Do Macrophages Have Any Role in Corpus Luteum Formation in the Bovine Ovary?

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Introduction and Aims
Cyclic ovarian function involves continual tissue remodelling due to follicle development, ovulation and the generation and regression of corpus luteum (CL). After each ovulation there is breakdown, repair and generation in the ovarian tissue. During ovulation macrophages invade the ovary and secret pro-inflammatory cytokines such as TNF-α and IL6 that have local actions on ovarian cells. The infiltration of the macrophages into the ovarian tissue as well as their presence in the female reproductive tract is strong evidence for their role in the female reproductive process. Here we investigated (1) The infiltration of macrophages in bovine ovary; (2) The effect of macrophages on granulosa, theca and stroma cell migration using an in vitro ‘wound healing’ assay.

Methodology

- Bovine ovaries were collected and the CL stages categorized according to gross morphology. After cryosectioning tissue was stained with macrophages markers CD68 and MHCII.

- Monocyte-derived macrophages were prepared from citrated blood. They were cultured with 10% of FCS for seven days then treated with LPS (10μg/ml). The cells were collected after 4 hours from the end of the treatment by removing medium and the cells were processed for RNA extraction and RT-PCR. For wound healing (‘scratch’) assays theca and stroma cells were cultured (10% serum) with/without macrophages for two days.

- For wound-healing (‘scratch’) assays TC and ovarian cortical SC were cultured (10% serum) with/without CFDA-labelled macrophages (10^3/well) for two days. A ‘scratch’ was made in the near confluent monolayer and cell migration (% wound closure) assessed over 24h. A further experiment examined the effect of treating the scratched cells with conditioned media from LPS-treated macrophages.

Results

- Macrophages were present in the ovarian sections with the greatest density in early CL tissue (Fig. 1).

- Macrophages significantly accelerated wound healing by luteinised theca and stroma cells (30% in comparison with control (p<0.01), however granulosa cell showed no migration activity with and without macrophages (Figure 2).

- Treatment of stroma cells with media from LPS-treated macrophages accelerated wound healing by 20% in comparison with control (p<0.01). In theca cells there was no significant difference between control and treated cells (Figure 3).

- LPS treatment had no effect on the wound healing rate in either theca or stroma (Figure 4). LPS caused a significant upregulation in the expression of TNF-α, IL6, GPR77 and TLR4 in macrophages, however there was no effect on IL1b expression (Figure 5).

Conclusions
We speculate the macrophages have a stimulatory effect on cell migration associated with CL formation, likely mediated by pro-inflammatory cytokines including TNF-α and IL6.

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