, T.S.; De Jong, F.H. & Weber, R.F. (1998). Serum Inhibin B as a Marker of Spermatogenesis.*J.Clin. Endocrinol. Metab.* 83:3110–3114.
15. **Anderson**, R.A. & Sharpe, R.M. (2000). Regulation of inhibin production in the human male and its clinical applications. *Int. J. Androl*. 23:136–44.
16. **Majdic**, G.; McNeilly, A.S.; Sharpe, R.M.; Evans, L.R.; Groome, N.B. & Saunders, P.T. (1997). Testicular Expression of Inhibin and Activin Subunits and Follistatin in the Rat and Human Fetus and Neonate and During Postnatal Development in the Rat. *Endocrinology* . 138(5): 2136-2147.
17. **Krummen**, L.A.; Moore, A.; Woodruff, T.K.; Covello, R.; Taylor, R.; Working, P. & Mather, J.P. (1994). "Localization of inhibin and activin binding sites in the testis during development by in situ ligand binding". *Biol. Reprod.* 50 (4): 734–44.
18. **Cheon**, M.; Park, D.; Kim, K.; Park, S.D. & Ryu, K. (1999). Homologous upregulation of GnRH receptor mRNA by continuous GnRH in cultured rat pituitary cells. *Endocrine*, 11: 49–55.
19. **Livak**, K.J. & Schmittgen, T.D. (2001). Analsis of relative gene expression data using real-time quantitative PCR and the 2(- Delta Delta C(T)) Method. *Methods*. 25: 402-410.
20. **Kondo**, M.; Udono, T.; Jin, W.; Shimizu, K.; Funakoshi, M.; Itoh, M.; Watanabe, G.; Groome, N.P. & Taya, K. (2000). Changes in plasma concentrations of inhibin A and inhibin B throughout sexual maturation in the male chimpanzee. *Endocrine Journal* .47: 707–714.
21. **De Kretser**, D.M.; Loveland, D.J.; Meehan, T.; O'Bryan, M.K.; Phillips, D.J. & Wreford, N.G.(2001). Inhibins, activins and follistatin: actions on the testis. *Molecular and Cellular Endocrinology* .180: 87–92.
22. **Simorangkir**, D.R.; Marshall, G.R. & Plant, T.M. (2003). Sertoli cell proliferation during prepubertal development in the rhesus monkey (Macaca mulatta) is maximal during infancy when gonadotropin secretion is robust. *J. Clin. Endocrinol. Metab*. 88:4984–4989.

23. **Andersson**, A.M.; Toppari, J.; Haavisto, A.M.; Petersen, J.H.; Simell, T.; Simell, O. (1998b). Longitudinal reproductive hormone profles in infants: peak of inhibin B levels in infant boys exceeds levels in adult men. *J. Clin. Endocrinol. Metab.*83: 675-681.
24. **Winters**, S.J.; Plant, T.M. (1999). Partial characterization of circulating inhibin-B and pro-alpha C during development in the male rhesus monkey.*Endocrinology* 140:5497-5504.
25. **Ramaswamy**, S.; Marshall, G.R.; McNeilly, A.S. & Plant, T.M. (1999). Evidence that in a physiological setting Sertoli cell number is the major determinant of circulating concentrations of inhibin B in the adult male rhesus monkey (Macaca mulatta.). *J. Androl*. 20:430–434.
26. **Risbridger**, G.P. & Cancilla, B. (2000). Role of activins in the malereproductive tract. *Rev. Reprod*. 5: 99-104.
27. **Ito**, I.; Minegishi, T.; Hasegawa, Y.; Shinozaki, H.; Nakamura, K.; Igarashi, S.; Nakamura, M.; Miyamoto, K. & Ibuki, Y. (1993). Developmental changes of testicular activin and FSH receptor mRNA and plasma FSH and inhibin levels in the rat. *Life Sci.* 53:1299-1307.

التقدير الكمي لمستوى تعبير جينات *Inha* و *Inhba* و *Inhbb* في خصى جرذان الوستر أثناء مرحلتي قبل وبع البلوغ

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الخلاصة:

صممت الدراسة الحالية بهدف التحري عن مستوى تعبير الجينات ***Inha*** **و** ***Inhba*** **و** ***Inhbb*** في خصى جرذان الوسترخلال مرحاتي قبل وبعد البلوغ. أستخدم في الدراسة خمسون ذكراً من سلالة الوستر (25 غير بالغة و 25 بالغة). تم تخدير خمسة حيوانات من كل مرحلة من مراحل قبل البلوغ (بعمر 25 و 30 و 35 و 40 و 45 يوماً) ومراحل بعد البلوغ (بعمر 55 و 60 و 65 و 70 و 75 يوماً) . بعد تخدير الحيوانات ، أخذت منها الخصى لغرض إجراء الدراسة الجزيئية لتقدير مستوى تعبير الجينات ***Inha*** **و** ***Inhba*** **و** ***Inhbb*** فيها باستخدام تقانة تفاعل سلسلة البلمرة الكمية. أظهر تعبير الجين ***Inha*** في الخصى انخفاضاً مع تقدم العمر الى حين 40 يوماً ثم ارتفع قليلا بعمر 45 يوماً. بعمر 55 يوماً، ارتفع التعبير معنوياً بينما لم يتغير التعبير بعمر 60 و 65 يوماً في حين ارتفع معنوياً بعمر 70 و 75 يوماً. أما الجين ***Inhba*** فقد أظهر تعبيره في الخصى إزدياداً معنوياً مع تقدم العمر خلال مراحل قبل البلوغ وخصوصاً بعمر 45 يوماً واستمر بالزيادة خلال مراحل بعد البلوغ، إذ سجلت أعلى التعابير بعمر 55 و 60 و 65 يوماً ثم مالت بالانخفاض بعمر 70 و75 يوماً. في حين أظهر الجين ***Inhbb*** ازدياداً تدريجياً في التعبير مع تقدم العمر خلال مراحل قبل البلوغ بينما لم تسجل تغيرات معنوية بعمر 55 و 60 يوماً بالمقارنة مع عمر 45 يوماً ثم سجل أعلى تعبير بعمر 65 يوماً ثم انخفض التعبير بعمر 70 و 75 يوماً. يمكن الاستنتاج أن التغيرات في تعبير جينات ***Inha*** **و** ***Inhba*** **و** ***Inhbb*** في الخصى مرتبط مع التغيرات في تراكيز الانهبينات والاكتيفينات في الأعمار المختلفة من مرحلتي قبل وبعد البلوغ من عمر ذكور جرذان الوستر.