Supplementary Data

Cholesterol supports bovine granulosa cell inflammatory responses to lipopolysaccharide

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Supplementary Figure 1. Schematic of experimental protocol

Granulosa cells isolated from 4–8 mm or > 8.5 mm diameter ovarian follicles were grown in granulosa cell culture medium supplemented with 10% fetal bovine serum in 24-well plates for 18 h to achieve 80% confluence. The medium was discarded, and the cells were then treated in a granulosa cell culture medium (with or without serum) for 24 h with the treatments specified in the Materials and Methods section. The cells were finally challenged with control medium or medium containing 1 µg/ml ultrapure lipopolysaccharide (LPS) for a further 24 h in the continuing presence of the treatments. Samples were collected at the end of each experiment, including culture supernatants for ELISA and cells for protein or cholesterol analysis, or cell viability was evaluated by MTT assay. Each experiment was performed with 3 to 6 independent cultures of granulosa cells.
Supplementary Figure 2. VLDL cholesterol does not alter granulosa cell inflammatory responses to LPS

Granulosa cells from 4–8 mm or > 8.5 mm diameter ovarian follicles were cultured for 24 h with the indicated concentrations of VLDL cholesterol and then challenged for 24 h with control medium (●) or medium containing 1 µg/ml LPS (○), in the continued presence of the treatment. Cell supernatant IL-1α, IL-1β or IL-8 concentrations were measured by ELISA. Data are presented as mean + SEM from 3 independent experiments; statistical significance was determined using ANOVA, and P values reported for the effect of VLDL cholesterol on responses to LPS.
**Supplementary Figure 3. LDL cholesterol does not alter oestradiol or progesterone secretion from granulosa cells**

Granulosa cells from > 8.5 mm diameter ovarian follicles were treated for 24 h in serum-free culture medium containing either vehicle or 50 µg/ml LDL cholesterol. Cell supernatant oestradiol and progesterone concentrations were measured by ELISA. Data are presented as mean + SEM from 3 independent experiments.
Supplementary Figure 4. Quantification of siRNA knockdown of HMGCR, FDPS and FDFT1 in granulosa cells

Pooled populations of granulosa cells from 4–8 mm diameter and > 8.5 mm diameter ovarian follicles were transfected for 48 h with scramble siRNA or with siRNA targeting HMGCR, FDPS or FDFT1. The mRNA expression of each gene was measured by qPCR, and normalised to two reference genes (ACTB and RLP19). Data are presented as mean ± SEM, from at least 3 independent experiments; statistical significance was determined using t-tests; values differ from scramble, * P < 0.05, ** P < 0.01, *** P < 0.001.
Supplementary Figure 5. FSH and inflammatory responses to LPS with serum

Granulosa cells from 4–8 mm or > 8.5 mm diameter ovarian follicles were cultured in granulosa cell culture medium supplemented with 10% fetal bovine serum and treated for 24 h with vehicle, $10^{-7}$ M androstenedione (A4), 2.5 µg/ml FSH, or androstenedione and FSH, and then challenged for 24 h with 1 µg/ml LPS in the continued presence of the treatments. Cell supernatant IL-1α, IL-1β or IL-8 concentrations were measured by ELISA. Data are presented as mean ± SEM from 3 independent experiments; statistical significance was determined using ANOVA and Dunnett’s post hoc test, values differ from vehicle * P < 0.05.
Supplemental Figure 6. Androstenedione increases oestradiol secretion from granulosa cells cultured with serum

Granulosa cells isolated from 4–8 mm or > 8.5 mm diameter ovarian follicles were treated in granulosa cell culture medium supplemented with 10% fetal bovine serum for 24 h with vehicle, 10⁻⁷ M androstenedione (A4), 2.5 µg/ml FSH, or androstenedione and FSH, and then challenged for 24 h with control medium (●) or 1 µg/ml LPS (○) in the continued presence of the treatments. Cell supernatant oestradiol and progesterone concentrations were measured by ELISA. Data are presented as mean ± SEM from 3 independent experiments. Statistical significance was determined using two-way ANOVA with Bonferroni’s *post hoc* test; *** P < 0.001.