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The Regulation of Ovarian Follicle Growth, Demise and The Ovulatory Response

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The laboratory deals with basic questions of mammalian ovarian physiology and endocrinology, the control of follicular growth and when a mature preovulatory follicle has evolved, its ovulatory response culminating in the release of a fertilizable ovum.

Follicular growth and selection.

Contrary to the naïve thought, degeneration rather than ovulation is the ultimate fate of the vast majority of oocytes. Of the approx. 2 million oocytes in the human ovary at birth only 400 reach ovulation during the fertile life. Each of these oocytes is in a "nest" of supporting cells forming the ovarian follicle. Thus, more than 99,9% of human follicles undergo degenerative changes, referred to as atresia, which involves apoptosis, or programmed cell death of follicular granulosa cells. Follicular apoptosis is currently examined in collaboration with A. Gross and K. Yacobi. In order to preserve reproductive potential of cancer patients treated by chemo- or radiotherapy there is a need to devise methods of ovarian cryopreservation and transplantation. In collaboration with M. Neeman and T. Israely we are examining the role of angiogenesis in implant and germ cell preservation, by transplanting rat ovaries into immunodeficient mice.

Ovulation.

At the present the laboratory works mainly on one aspect of the ovulatory response, the resumption of oocyte maturation. We were the first to establish an *in vitro* system that enabled the discovery of the basic facts of the hormonal regulation of oocyte maturation. Cyclic AMP (cAMP) plays a central role in the regulation of meiotic maturation of mammalian oocytes. High oocyte levels of cAMP were implicated in the maintenance of meiotic arrest, and a decrease in oocyte cAMP is necessary for resumption of meiosis. On the contrary, the stimulation of the ovulatory process by luteinizing hormone (LH), including the resumption of meiosis, is clearly associated with a rise in cAMP levels in the somatic cells of the follicle. In collaboration with Professor Conti from Stanford, we have provided a solution to this paradox by invoking the selective regulation of specific phosphodiesterases in the somatic and germ cell compartments of the preovulatory follicle. Differential regulation of PDEs in the somatic (containing PDE4) and germ cell (containing PDE3) compartments of the follicle, by gonadotropins seems to be involved in the regulation of their cAMP level. Stimulation of oocyte PDE may explain the paradoxical decline in cAMP levels in the oocyte, allowing resumption of meiosis, concomitantly with its rise in the somatic compartment of the follicle in response to stimulation of ovulation by LH. Furthermore, pharmacologic inhibition of oocyte PDE3 may allow the development of specific, midcycle contraceptive that does not affect the menstrual cycle.

Previous studies showed that EGF and TGFα mimic the action of LH on the resumption of oocyte maturation. Now, in collaboration with Prof. Conti, we have obtained evidence suggesting that ovarian EGF-like agents also mediate the LH stimulation of the ovulatory response in the rat. LH induced transient follicular expression of amphiregulin (AR), epiregulin (ER) and betacellulin (BTC) mRNA. Furthermore, the addition of ER, AR and BTC to the culture medium could mimic some of LH actions. AR and ER simulated LH-induced resumption of meiosis *in vitro*. The EGFR kinase inhibitor, AG1478, was used to examine the putative involvement of EGF-like factors in the mediation of the LH signal. AG1478 inhibited the resumption of meiosis induced by LH. In addition to the inhibition of resumption of meiosis as *in vitro*, AG1478 administration into the bursa (3

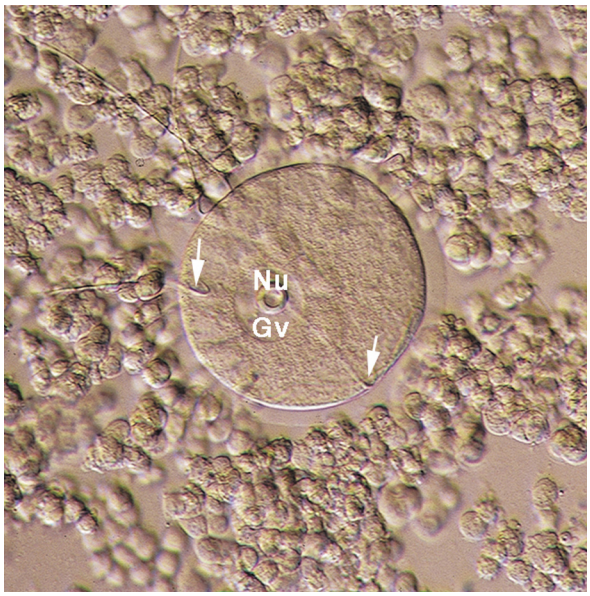

Fig. 1 *Immature oocyte obtained from the oviduct. Treatment with a PDE3 inhibitor did not affect endocrine changes, ovulation and mating, but it prevented the maturation of the oocyte (see the large nucleus, germinal vesicle-GV, and nucleolus-Nu) and consequently fertilization and embryonic development. Several sperm heads (arrows) are seen in the perivitelline space.*

µg/bursa) resulted in inhibition of ovulation in the treated ovaries. LH as well as ER induced follicular expression of rCOX-2, rHAS-2 and rTSG-6 mRNA, genes involved in the ovulatory response. AG1478 inhibited these effects of LH. A metalloprotease inhibitor, GM6001 inhibited LH induced oocyte maturation, but not ER induced oocytes maturation, supporting the notion that LH releases, through activation of follicular metalloproteases, follicle-cell membrane bound EGF-like agents. It appears, that locally produced EGF-like factors such as ER, AR and BTC mediate, at least partially, the LH stimulation of oocyte maturation, ovulatory enzyme expression and ovulation.

The recently suggested role of a meiosis activating sterol (MAS) in the mediation of LH induction of meiosis is being assessed by a combination of pharmacological and molecular biology approaches. These studies did not provide evidence for such a physiological role of MAS. (i) Specific inhibitors of MAS synthesizing enzymes did not prevent spontaneous or LH-stimulated meiosis at doses that have previously been shown to effectively suppress LDM activity. At higher doses they caused degeneration of oocytes. (ii) The timing of LDM expression in the ovary was incompatible with a role for MAS in meiosis. (iii) Finally, the time-course of resumption of meiosis by MAS stimulation revealed a significant delay as compared to oocytes maturing spontaneously or due to hormone-stimulated maturation in these species. Our studies question any role of FF-MAS as an obligatory mediator of LH activity on resumption of meiosis. Yet, MAS may be involved in the developmental competence of mammalian ova.

Selected Publications

Vaknin, K. M., Lazar, S., Popliker, M. and Tsafriri, A. (2001) The role of meiosis activating sterols in rat oocyte maturation: Effects of specific inhibitors and changes in the expression of lanosterol 14α demethylase during the preovulatory period. Biol Reprod, 64, 299-309.

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