Supplementary Information

Supplementary Materials and Results

To develop the GeXP platform for our study, RNA was extracted from a mixture of cells (i.e. aspirated and scrapped bovine follicles of varying size, plus sonicated CL tissue). These mixed cell populations included oocytes. This phase of the study concerned GeXP primer design. All genes in the master list (Table 1 of manuscript) were detected.

For GeXP validation, *ESR2* and *HIF1A* were chosen at random and quantified by quantitative, real time PCR (qRT-PCR) and by GeXP. Granulosa cells were collected from a selection of 10-14 mm diameter, abattoir derived bovine dominant follicles. 12µl of mRNA, extracted and purified as described in the materials and methods, was denatured at 70°C for 10 minutes using a thermal cycler (BioRad, Hemel-Hempstead, Hertfordshire, UK). Reverse transcription (RT) was performed at 37°C for 60 minutes using an Omniscript RT Kit (Qiagen Ltd., Crawley, West Sussex, UK) with RNase inhibitor (Bioline Ltd., London, UK). 1µl of the resulting cDNA was added to a PCR reaction mixture including SYBR Green (*Applied Biosystems, Warrington, Cheshire, UK*) and 20µM forward and reverse primers. The PCR reaction was performed within a LightCycler 480 (Roche Diagnostics GmbH, Mannheim, Germany) using the following program: Quantification – 1 cycle of 95°C for 10 minutes; 35 cycles of 95°C for 30 seconds, 60°C for 90 seconds, 72°C for 10 seconds; Melting – 95°C for 5 seconds; 65°C for 1 minute; Cooling – 40°C for 30 seconds. LightCycler 480 software was used to normalise expression relative to the house-keeping gene *H2AZ*, interpret and analyse the results. The same samples were also quantified by GeXP, as described in the materials and methods.

GeXP and qRT-PCR determined expression of *ESR2* and *HIF1A* was correlated (*R*²=0.515, *P*<0.001 and *R*²=0.453, *P*=0.006, respectively) (Supplementary Figure 1) so confirming GeXP as a suitable method for the quantification of gene expression.

Genes not expressed in experimental cells

Transcripts for all genes described in Table 1 of the manuscript (including those listed below) were expressed in a mixed population of cells during GeXP platform development. These cells were harvested from a mixed sample of abattoir derived CLs and follicles at various antral-stages of development (from 2 mm in diameter). Below we describe expression patterns and functions for those genes not detected in our experimental granulosa, theca and luteal cells. In some cases this may have arisen as a consequence of primer design and splice variant expression, because bovine variants for these genes are poorly described in the literature.

**AMH**: Important during pre-antral follicle development, regulating both the transition from primordial to primary follicle stages, and the response to FSH (Knight and Glister, 2006). Transcript expression for *AMH* is restricted to granulosa cells (Vigier et al., 1984; Takahashi et al., 1986) and declines beyond the early antral stages of follicle development, and is further reduced in granulosa cells from atretic follicles (Rico et al., 2009). Interestingly, blood AMH concentrations are also at their lowest between Days 4 and 6 of the oestrous cycle (Rico et al., 2011) when Group A animals were slaughtered in our study. Collectively these results may
explain why we did not detect AMH transcripts in selected somatic cells of the bovine ovary in the current study.

**BMP6:** Expressed and active in bovine granulosa and thecal cells at least up to approximately 6 mm in diameter where it can attenuate the actions of both FSH and forskolin *in vitro* (Kayani et al., 2009; Glisher et al., 2013). BMP6 mRNA is lost during selection of the dominant follicle in the rat (Erickson and Shimasaki, 2003), although transcripts for this transforming growth factor-beta superfamily member have been detected within the bovine CL (Kayani et al., 2009) and follicle up to 18 mm in diameter (Glisher et al., 2010). Established actions of BMP6 on bovine ovarian follicular cells have largely been confined to *in vitro* culture with cells from follicles < 6 mm in diameter. Inability to detect transcripts for BMP6 in the current study could have been due to low expression, especially in granulosa cells (Glisher et al., 2010).

**FGF1:** Transcripts for this fibroblast growth factor have previously been reported in theca and granulosa cells from antral (5 to 14 mm) follicles derived from abattoir recovered bovine ovaries (Berisha et al., 2004). Expression was relatively greater in theca than granulosa cells in that study and did not vary significantly between follicle size classes. FGF1 is generally known to exert mitogenic, anti-apoptotic and angiogenic effects in a variety of tissues. In cultured bovine granulosa cells (harvested from 2-5 mm follicles) FGF1 increased expression of Sprouty family members (SPRY2 and SPRY4), as well as orphan nuclear receptors (NR4A1 and NR4A3), thereby confirming functional activity in these cells (Jiang and Price, 2012). Inability to detect transcripts for FGF1 in the current study could have also been due to low expression, again especially in granulosa cells.

**IL6 and IL2:** Interleukin-6 is a pro-inflammatory cytokine involved in a variety of roles (including anti-apoptotic) within the ovary associated with ovulation, CL formation and demise (Bornstein et al., 2004; Richards et al., 2008). IL6 is produced predominantly by macrophages and activated T cells within the CL, particularly during luteolysis. IL6 mRNA expression is often barely detectable in the CL, with inhibition stemming from locally produced progesterone (Telleria et al., 1998). Similarly, Petroff et al. (1999) failed to detect transcripts for IL2 in the bovine CL at various stages of the luteal phase. However, interleukins are inducible. For example, exposure of bovine granulosa cells to lipopolysaccharide led to a rapid and sustained increase in transcripts for IL6 in cultured bovine granulosa cells (Bromfield and Sheldon, 2011). It’s possible that the necessary conditions for induction may have been absent in our cells.

**References**


Erickson GF, Shimasaki S 2003 The spatiotemporal expression pattern of the bone morphogenetic protein family in rat ovary cell types during the estrous cycle. Reproductive Biology & Endocrinology 19.

Glister C & Knight PG 2010 Changes in expression of bone morphogenetic proteins (BMPs), their receptors and inhibin co-receptor betaglycan during bovine antral follicle development: inhibin can antagonize the suppressive effect of BMPs on thecal androgen production. Reproduction 140 699-712.


Kayani AR, Glister C & Knight PG 2009 Evidence for an inhibitory role of bone morphogenetic protein(s) in the follicular–luteal transition in cattle. Reproduction 137 67-78.


**Supplementary Figure 1** A comparison of GeXP and qRT-PCR determined *ESR2* (A) and *HIF1A* (B) transcript expression in granulosa cells from bovine dominant ovarian follicles collected from a local abattoir. Expression is given in arbitrary fluorescence units relative to house-keeping gene expression (*GAPDH, H2AZ* and *RPLP0* for GeXP; *H2AZ* for qRT-PCR).

**Supplementary Figure 2** *LHCGR* transcripts, control genes (*GAPDH, H2AZ* and *RPLP0*) and internal standard (Kan') peaks detected using GeXP in granulosa cells from a sample of abattoir derived early luteal dominant follicles (A). Smaller fragments of the *LHCGR* with missing exons (indicated in brackets) were identified alongside three complete fragments (B) (not to scale).

**Supplementary Figure 3.** Relationships between ovulatory dominant follicle (DF) and 6-day old *corpus luteum* (CL) diameter (A), and CL diameter and progesterone (P4) synthesis (B). All DFs were scanned on experimental Day -1 (●) and the resulting CL were measured on experimental Day 6 by ultrasonography (Groups B and C) or following dissection (Group A). DFs present in Group B were scanned on experimental Day 6 (○) and the resultant CL measured following dissection on experimental Day 13. Day -1 DFs were positively correlated (*r* = 0.63; *P* = 0.001) with Day 6 CL diameter, whereas Day 6 DFs were not significantly correlated with Day 13 CL diameter. There was no significant difference in mean diameter between Day -1 and Day 6 DFs (13.4 vs 12.0 mm; *P* = 0.09). There was no relationship (*r* = 0.17; NS) between CL diameter and P4 synthesis for either Group A or B treatments.

**Supplementary Figure 4.** Ovarian follicular growth and plasma progesterone (P4) concentrations (ng/ml) in eight Group C Hereford x Holstein heifers following synchronised oestrus (ovulation occurred between 11 am on Day -1 and 11 am on Day +1), as determined by trans-rectal ultrasonography. Heifers 230 and 260 had not ovulated when initially scanned early on Day +1 but had later that morning. Solid black lines indicate ovulated dominant follicles (DFs) (heifers 230 and 260) and DFs present at slaughter. Coloured dashed lines indicate other DFs. Grey dotted lines indicate all other follicles greater than 4 mm in diameter. Plasma P4 concentrations are illustrated by yellow shading.