77

BARD

FINAL REPORT

PROJECT No. I-112-79

The Control of Meiotic Maturation in Mammals

A. Tsafiriri, C.P. Channing, S.H. Pomerantz

1983

BARD No. of Proposal: I-112-79

Title of Proposed Research: The Control of Meiotic Maturation in Mammals

Name(s) of Investigator(s)

Principal Investigator: A. Tsafiriri

Cooperating Investigators: C.P. Channing and S.H. Pomerantz

Name and Address of Affiliated Institutions

Principal Institution: Department of Hormone Research,
The Weizmann Institute of Science,
Rehovot, Israel.

Cooperating Institution: Departments of Physiology and Biological
Chemistry,
University of Maryland School of Medicine

Project's Starting Date: 1.4.1980

Type of Report: Final

SIGNATURE

PRINCIPAL INVESTIGATOR: INSTITUTION'S AUTHORIZING OFFICIAL

A. Tsafiriri, Ph.D.,

N. Guter,
Deputy Director, Research Contracts
and Projects

June, 1983.

Table of Contents

Introduction	1
(a) Further purification of OMI from porcine follicular fluid	2
(b) Physiological studies of OMI	4
(i) OMI in porcine follicular fluid at various stages of estrous cycle	4
(ii) Role of cumulus cells in OMI action	6
(c) The acquisition of meiotic competence by rat oocytes	7
(i) The role of gonadotropins in the acquisition of meiotic competence	8
(ii) Is the action of FSH on meiotic competence mediated by ovarian estrogen synthesis	9
References	11
Figures	13
Evaluation of research achievements	17
Description and results of cooperation	17
List of reprints appended	17

Abstract

Preovulatory surge of gonadotropins induces a series of changes culminating with the release of a fertilizable ovum. These include, resumption of the meiotic division, a process held in abeyance from a short time after birth. In general, the objective of this study was to gain a better understanding of the factors involved in the regulation of preovulatory resumption of meiosis, i.e. in the production of a fertilizable ovum. More specifically, the project was aimed on further characterization of a factor, oocyte maturation inhibitor (OMI) previously described by us and others to be involved in the regulation of resumption of meiosis. Considerable progress in the purification of OMI was achieved by a combination of QAE-Sephadex and Reversed Phase High Performance Liquid Chromatography on Whatman C₁₈ column. This shortened the procedure considerably and led to substantial purification at a higher yield.

OMI activity of porcine follicular fluid was not related to the stage of the estrous cycle, but was dependent upon the stage of follicular development. Fluid from small and medium sized follicles showed a significantly higher OMI activity when compared with fluid from large follicles. OMI was effective in inhibiting spontaneous maturation of rat oocytes only when the oocytes were cultured within their intact cumulus cell complex, oocytes mechanically denuded from their investing cumulus cells were not inhibited. Since OMI activity has been demonstrated only in antral follicles, we examined the development of the ability of rat oocytes to undergo spontaneous maturation during their growth phase in preantral follicles. We have found that the ability of rat oocytes to resume maturation ("meiotic competence") is acquired between days 20-26 post partum. By the use of hypophysectomy on day 15 of life and treatment with hormones and inhibitors we demonstrated that the acquisition of meiotic competence is dependent upon FSH stimulation and mediated, at least partially, by ovarian estrogen production. Our finding that oocytes from preantral follicles are meiotically incompetent suggests that the physiological role of follicular OMI is limited only to antral follicles, i.e. when the oocytes acquire meiotic competence. Furthermore, it appears that meiotic arrest around the time of birth is exerted by mechanisms differing from those keeping meiosis in abeyance in the antral follicle.

Introduction

Meiosis of mammalian oocytes is initiated during fetal life and usually becomes arrested shortly after birth at the stage of diplotene. The meiotic process is resumed in adult life following the preovulatory surge of gonadotropins, a few hours prior to ovulation. During most the the meiotic arrest the oocyte persists in the diffuse diplotene or dictyate stage. We shall use the terms "oocyte maturation" or "meiotic maturation" to denote the preovulatory resumption of the meiotic process, including the progress of dictyate oocyte to the metaphase of the second meiotic division, i.e. to a fertilizable ovum.

Maturation of mammalian oocytes in vivo is clearly dependent upon the preovulatory surge of gonadotropins (Ayalon et al., 1972; Vermedien and Zeilmaker, 1974). On the other hand, oocytes dislodged from their follicles resume meiosis spontaneously in culture (Pincus and Enzmann, 1935; Tsafriri, 1978). Therefore, it was suggested that the gonadotropic stimulus is probably needed to remove the inhibitory influence of the follicle rather than to trigger resumption of meiosis directly. In general, the objective of the proposed research was to gain a better understanding of the factors involved in the regulation of oocyte maturation, i.e. in the production of a fertilizable ovum.

Specifically, the project centered around the following three issues and the results are presented accordingly: (a) Further purification of the inhibitor of oocyte maturation (OMI) from porcine follicular fluid. OMI activity has been previously described by us and others (Tsafriri and Channing, 1975; Gwatkin and Anderson, 1976; Tsafriri et al., 1976; Sato and Ishibashi, 1977; Stone et al., 1978). (b) Physiological studies of OMI.

These included the following aspects: (i) Examination of OMI content of porcine follicles at various stages of follicle growth and estrous cycle; (ii) Role of cumulus cells in OMI action. (c) The acquisition of meiotic competence by rat oocytes. The progress of meiotic maturation is arrested, in most mammalian species, just after birth. Since OMI activity has been demonstrated only in antral follicles, we have examined the development of the ability of rat oocytes to undergo spontaneous maturation during their growth phase in preantral follicles.

(a) Further purification of OMI from porcine follicular fluid

At the beginning of this project the procedure of OMI purification involved six rather lengthy steps: Amicon PM-10 filtration of follicular fluid; lyophilization and extraction of the dry powder with 2N acetic acid in methanol, and solution of the dried extract in 0.1 N aqueous acetic acid; extraction with ether; chromatography of the aqueous phase on Sephadex G-25 column; two peaks of activity were eluted: A corresponding to a molecular weight of about 2000 daltons and a second peak, B, was eluted later and corresponded to less than 1000 (Tsafriri et al., 1976; Stone et al., 1978). Peak A was chromatographed on a CM Sephadex column eluted with 0.01 M NH_4HCO_3 . All the activity was either eluted with the solvent front or else only very slightly retarded; the active fraction was applied to a DEAE-Sephadex column and eluted, in succession, with 0.01 M NH_4HCO_3 , a gradient of 0.01 M NH_4HCO_3 - 0.1 M NH_4HCO_3 , and finally with 0.5 M NH_4HCO_3 .

In the course of this project this procedure was somewhat improved to include: Amicon PM-10 filtration of follicular fluid; lyophilization and extraction of the dry powder with organic solvents (1N acetic acid in methanol; methanol) and solution of the dried extract in 0.1 N aqueous acetic

acid; extraction with ether; chromatography of the aqueous phase on Biogel P2; and chromatography on CM-Sephadex (NH_4OAc , pH 5). This method confirmed our earlier work that there are several substances with OMI activity. One active fraction was purified 35,000-fold over follicular fluid (about 350-fold over the PM-10 fraction) in a yield of about 20%.

This procedure was still quite unsatisfactory because of its excessive length and the relatively large losses in obtaining a fraction which was still quite impure. We are pleased to report the following procedure which is shorter and leads to considerable purification and at a higher yield.

The PM-10 filtrate (1770 ml) from about 2400 ml of pig follicular fluid derived from small and medium-sized follicles is diluted with an equal volume of ice-cold water and passed onto a column of QAE-Sephadex (5 x 4.5 cm) suspended in 0.01 M NH_4HCO_3 , pH 8. The column is washed with 0.01 M NH_4HCO_3 (200 ml) and then eluted with a linear gradient of NH_4HCO_3 formed by passing 750 ml of 0.01 M NH_4HCO_3 , pH 8 into 750 ml of 0.7M NH_4HCO_3 , pH 8. Two peaks of activity are found, one, Fraction A, emerging between 0.3 and 0.35M NH_4HCO_3 and the second, Fraction B, between 0.4 and 0.45 M NH_4HCO_3 . Fraction A has been further purified on reversed phase high performance liquid chromatography (HPLC) using a gradient of 100% 0.1% trifluoroacetic acid (CH_3CN). One area of activity has been located. The data for one run is as follows:

<u>Fraction</u>	<u>mg/ml</u>	<u>units/mg</u>	<u>Total units</u>
1. Follicular fluid	Not determined		
2. PM-10 filtrate	8.2	0.18	2655
3. QAE-Sephadex			
Fraction A	1.24	430	775
Fraction B	0.688	776	775
4. Fract A on HPLC	0.174	5750	2800*

*This is an apparent recovery of 360%. Possibly a stimulator has been removed.

Fraction B from QAE-Sephadex has also been purified on HPLC but this run has not been analyzed as yet. From other results we expect that there will be two active fractions derived from fraction B.

It seems that we will have a good chance to obtain a homogeneous sample of at least one of these OMI fractions. Then the way will be open to determine its exact composition. Since we have evidence that crude OMI is peptide in nature we hope to be able then to obtain an amino acid composition and later a sequence.

Physiological studies of OMI

(i) OMI in porcine follicular fluid at various stages of estrous cycle.

Samples of porcine follicular fluid (N = 28) were collected from ovaries of 18 cyclic, 7-9 month-old crossbred Dutch Landrace x Great Yorkshire gilts according to the procedure described by Tsafriri and Channing (1975),

except that no antibiotics or Millipore filters were used and ovaries or follicular fluid were always kept on ice. Since the pig produces 16.4 corpora lutea on average after ovulation (Hansel, et al., 1973), follicular fluid from approximately 20 of the largest follicles was always combined, whereas fluid of the other follicles was pooled according to the size of the follicles (small < 3 mm; medium size 3-6 mm; large > 6 mm diameter). After centrifugation, the total volume of each pool was determined and samples were stored at -70°C. Follicular fluid OMI was determined as described by Tsafiriri et al. (1977) and correlated with inhibin and steroids. Inhibition of oocyte maturation in all samples taken at Day 5, 10, 15 and 18 of the estrous cycle are shown in Fig. 1. With one exception the percentage inhibition always decreased with increasing follicle diameter, and there was no relationship with the stage of the estrous cycle. If the sample with the negative value was excluded, mean OMI concentrations ($\pm$ S.D.) in follicles with diameter <3, 3-6, 6-9 and 9-12 mm were 29.3 $\pm$ 3.4, 16.7 $\pm$ 8.0, 8.4 $\pm$ 5.5 and 6.8 $\pm$ 3.3 respectively. There were low concentrations of OMI both in the largest follicles on Day 18 and in the follicles after the LH surge (9.2 $\pm$ 5.8 and 8.7 $\pm$ 4.0 respectively). When follicle classes were compared within individual animals, OMI concentrations decreased significantly ($p < 0.05$) with increasing follicle diameter.

A positive correlation ($r = 0.64$) was found between OMI and inhibin concentrations. No significant correlations were found between OMI and progesterone or estradiol concentration in follicular fluid. Inhibin concentrations were not related to follicular fluid estradiol concentrations, but a negative correlation ($r = -0.54$) was found between inhibin and log progesterone concentration.

Oocyte maturation inhibitor activity in follicular fluid, as measured in an assay utilizing oocytes isolated from rats, was not related to the stage of the estrous cycle, but was dependent on the size of the follicle. Follicular fluid from small or medium sized follicles showed a significantly higher OMI activity when compared with fluid from large follicles. Similar results were obtained when follicular fluid was collected from small, medium and large follicles of porcine ovaries collected at an abattoir (Stone et al., 1978) or when granulosa cells collected from such follicles were extracted (Centola et al., 1981). It therefore appears that the concentration of OMI in follicular fluid decreases with follicular growth, although sufficient OMI must remain within healthy preovulatory follicles to prevent the resumption of meiosis.

(ii) Role of cumulus cells in OMI action.

The close association between the cumulus cells and the oocyte was first described by Paladino (1890). Gap junctions between cumulus cell projections and the oocyte have been observed (Amsterdam et al., 1976) and transfer of ions and small molecules between the oocyte and cumulus cells was demonstrated (Gilula et al., 1978). Ionic coupling and transfer of molecules were maximal before gonadotropic stimulation and decreased afterwards. This led to the hypothesis that the release of the oocyte from suppression of meiosis is a result of dismantling or of functional disengagement oocyte-cumulus cell junctions, possibly induced by the ovulatory hormone (Lindner et al., 1974).

In order to test the possible role of cumulus cells in the action of OMI on meiotic maturation rat oocytes were cultured within their cumulus or after removal of adherent cells in the presence of the low molecular weight fraction of porcine follicular fluid.

OMI was effective in inhibiting spontaneous maturation only in oocytes cultured within their intact cumulus cell complex; denuded oocytes were not inhibited (Fig. 2). Moreover, addition of the low molecular fraction of porcine FF1 somewhat facilitated the maturation of denuded oocytes. It appears, therefore, that the inhibitory signal of OMI is communicated to the oocyte via the enveloping cumulus cells.

(c) The acquisition of meiotic competence by rat oocytes.

Oocytes were collected from rats 15 to 31 days post partum (p.p.) by the procedure described by Mangia and Epstein (1975) and cultured for 6 or 24 h. The majority of oocytes recovered from rats on Day 15 to 20 of age failed to resume maturation in culture; in older animals, the percentage of competent oocytes increased gradually until Day 26 p.p. (Fig. 3) and remained similar on Day 31 of life. Extension of the incubation period to 24 h did not increase the percentage of oocytes resuming meiosis.

The diameter of oocytes explanted between Days 15 and 26 p.p. increased with age in concert with the increase in the percentage of competent oocytes. In the pool of oocytes explanted between Days 15 and 31 p.p. there was a high correlation ($r = 0.98$) between the ability of oocytes to resume maturation in culture and their diameter. It appears, however, that the diameter of the oocytes is probably not the only determinant of meiotic competence. Thus, the average diameter of competent oocytes on Day 20 p.p. ($61.8 \pm 1.2 \mu\text{m}$) was significantly below the average diameter of incompetent oocytes from 26-day-old rats ($75.5 \pm 0.8 \mu\text{m}$; $p < 0.001$).

At histological examination of ovaries obtained from 20, 24 and 26 day-old rats, there was an increase in the percentage of antral follicles from 14.3 to 38.8% (Fig. 3). Thus the increase in the percentage of competent

oocytes and of antral follicles occurred concomitantly. These results served the basis for investigating the role of hormones in the development of meiotic competence and salient results are summarized below.

(i) The role of gonadotropins in the acquisition of meiotic competence

Rats were hypophysectomized on Day 15 or 20 post partum (p.p.) utilizing an intra-aural approach. Oocytes were explanted on Day 26 p.p. and examined for germinal vesicle breakdown (GVB) after 6 h of culture. Both the percentage of competent oocytes and number of oocytes that could be collected was reduced following hypophysectomy on Day 15. The results of hypophysectomy (Fig. 4) are presented as change in maturation rate:

$$\text{maturation rate} = \frac{\text{number of competent oocytes}_{(\text{treated})} \times 100}{\text{number of competent oocytes}_{(\text{control})}}$$

The reversal of the effect of hypophysectomy on Day 15 by either gonadotrophin or estradiol-17 (E_2) was tested by administration of PMSG (3 I.U.) on Days 25, 27 and 29 of age and by insertion of silastic tubing filled with estradiol-17 on Day 25 p.p. Administration of PMSG or E_2 resulted in an increase in the maturation rate (Fig. 5). Estrogen-treatment of hypophysectomized rats resulted in an increase in the percentage of competent oocytes from $27.8 \pm 2.4\%$ to 63.3 ± 2.3 within the first two days of treatment; this percentage of competent oocytes did not change with the prolongation of E_2 to six days. On the other hand, administration of PMSG resulted in a gradual increase in the percentage of competent oocytes during the six-day treatment, from 41.9 ± 3.9 on Day 27 to 68.9 ± 3.4 on Day 24 and to 94.5 ± 1.5 on Day 31. In addition to its action on the percentage of competent oocytes,

PMSG also increased the absolute number of competent oocytes (46.8 ± 0.5 oocytes per rat on Day 31) to the number found in intact controls (46.3 ± 3.9); by contrast E_2 was only partially effective in this respect (13.9 ± 1.9 competent oocytes per rat on Day 31).

The action of PMSG on the acquisition of meiotic competence appears to be primarily due to its FSH-like activity. Thus only FSH (NIAMD-rat-FS) but not LH (NIAMD, purified-ovine-LH, Batch 21), was able to elicit this response when injected on Days 25-28 to rats hypophysectomized on Day 15 (Fig. 6).

(11) Is the action of FSH on meiotic competence mediated by ovarian estrogen synthesis.

The action of PMSG (or FSH) in promoting acquisition of meiotic competence is, at least in part, mediated by ovarian estrogen production. Inhibition of steroidogenesis at the stage of cholesterol 20 α -hydroxylase, aromatase or 17:20 lyase by aminogluthetimide (Kahnt and Neher, 1966; Thompson and Siiteri, 1974), 17 β -formamidoandrost-4-en-3-one (Arth *et al.*, 1971), 1,4,6-androstatriene-3, 17-dione (Schwarzel *et al.*, 1973) or 4-hydroxy-4-androstene-3, 17-dione (Brodie *et al.*, 1979) prevented the effect of FSH on the acquisition of meiotic competence. When estradiol was administered together with these inhibitors of steroidogenesis, the effect of FSH on the development of meiotic competence was restored (Fig. 7).

These results strongly suggest that FSH is involved in the development of meiotic competence and that this action of FSH is mediated, at least in part, by follicular estrogen production. Though determined in an artificial system, it seems likely that meiotic competence reflects an important aspect of oocyte development. *In vivo*, however, resumption of meiosis is

also dependent upon the ability of the surrounding granulosa cells to respond to the triggering action of LH (see Tsafiriri et al., 1983). This is acquired in the course of growth and differentiation of antral follicles through the sequential action of steroid hormones and FSH, which jointly induce the LH receptor on granulosa cells (Richards, 1980). The responsiveness of granulosa cells to LH thus reaches its peak just before the preovulatory LH surge.

The finding that oocytes from preantral follicles are meiotically incompetent seems to indicate that follicular OMI has a physiological significance only in antral follicles, i.e. when the oocytes acquire meiotic competence. Studies on follicular atresia demonstrating resumption of meiosis only of oocytes from antral, but not preantral follicles, undergoing atresia lend support to this notion.

REFERENCES

- Amsterdam, A., Josephs, R., Lieberman, M.E. and Lindner, H.R. (1976) *J. Cell. Sci.* 21, 93-105.
- Arth G.E., Patchett, A.A., Jefopoulos, T., Bugianesi, R.L., Peterson, L.H., Ham, E.A., Kuehl, F.A. and Brink, N.G. (1971) *J. Med. Chem.* 24, 675-679.
- Ayalon, D., Tsafriri, A., Lindner, H.R., Cordova, T. and Harell, A. (1972) *J. Reprod. Fertil.* 31, 51-58.
- Brodie, M.H., Marsh, D.A., Wu, J.T. and Brodie, H.J. (1979) *J. Steroid Biochem.* 11, 107-112.
- Centola, G.M., Anderson, L.D. and Channing, C.P. (1981) *Gamete Res.* 4, 451.
- Gilula, N.B., Epstein, M.L. and Beers, W.H. (1978) *J. Cell Biol.* 78, 58-75.
- Gwatkin R.B.L. and Andersen, O.F. (1976) *Life Sci* 19, 527-536.
- Hansel, W., Concannon, P.W. and Lukoszevska, J.H. (1973) *Bioleprod.* 8, *Biol. Reprod.* 8, 222.
- Kahnt, F.W. and Neher, R. (1966) *Helvetica Chimica Acta* 49, 725-732.
- Lindner, H.R., Tsafriri, A., Lieberman, M.E., Zor, U., Koch, Y., Bauminger, S. and Barnea, A. (1974) *Rec. Prog. Horm. Res.* 30, 79-138.
- Mangia, F. and Epstein, C.J. (1975) *Devl. Biol.* 45, 211.
- Paladino, G. (1890) *Anat. Anz.* 15, 254-259.
- Pincus, G. and Enzmann, E.V. (1935) *J. Exp. Med.* 62, 655-675.
- Richards, J.S. (1980) *Physiol. Rev.* 60, 51-89.
- Sato, E. and Ishibashi, T. (1977) *Jpn. J. Zootech. Sci.* 48, 22-26.
- Schwarzel, W.C., Kruggel, W.G. and Brodie, H.J. (1973) *Endocrinology* 92, 866-880.
- Stone, S.L., Pomerantz, S.H., Schwartz-Kripner, A. and Channing, C.P. (1978) *Biol. Reprod.* 19, 585-592.
- Thompson, A.E. and Siiteri, P.K. (1974) *J. Biol. Chem.* 249, 5373-5378.

- Tsafriri, A. (1978) In: "Ovarian Follicular and Corpus Luteum Function" (eds. Channing, C.P. and Marsh, J.M.) Plenum Press, New York, pp. 269-282.
- Tsafriri, A., Bar-Ami, S. and Lindner, H.R. (1983) In: "Fertilization of the Human Egg In Vitro" (ed. Beier, H.M. and Lindner, H.R.), Springer-Verlag Berlin, Heidelberg, pp. 3-17.
- Tsafriri, A. and Channing, C.P. Endocrinology 96, 922-927.
- Tsafriri, A., Channing, C.P., Pomerantz, S.H. and Lindner, H.R. (1977) J. Endocrinol. 75, 285-291.
- Tsafriri, A., Dekel, N. and Bar-Ami, S. (1982) J. Reprod. Fert. 64, 541-551.
- Tsafriri, A., Pomerantz, S.H. and Channing, C.P. (1976) Biol. Reprod. 14, 511-516.
- Vermeiden, J.P.W. and Zeilmaker, G.H. (1974) Endocrinology 94, 341.

Fig. 1. Relation between follicular OMI activity, follicle size and stage of the estrous cycle. Follicular fluid was collected on days 5, 10, 15 and 18 of the cycle and OMI activity tested on rat oocytes in culture (Tsafriri *et al.*, 1977). Numbers 1 and 2 indicate pooled samples which were obtained from 5 and 2 gilts respectively. Samples obtained from the same animals are connected by lines. Data from days 10 and 15 represent the luteal phase of the estrous cycle and hence are combined.

Fig. 2. Role of the cumulus cells in OMI action upon maturation of rat oocytes. Oocytes were cultured within their cumulus (a) or after removal of adherent cells by repeated transfers through thin-bore glass capillaries (b). Culture was for 6 h in the presence of the indicated dilution of the low molecular weight fraction of porcine follicular fluid. Values are mean + S.E.M. *P < 0.05; **P < 0.005 compared with appropriate control cultured without follicular fluid.

Fig. 3. The development of meiotic competence and of antral follicles in immature rats. Oocytes were isolated from rats at the age indicated (days post partum) and cultured for 6 h; the percentage of oocytes resuming meiotic maturation (GVB, germinal vesicle breakdown) is indicated. The percentage of antral follicles among the healthy follicles with more than 80 granulosa cells per largest cross section were counted in every fifth section of the ovary (Braw and Tsafiriri, 1980). Number of oocytes examined at each age is shown on graph; mean $\pm$ S.E.M. are indicated.

Fig. 4. Hypophysectomy and maturation rate. Hypophysectomy was performed on Day 15 or 20 post partum (p.p.). Oocytes were explanted on Day 26 p.p. and examined after 6 h of culture. White column, intact rats, black columns, hypophysectomized rats. The number of rats examined is indicated on columns.

$$\text{Maturation rate} = \frac{\text{number of competent oocytes (treated rats)}}{\text{number of competent oocytes (intact rats)}} \times 100$$

$x_p < 0.01$ vs. hypophysectomized rats treated with FSH.

Fig. 5. Effect of hypophysectomy and oestrogen or PMSG treatment on maturation rate. Oocytes were isolated from rats on the day of age indicated from rats hypophysectomized on Day 15 (black columns), rats hypophysectomized on Day 15 and implanted on Day 25 p.p. with a capsule of silicone tubing containing estradiol-17 (E₂) or treated with PMSG (3 I.U.) on Days 25, 27 and 29 (PMSG). The oocytes (number on columns) were cultured for 6 h.

Fig. 6. Effect of gonadotrophins on maturation rate in hypophysectomized rats. Rats were left intact or hypophysectomized on Day 15 p.p. On Days 25-28 half of the indicated daily dose (μg) of LH or FSH, or both was injected on morning and evening 12 h apart. On the morning of Day 29 the oocytes were collected and cultured for 6 h.

Fig. 7. The effect of inhibitors of steroidogenesis on the action of FSH on meiotic competence. Rats were hypophysectomized (Hypox) on the day 15 of life. On the evening of day 25 some of them were implanted with a silastic capsule (6 mm long) containing 17 β -formylaminoandrost-4-en-3-one, 1, 4, 6 androstatriene-3, 17-dione, 4-hydroxy-4-androstene-3, 17-dione or injected with aminoglutethimide phosphate every 12 h. In addition, some of the rats were implanted with an E₂ capsule on the morning of day 25 and/or treated with FSH (20 μ g/day) every 12 h. The oocytes were collected on day 29 of age and cultured for 6 h. Since all the inhibitors of steroidogenesis exerted a similar effect, the data of all the inhibitors were pooled.

Evaluation of the research achievements with respect to the original research proposal.

While further purification of OMI was achieved and a simplified procedure for its purification has been established we are still in the process of preparation of monoclonal antibody to OMI. It is hoped that within a year we may have in our hands an antibody to be used for the development of a radioimmunoassay for OMI.

Our search for alternative source(s) of OMI was unsuccessful. Thus OMI activity of conditioned media of normal or transformed rat granulosa cells and in granulosa cell extracts was much lower than that obtained from batches of fluid obtained from small porcine follicles. Hence, porcine follicular fluid seems still to be the best source for further work on OMI.

Description and results of cooperation

The study on OMI has been conducted in both laboratories in parallel and coordinated by frequent exchange information and visits. Dr. S. Bar-Ami (whose thesis is appended) is now a post doctoral fellow in the laboratory of Dr. Channing. The results of the work of the Baltimore group were summarized in the following publications:

Channing, C.P., Anderson, L.D., Hoover, D.J., Kolena, J., Osteen, K.G., Pomerantz, S.H. and Tanabe, K. In: Recent Progress in Hormone Research, R.O. Greep (editor), vol. 38, 331-408, 1982. Academic Press.
Channing, C.P., Pomerantz, S.H., Bae I.H., Evans, V.W. and Atlas, S.J. In: Intraovarian Control Mechanisms, C.P. Channing and S.J. Segal (editors), pp. 189-210, 1982, Plenum Press (appended).

In addition (i) some of the samples prepared in the course of OMI purification were tested in parallel in the porcine oocyte assay (in Baltimore) and in the rat oocyte assay (in Rehovot). Very good agreement between the two assays was obtained in most of the cases; (ii) The Baltimore group provided us with porcine follicular fluid. In the course of the project we obtained follicular fluid also from Contraceptive Development Branch, NICHD, NIH. However, these batches contained very low OMI activity, being apparently aspirated from large follicles as well as from small ones.

List of reprints appended:

1. PH.D. Thesis of S. Bar-Ami: Factors involved in the control of oocyte maturation in the rat. (1982)
- ✓ 2. Tsafiriri, A. and Bar-Ami, S. (1981) The role of gonadotrophins and steroids in the acquisition of meiotic competence. In: Development and Function of Reproductive Organs, A.G. Byskov and H. Peters (eds.), pp. 225-233.
- ✓ 3. Tsafiriri, A. and Bar-Ami, S. (1982) Oocyte maturation inhibitor: A 1981 perspective. In: Intraovarian Control Mechanisms, C.P. Channing and S.J. Segal (eds), pp. 145-159, Plenum Press, New York.

- ✓ 4. Tsafriri, A., Dekel, N. and Bar-Ami, S. (1982) The role of oocyte maturation inhibitor in follicular regulation of oocyte maturation. *J. Reprod. Fert.* 64, 541-551.
- ✓ 5. Tsafriri, A., Bar-Ami, S. and Dekel, N. (1982) Follicular regulation of oocyte maturation. In: *Non-Steroidal Regulators in Reproductive Biology and Medicine*, T. Fujii and C.P. Channing (eds.), pp. 147-156.
- ✓ 6. Tsafriri, A. and Bar-Ami, S. (1981) The role of gonadotrophins and steroids in the acquisition of meiotic competence. In: *Development and Function of Reproductive Organs*, A.G. Byskov and H. Peters (eds), pp. 225-233.
- ✓ 7. Tsafriri, A., Bar-Ami, S. and Lindner, H.R. (1983) Control of the development of meiotic competence and of oocyte maturation in mammals. In: *Fertilization of the Human Egg In vitro*, H.M. Beier and H.R. Lindner (eds.), pp. 3-17.
- ✓ 8. Van de Wiel, D.F.M., Bar-Ami, S., Tsafriri, A. and de Jong, F.H. (1983) Oocyte maturation inhibitor, inhibin and steroid concentrations in porcine follicular fluid at various stages of the estrous cycle. *J. Reprod. Fertil.* 68, 247-252.
- ✓ 9. Bar-Ami, S., Nimrod, A., Brodie, A.M.H. and Tsafriri, A. (1983) Role of FSH and estradiol 17 in the development of meiotic competence in rat oocytes. *J. Steroid Biochem.* in press.