Control of the resumption of meiosis in mammals

Control of the Resumption of Meiosis in Mammals

H. R. Lindner, S. Bar-Ami, and A. Tsafriri

Department of Hormone Research, The Weizmann Institute of Science, Rehovot, Israel

Several reviews dealing with different aspects of oocyte maturation in mammals have been published recently (8,34,37,58). Therefore, this is not intended to be an exhaustive review of mammalian oocyte maturation, but a brief account with a special emphasis on the follicular factors involved in the regulation of the meiotic process. Oogenesis in mammals is a protracted process (Fig. 1). The meiotic division of the oocyte is initiated during fetal life and is arrested shortly after birth at the stage of diplotene. At this stage, the nucleolus and the nuclear membrane reappear and the so-called dictyate oocyte persists for a very long period. In humans, this period may reach 40 years or more. Meiosis is resumed in adult life following the preovulatory surge of gonadotrophins, a few hours prior to ovulation. We shall use the terms "oocyte maturation" or "nuclear maturation" to denote the preovulatory resumption of the meiotic process and its progress to the metaphase stage of the meiotic division, i.e., to a fertilizable ooctye (Fig. 2). Development beyond this stage, with the completion of the second meiotic division, will depend on the penetration of a fertilizing spermatozoon.

Oocyte maturation, like other ovulatory processes such as the increase in the ratio of progesterone–to–estrogen secretion and follicular rupture, is triggered *in vivo* by the preovulatory surge of luteinizing hormone (LH) (6,54,61). On the other hand, oocytes dislodged from their follicles resume the meiotic process *in vitro* even in hormone-free media (39; reviewed in 15,41,58). The meiosis-inducing action of gonadotrophins has been studied *in vitro* by explanting follicles prior to the preovulatory surge of gonadotrophins (51). This system has been exploited to define the role of gonadotrophin receptors, cyclic AMP, protein kinase, prostaglandins, steroid hormones, and glycolysis in the mediation of this response (31,60). The contrasting behavior of oocytes dislodged from their follicles and of follicular oocytes *in vivo* or *in vitro* led to the view that within the follicle oocyte maturation may be prevented by an inhibitor elaborated by follicle cells. In order to test this hypothesis, a third approach to the study of oocyte maturation *in vitro* was adopted, namely coculture of various follicular components with oocytes (18,53).

SPONTANEOUS MATURATION OF ISOLATED OOCYTES

The pioneering observation of Pincus and Enzmann (39) that rabbit oocytes explanted from their follicles undergo maturtion *in vitro*, even in hormone-free

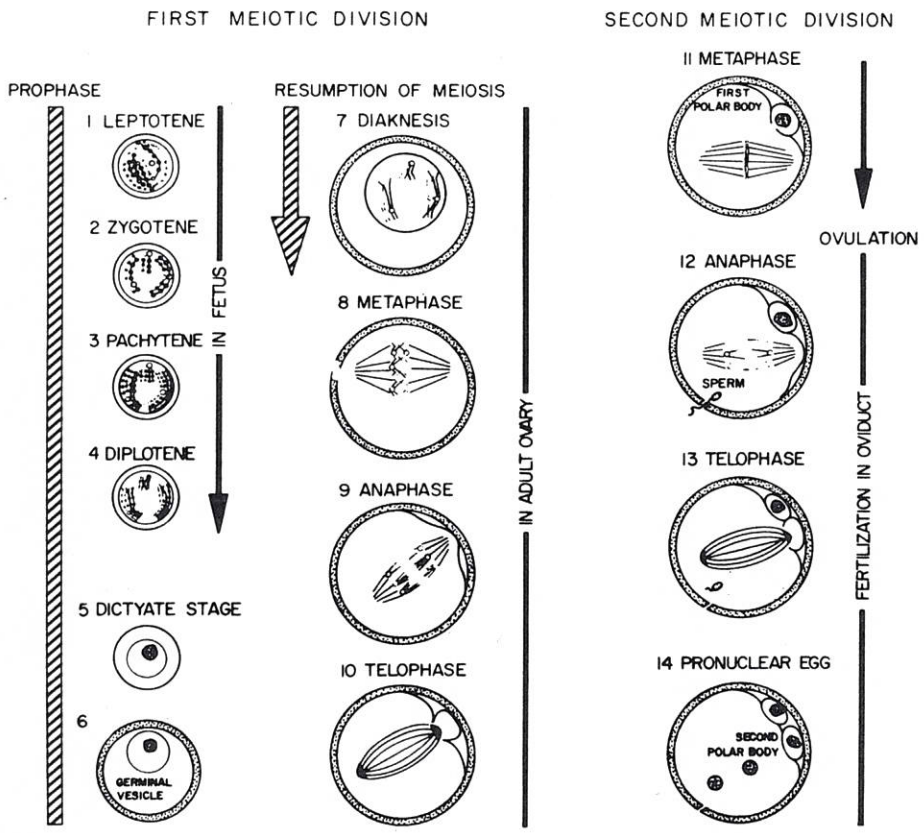

FIG. 1. Diagram of oocyte meiosis. For simplicity, only three pairs of chromosomes are depicted. 1–4, Prophase stages of the first meiotic division, which occurs in most mammals during fetal life. The meiotic process is arrested at the diplotene stage ("first meiotic arrest") and the oocyte enters the dictyate stage (5–6). When meiosis is resumed, the first maturation division is completed (7–11). Ovulation occurs usually at the metaphase II stage (11), and the second meiotic division (12–14) takes place in the oviduct only following sperm penetration. (From Tsafriri, ref. 58, with permission of Plenum Press.)

media, has been confirmed and was extended to many other mammalian species, including humans (11,15,40).

The spontaneous maturation of oocytes dislodged from their follicles results in morphologically normal secondary oocytes in most species. Nevertheless, the fertilization rate obtained after spontaneous maturation *in vitro* was very low in all of the species tested, including the human (45). The most common abnormality in such oocytes was the failure of the sperm nucleus to swell in the ovum cytoplasm, i.e., no normal male pronucleus was formed. Thibault and Gerard (48) suggested that the lack of a putative "male pronucleus growth factor" (MPGF) in such dislodged oocytes may be responsible for this failure of development of the male nucleus.

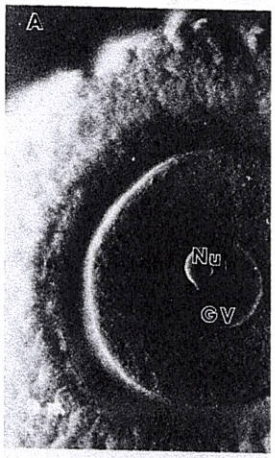

FIG. 2. Reinitiation of mei
contrast optics. **A:** Dictyate
breakdown. *Inset (M-I)*: biva
Inset (M-II): chromosomes (
cording to Tarkowski (1966),

Recent studies suggest
bring about physiologica
male pronucleus formati
treatment with estradiol
nucleus formation was o
phins, prolactin, estradio
(50).

In conclusion, if fertil
opment are adopted as t

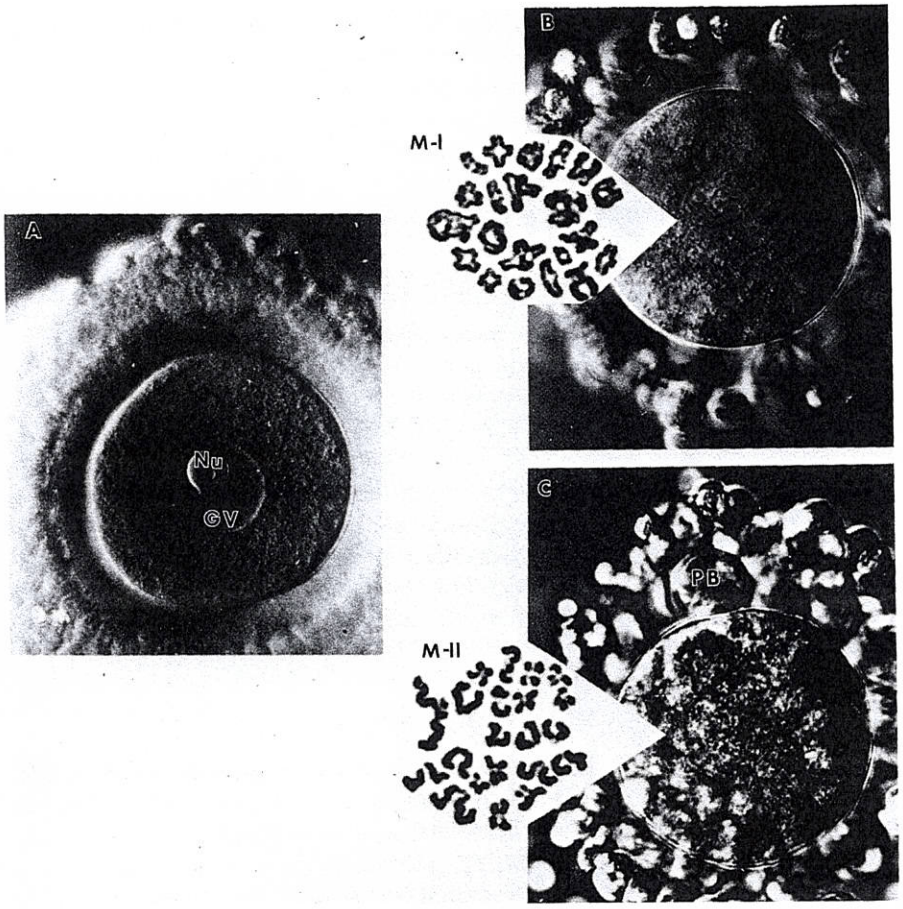

FIG. 2. Reinitiation of meiotic maturation in rat oocytes viewed with Nomarski interference contrast optics. **A:** Dictyate oocyte. GV, germinal vesicle; Nu, nucleolus. **B:** Germinal vesicle breakdown. *Inset (M-I)*: bivalents in metaphase I. **C:** Secondary oocyte. PB, first polar body *Inset (M-II)*: chromosomes (dyads) seen at metaphase II. Chromosomes were prepared according to Tarkowski (1966), *Cytogenetics*, Vol. 5, and were photographed under phase contrast.

Recent studies suggest that the addition of hormones to the culture medium may bring about physiological maturation of liberated oocytes. Soupart (45) described male pronucleus formation in human oocytes matured *in vitro* after sequential treatment with estradiol and 17α-hydroxyprogesterone. Some degree of male pronucleus formation was obtained upon fertilization when a mixture of gonadotrophins, prolactin, estradiol, and testosterone was added to rabbit oocytes in culture (50).

In conclusion, if fertilizability and the potential for normal embryonic development are adopted as the criteria for the normalcy of oocyte maturation, one is

forced to infer that the spontaneous maturation of oocytes dislodged from the follicle is largely an experimental artifact or, at least, is not a fully adequate model for studying the meiotic process. Hence, other model systems that more closely represent the physiology of oocyte meiosis *in vivo* and that yield normal, fertilizable ooctyes are needed.

MATURATION OF FOLLICLE-ENCLOSED OOCYTES

Hormonal Induction *In Vitro*

When preovulatory rat follicles are explanted before the endogenous surge of gonadotrophins and placed in organ culture without hormonal supplementation, the oocytes remain indefinitely in the dictyate state. The test system allowed us to study the meiosis-inducing action of gonadotrophins and other agents *in vitro*. Luteinizing hormone (LH), human chorionic gonadotropin (HCG), and immunochemically pure follicle-stimulating hormone (FSH) and prostaglandin E_2 (PGE_2) proved capable of triggering the maturation of such follicle-enclosed oocytes in culture (31,51). Similar results were obtained when ovarian fragments (10,38) or preovulatory follicles (22) of pregnant mare serum gonadotropin (PMSG)-treated mice or rats were cultured. Gonadotrophin-induced maturation of follicle-enclosed oocytes has since also been achieved in the rabbit (49) and hamster (21).

Mediation by Cyclic AMP

The feature common to all the agents that induced maturation of follicle-enclosed oocytes *in vitro* was the ability to stimulate the production of cyclic AMP (and hence to activate protein kinase) in the follicle (31). Indeed, introduction of dibutyrl cyclic AMP (dbcAMP) into the follicular antrum (51) or short-term incubation of follicles in a medium containing 8-bromo-cyclic AMP (24) triggered germinal vesicle breakdown, in stark contrast to the inhibitory action of cyclic nucleotide on isolated oocytes discussed above. It thus seems likely that cAMP does not act directly on the oocyte itself but rather on other cellular components of the follicle, perhaps terminating an inhibitory action exerted by these cells on the oocyte (see below).

Fertilizability: Variation Between Animal Models

Follicle-enclosed rabbit oocytes matured *in vitro* by stimulation with LH underwent normal fertilization *in vitro* and, upon transplantation into suitable recipients, developed into normal viable young. By contrast, normal meiotic maturation has not yet been achieved in explanted follicles of pigs (9), sheep (37), or women (10) by treatment with gonadotrophins *in vitro*.

Does this difference in behavior between follicle-enclosed oocytes of rodents and lagomorphs on the one hand, and those of the human, pig, and sheep on the other, indicate that the onset of meiosis in these species is controlled by a basically different

mechanism? An alternative possibility is that in experiments with the latter species, which have a long estrous cycle, the explanted follicles were not mature enough to respond, whereas in studies with rodents and lagomorphs, only late preovulatory follicles, shortly before the LH surge, were explanted. Indeed, Hunter et al. (26) demonstrated that administration of HCG to pigs on day 17 of the 21-day estrous cycle elicits premature ovulation of dictyate oocytes, whereas HCG on day 19 or 20 results in ovulation of normal oocytes at metaphase II stage.

Role of Steroids: Interspecific Differences?

Inhibition of steroid hormone synthesis by addition of cyanoketone or of aminoglutethimide to the culture medium did not impair the meiosis-inducing action of gonadotrophins on rat oocytes explanted within their follicles (28,52). In the rabbit, likewise, there appears to be no need for LH-induced steroidogenesis to achieve physiological maturation and fertilizability *in vitro* (49). By contrast, in cultured ovine follicle-enclosed oocytes, inhibition of steroidogenesis blocked LH-induced meiosis at the metaphase I stage. Addition of estradiol to such cultures together with the gonadotrophin significantly improved fertilizability of the oocyte and embryonic development upon transfer to foster ewes (35,37). Fertilizability has not yet been adequately tested in cultured rat oocytes matured within their follicles. The apparent difference between cultured rabbit and sheep oocytes in the degree of dependence on exogenous steroids for normal maturation *in vitro* may be related to differences in the dynamics of follicle growth and steroidogenesis, resulting in differential exposure of the oocytes to steroids *in vivo* prior to explantation. Little is known about the steroid requirements for maturation of the human oocyte, except that steroids (estradiol and 17α-hydroxyprogesterone) appear to promote cytoplasmic maturation and normal male pronucleus formation (45). Thus, while it is clear that the meiosis-inducing action of LH is not mediated by the enhancement of follicular steroidogenesis (28), it appears that the fertilizability of oocytes of several animal species is clearly dependent upon undisturbed follicular steroidogenesis (35).

FOLLICULAR CONTROL OF OOCYTE MATURATION

Inhibitory Effect of Granulosa Cells

The divergent behavior of dislodged oocytes, which mature spontaneously, and those cultured within the follicle, which remain in the dictyate stage unless stimulated by gonadotrophin, suggest that the granulosa cells may be responsible for maintaining meiotic arrest. Indeed, Foote and Thibault (18) demonstrated an inhibitory effect of porcine granulosa cells upon the resumption of maturation by porcine oocytes: oocytes cultured within the domes of dissected follicular wall did not mature, and theca alone was not inhibitory. Tsafriri and Channing (53) extended these findings to show that this effect of granulosa cells is dose dependent and that cells from small follicles were more potent in this respect than those from medium

or large ones. Disconcertingly, the inhibitory effect of porcine granulosa cells was not reversible by LH, FSH, PGE_2, or dbcAMP. It now seems probable that the failure of porcine oocytes cocultured with granulosa cells to respond to hormonal stimuli by resumption of meiosis is due to inadequate maturation of the follicles from which the cells were collected (see above).

When rat oocytes were added to 24-hr-old rat granulosa cell cultures, spontaneous oocyte maturation was suppressed, the degree of inhibition depending on the number of granulosa cells in the culture. This inhibitory effect was reversed when LH was added to the cultures together with the oocytes (58,59).

Oocyte Maturation Inhibitor

Porcine granulosa cell extract (55) as well as medium in which rat granulosa cells (58) had been cultured previously ("conditioned medium") exerted an inhibitory effect upon the resumption of meiosis by cultured oocytes. Follicular fluid (FFl) from rabbit, pig, cow, sheep, and hamster ovaries were shown to contain similar activity (13,21,23,27,53,57). This effect is not species-specific: porcine FFl inhibits the maturation of oocytes of the mouse (Channing and Tsafriri, unpublished observations) and rat (57); bovine FFl inhibits hamster oocytes (21); and human FFl inhibits the maturation of porcine oocytes (23).

The oocyte maturation inhibitor (OMI) from porcine follicular fluid appears to be a peptide with a molecular weight of less than 2,000 daltons (46,56). OMI activity was demonstrated in both frozen and freshly collected porcine FFl. The inhibitory action of OMI was reversed by transferring the oocytes to fresh medium devoid of OMI 20 to 24 hr after the initiation of culture. The OMI concentration of porcine FFl declined with follicular growth (46,56). By sequential Amicon PM-10 membrane filtration, Sephadex G-25 (46,58) and CM-Sephadex column chromatography, approximately 5,000-fold purification of OMI was achieved (Table 1). Immunization of rabbits or rats with the low molecular weight fraction of porcine follicular fluid (the Amicon PM-10 membrane filtrate) conjugated to bovine serum albumin (BSA) produced an antiserum able to neutralize OMI action on rat oocytes. When the antibodies were purified by affinity chromatography, OMI action was neutralized only by the specific antibody fraction and not by the absorbed serum (59,60).

TABLE 1. *Purification of oocyte maturation inhibitor from porcine follicular fluid*

Fraction	Volume (ml)	Peptide (mg/ml)	Units/ mg	Total units	Fold purification
FFl	900	2,000	0.001	1,800	—
Amicon PM-10 filtrate	24.5	105	0.48	1,225	480
Sephadex G-25 peak A	20	43.4	1.38	1,200	1,380
CM-Sephadex active peak	15	29	5.17	2,250	5,170

Significance of Communication Between Oocyte and Cumulus Cells

The density of LH-receptors is much higher on the surface of the mural granulosa cells of the mature graafian follicle than on cumulus oophorus cells. On the oocyte itself and the adjacent coronal cells, it is difficult to demonstrate LH-receptors by radioautographic techniques (4), yet the follicle-enclosed oocyte responds promptly to gonadotrophic stimulation. This suggested the existence of a system of intercellular communication that might propagate the hormone stimulus within the follicle. A structural basis for such communication was provided by the description of extensive gap-junctions between adjacent cells within the theca and granulosa layers (1,3) and the demonstration that similar specialized junctions exist between cytoplasmic extensions of the corona radiata cells that traverse the zona pellucida and the oolemma (2,5). Such junctions can facilitate the transfer of ions and small molecules, possibly including chemical messengers, up to about 2,000 daltons in size, between neighboring cells and bring about their electrical coupling, i.e., coordination of their membrane potential (7,20,43). Thus, Gilula, Epstein, and Beers (19) demonstrated bidirectional electrical coupling between cumulus cells and the oocyte as well as transfer of iontophoretically injected fluorescein dye from the oocyte to cumulus cells. Ionic coupling was maximal prior to HCG stimulation and it decreased as ovulation approached. Similarly, Moor et al. (36) used [^{3}H]-labelled choline, uridine, and inositol for measuring the intercellular coupling between cumulus cells and oocyte of sheep. They demonstrated that gonadotrophins *in vivo* or *in vitro* reduced, but did not totally eliminate, within 12 to 15 hr the coupling between these two cell types. The fact that both [^{3}H]-labelled uridine incorporation (16,62) and growth (17) of mouse oocytes have been shown to be dependent upon the presence of intact cumulus cells further attests to the intimate interaction between these cell types.

The close association between cumulus cells and the oocyte and the apparent disruption of this relationship following ovulation led to the hypothess (29,31) that the release of the oocyte from the inhibitory action of the cumulus cells and subsequent resumption of meiosis may result from the dismantling or functional disengagement of oocyte/corona cell junctions, possibly induced by the ovulatory hormone. This would bring about a functional sequestration of the oocyte within the preovulatory follicle, analogous to the physical separation achieved by surgery in the isolated oocyte culture model, and would explain why in the later model gonadotrophins are redundant.

It may be noted that a similar detachment of follicle cells from the oocyte accompanies maturation of starfish (40) and amphibian (41,42,44) oocytes. Nevertheless, the morphological dissociation of cumulus–oocyte junctions appears to follow, rather than precede, germinal vesicle breakdown in rat (14) and rabbit oocytes (47). Furthermore, the reduction in intercellular transport of choline from sheep cumulus cells to the oocyte occurred only *after* meiotic maturation had progressed to prometaphase or even first metaphase (36). It is possible, however, that physiological occlusion of cumulus–oocyte junctions precedes their morpho-

logical separation. Therefore, more detailed kinetic studies of the changes in physiological coupling between cumulus and oocyte cells in relation to the resumption of meiosis are needed before one can decide whether there is a causal relationship between these two processes.

An essential role of cumulus cells in the control of resumption of meiosis has recently been demonstrated by a different approach. Whereas the low molecular weight fraction of porcine FFl inhibited the maturation of oocytes cultured within their intact cumuli, it did not interfere with the maturation of fully denuded oocytes of the pig (25), rat (Fig. 3), or mouse (Bar-Ami and Tsafriri, unpublished observations). Moreover, addition of the low molecular weight fraction of porcine follicular fluid even tended to facilitate the maturation of denuded rat oocytes. It thus appears that OMI exerts its inhibitory action upon the resumption of meiosis not directly on the oocyte but through the mediation of the cumulus cells. Whether the maturation-inducing action of LH is exerted solely by cumulus-oocyte uncoupling or whether the hormone also suppresses the formation of OMI remains to be established.

CONCLUSIONS

We have considered three different *in vitro* models currently in use for the study of ovum maturation, namely, the culture of isolated oocytes, organ culture of follicles explanted intact, and coculture of oocytes with other cellular components of the follicle. These have been applied to material from a number of animal species. The three model systems serve to reveal different aspects of the meiotic process.

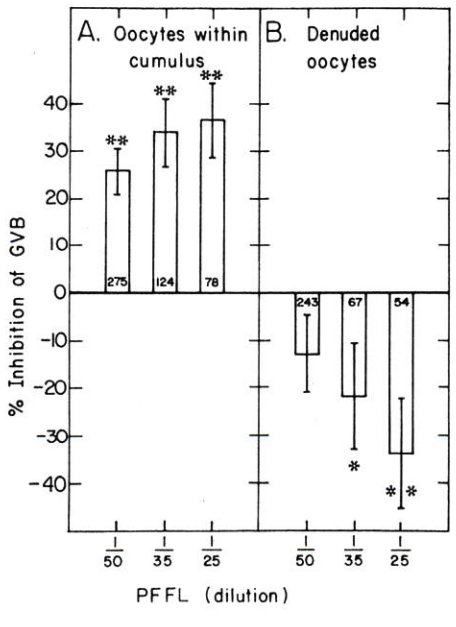

FIG. 3. Role of cumulus cells in OMI action upon maturation of rat oocytes. The oocytes were cultured either within their cumulus or after removal of adherent cells by repeated transfers through thin bore glass capillaries (denuded). Culture was for 6 hr in the presence of the indicated dilution of the low molecular weight fraction of porcine follicular fluid (PFFl). $^{**}p < 0.005$; $^{*}p < 0.05$ vs. appropriate control. (From Lindner et al., ref. 30.)

Isolated oocytes were su
maturation of mouse ooc
and to study changes in
maturation (32,33). Expl
monal factors involved in
mixed culture approach
control of oocyte maturati
systems seems most likel

The hypothesis of follic
first put forward in the 1
recent findings that suppo
follicle by a local factor,
of these experiments were
laboratories encountered
follicular constituents. It
apparently temporal nature
the instability of OMI, the
maintenance of cumulus-o
characterization of OMI a
ment of the physiological

A review covering sim
published by us in *Animal*
L. Martini, published by R
by generous grants (to H.
New York, and the Rocke
Agricultural Research and
was in partial fulfillment o
School for the Ph.D. degr
II Chair of Endocrinology
Science.

1. Albertini, D. F., and Anders during the ontogeny of the *Cell Biol.*, 63:234–250.
2. Amsterdam, A., Josephs, intramembrane particles in f analysis. *J. Cell Sci.*, 21:9
3. Amsterdam, A., Josephs, intrauterine particles of gra
4. Amsterdam, A., Koch, Y., sites for hCG in the preovu
5. Anderson, E., and Albertini cells in the mammalian ova
6. Ayalon, D., Tsafriri, A., I trophin levels in prooestrous *Fertil.*, 31:51–58.

Isolated oocytes were successfully used to demonstrate the role of pyruvate in maturation of mouse oocytes, to establish optimal media for oocyte culture (12), and to study changes in protein synthesis (63) and oxygen consumption during maturation (32,33). Explanted follicle-enclosed oocytes permit study of the hormonal factors involved in the induction and regulation of the meiotic process. The mixed culture approach was adopted to analyze the role of follicle cells in the control of oocyte maturation. Thus, the combined exploitation of a variety of model systems seems most likely to advance our understanding of the meiotic process.

The hypothesis of follicular control of the resumption of the meiotic process was first put forward in the 1930s by Pincus and Enzmann (39). We have reviewed recent findings that support the view that meiosis is prevented in the preovulatory follicle by a local factor, OMI, produced by granulosa cells. Nevertheless, some of these experiments were performed only by several groups, whereas a few other laboratories encountered difficulties in demonstrating OMI-like activity in some follicular constituents. It is possible that these difficulties are in part due to the apparently temporal nature of both oocyte sensitivity to OMI and OMI production, the instability of OMI, the rapid reinitiation of meiosis, and the need for continued maintenance of cumulus-oocyte coupling for keeping meiosis in abeyance. Further characterization of OMI and its purification to homogeneity will allow the assessment of the physiological role of OMI in the control of the meiotic process.

ACKNOWLEDGMENTS

A review covering similar ground from a different perspective was recently published by us in *Animal Models in Human Reproduction*, edited by M. Serio and L. Martini, published by Raven Press, New York (30). These studies were supported by generous grants (to H. R. L.) by the Ford Foundation, the Population Council, New York, and the Rockefeller Foundation and (to A. T.) by BARD U.S.A.-Israel Agricultural Research and Development Fund. The contribution of Mr. Bar-Ami was in partial fulfillment of the requirements of the Weizmann Institute's Graduate School for the Ph.D. degree. H. R. L. is the incumbent of the Adlai E. Stevenson II Chair of Endocrinology and Reproductive Biology at The Weizmann Institute of Science.

REFERENCES

1. Albertini, D. F., and Anderson, E. (1974): The appearance and structure of intercellular connections during the ontogeny of the rabbit ovarian follicle with particular reference to gap junctions. *J. Cell Biol.*, 63:234–250.
2. Amsterdam, A., Josephs, R., Lieberman, M. E., and Lindner, H. R. (1976): Organization of intramembrane particles in freeze-cleaved gap junctions of rat graafian follicles: Optical-diffraction analysis. *J. Cell Sci.*, 21:93–105.
3. Amsterdam, A., Josephs, R., Lieberman, M. E., and Lindner, H. R. (1976): Organization of intrauterine particles of granulosa cell gap junctions in rat ovarian follicles. *J. Cell Biol.*, 63:8a.
4. Amsterdam, A., Koch, Y., Lieberman, M. E., and Lindner, H. R. (1975): Distribution of binding sites for hCG in the preovulatory follicle of the rat. *J. Cell Biol.*, 67:894–900.
5. Anderson, E., and Albertini, D. F. (1976): Gap junction between the ooctye and companion follicle cells in the mammalian ovary. *J. Cell Biol.*, 71:680–686.
6. Ayalon, D., Tsafriri, A., Lindner, H. R., Cordova, T., and Harell, A. (1972): Serum gonadotrophin levels in prooestrous rats in relation to the resumption of meiosis by the oocytes. *J. Reprod. Fertil.*, 31:51–58.

7. Azarnia, R., Larsen, W. J., and Loewenstein, W. R. (1974): The membrane junctions in communicating and noncommunicating cells, their hybrids and segregants. *Proc. Nat. Acad. Sci. USA*, 71:880–884.
8. Baker, T. G. (1979): The control of oogenesis in mammals. In: *Ovarian Follicular Development and Function*, edited by A. R. Midgley and W. A. Sadler, pp. 353–364. Raven Press, New York.
9. Baker, T. G., Hunter, R. H. F., and Neal, P. (1975): Studies of the maintenance of porcine graafian follicles in organ culture. *Experientia*, 31:133–135.
10. Baker, T. G., and Neal, P. (1974): Organ culture of cortical fragments of graafian follicles from human ovaries. *J. Anat.*, 117:361–371.
11. Biggers, J. D. (1972): Metabolism of the oocyte. In: *Oogenesis*, edited by J. D. Biggers and A. W. Schuetz, pp. 241–251. University Park Press, Baltimore.
12. Biggers, J. D., Whittingham, D. G., and Donahue, R. P. (1967): The pattern of energy metabolism in the mouse oocyte and zygote. *Proc. Nat. Acad. Sci. USA*, 58:560–567.
13. Chang, M. C. (1955): The maturation of rabbit oocytes in culture and their maturation, activation, fertilization and subsequent development in fallopian tube. *J. Exp. Zool.*, 128:378–399.
14. Dekel, N., and Kraicer, P. F. (1978): Induction *in vitro* of mucification of rat cumulus oophorus by gonadotrophins and adenosine 3′,5′-monophosphate. *Endocrinology*, 102:1797–1802.
15. Donahue, R. P. (1972): The relation of oocyte maturation to ovulation in mammals. In: *Oogenesis*, edited by J. D. Biggers and A. W. Schuetz, pp. 413–438. University Park Press, Baltimore.
16. Eppig, J. J. (1976): Analysis of mouse oogenesis *in vitro*: Oocyte isolation and the utilization of exogenous energy sources by growing oocytes. *J. Exp. Zool.*, 198:375–382.
17. Eppig, J. J. (1977): Mouse oocyte development *in vitro* with various culture systems. *Dev. Biol.*, 60:371–388.
18. Foote, W. D., and Thibault, C. (1969): Recherches expérimentales sur la maturation *in vitro* des ovocytes de truie et de veau. *Ann. Biol. Anim. Biochim. Biophys.*, 9:329–349.
19. Gilula, N. B., Epstein, M. L., and Beers, W. H. (1978): Cell-to-cell communication and ovulation: A study of the cumulus-oocyte complex. *J. Cell. Biol.*, 78:58–75.
20. Gilula, N. B., Reeves, O. R., and Stienbeck, A. (1972): Metabolic coupling, ionic coupling and cell contacts. *Nature (London)*, 325:262–265.
21. Gwatkin, R. B. L., and Andersen, O. F. (1976): Hamster oocyte maturation *in vitro* inhibition of follicular components. *Life Sci.*, 19:527–536.
22. Hillensjö, T. (1976): Oocyte maturation and glycolysis in isolated pre-ovulatory follicles of PMS-injected immature rats. *Acta Endocrinol.*, 82:809–830.
23. Hillensjö, T., Batta, S. K., Schwartz-Kripner, A., Wentz, A. C., Sulewski, J., and Channing, C. P. (1978): Inhibitory effect of human follicular fluid upon the maturation of porcine oocytes in culture. *J. Clin. Endocrinol. Metab.*, 47:1332–1335.
24. Hillensjö, T., Ekholm, C., and Ahrén, K. (1978): Role of cyclic AMP in oocyte maturation and glycolysis in the preovulatory rat follicle. *Acta Endocrinol.*, 87:377–388.
25. Hillensjö, T., Kripner, A., Pomerantz, S. H., and Channing, C. P. (1979): Action of porcine follicular fluid oocyte maturation inhibitor *in vitro*: Possible role of cumulus cells. In: *Ovarian Follicular and Corpus Luteum Function*, edited by C. P. Channing, J. M. Marsh, and W. J. Sadler, pp. 283–290. Plenum Press, New York.
26. Hunter, R. H. F., Cook, B., and Baker, T. G. (1976): Dissociation of response to injected gonadotrophin between the graafian follicle and oocyte in pig. *Nature*, 260:156–158.
27. Jagiello, G., Graffeo, J., Ducayen, M., and Prosser, R. (1977): Further studies of inhibitors of *in vitro* mammalian oocyte maturation. *Fertil. Steril.*, 28:476–481.
28. Lieberman, M. E., Tsafriri, A., Bauminger, S., Collins, W. P., Ahrén, K., and Lindner, H. R. (1976): Oocyte meiosis in cultured rat follicles during inhibition of steroidogenesis. *Acta Endocrinol.*, 83:151–157.
29. Lindner, H. R., Amsterdam, A., Salomon, Y., Tsafriri, A., Nimrod, A., Lamprecht, S. A., Zor, U., and Koch, Y. (1977): Intraovarian factors in ovulation: Determinants of follicular response to gonadotrophins. *J. Reprod. Fertil.*, 51:215–235.
30. Lindner, H. R., Bar-Ami, S., and Tsafriri, A. (1980): Model systems for studying oocyte maturation. In: *Animal Models in Human Reproduction*, edited by M. Serio and L. Martini, pp. 65–85. Raven Press, New York.
31. Lindner, H. R., Tsafriri, A., Lieberman, M. E., Zor, U., Koch, Y., Bauminger, S., and Barnea, A. (1974): Gonadotrophin action on cultured graafian follicles: Induction of maturation division of the mammalian oocyte and differentiation of the luteal cell. In: *Recent Progress in Hormone Research*, edited by R. O. Greep, Vol. 30, pp. 79–138. Academic Press, New York.

32. Magnusson, C. (1980): Rc
Res., 3:133–140.
33. Magnusson, C., Hillensjö
sumption of maturing rat
34. Mangia, F., and Canipari
oocytes. In: *Development i*
Publishing Co., Amsterda
35. Moor, R. M., Polze, C., ar
and fertilization of mamm
36. Moor, R. M., Smith, M. W
between oocytes and cumi
37. Moor, R. M., and Warne
Control of Ovulation, edit
ming, pp. 159–176. Butte
38. Neal, P., and Baker, T. G
pregnant mare's serum g
critical time intervals. *J. I*
39. Pincus, G., and Enzmann
and *in vitro*. *J. Exp. Med*
40. Schuetz, A. W. (1967): C
factor and ovarian factor.
41. Schuetz, A. W. (1974): R
42. Schuetz, A. W. (1979): Th
to oocyte maturation. In:
ning, J. M. Marsh, and W
43. Sheridan, J. D. (1971): D
bryonic cells. *Dev. Biol.*,
44. Smith, L. D. (1975): Mo
Development, edited by R
45. Soupart, R. (1974): Fecon
J. Med. Bruxelles, 54:47
46. Stone, S. L., Pomerantz,
oocyte maturation from p
action. *Biol. Reprod.*, 19:
47. Szöllösi, D., Gérard, M.,
Corona cell-oocyte relatio
48. Thibault, C., and Gérard,
nucleus mâle dans l'ovoc
49. Thibault, C., Gérard, M.
oocyte maturation. *J. Rep*
50. Thibault, C., Gerard, M.,
oocyte maturation *in vitro*
Biol., 1:233–240 (Karger
51. Tsafriri, A., Lindner, H.
division in follicle-enclos
Fertil., 31:39–50.
52. Tsafriri, A., Lieberman, M
of ovum maturation and o
Role of RNA and protein
53. Tsafriri, A., and Channin
fluid upon porcine oocyte
54. Tsafriri, A., Lieberman, M
H. R. (1976): Capacity o
and steroidogenesis in gra
Endocrinology, 98:655–6
55. Tsafriri, A., Pomerantz, S
In: *Ovulation in the Hum*
Academic Press, New Yo

32. Magnusson, C. (1980): Role of cumulus cells for rat oocyte maturation and metabolism. *Gamete Res.*, 3:133–140.
33. Magnusson, C., Hillensjö, T., Tsafriri, A., Hultborn, R., and Ahrén, K. (1977): Oxygen consumption of maturing rat oocytes. *Biol. Reprod.*, 17:9–15.
34. Mangia, F., and Canipari, R. (1977): Biochemistry of growth and maturation in mammalian oocytes. In: *Development in Mammals*, edited by M. H. Johnson, vol. II, pp. 1–29. North Holland Publishing Co., Amsterdam.
35. Moor, R. M., Polze, C., and Willadsen, S. M. (1980): Effect of follicular steroids on the maturation and fertilization of mammalian oocytes. *J. Embryol. Exp. Morphol.*, 56:319–335.
36. Moor, R. M., Smith, M. W., and Dawson, R. M. C. (1980): Measurement of intercellular coupling between oocytes and cumulus cells using intracellular markers. *Exp. Cell Res.*, 126:15–29.
37. Moor, R. M., and Warness, G. M. (1979): Regulation of oocyte maturation in mammals. In: *Control of Ovulation*, edited by D. B. Crighton, G. R. Foxcroft, N. B. Haynes, and G. E. Lamming, pp. 159–176. Butterworths, London.
38. Neal, P., and Baker, T. G. (1973): Response of mouse ovaries to *in vivo* and in organ culture to pregnant mare's serum gonadotrophin and human chorionic gonadotrophin. I. Examination of critical time intervals. *J. Reprod. Fertil.*, 33:399–410.
39. Pincus, G., and Enzmann, E. V. (1935): The comparative behaviour of mammalian eggs *in vivo* and *in vitro*. *J. Exp. Med.*, 62:655–675.
40. Schuetz, A. W. (1967): Chemical properties and physiological actions of a starfish radial nerve factor and ovarian factor. *Gen. Comp. Endocrinol.*, 12:209–221.
41. Schuetz, A. W. (1974): Role of hormones in oocyte maturation. *Biol. Reprod.*, 10:150–178.
42. Schuetz, A. W. (1979): The somatic-germ cell complex: Interactions and transformation in relation to oocyte maturation. In: *Ovarian Follicular and Corpus Luteum Function*, edited by C. P. Channing, J. M. Marsh, and W. J. Sadler, pp. 307–314. Plenum Press, New York.
43. Sheridan, J. D. (1971): Dye movement and low resistance junctions between reaggregated embryonic cells. *Dev. Biol.*, 26:627–636.
44. Smith, L. D. (1975): Molecular events during oocyte maturation. In: *Biochemistry of Animal Development*, edited by R. Weber, vol. III, pp. 1–46. Academic Press, San Francisco.
45. Soupart, R. (1974): Fecondation humaine expérimentale etat de la question et perspectives d'avenir. *J. Med. Bruxelles*, 54:473–499.
46. Stone, S. L., Pomerantz, S. H., Schwartz-Kripner, A., and Channing, C. P. (1978): Inhibition of oocyte maturation from porcine follicular fluid: Further purification and evidence for reversible action. *Biol. Reprod.*, 19:585–592.
47. Szöllösi, D., Gérard, M., Ménézo, Y., and Thibault, C. (1978): Permeability of ovarian follicle: Corona cell-oocyte relationship in mammals. *Ann. Biol. Anim. Biochim. Biophys.*, 18:511–521.
48. Thibault, C., and Gérard, M. (1970): Facteur cytoplasmique nécessaire à la formation due pronucleus mâle dans l'ovocyte de lapine. *C. R. Acad. Sci.*, 270:2025–2026.
49. Thibault, C., Gérard, M., and Ménézo, Y. (1975): Preovulatory and ovulatory mechanisms in oocyte maturation. *J. Reprod. Fertil.*, 45:605–610.
50. Thibault, C., Gerard, M., and Ménézo, Y. (1976): Nuclear and cytoplasmic aspects of mammalian oocyte maturation *in vitro* in relation to follicle size and fertilization. *Sperm Action Prog. Reprod. Biol.*, 1:233–240 (Karger, Basel).
51. Tsafriri, A., Lindner, H. R., Zor, U., and Lamprecht, S. A. (1972): *In vitro* induction of meiotic division in follicle-enclosed rat oocytes by LH, cyclic AMP and prostaglandin E_2. *J. Reprod. Fertil.*, 31:39–50.
52. Tsafriri, A., Lieberman, M. E., Barnea, A., Bauminger, S., and Lindner, H. R. (1973): Induction of ovum maturation and of steroidogenesis in the isolated graafian follicle by luteinizing hormone: Role of RNA and protein synthesis. *Endocrinology*, 93:1378–1386.
53. Tsafriri, A., and Channing, C. P. (1975): An inhibitory influence of granulosa cells and follicular fluid upon porcine oocyte meiosis *in vitro*. *Endocrinology*, 96:922–927.
54. Tsafriri, A., Lieberman, M. E., Koch, Y., Bauminger, S., Chobsieng, P., Zor, U., and Lindner, H. R. (1976): Capacity of immunologically purified FSH to stimulate cyclic AMP accumulation and steroidogenesis in graafian follicles and to induce ovum maturation and ovulation in the rat. *Endocrinology*, 98:655–661.
55. Tsafriri, A., Pomerantz, S. H., and Channing, C. P. (1976): Follicular control of oocyte maturation. In: *Ovulation in the Human*, edited by P. G. Grosignani and D. R. Mishel, vol. 8, pp. 31–39. Academic Press, New York.

56. Tsafriri, A., Pomerantz, S. H., and Channing, C. P. (1976): Inhibition of oocyte maturation by porcine follicular fluid: Partial characterization of the inhibitor. *Biol. Reprod.*, 14:511–516.
57. Tsafriri, A., Channing, C. P., Pomerantz, S. H., and Lindner, H. R. (1977): Inhibition of maturation of isolated rat oocytes by porcine follicular fluid. *J. Endocrinol.*, 75:285–291.
58. Tsafriri, A. (1978): Oocyte maturation in mammals. In: *The Vertebrate Ovary*, edited by R. E. Jones, pp. 409–442. Plenum Press, New York.
59. Tsafriri, A. (1979): Mammalian oocyte maturation: Model systems and their physiological relevance. In: *Ovarian Follicular and Corpus Luteum Function*, edited by C. P. Channing, J. Marsh, and W. J. Sadler, pp. 269–281. Plenum Press, New York.
60. Tsafriri, A., Weinstein, Y., Bar-Ami, S., Channing, C. P., Pomerantz, S. H., and Lindner, H. R. (1979): The control of meiotic maturation of the rat oocyte. In: *Research on Steroids*, edited by E. Conti, vol. 8, pp. 193–198. Academic Press, London.
61. Vermeiden, J. P. W., and Zeilmaker, G. M. (1974): Relationship between maturation division, ovulation and luteinization in the female rat. *Endocrinology*, 95:341–351.
62. Wasserman, P. M., and Letourneau, G. E. (1976): RNA synthesis in fully-grown mouse oocytes. *Nature*, 261:73–74.
63. Wassarman, P. M., Schultz, R. M., Letourneau, G. E., LaMarca, M. J., Josefowicz, W. J., and Bleil, J. D. (1979): Meiotic maturation of mouse oocytes *in vitro*. In: *Ovarian Follicular and Corpus Luteum Function*, edited by C. P. Channing, J. M. Marsh, and W. J. Sadler, pp. 251–267. Plenum Press, New York.

This paper was completed in 1981.

Structural Ch and H

Department of Obstetrics c

The fundamental funct progresses through a ser physiologic and morpholo have appeared in the liter tional characteristic. Such granulosa cells, the numb antrum, and even the ove

A primary follicle is c cells. Depending upon th follicle, the actual morp although multilayered, ha A tertiary, graafian, or pr follicles are categorized a preovulatory follicles. Mo iologically and functiona functionally than the earl of tertiary follicles will b

Follicular development out the female's reproduct on the interactions betwee roles of the ovary.

This chapter presents a follicle from the primordi description of the hormo development (or maturati is a concomitant develop events may occur simulta

TI

The culmination of feta or primary follicle (34).