

**Abstracts - 37th Annual Meeting of the Association of Embryo Technology in Europe (AETE)****Contribution for Workshop I**

# Insights into the multifunctional roles of bovine host defence peptides in reproduction and immunity

Kieran Meade

University College Dublin, Ireland; kieran.meade@ucd.ie

Previous work by our group uncovered a panel of novel host defence peptides, consisting of both  $\beta$ -defensin and cathelicidin families which show evidence of expansion in the bovine genome compared to humans and mice. We hypothesised that the peptides these genes encode for would play important antimicrobial and immunomodulatory roles. Expression patterns suggested a role for the  $\beta$ -defensin genes in the male reproductive tract with higher levels of expression in the caudal epididymis. Analysis of evolutionary orthologues showed that one of these proteins,  $\beta$ -defensin 126, creates an 'invisibility cloak' on human sperm to prevent their recognition by the female immune system as they pass through the cervix and uterus. More recent analyses have also suggested that restoration of  $\beta$ -defensin levels on human sperm can improve antimicrobial function. Taking a lead from these human studies we assessed the antimicrobial and reproductive roles of the bovine peptides using genetic association studies, antibacterial assays and a range of *in vitro* functional assays. In an analysis of bulls used in artificial insemination with divergent field fertility, we uncovered a  $\beta$ -defensin haplotype representing a single nucleotide polymorphism (SNP) panel significantly associated with reduced fertility. Within the haplotype was the bovine  $\beta$ -defensin 126 gene and functional assays confirmed a role for this protein in sperm agglutination, motility and binding to oviductal epithelium. Three-dimensional analysis of the protein structure using the recently released artificial intelligence program *AlphaFold*, shows a very distinctive extended C-terminal tail on some  $\beta$ -defensins which may yield important insights into their precise biological roles. In contrast, a highly coiled  $\alpha$ -helical structure is apparent in the cathelicidin peptides. The reproductive tract of the male represents a very dynamic environment with sequential expression of specific peptides to equip sperm for transit and survival after ejaculation. Our research shows that these peptides play important reproductive and immunological roles, critical to protection of gametes within a highly regulated immune environment. Although more detailed functional investigation is required, these multifunctional peptides exhibit relevant species-specific adaptations and thereby offer exciting potential for targeting pathogenic bacteria and improving fertility via modulation of the semen microbiome.

**Keywords:** Defensins, Cathelicidins, immune, fertility