SRF Vacation Scholarship Report 2015

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Title: Disease takes its Toll on reproduction: Toll-like receptors and the bovine corpus luteum.

The fertility of dairy cows in the UK is reportedly on the decline (Hudson et al., 2010) with ensuing economic difficulties promising to cause problems. Whilst the endometrium and granulosa cells have been found to express TLRs, no studies have investigated the possibility of the corpus luteum expressing any TLRs. A cow's corpus luteum (CL) has a vital part in maintaining the pregnancy, secreting progesterone after ovulation has occurred (Lukaszewska and Hansel, 1980). Should there be a delay in the regression of the corpus luteum after parturition, for whatever reason, then the return to oestrus will be delayed.

Toll-like receptors (TLRs) are a family of 10 cellular receptors that are a type of pattern recognition receptor (PRR). They are vital mediators of the innate immune system and help with the distinction of self from non-self. TLRs detect and initiate the innate immune system by binding to specific conserved small molecular structures found on the surface of invading pathogens, known as pattern associated molecular patterns (PAMPs) (Woods et al., 2011, Chen et al., 2014). The binding of PAMPs by TLRs causes intracellular signals which induce gene expression of several inflammatory-related enzymes (for example COX2) (Bromfield and Sheldon, 2011, Zhou et al., 2009). Several chemokines and cytokines, such as IL-6, IL-8 and macrophage migration inhibitory factor (MIF) are also stimulated by the activation of TLRs and these act to produce a variety of host responses (Taghavi et al., 2014). Another response resulting from TLR binding to ligands is the production of IL-1β, TNFα and upregulation of scavenger receptors. The stimulation of these receptors result in phagocytosis of pathogens and the presentation of antigens to naïve T cells (Woods et al., 2009, Zhou et al., 2009).

TLRs are classified based on their cellular locations, such as on the cell membrane (TLRs 1, 2, 4, 5, 6 and 10) or in the cytoplasm (TLRs 3, 7, 8 and 9)(Taghavi et al., 2014). Those on the cell surface mainly recognise components from the pathogen's surface whereas the TLRs inside the cell will recognise ssRNA, dsRNA or dsDNA from viruses (Chen et al., 2014). TLR 4 identifies lipopolysaccharide (LPS) which is the prototypical PAMP of the outer cell wall of Gram-negative bacteria. TLR2 detects lipoteichoic acid which is on the cell surface of Gram-positive bacteria. TLR 5 detects the flagellin that is present in bacteria that have flagella (Price and Sheldon, 2013). TLRs are predominantly expressed in antigen presenting cells (APCs) such as macrophages and neutrophils but they are not restricted to them (Chen et al., 2014).

It has been discovered that there are no such immune cells to be found in the basement membrane of healthy ovarian follicles of cattle. One study reportedly found both TLR 2 and TLR 4 in bovine granulosa cells (Price and Sheldon, 2013). Another study has shown that granulosa cells are stimulated by LPS (which supports the finding of TLR 4 in bovine granulosa cells). This stimulation initiates a cellular response that suppresses oestradiol production (Magata et al., 2014). However it is uncertain if other bacterial PAMPs can cause granulosa cells to mount an inflammatory response (Price and Sheldon, 2013).TNFα (a major pro-inflammatory cytokine) has been shown to reduce androstenedione production of bovine theca cells *in vitro*, which indicates that perhaps bovine theca cells bring about an inflammatory response that inhibits steroid production of follicles (Magata et al., 2014).

Aim:

The aim of this study was to investigate whether the bovine corpus luteum expresses Toll like receptors (TLR), due to the critical role of the CL in pregnancy and the potential effect of TLRs on the return to oestrus once stimulated. The relevant literature suggests that TLRs 2 and 4 are the isoforms most likely to be expressed by the CL.

Investigation:

Immunohistochemistry was used to try to localise TLR2 and TLR4 protein in sections of bovine luteal tissue. Initial training was performed using a well characterised antibody against the endothelial cell marker Von-Willebrand Factor (vWF; Fig1A). The resulting staining was as anticipated and the protocol was then adapted for antibodies against TLR 2 and 4 in bovine CL. The commercial TLR antibodies were selected as they have been successfully used on bovine tissue in the literature; however, in our hands the resultant ovarian staining was too inconsistent to definitively determine whether TLRs 2 and 4 were present in the bovine corpus luteum, despite repeated optimisation of the protocol. Strong staining for both TLR2 and TLR4 was observed in sections of bovine mammary tissue, provided as a positive control tissue (Fig1C).

Subsequent analysis used RT-PCR to investigate the expression of mRNA encoding TLR 1 to 10 in the bovine CL. Total RNA was extracted from a bovine mid-stage CL sample and was reverse transcribed, before specific amplification.

This project has revealed for the first time that the bovine CL expresses mRNA encoding all the TLRs from 1 to 10 (Fig1D). Further experiments extended this work to include different stages of CL development (early, mid, late and regressed) and a large follicle.

The expression of TLR 1-10 mRNA by the bovine CL demonstrates that there is great potential for regulation of luteal function by members of the TLR family. Future studies will aim to investigate how TLR signalling might impact luteal function and how this might be altered in animals with concurrent disease.

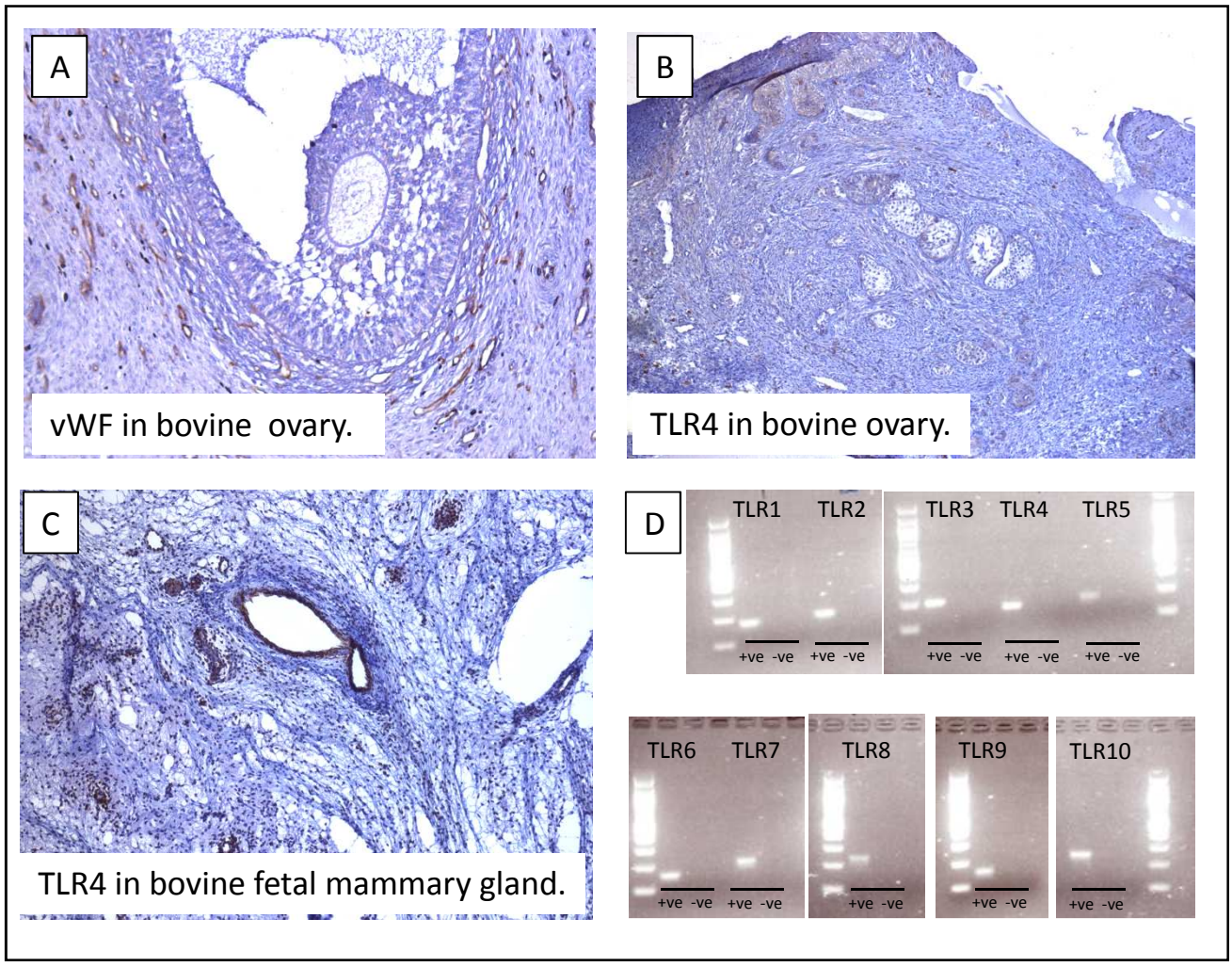

Figure 1: Immunohistochemical localisation of (**A**) von Willebrand factor (vWF) and (**B, C**) Toll like receptor (TLR) 4 in sections of bovine ovary (**A, B**) and mammary gland (**C**); positive staining is indication by a brown endpoint. Amplification of TLR1-10 by bovine mid-stage CL (**D**). Positive (+ve) and negative control (-ve; no reverse transcription) samples were prepared for each sample and amplification was always absent from contamination controls (water).

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