Expression Pattern of DMRT1 and STRA8 Genes During Postnatal Development of Rabbit Testes

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Abstract

Thirty New Zealand rabbits of various ages were used for this investigation. DMRT1 and STRA8 genes expression was recorded by RT PCR, completed by histological sections of testes in studied ages. DMRT1 and STRA8 genes have distinct roles during spermatogenesis in mammals. The present study hypothesized that the change in the level of specific genes expression during spermatogenesis could be related to the change in the complement of germ cell types. Distinct expression patterns were observed for DMRT1 reached the maximum level (peak or plateau) just before spermatogenesis process at age of 6.5th week and testicular STRA8 reached the maximum level at 6 months. The two genes expression patterns are consistent with their specific roles during spermatogenesis. The purpose of the present study was to determine the expression of DMRT1 and STRA8 genes throughout postnatal New Zealand rabbit testis that would serve as a reference for expression pattern of the studied genes.

Keywords: expression, testes, DMRT1, STRA8, postnatal, spermatogenesis, rabbit.

Introduction

The rabbit is an excellent model used in a variety of biomedical research fields including neuroscience, oncology, embryonic development, cardiovascular studies, dermatology, and reproduction (Ewuola & Equnike, 2010; Asano *et al*., 2011 and Guo *et al*., 2012). Rabbit is an attractive species for making gonad studies due to easily identification of the morphological changes of the seminiferous epithelium cycle (Ewuola and Equnike, 2010). The period of

pre-spermatogenesis exceeds that of other laboratory animals such as rat. This particular characteristic ma-kes the development of rabbit closer to that of human (Wu *et al*., 2003; Vigueras *et al*., 2006 and Culty, 2009).

Spermatogenesis is a complexly regulated process and any genetic disturbance in it leads to male infertility. The adult mammalian testis is among the body's most proliferative tissues, producing millions of highly specialized gametes, or spermatozoa each day. Spermatogenesis is carefully regulated, ensuring that spermatozoa are produced at a constant rate (Endo *et al*., 2015).

Double-sex and mab-3-related transcription factor1 (DMRT1) acts as the 'gatekeeper' that prevents uncontrolled entry of spermatogonia to meiosis (Don *et al*., 2011). DMRT1 is required for spermatogenesis as it is critical to maintain the balance between mitotic and meiotic germ cells. In addition, it has a prominent role in testicular differentiation in all vertebrates (Zarkower, 2012). DMRT1 is essential in spermatogonia to restrict retinoic acid responsiveness and directly repress STRA8 transcription, thereby preventing meiosis and promoting spermatogonial development by coordinating spermatogonial development and mitotic amplification with meiosis, as a result, DMRT1 allows abundant and continuous production of sperms (Matson *et al*., 2010). In mice, DMRT1 is expressed in all mitotic spermatogonia, but the expression decreases with the onset of spermatogonial differentiation and disappears at the initiation of meiosis (Matson *et al*., 2010). Moreover, Don *et al*. (2011) stated that DMRT1 is highly expressed in undifferentiated spermatogonia, less abundantly expressed differentiating spermatogonia, and absent from preleptotene spermatocytes or other meiotic or postmeiotic cells.

Stimulated by retinoic acid gene 8 (STRA8) protein plays roles for the progression of meiosis (Choi *et al*., 2010). Additionally, the gene STRA8 is essential for meiotic initiation, also promotes (but is not required for) spermatogonial differentiation (Endo *et al*., 2015). In mice, STRA8 expression begins immediately before spermatogonial differentiation as well as STRA8 expression begins in late undifferentiated spermatogonia and persists in differentiating spermatogonia (Endo *et al*., 2015). Additionally, STRA8 expression is increased in preleptotene spermatocytes (premeiotic cells) as well as in undifferentiated spermatogonia (Zhou *et al*., 2008 and Mark *et al*., 2015). Because STRA8 promotes spermatogonial differentiation and is required for

meiotic initiation, precisely timed increases in STRA8 expression might coordinate both transitions, ensuring their co-occurrence in stages of spermatogonial differentiation and meiotic initiation (Endo *et al*., 2015). To the best of our knowledge, this is the initial reference to analyze the expression pattern of rabbit DMRT1 and STRA8 genes during cellular development, using spermatogenesis as a model system.

Materials and Methods

Animals

The current study was carried out on 30 male New Zealand rabbits of various ages (0 dpp, 2w, 4w, 6w, 6.5w, 7w, 7.5w, 3m ,4m & 6m), three animals for each age were used to obtain their testes.

Histology

The animals were slaughtered according to guidelines of animal care committee of Faculty of Veterinary Medicine, Benha University. Testes were obtained from rabbits, fixed in Bouin's solution then dehydrated in graded ethanol. Tissues were then infiltrated and embedded in paraffin. Five micron sections were mounted onto gelatin-coated slides and stained with Haematoxylin & Eosin for general histological structures and periodic acid Schiff (PAS) for mucopolysaccharide using standard methods (Drury and Walington, 1980).

Quantitative RT-PCR

Rabbit testicular tissues were obtained and total RNA was extracted using total RNA Purification Kit following the manufacturer protocol (iNtRON Biotechnology, easy-REDTM Total RNA Extraction Kit). cDNA was synthesized from 5 µg of total RNA using M-MuLV Reverse Transcriptase enzyme following the manufacturer protocol (Thermo Scientific, Fermentas, # EP0451). Quantitative PCR was performed using 2X Maxima SYBR Green/ ROX qPCR Master Mix following the manufacturer protocol (Thermo scientific, USA, # K0221). The results were analyzed by the delta-delta CT method using H2AFx gene as an internal reference. Results were plotted as percentage of maximum expression+/- SE.

RT-PCR primer sequences were as following:

Gene	Forward primer (/5 ------ /3)	Reverse primer (/5 ------ /3)
DMRT1	GGAGCCTCCCAGCACCTTAC	TGCATCCT GTACTGC GAACTCA
STRA8	GACAACAATGAGGCTCCAAATG	TGATCTG-CAC-GTAGAGC TGAAAC
H2AFx	ACCTGACGGCCGAGATCCT	CGCCCAG-CAGCTT-GTTGAG

Results

Histological observations:

Histological analysis of (0dpp-4weeks) old rabbit testes revealed that the testicular cords contained two types of cell populations, a large number of dark polygonal cells with irregular nuclei, Sertoli cells, and a small number of large, light, round cells with relatively round nuclei, pre-spermatogonia or undifferentiated spermatogonia (Figs.1 A, B & C). Toward the end of the period of pre-spermatogenesis (6 - 6.5weeks), germ cells appeared large with pale cytoplasm and large vesicular nuclei with clear nucleoli, late undifferentiated spermatogonia (Figs.1D, E).

At 7-7.5weeks old rabbit testis, spermatogenesis began at which the late undifferentiated spermatogonia underwent maturation and transformed to spermatogonia. The latter were spherical or ovoid with clear cytoplasm and large round nuclei with condensed chromatin island peripherally seated and their nuclei were eccentric or central and vesicular (Fig.1F,2B).

Histological examination of 3 months-old rabbit testis revealed the appearance of primary spermatocytes in some seminiferous tubules (Fig.2B). Spermatids were first seen at 4-months- old rabbit testis that reacted positively to PAS stain (Fig.2C). All stages of spermatogenesis were evident at 6-months old rabbit and spermatozoa appeared in the tubular lumen at this stage (Fig.2D).

Analysis of Gene expression:

The data obtained from RT- PCR revealed a significant ($P \leq 0.05$) gradual increase in the expression level of the DMRT1 gene in testis of rabbit from the day of the birth (0dpp) till reach the maximum level (peak or plateau) at age of 6.5W (Fig. 3). Following 6.5W, the expression declined gradually till reached the age of 3M. After that, the expression became very low but still present. The highest significant up-regulation was at age of 6.5W, while the lowest down-regulation was seen at age 6M.

The data obtained from RT- PCR revealed a significant ($P \leq 0.05$) gradual increase in the expression level of the STRA8 gene in testis of rabbit from the day of birth (0D) till reached the maximum level (peak or plateau) at age of 6M (Fig. 4). Unlike DMRT1 gene, the highest significant up-regulation in STRA8 gene was noticed at age from 3M to 6M, while the lowest down-regulation was seen at age from 0dpp to 6W.

Discussion

Previous studies showed strong correlation between the two genes

(DMRT1, STRA8) and spermatogenesis (Kim *et al.,* 2007; Mark *et al*., 2008; Choi *et al*., 2010; Maston *et al*., 2010; Don *et al.*, 2011 and Endo *et al*., 2015).

The current investigation showed the expression analysis at histo-logical level as gene expression was associated with precise stages of the seminiferous epithelium cycle. To begin our survey of DMRT1 and STRA8 expression in rabbit testis, we used RT-PCR to investigate the level of mRNA production for the two genes in total testis samples from rabbit at various ages (0 dpp, 2w, 4w, 6w, 6.5w, 7w, 7.5w, 3m, 4m & 6m).

The present work analyzed DMRT1 expression level which was gradually increased immediately after birth (0day) to (4w) which might be due to gradual increase in mitosis, which suggested that DMRT1 may play role in mitotic process. This was in accordance with Kim *et al.* (2007). The present study indicated that the period of pre-spermatogenesis was mitotically active. This was in agreement with that mentioned by Iczkowski *et al*. (1991). Contrastingly, Gondos *et al*. (1973) stated that, the period of pre-spermatogenesis was a quiescent period during which germ cell mitosis ceases followed by a second postnatally mitotic stage just before the onset of spermatogenesis. However, in rat, cessation of mitosis occurred between day 18 and day 19 in the fetus, and division did not resume until one week later, 4 days after birth, before spermatogenesis (Huckins and Clermont, 1968).

According to Gondos *et al*. (1973) and Iczkowski *et al*. (1991) who noticed increasing of mitosis just before the onset of spermatogenesis process, the current study revealed that the expression of DMRT1 was dramatically increased at 6w and reached peak at 6.5w which suggested that this increase might be due to sharp increase of mitosis just before the onset of spermatogenesis process. Noticeable, the increase of gene expression coincides with the appearance of late undifferentiated spermatogonia in seminiferous cord. This proves that DMRT1 was required to allow mitotic amplification before spermatogenesis and therefore, providing large numbers of spermatogonia that enter meiosis allowing abundant, continuous production of sperms. This coincides with that mentioned by Maston *et al*. (2010) and Don *et al.* (2011). The results presented here establish DMRT1 as a key regulator of the mitosis.

It is worth to mentioning that, spermatogenesis begins in New Zealand rabbits at 7-8week and when we investigated the level of mRNA pro-

duction for DMRT1 gene at this period; the expression of the gene was decreased. This proves that by onset of spermatogenesis, the expression of DMRT1 was decreased. As mentioned before, the expression decreased with the onset of spermatogonial differentiation (Matson *et al*., 2010). Dissimilar situation has been reported in the rainbow trout (Marchand *et al*., 2000), where DMRT1 expression was found to be highly expressed throughout spermatogenesis. Notably, at the level of histological analysis, the present study found that the late undifferentiated spermatogonia underwent maturation and transformed to spermatogonia at 7-8weeks postnatally. On the other hand, examination of expression of DMRT1 revealed decrease in expression at that stage. This result can be interpreted by that *DMRT1* was less abundantly expressed in differentiating spermatogonia as reported by Don *et al*.(2011)

Furthermore, the histological examination of 3 months-old rabbit testis revealed the appearance of primary spermatocytes in some seminiferous tubules and when following the gene expression, the expression was decreased than the previous age. This result can be interpreted by absence of DMRT1 expression in primary spermatocytes. This was in agreement with that mentioned by Maston *et al*. (2010) and Don *et al*. (2011) by in situ-hyperdization study.

RT-PCR data for DMRT1 of 4 months and 6 months rabbit testis revealed marked low expression near background. This decrease might be due to increased meiosis at these ages. This result was in agreement with that reported by Maston *et al*. (2010). Also, Marchand *et al*. (2000) mentioned that, the expression of DMRT1 decreased in rainbow trout at spermiation.

From histological view at these ages, all spermatogenic cells (spermatogonia, spermatocyte, spermatid and spermatozoa) appeared and when the gene expression was analyzed, marked decrease in the level of expression of *DMRT1* was found. This result can be interpreted by absence DMRT1 in premeiotic cells and postmeiotic cells (Maston *et al*., 2010 and Don *et al.,* 2011). These cells were the major cell type in seminiferous tubules at this age. Also, Johnsen *et al*. (2010) mentioned that in the adult males, abundant dmrt1 mRNA was restricted to the periphery of the tubuli, whereas no signal was identified in the more central region harbouring the spermatocytes and mature spermatids. Moreover, the present study examined the timing of STRA8 expression during postnatal testis development. STRA8 expression was detected in

testis soon after birth. However, its expression appeared to remain relatively stable from 0day to 4w and was present in low level comparing with the following ages which suggests the low expression of this gene in the presence of undifferentiating spermatogonia. This interval revealed the presence of undifferen tiating spermatogonia by the histogical analysis of testis.

The present study observed that the expression of STRA8 was releatively increased just before and during spermatogensis process which might suggest that STRA8 may promote spermatogonial differentiation. This result was consistant was that reported by Endo *et al*. (2015).

Interestingly, the histological analysis of rabbit testis at the age of (6-6.5ws) revealed the presence of late undifferentiated spermatogonia. At the same time, the expression level of STRA8 was releatively increased than the previous ages which might suggest that by the appearance of late undifferentiating spermatogonia, the expression increased. This result could be interpreted by that STRA8 expression also occurs in late undifferentiated spermatogonia in accordance with that reported by Endo *et al*. (2015). Also, Oulad-Abdel-ghani *et al*. (1996) mentioned that, STRA8 expression begins immediately before spermatogonial differentiation in mice. Thus, we could conclude that STRA8 expression begins in late undifferentiated spermatogonia.

At 7-7.5weeks age, spermatogenesis began at which the late undifferentiated spermatogonia underwent maturation and transformed to spermatogonia. At this stage, the expression level of this gene was increased than in the previous ages which might suggest that by the appearance of differentiated spermatogonia, the expression of gene increased. This result was consistant with Oulad-Abdelghani *et al*.(1996). This indicated that STRA8 might promote spermatogonial differentiation as reported by Endo *et al*. (2015).

From histogical view of rabbit testis at the age of 3months, primary spermatocytes were appeared. At the same time, the expression level of STRA8 was increased than in the previous ages which might suggest by the appearance of primary spermatocytes, the ex-pression increased. As already reported by Zhou *et al*. (2008); Endo *et al*. (2015) and Mark *et al*. (2015), STRA8 expression increased in premeiotic cells (primary spermatocyte). This pattern suggests that STRA8 is required for meiosis of the primary spermatocytes and STRA8 protein

may be play a role in the premeiotic phase of spermatogenesis.

Adult rabbit testes at age of 4- 6 months are in waves of meiosis and continue throughout life, providing a continuous supply of sperm from spermatogonial stem cells at the periphery of the testis cords and sperms appeared in the center of seminiferious tubules at this stage. In the current study, RT-PCR recorded high level of expression of STRA8 at this stage and the rabbit testis of 6 months where the volume of testis increased and germ cells which required more miosis increased. This study revealed that the expression of *STRA8* reached its peak which might suggest that STRA8 may play role in meiosis process. As already said, STRA8 is essential for successful meiosis and normal spermatogenesis (Zhou *et al*., 2008). Also, Daniel-carlier *et al*. (2013) mentioned that, in testes, the STRA8 gene was clearly expressed when meiosis started until adulthood.

This data displayed an interesting trend for STRA8 mRNA levels indicating low level of expression in the juvenile testis and rising sharply in a linear fashion to adulthood. This expression pattern would be in accordance with that STRA8 being required in the initiation of meiosis.

References

Ahmed, Y. and Ali, G. K. M. (2012): Histological and histo-morphometric changes of the rabbit testis during postnatal development. *Res. J. Vet. Sei*, *5*, 42–50.

Aire, T. A. (1973): The development of the testis in the dwarf Nigerian ram lamb. Research in Veterinary Science, 14(1), 104.

Archana, P.; Katiyar, R. S.; Sharma, D. N.; Farooqui, M. M.and Prakash, A. (2014): Postnatal development of testis in Gaddi goat (Capra hircus). Int. J. Morphol, 32(1), 166–176.

Asano, Y.; Ishikura, T.; Kudoh, K.; Haneda, R.and Endoh, T. (2011): Prenatal developmental toxicity study of ethyl tertiary-butyl ether in rabbits. Drug and Chemical Toxicology, 34(3), 311–317.

Choi, Y. J.; Yoon, J. W.; Pyo, C. W.; Kim, J. A.; Bae, S. H. and Park, S. S. (2010): A possible role of STRA8 as a transcriptional factor. Genes and Genomics, 32(6), 521–526.

Culty, M. (2009): Gonocytes, the forgotten cells of the germ cell lineage. Birth Defects Research Part C: Embryo Today: Reviews, 87(1), 1–26.

Daniel-Carlier, N.; Harscoët, E.; Thépot, D.; Auguste, A.; Pailhoux, E. and Jolivet, G. (2013): Gonad differentiation in the rabbit: evidence of species-specific features. *PloS One*, *8*(4), e60451.

Don, J.; Nir, U.and Breitbart, H. (2011): DMRT1 at the border between mitosis and meiosis. Asian J Androl, 13(2), 189–190.

Drury, D.R. And Walington, E.A. (1980): Carleton·s histological Techniques.5th ed.Oxford.Univ.Press.

Endo, T.; Romer, K. A.; Anderson, E. L.; Baltus, A. E.; de Rooij, D. G.and Page, D. C. (2015): Periodic retinoic acid–STRA8 signaling intersects with periodic germ-cell competencies to regulate spermatogenesis. Proceedings of the National Academy of Sciences, 112(18), E2347–E2356.

Ewuola, E. O.and Egbunike, G. N. (2010): Effects of dietary fumonisin B1 on the onset of puberty, semen quality, fertility rates and testicular morphology in male rabbits. Reproduction, 139(2), 439–445.

Gondos, B.; Renston, R. H. and Conner, L. A. (1973): Ultrastructure of germ cells and sertoli cells in the postnatal rabbit testis. American Journal of Anatomy, 136(4), 427–439.

Guo, Y.; Zhang, Y.; Jin, N.; Klein, R.; Nicolai, J.; Lewandowski, R. J., Larson, A. C. (2012): Electro-poration-Mediated Trans-catheter Arterial Chemoemboli-zation (E-TACE) in the Rabbit VX2 Liver Tumor Model. Investigative Radiology, 47(2), 116.

Huckins, C., and Y. Clermont (1968): Evolution of gonocytes in the rat testis during late em- bryonic and early post-natal life. Ar
ch. Anat. Hist. Embry., 51: 343-354.

Iczkowski, K. A.; Sun, E. L. and Gondos, B. (1991): Morphometric study of the prepubertal rabbit testis: germ cell numbers and seminiferous tubule dimensions. American Journal of Anatomy, 190(3), 266–272.

Jess, F.B. (1979): Animal Anatomy and Physiology chapterB., 55-387.Reston publishing Company, Inc.,Reston,Virginia.Aprentice Hall Company.

Johnsen, H.; Seppola, M.; Torgersen, J. S.; Delghandi, M. and Andersen. (2010): Com-parative Biochemistry and Physio-logy , Part B Sexually dimorphic expression of dmrt1 in immature and mature

Atlantic cod (*Gadus morhua L* .). Comparative Bioche-mistry and Physiology, Part B, 156(3), 197–205.

Kim, S.; Bardwell, V.J. and Zarkower, D. (2007): Cell type-autonomous and non-autonomous requirements for Dmrt1 in postnatal testis differentiation. Dev. Biol. 307, 314–327.

Marchand, O.; Govoroun, M.; Cotta, H. D.; Mcmeel, O.; Lareyre, J.; Bernot, A., Guiguen, Y. (2000): DMRT1 expression during gonadal di ¡ erentiation and spermato-genesis in the rainbow trout , Onc-orhynchus mykiss. Develop-ment, 1493, 180–187.

Mark, M.; Jacobs, H.; Oulad-Abdelghani, M.; Dennefeld, C.; Féret, B.; Vernet, N., Ghyselinck, N. B. (2008): STRA8-deficient spermatocytes initiate, but fail to complete, meiosis and undergo premature chromosome conden-sation. Journal of Cell Science, 121(Pt 19), 3233–3242.

Mark, M.; Teletin, M.; Vernet, N.and Ghyselinck, N. B. (2015): Role of retinoic acid receptor (RAR) signaling in post-natal male germ cell differentiation. Biochimica et Biophy-sica Acta (BBA)-Gene Regu-latory Mechanisms, 1849(2), 84–93.

Matson, C. K.; Murphy, M. W.; Griswold, M. D.; Yoshida, S.; Bardwell, V. J.and Zarkower, D. (2010): The mammalian doublesex homolog DMRT1 is a transcriptional gatekeeper that controls the mitosis versus meiosis decision in male germ cells. Developmental Cell, 19(4), 612–624.

Oulad-Abdelghani, M.; Bouillet, P.; Decimo, D.; Gansmuller, A.; Heyberger, S. Dolle, P. and Cham-bon, P. (1996): Characterization of a premeiotic germ cell-specific cyto-plasmic protein encoded by Stra8, a novel retinoic acid-responsive gene. Journal of Cell Biology, 135 (2), 469-477.

Vigueras-Villaseñor, R. M.; Moreno-Mendoza, N. A.; Reyes-Torres, G.; Molina-Ortiz, D.; León, M. C. and Rojas-Castañeda, J. C. (2006): The effect of estrogen on testicular gonocyte maturation. Rep-roductive Toxicology, 22(3), 513–520.

Wu, J.; Jester, W. F.; Laslett, A. L.; Meinhardt, A. and Orth, J. M. (2003): Expression of a novel factor, short-type PB-cadherin, in Sertoli cells and spermatogenic stem cells of the neonatal rat testis. Journal of Endocrinology, 176(3), 381–391.

Zarkower, D. (2012): DMRT genes in vertebrate gametogenesis. Current Topics in Developmental Biology, 102, 327–356.

Zhou, Q.; Nie, R.; Li, Y., Friel, P.; Mitchell, D.; Hess, R. A., …

Griswold, M. D. (2008): Expression of stimulated by retinoic acid gene 8 (Stra8) in spermatogenic cells induced by retinoic acid: an in vivo study in vitamin A-sufficient postnatal murine testes. Biology of Reproduction, 79(1), 35–42.

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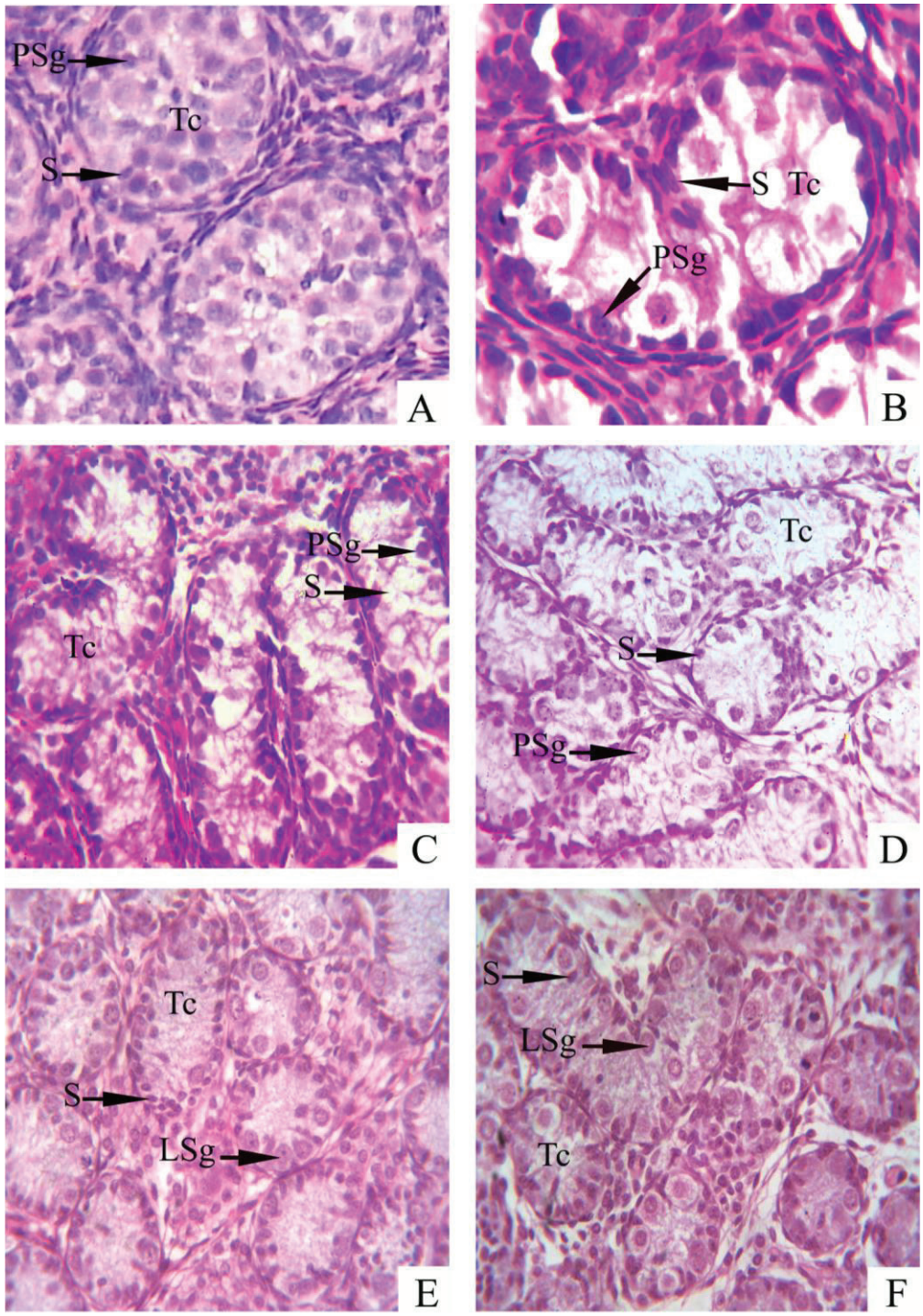
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Fig (1): (A, B & C): Photomicrographs of 0dpp, 2w and 4w old New Zealand rabbit testis respectively showing that: the testicular cords (Tc) contained two types of cell populations; small number of large, light, round cells with relatively round nuclei, prespermatogonia (PSg) and a large number of dark polygonal cells with irregular nuclei, Sertoli cells (S). **(D):** 6w and (**E)** 6.5w, the late undifferentiated spermatogonia appeared large with pale cytoplasm and large vesicular nucleus with clear nucleolus, (LPSg). H&E staining. X 40. Tc. testicular cord, PSg. Prespermatogonia, S. Sertoli cells, LPSg. Late undifferentiated spermatogoni

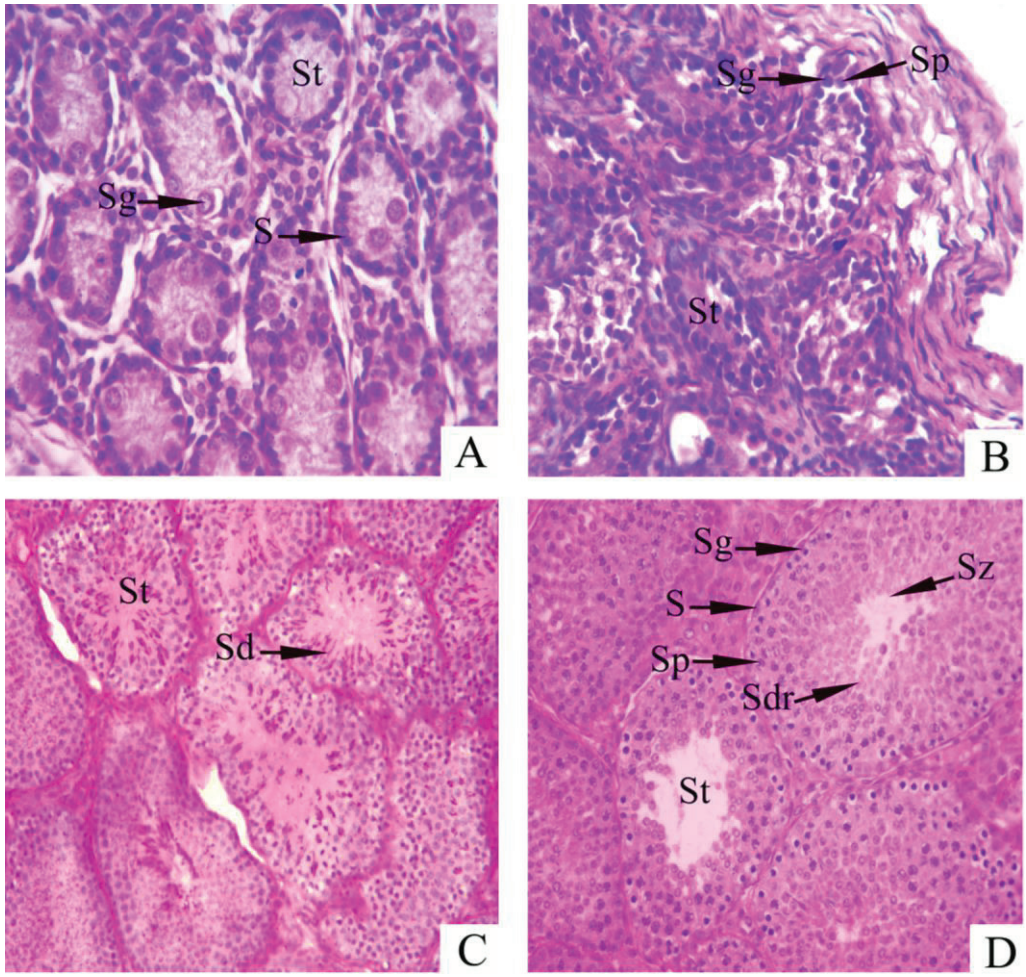

Fig (2): (A & B): Photomicrographs of 7w and 7.5 w old New Zealand rabbit testis respectively showing that: The spermatogenesis began at which the late undifferentiated spermatogonia (LSg) underwent maturation and transformed to spermatogonia (Sg) which appear spherical with clear cytoplasm and large round nuclei with condensed chromatin island peripherally seated and their nuclei were central or eccentric and vesicular. (B): 3m, the primary spermatocytes (Sp) observed in some seminiferous tubule. (C) 4m, Spermatids (Sd) were first seen at these stages. (D): 6m, all stages of spermatogenesis were evident and Spermatozoa (Sz) appeared in the tubular lumen at this stage. (A, B, D) H&E staining, (C) PAS staining. X 40. St seminiferous tubules, Sg. Spermatogonia, S. sertoli cells, Sp. primary spermatocyte, Sd spermatid, Sdr. round spermatid, Sz. Spermatozoa.

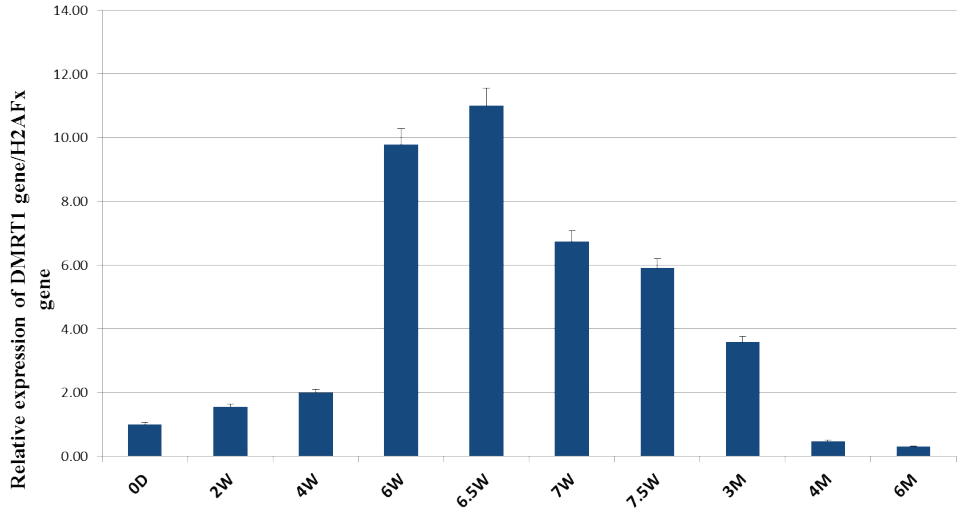

Fig (3): Graphical presentation of quantitative real-time PCR analysis of the expression of DMRT1 gene in rabbit testis from the age of 0dpp to 6M.

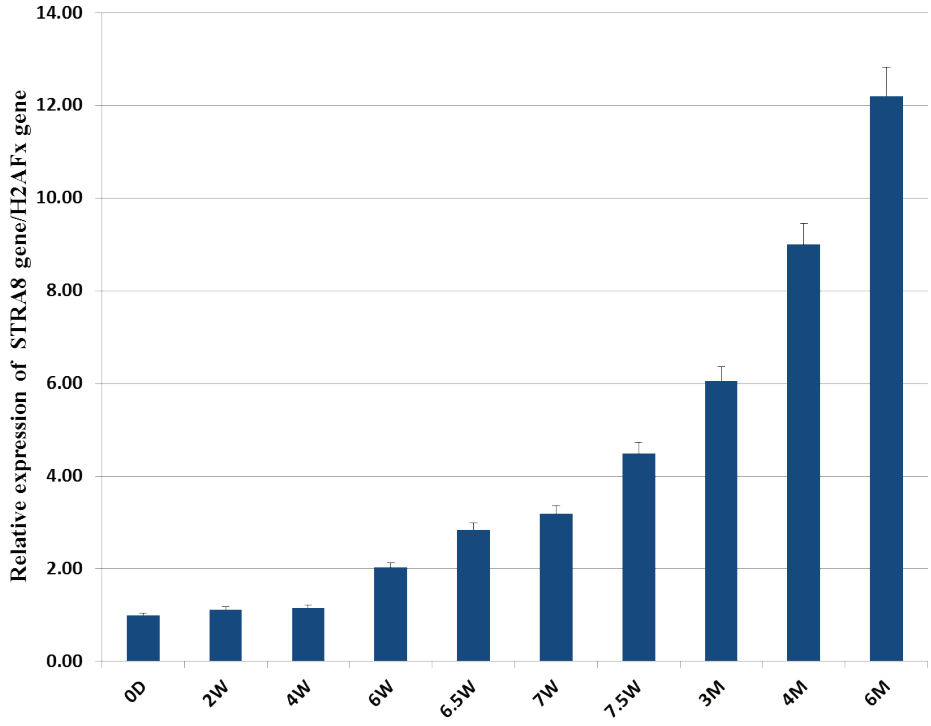

Fig (4): Graphical presentation of quantitative real-time PCR analysis of the expression of STRA8 gene in rabbit testis from the age of 0D to 6M.

Animals of this issue

Rabbit *(Oryctolagus cuniculus)*

Kingdom: Animalia & Phylum: Chordata & Class: Mammalia & Order: Lagomorpha & Family: Leporidae (in part) & Genus: *Oryctolagus*

The rabbit's long ears, which can be more than 10 cm (4 in) long, are probably an adaptation for detecting predators. They have large, powerful hind legs. The two front paws have 5 toes, the extra called the dewclaw. The hind feet have 4 toes. They are plantigrade animals while at rest; however, they move around on their toes while running, assuming a more digitigrade form. Wild rabbits do not differ much in their body proportions or stance, with full, egg-shaped bodies. Their size can range anywhere from 20 cm (8 in) in length and 0.4 kg in weight to 50 cm (20 in) and more than 2 kg. The fur is most commonly long and soft, with colors such as shades of brown, gray, and buff. The tail is a little plume of brownish fur (white on top for cottontails). Rabbits can see nearly 360 degrees, with a small blind spot at the bridge of the nose.

Source: Wikipedia, the free encyclopaedia