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# 論　文　内　容　の　要　旨

| 氏　名　（　WANG HAOTING　） | |
|---|---|
| 論文題名 | Elucidation of the Causes of Male Infertility with a Focus on the Sperm Flagellum<br>（精子鞭毛に着目した男性不妊症の原因究明） |

Infertility is a major global health issue, affecting an estimated 17.5% of couples worldwide, and male factors contribute to nearly half of all cases. Although significant progress has been made in reproductive biology, the causes of many cases of male infertility remain unexplained. One of the most frequent clinical manifestations is asthenozoospermia, characterized by severely reduced sperm motility. This condition may occur alone or together with multiple morphological abnormalities of the sperm flagellum, including coiled, bent, shortened, or absent tails. Because these defects are often linked to genetic abnormalities, identifying the molecular functions of flagellar proteins is essential for understanding and diagnosing male infertility.

Normal sperm motility is indispensable for successful fertilization, as spermatozoa must actively navigate through the female reproductive tract to reach the egg. Motility is driven by the sperm flagellum, which produces propulsive force through coordinated bending. After entering the female tract, activation of the CatSper calcium channel elevates intracellular $Ca^{2+}$ levels and induces “hyperactivation”, a shift from symmetrical, low amplitude beating to vigorous, asymmetrical movement. Hyperactivation enables spermatozoa to overcome physical and biochemical barriers, and defects in either basal motility or hyperactivation lead to infertility.

The core machinery that powers sperm motility is the axoneme, a conserved microtubule-based structure found in cilia and flagella across species. The axoneme exhibits a characteristic “9+2” arrangement composed of nine outer microtubule doublets surrounding a central pair of singlet microtubules. This structure contains specialized components, including inner and outer dynein arms that generate force, radial spokes that transmit regulatory signals, and the nexin-dynein regulatory complex that ensures structural integrity and coordinated bending. Recent cryo-electron microscopy studies have revealed new levels of structural complexity, identifying numerous conserved proteins whose functions in spermatozoa remain largely unknown.

To clarify the roles of these proteins in vivo, this thesis investigates several conserved and testis-enriched axonemal proteins using CRISPR/Cas9-generated mouse knockout (KO) models. By integrating morphological, biochemical, and functional analyses, we aimed to determine how defects in specific axonemal components contribute to impaired sperm motility and male infertility.

We analyzed the microtubule inner protein CFAP77. Although CFAP77 deficiency did not disrupt the general axonemal architecture, *Cfap77* KO males exhibited markedly reduced sperm motility and decreased fertility. These findings suggest that CFAP77 stabilizes outer doublet microtubules and supports effective flagellar beating. Moreover, MYCBPAP is a central apparatus-associated protein. *Mycbpap* KO spermatozoa displayed severe defects in tail assembly, including shortened or absent axonemes, leading to complete loss of motility. This demonstrates that MYCBPAP is essential for proper flagellar formation during spermiogenesis. Finally, we examined two additional proteins, CFAP91 and EFCAB5. *Cfap91* KO spermatozoa showed impaired flagellar elongation and disorganization of microtubules, resulting in reduced motility. In contrast, EFCAB5, a calcium-binding protein, was essential for triggering hyperactivation. *Efcab5* KO spermatozoa failed to convert to asymmetrical beating during hyperactivation, leading to diminished fertilization capacity in vivo.

Together, these findings demonstrate that CFAP77, MYCBPAP, CFAP91, and EFCAB5 function at distinct stages of axoneme assembly and motility regulation. Their loss results in reduced motility and impaired male fertility in mice. Because these proteins are conserved in humans, mutations in their human orthologs may underlie currently unexplained cases of asthenozoospermia. By characterizing the

in vivo functions of these axonemal proteins, this thesis contributes to a deeper understanding of sperm motility mechanisms and provides potential molecular targets for improving the diagnosis of male infertility.

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# 論文審査の結果の要旨及び担当者

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## 論文審査の結果の要旨

本論文は、CRISPR/Cas9ゲノム編集マウスを用いた解析により、精子鞭毛の形成および機能に必須な分子基盤を明らかにし、雄性不妊の理解を深めた研究である。

精子鞭毛に局在する複数の候補タンパク質についてCRISPR/Cas9を用いてKOマウスを作製し、交配試験を実施した。その結果、CFAP77、MYCBPAP、CFAP91およびEFCAB5の欠損が雄妊孕性の著しい低下を引き起こすことを示し、これらのタンパク質が精子形成および機能に必須であることを明らかにした。

1. *Cfap77* KOマウスの解析から精子運動能の低下が認められた。また、免疫沈降解析により、CFAP77が精子運動の維持に関与するTEKTL1と結合することを示した。さらに、TEKTL1欠損マウスにおいてCFAP77は精子に残存する一方で、CFAP77欠損マウスではTEKTL1が精子から消失することを明らかにした。これらの結果は、CFAP77がTEKTL1の上流で機能し、精子運動能の維持に関与している可能性を示唆するものである。

2. *Mycbpap* KOマウスの解析では、鞭毛形成異常および精子運動能の著しい低下が認められた。微細構造の解析から、MYCBPAPが精子鞭毛と核膜の結合に必須であることを明らかにした。さらに、免疫沈降を用いた解析により相互作用因子としてCFAP65およびCFAP70を同定し、MYCBPAPがこれらの分子と協調して精子鞭毛形成に関与する可能性を示した。

3. CFAP91に着目した解析では、免疫沈降解析により、精子鞭毛形成に重要なCFAP251と結合し、CFAP251を鞭毛内へリクルートすることを明らかにした。さらに、近接依存性ビオチン標識法を用いて精子特異的なラジアルスポークタンパク質としてEFCAB5を同定した。EFCAB5欠損マウスの解析により、EFCAB5が精子の超活性化運動の制御に関与する可能性を示した。

以上、本論文は、雄性不妊モデルマウスの表現型解析を通じて、精子鞭毛の形成および機能に必須な分子群を同定したことにより、博士（薬科学）の学位論文に値するものと認める。