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The study of changes in WNT2 and H-PGDS relative gene expression and the histological characteristics of seminiferous tubules during the puberty of rooster

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Abstract This research was conducted on 24 roosters in a completely randomized design with 4 treatments (age groups of 5, 6, 7 and 8 months) and 6 replications (roosters) in each treatment. At each sampling time, the roosters were slaughtered, and testicular samples were prepared to simultaneously examine changes in the relative expression of WNT2 and H-PGDS genes, as well as the histological characteristics of the seminiferous tubules. The results showed that the relative expression level of the H-PGDS gene decreased 0.02-fold at 6 months of age compared to 5 months of age and increased 45.54 and 12.69-fold at 7 and 8 months of age respectively, compared to 5 months. While the relative expression of the WNT2 gene increased significantly at 6 (47.77 folds), 7 (71.18 folds), and 8 (88.17 folds) months of age compared to 5 months of age ($P \leq 0.05$). In addition, the results of histological examination of the seminiferous tubules showed that the mean diameter of these tubules increased ($P \leq 0.05$) at the age of 7 months (405.90 µm and 112.4 µm, respectively) compared to the ages of 5 and 6 months (343.07 µm and 354.74 µm, respectively). Also, the maximum thickness of germinal layer (112.4 µm) was observed at the 7 months of age. Overall, the results showed a coordinated increasing trend in the relative expression of WNT2 and H-PGDS genes and the growth of seminiferous tubules during the puberty period of native roosters.

Keywords: H-PGDS, rooster, seminiferous tubules, sexual maturation, rooster, WNT2

Introduction

Puberty is a process that begins at a certain age in poultry and increases the activity of the gonads (Sturkie, 2016). With the onset of sexual maturity, the secretion of gonadotropin-releasing hormone (GnRH) increases, followed by the increased secretion of FSH and LH hormones from the pituitary gland, which causes the growth of the sexual organs. In male birds, testicular development leads to sperm production (Avital-Cohen et al., 2013). Studies have shown that several genes play important role in rooster fertility, and the expression levels of these genes in the testes of healthy roosters differ from roosters with reproductive problems and low fertility (Sun et al., 2019). The H-PGDS (Hematopoietic-prostaglandin D synthase) gene plays an important role in the production of prostaglandin D, which is critical in the embryonic and adult development of the testes, the process of spermatogenesis and sperm maturation in the seminiferous tubules, as well as in steroidogenesis and the development of Sertoli cells (Rossitto et al., 2015). The expression of H-PGDS gene varies greatly in different species. In mice, chickens and humans, H-PGDS gene

expression has been reported in many tissues, including adipose tissue, placenta, lung, liver, heart, skin, lymph nodes and fetal bone marrow. The high expression of this gene in the mammalian reproductive system also indicates a direct effect of this gene on the function of the reproductive system (Kamauchi and Urade, 2011). Changes in the expression level of the H-PGDS gene in Leydig cells and testicular mast cells in birds cause disruption in spermatogenesis and sperm maturation. On the other hand, with increasing age of the bird, low expression of this gene causes a decrease in sperm motility and disruption in spermatogenesis, which may be due to inhibition of prostaglandin D_2 synthesis (Schell et al., 2007).

The WNT2 gene (Wingless-type family member 2) is another gene that causes the development of the reproductive system during the embryonic period. In various species, it is involved in the development of accessory gonads such as the vesicular glands, Reduced expression of this gene causes abnormalities in the morphology of the testis in birds, resulting in reduced sperm motility and fertility (Sun et al., 2019). Interestingly, a deficiency in WNT2 gene expression even in the male testis results in genital defects and ultimately sterility (Holzem et al., 2019). Previous studies have shown that a deficiency in the expression of a similar gene, called WNT7A (Wingless-type family member 7), in male mice results in testicular defects and sterility (Parr, 1998). Simultaneous examination of the expression changes of H-PGDS and WNT2 genes in Beijing native roosters showed that the expression levels of both of these genes were much lower in low-fertility roosters than in roosters with good fertility (Sun et al., 2019).

The seminiferous tubules of the testes in birds contain irregularly shaped Sertoli cells, and a germinal epithelium layer that contains several layers of spermatogonia at different stages of spermatogenesis (Kouatcho et al., 2015). With increasing age, male bird fertility decreases rapidly, which is positively correlated with a decrease in the diameter of the seminiferous tubules and a decrease in the number of spermatogonia and Leydig cells (Wilson et al., 2018).

During puberty, growth and development of the seminiferous tubules continue with the increasing age of the roosters and continue until sometime after sexual maturity (Sturkie, 2016). Hormones such as FSH and LH (Zirkin and Papadopoulos, 2018), thyroxine (Fumel et al., 2012), and insulin-like growth factor-1 (IGF-1) affect the growth and development of the seminiferous tubules and the divisions of spermatogonia (Pitetti et al., 2013).

A histological study of the testes of Fars native roosters at puberty showed that, along with the increase in IGF-1 gene expression, the number of Sertoli cells and testicular size also increased (Hashmatipour et al., 2020). In a flock of broiler breeders, the number of Sertoli cells and testicular size also gradually increased with the age of the breeding roosters during puberty, and these changes were positively correlated with the number of Sertoli cells and spermatogonia, as well as the diameter of the seminiferous tubules (SarabiaFragoso et al., 2013).

Research on Japanese quail also showed that the average diameter of the spermatogenic tubules gradually increased with age, reaching a peak at the end of puberty (Kouatcho et al., 2015). The same results were observed in 9-month-old adult Nigerian roosters, but there was no significant difference between the diameter of the spermatogenic tubules in the left and right testicles (Udoumoh et al., 2020). Studies showed that the development of seminiferous tubules and sperm production was associated with the expression of H-PGDS and WNT2 genes, and that low levels of these genes caused abnormal testicular growth in birds and ultimately reduced the activity of the germinal layer of seminiferous tubules (Sun et al., 2019).

These genes also have a positive effect on the production of motile sperm in the testicles, which increases the fertility of the rooster, since during the native rooster's puberty, significant changes occur in the size of the testicles and their morphological characteristics (Heshmatipour et al., 2020). Therefore, it was of interest to investigate the relative expression of genes affecting testicular growth and development during this period. For this purpose, the present study was designed and conducted to simultaneously study the relative expression of WNT2 and H-PGDS genes, which have recently been identified as genes responsible for morphological changes in seminiferous tubules (Sun et al., 2019), during the sexual maturation period of native roosters (5 to 8 months of age).

Materials and methods

Breeding and maintenance of birds before the experiment

This study was conducted in a completely randomized design with 24 roosters. In this experiment, roosters were randomly selected and purchased from a Fars native chicken and breeding flock at the ages of 5 (onset of puberty) to 8 (at sexual maturity) months. The experimental treatments in this study included four sampling sessions at the ages of five, six, seven, and eight months with six replications (6 roosters).

During the experiment, the roosters were fed a special diet for breeding native productive roosters (Table 1) with an energy content of 2812 kcal/kg of diet and 16.64 % crude protein, and reared under a light schedule of 16 hours of light and 8 hours of darkness.

Ethical consideration

The breeding procedure, sampling and slaughtering of the roosters were carried out in accordance with the farm animal rights, based on the research proposal of M.Sc. student by the permission (980441005) of the postgraduate education department of Yasouj University.

Table 1. Feed ingredients and chemical composition of the experimental diet

Feed Ingredients (kg of DM)	
Corn	611.7
Barley	4
Wheat bran	100
Alfalfa hay	10
Soybean meal (44% CP)	219.5
Calcium carbonate	20
Dicalcium phosphate	18
NaCl	3.8
Lysine	1
Methionine	1
Vegetable oil	6
Mineral and vitamin supplement[1]	5
Chemical composition	
Metabolizable energy (kcal/kg)	2812
Crud protein (%)	16.64
Lysine (%)	1
Methionine (%)	0.5
Calcium (%0	0.92
Total phosphorous (%)	0.52
Available phosphorous (%)	0.29

[1]Mineral and vitamin premix composition per kilogram of diet: 11,000 IU vitamin A, 2300 IU vitamin D3, 121 IU vitamin E, 2.3 mg vitamin K3, 4 mg vitamin B1, 4 mg vitamin B2, 4 mg vitamin B6, 0.03 mg biotin, 1 mg folic acid, 0. 02 mg vitamin B12, 840 mg choline chloride. 100 mg Mn, 50 mg Fe, 169. 4 mg Zn, 100 mg Cu, 0.2 mg Se, 1 mg I, 0. 47 mg Co.

Testicular sample preparation for gene expression and histology

At each sampling time, six roosters were slaughtered and after emptying the viscera, the testicles were carefully removed from the abdominal area and the epididymis and associated tissues were carefully separated and after washing with physiological serum. A part of the testicular tissue was transferred into test tubes containing 10% formalin for histological analysis, and the remainder was transferred into capped test tubes and stored in a nitrogen storage tank until gene expression was carried out.

Relative gene expression analysis

For each evaluation, 50 mg of frozen testicular tissue were separated from the left and right testicles and quickly transferred into a porcelain crucible before complete thawing, and then gently ground and mixed well. The obtained sample was immediately poured into a 2 mL RNase-free microtube that had been previously cooled in liquid nitrogen. Before complete thawing of the sample, 1000 µL of RNA-Plus solution from the mRNA extraction kit (RNX-Plus, EX6101, Sinaclone-Iran) were added to the microtube containing the sample using a sterile sampler, and after 10 seconds, the microtube content was homogenized by vortexing. After performing all mRNA extraction steps according to the kit instructions, the final sample was stored in liquid nitrogen for cDNA preparation. The cDNA synthesis from mRNA was performed by reverse transcriptase with random hexamer primer. The WizScriptTM RT Master kit manufactured by Synaclone (Catalog number: W2203) was used to make cDNA. To examine the relative expression of the desired genes, specific primers for each gene were used. The primers were designed based on accession number information obtained from the Gene Bank by using Primer 3 software (Table 2).

Table 2. Characteristics of the primers for the WNT2, H-PGDS, and β-Actin genes

Gene	Sequence	Length	Accession No.
WNT2	F: 5'-aaatacaatggagcgattcaggtgg-3' R: 5'-ccaggtcattcttagttggcttctt-3'	97 bp	NM_205011
H-PGDS	F: 5'-caagaagtggtttgtagggaaatctgt-3' R: 5'-ctttgtaggacaggagcgttgtg-3'	83 bp	XM_416013
β-actin	F: 5'-ctgtgcccatctatgaaggcta-3' R: 5'-atttctctctcggctgtggtg-3'	139 bp	NM_205518

Subsequently, sterile deionized water was added to the tube containing each primer (Synthesized by Topagen Company) until the base concentration reached 100 micromoles. For use in the polymerase chain reaction, a concentration of 10 picomoles was prepared from the base solution and then stored at -20°C for further analysis. A real-time PCR device was used to examine the relative expression of the target gene. All reactions were performed in triplicate. The real time PCR amplification conditions were 95 °C for 10 min; 40 cycles of 95 °C for 5 s and 60 °C for 40 s. In order to confirm the absence of nonspecific products and primer dimmers, all samples were submitted to dissociation curve analysis (melting curve by 95 °C for 5 s, 65 °C for 15 s, and 95 °C for 0 s). The level of mRNA expression of the testicular genes (WNT2 and HPGDS) was normalized with the β-actin gene as a reference (Housekeeping) gene (Khostavaneh et al., 2025).

Histological measurements

The samples stored in formalin solution were washed with distilled water, and fixation, dehydration, and tissue preparation were performed using a tissue processor. The tissue samples were then embedded in paraffin and 5-micrometer sections were prepared using a rotary microtome. Two slides were prepared from each testicular tissue sample and stained with hematoxylin-eosin stain. Finally, the prepared slides were covered (mounted) and stored in a slide box until evaluation. Morphometric data were obtained from 10 to 15 photographs taken from each slide with an Olympus light microscope at ×40, ×10, and ×5 magnifications and evaluated with Dinolite software (Figure 1). The average diameter of the spermatogenic tubules in 10 photographs with a total area of 19 mm^2 with a lens of 4 and the thickness of the epithelial tissue of 50

spermatogenic tubules in 10 photographs with a total area of 3 mm^2 with a lens of 10 for each sample were obtained (Heshmatipour et al., 2020).

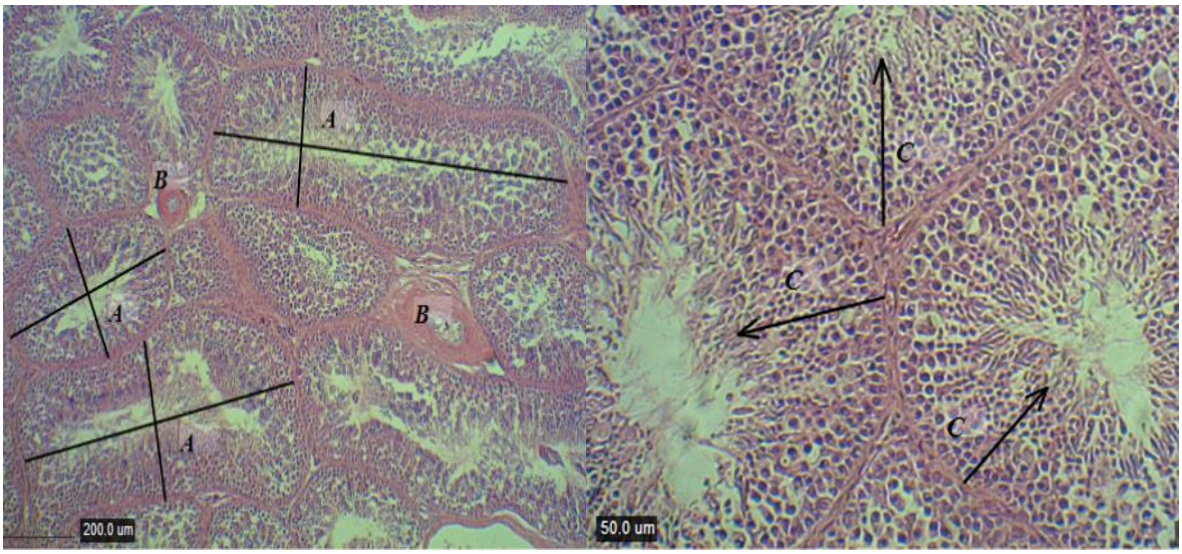

Figure 1. Cross-section of a rooster testicle. A: Diameter of the seminiferous tubule, B: Blood vessels. Magnification: ×5, scale bar: 200 µm. C: Thickness of the seminiferous tubule epithelium. Magnification: ×10, scale bar: 50 µm.

Statistical analysis

The data were analyzed in the form of a completely randomized design. The relative gene expression data were analyzed with the REST© 2009, V2.0.13 software (Pfaffl et al. 2002), which Pair Wise Fixed Reallocation Randomization Test apply to compare the differences in gene expression between groups. In addition, the PROC GLM (SAS, 2020) was used to investigate the characteristics of the seminiferous tubules and the germinal layer. The Duncan's multiple-range test was used to compare the means at different ages.

Results

Figure 2 (A and B) shows the relative expression of WNT2 and H-PGDS mRNA genes in the testis of 5- to 8-month-old roosters. The expression level of WNT2 gene in six-, seven-, and eight-month-old roosters increased significantly compared to five-month-old roosters, such that at 6, 7, and 8 months of age, it increased by 47.77, 71.18, and 88.14 times compared to that at 5 months of age, respectively.

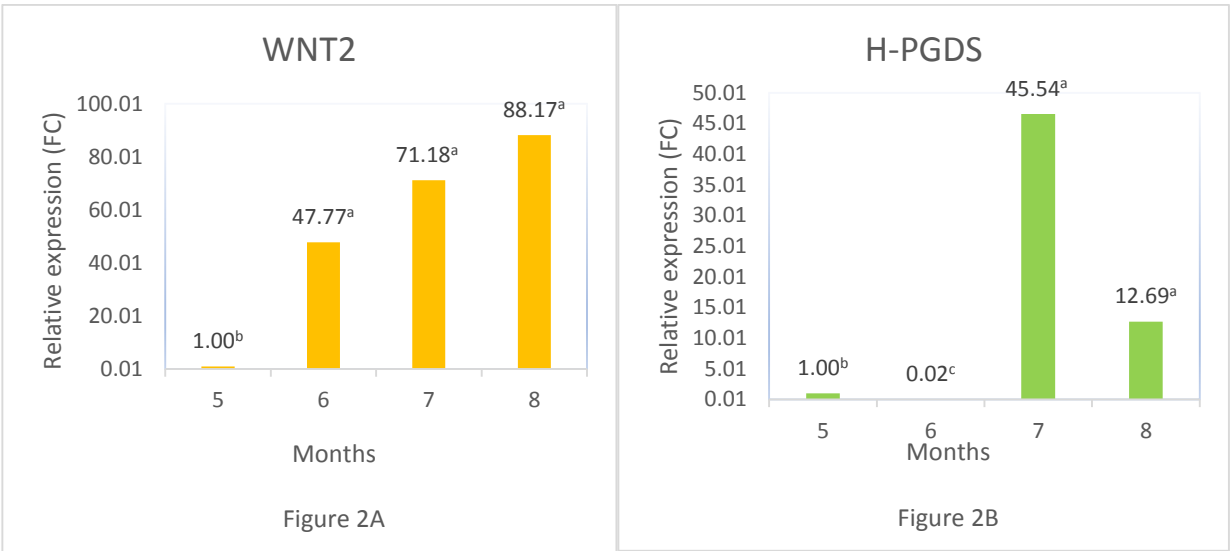

Figure 2. Comparison of relative mRNA expression of WNT2 and H-PGDS genes in the testes of 6, 7, and 8-month-old roosters with 5-month-old ones. FC: Fold change.
[a,b:] Means with common superscript (s) are not different (P>0.05).

The relative expression levels of the H-PGDS gene in six-, seven-, and eight-month-old roosters compared to five months followed a different pattern, such that its relative expression at 6 months of age was lower than in 5-month-old roosters (0.018 times); however, the relative expression levels of this gene at seven and eight months of age increased by 46.54 and 12.86 times compared to 5 months, respectively.

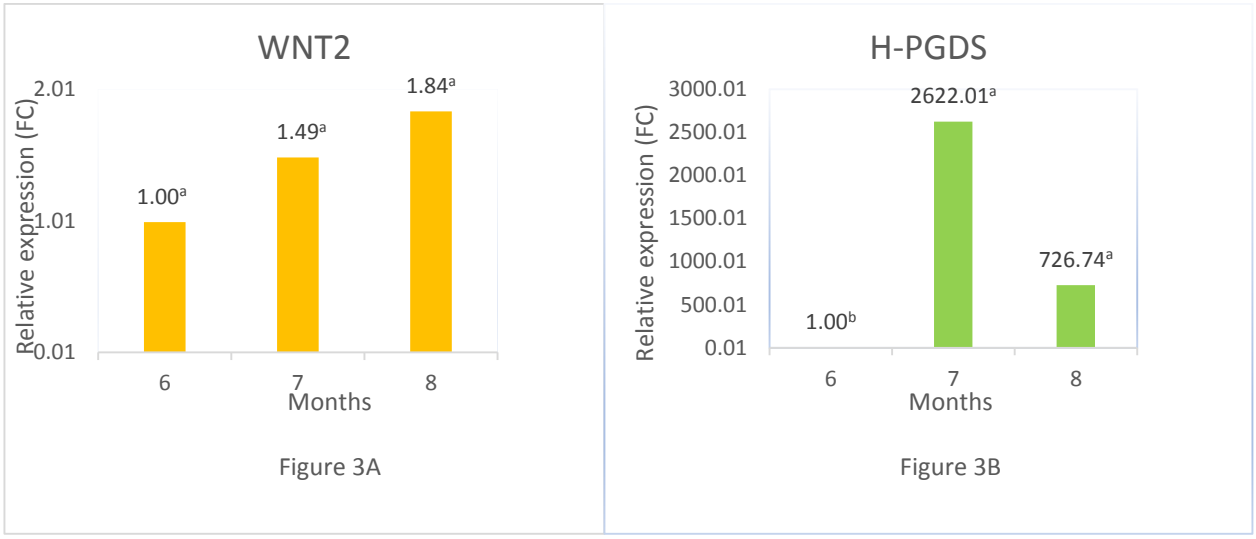

Figure 3. Comparison of relative mRNA expression of WNT2 and H-PGDS genes in the testes of 7 and 8 -month-old roosters with 6 -month-old roosters. FC: Fold change.
a,b: Means with common superscript (s) are not different (P>0.05).

Figure 3 (A and B) shows that the relative expression level of the WNT2 gene in six-month-old roosters was not significantly different from that in seven- and eight-month-old roosters, but the H-PGDS gene significantly increased by 2629.00-fold at 7 months and by 726.74-fold at 8 months compared to 6 months. The standard deviation of gene expression data obtained in real-time PCR studies is normally high. In addition, the PCR data in this study were obtained from different ages (5 to 8 months), which also contributed to high standard deviations, causing the means to be non-significant despite their large differences.

Figure 4 (A and B) shows that the relative expression levels of WNT2 and H-PGDS genes in the testes of 8-month-old roosters were not significantly different from those of 7-month-old roosters.

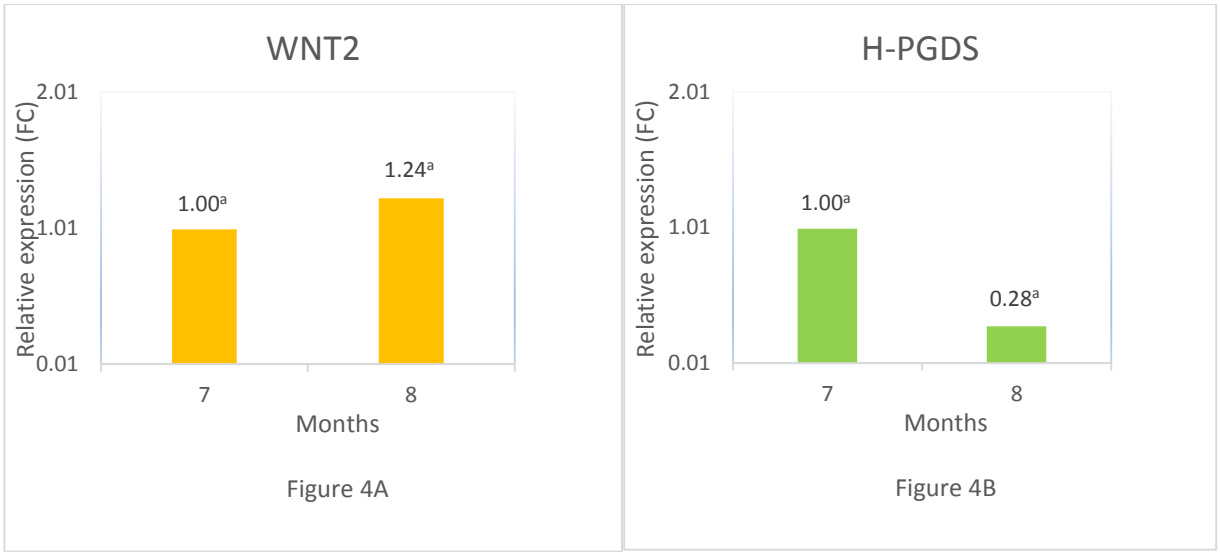

Figure 4. Comparison of relative mRNA expression of WNT2 and H-PGDS genes in the testes of 8-month-old and 7-month-old roosters. FC: Fold change.
a,b: means with common superscript (s) are not different (P> 0.05).

Histology of the seminiferous tubules

The average diameter of the seminiferous tubules and the thickness of the germinal layer of the seminiferous

tubules of 5, 6, 7 and 8-month-old native roosters are shown in Table 3. The average diameter of the seminiferous tubules at the age of 7 and 8 months was significantly greater than that at the ages of 5 and 6 months, but no significant difference was observed between the ages of 7 and 8 months. In general, the diameter of the seminiferous tubules increased significantly with increasing age during puberty (Table 3).

The thickness of the germinal layer of the seminiferous tubules did not differ significantly between the ages of 5 and 6 months, and the highest thickness of the germinal layer of the seminiferous tubules (112.4 µm) was observed in the testis of 7-month-old roosters (Table 2). The thickness of the germinal layer in 8-month-old roosters, although significantly higher than that of 5- and 6-month-old roosters, decreased compared to that of 7-month-old roosters. In short, the thickness of the germinal layer of the seminiferous tubules increased gradually with increasing age from 5 to 7 months, but this trend then decreased until 8 months of age.

Table 3. Mean (± standard deviation) of the diameter of seminiferous tubules and thickness of the germinal layer of seminiferous tubules of testicular spermatozoa jn native roosters aged 5 to 8 months

Characteristics[1]/Age (months)	5	6	7	8	P-value
Diameter of the seminiferous tubule (µm)	$343.07^{b} \pm 21.44$	$354.74^{b} \pm 26.30$	$405.90^{a} \pm 15.43$	$414.08^{a} \pm 25.41$	0.006
Thickness of the germinal layer (µm)	$96.24^{c} \pm 3.12$	$92.25^{c} \pm 5.43$	$112.4^{a} \pm 3.8$	$103.21^{b} \pm 4.01$	<0.001

[1] Characteristics were calculated based on the average cross-sections of 50 seminiferous tubules per bird.
[a,b:] Within row, means with common superscript (s) are not different (P> 0.05).

Discussion

Various factors such as breed, age, season, temperature, nutrition, and rearing and maintenance system affect the reproduction and fertility of roosters. In many birds, the size of the testes undergoes significant changes throughout the year. In native domestic birds such as roosters, changes in semen characteristics and testicular size during and after sexual maturity have been the focus of most researchers in this field (Meamar and Zamiri, 2005). The results of the study by Heshmatipour et al. (2020) on Fars native roosters aged 5 to 8 months also showed that the size of the testes and the population of Sertoli and Leydig cells reached their maximum values at the age of 8 months. The main purpose of the present study was to investigate the changes in the relative expression of the two genes, WNT2 and H-PGDS, the genes responsible for the growth and activity of the seminiferous tubules, during the puberty period in native roosters, and to complete another part of the information related to the factors affecting rooster reproduction during puberty. In general, it was shown that at the end of the puberty period (8 months), in addition to increasing the relative expression of these genes, the diameter of the seminiferous tubules and the thickness of the germinal layer also increased. In a similar study on the testis of roosters (Sun et al., 2019), it was also shown that the relative expression of the H-PGDS and WNT2 genes increased significantly at the end of the puberty period (9 months), which, considering the slight difference in the time of the beginning and end of the puberty period in different breeds, the results in these two studies are completely consistent in this respect.

With the onset of puberty in birds, the testes grow rapidly, and the increase in testicular size is positively correlated with the increase in the diameter of the seminiferous tubules and the population of the main cells (Sertoli and Leydig) in testes (Gonzalez-Maran Soria-Castro, 2010). In the present study, along with the gradual increase in the rooster age during puberty, the diameter of the seminiferous tubules and the thickness of the germinal layer also gradually increased, which was in line with the gradual increase in the relative expression of the H-PGDS and WNT2 genes during the puberty in these roosters. Similar results were obtained in similar studies regarding the increase in the expression of these genes in the testis and their effect on the activity of the seminiferous tubules, sperm motility, and sperm concentration (Sun et al., 2019). Growth and development of the testes and sperm production during puberty depend on several factors, the most important of which are gonadotropins and testosterone (Sarabia et al., 2013). Since the H-PGDS gene plays a key role in the synthesis of prostaglandin D in the production of steroids, especially testosterone in Leydig cells, therefore, this gene, through its role in testosterone synthesis, is directly involved in sperm maturation and the fertility of male birds during puberty (Schell et al., 2007).Previous study on Fars native roosters during puberty (5 to 8 months of age) showed that the highest serum testosterone concentration and androgen receptor gene expression in the testes gradually increased from 5 to 8 months of age and reached their highest value at 8 months of age (Lohari et al., 2020). In the present study, changes in H-PGDS gene expression were similar to changes in testosterone concentration in the blood of 5- to 8-month-old native roosters in previous studies (Lohari et al., 2010); therefore it may be concluded that one of the reasons for the increase in the diameter of the seminiferous tubules in the presence of testosterone is the simultaneous increase in H-PGDS gene expression and its specific effect on intratesticular steroidogenesis in the testis (Schell et al., 2007).

The relative expression of the WNT2 gene has been studied in species other than birds, such as mice and humans. The results have shown that, reduced the relative expression of this gene in the reproductive tract of these species was directly related to the reduced

fertility. Even reduced expression of this gene has caused abnormal testicular growth and an increase in the percentage of abnormal sperm (Linnemannstos et al., 2014).

The results of numerous studies have shown that there is a high correlation between the total number of sperm produced in the testes and the diameter of the seminiferous tubules and the thickness of the germinal layer (Hu et al., 2013). The results of the study by Sun et al. (2019) showed that in two groups of roosters of the same age with different sperm quality, the expression levels of the H-PGDS and WNT2 genes were different, meaning that in the group with lower sperm quality, the expression of these two genes was also lower. Which itself indicates the special effect of these genes on the process of producing motile and fertile sperm in the testes. Since the production of high-quality sperm in roosters, in terms of the total number of sperm produced, is directly dependent on the function of the seminiferous tubules, especially the germinal layer within these tubules (Froman and Rhoads 2013). Therefore, it can be concluded that another reason for the increase in the diameter of the seminiferous tubules and the thickness of the germinal layer at the end of the puberty period of the roosters in the present study, in addition to the effect of the main reproductive hormones (gonadotropins and sex hormones) on the growth and development of the testes during puberty, is the increase in the local expression of genes such as WNT2 in this organ, the effect of which on testicular activity and the production of quality sperm has been previously proven (Linnemannstos et al., 2014).

Conclusions

The results of the present study generally showed that during puberty in native roosters (5 to 8 months of age), the relative expression of genes related to the growth and development of testes, such as WNT2 and H-PGDs, increased gradually from the beginning to the end of puberty, and at the same time, the growth of spermatogenic tubules reached its highest rate with a trend similar to the changes in the relative expression of these genes at the end of the puberty.

Conflict of interest

The authors confirm that there are no conflicts of interest with the conduct or publication of this research.

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References

Avital-Cohen, N., Heiblum, R., Argov-Argaman, N., Rosenstrauch, A., Chaiseha, Y., Mobarkey, N., Rozenboim, I., 2013. Age-related changes in gonadal and serotonergic axes of broiler breeder roosters. *Domestic Animal Endocrinology* 44, 145-150.

Froman, D.P., Rhoads, D.D., 2013. Breeding and genetics symposium: a systems biology definition for chicken semen quality. *Journal of Animal Science* 91, 523-9.

Fumel, B., Guerquin, M.J., Livera, G., Staub, C., Magistrini, M., Gauthier, C., Fouchécourt S., 2012. Thyroid hormone limits postnatal sertoli cell proliferation *in vivo* by activation of its alpha1 isoform receptor (TR alpha1) present in these cells and by regulation of Cdk4/JunD/c-myc mRNA levels in mice. *Biology of Reproduction* 87, 16-1.

González-Morán, M.G. and Soria-Castro, E., 2010. Histological and stereological studies on leydig cells in the testes of *Gallus domesticus* from pre-hatching to sexual maturity*. Animal Reproduction Science* 120**,** 129-135.

Heshmatipour, Z., Meamar, M., Mohaghegh Dolatabady, M., Hashemi S.M.R., 2020. Investigation of insulin-like growth factor-1 (IGF-1) gene expression and histological changes in testes during puberty in native roosters*. Iranian Journal of Animal Science* 2, 121-128. (In Persian).

Holzem M., Braak N., Brattstrom O., 2019. WNT gene expression during early embryogenesis in the Nymphalid butterfly (*Bicyclus anynana*). *Frontiers in Ecology and Evolution* 8, 1-12

Kamauchi, S., Urade, Y., 2011. Hematopoietic prostaglandin D synthase inhibitors for the treatment of Duchenne muscular dystrophy. *Brain and Nerve* 63, 1261-1269.

Khostavaneh, R., Meamar, M., Habibizad, J., Muhaghegh, Dolatabady M., Naghiha Najafabadi R., 2025. Investigate the gene expression of interleukins 1 and 6 in the testis and sperm characteristics of roosters during puberty. *Iranian Journal of Livestock Sci*ence 1, 1-8.

Kouatcho, F.D., Kenfack, A., Ngoula, F., Teguia, A., 2015. Sexual maturity prediction based on hormonal profiles, testes and semen characteristics in male Coturnix quail (*Garsault, 1764*) in the western Highlands of Cameroon. *International Journal of Agronomy and Agriculture Research* 7, 143-154.

Linnemannstons K.C., Ripp, M., Honemann-Capito, K., Brechtel-Curth, M., Hedderich, M., Wodarz, A., 2014. The PTK7-related transmembrane proteins off-track and off-track 2 are co-receptors for Drosophila Wnt2 required for male fertility. *PLOS Genetics* 10, e1004443.

Lohari Yeylaghi, S., Meamar, M., Muhaghegh Dolatabady, M., Naghiha Najafabadi R., 2020. Investigation on the changes in sexual steroids concentrations and their receptors gene expression during the sexual maturity in native roosters*. Iranian Journal of Animal Science* 51, 37-44. (In Persian).

Meamar, M., Zamiri M.J., 2005. Seasonal distribution of semen characteristics and fertility of native Persian roosters. *Iranian Journal of Agriculture Science* 36,581-590. (In Persian).

Parr, B.A., McMahon, A.P., 1998. Sexually dimorphic development of the mammalian reproductive tract requires Wnt-7a. *Nature* 395, 707-710.

Pfaffl, M.W., Horgan, G.W., Dempfle, L., 2002. Relative expression software tool (REST©) for group-wise comparison and statistical analysis of relative expression results in real-time PCR. *Nucleic Acids Rese*arch 1;30, e36.

Pitetti, J. L., Calvel, P., Zimmermann, C., Conne, B., Papaioannou, M.D., Aubry, F., Cederroth, C. R., Urner, F., fumel, B., Crausaz, M., Docquier, M., Herrera, P.L., Pralong, F., Germond, M., Guillo F., Jegou, B., Nefs, S., 2013. An essential role for insulin and IGF- 1 receptors in regulating sertoli cell proliferation, testis size and FSH action in mice. *Molecular Endocrinology* 27, 814-827.

Rossitto, M., Ujjan, S., Poulat, F., Boizet-Bonhoure, B., 2015. Multiple roles of the prostaglandin D2 signaling pathway in reproduction. *Reproduction* 149, R49-R58.

Sarabia Fragoso, J., Pizarro Díaz, M., Abad Moreno, J.C., Casanovas Infesta, P., Rodriguez-Bertos, A., Barger K., 2013. Relationships between fertility and some parameters in male broiler breeders (body and testicular weight, histology and immunohistochemistry of testes, spermatogenesis and hormonal levels). *Reproduction in Domestic Animal* 1, 345-352.

SAS, 2020. SAS/STAT® 9.2. User's Guide. Statistical Analysis System,SAS Institute Inc. Cary, North Carolina.

Schell, C., Frungieri, M.B., Albrecht, M., Gonzalez-Calvar, S.I., Köhn, F.M., Calandra, R.S., Mayerhofer, A., 2007. A prostaglandin D2 system in the human testis. *Fertility and Sterility* 88, 233-236.

Sturkie, P.A., 2016. Sturkie's Avian Physipology, 6th ed. Academic Press, San Diago, USA. P. 675 (eBook ISBN: 9780124072435).

Sun,Y., Xue, F., Li ,Y., Fu, L., Bai, H., Ma, H., Chen, J., 2019. Differences in semen quality, testicular histomorphology, fertility, reproductive hormone levels, and expression of candidate genes according to sperm motility in Beijing-You chickens. *Poultry Science* 98, 4182-4189.

Udoumoh, A.F., Nwaogu, I.C., Igwebuike, U.M., Obidike, I.R., 2020. Histogenesis and histochemical features of gastric glands of pre-hatch and post-hatch broiler chicken. *The Thai Journal of Veterinary Medicin* 50, 17-25.

Wilson, F.D., Johnson, D.I., Magee, D.L., Hoerr, F.J., 2018. Testicular histomorphometries including sertoli cell quantitation for evaluating hatchability and fertility issues in commercial breeder-broiler roosters. *Poultry Science* 97, 1738-1747.

Zirkin, B. R., Papadopoulos, V., 2018. Leydig cells: formation, function, and regulation. *Biology of Reproduction* 99, 101-111.