RESPONSE OF MOUSE OVARIES *IN VIVO* AND IN ORGAN CULTURE TO PREGNANT MARE'S SERUM GONADOTROPHIN AND HUMAN CHORIONIC GONADOTROPHIN

I. EXAMINATION OF CRITICAL TIME INTERVALS

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(Received 12th April 1972)

Summary. A series of experiments was carried out to assess the effects of HCG *in vivo* and in organ culture, on mouse oocyte and follicle maturation following PMSG treatment *in vivo*. Preovulatory maturation and its relationship to time of injection and duration of hormone treatment was assessed using quantitative histological techniques.

The general pattern and timing of meiotic changes was similar in organ culture to that occurring in the intact animal, although fewer oocytes reached metaphase II and ovulation was a rare event. This deficiency in the cultured ovaries was related to the destruction of Graafian follicles during the preparation of explants.

The stage of meiosis reached by the oocyte was largely a factor of critical time intervals, but the size of the follicle also had an important limiting effect. Thus, medium-sized and small antral follicles contained oocytes whose meiotic maturation was seemingly 'blocked' at metaphase I, while Graafian follicles either reached metaphase II or persisted at the dictyate stage. These results are discussed in the light of current knowledge of oocyte/follicle cell interactions and the mode of action of gonadotrophic hormones.

INTRODUCTION

Gonadotrophic hormones have long been used for the induction of ovulation in intact and hypophysectomized rats and mice. Injections of FSH induce proliferation of follicle cells and thus increase the number of 'growing' follicles within the ovary. Subsequent treatment with either LH or HCG causes pre-ovulatory maturation affecting the oocyte and its follicle, which eventually ruptures at ovulation (e.g. Rowlands & Williams, 1943; Rowlands, 1944; Edwards & Gates, 1959). The number of secondary oocytes (at metaphase II) that are ovulated is dependent upon: (i) the age of the animal, (ii) which inbred or outbred strain is used, and (iii) the doses of FSH and LH (or HCG) that are

selected (Zarrow & Wilson, 1961). In practice, PMSG is often substituted for FSH since it is as effective in inducing superovulation and at suitable dose levels displays FSH- and LH-like activities (Zarrow, Caldwell, Hafez & Pincus, 1958).

The development of suitable techniques for the culture of whole ovaries or of ovarian fragments has enabled the precise effects of hormones to be assessed in the absence of those due to the animals' own endocrine organs (Fritz, Cho & Biggers, 1965; Baker & Neal, 1969; Ryle, 1969, 1970). Injections of PMSG and HCG have been used to induce preovulatory maturation and even ovulation of some follicles of mice: the changes observed are broadly similar to those occurring spontaneously in intact animals (Fritz *et al.*, 1965; Baker & Neal, 1972).

The purpose of the present investigation was to assess, both *in vivo* and in organ culture, the optimal time interval between PMSG and HCG injections and also the timing of oocyte maturation in relation to HCG treatment in the mouse.

MATERIALS AND METHODS

Mice of the Schofield albino strain aged 6 to 8 weeks *post partum* were used in the present study. They were randomly selected for stage of the oestrous cycle since it has been reported that this has little effect on the response of mice to gonadotrophic hormones at the doses selected (see Edwards, Wilson & Fowler, 1963).

The hormones used to induce superovulation were Pregnyl and Gestyl (HCG and PMSG, respectively; Organon Laboratories, London). They were dissolved in Eagle's medium (Flow Laboratories, Irvine, Scotland) to a concentration of 20 i.u./ml (PMSG) or 10 i.u./ml (HCG). The doses of hormones selected for injection (5 i.u. PMSG and 2 i.u. HCG), and for addition to the cultures (0.4 i.u./ml), were those shown in previous experiments to elicit a suitable response *in vivo* and *in vitro* for this strain of mouse (Baker & Neal, 1972).

The experiments were designed to investigate four possible sources of variation in the response of ovaries to gonadotrophic hormones.

1. The effect of varying the duration of PMSG treatment on the subsequent response of ovaries. Mice were treated with PMSG for 32, 40 or 48 hr, after which their ovaries were either fixed for histology or received HCG *in vivo* or *in vitro* for 24 hr.

2. The number of oocytes undergoing maturation at various times after treatment *in vivo* with HCG, which the mice received 48 hr after injection with PMSG. Their ovaries and associated oviducts were then fixed for histology at various intervals up to 60 hr later.

3. Assessment of the number of follicles destroyed when ovaries are cut into fragments for organ culture. Mice were killed 48 hr after injection with PMSG and their ovaries were fixed either intact or after being cut into pieces of approximately 1 to 2 mm³ in size.

4. The number of oocytes undergoing maturation at various times after treatment with HCG *in vitro*. Mice were killed 48 hr after injection with PMSG

and their ovaries were cultured with HCG for periods of up to 60 hr before fixation.

Procedure for organ culture

Ovaries, removed aseptically from freshly killed mice, were dissected free of adjoining tissue and cut into pieces of approximately 1 to 2 mm³ in size. All the fragments from one ovary were placed on a Millipore membrane filter supported on a stainless steel expanded metal platform in a plastic Petri dish. The technique used for organ culture has been published fully elsewhere and only the essential details will be presented here (see Baker & Neal, 1969). Eagle's medium, supplemented with calf serum (to 20%), glutamine, antibiotics and hormones (see above), was added to the Petri dishes at the onset of culture. At the end of the period of culture, the ovarian fragments were transferred to slips of card to which they adhered. By this means, all fragments derived from one ovary were kept together during their passage through various histological procedures. In the rare event of fragments being lost, the entire specimen was discarded, thus ensuring the validity of the quantitative procedures.

Histology

Ovarian fragments from organ culture, and also ovaries and oviducts from mice treated with hormones *in vivo*, were fixed in Bouin's aqueous fixative. They were subsequently dehydrated with ethanol, cleared in methyl salicylate, and embedded in paraffin wax. Serial sections cut at 5 to 7 μ m were stained with Harris's haematoxylin and eosin and mounted with DePeX.

The development of follicles and maturation changes in their oocytes was assessed histologically for each of the four treatment groups employed (see p. 400). Counts were made of those follicular oocytes in which meiosis had progressed beyond the dictyate stage. In the case of superovulated intact mice, the number of eggs in each oviduct was also recorded. Oocytes were arbitrarily classified into the four groups used in our previous study (Baker & Neal, 1972):

- 'Metaphase I' Included all oocytes in which no polar body could be detected (metaphase I, anaphase I, telophase I) but excluded those at the dictyate and 'germinal vesicle' stages.
- 'Metaphase II' Follicular oocytes with a distinct polar body and chromosomes arranged on a metaphase spindle.
- 'Ovulated eggs' Oocytes recovered from the oviducts.
- 'Free eggs' Seemingly 'ovulated' oocytes recovered from Petri dishes after organ culture procedures.

'Free eggs' were only occasionally observed and their incidence was insufficient to warrant inclusion as a separate group in the quantitative results: they were merely added to the total for 'Metaphase II'.

RESULTS

Histology

Response of intact mice to PMSG. Medium-sized follicles with three to five layers

of granulosa cells (Pl. 1, Fig. 6) are relatively constant in number irrespective of the length of time after injection with PMSG. The majority remain normal in appearance, although some undergo degeneration. By contrast, the population of follicles with antra (fluid-filled cavities) increases from the 7th hr onwards so that by 17 hr, numerous Graafian follicles are observed (Pl. 1, Fig. 1). Preovulatory maturation changes occur in a small proportion of the Graafian follicles: the oocyte passes through meiosis to metaphase II, the cumulus cells become spindle-shaped with their nuclei arranged distally to the oocyte (Pl. 1, Fig. 2), and the follicle develops a stigma in preparation for ovulation (see below). However, the appearance of the majority of the Graafian follicles remains unchanged up to 48 hr after treatment with PMSG: the oocyte persists at the dictyate stage and the cumulus cells remain round in form (Pl. 1, Fig. 1).

The size of the follicle often provides little indication as to the stage of maturation attained by the oocyte within it. Follicles with five layers of granulosa cells and those showing signs of antrum formation may contain oocytes at the dictyate stage of meiotic prophase or at metaphase I (Pl. 1, Figs 5 and 6). Although oocytes at metaphase II are occasionally seen in such follicles, their size appears in some way to be a limiting factor which inhibits further maturation of the oocyte. Similarly, oocytes in large antral follicles may persist at the dictyate stage (even after treatment with HCG), may only reach metaphase I, or they may be ovulated after progressing to metaphase II.

The appearance of oocytes in the oviducts of some intact mice indicates that ovulation occurs at three different times. The first 'crop' of oocytes seems to be ovulated before treatment with PMSG in response to the animal's own hormones: by 7 hr after injection, the oocytes show marked degenerative changes including fragmentation and loss of cumulus cells. The second 'crop' of eggs at metaphase II is ovulated in response to the injection of PMSG which acts on Graafian follicles present at the onset of treatment. These oocytes are normal in appearance when examined 20 to 32 hr after injection, but subsequently undergo degeneration before the HCG-induced ovulation occurs. The third 'crop' of oocytes is ovulated in response to injection of HCG. Thus, 48 hr after the PMSG treatment, these oocytes are still at the dictyate stage of meiotic prophase and are enclosed within Graafian follicles. Preovulatory maturation

EXPLANATION OF PLATE 1

Photomicrographs of histological sections of mouse ovaries treated with PMSG and HCG *in vivo*.

FIG. 1. Graafian follicle enclosing an oocyte at the dictyate stage of meiotic prophase (11 hr after HCG). $\times 230$.

FIG. 2. Oocyte at anaphase. Note the detachment of the cumulus from the membrana granulosa (11 hr after HCG). $\times 230$.

FIG. 3. Oocyte showing extrusion of the polar body 11 hr after treatment with HCG. $\times 230$.

FIG. 4. Oocytes at metaphase II within the oviduct. The pycnotic cumulus cells are typical of those surrounding such eggs (24 hr after HCG). $\times 230$.

FIG. 5. Small antral follicle with a normal oocyte at metaphase I (48 hr after HCG). $\times 230$.

FIG. 6. Preantral follicle enclosing an oocyte undergoing extrusion of the polar body (48 hr after HCG). $\times 470$.

PLATE 1

PLATE 2

(Facing p. 403)

commences after injection of HCG and large numbers of oocytes at metaphase II appear in the oviducts some 12 to 18 hr later.

Response of ovaries to PMSG and HCG in vivo. At the onset of treatment with HCG, the large Graafian follicles contain oocytes at the dictyate stage (Pl. 1, Fig. 1). Germinal vesicle breakdown rapidly ensues and the oocytes enter metaphase I from 6 to 11 hr after injection. During this period, some oocytes progress to metaphase II and their cumulus cells become spindle-shaped (Pl. 1, Fig. 2). The oocyte within its cumulus oophorus subsequently becomes detached from the membrana granulosa (Pl. 1, Fig. 3), after which it floats within the follicular fluid until ovulation ensues. Ovulated oocytes at metaphase II accumulate in the oviducts from 11 to 18 hr after injection of HCG (Pl. 1, Fig. 4). By the 48th hr, these oocytes show obvious signs of degeneration, including the loss of corona radiata cells. Some of the oviducts are devoid of oocytes by this time, although seemingly active CL are evident within the corresponding ovaries.

With the completion of the process of ovulation some 18 hr after the HCG injection, the Graafian follicles that remain in the ovary contain oocytes largely at the dictyate stage or at metaphase I. These follicles seem to persist for long periods, although some undergo degeneration. Medium-sized follicles are also normal in appearance and by 30 hr after injection with HCG, the number of granulosa cells undergoing mitosis appears to increase.

Effect of cutting the ovary into fragments. The procedure for cutting the ovary into explants of about 1 to 2 mm³ in size results in the destruction of most of the large Graafian follicles (Table 5). The few that remain generally contain oocytes at the dictyate stage, while the smaller antral and preantral follicles enclose oocytes at the dictyate stage or metaphase I.

Response of ovaries to HCG in organ culture. Apart from the marked reduction in the numbers of Graafian follicles mentioned above, the appearance of the explants at varying intervals of culture with HCG is similar to that of ovaries from intact mice. Thus, 6 hr after HCG treatment, oocytes in antral follicles have generally progressed from the dictyate stage to metaphase I while by 12 hr, the majority are at metaphase II (Pl. 2, Figs 7 to 10). As with ovaries stimulated with hormones *in vivo*, many of the medium-sized follicles contain oocytes at metaphase I, the further maturation of which appears to be blocked (Pl. 2, Figs 11 and 12).

EXPLANATION OF PLATE 2

Photomicrographs of histological sections of mouse ovaries treated with PMSG *in vivo* and HCG in organ culture.

FIG. 7. Graafian follicle enclosing an oocyte at the dictyate stage of meiotic prophase recovered 18 hr after treatment with HCG. $\times 230$.

FIG. 8. Oocyte at metaphase I within a large Graafian follicle (11 hr after HCG). $\times 230$.

FIG. 9. Oocyte at anaphase I (24 hr after HCG treatment). $\times 470$.

FIG. 10. Graafian follicle containing an oocyte which has progressed to metaphase II (18 hr after HCG). $\times 470$.

FIG. 11. Medium-sized follicle enclosing an oocyte at metaphase I (24 hr after HCG). $\times 470$.

FIG. 12. Oocyte at metaphase I in a small antral follicle. Note the normal appearance of the ovarian tissue 48 hr after the onset of HCG treatment. $\times 230$.

The appearance of the large antral follicles changes little between the 12th and 18th hr of culture. Although the oocytes reach metaphase II of meiosis, the changes in the follicle that are usually associated with ovulation (formation of the stigma; change in shape of cumulus cells) either fail to occur, or are poorly developed (Pl. 2, Fig. 10). 'Ovulation' appears to be a rare event in the cultured ovaries: preovulatory follicles persist for up to 48 hr before eventually undergoing atresia. Those Graafian follicles in which a stigma develops are sometimes ruptured and devoid of oocytes. In such cases, oocytes at metaphase II can usually be recovered from the Petri dishes at the end of culture.

Sixty hours after the onset of culture with media containing HCG, the ovarian fragments contain numerous preantral follicles, at least some of which remain normal in appearance. By contrast, most of the antral follicles are undergoing atresia.

Table 1. The effect of time after a single injection of PMSG on the number of oocytes maturing in mice

Time after PMSG (hr)	Ovaries/group	Oviducts containing eggs	No. of oocytes maturing/ovary*			
			Metaphase I	Metaphase II	Ovulated	Total
7	6	2	2.3 ± 0.5	3.7 ± 0.8	1.0 ± 0.8	7.0 ± 0.7
17	10	2	2.8 ± 0.8	1.2 ± 0.5	1.1 ± 0.7	5.1 ± 1.1
24	10	10	3.2 ± 0.7	2.5 ± 0.5	4.5 ± 0.7	10.2 ± 1.5
32	8	8	3.1 ± 0.4	3.4 ± 1.0	6.2 ± 0.5	12.8 ± 1.3
40	12	6	6.2 ± 1.2	1.4 ± 0.5	3.2 ± 1.3	10.8 ± 1.8
48	12	6	12.5 ± 1.6	2.8 ± 0.7	3.4 ± 1.2	19.5 ± 2.6

* No. of oocytes maturing/ovary expressed as mean ± S.E.

Table 2. Number of oocytes maturing *in vivo* in mice: variable duration of PMSG treatment followed by HCG for 24 hr

Duration of PMSG stimulation (hr)	Ovaries/group	Oviducts containing eggs	No. of oocytes maturing/ovary*			
			Metaphase I	Metaphase II	Ovulated	Total
32	14	10	10.1 ± 1.8	3.6 ± 0.8	4.5 ± 1.5	18.3 ± 3.2
40	14	13	8.6 ± 1.6	3.9 ± 1.1	8.6 ± 1.2	21.1 ± 2.3
48	12	10	10.2 ± 1.7	2.9 ± 0.6	7.0 ± 1.1	20.1 ± 2.2

* No. of oocytes maturing/ovary expressed as mean ± S.E.

Quantitative results

Response of intact mice to PMSG. The number of oocytes at metaphase I remains fairly constant up to 32 hr after treatment with PMSG, following which it rises sharply (Table 1). By contrast, oocytes which have progressed to metaphase II reach peak numbers by 32 hr and the number of ovulated eggs within the oviducts closely parallels this (20 to 32 hr after PMSG treatment).

In terms of the subsequent response of the ovary to HCG *in vivo*, an interval of 40 to 48 hr between injections of PMSG and HCG is more effective than one

of 32 hr (Table 2). However, this effect is less apparent when the ovaries receive HCG in organ culture (Table 3).

Response of ovaries to PMSG and HCG in vivo. Injection of HCG some 48 hr after treatment with PMSG is followed by a decline in the population of oocytes at metaphase I while the number of oocytes at metaphase II increases (Table 4). By 11 hr after treatment, oocytes at metaphase II reach peak proportions and ovulation ensues within the subsequent 12 hr (Table 4). Ovulation is accompanied by a sharp decline in the number of follicular oocytes at metaphase II.

Table 3. Number of mouse oocytes maturing *in vitro*: variable duration of PMSG treatment *in vivo* followed by HCG *in vitro* for 24 hr

Duration of PMSG stimulation (hr)	Ovaries/group	Ovaries containing oocytes at metaphase II	No. of oocytes maturing/ovary*		
			Metaphase I	Metaphase II	Total
32	12	10	12.8 ± 1.8	4.3 ± 1.2	17.2 ± 2.3
40	8	7	15.6 ± 1.8	3.5 ± 1.5	19.1 ± 2.8
48	11	11	16.0 ± 2.0	4.2 ± 0.7	20.2 ± 2.5

* No. of oocytes maturing/ovary expressed as mean ± S.E.

Table 4. The effect of time on the number of mouse oocytes maturing in response to HCG administration *in vivo* after pretreatment with PMSG

Time after HCG (hr)	Ovaries/group	Oviducts containing eggs	No. of oocytes maturing/ovary*			
			Metaphase I	Metaphase II	Ovulated	Total
0	12	6	12.5 ± 1.6	2.8 ± 0.7	3.4 ± 1.2	19.5 ± 2.6
6	8	2	11.0 ± 1.8	7.2 ± 2.4	0.9 ± 0.6	19.1 ± 2.9
11	7	6	7.3 ± 1.5	13.2 ± 3.1	2.7 ± 0.9	23.2 ± 3.1
18	8	7	5.5 ± 1.1	4.9 ± 1.3	6.4 ± 1.6	16.8 ± 1.8
24	12	10	10.2 ± 1.7	2.9 ± 0.6	7.0 ± 1.1	20.1 ± 2.2
30	11	7	7.1 ± 1.2	3.6 ± 1.0	3.7 ± 1.0	14.5 ± 2.0
48	11	4	9.5 ± 1.1	3.9 ± 1.4	2.5 ± 1.2	15.8 ± 2.2
60	8	4	3.4 ± 1.2	3.3 ± 0.9	4.1 ± 1.6	10.8 ± 2.9

* No. of oocytes maturing/ovary expressed as mean ± S.E.

By contrast, the population at metaphase I increases during the same period. From 30 to 48 hr after HCG injection, the number of oocytes at various phases of preovulatory maturation remains fairly constant and then declines.

Effect of cutting the ovary into fragments. Preparation of the ovarian explants for organ culture results in the destruction of many of the Graafian follicles. Those follicles containing oocytes at metaphase II appear largely unaffected while those at metaphase I decline sharply (Table 5).

Response of ovaries to HCG in organ culture. During the initial 6 hr of culture with HCG, the population of oocytes at metaphase I is partially restored while those that progress to metaphase II increase at a less dramatic rate, eventually reaching a peak at 18 hr (Table 6). The number of oocytes at metaphase I

reaches a maximum value 24 hr after the addition of HCG. The population of oocytes undergoing preovulatory maturation subsequently declines with increasing time and few maturation phases are evident 60 hr after the onset of culture.

Table 5. The effect of cutting up the ovary into small pieces on the number of mouse oocytes maturing

Treatment	No. of oocytes maturing/ovary*		
	Metaphase I	Metaphase II	Total
Intact ovaries	12.5 ± 1.6	2.8 ± 0.7	15.3 ± 2.0
Ovarian fragments	3.6 ± 0.6	3.0 ± 0.7	6.6 ± 1.0

The mice were given an intraperitoneal injection of 5 i.u. PMSG and killed 48 hr later.

* No. of oocytes maturing/ovary expressed as mean ± S.E.

Table 6. The effect of time on the number of mouse oocytes maturing in response to HCG administered *in vitro* after pretreatment with PMSG *in vivo*

Time after HCG (hr)	Ovaries/group	Ovaries containing oocytes at metaphase II	No. of oocytes maturing/ovary*		
			Metaphase I	Metaphase II	Total
0	12	11	3.6 ± 0.6	3.0 ± 0.7	6.6 ± 3.6
6	12	9	10.8 ± 1.6	3.7 ± 0.9	14.6 ± 2.2
11	12	11	10.3 ± 1.6	4.8 ± 0.8	15.2 ± 2.3
18	12	10	10.6 ± 1.6	5.8 ± 1.7	16.4 ± 2.2
24	11	11	16.0 ± 2.0	4.2 ± 0.7	20.2 ± 2.5
30	13	11	13.8 ± 1.8	4.5 ± 1.1	18.2 ± 2.8
47	8	8	11.0 ± 1.1	4.9 ± 0.9	15.9 ± 1.8
60	8	2	3.7 ± 1.3	0.5 ± 0.3	4.2 ± 1.5

* No. of oocytes maturing/ovary expressed as mean ± S.E.

DISCUSSION

The results of the present study confirm earlier reports that the optimal time interval (in terms of oocyte maturation and subsequent ovulation) between injections of PMSG and HCG is 40 to 48 hr for the mouse (Fowler & Edwards, 1957; Baker & Neal, 1972). By using suitable doses of hormones (e.g. 5 i.u. PMSG and 2 i.u. HCG), the number of ovulated oocytes can be increased above that occurring spontaneously in response to endogenous gonadotrophins (Fowler & Edwards, 1957; Zarrow & Wilson, 1961).

It is generally agreed that PMSG contains 'components' which act like FSH and LH (Zarrow *et al.*, 1958). The accumulation of multilayered follicles after injection of PMSG probably reflects the action of both of these components since FSH and LH act synergistically in inducing follicular growth (Hisaw, 1947; Labhsetwar, 1970). Moreover, endogenous gonadotrophic hormones are released simultaneously rather than sequentially and thus the ratio of the two

hormones may be an important factor (e.g. McClintock & Schwartz, 1968).

The present results also confirm that a single injection of PMSG induces ovulation in female mice (Burdick, Watson, Ciampa & Ciampa, 1943; Saunders, 1947). Stern & Schuetz (1970) have shown that ovulation occurs about 20 hr after injection of mice taken at random for stage of the oestrous cycle, and Kaufman & Whittingham (1972) have shown that such eggs can develop normally after fertilization *in vitro*. It has also been shown that 20-day-old rats ovulate after injection of HCG (Sugawara, Umezu & Takeuchi, 1969), and that mature rats ovulate in response to treatment with NIH FSH-S6 and -P1 (Harrington & Elton, 1969). It may well be that ovulation is induced largely in response to the LH activity within these hormones rather than to a non-specific action due to similar molecular structure (but see Harrington, Bex Elton & Roach, 1970). In either case, the gonadotrophins act on pre-existing Graafian follicles which grew in response to the animal's own gonadotrophins (e.g. Pedersen, 1970). Support for this conclusion derives from studies on hypophysectomized rats where HCG-induced ovulation only occurs if the atrophic ovaries are treated 56 hr previously with PMSG to induce follicular growth (Rowlands & Williams, 1943; Rowlands, 1944).

Some oocytes in 'growing' and Graafian follicles of intact rodents undergo preovulatory maturation and ovulation whether or not HCG is administered 48 hr after injection with PMSG (Klausing & Meyer, 1968; P. Neal and T. G. Baker, in preparation). This effect could be due to the LH-like activity in PMSG, but is more likely to be the result of release of endogenous LH.

In organ culture, medium-sized follicles and those with small antra contain oocytes in which meiotic maturation may commence only to be seemingly 'blocked' at metaphase I. By contrast, oocytes in the larger antral (Graafian) follicles either do not embark on preovulatory meiotic changes, or proceed directly to metaphase II without interruption (see also Baker & Neal, 1972). Similar results have been obtained when oocytes have been removed from their follicles into chemically defined media (e.g. Edwards, 1965). Such oocytes resume meiosis to varying degrees; a proportion remain at the germinal vesicle stage, others progress to metaphase II, and a proportion are 'blocked' at metaphase I. Information on the size of these oocytes and of the follicles from which they were removed is at present lacking, but these may in some way represent factors limiting meiosis (see below).

In contrast to the results of the present study, neither Foote & Thibault (1969), who cultured follicles from cows and pigs, nor Merker & Zimmerman (1970), using rat ovaries, were able to induce resumption of meiosis in follicular oocytes by gonadotrophic hormones, though the latter authors reported changes within the ooplasm ('membrane packages') which are similar to those normally accompanying nuclear maturation. The authors conclude that the follicle cells produce an inhibitor which prevents nuclear maturation and cytoplasmic changes *in vivo* but allows cytoplasmic maturation *in vitro*. They suggest that gonadotrophins remove this 'block' to meiosis by isolating the oocyte from the membrana granulosa, presumably by the development of the cumulus and corona cells.

There are a number of problems with this hypothesis, not the least of which

is that neither Foote & Thibault (1969) nor Merker & Zimmerman (1970) could detect a response to gonadotrophic hormones in their cultured follicles. The main problem, as shown by the results of the present study, is that oocytes may be 'blocked' at three different stages of meiosis (dictyate stage; metaphase I or II), depending upon the size of the follicle and on the hormones used (see also Baker & Neal, 1972).

The oocyte and its follicle seem to be in a delicate state of balance, each influencing or modifying the maturation of the other (Baker, 1971). The follicular contribution is probably related to the metabolic activity of the granulosa and cumulus cells (Biggers, Whittingham & Donahue, 1967), while that from the oocyte may be related to temporal changes in the type of ribonucleic acid produced by lampbrush chromosomes (Baker, 1972). Evidence that the oocyte exerts control over its follicles has been inferred from studies involving removal of the oocyte from the pig Graafian follicle on Day 19 of the cycle which induces formation of functional corpora lutea. Merely puncturing the follicle is apparently without effect (El-Fouly, Cook & Nalbandov, 1970; Nekola, Stoklosowa & Nalbandov, 1971), indicating that the oocyte and/or cumulus-corona cell complex inhibits the final maturation of the follicle (luteinization) until after ovulation and/or the LH-surge.

Preovulatory maturation changes in follicular oocytes are essentially similar whether HCG is administered *in vivo* or *in vitro*, and are completed within the same period of time (i.e. 18 hr), although the proportion of cells at each stage of meiosis varies considerably. More oocytes reach metaphase II *in vivo* than in organ culture: the reverse is true for metaphase I. This change in the population of meiotic stages is probably due to the destruction of the large follicles during preparation of the explants: such follicles would normally contain oocytes which mature to an ovulatory condition (metaphase II). Oocytes in medium-sized follicles seem to be recruited to replace those that were destroyed. Autoradiographic studies with [³H]thymidine to test this hypothesis have not so far been completed.

Noyes, Clewe & Yamate (1961), who transplanted rat ovaries to the anterior chamber of the eye in male recipients, also found fewer preovulatory follicles than in controls. Their conclusion that an inadequate milieu of steroids, together with an inadequate or deficient blood supply, prevents the final growth of the follicle may account for the situation in organ culture. It is noteworthy that Noyes *et al.* (1961) found ovulation to be a rare event in the transplanted ovaries and ovulation (if it occurs at all) is also rare *in vitro*. Baker & Neal (1972) found some Graafian follicles with structures resembling preovulatory stigmata, while others were devoid of an oocyte and the stigma was ruptured. It is conceivable that a proportion of these follicles may have undergone ovulation, although their incidence was small compared to (i) the numbers of eggs ovulated *in vivo* and (ii) the number of follicles with oocytes at metaphase II. The possibility cannot, at present, be excluded that some of these 'ovulated' follicles result from mechanical damage caused during the preparation of the explants. Nevertheless, the results of the present study clearly show that the technique of organ culture provides valuable information which may lead to a better understanding of the control of ovarian function.

ACKNOWLEDGMENTS

This work was partly financed out of a grant to Dr B. M. Hobson by Organon Laboratories Ltd, who also supplied the hormones, and also by a grant to T.G.B. by the Population Council.

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