

**P-258. Effect of IGFI and IGFI on PGE<sub>2</sub> and PGF<sub>2α</sub> release by human luteal cells**

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**Introduction:** The relevance of the insulin-like growth factor (IGFs) system in ovarian physiology has been extensively demonstrated. Moreover, IGFs have been shown to enhance steroidogenesis both in human granulosa and luteal cells. Taking into account the reports that prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) and prostaglandin F<sub>2α</sub> (PGF<sub>2α</sub>) have opposite (i.e. luteotropic and luteolytic) effects on the human corpus luteum, we aimed to study whether a correlation exists between IGFs effect on progesterone secretion and the activation of the prostaglandin system.

**Materials and methods:** Isolated human luteal cells were cultured for different times (12, 24 and 48 h) with IGFI, IGFI (10–100 ng/ml) and GH (100 ng/ml); PGE<sub>2</sub> and PGF<sub>2α</sub> release in culture media was assayed by radioimmunoassay.

**Results:** Both IGFI and II were able to induce PGE<sub>2</sub> synthesis in a time- and dose-dependent way while they both inhibited PGF<sub>2α</sub> production. GH was also found able to significantly reduce PGF<sub>2α</sub> synthesis; this effect was IGFI-mediated since it was reverted by increasing concentrations of anti-IGFI antibody. Conversely, GH had no effect on PGE<sub>2</sub> production.

**Discussion:** In a recent paper of ours, we showed that IGFI as well as II are able to stimulate progesterone secretion by human luteal cells, suggesting a direct luteotropic action exerted by these substances. In light of the evidence of a well-defined correlation between IGFs and prostaglandin release by luteal cells, it is possible to suggest that IGFs could influence luteal steroidogenesis by regulating the complex balance of luteotropic and luteolytic effects exerted by the two different prostanoids.

**P-259. Characterization of an immortalized human granulosa cell line (COV434) for the study of the development of ovarian follicles *in vitro*\***

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**Introduction:** The biological properties of an immortalized human granulosa cell line (COV434) are presented. The following characteristics were considered essential for normal functionality of granulosa cells: (i) increased synthesis and secretion of 17β-oestradiol after stimulation of cytochrome P450 aromatase with follicle stimulating hormone (FSH); (ii) establishment of intercellular connections between the immortalized granulosa cells and cells of a cumulus oophorus;

and (iii) the potential response to similar inducers of apoptosis as compared to natural granulosa cells.

**Material and methods:** The cell line of immortalized granulosa cells was established from a primary human granulosa cell tumour. Control granulosa cells were collected from patients undergoing *in-vitro* fertilization (IVF) or intracytoplasmic sperm injection (ICSI). The cells were studied under light microscopy, with transmission electron microscopy and with scanning electron microscopy under various conditions. To assess the aromatase activity the cells were cultured in Dulbecco's minimal essential medium (DMEM), supplemented with 10% fetal calf serum (FCS) and 4-androstene-3, 17-dione in presence of recombinant FSH, luteinizing hormone (LH) or urinary human chorionic gonadotrophin (HCG) and the concentrations of 17β-oestradiol and progesterone were measured in the supernatant. mRNA of apoptosis-associated genes were analysed by reverse transcription-polymerase chain reaction (RT-PCR) and various apoptotic protein expressions were examined by Western blotting.

**Results:** Aromatase activity in the COV434-granulosa cells was demonstrated by the rapid rise of the 17β-oestradiol concentration in presence of FSH. No rise of progesterone concentration was observed after the addition LH or HCG neither could the LH-receptor be induced with FSH and 17β-oestradiol. Expression of various apoptosis-associated genes was similar in both granulosa cell types. Multiple intercellular connections were formed during co-culture of COV434-granulosa cells with cumulus cells containing a viable oocyte but not with cumulus cells devoid of an oocyte. Co-cultured with COV434-granulosa cells, immature human oocytes remained viable for 8 days, but degenerated thereafter.

**Conclusions:** The immortalized human granulosa cell line COV434 contains many of the properties of natural granulosa cells and may be instrumentalized for experimental studies on follicular development.

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**P-260. Pattern of cyclo-oxygenase expression in the human endometrium during the menstrual cycle**

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**Introduction:** Recent attention has been focused on the implantation stage endometrial changes at the initiation of decidual reaction induced by prostaglandins (PGs). PGs are derived from arachidonic acid by the action of cyclo-oxygenase (COX), and are comprised of two isoforms, COX-1 and COX-2. COX-1 is a constitutional enzyme, and is present in many human tissues, whereas COX-2 is induced by endocrinological stimulation. The expression of COX-1 is relatively uniform in the oestrus cycle of mice, but the expression of COX-2 has