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Short Communication

DMRT1 Expression is Positively Correlated with the Development of Testes in Adult Mule Duck

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YY and PG conceived and designed the study. YY, SQY, LXW, XC and YGQ performed the experiments. YY and SQY wrote the paper. LXW, XC, YGQ and PG edited the manuscript.

Key words
Mule duck, DMRT1, Expression, Testes, Development.

ABSTRACT

DMRT1 expression has been followed in the developing testes of three groups (Testes weight: Body weight ratios (<1%, 1%-2%, 2%-3%)) of adult mule duck using immunohistochemical staining method. The result showed that DMRT1 was mainly expressed in spermatogonia nucleus of seminiferous tubules and had no expression in Leydig cells. It was indicated that the DMRT1 expression was positively correlated with the testes development of adult mule duck.

A conserved sequence in *Dmrt*1 (double-sex and mab-3-related transcription factor 1) gene is highly homologous in many species. Studies on *Dmrt*1 have been carried out in mammals, chickens, turtles, frogs and fish, and showed important role in gonad development in different species (Koopman, 2009; Chen *et al*., 2014). In chickens, the expression of *Dmrt*1 in testes was higher than that in ovaries in early embryos (Yang *et al*., 2013), and *Dmrt*1 was a necessary gene for male gonad development (Smith *et al*., 2009). In ducks, the expression pattern of *Dmrt*1 was similar to that in chickens (Koba *et al*., 2008; Wang *et al*., 2015), it's unclear whether *Dmrt*1 has similar functions.

Mule duck is sterile intergeneric hybrid, which is hybridized offspring of muscovy duck (*Cairina moschata*) and domestic duck (*Anas platyrhynchos f. domestica*). It's an important research model for gonadal development. Since the seventies of the 20th century, several researchers focused on the reason of gonad infertility using different methods in mule duck. Rigdon and Mott (1965) conducted a study on the differences in the content of the glycogen, lipid content and parental spermatocyte. Guan *et al*. (2010) identified the duck germ cells by using the specific antibody SSEA-1 of the germ cell. For the reason of infertility mule duck, more scholars believe that the sex chromosome difference (the chromosome of the domestic duck and the duck is 39 pairs, but the chromosome size is different), which leads to no synapsis during meiosis. Fhamida *et al*. (2013) showed that the cause of male sterility was related to the primary oocyte meiosis stagnated. However, with the development of molecular biology technology, there is no synchronous progress on the mule duck gonad development, it is necessary to synchronize the expression regulation of the important genes in mule duck gonadal development. During embryonic development, under the regulation of genes involved in sex determination and sex gland development, poultry gonad began from genital ridge differentiation into testis and ovaries, after hatch out of their shells to sexual maturity, maintain their gender characteristics under the control and regulation of sex hormones. In this study, the regularity and morphological characteristics in adult mule duck testis were conducted, and the expression pattern of DMRT1 in the testis were studied using IHC, to elucidate the role of DMRT1 in the mule duck gonads.

Material and methods

More than 50 mule ducks were raised by standard feeding process, and the feed was provided by Hua Mei Company. The mule ducks were weighed and slaughtered at the age of 300 days, and the left and right testis were taken and weighed respectively, and the growth and development of the testes were analyzed. The testes weight/body weight ratio (%) was (Gross testes weight×100) / body weight.

For histological studies, testes were placed in 4% paraformaldehyde (PFA) for more than 24 h, and

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6μm thick paraffin sections were cut, and stained with hematoxylin-eosin staining (HE). The histological sections of left and right testis were mounted on poly-l-lysine-coated glass slide, dried overnight at 37°C and proceeded for Streptavidin-biotin-peroxidase complex immunohistochemical (IHC) method (Gong *et al*., 2012; Yang *et al*., 2012) using the antibody of DMRT1 (sc 98341, Santa Cruz Biotechnology).

Results and discussion

The gross testes weight ranged from 9.4 g to 102 g, average weight of the left testis was lower than that of the right testis (Supplementary Table I). The testes weight/body weight ratio was 1.55±0.86. The sex gland index is an indicator of sexual maturity, and the testis weight, body weight and testes index of poultry are correlated with sexual maturity and fecundity. Shen and Tong (1991) found that the testes index of Shaoxing duck was 3.16% at 16 weeks, and 3.55% at 43 weeks. This study found that the testes index was only 2.98% at 43 weeks, far lower than that of Shaoxin duck and other poultry species, and testes weight was less than that of the Khaki Campbell duck in the same period (Snapir *et al*., 1998). A few individuals with high testicular index may produce sperm, which is consistent with the results of Tan *et al*. (1998).

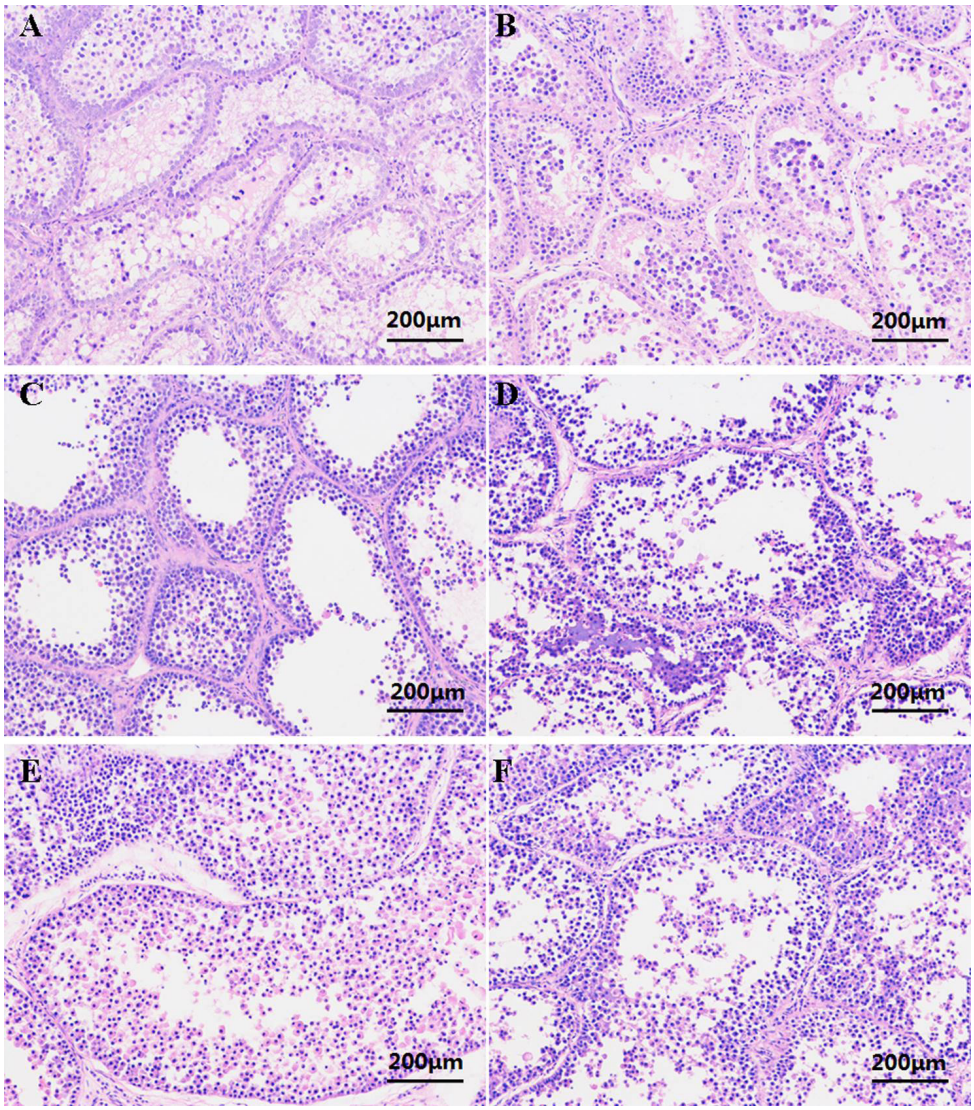

Fig. 1. Histological structure of adult mule duck testis (200×). A and B, the testes weight/body weight ratio <1%; C and D, the testes weight/body weight ratio1-2%; E and F, the testes weight/body weight ratio 2-3%. A, C and E, the left testis; B, D and F, the right testis. Staining: H&E.

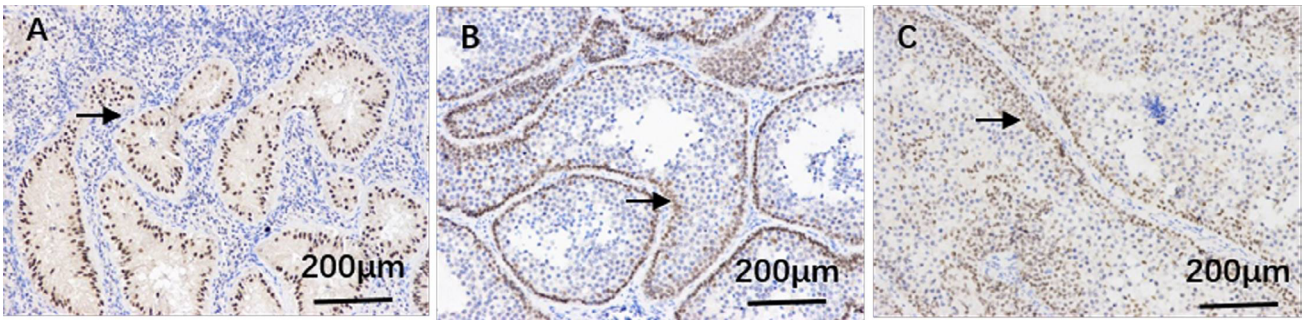

Fig. 2. The expression of DMRT1 in adult mule duck testis (200×). A, the testes weight/body weight ratio <1%; B, the testes weight/body weight ratio 1-2%; C, the testes weight/body weight ratio 2-3%. The arrow shows the expression of DMRT1 in seminiferous tubule.

In the testes weight/body weight ratio <1% sections, fewer interstitial cells between adjacent seminiferous tubule were observed, and 1-2 layer cells in typical seminiferous tubule, spermatgonia were arranged in seminiferous tubule, and no sperm cells or sperm were seen (Fig. 1). All levels of spermatogenic cells were disorganized, scattered and even filled in the entire lumen, with a large number of nuclei falling off. Part of the left testis seminiferous tubule has disappeared except spermatgonia. More than 2 layer cells in typical seminiferous tubule and a large space in the lumen were appeared in the testes weight/body weight ratio 1%-2% sections. More than 4 layer cells were observed, the permutations of spermatozoa were more regular in some testicular tissues in the testes weight/body weight ratio 2%-3% sections (Fig. 1). The occurrence of poultry sperm begins with the division of spermatogenic cells and ends with the formation of mature sperm. The structure of normal mature poultry testis has undergone significant changes during the spermatogenesis. Mature testicular seminiferous tubule has multiple layers of epithelium in different stages of spermatogenesis. The mesenchymal cells exist singly or in clusters, mainly in the larger interstitial spaces (Bacha and Bacha, 2000). This study found that mule duck testes is abnormal compared with general sexual maturity poultry, and showed significant individual difference. This is consistent with the testicular structure of the mule duck (Muscovy drake x Khaki Campbell) reported by Snapir *et al*. (1998). Li *et al*. (2017) screened multiple differentially expressed genes related to reproductive function in mule duck.

The IHC results showed that DMRT1 mainly expressed in seminiferous tubule, and there were significant differences in different testes weight/body weight ratio sections, with the lowest expression in the ratio <1% sections and the highest expression in ratio 2%-3% sections (Fig. 2). It has been confirmed that *Dmrt*1 is a necessary gene for the development of chicken embryo testis (Smith *et al*., 2009). It is speculated that *Dmrt*1 may play a role in the development of testicular development in mule duck. In this study, DMRT1 expression in the testis is positively correlated with the testes weight/body weight ratios, which is consistent with the results of Wang *et al*. (2015).

Conclusions

Mule duck testes is abnormal and showed significant individual difference. DMRT1 was mainly expressed in spermatogonia nucleus of seminiferous tubules in adult mule duck testes. The results indicated that DMRT1 expression is positively correlated with the testes weight/body weight ratios.

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Supplementary material

There is supplementary material associated with this article. Access the material online at: https://dx.doi.org/10.17582/journal.pjz/20200509030529

Statement of conflict of interest

The authors have declared no conflict of interests.

References

Chen, S.L., Zhang, G.J., Shao, C.W., Huang, Q.F., Liu, G., Zhang, P., Song, W.T., An, N., Chalopin, D., Volff, J.N., Hong, Y., Li, Q., Sha, Z., Zhou, H., Xie, M., Yu, Q., Liu, Y., Xiang, H., Wang, N., Wu, K., Yang, C., Zhou, Q., Liao, X., Yang, L., Hu, Q., Zhang, J., Meng, L., Jin, L., Tian, Y., Lian, J., Yang, J., Miao, G., Liu, S., Liang, Z., Yan, F., Li,

Y., Sun, B., Zhang, H., Zhang, J., Zhu, Y., Du, M., Zhao, Y., Schartl, M., Tang, Q. and Wang, J., 2014. *Nat. Genet.*, **46**: 253-260. https://doi.org/10.1038/ng.2890

Gong, P., Yang, Y., Lei, W.W., Feng, Y.P., Li, S.J., Peng, X.L. and Gong, Y.Z., 2012. *Sex. Develop.*, **6**: 178-187. https://doi.org/10.1159/000338471

Guan, W., Wang, Y., Hou, L., Chen, L., Li, X., Yue, W. and MA, Y., 2010. *Poult. Sci*., **89**: 312-317. https://doi.org/10.3382/ps.2009-00413

Li, L., Miao, Z.W., Xin, Q.W., Zhu, Z.M., Zhang, L.L., Zhuang, X.D. and Zheng, N.Z., 2017. *Sci. Agric. Sin.*, **50**: 3608-3619.

Fhamida, B.I., Satoshi, I., Yoshinobu, U., Kornsorn, S. and Yoichi, M., 2013. *Jap. Poult. Sci.*, **50**: 311-320.

Koba, N., Ohfuji, T., Ha, Y., Mizushima, S., Tsukada, A., Saito, N. and Shimada, K., 2008. *J. Poult. Sci.*, **45**: 132-138. https://doi.org/10.2141/jpsa.45.132

Koopman, P., 2009. *Trends Genet.*, **25**: 479-481. https://doi.org/10.1016/j.tig.2009.09.009

Rigdon, R.H. and Mott, C., 1965. *Pathol. Vet.*, **2**: 553-565. https://doi.org/10.1177/030098586500200603

Shen, Y.X. and Tong, L.F., 1991. *J. Zhejiang Agric. Univ.*, **17**: 283-289.

Smith, C.A., Roeszler, K.N., Ohnesorg, T., Cummins, D.M., Farlie, P.G., Doran, T.J. and Sinclair, A.H., 2009. *Nature*, **461**: 267-271. https://doi.org/10.1038/nature08298

Snapir, N., Rulf, J., Meltzer, A., Gvaryahu, G., Rozenboim, I. and Robinzon, B., 1998. *Br. Poult. Sci.*, **39**: 572-574. https://doi.org/10.1080/00071669888791

Tan, J.Z., Chen, H., Song, J.J. and Liu, Y.T., 1998. *Fujian J. agric. Sci.*, **68**: 553-560. https://doi.org/10.1002/(SICI)1097-4628(19980425)68:4<553::AID-APP6>3.0.CO;2-L

Wang, Q., Weng, H., Chen, Y., Wang, C., Lian, S., Wu, X., Zhang, F. and Li, A., 2015. *Br. Poult. Sci.*, **56**: 390-397. https://doi.org/10.1080/00071668.2015.1027172

Bacha, W.J. and Bacha, L.M., 2000. *Color atlas of veterinary histology,* 2nd Edition. Blackwell Publishing Ltd., Oxford.

Yang, Y., Gong, P., Feng, Y.P., Li, S.J., Peng, X.L., Ran, Z.P., Qian, Y.G. and Gong, Y.Z., 2013. *Acta Biol. Hung.*, **64**: 161-168. https://doi.org/10.1556/ABiol.64.2013.2.3

Yang, Y., Gong, P., Feng, Y.P., Yang, Y.P., Li, S.J., Peng, X.L. and Gong, Y.Z., 2012. *J. Anim. Vet. Adv.*, **11**: 561-565.