Animal Reproduction

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 immunitye 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 β-defensin and cathelicidicin 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 β-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, β-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 β-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 β-defensin haplotype representing a single nucleotide polymorphism (SNP) panel significantly associated with reduced fertility. Within the haplotype was the bovine β-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 β-defensins which may yield important insights into their precise biological roles. In contrast, a highly coiled α-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

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Regulation of sperm transit across the ovine cervix

Laura Abril-Parreño[1], Xavier Druart[2], Anette Krogenæs[3], Sean Fair[1]

[1]Laboratory of Animal Reproduction, School of Natural Sciences, Bernal Institute, Faculty of Science and Engineering. University of Limerick, Limerick, Ireland; [2]UMR 6175 INRA, CNRS-Université de Tours-Haras Nationaux, Station de Physiologie de la Reproduction et des Comportements Institut National de la Recherche Agronomique, Nouzilly, France.; [3]Faculty of Veterinary Medicine, Norwegian University of Life Sciences, Ås, Norway; laura.parreno@ul.ie

In species where semen is deposited in the vagina, the cervix and its secretions are the major barriers for sperm transport. The ewe is an excellent model for investigating how sperm are selected in the cervix as semen is deposited vaginally and cervical artificial insemination (AI) is limited due to poor pregnancy rates when frozen–thawed semen is used. Worldwide, pregnancy rates rarely exceed 30% when frozen–thawed semen is used in conjunction with cervical AI. However, Norway is the exception to this, since they routinely achieve pregnancy rates of 60–70% with vaginal (shot-in-the-dark) AI to a natural oestrus. This has been reported to be due to the breed of the ewe used in Norway and specifically the inability of frozen–thawed sperm to traverse the cervix of some ewe breeds. During oestrus the cervix is filled with mucus, which is a gel made up predominantly of mucins, which are heavily *O*-glycolysated proteins that can be modified by the addition of terminal sugars such as either fucose or sialic acid. In order to identify the components in the cervical mucus that could explain why frozen-thawed ram sperm can traverse the cervix of some ewe breeds but not others we used a novel sheep model composed of six ewe breeds with known differences in cervical sperm transport following cervical AI with frozen–thawed semen. These were Suffolk and Belclare (low and medium fertility, respectively) in Ireland, Ile de France and Romanov (both with medium fertility) in France and Fur and Norwegian White Sheep (NWS), both with high fertility in Norway. Cervical mucus was collected at the follicular phase of both a synchronised and a natural oestrous cycle. By combining ultra-performance liquid chromatography (UPLC), exoglycosidase digestions and mass spectrometry, a total of 124 *O*-glycans were identified across a range of mucin-type cores, from which core 2 and core 4 glycans had higher abundance in the low fertility Suffolk breed compared with high fertility ewe breeds (Fur and NWS). Differences in sialylated glycans were also identified between high and low fertility ewe breeds. For example, the sialylated glycan (2,3)-sialyl-T-antigen had lower abundance in the low-fertility, Suffolk, compared with Fur (high fertility). Using other biochemical techniques such as reverse phase UPLC and weak anion exchange UPLC, Suffolk had higher levels of sialic acid compared to high fertility ewe breeds (NWS and Fur). From over 50 different sialic acid structures in nature, we characterised seven. The two most prevalent were *N*-acetyl-neuraminic acid (Neu5Ac) and *N*-glycolyl-neuraminic acid (Neu5Gc), acetylated and glycosylated, respectively. There was no effect of oestrous synchronisation on sialic acid species although there was an effect of ewe breed, which was represented by Suffolk having higher levels of Neu5,9Ac2 compared to NWS. The results of this study suggest that cervical sperm transport is regulated by sialylated cervical mucins, which could be also involved in the regulation of the cervical immune response against sperm.

Keywords: cervical mucus, sperm interaction, sialic acid

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Regulation of the uterine environment by paternal factors: Insights from the bovine model

Beatriz Fernandez-Fuertes[1], José María Sánchez[1], Pat Lonergan[2]

[1]National Institute for Agricultural and Food Research and Technology (CSIC-INIA), Madrid, Spain; [2]University College Dublin, Belfield, Ireland; beatriz.fernandez@inia.csic.es

During transit through the male reproductive tract, mammalian sperm are sequentially bathed in testicular, epididymal and accessory gland (AG) secretions which collectively constitute seminal plasma (SP). It has been suggested that SP components can modulate the maternal immune response, creating a tolerogenic environment for the semi-allogeneic embryo and leading to improved fertility. Indeed, exposure of sows and mice to SP has a positive effect on embryo survival and implantation. Due to the characteristics of mating in these species, the ejaculate comes into direct contact with the endometrium. However, bulls ejaculate in the vagina, and it is questionable whether any SP reaches the uterus. This makes cattle an interesting model for the study of the regulation of the uterine environment by paternal factors in species that ejaculate intravaginally. As a first approach to study this interaction, we generated endometrial explants from heifers in oestrus, and incubated them with sperm or SP. Interestingly, SP had a detrimental effect on endometrial RNA integrity which was blocked by addition of an RNase inhibitor, demonstrating a role for a SP-RNase. Both cervical and vaginal explants were more resilient to the SP-induced RNA degradation. These data gave weight to our hypothesis that, in species that ejaculate intravaginally, paternal modulation of the uterine environment is not due to direct contact with SP. To determine whether SP can have an indirect effect on the uterus and/or whether the presence of sperm can affect the endometrial response, we designed an in vivo study where heifers were mated to an intact bull (that ejaculate sperm and SP), a vasectomized bull (that only ejaculate SP), or were left unmated. Surprisingly, no differentially expressed genes (DEG) were observed in the endometrium of unmated heifers and those mated to vasectomized bulls, 24 h after mating. In contrast, the endometrium of heifers mated to intact bulls exhibited 22 and 24 DEGs in comparison with unmated heifers and heifers mated to vasectomized bulls, respectively. These data suggested that sperm might play a more critical role than SP in the regulation of the uterine environment in species that ejaculate in the vagina. To determine whether the effect observed was driven by intrinsic sperm factors, or rather, by AG factors that bind to sperm at ejaculation, we incubated endometrial explants alone or with epididymal (which have had no exposure to AG secretions) or ejaculated sperm. Both epididymal and ejaculated sperm induced changes in the endometrial transcriptome. However, the response elicited by the former was more dramatic than the later (1912 vs. 115 DEGs, respectively). In both cases, the top pathways associated with these genes included T cell regulation and NF-KB and IL17 signaling. Altogether, the data derived from our studies demonstrate the different ability of AG factors, and those acquired during spermatogenesis and epididymal maturation, in the regulation of the bovine endometrial transcriptome by sperm. At the same time, it highlights the species-specific nature of the modulation of the female environment by paternal factors in mammals.

Keywords: Seminal plasma, sperm, endometrium, bovine