Effect of steroids on oocyte maturation and atresia in mouse ovarian fragments *in vitro*

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Summary. Ovaries from pubertal mice were dissected into fragments containing 1–4 antral follicles which were cultured on the surface of a chemically defined, modified Eagle's Minimum Essential Medium (MEM). After 17–20 h oocytes were released from the cultured follicles and their meiotic status recorded. In the presence and absence of exogenous gonadotrophins, oocytes in these cultures behaved meiotically as expected if they had been studied *in vivo*. Medium containing progesterone (10 μM), testosterone (100 μM), or androstenedione (100 μM) increased the incidence of oocytes remaining meiotically inactive compared to follicles cultured in steroid-free medium. Lower concentrations of the above steroids or pregnenolone (1–100 μM) did not affect the meiotic status of cultured follicle-enclosed oocytes. Most (61%) of the dictyate oocytes released from steroid-treated follicles resumed and/or completed the first meiotic division when cultured further in steroid-free medium. Progesterone (100 μM) caused atresia of oocytes and follicle cells which increased as the period of follicular exposure to progesterone was increased.

Introduction

Mammalian oocytes removed from antral follicles resume meiosis spontaneously *in vitro* (reviewed by Donahue, 1972). However, the functional normality of oocytes matured in this fashion has been questioned (Thibault, 1977; Biggers & Powers, 1979) since the physical, morphological and hormonal relationships between the oocyte–cumulus–granulosa complex and follicle wall are disrupted to obtain the material. If intact follicles from several species are suitably cultured, however, resumption of meiosis must be initiated by gonadotrophin stimulation, as occurs *in vivo*, and isolated oocytes will mature spontaneously when released from follicles cultured in gonadotrophin-free medium (reviewed by Tsafiriri, 1978). The technique of whole follicle culture may therefore provide a solution to the problem of maintaining follicular integrity while examining oocyte maturation.

The role of steroids in the mediation of the gonadotrophic stimulus on the resumption of meiosis in amphibians is well established (reviewed by Schuetz, 1972). Various studies using cultured rat follicles, however, suggest that in mammals the gonadotrophic stimulus for resumption of meiosis is not mediated by steroid synthesis (reviewed by Tsafiriri, 1978). Nevertheless, within the preovulatory follicle the oocyte is exposed to high concentrations of steroids (Chang, Jones, Ellefson & Ryan, 1976; see also McNatty, 1978, for review). The effect of such exposure on the meiotic process is not known. Various steroids (Smith & Tenney, 1978; Smith, Tyler & Erickson, 1978) and prostaglandins (Cho, 1976) inhibit the spontaneous meiotic maturation of mouse oocytes *in vitro* and in the highest concentrations tested cause oocyte degeneration. Steroids might therefore play a part in controlling the rate of progression of oocytes through meiosis or be involved in follicular atresia.

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The aims of the present study were (1) to develop a simple technique for culturing whole follicles explanted from mice such that follicle cells did not degenerate and oocytes did not resume meiosis unless appropriately stimulated; (2) to observe the effects of various concentrations of different steroids on the stage of meiotic maturation and morphology of follicle-enclosed oocytes; and (3) to determine whether steroid treatment produced atresia in cultured follicles.

Materials and Methods

Animals

Random-bred CD1 Swiss albino mice (Charles River Breeding Laboratories) were killed at 29–31 days of age by cervical dislocation and their ovaries were removed aseptically for culture. Although mice were chosen without determining the stage of oestrus, corpora lutea were rarely seen in the dissected ovaries, indicating that most mice had not experienced the first ovulatory cycle.

Culture procedures

The ovaries from 3 or 4 mice were pooled in a Petri dish (Falcon Plastics) containing control medium equilibrated in the culture environment for 1–2 h. The ovaries were dissected with fine cataract knives until fragments containing 1–4 antral follicles (each 300–600 μm in diameter) were obtained. In each experiment, these fragments were randomly distributed to the various treatment groups. The follicles were cultured on the surface of stainless steel, 60-mesh wire rafts in organ culture dishes (Jensen, Gwatkin & Biggers, 1964) using Falcon Plastics dishes (60 $\times$ 15 mm with absorbent ring) partly filled with medium and placed in an incubator at 37°C in an atmosphere of 5% CO_2 in air and 100% humidity. The dissection and preparation of cultures from one group of mice was completed in less than 20 min.

Three commercially available media were evaluated: Eagle's minimum essential medium with Earle's balanced salt solution (MEM) (Microbiological Associates), McCoy's 5a (Microbiological Associates), and Trowell's T8 (Grand Island Biological Co.). In addition, a simple medium used for mouse ovum culture (MOC) described by Biggers, Whitten & Whittingham (1971) and modified slightly (see below), as well as modified Waymouth's medium MAB 87/3 (Eppig, 1977), were used. All the commercially prepared media were supplemented with sodium pyruvate, 0.055 mg/ml (Type II crystalline, Sigma Chemical Co.), glutamine, 0.22 ng/ml (Bae & Foote, 1975), penicillin, 100 i.u./ml and streptomycin, 50 $\mu\text{g}/\text{ml}$. The modified MOC also contained the above concentrations of pyruvate, glutamine and antibiotics. Whole bovine serum albumin (BSA) (crystallized and freeze dried, Sigma Chemical Co.) at a concentration of 4 mg/ml, or polyvinylpyrrolidone (PVP), 2 mg/ml (Sigma), were included in all media. Serum was not used in any of the experiments. All media were sterilized by filtration through a Millipore membrane (pore size 0.03 μm).

Pregnenolone, progesterone, androstenedione, testosterone and oestradiol-17 β were obtained from Sigma Chemical Co. and kept as 10 mM stock solutions in ethanol at 4°C. Steroids were added to the culture medium at a concentration so that the final amount of ethanol was less than 1% of the total volume. This concentration of ethanol had been shown not to affect the system. PVP was used to replace the physical properties of albumin which appear to be required for preimplantation development of cultured embryos (Brinster, 1965). When substituted for BSA in MOC, PVP did not reduce the rate of spontaneous mouse oocyte maturation but did reduce the concentration of progesterone required to block maturation compared with an albumin-containing medium (D. M. Smith, unpublished results). In each of 4 or more replicate experiments, follicles were cultured in control (ethanol- and steroid-free) medium containing PVP and in the same medium with 1, 10 or 100 μM concentrations of 1 of the 5 steroids. In one

experiment the simultaneous effect of progesterone (100 μM) and oestradiol-17 β (100 μM) was examined. Some explanted ovarian fragments were also exposed to 5 i.u. Pergonal (Cutter Laboratories, Berkeley) (50% FSH, 50% LH activity) in the culture medium.

Follicles were cultured for 17–20 h. Any free oocytes in the bottom of the culture dish (few) were removed. Follicles were then moved from the supporting grid into the medium of the culture dish and opened with a cataract knife. The enclosed oocytes were gently expressed and the surrounding cumulus cells removed by repeated agitation in an orally-controlled, fine-bore capillary pipette. Oocytes were examined under a dissecting microscope and classified into: those with a germinal vesicle (GV) which indicated failure to resume meiosis; those that had extruded a polar body (PB) and had therefore completed the first meiotic division; those without a GV or PB, indicating resumption but not completion of the first maturation division; and those that were degenerating, necrotic, cytolysed, abnormal in shape or fragmented. The oocytes in the third category were assumed to be at one of the stages between diakinesis of first prophase and telophase I and have been designated metaphase I, even though the actual stage of meiosis was not determined cytologically.

When ovarian fragments were cultured in steroid-containing media, oocytes which had intact GVs after 17–20 h were cultured for another 20–22 h in steroid-free medium to determine their ability to mature spontaneously.

Statistical analysis

The effects of the treatments on maturation were examined statistically by comparing the number of oocytes retaining a GV with those in which GVBD occurred in a $2 \times c$ contingency table where c represents the number of replicate experiments. Similarly, the effect of the treatments on completion of meiotic maturation were examined by comparing the number of oocytes extruding PBs with those which remained at metaphase I in a $2 \times c$ contingency table. Overall χ^2 values were calculated for $2 \times c$ contingency tables and partitioned using the method described by Kimball (1954).

Histological procedures

Some cultured follicles were fixed in Bouin's fluid, embedded in paraffin wax serially sectioned (7 μm) and stained with haematoxylin and eosin. The degree of atresia was estimated according to the relative numbers of granulosa cells showing pyknotic nuclei and the degree of disaggregation of the follicle wall (Hay, Cran & Moor, 1976).

Results

Preliminary experiments defining the culture system

In a series of pilot experiments ovarian fragments were cultured in MEM, McCoy's 5a, T8, MOC or MAB 87/3 media containing 4 mg BSA/ml. The results indicated that media MEM and MAB 87/3 maintained the oocytes in the most stable condition. Since MEM was commercially available it was chosen for further use. Sections of follicles cultured in MEM contained few granulosa cells with pyknotic nuclei (Pl. 1, Fig. 1) except when ovarian fragments contained more than 4 follicles. Most cells in the central follicles of these large fragments were necrotic so in all further studies only small fragments were cultured.

The effect of Pergonal on ovarian fragments *in vitro* is shown in Table 1. In both experiments a significant increase ($P < 0.001$) in GVBD occurred in response to Pergonal, showing that the cultures responded in the expected way to an LH stimulus. However, pretreatment of the

Table 1. Effect of Pergonal *in vitro* on the stage of oocyte maturation in cultured follicles obtained from PMSG-primed or untreated mice

Treatment of:		Oocytes				
		No. recovered from cultured fragments	No. without signs of degeneration (%)*	Stage of meiosis‡		
Mouse	Follicle			GV(%)†	M I	PB
None	Culture	192	171 (89)	98 (57)	21	52
	Culture + Pergonal	171	161 (94)	24 (15)	46	91
PMSG	Culture	233	183 (79)	99 (54)	27	57
	Culture + Pergonal	231	183 (80)	45 (25)	56	82

* Of oocytes recovered.

† Of oocytes which had not degenerated.

‡ GV = germinal vesicle; M I = metaphase I; PB = polar body.

animals with PMSG increased the incidence of necrotic and abnormal oocytes and subsequent donor mice were not primed with gonadotrophins.

Replacement of bovine serum albumin with polyvinylpyrrolidone

Although the inclusion of BSA in the medium is beneficial in many culture systems its presence complicates dose-response studies because of its ability to bind many small molecules. The effect of replacing BSA with PVP on the behaviour of oocytes in ovarian fragments was therefore examined. Duplicate experiments were carried out in which the two media were compared simultaneously. The results are summarized in Table 2. In both experiments there was no statistically significant difference in the maintenance of the GV between the two media (average 62%). There was also no statistically significant difference between the two media in the incidence of PB formation in those oocytes in which GVBD occurred (average 25%) or in the number of abnormal or degenerating oocytes observed after culture (<10%). Histological examination also showed little difference in the degree of atresia in follicles cultured in either medium. Consequently, PVP (2 mg/ml) was substituted for BSA in experiments in which follicles were cultured in the presence of exogenous steroids.

Table 2. Stages of maturation and frequency of degeneration of mouse oocytes released from follicles cultured in medium containing bovine serum albumin (BSA) or polyvinylpyrrolidone (PVP)

		Oocytes			
		No. recovered from cultured fragments	No. without signs of degeneration (%)*	Stage of meiosis‡	
				GV(%)†	M I PB
BSA (4 mg/ml)	162	146 (90)	99 (68)	34	13
PVP (2 mg/ml)	159	146 (92)	82 (56)	49	15

* Of oocytes recovered.

† Of oocytes which did not degenerate.

‡ GV = germinal vesicle; M I = metaphase I; PB = polar body.

Effects of exogenous steroids

In all experiments in which the effects of exogenous steroids were studied, a group of oocytes cultured in steroid-free medium (control) was included. When the results from the control experiments were tested for homogeneity using χ^2 tests it was found that the behaviour of the oocytes varied between experiments, but this could only be explained as variation between the batches of pubertal mice. Consequently, each experiment was analysed separately using its own control data. In most cases a series of experiments gave similar results and therefore the corresponding χ^2 values were summed to test the significance of the entire results. In a few cases there was heterogeneity in the results of a series of experiments. These results are indicated in the text but not given in detail as no useful conclusions can be reached.

Androgens. The results of the addition of 1, 10 or 100 μM -androstenedione or -testosterone on the stage of maturation and frequency of degeneration of oocytes cultured in intact follicles are shown in Table 3. The incidence of degeneration of follicle-enclosed oocytes was 20% for those cultured with either androgen or in steroid-free medium. At 100 μM , both androgens caused a significant decrease in GVBD. There were no significant differences in the status of oocytes removed from follicles cultured in 1 or 10 μM -androstenedione or -testosterone when compared to controls or each other. Of those oocytes that did resume meiosis the rate of PB formation was not significantly different at any of the doses tested. The results of

Table 3. The effect of androgens and progestagens on the stage of maturation and frequency of degeneration of mouse oocytes cultured in intact follicles

Steroid concentration (μM)	No. of ovaries	Oocytes				
		No. recovered from cultured fragments	No. without signs of degeneration (%)*	Stage of meiosis†		
				GV (%)†	M I	PB
Androstenedione						
0 (control)	14					
1		87	67 (77)	37 (55)	18	12
10		81	64 (79)	33 (52)	17	14
100		95	81 (85)	42 (52)	24	15
		110	89 (82)	82 (92)§	3	4
Testosterone						
0 (control)	14					
1		102	85 (83)	23 (27)	25	37
10		124	105 (85)	34 (32)	17	54
100		125	92 (74)	38 (41)	19	35
		145	112 (77)	100 (89)§	6	6
Pregnenolone						
0 (control)	12					
1		86	77 (90)	44 (57)	15	18
10		98	85 (87)	57 (67)	20	8
100		88	74 (84)	38 (51)	24	12
		80	64 (80)	40 (63)	15	9
Progesterone						
0 (control)	34					
1		383	347 (91)	150 (43)		
10		249	239 (96)	82 (34)		
100		248	215 (87)	127 (59)		
100 + oestradiol (100)		143	1 (1)	1 (100)		
		26	0 (0)	0 (0)		

* Of oocytes recovered.

† Of oocytes which did not degenerate.

‡ GV = germinal vesicle; M I = metaphase I; PB = polar body.

§ $P < 0.001$ compared with control.

culturing dictyate oocytes from androgen-treated follicles in steroid-free medium are shown in Table 4. The majority (>80%) did not degenerate and underwent GVBD spontaneously, indicating that the effects of androgen treatment of the follicle on meiosis were reversible. The rate of polar body formation was highest in those oocytes from follicles previously cultured in testosterone.

Table 4. Maturation *in vitro* of dictyate oocytes from intact follicles previously cultured in different concentrations of 2 androgens and 2 progestagens

Steroid concentration (μM)	Oocytes				
	No. recovered from cultured fragments	No. without signs of degeneration (%)*	Stage of meiosis‡		
			GV(%)†	M I	PB
Androstenedione					
0 (control)	37	37 (100)	11 (30)	19	7
1	33	32 (97)	4 (13)	17	11
10	42	41 (98)	10 (24)	17	14
100	81	79 (98)	33 (42)	26	20
Testosterone					
0 (control)	23	19 (83)	0 (0)	0	19
1	34	33 (97)	2 (6)	2	29
10	36	32 (89)	6 (19)	10	16
100	92	85 (92)	3 (4)	20	62
Pregnenolone					
0 (control)	41	38 (93)	16 (34)		
1	54	51 (94)	8 (16)		
10	38	38 (100)	15 (40)		
100	40	39 (98)	14 (36)		
Progesterone					
0 (control)	69	60 (87)	7 (12)	11	42
1	30	29 (97)	4 (14)	5	20
10	91	82 (90)	5 (6)	24	53

* Of oocytes recovered.

† Of oocytes which did not degenerate.

‡ GV = germinal vesicle; MI = metaphase I; PB = polar body.

Progestagens. The results of the addition of 1, 10 or 100 μM -pregnenolone on the stage of maturation and frequency of degeneration of oocytes cultured in intact follicles are shown in Table 3. Most (84%) of the follicle-enclosed oocytes appeared healthy after culture at all concentrations of pregnenolone tested and this value was similar to that for follicles cultured in steroid-free medium (90%). When the maintenance of dictyate oocytes was examined statistically there were no significant differences between any of the concentrations used and the controls. Similarly, of those oocytes that did resume meiosis the rate of PB formation was not significantly different between any of the groups. Table 3 also shows that progesterone had the most marked effect on the status of follicle-enclosed oocytes when added to the culture medium at a concentration of 100 μM . Of 143 oocytes recovered, 99% were mishapen or necrotic after 17 h in culture. The addition of 100 μM -oestradiol-17 β did not alter this result and no healthy looking oocytes were recovered from 26 follicles. Similarly, histological preparations of follicles cultured in 100 μM -progesterone showed large numbers of granulosa cells with pyknotic nuclei (Pl. 1, Fig. 2). At lower doses of progesterone (1 and 10 μM), most oocytes recovered from cultured follicles appeared normal and the average incidence of degeneration (9%) was not different from that in controls (9%). Statistical analysis of the combined data from 4 individual

Fig. 1. Section through a small antral follicle from a 1-follicle ovarian fragment cultured for 17–20 h in MEM. Few granulosa cells show pyknotic nuclei and the dictyate oocyte does not appear to be degenerating. H & E, $\times 160$.

Fig. 2. Section through a small antral follicle from a 3-follicle fragment cultured for 17–20 h in MEM containing 100 μM -progesterone. Atresia is pronounced in the oocyte and granulosa cells. H & E, $\times 160$.

experiments showed that, at a concentration of 10 μM , but not 1 μM , progesterone significantly inhibited GVBD in follicle-enclosed oocytes ($P < 0.005$). The data for the relationship between PB formation and GVBD, however, were not homogeneous, and the χ^2 values could not be combined for final analysis. Consequently, these data have not been included in Table 3.

The capacity of dictyate oocytes recovered from follicles cultured with progestagens to mature when cultured further in steroid-free medium is shown in Table 4. Most (93%) oocytes recovered from follicles cultured in the 3 concentrations of pregnenolone did not degenerate during further culture and the majority ($>60\%$) resumed meiosis. However, the data showing the stage of maturation reached by oocytes in which GVBD occurred were not homogeneous and conclusions regarding the frequency of polar body formation were not possible. The χ^2 values have not been combined and the data are not included in Table 4. In contrast, the results for the ability of dictyate oocytes recovered from follicles cultured in 10 μM -progesterone to mature spontaneously in steroid-free medium were homogeneous and showed that most oocytes (average 90%) underwent GVBD and, of these, approximately 74% formed polar bodies. The inhibitory action of 10 μM -progesterone on the meiotic process was therefore reversible.

Oestradiol-17 β . The combined data from the 4 experiments involving the addition of 1, 10 or 100 μM -oestradiol-17 β were not homogeneous. The χ^2 values were therefore not combined. Further work is required before the effects of oestradiol-17 β can be unequivocally demonstrated.

Duration of exposure to 100 μM -progesterone. Since oocytes cultured within their follicles in medium containing 100 μM -progesterone became atretic after 17–20 h, additional experiments were carried out to study the effects of varying the time of exposure to medium containing 100 μM -progesterone. The results are shown in Table 5. All follicles were cultured for a total of 17–20 h before examination. After different periods, the medium containing 100 μM -progesterone was replaced with steroid-free medium for the remainder of the culture period. As the time of exposure was increased, the incidence of degeneration increased until, after 17–20 h, almost all recovered oocytes were necrotic.

Table 5. Effect of duration of exposure of follicles to 100 μM -progesterone in a 17–20 h culture on the stage of maturation and frequency of degeneration of the oocytes recovered

Duration of exposure to progesterone (h)	No. recovered from cultured fragments	Oocytes			No. with evidence of degeneration (%)†
		Stage of meiosis of those without signs of degeneration			
		GV(%)*	M I	PB	
0 (control)	126	81 (80)	18	2	25 (20)
3-4	136	98 (93)	6	1	31 (23)
7-8	107	44 (92)	4	0	59 (55)
17-20	105	1 (100)	0	0	104 (99)

* Of oocytes without signs of degeneration.

† Of oocytes recovered.

‡ GV = germinal vesicle; M I = metaphase I; PB = polar body.

Discussion

A simple technique for short-term culture of mouse ovarian fragments has been developed in which, by simple morphological criteria, antral follicles remain healthy for at least 24 h. The following observations support this contention. First, atresia appeared minimal in sections of

cultured control follicles, except when the ovarian fragments were too large. Secondly, most of the fragments cultured in control medium, free of gonadotrophins, contained oocytes which had not resumed meiosis, while addition of Pergonal initiated meiotic activity. Finally, dictyate oocytes recovered from cultured follicles were capable of resuming meiosis and releasing PBs after continued culture, just as oocytes released from antral follicles *in vivo* mature spontaneously *in vitro*. However, the *in-vitro* culture conditions did not duplicate fully the environment of the follicles *in vivo* as the incidence of GV maintenance was reduced to 50–60% after culture for 17–20 h in steroid-free medium containing PVP. This probably reflects a number of factors, such as the inadequacy of PVP as a total replacement for serum in culture media, and the loss of vasculature with concomitant changes in steroid delivery and removal, and gaseous exchange. Since in these experiments the follicles were cultured for relatively short periods (PB formation requires only 11–13 h in the mouse), it has yet to be determined whether physiologically competent follicles can be maintained in culture for prolonged periods using such a simplified technique. A hyperbaric environment may be required to culture intact mouse follicles and corpora lutea for extended periods (T. G. Baker, unpublished results).

The concentrations of steroids in human follicular fluid are far greater than in peripheral plasma (Sanyal, Berger, Thompson, Taymor & Horne, 1974; McNatty, Hunter, McNeilly & Sawers, 1975; Baird & Fraser, 1975; Kemeter, Salzer, Breitenacker & Friedrich, 1975; McNatty *et al.*, 1976). From the data of McNatty *et al.* (1975), it can be calculated that in late follicular-phase Graafian follicles the concentrations of testosterone, androstenedione, progesterone and oestradiol-17 β in follicular fluid may be respectively 80, 120, 750 and 10 000 times greater than in peripheral plasma. There is, however, little information regarding the concentration of steroids in peripheral venous plasma of mice during the oestrous cycle, and no data concerning steroids in follicular fluid. Progesterone has been measured in peripheral plasma throughout the oestrous cycle in mice by Michael (1976) who demonstrated a maximum concentration (204 nM) on the evening of pro-oestrus, and a second smaller increase (86 nM) during the day of metoestrus. This pattern of plasma progesterone levels is similar to that found in the rat (Smith, Freeman & Neill, 1975). In an attempt to estimate concentrations of steroids in mouse peripheral blood, radioimmunoassay of plasma, pooled from 20 dioestrous mice, gave the following values: progesterone, 50.3 nM; oestradiol-17 β , 118 pM; testosterone, 860 pM. Androstenedione could not be measured as values were below the sensitivity of the assay (J. P. Tyler, unpublished results). Using these values, and the human follicular fluid to peripheral plasma ratios calculated above, tentative estimates of steroid concentrations in mouse follicular fluid were derived. They are 38 μ M for progesterone, 1.2 μ M for oestradiol-17 β and 0.069 μ M for testosterone. These estimates are at best an approximation since peripheral venous plasma levels in women are largely dependent on one preovulatory follicle whereas in mice they represent the product of steroid activity in 8–10 follicles. Nevertheless, they may provide some guidelines for determining whether or not the concentrations of steroids used in the present experiments represent those found physiologically. No values for the concentration of pregnenolone in mice are available, but this steroid had no effect on the amount of degeneration or meiotic status of oocytes in cultured follicles in comparison to control data.

When follicles were cultured in androstenedione or testosterone, only the highest concentration (100 μ M) had a significant effect, and the incidence of GV maintenance after culture increased. However, these concentrations are 1000 times greater than those estimated above for these steroids in follicular fluid and are likely, therefore, to be greatly in excess of those found under normal conditions. Nevertheless, these concentrations did not appear toxic, since the majority of oocytes with GVs were capable of resuming meiosis after removal from the follicles cultured with androgens. These results do not support the evidence that androgens may act locally to produce follicular atresia (Payne, Hellbaum & Owens, 1956; Speroff & van Wielle, 1971).

In contrast to androgens, oestrogens have been shown to decrease atresia (Ingram, 1959;

Harman, Louvet & Ross, 1975). In the present experiments, however, the results with oestradiol-17 β were extremely variable and further experiments are required to determine whether oestrogens affect the status of oocytes cultured in intact follicles. Nevertheless, 100 μ M-oestradiol-17 β could not overcome the atresia of oocytes in follicles cultured in 100 μ M-progesterone.

All the concentrations of progesterone used in the present experiments are close to, or less than, the estimated physiological value in follicular fluid. Even so, 10 μ M-progesterone significantly inhibited GVBD in follicular oocytes, and at 100 μ M caused all oocytes and most other follicle cells to degenerate. Atresia occurred within 17–20 h of exposure to a concentration of progesterone (100 μ M) assumed to be of physiological proportions. Similar atretic changes have been reported for hamsters injected subcutaneously with 50 or 100 μ g progesterone or medroxy-progesterone acetate (Williams, Runyan & Hagen, 1972). These data lead us to suggest that sustained high concentrations of progesterone within the follicle might be involved in the atresia which ultimately occurs in all unovulated follicles. This hypothesis is, however, in apparent conflict with the fact that there is a pro-oestrous rise in plasma progesterone concentration in the mouse (Michael, 1976) which obviously does not destroy oocytes destined for ovulation within the next 10–15 h. Since follicular fluid progesterone concentrations were not reported in the above study, it is impossible to determine whether oocytes were exposed to high progesterone during the pro-oestrous period or whether it was produced by the thecal or stromal compartments and removed by the ovarian circulation, thus preventing accumulation in the antral fluid. Szoltys (1976) has shown in homogenized preovulatory follicles obtained from rats every 2 h during pro-oestrus that the peak concentration of progesterone is transitory, lasting less than 3 h. These data are in keeping with the results shown in Table 5 which suggest that atresia might only be initiated after exposure to progesterone for about 4 h.

In conclusion, the results of these experiments have confirmed that steroids do not induce meiotic maturation and that, at concentrations which are assumed to be physiological, androstenedione, testosterone, and pregnenolone neither inhibited nor induced GVBD in follicle-enclosed oocytes in culture. At concentrations assumed greater than physiological (but still not toxic) androstenedione and testosterone (100 μ M) inhibited GVBD. With progesterone at concentrations which may be in the physiological range GVBD was inhibited, and at higher concentrations (100 μ M) oocytes and follicle cells became atretic if intact follicles were exposed for more than 4 h. A complication of this type of experiment is not knowing how much exogenous steroid enters the follicular fluid of follicles in culture, or how that steroid affects the dynamics of steroid interconversions in a system already primed with hormones and steroid-converting enzymes. It remains to be determined whether these effects of steroids are physiologically significant in relation to the cyclic fluctuations of hormones in the follicular environment.

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