Immunological aspects of ovarian follicle ovulation and corpus luteum formation in cattle

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INTRODUCTION

Ovarian follicle ovulation and subsequent luteinization have been described as a controlled inflammatory event, comprising of tissue damage and repair. To elaborate this further in cattle, the contribution of immune cells to dominant follicle luteinization, ovulation and corpus luteum formation was investigated.

HYPOTHESIS:

Terminal follicle differentiation, ovulation and corpus luteum (CL) formation is characterized by changes in the number, type and activity of the local immune cell population.

OBJECTIVE:

To characterize the contribution of the immune system to ovulation and CL formation in cattle in terms of:

- The number and type of immune cells present
- The gene expression profile of factors associated with inflammation & immune cell signalling.

EXPERIMENTAL MODEL

Ovulation was controlled in cycling beef heifers using an 8-day progesterone-based oestrous synchronization program (Fig 1.A):

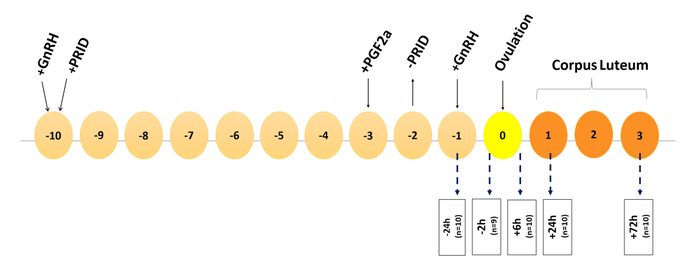

Figure 1: **A)**. Diagram of oestrous synchronization protocol. **B).** Heifers were slaughtered at a local abattoir at 5 timepoints (T): (T1) 24h prior to ovulation (n=10); (T2) 2h prior to ovulation (n=9); (T3) 6h post-ovulation (n=10); (T4) 24h post-ovulation (n=10); (T5) 72h post-ovulation (n-=10) and ovarian tissue was collected and returned to the laboratory on ice.

MATERIALS AND METHODS:

Follicular fluid, theca, granulosa and CL -tissues were recovered by dissection and processed for analysis (Fig 1.B):

- The concentrations of a panel of cytokines were measured using an antibody-conjugated magnetic bead immunonoassay.
- The abundance of T-lymphocytes, mast cells, neutrophils, eosinophils, monocytes, macrophages and dendritic cells was determined by immunohistochemistry. The slides were scanned using Aperio AT2 Digital Slide Scanner (Leica Biosystems) at 20X magnification and the images were viewed, annotated and analysed with Aperio ImageScope 12.3 software (Leica Biosystems) to recognise DAB positive cells specific to the stain/biomarker of interest.
- The mRNA relative abundance of candidate genes including, angiogenic growth factors, adhesion factors, chemokines and cytokines, was determined by quantitative real time PCR-analysis.

STATISTICAL ANALYSIS:

The resulting data sets were analysed using the linear mixed model procedure of SAS; differences were deemed significant at P≤0.05. Tukey's multiple comparison test was performed.

RESULTS

A. Follicular Fluid Cytokine Concentrations:

The cytokines IFNy, IP-10, IL-10, IL-36RA, MCP-1, MIP-1a, MIP-1b and VEGF-A were detected in the follicular fluid. The concentrations (pg/ml) of IL-10 and VEGF-A were significantly higher in T1 follicular fluid samples compared to T2 (7.70 v 0.86 and 2193.33 v 293.93, respectively), see Fig. 2. below.

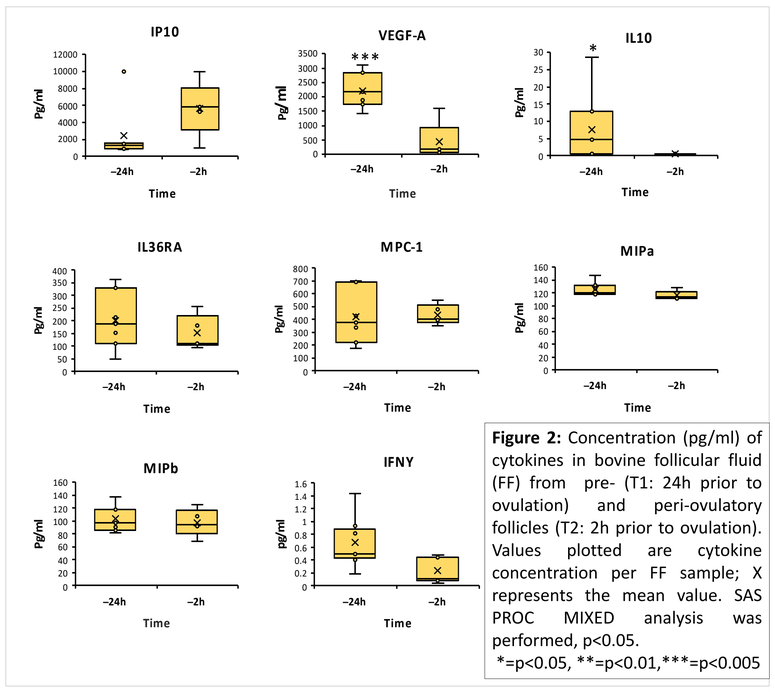

Figure 2: Concentration (pg/ml) of cytokines in bovine follicular fluid (FF) from pre- (T1: 24h prior to ovulation) and peri-ovulatory follicles (T2: 2h prior to ovulation). Values plotted are cytokine concentration per FF sample; X represents the mean value. SAS PROC MIXED analysis was performed, p<0.05.
*=p<0.05, **=p<0.01,***=p<0.005

B. Immune Cells Distribution:

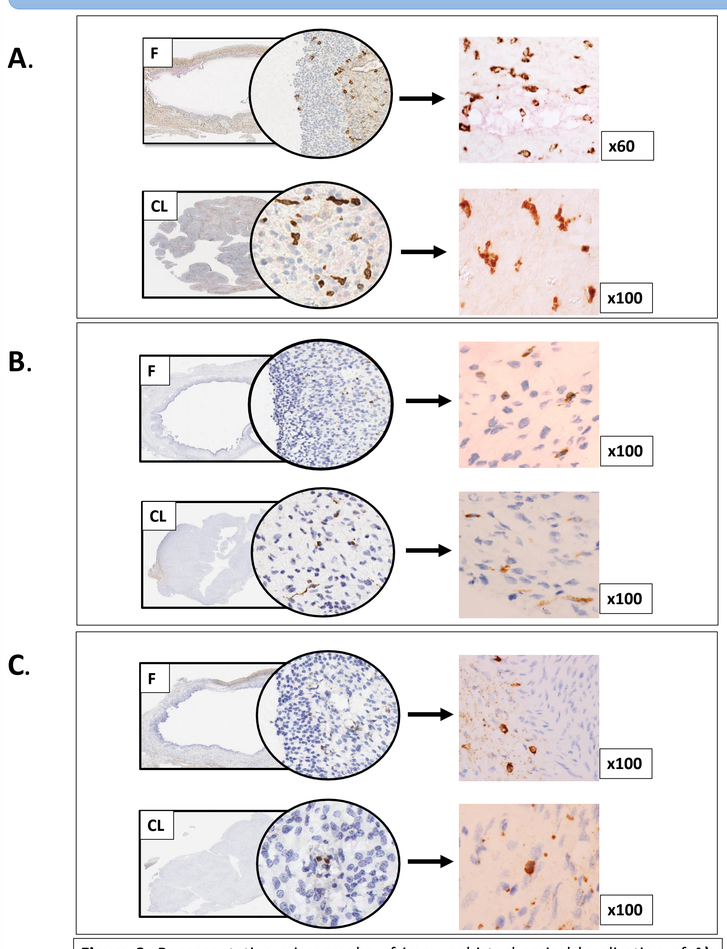

Figure 3: Representative micrographs of immunohistochemical localization of **A).** CD172a-CD11c⁺ Dendritic cells; **B).** CD68⁺ Macrophage cells and **C)**. Myeloperoxidase⁺ Neutrophil cells, localization in in periovulatory follicle (F) and corpus luteum (CL) tissue sections.

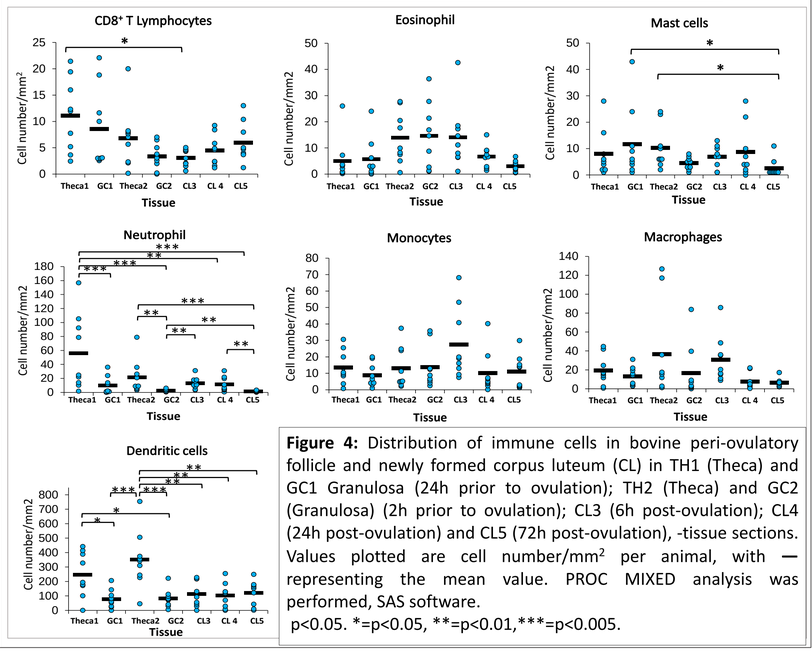

Figure 4: Distribution of immune cells in bovine peri-ovulatory follicle and newly formed corpus luteum (CL) in TH1 (Theca) and GC1 Granulosa (24h prior to ovulation); TH2 (Theca) and GC2 (Granulosa) (2h prior to ovulation); CL3 (6h post-ovulation); CL4 (24h post-ovulation) and CL5 (72h post-ovulation), -tissue sections. Values plotted are cell number/mm² per animal, with — representing the mean value. PROC MIXED analysis was performed, SAS software.
p<0.05. *=p<0.05, **=p<0.01,***=p<0.005.

Dendritic cells, were the most abundant immune cells in bovine ovulatory follicular and early corpus luteum -tissue at all time points (p<0.05); their numbers peaked in ovulatory (T2) thecal tissue (261.5 cells/mm²). The greatest number of neutrophils were identified in thecal tissue at T1 (45/mm²), thereafter their numbers declined to 1.1/mm² in CL tissue by T5. Similarly, the numbers of T-lymphocytes, mast cells, monocytes and macrophages, declined in CL tissue at T4 and T5. The data is summarized in Fig. 4.

C. mRNA expression

Candidate gene mRNA expression profiles appeared to be time and tissue specific, e.g., IFNA was highest in the pre-ovulatory granulosa tissue (T1), IL8 was highest in peri-ovulatory thecal tissue (T2), VEGFA and MMP9 was highest in the early CL tissue (T4 & T5), MMP1, TIMP1 and VCAM1 expression was highest in theca, granulosa and CL tissue collected on or after ovulation (T2, T4, T5), the expression of the prostaglandin related genes PTGES and PTGS2 was lowest in CL tissue, while PTGIS, was highest. The data is summarized in Fig. 5. below.

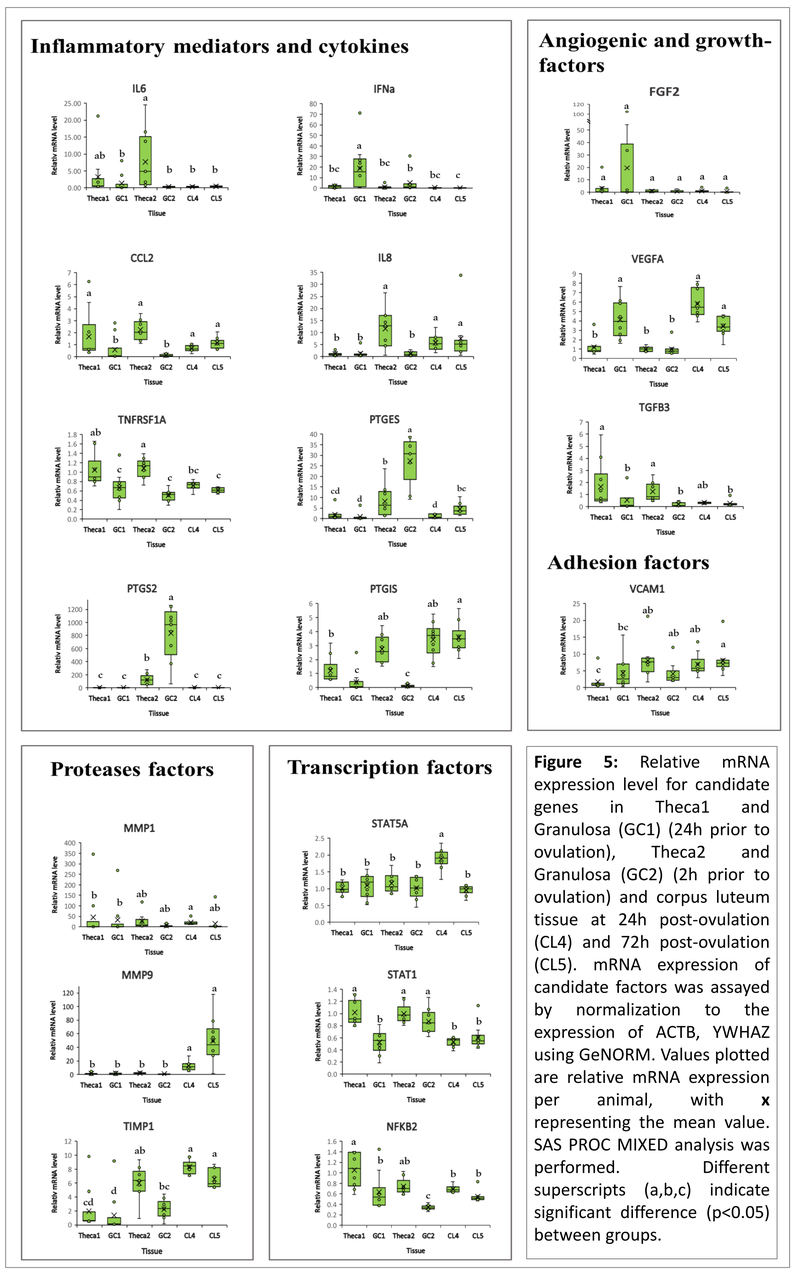

Figure 5: Relative mRNA expression level for candidate genes in Theca1 and Granulosa (GC1) (24h prior to ovulation), Theca2 and Granulosa (GC2) (2h prior to ovulation) and corpus luteum tissue at 24h post-ovulation (CL4) and 72h post-ovulation (CL5). mRNA expression of candidate factors was assayed by normalization to the expression of ACTB, YWHAZ using GeNORM. Values plotted are relative mRNA expression per animal, with **x** representing the mean value. SAS PROC MIXED analysis was performed. Different superscripts (a,b,c) indicate significant difference (p<0.05) between groups.

CONCLUSION:

The current findings support the hypothesis that ovulation in heifers is characterized by an initial proinflammatory cascade followed by a dramatic switch to tissue repair, growth and remodelling, all occurring within a 72h period and commencing with the LH surge. Moreover, our results highlight the roles of neutrophils, dendritic cells and macrophages as the key actors in this process.

ACKNOWLEDGEMENT:

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