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学位論文題目	Behavior of ACRBP-deficient mouse sperm in the female reproductive tract (ACRBP KO マウス精子の雌性生殖器内における挙動解析)

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Abstract of thesis

In this dissertation, the author describes how proacrosin-binding protein (ACRBP)-deficient mouse sperm migrate through the female reproductive tract.

Previously, depletion of ACRBP in mouse sperm has been reported to exhibit continuous variation in the head morphology. Although ACRBP-null male mice show a severely reduced fertility, the behavior of mutant sperm in the female reproductive tract remains unknown. Thus, this study focused on the role of the uterus and the oviduct in migration of ACRBP-null mouse sperm. The author initially examined the behavior of *Acrbp*$^{-/-}$ (KO) mouse sperm in the uterus and oviduct after coitus. The number of KO mouse sperm deposited in the uterus was comparable to that of *Acrbp*$^{+/+}$ (WT) mouse sperm. However, the number of KO sperm migrated into the oviduct was much smaller than that of WT sperm. Next, epididymal KO sperm in cauda epididymis were subclassified into four cell types (types 1, 2, 3, and 4), according to the shapes of the nucleus and acrosome. Type-1 KO sperm possessed slightly deformed acrosome on the nucleus, which was essentially similar to that of WT sperm. The heads of type-2 and type-3 KO sperm were moderately and severely affected, respectively, while type-4 KO sperm displayed a round-headed shape with a coiled midpiece around the nucleus. The ratios of type-1, type-2, type-3, and type-4 KO sperm recovered from

the uterus were approximately 53, 37, 8, and 1%, respectively. Notably, more than 80% of KO sperm, which were already migrated into the oviduct, were classified into “type 1”, whereas the ratios of type-3 and type-4 KO sperm were negligibly low. When the motility of type-1/type-2 KO sperm was assessed by computer-assisted semen analysis (CASA), these KO sperm deposited in the uterus exhibited irregular swimming patterns of frequent beating and head rotations. However, the CASA scores of oviductal KO sperm were comparable to those of oviductal WT sperm. These data suggest that fertilizing sperm may be selected by the morphology and motility during sperm migration from the uterus to the oviduct through the uterotubal junction (UTJ).

To understand migration of KO sperm in the oviduct further, *Acrbp*$^{-/-}$*/Tg*^{RBGS} transgenic mice expressing enhanced green fluorescent protein (EGFP) and red fluorescent protein (RFP) in the acrosome and the mitochondria, respectively, were generated. The number of *KO/Tg*^{RBGS} mouse sperm, which reached the ampulla, was smaller than that of WT mouse sperm. Importantly, *Acrbp*$^{-/-}$*/Tg*^{RBGS} mouse sperm displayed a marked reduction in the ability to successfully gain access to unfertilized oocytes. Thus, the male subfertility of KO mice may be attributed to incompleteness of the acrosome reaction rather than impairment in the sperm migration from the uterus to the oviduct.

Abstract of assessment result

【Review】

The present study demonstrates the cause of subfertility in ACRBP-deficient male mice in a very thorough manner. The materials and methods developed in this study, which could visualize and quantify the sperm motility and migration in vivo, are unique and valuable for future analysis of subfertile sperm in the female reproductive tract. This work would contribute to our further understanding of pathophysiology of subfertile sperm *in vivo*.

【Result】

The final examination committee conducted a meeting as a final examination on 25 01, 2019. The applicant provided an overview of dissertation, addressed questions and comments raised during Q&A session. All of the committee members reached a final decision that the applicant has passed the final examination.

【Conclusion】

Therefore, the final examination committee approved that the applicant is qualified to be awarded a Doctor of Philosophy in Human Biology.