Developmental Programming: Granulosa cell mRNA expression of differentiation, growth and apoptosis genes in abnormally large progestagenic follicles from androgenized ewes.

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In utero exposure of female lambs to excess testosterone, which causes androgenization, can affect ovarian function such that the ovaries contain one or more abnormally large antral follicles before and after puberty. Such altered follicular growth may be due to persistence in the absence of ovulation, and we have shown previously that the larger size is associated with increased progesterone (P) and estradiol (E) synthesis in individual large follicles. To study the mechanisms mediating excessive growth and altered steroidogenesis in follicles from androgenized ewes, this study aimed to characterize granulosa cell mRNA expression for genes known to be involved in the final stages of antral follicle development. Pregnant Poll Dorset ewes were injected twice weekly from days 30–90 of gestation with either 100 mg of testosterone propionate (n=10; A) or vehicle (vegetable oil) only (n=7; C). Ewe lambs from treated mothers were euthanased at 10 months of age and the ovaries were removed. The largest antral follicles >3.5mm in diameter were excised (A: n=15, C: n=11 follicles), their diameter measured, follicular fluid aspirated for steroid radioimmunoassay, and granulosa cells (GC) recovered for real-time Q-PCR analysis of transcripts encoded by genes regulating gonadotrophin responsiveness (*LHCGR*, luteinizing hormone receptor; *FSHR*, follicle-stimulating hormone receptor), cell differentiation (*CYP19A1*, aromatase; *HSD3B1*, 3-beta hydroxysteroid dehydrogenase; *INHA*, inhibin subunit alpha; *INHBA*, inhibin subunit betaA; *FST*, follistatin), proliferation (*CCND2*, cyclin D2; *MCL1*, myeloid cell leukaemia factor 1) or apoptosis (*BGCAN*, betaglycan; *MIF*, macrophage migration inhibitory protein; *CASP3*, Caspase 3; *BAX*, BcL 2 associated protein). When 2 or 3 follicles were recovered per ewe (in 5 A and 4 C) the follicle with highest intrafollicular E was analyzed. As reported previously, A follicles were 2 mm larger and had 5-fold higher P and a 6-fold higher E concentration in follicular fluid compared with C follicles. Expression of mRNA for *LHCGR* was upregulated ($P<0.01$), while mRNA expression for *FSHR*, *CCND2* and *MIF* was downregulated ($P\leq0.05$) and for *CASP3* tended to be downregulated ($P=0.08$) in GC of follicles from A compared with C ewes. No differences were detected between A and C follicles in GC mRNA expression for the other transcripts analyzed. In conclusion, GC from large persistent follicles in A ewes appear to develop an enhanced ability to respond to the elevated systemic LH concentrations characteristic for androgenized ewes, which may also induce a degree of cellular luteinization despite high E synthesis. In such follicles, FSH-responsiveness and expression of FSH-regulated genes (for example *CCND2*) may be reduced compared with the most estrogenic large C follicles. Consistent with the observation of lack of atresia and prolonged growth of A follicles, this investigation provides new molecular evidence that GC of individual A follicles may have reduced apoptosis. Research supported by the BBSRC, MRC and Pfizer Animal Health CASE Award.