9. APOPTOSIS IN HUMAN CUMULUS CELLS

L Bosco[a], G Ruvolo[b], G Morici[a], F Curatolo[a], A Romano[a], G Daunisi[a], A Pane[b], E Cittadini[c], MC Roccheri[a] *
[a]*Dipartimento di Biologia Cellulare e dello Sviluppo, University of Palermo, Viale delle Scienze ed.16, 90128 Palermo, Italy;* [b]*Centro di Biologia della Riproduzione, Palermo, Italy;* [c]*Dep. of Obstetrics and Gynecology, University of Palermo, Italy. Email: mariroc@unipa.it*

Normally functional ovarian activity involves the synergic action of the two pituitary gonadotrophins, FSH and LH, which determine follicular growth and maturation, ovulation and luteinization. Their combined action on granulosa cells and on the internal theca is expressed in the "two cells-two gonadotrophins" theory. Years of experience have shown that ovarian follicular growth proceeds normally in the presence of very small quantities of endogenous LH that, combined with pharmacological doses of FSH, can allow adequate follicular steroidogenesis.

Our interest was to investigate normogonadotrophic women who, when undergoing induction of multiple follicular growths, show an insufficient ovarian response in terms of follicular growth, defined as "low responders", despite the increase of the units of gonadotrophin administered. So, the administration of Gn-Rh could completely suppress the secretion of endogenous LH, without any exogenous support to maintain the biological activity of the LH.

In this study we tried to verify the efficiency of supplementation with exogenous LH, during ovarian stimulation, in patients that had previously experienced failed attempts at assisted fertilization. The clinical effect was assessed in terms of ovarian response, especially the influence on the quality of oocytes that were collected. Our aim was to identify indicators that were not the classic morphological assessment criteria for oocyte quality, but we tried rather to establish better oocyte quality through chromatin analysis of the oophorous cumulus cells, which play a vital role in the maturation of oocytes during folliculogenesis. Granulosa and cumulus cells are highly sensitive to apoptosis, on which the atresia process is based in most follicles that are selected during a woman's reproductive life.

We verified whether administrating LH to the patients involved in the study during ovarian stimulation may have conditioned the chromatin pattern of the oophorous cumulus cells, considering this as an important marker in defining adequate biological activity that would allow an optimum synchronization of cytoplasm and nuclear maturation of the oocyte.

Purpose of the study: Comparison of the rate of DNA fragmentation and Caspase-3 activity in cumulus cells in women stimulated with r- LH and r-FSH vs patients treated with r-FSH alone (control).

Summary of methods: 40 patients undergoing assisted fertilization program (IVF) were treated with a GnRh agonist and r-FSH, the treatment started on day 3 of the cycle (control). From day 8 of gonadotrophin stimulation 150 UI/day of r-LH were administered to women of the r-LH group.

Apoptosis in the cumulus cells was examined using TUNEL assay and anti-caspase-3 cleaved immunoassay.

Results and Conclusions: No differences were observed between the two groups in the total amount of r-FSH administered and in the number of retrieved oocytes/patient. A statistically significant increase of the immature oocytes number and in E_2 serum peak was observed in the control group. The number of transferred embryos resulted significantly higher in r-LH group. Pregnancy and implantation rates resulted higher in the r-LH group. As regards to the apoptosis rate in cumulus cells, it was higher in the control group than in the r-LH group.

This study suggests that supplementation with recombinant luteinizing hormone improves the chromatin quality of the cumulus cells, involved in control of oocyte maturation.

10. CHARACTERIZATION OF SPERM BINDING GLYCOCONJUGATES LOCATED AT THE FERTILIZATION SITE OF *D. PICTUS* EGG

C Campanella[1], M Caputo[1], L Tretola[1], R Carotenuto [1], N De Marco[1], MC Vaccaro[1], L Iannone, M Romano[2], E Limatola[2], M Prisco [2], L Camardella [3], A Flagiello[4] and V Infante[1]
[1] *University of Naples Federico II, Department of Structural and Functional Biology, MSA, Naples, Italy;*[2] *University of Naples Federico II Department of Biological Sciences, Mezzocannone 8, Naples, Italy;* [3]*IBPE, CNR Naples, Italy;* [4]*CEINGE Naples, Italy*

Little information is known about the molecules that permit adhesion and fusion of sperm on the egg plasma membrane. In *Discoglossus pictus* egg, the dimple is the only site where sperm fuse with egg and the activation current is started (Nuccitelli et al, 1988, Dev. Biol. 130: 120). The dimple membrane, decorated by an antennular glycocalyx, is far apart from the vitelline envelope, being ideal for studying molecules responsible for sperm adhesion and fusion without contamination from the external coats. Fucose, as detected by its ability to bind *Ulex europaeus agglutinin-I* (UEA-I), is a marker of gps located at the dimple surface. Following labelling of the egg surface proteins with the membrane impermeable sulfo-NHS-biotin, gps binding UEA-1 (of 200, 230, 260, 270 kDa) were found only at the dimple surface, although present in the cortex of the whole egg. The 270 kDa band reacted with specific lectins only in the dimple, suggesting that this band is differently glycosylated according to its localization. Gel-eluted gps 200 and 270/260 were adsorbed to polystyrene beads. Sperm stuck to the gp200-beads before Ca-ionophore treatment. For 270/260-beads the binding occurred after ionophore treatment suggesting that glycosylation and in particular fucosylation is crucial for modulating sperm binding (Maturi et al, 1998, 204:210). In the present work, we charged polyacrylamide gels with gp 200 or 270/260 bands excised from gels. As a result, we obtained two main bands of 120 and 66 kDa in both 200or 270/260 gps lanes. Data indicated that the 120 kDa is part of the 200-270/260 gps. The N-terminus of the 120 kDa protein showed high homology with *Xenopus laevis* vitellogenin (VTG) and lipovitellin 1. The latter is part of *Xenopus* VTG A2 and is located at the N-terminus of the molecule. VTG is a liver-derived high molecular weight glycoprotein containing two main proteins, lipovitellin and phosvitin. VTG is released into the blood stream and endocytosed by the oocyte, where it splits into its components and then incorporated into yolk platelets. Several cases of oocyte-derived vitellogenins have been also reported. In our studies, N-terminus sequencing showed that the dimple 200 and the 270/260 gps have the same N-terminus as the 120 kDa lipovitellin. MALDI spectra of lipovitellin, gps 200 and 270/260 share the same peaks. Moreover, liver VTG recovered from *Discoglossus* serum is a doublet of 200/210. Higher Mr gps, were not found in *Discoglossus* serum. From previous studies we know that the dimple forms in a specific territory of the oocyte following ovulation. A new membrane is inserted in the forming dimple, endowed with antennular glycocalyx and UEA-I affinity. Here, staining with anti-VTG and gold-conjugated secondary antibodies shows gold particles in the dimple glycocalyx. In toto immunofluorescence with the same anti-VTG shows immunofluorescence only in the dimple, co-localizing with UEA-I.The labelling is lost after fertilization..In conclusion, *D. pictus* 200 and 270/260gps, able to bind sperm in *in vitro* assay, are glycoconjugates of the VTGs family, found only at the dimple surface from where disappear after fertilization. Given that gp 270/260 is not released from the liver, we hypothesized that the VTGs came from the oocyte and are exposed to the external aspect of the plasma membrane at the time of dimple formation and acquisition of fertilizability.

11. A KEY ROLE FOR POU TRANSCRIPTION FACTORS IN THE NEURAL DEVELOPMENT OF *AMPHIOXUS*

Simona Candiani, Diana Oliveri, Manuela Parodi, Mario Pestarino*
Department of Biology, University of Genoa, viale Benedetto XV 5, 16132 Genova, Italy

POU proteins are an important class of transcription factors and play critical roles in determination and maintenance of a wide variety of cell types such as neural cells, endocrine cells, blood cells, skin cells and oocytes. The modular structure of the POU domain imparts a functional versatility that allows the domain to participate in transcriptional regulation of a variety of ubiquitous and tissue-specific genes. Furthermore, several members of the POU homeobox gene family play essential roles in establishing neuronal phenotypes in most, if not all, multicellular organisms. In vertebrates, the pituitary transcription factor Pit-1define the type I class, and is the sole example. The ubiquitously expressed Oct-1 and tissue-specific immune system's B cells Oct-2 are representative of the second category. Brn1,2,4 and Tst-1, which are