Animal Reproduction

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Regulation of sperm transit across the ovine cervix

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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