The regulation of ovarian follicle growth, demise and the ovulatory response

Department of
Biological Regulation

Prof. Alex Tsafriri

Shmulik Motola

☎ 972 8 934 4526

FAX 972 8 934 4116

@ alex.tsafriri@weizmann.ac.il

www.weizmann.ac.il

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 reaches 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.

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 oocytes 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 one solution to this paradox by invoking the selective regulation of specific phosphodiesterases (PDEs) in the somatic and germ cell compartments of the preovulatory follicle. Differential regulation of PDEs in the somatic (containing PDE4) and germ cell (containing PDE3A) 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 PDE3A may allow the development of specific, midcycle contraceptive that does not affect the menstrual cycle (Fig. 1). Previous studies showed that EGF and TGFa mimic the action of LH on the resumption of oocytes maturation. In collaboration with Prof. Conti, we have obtained evidence suggesting that ovarian EGF-like agents like epiregulin (ER), amphiregulin (AR) and betacellulin (BTC) also mediate the LH stimulation of the ovulatory response in the rat.

Gonadotropin-releasing hormone (GnRH) has been shown to mimic the actions of LH/hCG on oocyte maturation and ovulation. Recent studies demonstrated that induction of ovulation by LH/hCG is mediated, at least in part, by transactivation of epidermal growth factor receptors

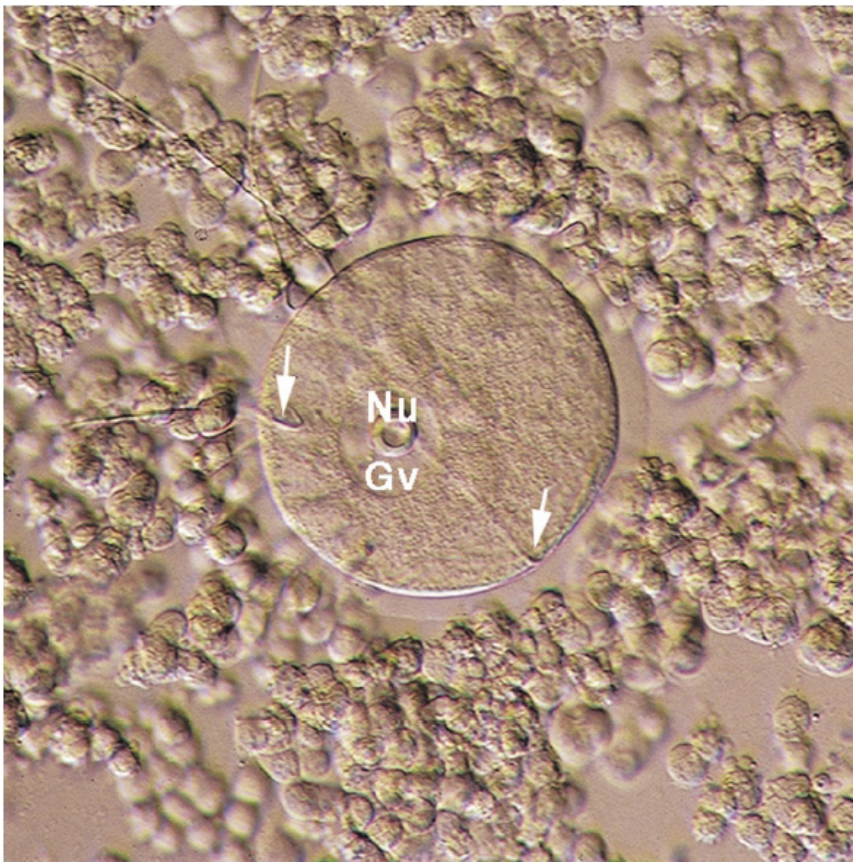

Fig. 1 *Immature oocyte obtained from the oviduct. Treatment with a PDE3 inhibitor did not affect endocrine changes, ovulation and mating, but it prevented the maturation of the oocyte (see the large nucleus, germinal vesicle-GV, and nucleolus-Nu) and consequently fertilization and embryonic development.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

(EGFR) by autocrine/paracrine EGF-like factors activated by metalloproteases. We have examined whether the action of GnRH on the preovulatory follicles is exerted through similar mechanisms involving activation EGFR. The EGFR kinase inhibitor, AG1478, inhibited GnRH-induced oocyte maturation in explanted follicles in vitro. Its inactive analog, AG43, did not affect GnRH-stimulated resumption of meiosis. GnRH, like LH, stimulated transient follicular expression of EGF-like agents, as well as rat cyclooxygenase-2 (rCOX-2), hyaluronan synthase-2 (rHAS-2) and tumor necrosis factor--stimulated gene-6 (rTSG-6) mRNAs, known ovulatory enzymes. Likewise, GnRH stimulated follicular progesterone synthesis. Conversely AG1478 inhibited all these actions of GnRH. Furthermore, Galardin, a broad spectrum metalloprotease inhibitor, blocked GnRH-induced oocyte maturation and follicular progesterone synthesis. In conclusion, we have demonstrated that follicular EGF-like factors mediate also the GnRH-stimulation of ovulatory changes, like these of LH/hCG.

Steroids mediate the gonadotropic stimulus of oocyte maturation in fish and amphibians. Such role of steroids in mammals has not been confirmed until recently. A series of studies presented data suggesting that steroids might be involved in meiosis of mouse oocytes. Here we examined this suggestion using in vitro cultures of rat and mouse follicle-enclosed oocytes (FEOs) and cumulus-enclosed oocytes (CEOs). In FEOs that mature only in response to gonadotropins or other stimuli we tested the ability of steroids to trigger meiosis and whether addition of steroid receptor antagonists blocks LH/hCG stimulation of meiosis. In CEOs that mature spontaneously, we tested whether steroid antagonists block maturation and whether steroids overcome the inhibition of maturation by hypoxanthine (Hx), a mild inhibitor of phosphodiesterases. The progesterone antagonists mifepristone (RU 486) and Org 31710, as well as the estrogen antagonist faslodex did not prevent LH-triggered maturation of rat or mouse FEOs or the spontaneous maturation of CEOs. In accordance, the progesterone agonist, promegestone (R5020) and estradiol did not stimulate the resumption of meiosis in rat and mouse FEOs, and both did not overcome the Hx inhibition of meiosis in rat and mouse CEOs. Flutamide, an androgen antagonist, did block meiosis in rat FEOs, but this action could not be affected by adding dihydrotestosterone (DHT), suggesting that it was not androgen receptor mediated. Flutamide did not affect spontaneous maturation of rat CEOs and DHT could not stimulate meiosis inhibited by Hx. Thus, in contrast to lower vertebrates, in mammals steroids do not seem to serve as an obligatory signal by which the somatic cells of the follicle transfer the gonadotropic stimulation of meiosis to the oocyte.

Cultures of mural granulosa cells (mGCs) and COCs were employed to investigate various aspects of follicle cell function and response to gonadotropins. Yet, such studies do not reveal the intricate cell-to-cell interactions in the whole follicle. Recently we compared the ovulatory responses to LH/hCG or ER of rat preovulatory follicles and of mGC and COC whether they were stimulated within the follicle or in primary cell cultures.

The expression of TSG-6 and COX-2 mRNA varied according to the culture system and mode of stimulation. In primary cultures stimulated with LH or ER resulted in their lower expression as compared to stimulation of follicles. LH/hCG stimulated higher follicular and mGC AR, ER and EGFR mRNA levels than in primary mGC cultures. COCs stimulated by LH/hCG *in vivo* responded with AR, ER and EGFR mRNA expression, but not in culture where only EGFR mRNA was stimulated. The differences in gene expression of mGCs and COCs when stimulated within their intact follicle or in primary cultures revealed here underscore the important role of cell–cell interactions in follicle physiology. Therefore, results obtained in primary mGC cultures need careful validation in models reproducing such in situ interactions for revealing mGC activity within the intact follicle.

In the mouse and the rat, MAPK activity in cumulus cells is necessary for gonadotropin induced, but not spontaneous, resumption of meiosis. Meiosis activating sterol (MAS), an intermediate in the cholesterol biosynthetic pathway, was suggested to be an obligatory intermediate of gonadotropins-stimulation of oocyte maturation. We aimed to compare the involvement of MAPK in LH and MAS induced resumption of meiosis in rat and mouse oocytes. Using rats, and w.t. and mos-/- mice, the latter lacking oocytes MAPK, with in vitro models requiring hormonal stimulation for oocyte maturation like FEO, CEO and denuded oocytes (DO) that mature spontaneously, we have compared MAPK activation and oocytes maturation stimulated by LH or MAS. Rat FEOs responded to LH and AY9944 (that stimulates accumulation of endogenous MAS) by MAPK activation and resumption of meiosis (germinal vesicle breakdown-GVB). Both responses occurred earlier when stimulated by LH and were blocked by U0126. This finding is inconsistent with the suggested role of MAS in mediating LH action. MAS did overcome the inhibition of spontaneous maturation by Hx in w.t and mos+/- DOs, but failed to do so in Mos-null mice. In w.t and mos- /- mouse CEOs cultured with Hx, AY9944 induced resumption of meiosis compared to Hx alone. Thus, MAPK activation is required for both LH- and MAS-induced resumption of meiosis. Normally, this is provided by phosphorylation of cumulus cell MAPK. DO of w.t., but not mos-/- mice, respond to MAS by maturation, proving that Mos provides in the absence of cumulus cells the necessary MAPK activity.

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