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Oocyte Maturation in Some Vertebrates ADN Mammals

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1. INTRODUCTION

The meiotic process in mammals commences during fetal life and usually becomes arrested shortly after birth (First meiotic arrest). This arrest stage continues to until puberty or until animal shows estrus cycle. The meiotic process is resumed again a few hours to 20 hours prior rto ovulation following the preovulatory gonadotropin surge depending on the species of mammalian species, meiosis is resumed within the mature, preovulatory follicle(s). At ovulation, a number of oocytes, depending on the species, are released at the metaphase of the second meiotic division (M II). However, in a few, mammals, dictyate oocytes are normally ovulated and maturation takes place in the oviduct (dog, Evans and Cole, 1931; fox, Pearson and Enders, 1943; horse, Hamilton and Day, 1945). In a group of insectivores (subfamily Centitidae) fertilization takes place within the ovarian follicle, and, consequently, pronuclear zygotes are ovulated (Strauss, 1938).

Any developmental change during the entire gametogenic process may be regarded as "oocyte maturation". In this minireview, however, the use of this term will be restricted to the preocess, i.e., to the preogress of the dictyate oocyte to the metlphase of the second meiotic division. I will discuss the oocyte during the time interval between the diplotene and second meiotic arrest, and introduce several hypothsis on the mechanism of oocyte maturation and its inhibition in vivo and in vitro. Before the introduction of several hypothesis I will briefly describe some of the morphological features and the physiological and biochemical activities of the dictyate oocyte in the first part, and then introduce some new experimental models elaborating the mechanism controlling oocyte maturation. To limit the length of the review, I have restricted the period covered to 1975-1982, although some earlier key papers have been included. Pre-1975 references can be found from recent reviews (Moor & Warnes, 1979; Tsafriri, 1978; Tsafriri et al., 1982).

2. THE DICTYATE OOCYTE

Before or just after birth the oocyte persists at the dictyate stage for a very long period; in human, this period may reach 40 years or more. Although meiotic process is suspended, oocyte growth and synthetic activity take place during this time period. This period hence cannot regarded as a resting stage. As this quiescent stage, the oocyte is a spherical cell enclosed within unilaminar follicles (primordial follicle). The plasma membrane (oolemma) is smooth

over most of the cell surface. It is closely apposed to the cell membranes of a single layer of flattened follicle cells which are resting on the basement membrane (Zamboni, 1976). The oocyte consists of a large vesicular nucleus (germinal vesicle) and ooplasm. The oocyte nucleus is large and spheroided with one or more nucleoli present. The nucleoli appear either as compact structures with many electron-lucent cavities or as an open network of nucleonema threads (Baker & Franchi, 1972). The chromatin organization within the ooplasm differs in mammalian species. In redent oocytes it is dispersed uniformly, whereas in primate and most other mammalian oocytes it shows some degree of condensation. In human and monkey oocytes, such chromosomes posses an electron-dense core enveloped by fibrillar material in the form of lateral loops (Baker & Franchi, 1972). The rodent dictyate oocyte represents an extreme case of the fibrillar elements of the lampbrush chromosomes (Baker et al., 1969). It was also shown that oocytes with compact lampbrush loops (e.g., human, monkey and guinea pig) are more resistant to radiation than with highly diffuse chromosomes (mouse and rat) (Baker, 1973). The majority of cytoplasmic organelles, such as mitochondria, prominent Golgi compex, elements of the endoplasmic reticulum, and lysosomes, are condensed into a crescentlike region around the nucleus. In the past this areas was referred to as "Balbiani's vitelline body" or "Yolk nuleus body". Mitochondria have a matrix of low electron opacity and a few cristae, which are shelflike, archlike or parallel to the outer mitochondrial membrane (Weakley, 1976). The Golgi complex is very prominent and consist of vesicular and tubular profiles close to the nucleus. Annulate lamellae are observed only in human and chimpanzee. The possible origin and function of this structure have been reviewed (Wischnistzer, 1970). The distribution of cytoplasmic organelles and their number change considerably in growing oocytes. The originally single Golgi complex subdivides into multiple aggregates which migrate to the cell periphery. These changes seem to be closely related to the synthesis and formation of cortical granules (Zamboni, 1970). The number of mitochondria increases considerably, and they become uniformly distributed throughout the ooplasm. Their size increases and they show great diversity in structure. Mitochondrial size and complexity of cristae were maximal at the beginning of the stage of rapid oocyte growth and therafter declined (after antrum formation the mitochondria became rounded and cristae were seldom seen (Weakley, 1976). In the ooplasm of rodent oocytes, highly ordered arrays of parallel chains or latticelike structure were observed. Zamboni (1970) suggested that they represent lattices of fibrillar RNA. With the results of enzyme experiments, these lattices are shown to be highly ordered ribosomes in an inactive storage form. The presence of actin and mhosin in the circumference of large rat oocytes (80-120 um in diameter) has been demonstrated by staining of cryostat section with antibodies to smooth muscle myosin and actin (Burkholder, 1971). On the other hand, only slight staining was observable in small (30-40 um) oocytes (Amsterdam, et al., 1977).

3. ZONA PELLUCIDA FORMATION

The zona pellucida appears first in follicles with a single layer of cuboidal granulosa cells, when the early stage of oocyte growth has been completed. Initially, the zona pellucida appears as a fibrillar materials situated in lacunae between adjacent granulosa cells and the oocytes surface. These areas zona pellucida enlarge and coalesce until a continuous layer formed. The zona pellucida consists of mucopolysaccharide and trypsin-digestable material which seems to be the only specific antigen in the ovary. Recently active studies are being made to develop a contraceptive drug, anti-zona pellucida by Sacco

(1978 & 1979) and Dunbar et al. (1980 & 1981). Formation of the zona pellucida bring about the separation of the oocyte and follicle cells. Simultaneously, both the oocyte and the surrounding follicle cells facing the oocyte develop cytoplasmic projections, microvilli. The coplasma develops short microvilli, and the number of oocyte microvilli increase with follicular growth (Zamboni, 1976). Recently, many of the junctions between the oocyte and granulosa cells were identified as gap junction (Amsterdam et al, 1976). Gap junctions were identified even in primordial follicles. However, their abundance increases with follicular development (Anderson and Albertini, 1976). Substances may enter intc the oocyte by membrane diffusion, by active pinocytosis or endocytosis, and even by phagocytosis (Zamboni, 1976). Gap junctions have been shown to be highly permeable to small molecules and to facilitate electrical coupling between cells (Gilula et al., 1978; Moor et al, 1980; Dekel et al., 1981).

4. BIOCHEMICAL STUDIES

Very few biochemical studies have been performed on oocytes in RNA, enzyme activity and metabolism of few studies done, RNA synthesis was measured auto-radiographically by the uptake of labeled nucleotides. These studies showed that RNA synthesis increased during bocyte growth, reaches a peak in fully grown oocytes (Moore et al., 1974). However, a low rate of RNA synthesis remains until the initiation of meiotic maturation (Rodman & Bachvarova, 1976; Wasserman & Letourneau, 1976; Crozet et al, 1981). The bulk of the RNA label in growing oocytes appears to be ribosomal RNA and transfer RNA, whereas low labels of heterogenous RNA have been demonstrated (Rodman and Bachvarova, 1976; Crozet et al., 1981), which is estimated 20% by sucrose gradient sedimentation (Jahn et al., 1976). It was shown that the RNA of growing oocytes all stages as well as of preovulatory oocytes if unusally stable and these RNA synthesized during growth at least during very early embryogenesis. A few studies on enzyme activity of and metabolic requirement of ovarian oocytes have been done; glucose-6-phosphate dehydrogenase (G6PD), lactate dehydrogenase (LDH), succinate dehydrogenase (SDH) (Vivarelli et al., 1976) and glycolysis (Hillensjo et al., 1978). Carbon dioxide evolution from pyruvate increased logarithmically with oocyte growth and plateaued when oocyte reached to maximum diameter (Eppig, 1976).

5. GONADOTROPIN AND OOCYTE MATURATION IN VIVO

Mammalian oocyte enter upon the process of meiosis during fetal life and nuclear development subsequently becomes arrested at the diplotene stage close to the time of birth. Meiosis dose not resume until the onset of preovulatory maturation in the mature Graafian (antral) follicle. During the period of arrested development the oocyte grows in size and gains more complex cytoplasmic organelles, and the follicle in which it resides develops from a single layer of granulosa cells (primary follicle) into a structure consisting of a great many cells layer (theca interna, theca externa, murine granulosa cell layer, and cumulus cells layers surround oocyte except the antrum) and a cavity (antrum) filled with follicular fluid related in composition to the blood plasma. During the growth phase, nucleus size becomes much bigger and forms a large nucleus "germinal vesicle (GV)". The initiation of preovulatory maturation within follicles in mammalian ovaries is related to at least two major factors; 1) the size of the follicle in which the oocyte resides; and 2) the specific "surge" of gonadotropic hormones which occurs prior to ovulation. Only those oocytes which are enclosed in normal Graafian follicles are, therefore, capable of responding to the so-called "LH-

surge" by proceeding from the germinal vesicle or diplotene stage to metaphase of the second meiotic division: those in pre-antral follicles persist at the GV stage, while those in follicles with small antra become "blocked" at metaphase I. (Baker & Neal, 1972). However, the results of numerous experiments on intact mammals have provided little precise information on the mode of action of gonadotropins in promoting maturation of the oocyte and ovulation. It should be point out here that pituitary hormones are required for inducing resumption of meiosis only when the oocyte resides within its follicles. When oocytes are removed from their follicles and placed in appropriate chemically defined media, resumption of meiosis occurs spontaneously, although the stage of meiosis attained by those oocyte is governed by the size of the follicle from which they were obtained (Moore & Lintern-Moore, 1974).

It is only those oocytes recovered from Graafian follicles of normal mature size which seemingly can reach metaphase II. (Cross & Brinster, 1971). It is becoming apparent that both the oocyte and the somatic cells of the follicle have to undergo forms of maturation in response to hormones before ovulation can occur. In response to LH, the follicular cells produce steroid hormones and the maturation. Changes also occur in the nature of the cumulus cells and in the pattern and concentration of hormones in follicular fluid (McNatty et al., 1974). These events can be dramatically shown from experiments on intact pig. When domestic pigs are injected with HCG (human chorionic gonadotropin) on Day 20 of a 21-day estrous cycle, ovulation occurs 42 hrs later and the chromosomes are arranged on the spindle at 2nd metaphase. In these animals, the sequence of meiotic events and the alternations in ovarian steroid concentrations are similar to those occuring spontaneously at estrous in uninjected control (Hunter et al., 1976). By contrast, animals injected with the same dose of HCG on Day 17 of the cycle also undergo ovulation, but the oocyte (recovered from the Fallopian tubes) remain "blocked" still at the diploten stage. Following insemination, these oocytes become penetrated by large numbers of spermatozoa which fail to undergo significant swelling of the sperm head in preparation for the formation of the male pronucleus. Thus, the majority of oocytes which are ovulated in response to treatment with HCG Day 17 show no block to polyspermy, they do not contain available male pronucleus growth factos (MPGF) (Thibault et al., 1975), and the level of oestrogen detected in follicular fluid are only 25% of those of the control. These results clearly show that there are a number of cellular and endocrine events occuring simulataneously during preovulatory maturation, one of which is the resumption of meiosis of the oocyte.

What makes it difficult in interpretation of oocyte maturation in vivo system is that there are so many components already detected and still undetected in the follicular fluid and so much changes in compositions as well as in the events in the follicle, and so much different mechanisms depending on the mammalian species (Neal & Baker, 1975; Hay & Moor, 1975; Baker & Neal, 1974). Human follicular fluid contains large amount of FSH, LH, prolactin, estrogens, etc., and thus it is somewhat suprising that human oocytes do not undergo spontaneously meiotic maturation (McNatty et al., 1974). It would seem that there is an inhibitor substance (Silverman et al., 1982) which either prevent the oocyte responding to those hormone, or which effectively "binds" the hormones such that they are no larger available to the oocyte. The results of recent studies certainly support the latter concept (diZierega & Hodgen, 1980 and 1981). Along with this dominant follicle theory in the primate (diZierega & Hodgen, 1981), prolactin seems to be implicated in this effect. McNatty et al. (1974) found that the only dramatic change in the hormonal composi-

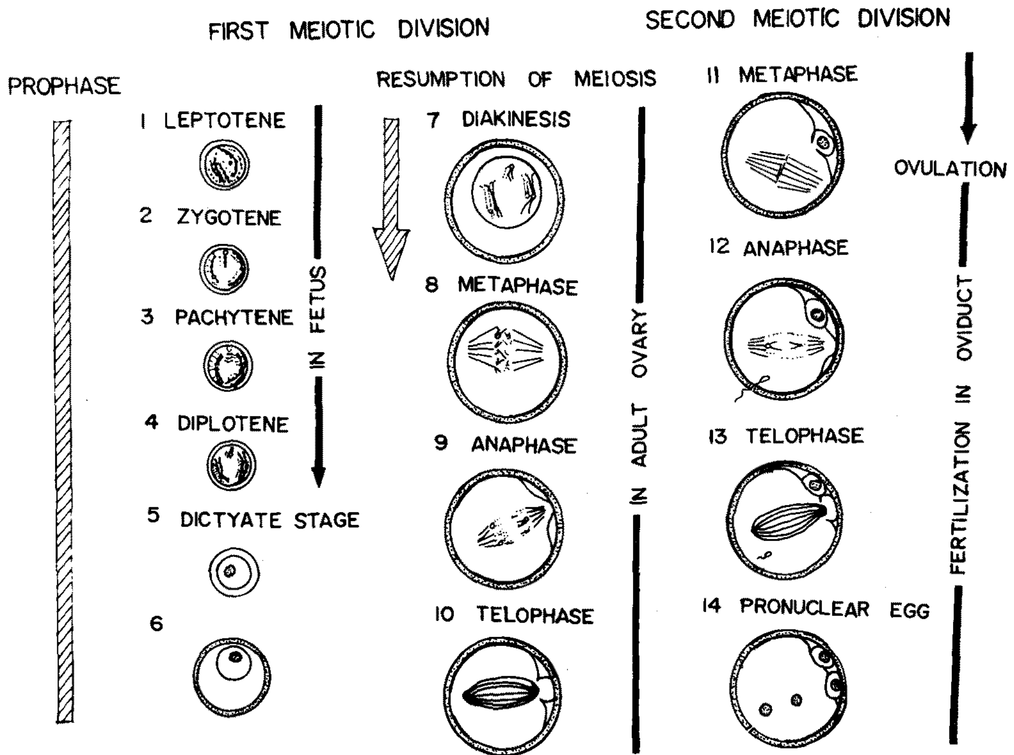

Figure 1. Oocyte meiosis

For simplicity, only three pairs of chromosomes are dipicted. 1-4, prophase stages of the first meiotic division which occur is most mammals during fetal life. For detailed description of these stage, see "The vertebrate ovary" (edited by Ricards E. Jones, Plenum publishing Corporation, 1978). At zygotene (2) the homologous maternal and paternal chromosomes begin to pair, and at pachytene, each homologous cleaves longitudinally to form two sister chromatids, so that each bivalent forms a tetrad. During this stage, interchange of genetic material between maternal and parternal chromatids occurs by crossing over. At diplotene (4) the chromosomes begun to separate, remaining united at points of interchange, the chiasmata. The meiotic process is arrested at this stage (first meiotic arrest), and the oocyte enters the dictyate stage. When meiosis is resumed, the first maturation division is completed (7-11). Ovulation occurs usually at the metaphase I stage (II), and the second meiotic division (12-14) takes place in the oviduct only following sperm penetration.

tion of human follicular fluid during the menstrual cycle was a sharp fall in the level of prolatin at the time of the mid-cycle LH surge. If porlactin, either directly or indirectly, prevents the oocyte from responding tp the FSH and LH in follicular fluid, antiprolactin antiserum would be expected to induce the resumption of meiosis in follicular oocytes. Yes, it is in both in vivo

system of organ culture of the follicle. Antiserum specifically raised in rabbits against prolactin was by far the most efficient agent in inducing the resumption of meiosis in follicle enclosed oocytes in vitro. It remains unclear why only a portion of the oocytes respond to treatment with the antiserum, since the majority would be expected to be ovulated at metaphase II if they had remained in the pig (Baker et al., 1975). Immediately before ovulation some oocytes of fully grown preovulatory follicles resume meiosis and undergo a series of structural and synthetic changes resulting in the maturation of the oocyte and ovulation. These changes include a relocation of intracellular organelles and structural changes (Masui & Clarke, 1979; Moor et al., 1981), a cessation of RNA synthesis (Rodman & Bachvarova, 1976) and specific qualitative and quantitative changes in polypeptide synthesis (Golbus & Stein, 1976; Van Blerkom & McGaughey. 1978; Moor & Smith, 1979). Changes in the oocyte membrane parallel those intracellular events. Gap junctional complexes between the oolemma and surrounding follicle cells become disrupted (Anderson & Albertini, 1976; Gilula et al., 1978), whereas others are still suggesting that complete interconnections between the oocyte and the cumulus mass until the postovulatory process before entering the oviduct (Dekel et al., 1978). However, evidence exists that gap junctions between the fully grown dictyate oocyte and its attached cumulus cells are functional. Cumulus cells and oocytes are ionically coupled and iontophoretically injected fluorescein dye is transferred between the oocyte and cumulus cells. A coordinate decrease in the number of gap functional contacts and extent of ionic coupling is observed as ovulation approaches. After ovulation, cumulus cells surround the oocyte but cumulus cell microvilli have retracted from the oocyte surface and ionic coupling is no longer observed (Gilula et al., 1978). Results from several previous studies suggested that naturally occuring matabolites pass from cumulus cells via gap junctions to the fully grown oocyte (Wasserman & Letourneau, 1976; Moor et al., 1980; Heller & Schultz, 1980; Heller et al., 1981).

Apart from these views, recent studies have shown that in association with LH-inducing luteinization of antral follicles, there is a marked loss of LH and FSH receptor content and that this loss occurs by mechanisms other than by occupation of binding sites (Richards et al., 1976; Rao et al., 1977). Similar effects of ovulatory doses of LH on other target tissue (Conti et al., 1976; Hsueh et al., 1976) have indicated that LH may act generally on gonadal cells to reduce its own receptor and to decrease target cell responsiveness. Therefore, it would seem reasonable to propose that LH surge might act on some preantral follicles to cause a loss of gonadotropic hormone (FSH and/or LH) receptors, to decrease responsiveness of these follicules to hormonal stimulaton, and possibly to initiate atresia. Along with these changes, it is also known that gonadotropins or coupluation before ovulation, stimulate steroidogenesis in the follicle. It is also known that an ovulatory surge of LH causes a conspicuous change in the ovarian vasculature. Within a few minutes after gonadotropin stimulation there is a significant increase in ovarian circulation (Lee & Novy, 1978). This elevation in ovarian blood flow lasts for at least 9 hr, (in rabbit) with a distinct peak occuring 4 hr after gonadotropin stimulation (Blasco et al., 1975). This increase in circulation with the hyperemic condition that develops in follicles which respond to LH (Cherney et al., 1975). Along with vasodilatation, there is an increase in vascular permeability in the follicles. These vascular changes cause the follicle to become edematous, a condition which persists through the time of follicular rupture and erythrocytes permeate the walls of the vessels (theca interna) and form petechiae in the interstitial spaces of the follicle (Espey, 1978). This is why hemolysed follicle can be seen in most mam-

Proposed model for oocyte maturation

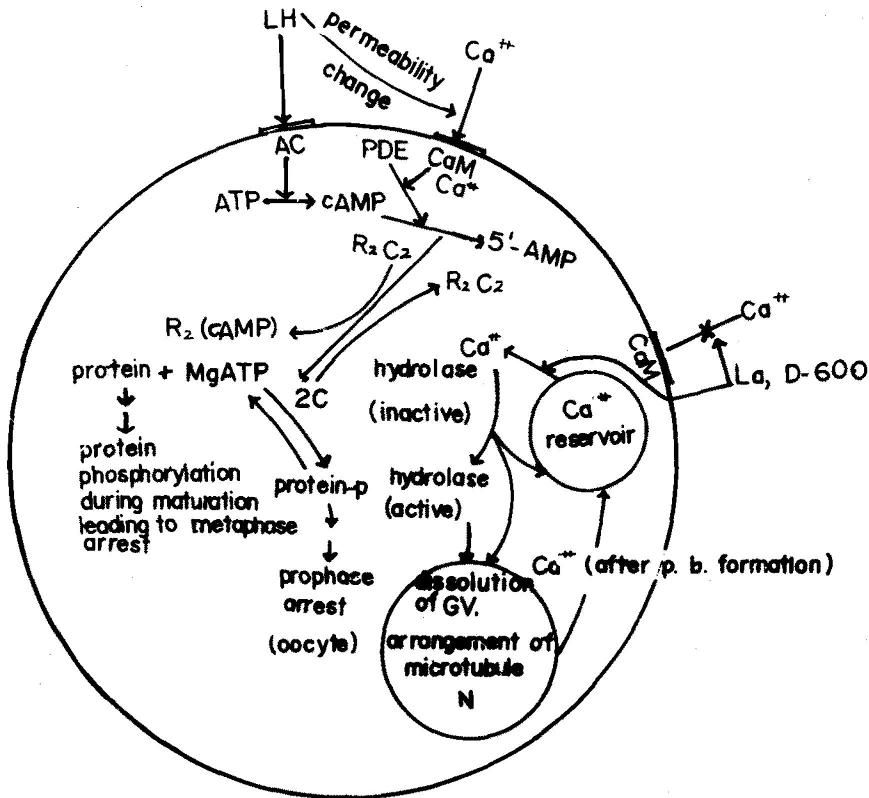

Figure 2. Proposed model for the maturation of the mammalian oocytes in general

Oocytes are maintained in the diplotene stage (prophase arrest, or the first meiotic arrest) by a protein phosphorylated (protein-P) by the catalytic subunit of cyclic-AMP-dependent protein kinase (C). After progesterone stimulation, free calcium concentrations increase, activate the Ca^{++}-dependent regulatory sununit of phosphodiesterase (PDE), decrease intracellular cyclic AMP levels and thereby decrease the catalytic subunit of C. by promoting the formation of the inactive unit R202.

Phosphorylation is inhibited and the fall in the concentration of the inhibitory phosphoprotein permits resumption of meiosis. Additional protein phosphorylation occurs during maturation and results in the second meiotic block metaphase II.

malian obary. With increase in ovarian circulation, the dependency of ovulation on vascular hydrostatic pressure may explain why the intentional "bleeding" of rabbits later than 1 hr after coitus will inhibit ovulation (Westman & Jacobson, 1936). This effect of LH on the ovarian vasculature can be mimicked by histamine, but not by FSH or serotonin. This vascular

layer is occasionally absent from the stigma just before rupture. Thus, LH induces ovarian hyperemia, vasodilation, edema and even extravasation of blood in ovulatory follicles. Along with vasculature, increased fluxes of amino acids are under gonadotropic regulation (Moor & Smith, 1979).

6. MODEL FOR MEIOSIS RESUMPTION STUDIES IN VITRO.

Studies on maturation of mammalian oocytes in vitro were pioneered by Pincus & Engman (1935) by liberating the rabbit follicular oocytes from their follicles, which resumed meiosis spontaneously and mature in culture without any requirements for hormonal stimulation. Up to date, three major models have been used by many investigators.

a) Follicle or whole ovary culture.
b) Oocyte culture.
c) Co-culture of granulosa cells or other type and oocytes.

a) Follicle culture

A various size of follicles can be isolated from the ovaries which were separated from the animal before preovulatory LH surge. Once the preovulatory size of follicle is stimulated by the gonadotropin de novo the oocytes of the follicle even in vitro system resume meiosis in the follicle. Thus, follicle isolation should be done before gonadotropin surge, and culture in appropriate medium in vitro by the addition of various hormones, drug, antiserum or antihormone, etc. After constant hours of culture, the oocytes should be liberated from the follicles, fixed and stained. In preparation of culture media, a few important factors should considered in 1) osolality 2) pH 3) culture dish or device and incubator set-up. Depending on the animal species, there is an optimum range of osmolality of the culture medium (Bae & Foote, 1980; Gwatkin, 1976; McGaughey, 1977). Another important point should be emphasized in oocyte reading after ataining. Without staining, degenerating oocytes with a delicate configuration of even germinal vesicle staged oocyte can not be exactly read, according to experiences of many year's oocyte reading. However, the follicles culture model has been developed to mimic ovarian culture but minimize th defects of ovarian culture.

i) Oocyte maturation in rodents

When large preovulatory follicle of adult cycling rats are explanted on the day of proestrous, the behavior of the oocytes in a hormone-free medium depends on the time of follicle isolation. OOcytes explanted before 14:00 PM., that is, before the preovulatory LH surge, remain in the dictyate stage throughout a 24 hr culture period. On the other hand, isolation of follicle later in the same day, following the endogenous LH surge, results in the resumption of meiosis in the majority of such follicle-enclosed oocytes (Ayalon et al., 1972). A similar relationship between the maturation of follicle-enclosed oocytes in culture and LH secretion was found in prepubertal rats treated with PMSG (Hillensjo, 1976). Resumption of meiosis can be triggered in rat follicles explanted on the day of proestrous by LH, HCG, immunochemically pure FSH or PGE (Shen et al., 1978). All of these hormones induce an immediate rise in cyclic AMP accumulation, even though a transient rise (Moor & Heslop, 1981) and a somewhat later increase in progesterone synthesis (Lindner et al., 1974; Moor et al., 1980; Moor, 1978). Gonadotropin-induced maturation of follicleenclosed oobytes was achieved in rabbit (Thibault & Gerad, 1973), calf and macaque monkey (Thibault et al., 1975) and hamster (Gwatkin & Anderson, 1976). There can be little doubt that FSH can induce the resumption of meiosis in follicular oocytes, although in vivo the concentration of this hormone compared to LH may be too low to be effective. Indeed, FSH appears to be effec-

tive than LH when added to mouse follicles in organ culture (Neal & Baker, 1975). By contrast, treatment of LH with an anti-serum to the β- subunit of LH prevents the action of this hormone on rat oocyte maturation. Whereas treatment of FSH with the antiserum has no effect (Lindner et al., 1974; Tsafriri et al., 1976). The demonstrated capability of FSH to induce oocyte maturation and ovulation seems to be regarded as merely a pharmacological effect. Additional studies are needed in order to determine whether this is true in other mammalian species as well. A number of substances can seemingly mimic the action of gonadotropins on oocyte maturation, including prostaglandin E2 (PGME 2), cyclic AMP and PGF 2 (although the latter probably only facilitates oocyte maturation at pharmacological doses (Neal et al., 1976; Neal et al., 1975) and induces GVBD even in denuded mouse oocytes, but blocks at the telophase (Cho, 1976).

ii) Oocyte maturation in ungulates and primates

Neither FSH nor LH seem to be able directly to induce the resumption of oocyte maturation when sheep (Hay & Moor, 1975; Moor et al., 1981); porcine (Baker et al., 1975) and human (Baker & Neal, 1974) Graafian follicles are maintained in organ culture. There can be little, however, that LH would normally induce the resumption of meiosis and steroidogenesis in the follicle in vivo, indicating that the mechanism of action of gonadotropins in these species may be slightly different from that of rodents. The lack of action of LH on the follicle in culture might be due to an inadequate number of binding sites since PGE 2 (which, like LH, acts on the adenyl cyclase system) can induce the resumption of meiosis in follicle-enclosed pig ocytes (Baker et al., 1975). The action of PGF 2 on this process is more difficult to explain but may be a pharmacological effect as it seems to be in rodents.

Culture of explanted Graafian follicles made possible, for the first time, the study of in vitro hormonal effects on the resumption of meiosis. However, despite the many studies directed forwards the nuclear, cytoplasmic and membrane changes associated with oocyte maturation, very little is known about the intrafollicular mechanisms involved in the regulation of these events. That the follicles has a controlling influence on oocyte maturation and embryonic development is clear from experiments which that oocytes matured in vitro within the follicle undergo full maturation and after fertilization develops into viable offsprings (Eppig, 1978; Trounson et al., 1977).

It was concluded that oocytes require a specific intra ollicular steroid environment for the completion of the full maturation process. Alterations to the steroid profile during maturation induce changes in the oocyte which are expressed as gross abnormalities at fertilization (Moor et al., 1980).

b) Oocyte-cumulus complex culture

This model has been adopted for a long time by many investigators, since Pincus and Enzman (1935) and Chang (1955) had studied the capacity of rabbit follicular oocytes to resume meiosis outside of the follicles. Since much of the biochemical data on oocyte maturation cited has been derived from studies using extrafollucular oocytes, it is probable that many of the observed changes relate more to the resumption of meiosis than to the acquisition of developmental capacity. Two separate events may therefore be necessary for the full physiological maturation of the oocyte, namely the suppression of inhibitory factors and the generation of inductive stimuli from the follicle cells. Differently from the maturation of mammalian oocytes, in lower animals, it has been demonstrated the production of hormonal substance, often from the surrounding follicle cells, provides the positive stimulus required for full maturation of oocytes (Wasserman et al., 1980; Balieu et al., 1978; Kanatani

& Nagahara, 1980; Kostellow & Morrill, 1980). For example, 1-methyladenine acts as the positive stimulus in starfish, while progesterone or its 17α, 20β metabolites initiates meiotic maturation in fish and amphibia.

i) Follicular factors of meiosis

It is now accepted that the follicle cells are responsible for inhibiting meiosis and maintaining the oocytes in the germinal vesicle stage. The basis for this realization was the discovery by Pincus and Enzmann (1935) that oocyte retained within the follicle remain arrested at the germinal vesicle stage whereas extrafollicular oocytes completed meiosis even in the absence of hormones. The first direct evidence for the presence of an inhibitor in the follicular fluid was provided by Chang (1955) and this first observation of the inhibitory effect of rabbit follicular fluid on the spontaneous maturation of rabbit oocytes went unnoticed for years. Furthermore, in some studies, follicular fluid was used to supplement the medium in which oocytes were cultured (Cho et al., 1971; Edwards, 1974; Jagiello et al., 1977).

In more recent studies, the inhibitory effect of porcine follicular fluid on the maturation of porcine oocytes (Tsafriri & Channing, 1975) and of hamster follicular fluid on hamster oocytes (Gwatkin & Anderson, 1976) was demonstrated. However, this inhibitory effect of follicular fluid ons spontaneous maturation of oocytes has subsequently supported by some workers mentioned above and at the same time disputed by others (Leibfried & First, 1980; Bae & Channing, 1982 unpublished). The demonstration that the granulosa cells are the source of the meiotic inhibitor was provided by the in vitro experiments of Foote and Thibault (1969) and subsequently supported by Sato et al. (1980).

In addition, this inhibitory action requires a close contact between the oocytes and the granulosa cell layer (Foote & Thibault, 1969; Sato et al., 1980), whereas, Tsafriri et al. (1976) shows that this inhibitory action is not necessary exerted by cell contact, since both granulosa cell extract (Tsafriri et al., 1976), follicular fluid and a conditioned medium (i.e., medium in which granulosa cells were cultured previously for 48 hr) inhibit the resumption of meiosis. Even though they insist (Tsafriri & Channing, 1975; Gwatkin & Anderson, 1976) that the follicular fluid of oocyte maturation is not species specific, in co-culture of mice granulosa cells with oocytes, spontaneous maturation occurred undisturbed (Nekola & Smith, 1974), aand subsequently Leibfried and First (1980), by adopting the same methods and materials of those of Tsafriri and Channing (1978) could not observe any inhibitory effect of conditioned medium and follicular fluid fraction (the follicular fluid fraction made by Channing's lad. was donated to Leibfried & First, 1979). However, it is known that this putative inhibitor, a low molecular weight peptide may require the active involvement of the cumulus cells for its suppressive action (Hillensjo et al., 1979). The precise means by which the inhibitor acts on the oocyte are uncertain, but could involve the suppression of specific RNA synthesis. Removal or neutralization of the inhibitor would initiate the burst of transcriptional activity observed immediately after the resumption of one component of follicular fluid has already been shown to suppress the synthesis of DNA-dependent RNA both in ascites tumor cells (Moore et al., 1975) and in granulosa cells (Bernard & Psychoyos, 1977) and two macromolecular components of the porcine follicular fluid have already been shown to suppress mouse oocyte maturation (Bae et al., 1981). It is possible that this factor (and/or these factors) may exert a similar action on the oocyte by inhibiting the transcriptional activity that is essential for the resumption of meiosis.

ii) Steroid involvement in maturation

Extrafollicular oocytes, once removed from the follicle and, therefore, also from the influence of the meiotic inhibitor, resume meiosis in the absence of gonadatropins and steroids. It has been claimed that oocytes within the follicle require gonadotropins but do not require gonadotropins but do not require sterois for th et al., 1974; Channing & Tsafriri; 1977). However, in mammals, the preovulatory gonadotropic stimulus elicits a prompt rise in follicular steroidogenesis (Tsafriri et al., 1976). This rise in progesterone synthesis could admit a possible role for progesterone in mediating the action of LH on oocyte maturation. Indeed, it was reported that progesterone facilitate maturation of denuded rabbit and bovine oocytes (Robertson & Baker, 1969). In addition, it was found that progesterone hastened the maturation of rabbit oocytes from medium-sized follicles (Bae & Foote, 1975). Furthermore, Moor (1978) support this contention and indicate that steroids are probably involved both in the late stages of nuclear maturation and in the regulation of cytoplasmic maturation. Steroids do not, however, seem to be important regulators of the rate of amino acid transport across the membrane of the oocyte during maturation (Moor & Smith, 1978). Evidence for the invelvement of steroids in cytoplasmic maturation has been obtained be maturing oocytes within the intact follicle in vitro. A high proportion of oocytes that mature within the cultured follicle in the presence of gonadotropins do so normally and subsequently develop into viable offspring (Moor & Trounson, 1977). However, when either the 20α-cholesterol oxidase (aminoglutethimide) or 17α-hydroxylase (SU10603) enzyme systems are inhibited in such cultures, important abnormalities in the maturation of both the cytoplasm and nucleus are observed. (Moor & Trounson, 1977). Administration of exogenous steroids to cultures in which endogenous steroidegenesis has been inhibited significantly reduces the portion of oocytes with cytoplasmic and nuclear abnormalities (Moor et al., 1980). The above findings on the involvement of steroids in nuclear maturation accord well with those of McGaughey (1977), who reported that presence of steroids reduced the incidence of chromosomal abnormalities at telophase and metaphase of meiosis in oocytes matured outside the follicle. Support for the hypothesis that steroids are essential for the normal maturation of the cytoplasm can be found in a variety of experiments summarized by Thibault (1977). By contrast, no facilitory effect of progesterone was observed on maturation of human (Shea et al., 1975), porcine (McGaughey, 1977) liberated oocytes. Similarly, follicular inhibitor may be an estrogenic steroid (Richter & McGaughey, 1981). Addition of steroids to the culture medium did not induce the maturation of bovine, porcine (Foote & Thibault, 1969) and rabbit (Smith et al. 1978). Smith et al. (1978) showed that progesterone at a concentration of 10 uM did not affect maturation, but 100 uM-progesterone blocked germinal vesicle breakdown in oocyte from medium-sized follicles and reduced both germinal vesicle breakdown and polar body formation in oocytes from large follicles of rabbit. Furthermore, a concentration of 100 uM progesterone did not appear deleterious since the rabbit oocytes in this experiments appeared normal after 16-20 hr in culture and were able to form polar bodies or resume meiosis with the expected frequency when washed and transferred to control medium. Progesterone concentrations in rabbit follicular fluid increase sharply to a peak at 3 hr after coitus or LH injection and fall rapidly to values less than those in precoital or unstimulated follicles by 8 or 9 hr (Younglai, 1972; Patwardhan & Lanthier, 1976). In addition, it is suggested that progesterone can contribute to the final sequence of follicular events, ending in vitro ovulation of an appropriately stimulated follicle (Baranczuk & Fainstat, 1976). A similar

pattern is seen in peripheral blood progestagens (Waterson & Mills, 1976), ovarian venous plasma (Hilliard & Eaton, 1971), and in cultured intact follicles treated with LH (Mills & Savard, 1973). The precitous drop in follicular progestagen production approximately 7 hr before ovulation is not inconsistent with the results of Smith et al. (1978) that progesterone blocks apontaneous maturation in vitro and that oocytes removed from progesterone containing medium can resume and complete the first meiotic division.

In conclusion, it is clear that while steroids are important during the obligatory inductive period within the follicle, they are alone unable to induce full maturation. These additional inductive factors are gonadotropin-dependent and are thought to act primainly in the regulation of cytoplasmic maturation: their identification would contribute substantially to our understanding of the regulation of full oocyte maturation.

iii) Cyclic AMP involvement in maturation

Various actions of LH on the ovary are mediated by cyclic AMP (reviewed by Marsh, 1976; Channing & Tsafriri, 1977). The effect of cyclic AMP and of phosphodiesterase inhibitors on the maturation of liberated mammalian oocytes is much clearer: dbc AMP and theophylline inhibit the maturation of mouse oocytes (Cho et al., 1974). Since this finding, all derivatives of cyclic AMP and phosphodiesterase inhibitors have been good tools for the investigation of spontaneous maturation of extrafollicular oocytes in mammals. Later, dbc AMP, 8-bromocyclic AMP and isobutylmethylxanthine (IBMX) were found to inhibit the maturation of rat oocytes (Hillensjo, 1977) and an alternative hypothesis is that meiotic arrest is maintained by the passage of cyclic AMP from the follicle cells into the oocyte through permeable junctions (Dekel & Beers, 1978, 1980; Beers & Dekel, 1981; reviewed by Tsafriri et al., 1982). The inhibitory effect of dbc AMP on the maturation of mouse oocytes was not due to alternation of the apparent rate or pattern of protein synthesis by the cultured oocytes (Stern & Wasserman, 1974). However, by the use of high-resulution two-dimensional electrophoresis, Schultz and Wasserman (1977) detected a change in the pattern of proteins synthesized by maturing mouse oocytes. This change in pattern was prevented by dbc AMP. The studies in explanted follicles imply the involvement of cyclic AMP in the mediation of the LH action of oocyte maturation. The inhibitory effect of theophylline or IBMX in maturation may be unrelated to their action as phosphodiesterase inhibitors. Alternatively, the contrasting effects of dbc AMP and of phosphodiesterase inhibitors may be interpreted as compartmentalization of cAMP in the follicle. It is possible that LH (and other peptide hormones that induce both rise in cAMP and resumption of meiosis) induced a rise of cAMP only in granulosa cells, whereas phosphodiesterase inhibitors may be assumed to cause cAMP accumulation indiscriminately in all compartments of the follicle, including the oocyte itself, and the increased concentration of the nucleotides in the germ cell may be inhibitory to the resumption of meiosis. Dibutyriyl cAMP has been shown to inhibit mitotic division of a great number of normal and malignant cells (reviewed by Ryan & Heidrick, 1974). By contrast, injection of the cAMP derivarive dbc AMP into the follicular antrum or short-term exposures of follicles to a medium containing 8-bromo cyclic AMP (Hillensjo et al., 1978), dbc AMP or IBMX triggered germinal vesicle breakdown (GVBD) (Dekel et al., 1981). However, the continuous presence of cAMP derivatives, or of most of the phosphodiesterase inhibitors prevented the LH-induced maturation of follicle-enclosed oocytes (Lindner et al., 1974; Hillensjo et al., 1978; Dekel et al., 1981). It is suggested that only cAMP serve as the physiological inhibitor of meiosis which is transmitted from the cumulus cells via gap junction (Dekel & Beers, 1978

and 1980). The adenylate cyclase activator, chorela enterotoxin (Cuatrecaseas et al., 1975), inhibit spontaneous maturation in oocytes, cultured within the cumulus but not in denuded oocytes (Dekel & Beers, 1980). Differently from the results of Dekel and Beers (1978, 1980 and Dekel et al., 1981) in rat follicular oocyte culture, the inhibitory effect of dbc AMP on completely denuded mouse oocytes is still effective (Cho et al., 1974). However, a recent conclusive study done by Moor and Heslop (1981) dose not support the hypothesis that cAMP is transmitted into the oocyte and blocks the maturation of the oocyte. They measured cAMP production from cultured oocyte, but they could not observe any significant increments of cAMP. Furthermore, another recent report (Powers & Paleos, 1982) shows that treatment that inhibited calcium uptake potentiated the inhibitory effect of dbc AMP and treatments which stimulated cellular calcium uptake overcame the inhibitory effect of dbc AMP on mouse oocyte maturation. Elevated extracellular calcium (> 10 mM) significantly decreased the inhibitory effect of concentrations of dbc AMP up to 150 uM when compared to control levels of calcium (1.7 mM). In addition, the calcium ionophore A23187 (> 1 uM) significantly overcame the effect of dbc AMP in media that contained 1.7 or 20 mM calcium. In the presence of 41 mM-dbc AMP the calcium antagonist verapamil increased (in a dose-dependent fushion) the percentage of oocytes blocked at the germinal vesicle stage. A similar dose-dependent, reversible potentiation of the effect of dbc AMP was found with tetracaine, which also lowers cytoplasmic calcium concentrations. This results (Powers & Paleos, 1982) suggest that cytoplasmic calcium has a central regulatory role in germinal vesicle breakdown (GVBD) of the mouse oocyte in vitro. While intracellular reservoirs of calcium are apparently sufficient to supply calcium for GVBD in vitro without dbc AMP, exogenous calcium is required to stimulate GVBD in the presence of dbc AMP. This suggests that dbc AMP may act by reducing cytoplasmic calcium, perhaps by stimulation of a membrane-bound calcium pump, as found in other systems (Berridge, 1975; Morrill et al., 1981). Recently, Richter and McGaughey (1981) shows that dbc AMP affects the synthesis of four polypeptide by reversively inhibiting the synthesis of one polypeptide, and irreversibly inhibiting the synthesis of three other polypeptides, In addition, dbc AMP also reversibly inhibits the synthesis of at least two specific nondevelopmental polypeptides. This study shows that during the blockade of maturation by dbc AMP a few developmental specific polypeptide are not synthesized, which may be essential polypeptide for GVBD or maturation of the oocyte. Even though this is done in amphibia, it is well known that progesterone inhibits adenylate cyclase activity in Xenopus oocyte and, in addition to this, progesterone stimulate calcium fluxes at the same time. There might be the same mechanism in mammalian oocytes as in amphibia (Sadler & Maller, 1981; Kostellow & Morrill, 1980). Along with the results mentioned above by many investigators, further study is still required whether cAMP or its derivatives serve as an inhibitory signal at the level of oocyte in vivo and in vitro.

iv) Macromolecule synthesis involvement in maturation

Studies on synthesis of macromolecules by the dictyate oocytes has been a consistant target for oocyte maturation in both amphibia and mammals. RNA synthesis, which regularly increases during the growth of oocytes, reaching a peak at the trilaminar follicle stage decrease drastically when antrum formation is initiated (Oakberg, 1967; Moore et al., 1974). However, a low rate of RNA synthesis remains until the initiation of meiotic maturation (Rodman & Bachvarova, 1976; Wasserman & Letourneau, 1976). Part of the RNA synthesized during the

growth period metabolized, while another part, despite turnover, is stored in the cytoplasm and constitutes developmental information for early embryogenesis (Oakberg, 1967; Bachvarova, 1974; Jahn et al., 1976). In addition, Wasserman and Letourneau (1976) suggest that nuclear RNA synthesis occurs early in the maturation period but is terminated after the breakdown of the germinal vesicle. Mitochondrial RNA synthesis, by contrast, continuous throughout the entire maturation process. Moreover, there is probably a reprogramming in gene expression at the stage of antrum formation at seem to indicate the morphological changes in nuclear RNA structures observed on frozen sections of mouse oocytes by Palombi and Viron (1977). Recent studies revealed that RNA synthesis during the early stage of maturation is necessary for the resumption of meiosis, since actinomycin D prevents meiosis in mouse oocytes (Crozet & Szollosi, 1980; Rodman & Bachvarova, 1976; Wasserman & Letourneau, 1976). In oocyte stimulated to mature by liberation from their follicles, or after HCG administration to PMSG-treated immature mouse, 3H uridine incoporation could be detected during the 2 hr preceding germinal vesicle breakdown (Bloom & Mukherjee, 1972; Rodman & Bachvarova, 1976; Wasserman & Letourneau, 1976). Maturation of liberated mouse oocytes was prevented by 0.1 μg/ml of actinomycin D (Bloom & Mukherjee, 1972). On the other hand, Jagiello (1969) observed chromosomal damage to mouse oocytes cultured in the presence of 0.2-1.0 μg/ml actinomycin D : the resumption of meiosis was not prevented, but it was blocked at metaphase I (Jagiello, 1969; Golbus & Stein, 1976). However, the arrest of nuclear progression dose not necessarily mean an arrest of cytoplasmic maturation or of progression of the pattern of protein synthesized. Golbus and Stein (1976) found that a metaphase II-like pattern of protein synthesis existed in oocytes cultures in medium with actinomycin D even though the nucleus was arrested at metaphase I. They suggested that the changes in the pattern of proteins synthesized during mouse oocyte meiosis were dictated by mRNA already present and not by mRNA synthesized during meiosis. By contrast, other reports show that meiosis is not inhibited by actinomycin D at low concentrations (Crozet & Szollosi, 1980; Hillensjo, 1976) but that high concentrations result in chromosomal abnormalities (Alexander & Gerin, 1977).

These conflicting observations may result from the fact that low concentration of actinomycin D inhibit the synthesis of ribosomal but not mRNA and that the higher concentrations did to inhibit mRNA synthesis. Actinomycin D has deleterious side effects. Studies utilizing alpha-amanitin, a more specific inhibitor of mRNA synthesis have been undertaken to clarify this question. It has been claimed that alpha-amanitin dose not inhibit the resumption of meiosis in extrafollicular oocytes and that RNA synthesis at the beginning of maturation is not necessary for the initiation of nuclear maturation. RNA already synthesized during the growth phase may be used for the reprogramming of protein synthesis occurring during nuclear maturation (Crozet & Szollosi, 1980). In addition, there is no detectable difference in majority of mRNA molecules between unfertilized matured oocyte and fertilized egg (Schultz et al., 1980). The continuity of gene expression throughout the period of meiotic arrest is accompanied by considerable metabolic activity with the oocyte cytoplasm. In the mouse, for example, the volume of the oocyte increase about 70-fold during the growth period; the protein required for this considerable growth is derived almost entirely from endogenous synthesis and not from the uptake of maternal proteins, as occurs in lower vertebrates. Protein synthesis occurs throughout the entire germinal vesicle stage but shows little evidence of any substantial changes in the pattern of polypeptides produced during this extended phase. It is probable, however, that the overall

rate of protein synthesis increase during the growth of the oocyte. For example, synthesis of glucose-6-phosphate dehydrogenase and lactate dehydrogenase increase progressively until the oocyte reaches its maximum diameter; the acticity of those enzymes in the fully grown germinal vesicle oocyte remains constant (Mangia & Epstein, 1975). Pyruvate provides the essential energy source for the normal metabolic activity in the resting oocytes (Eppig, 1977). The apparent rate of protein synthesis during oocyte maturation varies with the type of amino acid used in the incorporation experiments. A constant rate of synthesis is suggested by the linear incorporation of valine and leucine into protein. A complex and changing rate of synthesis is observed with tyrosine. By carrying out pulse chase experiments it was found that short-lived protein is made during the early maturation stages, and that this rapidly degraded material is preferentially labelled by tyrosine. Measurements using 35S methionine have indicated that the absolute rate of protein synthesis, using this amino acid as a marker, declines from 43 pg/hour to 31 pg/hour in each oocyte between the germinal vesicle and metaphase II stage of meiosis (Wassarman et al., 1979). In addition to changes in the rate of synthesis, important qualitative changes occurs in the nature of the proteins synthesized during oocyte maturation (Golbus & Stein, 1976; Warnes et al., 1977; Van Blerkom & McGaughey, 1978). Golbus and Stein (1976) showed that the protein pattern in oocyte cultured in the presence of actinomycin D progressed to metaphase II pattern in spite of the nuclear maturation arrest, indicating a dissociation between meiotic maturation and the changes in the pattern of proteins synthesized at different stages of maturation (Golbus & Stein, 1976). In addition, Tsafriri et al. (1973) showed in cultures of explanted rat follicles that addition of actinomycin D, at dose level (8 μg/ml) which dose not inhibit incorporation into protein, failed to prevent the stimulatory action of LH on oocyte maturation, although it blocked the effect of LH on steroidogenesis. These findings suggests that LH may stimulate the translation of preformed mRNA to give rise to a specific protein(s) essential for oocyte maturation. Furthermore, the change in protein synthesis observed by Warnes et al. (1977) dose not seem, therefore, to be essential for the completion of nuclear maturation. Nevertheless, the change in protein pattern may be related to cytoplasmic maturation essential for normal fertilization and subsequent development. Furthermore, a recent study done by Richter and McGaughey (1981) shows that oocytes synthesize stage-specific polypeptides (i.e., developmental polypeptides) during meiotic maturation of mouse oocyte, and dbc AMP affects the synthesis of four of these developmental polypeptides by reversibl inhibiting the synthesis of one polypeptides, and irreversibly inhibiting the synthesis of three of other polypeptides. In addition to this, dbc AMP also reversibly inhibited the synthesis of at least two specific nondevelopmental polypeptides. However, whether or not oocytes which have been allowed to mature following inhibition by dbc AMP are competent for fertilization and embryonic development has not been determined. Nevertheless, it is clear that as with the studies on RNA synthesis, further studies are necessary to provide more definite evidence on the role of protein syntheisi in the regulation of meiosis and normal fertilization process.

v) Metabolic requirements for oocyte maturation.

In denuded mouse oocytes in culture, only pyruvate and oxaloacetate substain maturation (Biggers et al., 1967). A similar pattern of energy source utilization is observed in growing mouse oocyte (Eppig, 1976) and preimplantation embryos of mouse, rabbit and rhesus monkey (Brinster, 1968 and 1969; Quinn & Wales, 1973). The inabilityof mouse embryos to utilize glucose as an energy source has been attributed principally to a block in glycolysis at the phospho-

fructokinase step (Barbehenn et al., 1974). In view of the abundance of lactate dehydrogenase in mouse oocyte (Mangia & Epstein, 1975), the failure to utilize lactate is puzzling. It has been suggested that this failure to utilize lactate is due to NAD^+ deficiency (Sorenson, 1972; Zeilmaker et al., 1972). However, in mouse embryos, no NAD^+ deficiency was found (Wales, 1974). The dependency of the resumption of maturation on pyruvate led to the suggestion that the preovulatory LH surge induces maturation by the supply of the essential nutrient for the oocyte by follicular cells (Biggers, 1972). In fact, LH markedly increase respiration of isolated granulosa cells (Ahren et al., 1969) and stimulates aerobic glycolysis in isolated rat follicles (Tsafriri et al., 1976). Oocytes from some other species have a less restricted energy requirement. Denuded rat oocytes utilize pyruvate or lactate for maturation, while cumulus-enclosed rabbit oocyte utilize amino acids, e.g., glutamines as a source of energy (as well as for protein synthesis) (Bae & Foote, 1975a, b, and 1980; Smith et al., 1978; Chung & Bae, 1972) while hamster oocytes utilized some other amino acids (Gwatkin & Haidri, 1973). However, in mouse, any single amino acid (e.g., glutamine) can not be utilized as a sole energy source different from that of rabbit. It has been postulated that a deficientcy of oxygen or energy substrate block the resumption of meiosis in vivo (Zeilmaker & Verhamme, 1974). These limitations could be overcome by the known stimulatory effect of luteinzing hormone on aerobic glycolysis within the follicle (Nilsson, 1974). It has, however, been shown that LH reduces the oxygen consumption of the oocyte and cumulus (Hillensjo et al., 1975). The increase in oxygen consumption of the denuded oocyte is in contrast to the depression by LH of oxygen consumption by oocyte-cumulus cell complexes (Dekel et al., 1976). However, it is not known at the present whether this decrease in cumulus cell respiration is essential for oocyte maturation. Even in large mammals, pig and bovine, a few carbohydrates, pyruvate, lactate and glucose, or combination of these could be utilized as energy source in simple chemically defined medium (McGaughey, 1977; Sato et al., 1980; Bae, 1981; Bae & Channing, 1982), but to the present time human follicular oocytes or primate follicular oocytes have been tested for maturation in a simple chemically defined medium with a few carbohydrates or its combinations (pyruvate, lactate and glucose) as an energy source or with any single amino acid as done by Bae and Foote (1975, 1980).

Further studies are required to investigate human or primate follicular oocytes in a simple chemically defined medium with an energy source like carbohydrate or amino acids. Furthermore, additional studies are required in order to reveal the metabolic changes within the oocyte and the follicle cell associated with oocyte maturation and their involvement in the regulation of the meiotic process.

vi) Plasma membrane changes and metal ions involvement in maturation

The membrane of the mammalian oocytes undergoes a number of important changes during maturation. The most apparent of these is the disruption of the junctional complexes between the oolemma and the surrounding follicle cells (Zamboni, 1972). It has recently been suggested that the disruption of these site of intercellullr comminication is necessary for the migration and aligment of the cortical granules along the cell membrane (Szollosi, 1978) and for the blockade of migration of cAMP or its derivative product from the cumulus cells via gap junction into the oocyte (Dekel & Beers, 1978; Dekel et al., 1981). The adenylate cyclase activator, cholera euterotoxin (Cuatrecasas et al., 1975), inhibits spontaneous maturation in oocytes, cultured with the cumulus but not in denuded oocytes (Dekel & Beers, 1980) This may suggest that cAMP produced by the cumulus

cells is transferred to the oocytes and blocks the resumption of meiosis. The effect of LH, which partly relieves this inhibitory effect, is probably due to its effect on breakdown of communication and hence blockade of transfer of cAMP to the oocyte. Furthermore, there is a sharp decrease in cAMP production in the whole follicle within a few hour after LH surge, whereas at the same time there increases a very high concentration of steroids, especially progesterone in the follicle which is destined to ovulation (McNatty et al., 1975; Hunter et al., 1976) or follicular oocytes complex after gonadotropin treatment in vitro (Bae & Channing, 1981) It is a well known fact that progesterone inhibit adenyl cyclase activity and also stimulate calcium fluxes in the amphibian follicles (Sadler & Maller, 1981). There is at the same time a high increase of prostaglandings after LH surge as well as permeability increase in the follicle by the production of histamine from the endothelial and mast cells after LH surge (Ainsworth et al., 1975; Espey, 1980). These combined actions, permeability increase in the follicle, steroidogenesis, prostaglandin production, histamine production, calcium fluxes in the oocytes seem to be a cause for resumption of oocyte maturation. Furthermore, recent studies done by Paleos and Powers (1980, 1981), Bae and Channing (1982), and Sato et al. (1980) show that calcium ion is an essential requirement for the resumption of oocyte maturation. In addition, it is shown by Paleos and Powers (1982) that high concentration of calcium in a culture medium surely overcame the inhibition of dbc AMP for the resumption of oocyte maturation in mouse in vitro system, and free-calcium in a culture medium is a cause inhibition of GVBD or degeneration of swine oocyte cumulus complex (Bae & Channing, 1982). In addition, the effect of calcium on oocyte maturation was proved even in amphibia that the ionophoresis of Ca^{++} into cortex of oocytes (Moreau et al., 1976; Kostellow & Morrill, 1980; Morrill et al., 1981) could induce Xenopus laevis oocyte maturation in vitro. The timing of GVBD induced was the same as that for progesterone. Furthermore, Wallace and Steinhardt (1977) confirmed the general time course of depolarization of oocyte, but found that the magnitude of the change depended on whether or not oocytes were dissected from their folliclesprior to progesterone treatment. Oocytes maintained within their follicles exhibited resting potentials of about-40 mV. However, if the follicle epithelium was removed, the oocyte hyperpolarized and established a new resting potential of -60 mV to -80 mV. Wallace and Steinhardt suggest that the hyperpolarization reflects and activation of an electrogenic Na-K active transport process not normally active in oocytes maintained within their follicles. Thus, observation on the whole follicle probably more closely reflect the situation as it exists in vivo. Changes in membrane potential could reflect a change in the oocyte permeability to various ions. In this regard, O'Connor et al., (1977) recently have measured changes ini ion fluxes of Na^{+}, K^{+} and Ca^{++} before and during progesterone-induced maturation of Xenopus laevis oocytes. The results demonstrated that soon if not immediately after exponsure of oocytes to progesterone, both the possible sodium and potassium fluxes became elevated over control levels. This changes was calcium influx. Hence, progesterone treatment results in a specific sequence of changes in the membrane selectivity to all ions. Most striking progesterone treatment results in a rapid increase in the rate of calcium efflux (O'Connor et al., 1977; Kostellow & Morrill, 1980). However, this kind of membrane permeability changes to ions has not been proved in mammalian follicle or follicular oocytes as far as this reviewer has investigated, but indirect evidence of membrane permeability changes in mammals and requirements for calcium ion for resumption of mammalian oocyte maturation can be found in mouse studies done by Paleos and Powers (1981)

and Powers and Paleos 1982), and in pig (Bae & Channing, 1981 and 1982; Sato et al., 1980).

In addition, there is a few indirect evidences of permeability changes of the mammalian follicles after gonadotropin treatments in mammals. There is a difference in calcium concentration of the follicular fluid as well as a increase (Burgoyne et al., 1979) about 60% of follicular fluid after gonadotropin treatment due to the accumulation of solutes and water in the antrum in the rabbit (Rondell, 1964 and 1970). After gonadotropin (HCG) treatment there was a significant drop in follicular fluid calcium relatives to blood during the 10 hr period which may reflect calcium involvement in the regulation of oocyte maturation (Burgoyne et al., 1979). By contrast, there is a sharp increment of calcium in the oocyte cumulus complex after gonadotropin treatment. This increase of calcium concentration and serum LH levels occurred at a same time of an increase in germinal vesicle breakdown in the rat (Batta & Knudsen, 1980). This calcium changes, a drop in the rabbit follicular fluid and an increase in oocyte cumulus complex in the rat after gonadotropin treatment suggest that the permeability of follicle was changed due to the treated gonadotropin. These changes, however, explains the increased permeability of preovulatory follicles to Evans blue observed by Zachariae (1958) and observation that large healthy follicles in the cow are permeable to even the large serum proteins (Anderson et al., 1976), although the permeability of smaller follicles to serum proteins is related to their molecular weight. (Shalgi et al., 1973; Anserson et al., 1976). In relation to some suggestions for the involvement of calcium in oocyte maturation made by Burgoyne et al. (1979) there was a drop of calcium concentration in the rabbit follicular fluid at a time of GVBD after HCG treatment, and also there was an increase of calcium in the rat oocyte cumulus complex at a time of GVBD after LH surge (Batta & Kundsen, 1980) In addition, Sato et al. (1980) showed that there was a sharp decrease of GVBD in a calcium-free medium in the pig. Also there is another evidence that there was no GVBD or any further maturation of pig follicular oocytes in a calcium-free medium (Bae & Channing, 1981 and 1982). Furthermore, Powers and Paleos (1982) demonstrated that elevated levels of extracellular calcium can overcome the block of GVBD caused by dbc AMP in the mouse oocytes in vitro. In addition, the calcium ionophore A23187 (> 1 uM) significantly overcame the inhibitory effect of dbc AMP for the oocyte maturation.

In conclusion, it is not too much to say that however hard we emphasize the involvement of calcium for the regulation of oocyte maturation in vivo as well as in vitro system.

7. CONCLUSION

In this review, I have discussed the possible nature of control mechanisms underlying the follicular regulation of oocyte maturation. However, despite the many studies directed towards the nuclear, cytoplasmic and membrane changes associated with oocyte maturation, very little is known about the intrafollicular mechanisms involved in regulation of these events. That the follicle has a controlling influence on oocyte maturation and embryonic development is clear from experiments which show that oocytes matured in vitrollithin the follicle undergo full maturation and, after fertilization, develop into viable offsprings, whereas oocytes cultured outside the follicle undergo spontaneous meiotic maturation. From another viewpoint in vivo and in vitro from geometrical considerations, it is clear that the total surface of the coupled cumulus cells is far greater than of the oocyte.

As a result, the membrane potential of the oocytes may be controlled primarily by the permeability properties of the cell membranes of the cumulus. Any agent that modifies the permeability properties of the cumulus cells may

automatically cause similar changes to be propagated to the oolemma. Alternatively, an agent or gonadotropins that cause uncoupling of the oocyte and cumulus cells and of the follicle may allow the oocyte undergo membrane changes reflected by depolarization.

Since much of the biochemical data on oocyte maturation cited in this review has been derived from studies using extrafollicular oocytes, it is probable that many of the observed changes relate more to the resumption of meiosis than to the acquisition of developmental capacity.

It is now accepted that the follicle cells are responsible for inhibiting meiosis and maintaining the oocytes in the germinal vesicle stage until gonadotropin(s) triggers a series of events in the follicle. Additional in vivo and in vitro studies concurrently utilizing various model systems described may resolve some of these events.

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