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***Escherichia coli* lipopolysaccharide modulates bovine luteal cell function**

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An important cause of subfertility in cattle is prolonged luteal phases of the oestrous cycle associated with postpartum metritis or endometritis (Opsomer and others, 2000). This uterine disease is commonly associated with the presence of *Escherichia coli* and there are high concentrations of bacterial lipopolysaccharide (LPS) in the uterine fluid and peripheral plasma of these animals (Dohmen and others 2000, Mateus and others 2003, Williams and others 2005). The duration of the oestrous cycle is determined by the presence of a corpus luteum in the ovary. The corpus luteum is a temporary endocrine gland formed after ovulation that secretes progesterone under the regulation of luteinising hormone (LH) until luteolysis initiates the next follicular phase (Schams and Berisha, 2004). The mechanisms underlying the extended lifespan of the corpus luteum by uterine infection could include disruption of luteolysis associated with changes in endometrial secretion of prostaglandins by LPS (Herath and others, 2006). Alternatively, there may be a direct effect of LPS on the function of luteal cells, so the present study used *in vitro* culture to test this paradigm.

Corpora lutea were collected from cattle immediately after slaughter at an abattoir. The stage of the oestrous cycle was determined using the criteria of Ireland and others (1980) and only corpora lutea from the mid-luteal phase were used in the present study. The method of luteal cell isolation and culture was as previously described (Pate and Condon 1982). Briefly, cells were isolated by enzymatic digestion of luteal tissue, washed and plated in 24 well plates at a density of 2.5×10^5 cells/ml in supplemented Dulbecco's Modified Eagle's medium nutrient mixture F-12 Ham (Sigma, Poole, UK). The media was changed after 36 hours and treatments applied to the cells for 24 to 48 hours, as indicated in the results. At the end of the treatment period, the supernatants were collected and stored at -20°C until progesterone concentrations were measured by radioimmunoassay, as previously described (Barret and Wathes 1990). The cells in each well were washed twice with saline, trypsin (Accutase, Sigma) was applied, the cells suspended in media, and the number of live cells counted using the Trypan Blue exclusion method and a haemocytometer. For each experiment, cultures were conducted in triplicate from at least 3 corpora lutea and maintained at 37°C , 5% CO_2 in air, in a humidified incubator. To test if the luteal cells were functional *in vitro*, they were treated with LH (at final concentrations of 1, 3, 10 and 30 ng/ml; LH courtesy of Dr. Parlow, NIDDK, USA); control cultures were supplied with media. To determine the effect of LPS, *E. coli* O55:B5 LPS (0.1, 0.3, 1.0 and 3.0 $\mu\text{g/ml}$; Sigma, Poole, UK) was applied alone, or in the presence of 10 ng/ml LH or the LPS antagonist, polymyxin B (1, 2, 4, 8 $\mu\text{g/ml}$; Sigma, Poole, UK). Data were analysed using a general linear model in SPSS ver 14.0 (SPSS, Chicago, USA). Data are reported as mean $\pm$ SEM, and significance attributed when $P < 0.05$.

Bovine luteal cells were functional *in vitro* as determined by a concentration dependent secretion of progesterone in response to 48 hours treatment with LH ($P < 0.05$, Fig. 1), without affecting cell survival, as previously reported (Pate and Condon 1982). When luteal cells were treated with LPS for 24 hours they secreted more progesterone than control cultures ($P < 0.05$, Fig. 2). The increased progesterone production by luteal cells in response to LPS was abrogated by addition of polymyxin B ($P < 0.01$, Fig. 3). The number of cells after treatment did not differ significantly from control for 0.1 and 0.3 $\mu\text{g/ml}$ LPS (Fig. 2) but was less for 1 and 3 $\mu\text{g/ml}$ LPS ($P < 0.05$), although this effect was also abrogated by addition of 2 $\mu\text{g/ml}$ Polymyxin B (Fig. 3). Luteal cells treated concomitantly with LH and 3 $\mu\text{g/ml}$ LPS for 24 hours did not produce any more progesterone than cells treated with LH alone (26.6 ± 1.8 vs. 29.2 ± 4.1 ng/ml).

The present study confirmed that luteal cells cultured *in vitro* appeared to function normally by secreting progesterone in response to LH (Pate and Condon 1982). The cells secreted progesterone in response to LPS across the range of concentrations tested, although at the higher concentrations of LPS there was evidence of cell death. However, the concentrations of LPS required for cell death in the present study (1 $\mu\text{g/ml}$) were greater than the LPS concentrations of < 10 ng/ml measured in postpartum cattle with uterine infection (Mateus and others 2003). The specificity of the luteal cell response to LPS was confirmed by abrogating the effects on progesterone and cell survival using an LPS antagonist polymyxin B (Jacobs and Morrison 1977). It was interesting to note that the effect of LH and LPS on progesterone secretion was not summative, which begs the question as to whether LH and LPS act through similar intra-cellular pathways or whether the luteal cells had reached their maximum capacity for progesterone production. In the whole animal, the effects of LPS could be mediated directly or through the inflammatory response to LPS, which includes the production of inflammatory cytokines (Herath and others 2006). Indeed, it has already been established that inflammatory cytokines modulate luteal cell steroidogenesis (Pate 1996). The contribution of the increased progesterone secretion by luteal cells stimulated by LPS *in vitro*, to the prolongation of luteal phases associated with uterine disease in postpartum cattle, warrants further investigation.

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Figure 1. Progesterone concentrations (bars) in supernatants of luteal cells cultured for 48 hours with medium as control, or 1, 3, 10 or 30 ng/ml LH. Cell numbers are indicated (■). Values differ significantly from control * $P < 0.05$

Figure 2.

Progesterone concentrations expressed as percent of control (bars) in supernatants of luteal cells cultured for 24 hours with medium as control, or with 10 ng/ml LH, or 0.1, 0.3, 1 or 3 µg/ml LPS. Cell numbers are indicated (■). Values differ significantly from control * $P < 0.05$, *** $P < 0.001$

Figure 3. Progesterone concentrations (bars) in supernatants of luteal cells cultured for 24 hours with medium as a control, 1 or 3 $\mu\text{g/ml}$ LPS alone or in the presence of 2 $\mu\text{g/ml}$ Polymixin B. Cell numbers are indicated (■). Values differ significantly from control * $P < 0.05$, *** $P < 0.001$