Yonsei Medical Journal
Vol. 16, No. 1, 1975

The in Vitro Maturation of the Mouse Oocyte

In-Ha Bae and Soon-O Chung

Division of Reproductive Physiology, The Center for Population and Family Planning, Yonsei University, Seoul, Korea

ABSTRACT

Mouse follicular oocytes, denuded and intact, were cultured in pyruvate salt sol and glutamine salt sol supplemented bovine serum albumin to compare the maturation rate.

Glutamine has no effect on maturation of the denuded mouse oocyte but has an effect on maturation of the intact oocyte by increasing the maturation rate, depending on the increased concentration of glutamine (0.4 mM to 2 mM). Changes in osmolarity of the operation medium from 280 mOsm to 310 mOsm has no discernible effect on the oocyte maturation.

A high frequency of abnormal 1st polar bodies was observed in pyruvate salt sol. and this may be due to the increased energy source in the cytoplasm of the 1st polar body when the polar body was extruded into the perivitelline space after the 1st meiosis.

INTRODUCTION

Since Pincus and Enzmann (1935) introduced the in vitro culture of rabbit ovarian oocytes, a number of the mammalian species have been cultured by many investigators in order to investigate the possibility of the in vitro or in vivo fertilization of the cultured oocytes, the chromosomal and abnormal development frequency and metabolic requirements for maturation process up to the metaphase second stage of the second meiosis. A few reports have been made on requirements for resumption of meiosis in the dictyate prior to ovulation after variable periods of storage in the dictyate condition. Such investigations in various species, the mouse (Biggers et al, 1967; Donahue, 1968; Cross and Brinster, 1970) the human (Kennedy and Donahue, 1969; Cho et al. 1971) the rabbit (Chung et al, 1974; Bae and Foote, 1975 a,b,c) and the hamster (Gwatkin and Haidri, 1973) have been done. In the mouse it has been known that pyruvate is the most important and major component in supplying energy to the oocyte for maturation. Furthermore, mouse follicular cells, when included in the culture system, utilize other energy sources to support maturation, probably by liberating pyruvate into the culture medium (Donahue and Stern, 1968). Similar work has been done by Cross and Brinster (1970). The benefit of cumulus cells to oocytes was shown in that even in humans the removal of the cumulus might injure the oocyte. Secondly the cumulus might be required to supply unique substances to the oocyte.

Cross and Brinster (1970) found that the fertilization rate of the cultured oocytes decreased to a very low percentage, when

This study was supported by the Population Council in N.Y. Grant No. M71, 0136C.

the cumulus cells were removed. Later, in contrast to the above Cross (1973) found that the cumulus cells do not significantly improve physiological oocyte maturation in vitro or the in vitro fertilization of oocytes matured in vitro if calf serum components are added to the culture system. The absence of an effect of cumulus cells matured in vitro on fertilization in vitro contrasts with his previously reported positive effect of cumulus cells matured in vivo on fertilization in vitro (Cross and Brinster, 1970; Pavlik and McLaren, 1972; Miyamoto and Chang, 1972). Also, he has explained that this difference between cumulus cells matured in vivo versus in vitro suggested that these cells undergo an important change during maturation in vivo which renders them competent to contribute to normal oocyte development, cumulus cells not exposed to the in vivo stimulus would not able to function as well in either maturation or fertilization in vitro.

It is true that the major energy source of carbohydrate (pyruvate, lactate and glucose) has been added to the components of the culture media used for microorganisms up to the embryos of mammals. Contrary to this, Bae and Foote (1975 a) have developed a culture medium (glutamine salt sol.) for rabbit follicular oocytes in vitro in which glutamine is the only defined organic component. It was found that some amino acids could be good sources of energy for the maturation of the rabbit oocyte, which has many layers of compacted cumulus cells surrounding it and those could not be easily removed even by vigorous shaking or other methods (Bae and Foote, 1975 a, b).

The present study aims to compare the effect of glutamine salt sol and pyruvate salt sol (Donahue, 1968) on maturation, maturation rate of the denuded oocyte and the intact oocyte and lastly to investigate how long pyruvate is needed during the maturation process.

MATERIALS AND METHODS

Mice used in the present study were mixed bred immature albinos, 4 to 8 week old. The culture system used is the same adopted by Brinster (1963). Types of media were used, operation medium and culture medium. The operation medium in which oocytes were released from the follicles and pooled prior to incubation, was controlled to 280 mOsm or 308mOsm depending on the experimental conditions. In this operation medium glutamine or pyruvate were not added to the component but supplemented together with bovine serum albumin (0.1%). Depending on the conditions used, various concentrations of glutamine (0.0 mM ~2 mM) were adopted and the concentration of pyruvate increased to 0.33 mM, differently from the concentration (0.25 mM) used by Donahue (1968). Incubation time was 9 to 24 hours depending on the culture conditions. Components of media and detailed information are given by Bae and Foote (1975 a,b,c) and Donahue (1968).

RESULTS

As a preliminary test both glutamine salt sol. and pyruvate salt sol. as culture media and both types of oocytes, intact and denuded, were used and cultured. The results are shown in Table I. Of the total of 35 oocytes, 22 of them reached the 2nd meiotic division metaphase, MII in the pyruvate salt sol,

Table I. Comparison of Oocyte Maturation between Pyruvate Salt Sol. and Glutamine Salt Sol.*

Medium	Incubation hour	Chromosomes phase of oocytes +						
		D	P	M I	A-T	M II	Deg	othes
Pyruvate	18		1	11		22	1	
Glutamine	18	13	23	12	1	6	4	2

*: Oocytes: denuded and intact oocytes,
Operation medium: 308 mOsm; pyruvate saltsol: 308mOsm; glutamine saltsol: 280mOsm
+: D, dictyate; P, prophase; M I, 1st metaphase; M II, 2nd metaphase; Deg, degenerating oocyte; Other, non-classified.

compared with 6 of the total 61 oocytes and 36 remained at eiter dictyate or prophase I in the glutamine salt sol. It was found in both media that immature secondary whole follicles were cultured. This was found by the recovery of them after cultivation. About 90% of the oocytes cultureed in the glutamine salt sol (280 mOsm) showed either fragmentation or shrinkage of cytoplasm from the zona pellucida, although it is not certain if such phenomenona might be caused by non similarity of the osmols of the culture medium (glutamine salt sol.) or by some unknown reason. However, such fragmentation has never been observed in the oocytes cultured in the pyruvate salt sol. throughout the present study. In the next experiment only denuded oocytes were cultivated in either the pyruvate salt sol. or the glutamine salt sol. and the results are shown in Table II. Most oocytes cultured in the glutamine salt sol. remained at the dictyate or prophase I as in table I. Also, many showed fragmentation or shrinkage of cytoplasm from the zona pellucida as in the previous table. Results of Table I and II show that most of the oocytes cultured in the pyruvate salt sol. reached MII as reported by Donahue (1968), while not in the glutamine salt sol. and the glutamine salt sol does not seems to be a proper medium for the cultivation of denuded mouse oocytes.

As explained in the text, glutamine salt sol. was developed for rabbit oocyte culture and proved to be a good medium for this speices, in which the cumulus cells around the oocytes could not be easily removed from the oocyte. So in the next experiment both types of oocytes, intact and denuded. were cultured by adopting different concentrations of glutamine or different osmols of the operation media and the results are shown in Table III to VI. Denuded oocytes were cultivated by adopting 280 mOsm of the operation medium (Table III). Except for only one oocyte in the prophase II (Table III), most of the cultured oocytes remained in dictyate or prophase I, and cytoplasm showed either fragmentation or shrinkage from the zona pellucida as in the

Table II. Comparison of Oocyte Maturation between Pyruvate Salt Sol. and Glutamine Salt Sol.*

Medium	Incubation hour	D	P	M I	P II+	M II	Deg
Pyruvate	18	3		10	6	62	
Glutamine	18	3	61				10

*: Oocytes: all denuded oocytes.
+: P II, prophase II.
Operation medium: 308 mOsm.
Pyruvate saltsol: 308 mOsm
Glutamine saltsol: 280 mOsm

Table Ⅲ. Effect of Different Concentrations of Glutamine on Maturation of Oocytes in Vitro*

Medium		Incubation hour	D	P	P Ⅱ	Dg
glutamine salt sol.	0.0 mM	24	23	12		17
〃	0.08 mM	〃	24	18		9
〃	0.4 mM	〃	18	26	1	10
〃	2 mM	〃	9	29		14

*: Oocytes: all denuded oocytes.
Operation medium: 280 mOsm.
Culture medium: 280 mOsm.

Table Ⅳ. Effect of Different Concentrations Glutamine on Maturation of Oocytes in Vitro*

Medium		Incubation hour	D	P	P Ⅱ	Deg
Glutamine salt sol.	0.0 mM	18	15	7		3
〃	0.08 mM	〃	23	4		7
〃	0.04 mM	〃	18	8	1	5
〃	2 mM	〃	10	8		3

*: Oocytes: all denuded oocytes. Operation medium: 308 mOsm. Culture medium: 280 mOsm.

previous tables. Also, degenerating oocytes increased sharply by not entering the maturation process. Even though different osmols of operation medium were used in Table Ⅳ, the results are very similar to that of the Table Ⅲ. From the previous results it seems that even though different osmols were used from the rupture of the follicles to incubation in the culture media, the effect of different osmols was not found (Table Ⅱ～Ⅳ), and it seems that, differently from the rabbit, glutamine is not utilized to supply energy for the denuded oocyte of the mouse.

So in the next experiment only intact oocytes were cultivated and the results are shown in the Table Ⅴ and Ⅵ. In contrast to the previous results a slight trend toward the maturation process was shown in Table Ⅴ and such a trend is clear in Table Ⅵ in proportion to the increase of glutamine concentration to 0.4 mM and 2 mM. Such a difference between a slight maturation trend in Table Ⅴ and a clear increase of maturation rate at the concentration of 0.4 mM and 2 mM in the Table Ⅵ may or may not result from the different osmols of the operation media. Originally mammlian body fluid is 308 mOsm and the osmols of the pyruvate salt sol. were controlled to equal that of the body fluid. As in Table Ⅵ, if the operation and culture media are controlled to

Table Ⅴ. Effect of Different Concentrations of Glutamine on Maturation of the Intact Oocytes in Vitro*

Medium		Incubation hour	D	P	M I	A-T	Deg
Glutamine salt sol	0.0 mM	18	5	8			4
〃	0.08 mM	〃	2	4	1		6
〃	0.4 mM	〃	5	5	2		3
〃	2 mM	〃	5	1		1	

*: Oocytes: all intact oocytes. Operation medium: 280 mOsm. Culture medium: 280 mOsm.

Table Ⅵ. Effect of Different Concentrations of Glutamine on Maturation of the Intact Oocytes in Vitro*

Medium		Incubation hour	D	P	M Ⅰ	P Ⅱ	M Ⅱ	Deg
Glutamine salt sol	0.0 mM	18	6	5				7
〃	0.08 mM	〃	9	5				4
〃	0.4 mM	〃		10	5	1	1	5
〃	2 mM	〃	6	7	10	2		1

*: Oocytes: all intact oocytes.
Operation medium: 308 mOsm.
Culture medium: 308 mOsm.

308 mOsm, they seem to make more a favorable condition for the cumulus cells as well as for the oocytes. The increase of maturing oocytes in Table Ⅴ seems to originate from such favorable culture conditions. An interpretation is that the cumulus cells and oocyte of the mouse might be more susceptible to the effect of osmols than those of the rabbit and the cumulus cells of the mouse may supply metabolites needed by the oocyte through converting from unsuitable metabolites to ones more essential for maturation. In any cases, the concentration of glutamine originally developed for rabbit oocyte culture can be increased for the mouse beyond the optimum level (0.08–2mM) shown in the rabbit. However, in the present work such an extension of the experiment was not attempted beyond the optimum concentration for the rabbit only to compare the effect of both culture media. There are no reports showing whether pyruvate, the most important energy source for the mouse oocyte, is needed throughout maturation from the beginning of the germinal vesicle break-down to the MII stage or not. In the present work three different steps of transfer experiment were done to investigate the need of adding pyruvate salt sol. to the medium without pyruvate for the three main events of the meiosis. The results are shown in Table Ⅶ–Ⅸ. In Table Ⅶ germinal vesicle break- down was the criterion for the transfer of the oocytes. After 4–5 hour's initial cultivation in pyruvate salt sol., the oocytes were transferred to the medium without pyruvate after the confirmation of germinal vesicle break-down under a stereomicroscope and further cultivation was done in the medium without pyruvate for the remaining hours corresponding to the culture hours of the control experiment.

The general trend shown from Table Ⅶ, if the oocytes are transferred to the medium

Table Ⅶ. Effect of Pyruvate on Maturation of Oocyte in Vitro*

Medium	Incubation hour	P	M Ⅰ	A-T	M Ⅱ	Deg	Other
Pyruvate salt sol	24	2	6	2	21		an** 1
Pyruvate for 5 hr and transferred to w/o pyruvate	24	2				26	

*: Oocytes: all denuded oocytes.
Operation medium: 308 mOsm.
Culture medium: 308 mOsm.
**: an: Abnormal oocyte (see text).

without pyruvate, is that most oocytes are degenerating after the germinal vesicle breakdown in the experimental group, while most oocytes of the control group are in the maturation process from metaphase I to the M II stage of meiosis. This may means that even after the germinal vesicle breakdown the oocytes still need an energy source continuously afterwards. A special note should be pointed out here. Seven oocytes among 21 (Table VII) show the metaphase pattern of the 2nd meiosis and at the same time the 1st polar bodies of the seven oocytes also show the metaphase pattern. Furthermore, two among the same 21 oocytes have 1st polar bodies in which the chromosomal phase is telophase in contrast to the normal pattern in which the chromosomes of the 1st polar body are in condensed form or in scattered form. A third anomaly is an oocyte showing the metaphase pattern having two 1st polar bodies but one of the two polar bodies is showing the metaphase pattern while the other polar body has no chromatin materials at all. The 1st polar body usually degenerates after its formation both in vivo and in vitro: a spindle fails to form, the chromosomes remain scattered and eventually both the cytoplasm and chromatin are lost, presumably by lysis into the perivitelline space. However, the frequency of these abnormal oocytes is considered to be very high and this is very interesting. In Table VIII the oocytes were cultivated for 9 hours in pyruvate salt sol and then afterwards transferred into the medium without pyruvate for a further 15 hours. As shown in the table most of the oocytes except only one were in 1st metaphase and this result is very similar to that done by Donahue (1968). In another control group (24 hours cultivation in the pyruvate medium), 26 oocytes among a total of 41 (63%) showed metaphase second and this maturation rate was about 20% less than that of Donahue (1968). Such a difference between the present work and that of Donahue might be due to two reasons; one is that mice used in the present study were about 4 weeks old, very immature, and another reason is that the mice were raised in indaequate nutritional conditions. Four of the 26 oocytes showing the metaphase second were the ones in which the 1st polar bodes showed metaphase pattern. Another two abnormal oocytes were observed; one was an oocyte which had 4 polar bodies and all chromatin materials were shared among the 1st polar bodies but not in the oocyte at all. The other was the one in which the 1st polar body had all the chromosomes and showed the metaphase pattern but no chromatin materials in the oocyte. The former is considered to be caused by non-separation at the 1st metaphase and all

Table VIII. Effect of Pyruvate on Maturation of Oocyte in Vitro*

Medium	Incubation hour	D	P	M I	A-T	M II	Deg	Other
Pyruvate salt sol.	24	1	1	8	3	26		an** 2
Pyruvate for 9 hr	9		1	19				
Pyruvate for 9hr and transferred to w/o pyruvate	24		3		1		35	

*: Oocytes: all denuded oocytes.
Operation medium: 308 mOsm.
Culture medium: 308 mOsm.
**: an: abnormal oocytes (see text).

Table Ⅸ. Effect of Pyruvate on Maturation of Oocytes in Vitro*

Medium	Incubation hour	P	M I	A-T	P Ⅱ	M Ⅱ	Deg
Pyruvate salt sol.	14		4		1	5	2
Pyruvate for 12 hr	12	1	14	3	3	2	
Pyruvate for 12 hr and transferred to w/o pyruvate	14		5	5	11	4	2

*: Oocytes: all demided oocytes,
operation medium 308 mOsm
Culture medium: 308mOsm

chromosomes moved to the polar body.

In the experimental group the oocytes were initially cultivated in the pyruvate medium and then afterwards were transferred and further cultivated in the medium without pyruvate for another 15 hours. Three oocytes remained at prophase I and one at the stage between anaphase I and early telophase I. Thirty-five oocytes of the total 41 were in the process of degeneration. Twenty-one among 35 degenerating oocytes had all 1st polar bodies which all chromosomes in the oocyte were degenerating as well as in the 1st polar bodies. It seems that 21 oocytes had already reached metaphase second and then degenerated with an insufficient energy source for a viable condition. According to the result obtained by Donahue (1968) the period from metaphase I to M Ⅱ is very short, about 2 hours. On this basis, these 21 oocytes having 1st polar bodies had remained in a viable state at least for 2 hours and then reached M Ⅱ. Finally they might have degenerated because of insufficient energy source materials for a viable state. This means that the oocytes having M I could remain in a viable state without any further supply of energy at least for two hours with energy materials previously absorbed from the medium. Such a viewpoint that the oocytes in post-metaphase I can remain in a viable state and further proceed to maturation with an energy source previously absorbed is proved in Table Ⅸ. As shown in Table Ⅸ the oocytes cultivated in pyruvate medium for 12 hours were I in prophase I; 14 in metaphase I; 3 in anaphase I-telophase I; 3 in prophase Ⅱ and 2 in M Ⅱ. On the other hand, in the experimental group in which the oocytes were cultivated for 12 hours in pyruvate medium and then afterwards transferred and further cultivated for 2 hours in the medium without pyruvate, 25 oocytes among 27 were between metaphase I and metaphase Ⅱ, that is, most of the oocytes were still in a viable state for 2 hours without any energy source except for only 2 oocytes in degeneration. Furthermore, these oocytes were in a more advanced stage of meiosis than those in pyruvate for 12 hours. About 15 oocytes were lost at recovery, so no comparison could be made between the experimental group and the control group (14 hours cultivation in pyruvate medium). From the results from Table Ⅷ and Ⅸ the oocytes in post-metaphase I seemed to be in a viable state and proceeded to a more advanced maturational stage of meiosis with previously absorbed energy at least for 2 hours in the medium without energy source.

DISCUSSION

In the present study glutamine salt sol. was tested for the effect of maturation of the mouse oocytes, denuded and intact. Glutamine has no effect on denuded oocytes. but has on

intact oocytes. Furthermore, the present study shows that the maturation rate seems to increase in proportion to the increase of glutamine concentration. Such a difference in the effect of glutamine on maturation between denuded and intact oocytes seems to be caused by the matabolic pattern of the cumulus cells surrounding the oocyte. The cumulus cells may support the maturation of the mouse oocyte by providing the oocyte with energy by utilizing glutamine in the medium. The pathway of glutamine has not been shown even in the rabbit, in which the effect of glutamine appeared to exceed that of carbohydrates or the combination of glucose, pyruvate and lactate, but it was proved that glutamine can be utilized as an energy source as well as for protein synthesis material in the rabbit in vitro (Bae and Foote, 1675 b). However a study for the pathways of the end product of glutamine should be made. As Meister(1973) hypothesized the r-glutamyl cycle in the transport of free amino acid between the inside and the outside of cytoplasmic membrane, glutamine might have easier access to the TCA cycle than to glycolysis for an energy producing mechanism. Such an investigation should be further extended at the enzyme level. Furthermoret the close relationship between the cumulus cells and the oocyte should be emphasized in the effect of glutamine on maturation, in contrast to the recent assumption on the role of the cumulus cells (Cross, 1973). On the other hand, the mouse oocyte seems to be very sensitive to the osmols of the operation and culture media, if the oocytes are wholly denuded in the media. At least throughout the present study osmols of media closer to the level of the body fluid are recommended both in handling and cultivation. The effect of the media seems strongly to affect transportation of utilizable substances into and from the oocyte, according to previous results on the osmolarity effect on oocytes and preimplantational embryos (rabbitt oocyte, Bae and Foote, in preparation: mouse 2-cell embryo, Brinster, 1965; rabbit 2-and 4-cell embryo, Naglee et al, 1969). Fragmentation or shrinkage of cytoplasm of the rabbit oocyte could not be observed at the range of 280 mOsm or so. In the present study, cytoplasmic fragmentation and shrinkage in 280 mOsm of media (operation and culture media) were taking place in denuded oocytes only and the reason is not clear. In contrast to cytoplasmic fragmentation or shrinkage of the denuded oocytes these could not be observed in the intact oocyte cultivation in the present study. Such a difference in these phenomena between the denuded and the intact could be due to a buffer action of the cumulus cells of the intact oocytes. Thus the effect of osmols would not affect directly the oocyte, as shown in Table V and Ⅵ, if the intact oocytes are cultured.

In the cultivation of the intact oocyte, the increase in the maturation rate in proportion to the increase of glutamine concentration is very interesting and further experiments on the effect of the increased concentration of glutamine are indicated.

A high frequency of abnormal oocyte development is observerd in the pyruvate salt sol. The 1st polar body usually degenerates after its formation both in vivo and in vitro; a spindle fails to form, the chromosomes remain scattered or condensed and eventually both the cytoplasm and chromatin are lost, presumably by lysis into the perivitelline space (Donahue, 1970). The 1st polar body divides into two so that three polar bodies are eventually formed in many non-mammalian animals; this is very rare in mammalian eggs, but a few cases has been

reported(Sobotta, 1895; Rubaschkin, 1905; Kressovskaja, 1934; Odor, 1955). The frequency rate of the metaphase or telophase pattern on the 1st polar body is as much 10 times as high (reported by Donahue) (1970). The second anomaly is the oocyte which has two polar bodies having chromosomes in only one polar body, and the chromosomal phase of the 1st polar body is metaphase. A third anomaly is the oocyte which has 4 polar bodies in one egg, but no chromatin materials in the oocyte at all. These 4 polar bodies were degenerating and in condensed form. A fourth anomaly is the oocyte which has one polar body having the metaphase chromosomes pattern. No chromatin materials could be observed in the oocyte. Donahue (1970) classified the anomaly oocytes in the mouse as explained above. Several investigators suggest that the formation of an exceptionally large polar body may be due to the depth of the spindle within the egg; the deeper the spindle, the more nearly the division will result in equal-sized cells. Such a large polar body can be induced by centrifuging the egg and the maturation spindle moves toward the center of the oocyte, resulting in an exceptionally large 1st polar body (Austin, 1961). Less clear is the nature of the disturbance producing two 1st polar bodies which share equally all the the egg chromatin. It may be that after spindle transection has begun the single furrow becomes double and encircles both chromatin groups. This would account for the simultaneous separations of both chromatin groups and formation of two polar bodies (Donahue, 1970). On the other hand, nobody has explained the metaphase or telophase pattern of the 1st polar body. However, the nature of the metaphase or telophase of the Ist polar body is even less clear up to now. The authors of the present study would firstly assume that the divisional pattern of the 1st polar body, the metaphase and telophase pattern in the present studys may be due to the transfer of much energy source material into the cytoplasm of the 1st polar body from the cytoplasm of the oocyte when segragation takes place between the 1st polar body and the oocyte. As mentioned in the text of the present study pyruvate concentration was increased to 0.33 mM from 0.25 mM as used by Donahue (1968). Usually in normal lst polar body formation very little cytoplasm moves to the polar body and it is assumed that the cytoplasm of the 1st polar body carries very little energy source or nutritional material for its own further viable state. Frequent degeneration of the 1st polar body after its formation may be accounted for by insufficient energy in the polar body and in addition this may be why the 1st polar body can not be present in the oocyte for longer in most animal species. Further experiments should be carried out in the association of the increased concentration of pyruvate with the abnormal chromosomes pattern of the 1st polar body.

REFERENCES

Austin, C.R.: *The mammalian egg. Blackwel Scientific Publications, Oxford, England, 1961.*

Bae, I-H and Foote, R.H.: *Carbohydrate and amino acid requirement and ammonia production of rabbit follicular oocytes matured in vitro. Experimental Cell Res. 91:113-118, 1975.*

Bae, I-H and Foote, R.H.: *Utilizatoni of glutamine for energy and protein synthesis by cultured rabbit follicular oocytes. Experimental Cell Res., 90:432-436. 1975.*

Bae, I-H and Foote, R.H.: *Effects of hormone on the maturation of rabbit oocytes recovered*

from follicles of various size. J. Reprod. & Fert., 42:357-360, 1975.

Biggers, J.D., Whittingham, D.G. and Donahue, R.P.: *The pattern of energy metabolism in the mouse oocyte and zygote. Proc. Nat. Acad. Sci. U.S.A., 58:560-567, 1967.*

Brinster, R.L.: *A method for in vitro cultivation of mouse ova from two-cell to blastocyst. Experimental Cell Res., 32:205-208, 1963.*

Brinster, R.L.: *Studies on the development of mouse embryos in vitro. I. the effect of osmolarity and hydrogen ion concentration. J. Exp. Zool., 58:49-57, 1965.*

Cho, W.K., Kim, M.K. and Chung, S.O.: *In vitro maturation of human follicular oocytes in the presence of follicular fluid (abstract). International Congress Series No. 278. Fertility and Sterility. Proceedings of the & the World Congress, Oct. 17-25 1971. Tokyo & Kyoto, Japan.*

Chung, S.O., Bae, I-H. and Cho, W.K.: *The maturation in vitro of the rabbit oocytes. I. amino acids support the maturation in vitro of the rabbit oocytes. Yonsei Med. J., 15: 11-16, 1974.*

Cross, P.C. and Brinster, R.L.: *In vitro development of mouse oocytes. Biol. Reprod., 3:298-307, 1970.*

Cross, P.C.: *The role of cumulus cells and serum in mouse oocyte maturation in vitro. J. Reprod. & Fert., 34:241-245, 1973.*

Donahue, R.P.: *Maturation of the mouse oocytes in vitro. I. sequence and timing of nuclear progression. J. Exp. Zool., 69:237-250, 1968.*

Donahue, R.P. and Stern, S.: *Follicular cells support of oocyte maturation: production of pyruvate in vitro. J. Reprod. & Fert., 17: 395-398, 1968.*

Donahue, R.P.: *Maturation of the mouse oocyte in vitro. II. anomalies of first polar body formation. Cytogenetics, 9:106-115, 1970.*

Gwatkin, R.B.L. and Haidri, A.A.: *Requirements for the maturation of Hamster oocytes in vitro. Experimental Cell Res., 76:1-7, 1973.*

Kennedy, J.F. and Donahue, R.P., *Human oocytes: maturation in chemically defined media. Science, 164:1292-1293, 1969.*

Krassovskaja, O.V.: *Fertilization of rabbit egg outside the organism. Russk. Arkh. Anat., 13:415, 1934.*

Long, J.A. and Mark, E.L.: *The maturation of the egg of the mouse. Publ. No. 142, Carnegie Institution of Washington, D.C., 1911.*

Meister, A.: *On the enzymology of amino acid transport. Science, 180:33-39, 1973.*

Miyamoto, H. and Chang, M.C.: *Fertilization in vitro of mouse and hamster eggs after the removal of follicular cells. J. Reprod. & Fert., 30:309-312, 1972.*

Naglee, D.L., Maurer R.R. and Foote, R.H.: *Effect of osmolarity on in vitro development of rabbit embryos in a chemically defined medium. Experimental Cell Res., 58:331-333, 1969.*

Odor, D.L.: *The temporal relationship of the first maturation division of rat ova to the onset of heat. Amer. J. Anat., 97:461, 1955.*

Pavlok, A. and McLaren, A.: *The role of cumulus cells and the zona pellucida in fertilization of mouse eggs in vitro. J. Reprod. & Fert, 29:91, 1972.*

Pincus, G. and Enzmann, E.V.: *The comparative behavior of mammalian eggs in vivo and in vitro. J. Exp. Med., 62:665-675, 1935.*

Robertson, J.F. and Baker, R.D.: *Role of female sex steroids as possible regulators of oocyte maturation (abstract). Soc. for the study of reproduction. 2nd annual meeting, University of California, Davis, Ca. Acadmic Press, 1969.*

Rubaschkin, W.: *Uber die Reifungs-und Befruchtungsprozesse des Meer Meerschweinchenies. Anat. Hefte., 29:509, 1905.*

Sobotta, J.: *Die Befruchung und Fruchtunng der Eies der Maus. Arch. Miker. Anat., 45:15, 1895.*

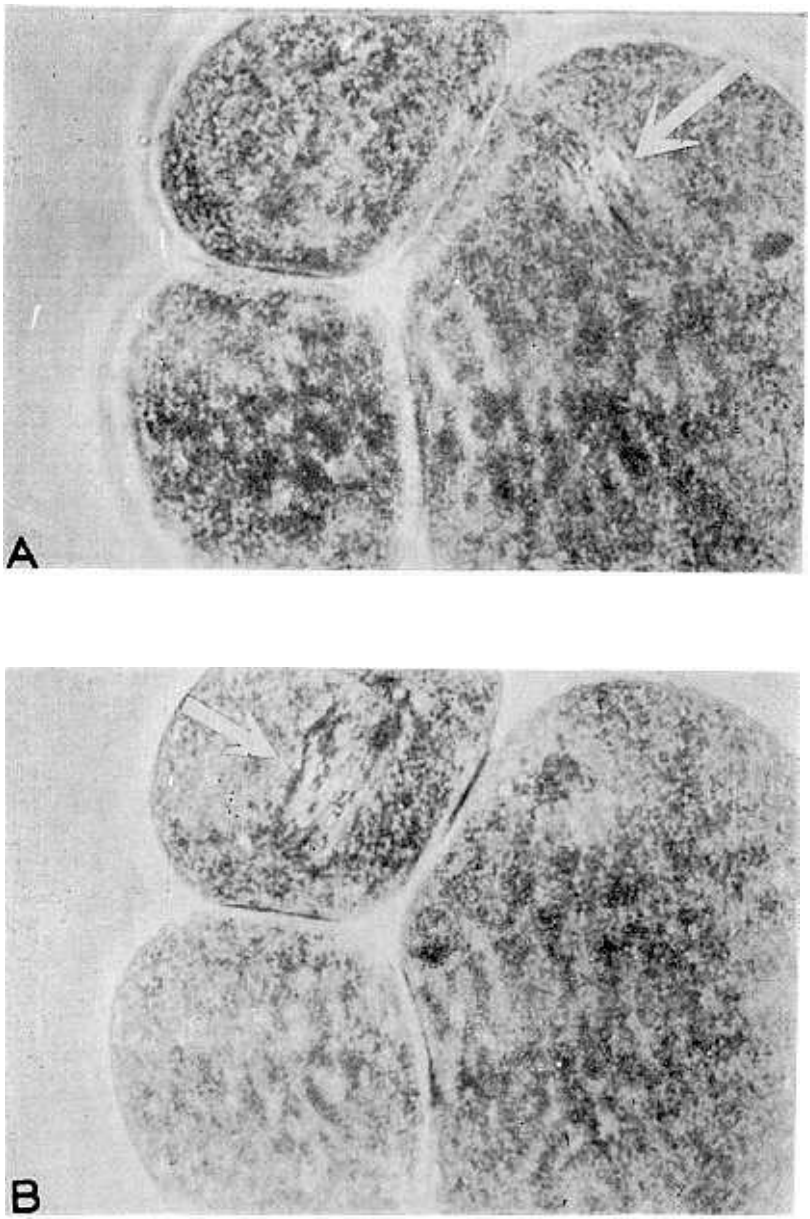

Fig. 1. An example of abnormal oocytes. This is the oocyte which has two polar bodies. One of the 1st polar bodies shows a metaphase (B) while there is no chromatin material in the other polar body. The oocytes shows a metaphose (A).