HAMMOND MEMORIAL LECTURE

Are follicular maturation and oocyte maturation independent processes?

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I wish to thank the members of the John Hammond Memorial Committee for their kind invitation to deliver this lecture, which expresses very personal feelings. When, in 1949, as a recently graduated Ph.D. from the University of Paris, I was seeking my future orientation in research, I had the good luck to be in Ghent when John Hammond presented a prospective survey of the improvement of fertility in domestic animals by hormonal treatment. Some months later, I visited Hammond's laboratory in Cambridge and studied his books and papers on reproduction in the cow and rabbit. Today, looking back over the past 26 years, I have the feeling that my work as a scientist, and as a Professor, found much of its inspiration in Hammond's thought and teaching.

I have chosen to speak today on the maturation of the female gamete because I consider that improvement in the availability of the female gamete in mammals remains the main objective of research related to animal production and the treatment of human infertility. Calves may be born after intracervical insemination with as few as 25,000 living spermatozoa, and in 3 years one bull may sire approximately 10 million calves; the cow, during the same 3 years, gives birth to three calves, or could be responsible for about a dozen calves if egg-transfer techniques are used.

The dream of maturing at will the thousands of oocytes in the ovary is certainly not realizable at present. Hormonally induced superovulation remains a lottery, and individual responses in sheep and cattle, as shown by John Hammond and his students, are highly variable. During the past 10 years there have been many papers on follicular development and oocyte growth and maturation, but authors have failed to mention the ability of experimental oocytes to continue normal development. My present intention is therefore to shed some light on the gaps in our knowledge of these vital processes.

Maturation of the follicle and oocyte of lower vertebrates

In lower vertebrates, as in mammals, the first steps of follicle and oocyte growth are almost completely independent of gonadotrophins or steroids. In hypophysectomized fish, oögonia will enter meiotic prophase. Goldfish ovarian fragments cultured without hormones show mitosis, meiotic prophase and previtellogenesis (Remacle, Delaere & Jacquet, 1976).

Later, gonadotrophins are essential for the stages from vitellogenesis to ovulation. They become detectable in the blood at the beginning of vitellogenesis and culminate around the ovulation period with plasma levels 10–100 times higher than during the anovulatory period (Crim, Mayer & Donaldson, 1973; Crim, Watts & Evans, 1975). There is apparently a gonadotrophin surge in the female similar to that which is known to occur in higher vertebrates before ovulation (Breton, Billard, Jalabert & Kann, 1972). Gonadotrophic action is mediated through steroids. In fish and amphibians, oocytes removed from their follicles do not resume meiosis spontaneously in culture. Steroids are necessary to initiate germinal vesicle breakdown (GVB), and there is some evidence of species specificity for progestagens, as shown in Tables 1 and 2. $17\alpha,20\beta$ -Hydroxyprogesterone has been shown to be the most potent progestin in pike, trout and goldfish (Jalabert, 1976). In amphibians it has been accepted that progesterone is the more active progestin. However, the concentrations of progesterone necessary to induce 100% GVB in the frog (Schuetz, 1972) are also able to induce GVB

Table 1. Maturation of fish oocytes (trout, pike and goldfish) *in vitro* by progestins

Steroid	Conc. (ng/ml)	GVB (%)	Conc. (ng/ml)	GVB (%)
Progesterone	125	5	—	—
17 α -Hydroxyprogesterone	125	30	12.5	0
20 β -Hydroxyprogesterone	125	100	12.5	10
17 α ,20 β -Hydroxyprogesterone	125	100	12.5	100

From Fostier *et al.*, 1973; Jalabert, 1976.

Table 2. Maturation of amphibian oocytes *in vitro* by steroids

Species	Steroid	Conc. (mol/l)	GVB (%)	Conc. (mol/l)	GVB (%)
*Frog	Progesterone	10 ⁻⁶	93	10 ⁻⁷	68
	5 α -Pregnane-3,20-dione	10 ⁻⁶	83	10 ⁻⁷	46
	3 α ,3 β ,5 α -Pregnane-20-one	10 ⁻⁶	70	10 ⁻⁷	3
		Conc. (ng/ml)	GVB (%)	Conc. (ng/ml)	GVB (%)
† <i>Xenopus</i>	Progesterone	100	93	10	14
	Testosterone	100	100	10	38
	Aldosterone	100	100	10	100
		Conc. (pmol/oocyte)	GVB (%)		
‡ <i>Xenopus</i>	Progesterone	1	10		
		3	38		
		10	68		
		30	91		

* From Sanyal & Sibre (1975).

† From Jacobelli *et al.* (1974).

‡ From Bellé *et al.* (1976).

in fish, and 17 α ,20 α - or 17 α ,20 β -hydroxyprogesterone is one of the main metabolites of progesterone in the *Xenopus* oocyte (Thibier-Fouchet, Mulner & Ozon, 1976). Thus, 17 α ,20 β -hydroxyprogesterone is perhaps the common final activator of fish and amphibian oocytes.

Membrane, cytosolic or nuclear progestin receptors have not been isolated from amphibian oocytes. Progestins penetrate the egg membrane and fix on peripheral pigment granules, the melanosomes (Ozon & Bellé, 1973; Jacobelli, Hanocq, Baltus & Brachet, 1974). It would seem that gene transcription is not involved because actinomycin D does not prevent GVB.

As in mammals, prostaglandins (PG) E-2 and F-2 α are synthesized during follicular maturation and PGF-2 α plays an active role in the ovulation process (trout: Jalabert & Szöllösi, 1975).

Since all these studies have been done *in vitro* and the results are therefore indisputable, it is tempting to postulate that steroid-dependent maturation of the oocyte is a general rule for vertebrates. However, physiological events related to oocyte maturation are so different in lower and higher vertebrates that this hypothesis cannot be accepted without critical analysis. Of these differences, the absence of intraovarian interaction between compartments with different steroidogenic activities, as is the rule in birds and mammals, may be mentioned.

Finally, we must remember that the biological endpoint which is usually taken is GVB. However, normal embryos do result from fertilization of in-vitro progestin-matured oocytes (*Rana pipiens*: Smith, Ecker & Subtelny, 1968; *Xenopus*: Brun, 1975; trout: B. Jalabert, personal communication).

Maturation of follicle-free oocytes in mammals

Oocytes from all the mammalian species studied are able to resume meiosis from the dictyate stage to metaphase II when cultured in a suitable medium (Table 3). Metabolic by-products from the cumulus cells are not involved in the initiation of maturation *in vitro* since cumulus-free oocytes also undergo maturation to metaphase II (rat: Zeilmaker & Verhamme, 1974; mouse: Cross, 1973). It is clear from many studies (Table 3) that while the majority of the oocytes resume meiosis, as indicated by GVB, only a variable proportion reach metaphase II. This general observation has stimulated many attempts to discover, among polypeptide and steroid hormones, prostaglandins and cyclic AMP, those which induce the resumption of meiosis. This type of screening does not necessarily yield any valuable conclusions (Jagiello, Ducayen, Miller, Graffeo & Fang, 1975).

Table 3. In-vitro maturation of extra-follicular oocytes in mammals

Species	Duration of culture (h)	GVB (%)	Oocytes in metaphase II (%)	Reference
Mouse	?	94	85	Sorensen & Wassarman, 1976
Mouse	17	94	88	Donahue, 1968
Mouse	18	91	67	Erickson & Sorensen, 1974
Rat	17	98	83	Erickson & Ryan, 1976
Guinea-pig	20–24	90	68	Yanagimachi, 1974
Hamster	12	?	90–95	Haidri & Gwatkin, 1973
Rabbit	9	100	100	Thibault & Gérard, 1971
Pig	43–48	88	80	Tsafiri & Channing, 1975a, b
Cattle	29–30	96	73	Shea <i>et al.</i> , 1976
Cattle	48	100	60	Cho & Lim, 1975
Cattle	29	100	91 (M1)	Hunter <i>et al.</i> , 1972
Cattle (calf)	29	100	97	Thibault <i>et al.</i> , 1975b
Sheep	40	?	91	Quirke & Gordon, 1971
Dog	48	?	22.5 (M1 + M2)	Mahi & Yanagimachi, 1976
Macaque	46–48	72	55	Suzuki & Mastroianni, 1966
Macaque	23–31	50	23	Jagiello <i>et al.</i> , 1975
Macaque	29–36	55	10	Thibault <i>et al.</i> , 1977
Man	42	43	31	Shea <i>et al.</i> , 1975
Man	43–47	51	41	Kennedy & Donahue, 1969
Man	42–49	45	42	Jagiello <i>et al.</i> , 1975

In fact, three situations involving specific oocyte stages have been used. Firstly, in the juvenile female, maximum oocyte size is much less than that in pubertal and adult animals (man: Lintern-Moore, Peters, Moore & Faber, 1974; hamster: Iwamatsu & Yanagimachi, 1975; mouse: Szybek, 1972; Sorensen & Wassarman, 1976). The oocytes from juvenile mammals are generally incompetent to resume meiosis, even if they come from antral follicles (hamster: Iwamatsu & Yanagimachi, 1975; macaque: Gould & Graham, 1976).

Secondly, all oocytes collected in preovulatory follicles, from either normal adults or PMSG-pretreated prepubertal females, resume meiosis when culture conditions are favourable. Let us examine the criteria for an appropriate in-vitro environment. 'Suitable conditions' mean that not only must meiosis resume, but that the cumulus cells should continue to divide and pyknotic nuclei should not appear. As shown in Table 4, oxygen requirements differ somewhat according to the species. The chronology of successive events should also be absolutely similar to that *in vivo* (Thibault & Gérard, 1971; Thibault, Gérard & Menezo, 1975a). Changes in plasma membrane microvilli and an increase in the number of cortical granules and multivesicular bodies are observed simultaneously in mouse oocytes maturing *in vivo* and *in vitro* (Merchant & Chang, 1971). Studies to demonstrate the positive effect of hormones or mediators on the percentage of oocytes reaching metaphase II must be discarded if the conclusions are based on the abnormal behaviour of the control (untreated) oocytes.

Table 4. Oxygen tension *in vitro* and first polar body (PB) formation in extrafollicular oocytes

Species	Oxygen (%)	Medium	Osmotic pressure (mosmol)	1st PB (%)	Reference
Hamster	5	GH2	285–295	100	Gwatkin & Haidri, 1973
	10	GH2	285–295	18	
	20	GH2	285–295	0	
Hamster	5	GH2	310	90	Iwamatsu & Yanagimachi, 1975
Hamster	5 or 20 (under oil)	GH2	285	66–100	Plachot & Mandelbaum, 1977
Rat	95 then 5	mKRB		80	Zeilmaker <i>et al.</i> , 1972
	0 then 5			68	
Rabbit	20	TC 199 + FCS	290	100	Thibault & Gérard, 1971
Cattle (calf)	20	TC 199 + FCS	290	95–100	Thibault <i>et al.</i> , 1975b
	5	TC 199 + FCS	290	100	
Pig	20	TC 199 + PS	?	53	Tsafiriri & Channing, 1975a, b
	45			44	
	95			37	

GH2, Medium 2 of Gwatkin & Haidri (1973); mKRB, modified Krebs–Ringer bicarbonate; FCS, fetal calf serum; PS, pig serum.

Finally, there is a third group of oocytes, collected from antral follicles of different sizes at any stage (usually unknown) of the ovarian cycle. (There is a close correlation between the capacity of the mouse oocyte to resume meiosis and the appearance of the antrum in the developing follicle (Erickson & Sorensen, 1974). However, rat oocytes from preantral follicles can resume meiosis, but only up to metaphase I (Erickson & Ryan, 1976).) The physiological and morphological heterogeneity of oocytes and cumulus cells (Swartz & Schuetz, 1975) renders this material unsuitable for planning pertinent experiments unless steroids–gonadotrophin or prostaglandin–gonadotrophin pretreatments have been used to synchronize and stimulate the growth of a wave of antral follicles. We have used this technique extensively with success in the 3-month-old calf; all the oocytes achieved first metaphase during culture (Table 5). By contrast, only 20% of monkey oocytes collected in the early follicular phase reached metaphase II (Table 9), irrespective of the pretreatment or the amount of cumulus or cumulus and granulosa cells encompassing the oocyte during culture. This is perhaps a consequence of the long follicular phase in primates.

Table 5. Maturation of calf oocytes cultured *in vitro* for 30 h

O ₂ tension (%)	Hormones added	No. of oocytes	Nuclear stage	
			Metaphase I	Metaphase II
20	None	3	0	3
20	FSH or LH	27	3	24
20	FSH + LH	38	2	36
5	+oestradiol-17 β	11	0	11
	FSH + LH			
	+oestradiol-17 β			

From Thibault *et al.*, 1975b.

We can conclude that preovulatory oocytes, or even oocytes from small antral follicles, resume meiosis spontaneously when isolated in culture in appropriate media (Menezo, 1976): hormones are not involved and final oocyte nuclear maturation in mammals is not follicle-dependent.

We now need to ask if nuclear maturation is the only event preparing the oocyte for further development; in other words, do other aspects of maturation need the presence of the follicle. It seems that in one species, the mouse, the extrafollicular oocyte can complete all aspects of its matu-

ration *in vitro*, as shown by the presence of 15-day-old living fetuses (Cross & Brinster, 1970) and the birth of young (Mukherjee, 1972) after complete maturation and fertilization *in vitro* before transfer to foster mothers. As far as I know, *in-vitro* maturation of extrafollicular oocytes in other species has not been followed by normal embryonic development (Sreenan, 1970). Depending on the species, this failure may be related to unfertilizability of the oocyte, delayed and incomplete sperm head swelling or abnormal protein synthesis.

Fertilizability of the extrafollicular oocyte

Recently, Plachot & Mandelbaum (1977) have shown that hamster oocytes matured *in vitro* cannot be fertilized *in vitro*. A large proportion of preovulatory hamster oocytes resumed meiosis when they were cultured with their attached cumulus in the conditions described by Gwatkin & Haidri (1974) (medium GH2, in paraffin oil, gas phase = 5% O₂ or air) and then mixed with epididymal spermatozoa, but fertilization had not occurred 24 h later. Tubal oocytes cultured with the same sperm sample were fertilized (Table 6). Inside the follicle, therefore, the zona pellucida had become sensitive to sperm enzymes, but it would not react if the oocyte had matured outside the follicle. Collection of oocytes at increasing times after HCG injection of the female shows that the capacity of the zona to be penetrated is acquired between 5 and 6 h after gonadotrophin injection (Table 7).

Table 6. Fertilization rates of hamster oocytes ovulated or matured *in vitro* (from Plachot & Mandelbaum, 1977)

Table 7. Partial maturation *in vivo* and fertilization rates of hamster oocytes (from Plachot & Mandelbaum, 1977), and rat oocytes (from Niwa & Chang, 1975)

Species	Recovery after HCG injection (h)	Duration of complementary culture with sperm. (h)	Fertilization <i>in vitro</i> (h)
Hamster	2	12	0 (0/37)
	4	10	0 (0/26)
	6	8	24 (6/26)
Rat	6	4	7 (8/108)
	8	4	52 (46/89)
	10	4	72 (46/89)

The data of Niwa & Chang (1975) indicate to me that the rat oocyte must also remain in the follicle for 6 or 7 h before becoming fertilizable. However, these authors did not draw this conclusion (Table 7), and Niwa, Miyake, Iritani & Nishikawa (1976) concluded that sperm penetration of oocytes matured *in vitro* from any stage of maturation was possible, although the final stage of fertilization was abnormal in oocytes recovered earlier than 6–8 h after HCG.

Ability of extrafollicular oocytes to change the sperm head into male pronucleus

In-vitro-matured oocytes from rabbits, pigs, cows and women can be penetrated by spermatozoa. However, a striking difference is visible between oocytes matured *in vivo* and *in vitro*: the male nucleus does not dissociate and swell immediately after penetration of in-vitro-matured oocytes as it does in freshly ovulated eggs. The sperm head remains unchanged for some hours, and when swelling does occur, grains of condensed chromatin remain in the male pronucleus. Finally, such eggs cleave but do not develop further (Thibault & Gérard, 1970; Thibault & Gérard, 1973; Soupart, 1973; Motlik & Fulka, 1974a, b; Thibault *et al.*, 1975a).

In the follicle *in vivo* the rabbit oocyte becomes able to assume immediate changes in sperm nucleus structure and composition only 6 h after an HCG injection or the postcoital LH surge (Thibault & Gérard, 1973). It is striking that this delay is so similar to that necessary for hamster and rat oocytes to become fertilizable (Table 8); such oocytes acquire, in the follicle, both zona sensitivity to spermatozoa and the ability to assume sperm nucleus decondensation. Niwa & Chang (1975) mention abnormality of sperm head transformation when rat oocytes were fertilized *in vitro* after removal from the follicle less than 6 h after the HCG injection. Usui & Yanagimachi (1976), with no reference to previous studies and using zona-free oocytes, confirmed that the male pronucleus growth factor (MPGF) in hamsters only appears *in vivo* about 8 h after HCG injection.

Table 8. Partial maturation *in vivo* and sperm nucleus decondensation of rabbit oocytes (from Thibault & Gérard, 1973)

Time of recovery after HCG (h)	Duration of complementary culture (h)	Sperm head change (%)	
		Abnormal	Normal
2	12	100	0
3	12	100	0
5	12	100	0
7	5	20	80

Do gonadotrophins or steroids mediate these two aspects of oocyte maturation?

Gonadotrophins act directly on cumulus cells (Thibault *et al.*, 1975a; Hillensjö, Dekel & Ahrén, 1976b), changing their metabolic pathways towards pyruvate and lactate formation, increasing the production of progesterone (Nicosia & Mikhail, 1975), enhancing the synthesis of glycoproteins by the Golgi apparatus (Moricaud, 1968) and probably breaking gap junctions. However, gonadotrophins are completely unable to promote cytoplasmic maturation of extrafollicular cumulus-enclosed oocytes, at least in experiments performed with rabbit, cow, hamster and human oocytes.

In contrast, some ovarian steroids, added to the culture to mimic the sequences and levels reached *in vivo* during final follicle maturation, can stimulate sperm nucleus decondensation in human and rabbit oocytes. The clearest results have been obtained for human oocytes by Soupart (1974); when oestradiol-17 β and then 17 α -hydroxyprogesterone were added during maturation of oocytes *in vitro*, decondensation of the sperm nucleus occurred immediately after fertilization.

In our studies, oestradiol and testosterone seem to have a similar action on rabbit eggs, but the results were not consistent (Thibault *et al.*, 1975b).

Because cumulus-enclosed oocytes have been used, there is no evidence for or against a direct effect of steroids on the oocyte itself, as in amphibians. It is evident that only steroids and steroid levels found in the follicular fluid should be taken into consideration. We have recently shown that the LH surge or injection of HCG stimulates a rapid increase of progesterone production with a simultaneous drop in oestradiol in sow follicular fluid, although no increase of progesterone can be detected in the blood plasma (P. Rombauts & J. Bertin, unpublished).

What happens in the follicle?

From the preceding studies *in vitro* it appears that three main events occur in the oocyte during the preovulatory phase. These are resumption of meiosis, cytoplasmic maturation, and maturation of the oocyte membrane.

Culture of the intact follicle has been shown to be a useful method for studying follicular regulatory mechanisms of oocyte maturation. There is general agreement in the recent literature that when carefully dissected follicles are cultured, meiosis resumes in the intrafollicular oocyte if gonadotrophins are added to the culture medium (rat: Tsafirri, Lindner, Zor & Lamprecht, 1972; Hillensjö *et al.*, 1976b; hamster: Gwatkin & Andersen, 1976; Mandelbaum, Plachot & Thibault, 1977; cow: Thibault *et al.*, 1975a, b; rabbit: Thibault & Gérard, 1973; monkey: Thibault *et al.*, 1976; sheep: Miller & Jagiello, 1973; Moor & Trounson, 1977; pig: Daguet, 1977).

Cytoplasmic and membrane maturation of the oocyte are also acquired during the culture of follicles in the presence of gonadotrophins. Hamster intrafollicular oocytes are fertilizable (Mandelbaum *et al.*, 1977) and sperm head swelling is immediate in rabbit intrafollicular oocytes (Thibault & Gérard, 1973). When fertilized, these oocytes develop normally up to the birth of normal young (Thibault *et al.*, 1975a, b). Moor & Trounson (1977) have recently described the birth of lambs from follicular oocytes recovered after culture of large antral sheep follicles in the presence of gonadotrophins. This shows that the antral follicle represents a physiological entity capable of supporting nuclear and cytoplasmic oocyte maturation *in vitro*.

Rat preovulatory follicles have been extensively used in studies on the mode of gonadotrophin action *in vitro*. Meiotic resumption occurs even if steroidogenesis is suspended by the addition of specific steroid inhibitors to culture media (Lieberman *et al.*, 1976). Similarly, the enhancement and change in metabolic pathways induced by gonadotrophins are unrelated to activation of the oocyte nucleus (Lieberman, Ahrén, Tsafirri, Bauminger & Lindner, 1975; Tsafirri, Lieberman, Ahrén & Lindner, 1976; Hillensjö, 1976).

Unfortunately, no recent data are available for the effect of steroidogenic or metabolic blockade on cytoplasmic maturation of the oocyte. Similarly, we do not know if cyclic AMP and PGE-2 sustain both nuclear and cytoplasmic maturation. More refined criteria than GVB must be introduced in subsequent biochemical and molecular research.

Follicular development and oocyte maturation

In the two successful experiments mentioned above, the rabbit (Thibault *et al.*, 1975a) and lamb (Moor & Trounson, 1977) material consisted of oocytes from relatively large antral follicles. Such follicles are present in rather limited numbers in the ovary. It is tempting to induce a larger population of small or medium-sized follicles, but this poses many questions, such as: are these follicles also able to resume meiotic maturation; how can the ovary be induced to provide a physiologically homogeneous population of such follicles; and are present culture techniques suitable for a long prematuration phase *in vitro*, if necessary?

Studies on the principal cytological and biochemical characteristics of the follicle show that follicles of the same size may differ physiologically, mainly with the sexual cycle period. The nature of the steroids secreted in culture (sheep: Seamark, Moor & McIntosh, 1974), FSH and LH titres in the follicular fluid vary (man: McNatty *et al.*, 1975), depending on whether they are collected during the follicular or luteal phase. Thus, the prerequisite is the preparation of an homogeneous population of medium-sized follicles by hormonal treatment. We successfully use a sequential pretreatment on 3-month-old calves and adult macaques (*M. fascicularis*). Basically, these animals receive repeated injections of progesterone followed by PMSG. The percentage of non-atretic follicles increases, and if ovaries are not removed after pretreatment, spontaneous super-ovulation occurs in the calf; a high pregnancy rate after mating for 2 days is the rule in the subsequent cycle of the macaque (Dang, 1975).

From serial experiments developed with 3–4-mm calf follicles cultured by superfusion in synthetic medium (Menezo, Gérard & Thibault, 1976b), in the presence of different combinations of gonadotrophins, we observed that only one-third of the oocytes reached metaphase II and that cumulus cell dissociation seldom occurred. This gonadotrophic response is constant *in vivo* after an LH surge or in preovulatory follicles cultured with gonadotrophin (pig: Daguet, 1977; rat: Hillensjö *et al.*, 1976b; rabbit: Thibault *et al.*, 1975a). Moreover, control culture experiments of extra-follicular oocytes recovered from the same follicular population show both nuclear maturation and a beautiful gonadotrophic reaction of the corona cells. The lack of response when the oocyte and its cumulus are left inside a medium-sized follicle may be due to insufficient penetration of gonadotrophin or more probably to absence of LH receptors, so that the inhibitory control of granulosa cells on the oocyte and cumulus cells is not turned off.

In the macaque, however, control extrafollicular oocytes show the same behaviour as intra-follicular oocytes; many of them remain either at the dictyate stage or in prometaphase and corona cell dissociation is rare and irregular (Table 9). More refined criteria than follicle size must be used for assessment of a physiologically homogeneous follicle population. In the macaque, the incompetence of oocytes from antral follicles, at least during the early follicular phase, raises the question of the importance of changes in the dictyate nucleus before the resumption of meiosis becomes possible. It has been shown in the urodele, *Notophthalmus viridescens* (Barsacchi-Pilone & Humphries, 1975), that shortening of the lampbrush chromosomes, contraction of the chromosomes and nucleoli in the central part of the germinal vesicle, together with migration of the germinal vesicle to the oocyte periphery, necessarily precede meiotic resumption. These processes do not depend on progesterone but are gonadotrophin-dependent. In the carp, the migration of the germinal vesicle to the periphery of the oocyte is also gonadotrophin-dependent, and 17 α ,20 β -hydroxyprogesterone is unable to induce meiotic resumption in oocytes which have not been primed with gonadotrophins (Jalabert, Breton, Brzuska, Fostier & Wieniawski, 1977).

Table 9. Maturation of macaque oocytes *in vitro*

	Total	Dictyate nucleus	MR*	MI	MII	MI + MII %
Extrafollicular oocytes	103	42	17	23	21	43
Intrafollicular oocytes	16	4	2	5	5	60

* Meiotic resumption, early chromosome formation before GVB.

Observations of calf, pig and macaque germinal vesicles seem to show the same preparation of the dictyate nucleus before meiotic resumption can occur. Five days before oestrus in the pig, oocytes in the largest healthy follicles show a typical dictyate nucleus; on the following days the lampbrush loops disappear and condensed chromatin masses progressively aggregate around the central nucleolus. The germinal vesicle reaches the oocyte periphery (Daguet, 1977). This final event has been described by Hunter & Polge (1966) and by Motlik & Fulka (1976) in pro-oestrous sows. It must be remembered that oocytes which are ovulated after HCG injection on Day 17 of the cycle remain in the dictyate stage (Hunter, Cook & Baker, 1976), and this probably means that germinal vesicle change is a prerequisite for resumption of meiosis.

In calves treated with gonadotrophins, oocytes in 3–4-mm follicles are at different stages of germinal vesicle maturation, even up to the time of disappearance of the nucleolus. It is tempting to correlate this heterogeneity with the variable rate of meiotic resumption when such follicles are cultured with gonadotrophins. In macaques treated with gonadotrophins, oocytes in the largest follicles are always at exactly the same dictyate stage, and this perhaps correlates with a very low percentage of meiotic resumption *in vitro* (Thibault *et al.*, 1978).

How follicular cycles *in vivo* can be understood

Using data from the impressive amount of recent research *in vivo* and *in vitro*, I have attempted to make a general plan of the main mechanisms and hormonal interactions involved in follicle maturation and oocyte maturation *in vivo* (Text-fig. 1).

The simplest analyses can be drawn from rodents which have an ovarian cycle without a luteal phase. During pro-oestrus, the rise of oestradiol to a level at least 10 times higher than the basal level has two effects. One, the induction of a gonadotrophin surge, is classical; the other (generally omitted), is the stimulation of a population of preantral follicles by increasing the mitotic rate of granulosa and theca cells (Goldenberg, Vaitukaitis & Ross, 1972; Louvet & Vaitukaitis, 1976) and by multiplying the number of gap junctions between granulosa cells (Merk, Botticelli & Albright, 1972; Albertini, Fawcett & Olds, 1975; Anderson & Albertini, 1976). These next permit passage of cyclic AMP from one cell to the other so that activation of the adenyl cyclase system in a peripheral cell may induce a general response of all the granulosa cells. Subsequently, on Day 1 the FSH surge occurs without an LH surge, and it has been beautifully shown for the hamster that there is a close relationship between the level of FSH (or FSH-like PMSG) at this time and the number of preantral follicles forming antra (Greenwald, 1974; Bast & Greenwald, 1974).

Text-fig. 1. Probable origin and fate of the main follicular waves occurring during the female cycle as related to FSH levels. Hamster FSH from Bast & Greenwald (1974), Bex & Goldman (1975); human FSH from Wide *et al.* (1973); sheep FSH from L'Hermite *et al.* (1972), Salamonsen *et al.* (1973), Hopkinson & Pant (1973), Pant *et al.* (1977). The FSH variations of pseudopregnant rats (Welschen *et al.*, 1975) are similar to those observed in sheep. The hamster follicular waves are according to Greenwald (1974) and Bex & Goldman (1975); the human and sheep follicular wave are as suggested in the present paper. PA → A, transition of preantral to antral follicles; —, FSH changes; hatched area, LH surge. Pro, pro-oestrus; O, oestrus; D₁, D₂, 1st and 2nd day of dioestrus; M, menstruation.

The only role for the FSH surge is the stimulation of preantral oestrogen-primed follicles; anti-FSH serum given on the day of pro-oestrus does not prevent ovulation but postpones formation of antral follicles. By contrast, when an antiserum to LH is given in the same conditions, neither ovulation nor meiotic resumption occur but antral follicles develop (rat: Welschen & Dullaart, 1976). During the following 2 days, FSH levels progressively descend to reach a nadir on the morning of the next day of pro-oestrus (Bast & Greenwald, 1974; Bex & Goldman, 1975), probably because of the cumulative effects of oestradiol and an inhibin-like substance from the growing follicles.

At this time, FSH levels are insufficient to support all preovulatory follicles. If the FSH level is increased artificially by hemicastration or FSH injections to intact animals, atresia is partly prevented, and a higher ovulation rate per ovary follows (rat: Welschen, 1972; Welschen & Dullaart, 1974; hamster: Greenwald, 1962; Grady & Greenwald, 1968; Bex & Goldman, 1975). According to our observations of atresia *in vitro*, we think that the main role of FSH at this critical period is to facilitate increased metabolic exchange, either directly or via oestradiol secretion.

FSH also induces the formation of LH receptors in granulosa cells, and the number of receptors increases tremendously during final growth of the follicle (Channing & Kammerman, 1974; Zelesnik, Midgley & Reichert, 1974). Oestradiol enhances this FSH effect and prepares granulosa cells to luteinize in response to the LH surge. In hypophysectomized rats, LH induces luteinization or atresia regardless of whether oestradiol is present (Richards & Midgley, 1976).

The discovery of the importance of the high gonadotrophin level during the preovulatory surge is fascinating. This high level is absolutely necessary for temporary desensitization of the adenyl cyclase system (rat: Zor, Lamprecht, Misulovin, Koch & Lindner, 1976; rat, rabbit: Hunzicker-Dunn & Birnbaumer, 1976a, b). After a burst of steroidogenesis, consisting mainly of progesterone (Hillensjö, Bauminger & Ahrén, 1976a) because LH secondarily initiates inhibition of 17-20 desmolase and 17 α -hydroxysteroid dehydrogenase, there is a general decrease in the steroid titre in the follicle before ovulation. We have shown previously that oestradiol, 17 α -hydroxyprogesterone or testosterone play a role in the cytoplasmic maturation of human and rabbit oocytes. This is probably the main function of the temporary increase of intrafollicular steroids after the LH surge.

Studies of intrafollicular oocyte maturation *in vitro* have confirmed the importance of a high gonadotrophin level in the medium to prime GVB. Turning now to primates, it is clear that during the follicular phase, gonadotrophin profiles may be superimposed on those observed in rodents with short cycles. The only differences are the duration of the phase (4 versus 12 days) and the absence of high oestradiol levels preceding high FSH levels. It is suggested that the transition from the preantral to the antral stage in the 'wave' of ovulatory follicles occurs at the beginning of the follicular phase. Regulation of ovulation rate by atresia seems to take place 3 days before the LH surge (A. Gougeon, personal communication). It is probable that a secondary 'wave' of follicles is induced at mid-cycle after the oestradiol and FSH surges, and the resulting antral follicles undergo atresia before the next 'wave' of ovulatory follicles occurs.

Finally, if we look at the sparse data on domestic mammals, partial overlapping of the luteal and follicular phases renders analysis and comparison more difficult. The presence of a permanent population of variable-sized follicles at all stages of the cycle reflects the regular follicular outflow from the 'pool' of primordial follicles; this population obscures the real lineage of the ovulatory follicles. As we are unable to identify key mechanisms, I shall try to produce an hypothesis which fits the preceding description of events. As in rodents, the 'wave' of development of antral follicles occurs at oestrus as a result of an oestradiol-FSH surge. However, because the corpus luteum is active, stimulated follicles become atretic after a rise in oestradiol secretion before mid-cycle. Greater efficiency of PMSG-prostaglandin treatment at this time of the cycle seems to indicate that PMSG can 'rescue' such follicles.

It has been postulated that the preovulatory follicles are derived from the second 'wave' of growth which occurs at mid-cycle. This assumption has no experimental basis, although there are some supporting data for this hypothesis. If a relatively high level of FSH is the necessary gonadotrophic stimulus for the transition from preantral to antral follicle, the FSH profile in sheep during the oestrous cycle indicates that this stimulation can occur when FSH returns to high levels around Day 7-8 of the cycle, i.e. approximately 1 week before the gonadotrophin surge at oestrus. The titre

of oestradiol in large follicles is also higher at this time than during the following days, and blood levels increase simultaneously. In the cyclic cow also, there are two periods of relatively high levels of FSH, at Days 4–9 and Days 16–21. However, it is not possible at present to establish any correlation between these profiles and waves of follicular growth, especially as the profiles differ profoundly from one cow to another (Schams & Schallenberger, 1976). It is tempting to ask whether these variations are related to the different degrees of superovulation after PMSG treatment.

It is rather difficult to obtain morphological information from papers dealing with follicle populations during the cycle because most authors follow the fate of follicles larger than those of most interest, i.e. those in transition from the preantral to the antral stage which have a diameter of about 200–300 µm in sheep and cattle (see Hay & Moor, 1975).

It has been shown that superovulation is induced in sheep when PMSG (Thibault, Ortavant & Laplaud, 1948) or an LH-RH analogue (Findlay & Cumming, 1976) is injected 3–4 days before oestrus. This increased ovulation rate may be attributed to the preservation of some preovulatory follicles which would otherwise have become atretic rather than to the stimulation of transition from the preantral to the antral stage; PMSG given 3–4 days before ovulation in ruminants would play the same role as PMSG treatment on the 2nd day of dioestrus in the hamster. Recent observations on the pig which support this interpretation must be mentioned. After methallibure treatment, follicles grow for 5 days and then ovulate around Day 6. Atresia affects some of the growing follicles around Day 2 (Daguet, 1977), a situation very similar to that occurring on the evening of dioestrus 2 in the hamster. It is interesting to remember that Hunter *et al.* (1976) observed superovulation after a single HCG injection only when this hormone was given on Day 17 of the cycle. This would be equivalent to the time (2–3 days) after methallibure treatment before partial atresia began. It seems highly likely, therefore, that in domestic ruminants ovulatory follicles leave the preantral stage approximately 1 week before the oestrous LH surge, and that final regulation of ovulation rate takes place by atresia 3–4 days before oestrus.

Conclusions

As far as meiotic resumption is concerned, oocyte competence is acquired long before the preovulatory gonadotrophin surge, before antrum formation (rat: Erickson & Ryan, 1976), immediately afterwards (mouse: Erickson & Sorensen, 1974), or later on (pig: Hunter *et al.*, 1976).

Competent oocytes resuming meiosis when isolated from the follicle are able to cleave after parthenogenetic activation (rabbit: Thibault, 1972; cow: Menezo *et al.*, 1976a). However, protein synthesis does not change during oocyte meiotic resumption *in vitro* in the same way as it does intrafollicularly (Warnes, Moor & Johnson, 1977).

When considering the ability of the oocyte to associate with the male gamete, to recognize its enzymes or to support its participation, it must be remembered that the oocyte remains dependent on the follicle almost up to the moment of follicle rupture. This role of the follicle is probably achieved *in vivo* by a classical sequential steroid action, when the LH surge dramatically changes the intra-follicular steroid content.

In the ovary, cyclic changes in oestradiol and FSH levels stimulate one or two waves of follicular growth. *In vivo* FSH and oestradiol play a major role in the regulation of ovulation rates (a) during the transition from the preantral to the antral stage and (b) some days before ovulation, when atresia reduces the number of preovulatory follicles. The ovulation of increased numbers of completely matured oocytes, or the preparation of a large population of LH-sensitive follicles for maturation *in vitro*, needs a better understanding of follicular cycles. An approach is suggested in rodents, ruminants and primates.

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