Interrelationships between the oocyte and somatic cells within the Graafian follicle of mammals

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Summary. Factors involved in the maturation of the oocyte and especially in the resumption of meiosis following the pre-ovulatory surge of gonadotrophin have been reviewed for a number of mammals. Follicular size has been shown to be an important physiological regulator of meiotic potential in several species, although when the oocytes are released into a chemically defined culture medium the influence of follicular diameter may be lost and meiosis can resume spontaneously in a high proportion of oocytes. Turning to studies of Graafian follicles in culture, a contrast was drawn between the requirements for meiotic maturation in oocytes of laboratory rodents and those in larger domestic species and women : gonadotrophins and added steroids seem necessary to facilitate maturation in the latter group. This finding is in accord with recent work on a porcine experimental model in which the process of HCG-induced ovulation can be dissociated from nuclear, cytoplasmic and membranous components of oocyte maturation when oestrogen concentrations in the follicular fluid are « low ». However, the precise mode of action of intra-follicular oestrogens on the somatic cells (and on the oocyte ?) remains to be clarified. A possible involvement of prolactin in these follicular events is also considered.

Introduction.

The female mammal is born with a finite stock of germ cells which reach the diplotene (arrested) stage of meiotic prophase either by the time of, or shortly after birth (e.g. see Baker, 1971). Irrespective of age or the stage of the reproductive cycle, about 90 p. 100 of these arrested oocytes are enclosed within primordial follicles consisting of a single layer of granulosa cells. The early stages of follicular growth are independent of gonadotrophic support, although the precise regulatory processes involved remain obscure (Zuckerman and Baker, 1977). The subsequent development of the antral follicle requires an adequate supply of follicle stimulating hormone (FSH) followed by the trophic influence of luteinizing hormone (LH). These hormones cause the expansion of the antrum and maturational changes in the theca and granulosa cells, although the oocyte nucleus remains morphologically unaffected until the onset

of the so-called « LH-surge ». FSH and LH — either directly or indirectly through steroid intermediates (especially œstrogen) — also regulate the proportion of follicles which undergo ovulation or atresia (e.g. Harman *et al.* 1975 ; Hay and Cran, 1978).
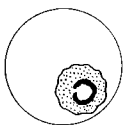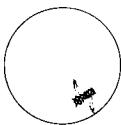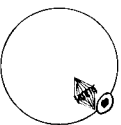
There can be little doubt that the onset of pre-ovulatory maturation of the oocyte and its follicle *in vivo* is related to the « LH-surge », although the precise sequence of events that follows this stimulus remains to be established. The purpose of the present communication is to discuss the available experimental evidence derived largely from studies of rodent, sheep, pig and primate follicles both *in vivo* and in organ culture.

Evidence from *in vivo* studies

The period of meiotic arrest at the diplotene stage of first prophase persists until a time (which is constant for each species) shortly before ovulation when a surge of gonadotrophic hormones induces germinal vesicle breakdown (GVB) (table 1). It should be noted, however, that gonadotrophins are only required when the oocyte resides within its follicle : when oocytes are removed into a chemically defined medium they resume meiosis spontaneously to metaphase II (but see below).

TABLE I

Time-response in hrs of the oocyte nucleus to the LH surge or a systemic injection of LH/HCG

Species	Latent Period After Stimulus	M_1	A_1	T_1	M_2	Ovulation
Rabbit	3 – 4	5 – 8	9	9 ½	10	10 – 12
Sheep	10 – 11	12 – 20	21	22	24	25 – 26
Pig	17 – 18	26 – 34	35	36	37	40 – 42
Human	18 – 20 ?	25 – 35	36	37	38	40 ?

It is well established that the pre-ovulatory « surge » of gonadotrophins above the tonic values found in plasma during the rest of the reproductive cycle consists of both FSH and LH, although the levels of LH are by far the highest (e.g. Speroff and Vande Wiele, 1971 ; Schwartz, 1974). Yang and Papkoff (1973) have shown that PMSG, HCG, FSH and LH can all induce ovulation in intact rats, and the activity of

FSH in this context is not due to contamination with LH : treatment of LH with anti-serum to the β-sub-unit of LH abolishes its effect on oocyte maturation and ovulation whereas the influence of FSH remains unaffected by this antiserum (Yang and Papkoff, 1973 ; Lindner et al., 1974).

The effect of treatment with gonadotrophins on pre-ovulatory maturation in mice is dependent upon the size of the follicle. The stage of meiosis attained by the oocyte varies from germinal vesicle stage (pre-antral and abnormally large Graafian follicles) through metaphase or anaphase I (small antral follicles) to metaphase II (only when the oocyte resides in a mature Graafian follicle) (Baker and Neal, 1972). When oocytes are removed from their follicles into chemically defined media, the oocyte resumes meiosis only if it is derived from an antral follicle (e.g. Sorensen and Wasserman, 1976). This implies that the follicle undergoes a process of maturation which affects the ability of its oocyte to undergo meiosis.

Further evidence for the maturation of somatic components of the follicle influencing the oocyte derives from an experiment in which mature pigs were injected with HCG either on the day preceding the « LH-surge » (day 20 of the cycle), or when the follicle is much smaller and immature (day 17). Injection of HCG on day 20 results in the ovulation of normal oocytes at metaphase II which, when exposed to spermatozoa, show the normal block to polyspermy and full development of a single male and female pronucleus. By contrast, injection of pigs on day 17 of the 21 day œstrous cycle induces ovulation of immature eggs at the germinal vesicle stage : these oocytes

RESPONSE TO A SINGLE INJECTION OF GONADOTROPHIN
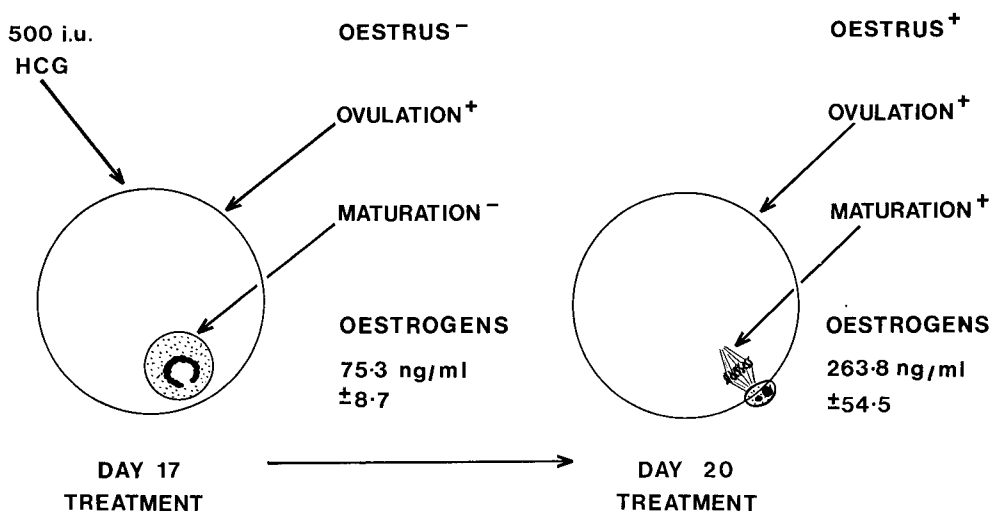IN EARLY OR LATE FOLLICULAR PHASE

FIG. 1. — A semi-diagrammatic illustration of ovarian and behavioural responses of mature pigs to a single injection of HCG given at different times of the oestrous cycle. Animals treated on day 17 ovulate in response to the injection, but the oocytes so released are completely immature (see text), the animals fail to show oestrus (lordosis), and the concentration of oestrogens in the follicular fluid is depressed compared with that following treatment on day 20 (After Hunter et al., 1976.)

show no block to polyspermy, sperm heads in the vitellus do not swell to form male pronuclei, and the cumulus cells remain immature and spherical in shape (Hunter, Cook and Baker, 1976). The immaturity of the follicles on day 17 of the cycle is not confined to the size of the follicle and the shape of cumulus cells : the concentrations of œstrogens in follicular fluid are only one third of those in control animals or those injected with HCG on day 20 (fig. 1). Thus these results show clearly that there are a number of cellular and endocrine events occurring simultaneously in the follicle during pre-ovulatory maturation, only *one* of which is the resumption of meiosis in the oocyte (fig. 1).

Maturation of Graafian follicles in organ culture

Studies of isolated mouse ovaries, or of rat Graafian follicles treated *in vitro*, have clearly indicated that both FSH and LH can induce pre-ovulatory maturation of the follicle, although in the absence of these supplements to the culture medium, resumption of meiosis in oocytes is a rare event (Baker and Neal, 1972 ; Lindner *et al.*, 1974). Indeed, FSH is more effective than LH (Neal and Baker, 1975) and a number of substances can mimic the action of gonadotrophins (prostaglandin E2, PGF2 α, cyclic AMP, etc..., but not prolactin : see Lindner *et al.*, 1974 ; Neal *et al.*, 1975, 1976 ; McNatty *et al.*, 1976). It is often suggested that steroid hormones are the intermediates affecting the oocyte or its cumulus oophorus (Schuetz, 1977), although there is evidence that steroids do not have to be *produced* in response to the « LH-surge » since amino-glutethimide and cyanoketone do not block LH-induced oocyte maturation (Lindner *et al.*, 1974). The possibility remains, however, that the high levels of steroids in the follicle prior to the « LH-surge » are sufficient to act as intermediates without the necessity for further steroid synthesis (Baker *et al.*, 1977).

By contrast, neither FSH nor LH seem to be able directly to induce the resumption of meiosis in oocytes within sheep, pig or human Graafian follicles maintained in organ culture (Baker and Neal, 1974 ; Hay and Moor, 1975 ; Baker *et al.*, 1975 ; Biggs, Baker and Hutcheon, 1977). It seems likely, therefore, that the physiological requirements for oocyte maturation in these species may be somewhat different to those in rodents and lagomorphs (see above ; also Thibault *et al.*, 1975). The lack of an *in vitro* action of gonadotrophins in ungulates and primates may be due to an inadequate number of binding sites since prostaglandin E2 (which like LH acts on the adenyl cyclase system) can induce the resumption of meiosis in follicle-enclosed porcine oocytes (table 2). However, cyclic-AMP itself was without effect and it is more difficult to explain the action of prostaglandin F2 α (table 2).

In porcine and ovine Graafian follicles maintained in organ culture, meiotic maturation of oocytes can be induced if FSH, LH and œstradiol-17 β are administered simultaneously (Moor and Trounson, 1977 ; see also table 2). There can be no doubt that the LH component is required for steroidogenesis although the FSH may promote the formation of suitable binding sites in the granulosa cells. Whether it is the FSH or LH which « triggers » the resumption of meiosis within the oocyte remains uncertain, although it is tempting to draw parallels with the results for mouse follicles (see above). The precise role of follicular œstrogen is also obscure : it is conceivable that it is an intermediate and augments the action of the FSH or LH in the culture

system : or it may compensate for an inadequate synthesis of steroid by the follicle *in vitro*. It is interesting to note that high levels of œstradiol-17 β are found in the antral fluid of human Graafian follicles *in vivo* (McNatty *et al.*, 1975), although the levels decline shortly before the onset of the « LH-surge » when progesterone is accumulated in the fluid.

TABLE 2

Stage of meiosis attained following treatment of pig Graafian follicles * in organ culture ** with various hormones. Oocytes were recovered 24-48 h after onset of culture and examined with phase contrast optics ***

Stage of cycle	Number of animals	Treatment	Nuclear status at onset of culture	No follicles cultured	No. oocytes recovered	Nuclear status of oocytes at end of culture
pro-œstr. ..	12	none	GV	103	87	GV or atretic
pro-œstr. + HCG (1-2 h)	4	none	GV	35	32	30 GV, 1 MI, 1 atretic
25 h post-HCG	1	none	MI	12	11	10 MI, 1 TI
25 h post-HCG	1	dibutyryl C-AMP	MI	7	7	1 GV, 6 MI
25 h post-HCG	3	PGF2 α	PM/MI	28	28	3 PM, 20 MI, 5 MII
25 h post-HCG	3	PGE2	PM/GV	11	11	5 GV, 2 PM, 4 MI
late pro-œstrus ...	4	prolactin antiserum	GV/PM	28	26	12 GV, 4 PM, 4 MI, 6 MII
23 h post-HCG	1	prolactin antiserum	PM	9	9	6 MI, 3 MII
late pro-œstrus	3	100 µg FSH 60 µg LH 60 µg OE2	GV	46	44	20 GV, 6 MI, 18 MII
late pro-œstrus	2	30 µg FSH 10 µg LH 10 µg OE2	GV	20	20	19 GV, 1 MI
late pro-œstrus	1	60 µg FSH 100 µg LH 1 µg OE2	GV/MI	10	10	3 MI, 1 PM, 2 GV, 2 AI, 2 MII
late pro-œstrus	1	100 µg FSH 30 µg LH 10 µg OE2	GV	5	5	2 GV, 2 MI, 1 MII

GV = germinal vesicle stage ; PM = pro-metaphase ; MI = metaphase I ; AI = anaphase I ; MII = metaphase II.

* Individual follicles measuring 6-10 mm diameter were set up in organ culture within 1 h of ovariectomy performed on day 20 or 21 of the œstrous cycle. When administered, HCG was always injected in pro-œstrus.

** The method used for organ culture has been described elsewhere (Baker, Hunter and Neal, 1975). Follicles presumed to contain oocytes at the GV stage were cultured for 36-40 h.

*** Oocytes were dissected from the follicles, denuded with hyaluronidase, fixed and cleared in 25 p. 100 acetic alcohol, and stained with 0.5 p. 100 orcein in 45 p. 100 acetic acid.

Perhaps the most significant change in the composition of human follicular fluid at the time of the « LH-surge » is a significant and sharp decline in the concentration of prolactin (McNatty *et al.*, 1975). It is therefore noteworthy in the present results that an antiserum to prolactin (table 2), when added to porcine Graafian follicles in organ culture, apparently induced meiotic maturation in the oocyte. It is doubtful that prolactin itself plays the role of an inhibitor of oocyte maturation, or of the action of gonadotrophic hormones, but it might prevent the availability of LH-binding sites or induce the binding to protein of intra-follicular steroids. On the other hand, prolactin may play a purely passive role, its elimination from the follicular compartment normally reflecting a change in the permeability of the basement membrane of the follicle, seemingly induced by LH.

Even if prolactin is not in itself an inhibitor of oocyte maturation, it may be involved in the regulation of the inhibitor substance believed to be produced by the granulosa cells (Tsafriri and Channing, 1975). The inhibitor is said to be produced when the follicle is immature, while another protein (antagonist) which nullifies the effects of the inhibitor is seemingly produced in response to gonadotrophins at their surge levels. This speculation, and the possible involvement of prolactin and/or LH in the control of the process, needs to be investigated further by means of carefully controlled experiments *in vivo* as well as in organ culture. However, a potential inhibitor has recently been recovered from follicular fluid and this substance appears not to be species specific (Tsafriri, 1978).

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Résumé. Nous avons passé en revue, chez plusieurs mammifères, les facteurs physiologiques qui gouvernent la maturation de l'ovocyte et, en particulier qui provoquent la reprise de la méiose après la décharge ovulante. La taille du follicule est un facteur limitant très important chez plusieurs espèces, bien que lorsqu'on extrait les ovocytes pour les placer dans différents milieux de culture classique, l'influence du diamètre folliculaire ne semble plus jouer, puisqu'une forte proportion des ovocytes reprennent spontanément leur méiose. Dans les follicules de de Graaf, cultivés dans des conditions variables, les conditions pour la reprise de la méiose chez les rongeurs diffèrent de celles nécessaires pour les animaux domestiques et l'homme. Pour ces derniers, les gonadotropines et aussi des stéroïdes tel que l'estradiol, semblent nécessaires pour provoquer la maturation. Il est rappelé la dissociation observée, chez la truie, entre l'ovulation obtenue par l'injection d'HCG au 17e jour du cycle et la reprise de la méiose qui ne se produit pas à ce moment du cycle quand la teneur en estradiol intrafolliculaire est basse. Le rôle possible de l'estradiol sur les cellules somatiques (ou sur l'ovocyte ?) reste à préciser. Une contribution de la prolactine doit aussi être considérée.

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