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11.15–11.30

O-020. Initiation of human primordial follicle growth in vitro in ultrathin slices of ovarian cortex

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Introduction: The human ovary is endowed with hundreds of thousands of primordial follicles at birth, but very little is known about the biology of the events that lead to initiation of their growth. follicles in vivo are closely surrounded by a complex and dynamic milieu, including ovarian stroma and branches of the systemic circulation, nervous system, and scavenger cells. Furthermore, the architecture of the ovary shows that quiescent primordial follicles are located in a thin, relatively avascular layer in the ovarian cortex, whereas growing follicles are found in the corticomedullary border which is richly vascularized. This suggests that primordial follicle activation and growth may depend on the provision of nutrients, hormones, and/or growth factors from the surrounding tissue. These observations could account for why conventional methods of isolated follicle culture have proved incapable of supporting human primordial follicle growth in vitro. A simpler alternative strategy is to grow early follicles (<190 μm in diameter) in situ in cortical slices (CS) which are sufficiently thin to avoid diffusion limitation. This approach provides a complex support system which more closely resembles the ovary in vivo. The objective of the current study was therefore to investigate the influence of the ovarian environment on the initiation of primordial follicle growth in the human ovary.

Materials and methods: Ovarian biopsies (20 mm thick) were obtained under ethically approved protocols from 20 women (age 29.1±1.3 years) undergoing Caesarean section or routine gynaecological surgery. Cortical tissue was sectioned to 80–100 μm thickness using a Vibratome and strips were cultured under defined serum-free medium [Wynn et al.,(1998); Hum. Reprod. 13, 3132–3138] on Milli-Cell inserts at 37 °C, in 5% CO2 in air. Tissue was cultured for 0, 4, 8 or 16 days with or without FSH (1.0 mIU/ml). At the end of culture the tissue was fixed, and sectioned. Follicle number and developmental stages were recorded and growth initiation indicated by staining for the cell cycle marker proliferating cell nuclear antigen (PCNA). Results were compared with growth data from 276 follicles isolated by enzymatic digestion and manual dissection. Individual follicles were cultured in medium containing FSH and follicle diameters were measured at regular intervals to determine in-vitro growth rates.

Results: The results indicate that primordial follicle growth was initiated in CS after only 4 days of culture. In contrast, in the same time-frame, 80% of isolated primordial follicles degenerated. Irrespective of FSH, a significant (P < 0.05) up-regulation of primordial follicle growth initiation was observed between day 0 (33.8% growing follicles) and day 16 (75.7% growing follicles) of culture. After 16 days multilayer layer growing follicles were observed in the CS. For isolated follicles, irrespective of their initial developmental stage, only limited growth was observed (% growing follicles: day 0 = 17.4%; day 4 = 27.6%; day 8 = 8.0%; day 16 = 2.4%) and this growth was not maintained. Furthermore, 89% of follicles of <180 μm at the start of culture degenerated due to rapid detachment of granulosa cells and/or extrusion of the oocyte. In contrast, isolated follicles in the preantral size range (90–220 μm diameter) at the start of culture increased in diameter from 216 ± 12.1 μm on day 0 to 348.8 ± 83.0 μm on day 16 of culture.

Conclusion: These data confirm that a research strategy for the in-vitro growth of human follicles must be tailored to the size of follicle. Furthermore, the integrity of the pregranulosa oocyte complex and the surrounding stromal tissue must be maintained to stimulate the initiation of primordial follicle growth but not antral cavity formation in vitro. Primordial follicle activation does not appear to be dependent on the provision of gonadotrophic support.

Andrology 1

Monday 28 June 1999
Room 04–Hall 4 + 5

10.00–10.15

O-021. Modulation of mammalian sperm adenylyl cyclase by FPP and adenosine involves first stimulatory and then inhibitory adenosine receptors and G proteins

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Background: Current evidence indicates that FPP (fertilization promoting peptide; p-Glu-Glu-Pro-NH2) and adenosine act via separate receptors to modulate adenylyl cyclase (AC)/cAMP, resulting in stimulation of capacitation in uncapacitated mammalian spermatozoa and then inhibition of spontaneous acrosome loss in capacitated cells. Responses to FPP appear to involve interaction between FPP and adenosine receptors, the biphase nature of responses suggesting that first stimulatory and then inhibitory receptors are involved; preliminary evidence suggests that G proteins are also play a role. This study was designed to examine these possibilities further.
Discussion and Conclusions: This study provides direct evidence that responses to FPP and adenosine involve first stimulation of AC, while inhibition of spontaneous acrosome loss involves inhibition of AC; additional evidence indicates that G proteins are involved in these responses. Cholera toxin's ability to stimulate AC/cAMP suggests the presence of Gt subunits, whereas pertussis toxin's ability to block FPP's inhibition of AC/cAMP suggests the presence of Gq subunits. Although earlier studies have identified Gq subunits but not Gt subunits in mammalian spermatozoa, we have preliminary immunolectrophoretic evidence for the presence of both, consistent with the functional responses observed in this study. The presence of both FPP and adenosine in seminal plasma of many mammals suggests they could play important roles in regulating sperm function in vivo. By modulating AC/cAMP to first stimulate capacitation and then prevent capacitated cells from undergoing spontaneous acrosome loss, FPP and adenosine could help the few spermatozoa that reach the site of fertilization in vivo to maintain and thus maximize their fertilizing potential.

Results: CGS-2160 (1 μM), an agonist for stimulatory A2a adenosine receptors, significantly stimulated capacitation and cAMP production in uncapacitated cells, but had no effect on capacitated cells. Consistent with this, 8-Cl-chlorostyryl caffeine, a selective A2a antagonist, inhibited responses to both adenosine and FPP in uncapacitated suspensions. In contrast, cyclopentyl adenosine (1 μM), an agonist for inhibitory A1 adenosine receptors, had no effect on uncapacitated spermatozoa but significantly inhibited spontaneous acrosome loss in capacitated spermatozoa, while 8-cyclopentyl-1,3-dipropylxanthine, a selective A1 antagonist, inhibited adenosine and FPP's ability to block spontaneous acrosome reactions. Modulation of AC activity by adenosine receptors usually involves G proteins, with activation of A2a receptors being associated with G proteins containing cholera toxin-sensitive (stimulatory) Gαs subunits and activation of A1 receptors being associated with G proteins containing pertussis toxin-sensitive (inhibitory) Gαi subunits. Cholera toxin (100 ng/ml) only affected uncapacitated cells, stimulating both capacitation (determined by CTC) and cAMP production. In contrast, pertussis toxin when used in conjunction with FPP only affected capacitated cells, preventing FPP's inhibition of both spontaneous acrosome loss and cAMP production. Consistent with these data, electrophoresis, Western blotting and enhanced chemiluminescence evaluation of sperm membrane preparations using antibodies specific for G subunits revealed the presence of proteins with molecular weights similar to both Gαs and Gαi.

Materials and Methods: Epididymal mouse sperm suspensions were prepared in complete modified Tyrode's medium and incubated for the first 40 min and/or from 90 to 130 min in the presence of various reagents. Effects on capacitation and/or acrosome loss were evaluated using chlorotetracycline (CTC) fluorescence and effects on AC activity were determined by measuring cAMP production. Electrophoresis/Western blotting was also done.

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