FHL2 deletion reduces male fertility by impairing the sperm meiosis

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To improving the fertility decline worldwide, this study investigated the spermatogenesis regulating mechanism. Guohua Hua affiliated with the college of Animal Science and Technology, is a faculty at Huazhong Agricultural University.

Fertility decline is a raising issue and about 1 in 6 people worldwide would be affected by infertility. The idiopathic azoospermia and oligospermia are the common cause of male infertility, which displays as spermatogenesis disorders with unknown underlying molecular mechanisms. Spermatogenesis refers to the process in which spermatogonia proliferate and differentiate in the testicular seminiferous tubules, which determines male reproductive physiology and fertility. Our previous study showed that FHL2 deficiency reduced female fertility by impairing follicular development. Here, we reported that FHL2 deletion also impaired male mice fertility. The female mice mated with $FHL2^{-/-}$ male mice displayed smaller litter size and less total offspring number in a 1-month breeding trail, comparing with age matched wildtype (WT) mice. Furthermore, the number of embryo implanted in female mice mated with $FHL2^{-/-}$ male mice was also significantly lower than that in the WT group. Although no significant difference was found in circulation serum testosterone and inhibin B from 5- to 18-week-old mice, FHL2 deletion induced smaller testicular size and organ index, as well as lower epididymis wight and epididymis organ index. Consistently, the sperm density in the epididymis tail of $FHL2^{-/-}$ mice was significantly lower than that of WT mice. But the sperm abnormality rate of $FHL2^{-/-}$ mice significantly increased. These data indicated that FHL2 deletion induced poor

sperm quality which may related with spermatogenesis disorders.

HE staining was then performed to exploring the histological morphology of testicular seminiferous tubules isolated from 3 to 12-week old mice. Both the seminiferous tubules diameter and the seminiferous epithelium thickness of FHL2$^{-/-}$ mice were smaller than those in WT mice, indicating an impaired spermatogenic ability by FHL2 deficiency. Importantly, round spermatids emerged at 3 weeks, and elongated spermatozoa showed at 5 weeks in WT mice seminiferous tubules, however, round spermatids and elongated spermatozoa emerged 1 week later in FHL2$^{-/-}$ mice. HE staining of caput epididymides further confirmed that FHL2$^{-/-}$ mice showed a delayed ejaculation time about 1 week. Further, Immunofluorescence staining of seminiferous tubules isolated from different ages of mice testis were performed to investigated the spermatogenesis. These results showed that, no difference was found in the expression of c-kit (spermatogonia marker) and PLZF (spermatogonial stem cell maker) between P10 and P14 FHL2$^{-/-}$ mice and age matched WT mice, which indicated FHL2 deletion showed imperceptible effect on the spermatogonia stage. However, the total spermatogenic cells (indicated by DDX4 staining) in each seminiferous tubule was significantly decreased in FHL2$^{-/-}$ mice. The expression of SCP3, which involved in the formation of synaptosome complexes was also downregulated on days 10, 14, and 21 in FHL2$^{-/-}$ mice. Besides, the expression of Stra8, which symbolizes the initiation of meiosis, was decreased in the FHL2$^{-/-}$ testes on day 21. Consequently, round sperm (indicated by ACRV1) and elongated sperm (indicated by TNP1) were significantly less in FHL2$^{-/-}$ seminiferous tubule compared with WT mice'. Collectively, these data

illuminated that FHL2 deletion impaired meiosis and delayed spermatogenesis, which leading to idiopathic azoospermia and oligospermia of male mice. FHL2 may serve as a new potential target to improve of male spermatogenesis.