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# THE EVOLUTIONARILY CONSERVED PROTEIN ANKEF1 CONFERS SPERM MIDPIECE FLEXIBILITYAND IS ESSENTIAL FOR MALE FERTILITY

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## OBJECTIVE:

The ankyrin repeat and EF-hand domain containing protein 1 (Ankef1) has been found to be highly enriched in the testes, yet its function remains uncharacterized in mammals. An Ankef1knockout (KO) mouse model was created by the CRISPR-cas9 system to help elucidate its function. The male mice homozygous for the Ankef1 knock out mutation were found to be sterile, however the sperm count and morphology was similar to wild type (WT) mice. The objective of this study is to characterize the function of Ankef1 and its role in male fertility.

## MATERIALS AND METHODS:

The sperm from Ankef1 deficient mice was examined by performing invitro fertilization (IVF) studies using both heterozygous (Ankef1) and homozygous knockout (Ankef1) mouse sperm. IVF was performed using sperm that were collected from caudae epididymides into FHM medium and capacitated during incubation. Ovulation was induced by sequential intraperitoneal injection of Pregnant Mare Serum Gonadotropin (PMSG) and human chorionic gonadotropin (hCG). On the day of the IVF, the cumulus masses were recovered from the oviducts and incubated with sperm in Cooks medium for 4 hours. The oocytes/zygotes were rinsed in FHM media and incubated in KSOM + amino acids overnight at 37°C. The following day, the number of 2 cell embryos was recorded. Zona Free IVF was performed as before but WT cumulus masses were first incubated in hyaluronidase to remove cumulus cells and zona pellucida (ZP). A sperm-ZP binding assay was performed to observe if Ankef1KO mouse sperm were able to bind the ZP of WT oocytes. Flagellar waveform analysis was performed using non-capacitated or capacitated spermatozoa from the cauda epididymis of Ankef1 and Ankef1 males. Spermatozoa were plated on fibronectin-coated coverslips and sperm motility was recorded for2s with 200 fps on a Zeiss Axioimager Z2M.

## RESULTS:

The initial IVF experiments showed that while fertilization rates remained high for Ankef1 sperm, Ankef1-deficient sperm were not able to fertilize WT oocytes. The ZP binding assay showed that Ankef1 sperm were able to bind to the ZP, but they were unable to penetrate it, thus fertilization did not occur. Ankef1 sperm was not visualized in the perivitelline space in these experiments. However, when the ZP was removed and zona-free IVF performed, fertilization was then restored. Flagellar waveform analysis revealed that Ankef1 sperm had a rigid midpiece that rendered their flagella unable to perform the high amplitude bends that define hypermotility and were thus unable to penetrate the ZP).

**CONCLUSIONS:**

These experiments demonstrate that Ankef1 is required for sperm midpiece flexibility, which is required for sperm hyperactivation, and Ankef1 knockout blocks ZP penetration and prevents successful fertilization. Therefore, Ankef1 plays a critical role in sperm function and male fertility.

**IMPACT STATEMENT:**

The Ankef1 protein is required for sperm midpiece motility and therefore hyperactivation and ZP penetration.

**REFERENCES:**

N/A