SEMINAL INFLUENCE ON THE OVIDUCT

Mating and/or semen components induce gene expression changes in the pre-ovulatory functional sperm reservoir in poultry and pigs

Mohammad Atikuzzaman

Unit of Obstetrics and Gynaecology, Division of Clinical Sciences, Department of Clinical and Experimental Medicine, Linköping University, SE-581 85, Linköping, Sweden.

E-mail: dr.atikuzzaman@gmail.com

ABSTRACT

Internal fertilization occurs in birds and eutherian mammals. Foetal development, however, is either extra- respectively *intra-corpore* (egg vs uterus). In these animal classes, the female genital tract stores ejaculated spermatozoa into a restricted oviductal segment; the functional pre-ovulatory sperm reservoir, where they survive until ovulation/s occur. Paradoxically, this immunologically foreign sperm suspension in seminal fluid/plasma, often microbiologically contaminated, ought to be promptly eliminated by the female local immune defence which, instead, tolerates its presence. The female immune tolerance is presumably signalled via a biochemical interplay of spermatozoa, as well as the peptides and proteins of the extracellular seminal fluid, with female epithelial and immune cells. Such interplay can result in gene expression shifts in the sperm reservoir in relation to variations in fertility. To further aid our understanding of the underlying mechanisms, this thesis studied the proteome of the seminal fluid (using 2D SDS-PAGE and mass spectrometry) including cytokine content (using Luminex and/or ELISA) of healthy, sexually mature and fertile boars and cocks. As well, gene expression changes (using cDNA microarray) in the oviductal sperm reservoirs of sexually-mature females, mated or artificially infused with homologous sperm-free seminal fluid/plasma were studied. Pigs were of commercial, fertility-selected modern breeds (Landrace), while chicken belonged to the ancestor Red Junglefowl (RJF, low egg laying-capacity), a selected egg-layer White Leghorn (WL) and of their Advanced Intercross Line (AIL). Ejaculates were manually collected as single sample in cocks or as the sperm-rich fraction [SRF] and the post-SRF fraction in boars to harvest seminal fluid/plasma for proteome/cytokine and infusion-studies. Oviducts were retrieved for gene-expression analyses via microarray immediately post-mortem (chicken) or at surgery (pig), 24 h after mating or genital infusion. In pigs, the protein-rich seminal plasma showed the highest amounts of cytokines [interferon-γ, interferon gamma-induced protein 10 (IP-10/CXCL10), macrophage derived chemokine (MDC/CCL22), growth-regulated oncogene (GRO/CXCL1), granulocyte-macrophage colony-stimulating factor (GM-CSF), monocyte chemo-attractant protein-1 (MCP-1/ CCL2), interleukin (IL)-6, IL-8/CXCL8, IL-10, IL-15, IL-17 and transforming growth factor (TGF)-β_{1-3}) in the larger, protein-rich and sperm-poor post-SRF, indicating its main immune signalling influence. Chicken showed also a plethora of seminal fluid proteins with serum albumin and ovotransferrin being conserved through selection/evolution. However, they showed fewer cytokines than pigs, as the anti-inflammatory/immune-modulatory TGF-β_2 or the pro-inflammatory CXCL10. The RJF contained fewer immune system process proteins and lacked TGF-β_2 compared to WL and AIL, suggesting selection for increased fertility could be associated with higher expression of immune-regulating peptides/proteins. The

oviductal sperm reservoir reacted *in vivo* to semen exposure. In chicken, mating significantly changed the expression of immune-modulatory and pH-regulatory genes in AIL. Moreover, modern fertile pigs (Landrace) and chicken (WL), albeit being taxonomically distant, shared gene functions for preservation of viable sperm in the oviduct. Mating or SP/SF-infusion were able to change the expression of comparable genes involved in pH-regulation (*SLC16A2*, *SLC4A9*, *SLC13A1*, *SLC35F1*, *ATP8B3*, *ATP13A3*) or immune-modulation (*IFIT5*, *IFI16*, *MMP27*, *ADAMTS3*, *MMP3*, *MMP12*). The results of the thesis demonstrate that both mating and components of the sperm-free seminal fluid/plasma elicit gene expression changes in the pre-ovulatory female sperm reservoir of chickens and pigs, some conserved over domestication and fertility-selection.

Key words: seminal plasma, proteome/peptidome, oviduct, gene expression, chicken, pig.