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In Vitro Development of Mouse Oocytes[1]

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Several aspects of the early *in vitro* development of the laboratory mouse were examined: oocyte nuclear maturation, fertilization, fertilization of oocytes matured *in vitro*, and fetal development of oocytes matured and fertilized *in vitro*.

Initially, optimum conditions for both oocyte nuclear maturation and fertilization were established. Oocyte nuclear maturation was greatest (91.7%) when oocytes were cultured with cumulus cells. The reduction in maturation when oocytes were cultured without cumulus cells (to 59.2%) was significant ($p < 0.02$) only when serum was not present in the medium. Fertilization *in vitro* of oviducal oocytes, measured by the number of two-cell ova formed, was greatest (90.1%) when the medium contained 30 mg/ml protein and the oocytes were fertilized with adhering cumulus cells. The removal of the cumulus cells from the oocytes prior to fertilization reduced ($p < 0.001$) the number of two-cell ova formed (90.1% to 46.3%).

Using the optimum conditions which produced 91.7% nuclear maturation and 90.1% fertilization, 37.5% of oocytes matured *in vitro* were fertilized *in vitro*. When serum was omitted from the maturation medium a significant drop ($p < 0.01$) to 22.5% fertilization occurred. When oocytes matured and fertilized *in vitro* were transferred into foster mothers, 3.2% developed into 15-day-old fetuses, thus demonstrating that normal oocyte maturation processes can occur *in vitro*.

Since 1935 when mammalian oocytes were matured *in vitro* for the first time (Pincus and Enzmann), maturation *in vitro* has been obtained in a large variety of species (see Schuetz, 1969). Only a few attempts, however, have been made to define the specific requirements for maturation. Oocytes of the mouse (Biggers, Whittingham, and Donahue, 1967) and human (Kennedy and Donahue, 1969) have been matured in defined media, establishing pyruvate as the central energy source in the mouse and possibly also in the human. The function of the cumulus cells, if any, in the nutrition of the mammalian oocyte during maturation remains unknown; however, in the mouse, cumulus cells added to an *in vitro* culture system are capable of utilizing glucose to supply pyruvate to the oocytes for maturation (Donahue and Stern, 1968). It has further been shown that the removal of the cumulus cells in the human (Kennedy and Donahue, 1969) and the rabbit and cow (Robertson and Baker, 1969) reduces the number of oocytes maturing *in vitro*.

In all of the above studies the criterion for successful oocyte maturation *in vitro*

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was the completion of nuclear maturation (formation of the first polar body and second metaphase). Nuclear maturation is not, however, necessarily an adequate criterion for normal development since previous studies have shown that the developmental potential of cytologically mature oocytes is limited. Chang (1955a) in the rabbit and Edwards, Bavister, and Steptoe (1969) in the human have obtained some fertilization of oocytes matured *in vitro*, but the attempts to obtain viable fetuses have failed (rabbit, Chang, 1955b; mouse, Donahue, 1968a).

The present investigation was undertaken primarily to develop an *in vitro* culture system capable of supporting normal oocyte maturation. The success of oocyte maturation *in vitro* was examined at three levels: (1) the completion of nuclear maturation, (2) the formation of two-cell ova from oocytes matured and fertilized *in vitro*, and (3) the formation of viable fetuses from oocytes matured and fertilized *in vitro*. The effects of complex and simple media, serum and cumulus cells on oocyte maturation were investigated. Since fertilization *in vitro* was used as one of the criteria for normal maturation, optimum conditions for this technique were also determined. In an attempt to demonstrate conclusively that maturation processes *in vitro* are normal, oocytes with cumulus cells, matured in a medium containing serum, were fertilized *in vitro* and transferred into genetically marked foster mothers. The development of viable fetuses from oocytes matured and fertilized *in vitro* would represent the first instance of the postimplantation development of oocytes matured *in vitro*.

MATERIALS AND METHODS

Maturation in Vitro

Oocytes were obtained from 6–8-week-old Swiss mice (A. R. Schmidt, Millerton, New York), injected 46–48 hr previously with 5 IU serum gonadotrophin (PMS) (Equinex, Ayerst). The injection of PMS was used to increase the number of immature oocytes with adhering cumulus cells (Cross, P., unpublished). Throughout this report the term "cumulus" will be used to include the corona radiata cells. Each ovary was placed in a watch glass containing 1 ml of Medium BMOC-2 [Brinster, 1965b: 94.88 mM NaCl, 4.78 mM KCl, 1.71 mM $CaCl_2$, 1.19 mM KH_2PO_4, 1.19 mM $MgSO_4 \cdot 7H_2O$, 25.07 mM $NaHCO_3$, 0.25 mM sodium pyruvate, 25.00 mM sodium lactate, 1 mg/ml bovine serum albumin (BSA), penicillin and streptomycin] under 2 ml of paraffin oil, and the follicles were punctured to release the oocytes. Immature oocytes were pooled in a single watch glass of medium under oil. When the collection period extended approximately 15 min, the oocytes were stored at 37 C in an atmosphere of 5% CO_2 in air. The immaturity of oocytes with cumulus cells was determined by the ability to distinguish a nucleolus through the granulosa cell layers. On several occasions, when the reliability of this criterion for oocyte immaturity was tested by removing the cumulus cells, all of the selected oocytes contained intact germinal vesicles. This method assured that oocytes had not undergone any grossly observable maturation prior to culture.

The oocytes were cultured in drops of media under oil at 37 C in 5% CO_2 in air (Brinster, 1963), 10 oocytes in each drop. The oocytes were matured in one of four media, itemized and described in Table 1: complex + serum, complex + BSA, simple + serum, and simple + BSA. The complex medium was a Medium 199 with Earle's salts (GIBCO), modified by the addition of sodium pyruvate. The simple medium was a BMOC-2 modified by the absence of lactate, an increase in pyruvate concentration, and the addition of glucose. Either 15% calf serum (GIBCO) or 30 mg/ml BSA (Sigma) was added to make up the final four media. In order to maintain the pH of all media at 7.4, 1 N NaOH was added to the media containing BSA.

In studies examining the completion of nuclear maturation, after 12–13 hr of culture the oocytes were squashed under a cover slip (see Austin, 1961) and examined under phase contrast for the first polar body and second metaphase. When the oocytes were cultured with adhering cumulus cells, it was necessary to remove these cells prior to phase contrast examination using hyaluronidase (300 unit/ml in a phosphate-buffered salt solution) and/or mechanical disturbance by suction and release of the oocytes in small bore pipettes. In studies examining the ability of *in vitro* matured oocytes to undergo fertilization, at the end of 12–13 hr of culture the oocytes were placed with sperm. In initial experiments, some oocytes were matured *in*

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TABLE 1
CULTURE MEDIA FOR OOCYTE MATURATION

	Modified Medium 199 (mM)[a]	Modified BMOC-2 (mM)[b]
NaCl	116.36	119.88
KCl	5.36	4.78
$CaCl_2$	1.80	1.71
KH_2PO_4	—	1.19
$NaH_2PO_4 \cdot H_2O$	1.02	—
$MgSO_4 \cdot 7H_2O$	0.81	1.19
$NaHCO_3$	26.19	25.07
Na lactate	—	—
Na pyruvate	0.50	0.50
Glucose	5.56	5.56
Penicillin	100 U/ml	100 U/ml
Streptomycin	50 μg/ml	50 μg/ml

[a] A complex medium with additional components not itemized, including amino acids, vitamins and DNA and RNA percursors. This medium was modified by the addition of pyruvate.

[b] A simple medium modified by the omission of lactate, increase in pyruvate and the addition of glucose. Either 15% calf serum or 30 mg/ml bovine serum albumin was added to the simple and complex media to make up the final four media used for oocyte maturation.

vitro and placed in the fertilization medium without sperm to determine the extent of parthenogenesis. Neither pronuclei nor cleavage was observed.

Fertilization in Vitro

The fertilization *in vitro* technique was basically that developed by Whittingham (1968); however, the medium was a variation of BMOC-2: a modified Krebs–Ringer bicarbonate (94.88 mM NaCl; 4.78 mM KCl; 1.71 mM $CaCl_2$; 1.19 mM KH_2PO_4; 1.19 mM $MgSO_4 \cdot 7H_2O$; 25.07 mM $NaHCO_3$) containing 0.50 mM sodium pyruvate, 25.00 mM sodium lactate, and 5.56 mM glucose as energy sources, 30 mg/ml BSA, penicillin, and streptomycin. The pH was adjusted to 7.4 with 1 N NaOH. Initial attempts to fertilize oocytes in Medium 199 supplemented with 0.50 mM sodium pyruvate and 15% calf serum failed, and therefore only the defined medium was used for fertilization.

Tubal oocytes (matured *in vivo*) were removed from the oviducts of superovulated 6–8-week-old Swiss mice (see Brinster, 1970) 14–15 hr after the injection of chorionic gonadotrophin (HCG) (Pregnyl, Organon). In the experiment to determine the optimum concentration of BSA for fertilization, the cumulus cells were removed with hyaluronidase (300 unit/ml in a phosphate-buffered salt solution) and the oocytes were washed three times in BMOC-2. The oocytes to be fertilized were placed in watch glasses containing 1 ml of the fertilization medium under oil. Either 10 or 20 oocytes were placed in each fertilization dish depending upon the experiment. The sperm were obtained from the uteri of a superovulated Swiss female injected with HCG 11–12 hr previously and mated to a Swiss male 1–2 hr previously. Both uteri of one female were stripped into 0.5 ml of the fertilization medium under oil, and approximately 50 μl of sperm suspension was transferred to each fertilization dish (Whittingham, 1968). The sperm collected from one female were used to fertilize all groups of eggs in any single experiment. The oocytes and sperm were incubated at 37 C in 5% CO_2 in air for 26 hr at which time the number of two-cell ova was recorded.

Transfers

Two-cell ova obtained from the fertilization *in vitro* of oocytes matured *in vivo* or *in vitro* were transferred into foster mothers on day 1 of pregnancy (day of vaginal plug). The foster mothers, homozygous dark-eyed C3D2F1 hybrids (C3H/HeJ ♀ × DBA/2J ♂, Jackson Laboratory), were mated with either vasectomized or intact Swiss males. Five two-cell ova were transferred into one oviduct of each recipient. Fifteen days after the transfer, the foster mothers were sacrificed and the eye color of all fetuses was recorded. All of the transfers consisted of two-cell ova resulting from the maturation and fertilization *in vitro* of oocytes cultured with their adhering cumulus cells, matured in the modified Medium 199 plus 15% calf serum, and fertilized in the defined medium.

For statistical analysis the data were converted to angles as described by Biggers and Brinster (1965). Either the analysis of variance or Student's *t* test was

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used to determine significance (Fisher and Yates, 1957; Snedecor, 1956).

RESULTS

Maturation in Vitro

The initial experiments on *in vitro* maturation examined the effect of different media and also the effect of the cumulus cells on the completion of oocyte nuclear maturation (first polar body and second metaphase). Table 2 gives the results of the effects of complex and simple media, either in the presence or absence of serum, on the maturation of oocytes with adhering cumulus cells. All four media supported a high percentage of oocyte maturation (86.7–96.7%). An analysis of variance of the data in Table 2 demonstrated no significant difference between the four media in the number of oocytes completing maturation; however, the behavior of the cumulus cells during maturation varied depending upon the culture media. In both media containing serum the cumulus cells attached to the bottom surface of the culture dish, and at the end of the culture period the removal of these cells from the oocytes required hyaluronidase and continual manipulation. When the oocytes were cultured without serum, the cumulus cells did not attach to the surface and were easily removed with gentle manipulation.

The results of the effects of the same media on the maturation of oocytes without cumulus cells are shown in Table 3. In contrast to the group with cumulus cells, an analysis of variance of the data on oocytes without cumulus cells (Table 3) does demonstrate a significant effect ($p < 0.01$) of serum on maturation. In the media containing serum, 43 out of 60 (71.7%) oocytes completed nuclear maturation in comparison to 28 out of 60 (46.7%) oocytes cultured in media not containing serum. The experiments shown in Tables 2 and 3 also demonstrate an effect of the cumulus cells on maturation. When only the difference between oocytes with and without cumulus cells is considered, a significantly greater number of oocytes ($p < 0.001$) complete maturation when cultured with cumulus cells (110/120, 91.7%) than without cumulus cells (71/120, 59.2%). The effect of the different media on oocytes cultured with and without cumulus cells can be summarized as follows. Cumulus cells improved the

TABLE 2

EFFECT OF DIFFERENT MEDIA ON OOCYTE NUCLEAR MATURATION WHEN OOCYTES ARE CULTURED WITH CUMULUS CELLS

Media	Response[a] Experiment			Mean angular response	Response (%)
	1	2	3		
Complex + serum	10	7	10	72.87	90.0
Complex + BSA	7	10	9	69.77	86.7
Simple + serum	10	9	10	77.80	96.7
Simple + BSA	9	10	9	74.70	93.3

[a] Number of oocytes in metaphase II after 12–13 hr culture out of a total of 10 oocytes initially placed in culture. Complex = modified Medium 199, see Table 1; Simple = modified BMOC-2, see Table 1; BSA = bovine serum albumin.

TABLE 3

EFFECT OF DIFFERENT MEDIA ON OOCYTE NUCLEAR MATURATION WHEN OOCYTES ARE CULTURED WITHOUT CUMULUS CELLS

Media	Response[a] Experiment			Mean angular response	Response (%)
	1	2	3		
Complex + serum	8	6	6	55.00	66.7
Complex + BSA	5	5	4	43.07	46.7
Simple + serum	8	9	6	61.93	76.7
Simple + BSA	5	5	4	43.07	46.7

[a] Number of oocytes in metaphase II after 12–13 hr of culture out of a total of 10 oocytes initially placed in culture. Complex = modified Medium 199, see Table 1; Simple = modified BMOC-2, see Table 1; BSA = bovine serum albumin.

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TABLE 4
EFFECT OF PROTEIN CONCENTRATION ON FERTILIZATION *in Vitro* OF OOCYTES MATURED *in Vivo*

Protein concentration (BSA, mg/ml)	Response[a] Experiment				Mean angular response	Response (%)
	1	2	3	4		
10	4	3	3	12	30.75	27.5
20	11	7	7	9	40.65	42.5
30	11	9	10	7	42.83	46.3
40	4	3	8	12	34.85	33.8
50	4	3	3	4	24.70	17.5
60	6	4	4	12	34.30	32.5

[a] Number of two-cell ova out of a total of 20 oocytes. BSA = bovine serum albumin; Medium was modified BMOC-2, see text. The cumulus cells were removed prior to fertilization.

response in all media, but the only significant difference ($p < 0.02$) in the oocytes matured with and without cumulus cells occurred in media not containing serum. Out of the 60 oocytes initially placed in media not containing serum, 54 (90.0%) completed nuclear maturation when cultured with cumulus cells, and only 28 (46.7%) completed nuclear maturation when cultured without cumulus cells. There was no significant difference between the effect of complex versus simple media on maturation with or without cumulus cells.

Fertilization in Vitro

An attempt was made to determine the optimum conditions for fertilization *in vitro*, since the success of maturation was to be determined in subsequent studies by the ability of oocytes matured *in vitro* to be fertilized and cleave to two-cell ova *in vitro*. Such a physiological check was considered a much more accurate assessment of maturation than the completion of nuclear maturation (Chang, 1955b). The initial experiments examined the effect of protein (BSA) concentration on fertilization *in vitro*. When tubal oocytes with adhering cumulus cells were used, the number of oocytes completing fertilization and the first cleavage division varied considerably between treatments. Since ovulated unfertilized oocytes from a single female clump together into one or two masses, the variation was probably related to differences between individual mice. When the cumulus cells were removed and the oocytes completely randomized, variation was reduced. The results of experiments to examine the effect of protein on fertilization *in vitro* are shown in Table 4. An analysis of variance of the data presented in Table 4 combined with the J. W. Tukey method of comparing means (Snedecor, 1956) demonstrated the following significant differences ($p < 0.01$): (1) 20 and 30 mg/ml BSA produced a greater response than 10 mg/ml; and (2) 20, 30, 40, and 60 mg/ml BSA produced a greater response than 50 mg/ml. Since the maximum fertilization rate was achieved in a concentration of 30 mg/ml, this concentration was used in the remaining experiments.

From the experiment on protein concentration it was evident that oocytes without cumulus cells could be fertilized *in vitro;* however, when the cumulus cells were removed, the number of two-cell ova formed (37/80, 46.3%) was significantly less ($p < 0.001$) than when oocytes were fertilized with adhering cumulus cells (137/152, 90.1%). These data are presented in Table 5.

Maturation and Fertilization in Vitro

In the next series of experiments, the same four media were used to culture oocytes, but in these experiments, rather than examining the oocytes at the end of maturation, the oocytes were tested for their ability to undergo fertilization and the first cleavage *in vitro* in the modified BMOC-2. Only oocytes with adhering cumulus cells were used in these experiments since preliminary studies indicated that maturing and fertilizing oocytes *in vitro* without adhering cumulus cells drastically reduced the number

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TABLE 5
EFFECT OF CUMULUS CELLS ON FERTILIZATION *in Vitro* OF OOCYTES MATURED *in Vivo*

	Response[a] Experiment					Total	Mean angular response	Response (%)
	1	2	3	4	5			
Without cumulus cells	11/20	9/20	10/20	7/20		37/80	42.83	46.3
With cumulus cells	32/33	18/22	19/24	35/38	33/35	137/152	71.41[b]	90.1

[a] Number of two-cell ova/number of oocytes.
[b] Significantly greater than without cumulus cells ($p < 0.001$).

TABLE 6
FERTILIZATION *in Vitro* OF OOCYTES MATURED IN DIFFERENT MEDIA

Media	Response[a] Experiment I			Experiment II			Mean angular response	Response (%)
	1	2	3	1	2	3		
Complex + serum	3	5	4	1	2	5	34.57	33.3
Complex + BSA	4	3	6	1	0	1	28.18	25.0
Simple + serum	5	5	3	2	5	5	39.97	41.7
Simple + BSA	4	3	2	1	1	1	25.70	20.0

[a] Number of two-cell ova out of a total of 10 immature oocytes initially placed in culture. Complex = modified Medium 199, see Table 1; Simple = modified BMOC-2, see Table 1; BSA = bovine serum albumin. The cumulus cells were left on for both maturation and fertilization.

of resulting two-cell ova. The results of these experiments are presented in Table 6. An analysis of variance of the data demonstrated an effect of serum ($p < 0.01$). More two-cell ova were formed from oocytes matured in media containing serum (45/120, 37.5%) than in media not containing serum (27/120, 22.5%). Observations under phase contrast did not demonstrate any gross differences between the two-cell ova resulting from maturation in the four media.

The final experiments specifically relate to the relative ability of oocytes matured *in vitro* and *in vivo* to undergo further development. Data presented in Table 7 compare the number of two-cell ova obtained from maturation *in vitro* and fertilization *in vitro* (33/100, 33.0%) with the number of two-cell ova obtained from maturation *in vivo* and fertilization *in vitro* (137/152, 90.1%). Representative two-cell ova are shown in Fig. 1. It is clear that the ability of oocytes to undergo fertilization and the first cleavage *in vitro* is reduced ($p < 0.001$) when maturation *in vitro* precedes fertilization.

Fetal Development

Out of 95 two-cell ova matured and fertilized *in vitro*, 3 (3.2%) (Fig. 2) developed into 15-day-old fetuses when transferred into foster mothers; whereas, out of 55 two-cell ova matured *in vivo* and fertilized *in vitro*, 13 (23.6%) developed into 15-day-old fetuses when transferred into foster mothers. The three fetuses which developed from maturation and fertilization *in vitro* were found within three separate foster

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TABLE 7

FERTILIZATION *in Vitro* OF OOCYTES MATURED *in Vitro* IN COMPARISON TO OOCYTES MATURED *in Vivo*

	Response[a] Experiment					Total	Mean angular response	Re-sponse (%)
	1	2	3	4	5			
Matured *in vitro*[b]	6/20	6/20	4/20	10/20	7/20	33/100	34.86	33.0
Matured *in vivo*	32/33	18/22	19/24	35/38	33/35	137/152	70.41[c]	90.1

[a] Number of two-cell ova/number of oocytes initially placed in culture.

[b] Complex + serum = modified Medium 199 + 15% calf serum, see Table 1.

[c] Significantly greater than oocytes matured *in vitro*, $p < 0.001$. The cumulus cells were left on for both maturation and fertilization.

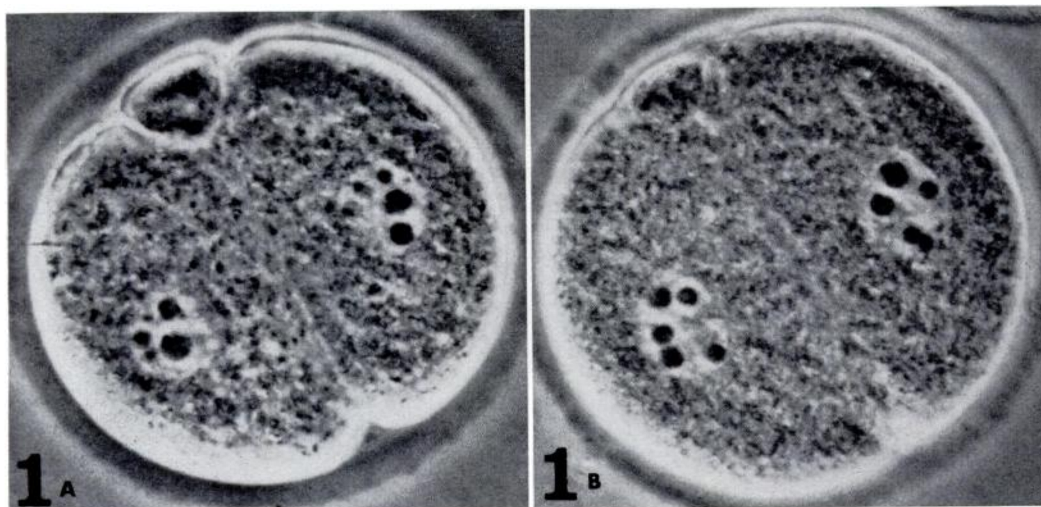

FIG. 1. A. Two-cell ovum produced from *in vitro* maturation in Medium 199 and 15% serum and *in vitro* fertilization. B. Two-cell ovum produced from *in vivo* maturation and *in vitro* fertilization. Both two-cell ova were from the same experiment, fertilized with sperm from one male. Only one polar body can be seen in this plane of focus. The ova were compressed under a coverslip and examined under phase contrast. ×800.

mothers and were the result of three separate experiments (days). The data demonstrate that oocytes matured *in vitro* are capable of developing into viable fetuses even though maturation in the culture system is inferior to *in vivo* maturation.

DISCUSSION

A high percentage of oocytes cultured with cumulus cells (90.7%) completed nuclear maturation at the same time (12–13 hr) as occurs *in vivo* (12.5–13 hr) (Edwards and Gates, 1959). The beneficial effect of cumulus cells on maturation observed in the present study has also been reported in the human (Kennedy and Donahue, 1969) and the rabbit and cow (Robertson and Baker, 1969). When oocytes without cumulus cells were cultured, the addition of serum to the medium enhanced maturation; however, serum apparently contributes no additional factors to those provided by

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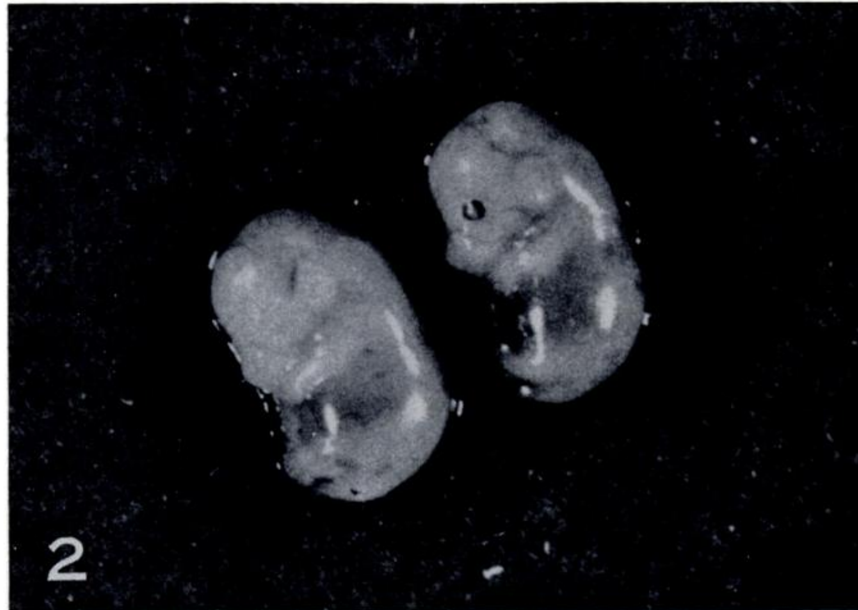

FIG. 2. Two 15-day-old fetuses obtained from the same foster mother. The dark-eyed foster mother (C3D2F1) was mated with a normal Swiss male and thus produced offspring with eye pigmentation. In addition, one fetus without eye color developed from the transfer of a Swiss × Swiss two-cell embryo matured and fertilized *in vitro*. ×3.3.

the cumulus cells, since as many oocytes cultured without serum and with cumulus cells complete nuclear maturation as oocytes cultured with both serum and cumulus cells. Kennedy and Donahue (1969) also found that serum does not improve maturation when human oocytes are cultured with adhering cumulus cells. Cumulus cells and serum are probably affecting the rate of maturation rather than the ability of the oocytes to mature, since a high percentage (88%) of mouse oocytes without cumulus cells or serum in the medium are capable of completing maturation after 18 hr of culture (Donahue, 1968b). Even though the nature of the total contribution of the cumulus cells and serum has not been determined, there is evidence to suggest that the cumulus cells can provide an energy source for oocyte maturation (Donahue and Stern, 1968) and may produce hormones capable of regulating maturation (Robertson and Baker, 1969).

The optimum fertilization *in vitro* of tubal oocytes reported in this study (90.1%) is similar to that obtained *in vivo* (85.2%) (Gates, 1965) and greater than previously obtained *in vitro* (40.9%) (Whittingham, 1968). Two factors were found to contribute to the success of fertilization: the concentration of protein in the medium and the use of ova with adhering cumulus cells. The optimum concentration of protein for fertilization was relatively high (30 mg/ml) in comparison to the 1 mg/ml routinely used to culture two-cell ova (Brinster, 1965a); however, the amount of protein required by ova may vary during different stages of development. Biggers (1970) improved the development of certain hybrid one-cell ova to blastocysts by increasing the concentration of protein from 1 to 4 mg/ml. In the present study, it is possible that the protein primarily affected the first cleavage division rather than fertilization itself, although the fertilization technique used could introduce

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unique factors which may require extra protein.

A beneficial effect of cumulus cells (defined to include the corona radiata) on fertilization *in vitro* has also been observed in the rabbit by Chang and Bedford (1962). However, other studies in the rabbit have have failed to demonstrate any reduction in fertilization when the cumulus cells are removed (Bedford and Chang, 1962; Harper, 1970); therefore, this effect on fertilization, at least in the rabbit, apparently depends upon the experimental conditions.

Combining the optimum conditions for maturation *in vitro* with those established for fertilization *in vitro*, it was found that oocytes matured *in vitro* could undergo fertilization and the first cleavage division *in vitro*. This study represents the first time that mouse oocytes matured *in vitro* have been fertilized *in vitro* and the first time in any species that normal cleavage has been obtained from oocytes matured *in vitro*. Pincus and Enzmann (1935) reported fertilization *in vitro* of rabbit oocytes matured *in vitro;* however, since the source of the sperm was not identified and we know that in this species capacitation is essential, the success of the fertilization reported remains questionable. More recently in the rabbit, attempts were made to fertilize *in vivo* oocytes matured *in vitro* (Chang, 1955a). Although in these studies sperm were found in the perivitelline space, two polar bodies were observed, and cleavage occurred, the blastomeres observed in the cleaved ova were of unequal size and the cytoplasm had fragmented. In the human, Edwards, Bavister, and Steptoe (1969) observed fertilization *in vitro* of oocytes matured *in vitro*. Eighteen out of 34 ova had either sperm in the zona pellucida, sperm in the perivitelline space or pronuclei. In a subsequent study, midpieces and tails of sperm were identified in the vitellus of some oocytes (Bavister, Edwards, and Steptoe, 1969), but in no case did normal cleavage occur.

In the present study we found that the appearance of a normal morphological nuclear maturation did not always indicate that the oocytes matured *in vitro* were capable of being fertilized. Thus 90% of the oocytes initially placed in culture completed a normal appearing nuclear maturation by 12–13 hr and yet only 33.0% were capable of being fertilized *in vitro*. This low percentage of fertilization must be due to abnormal processes which occurred during maturation *in vitro* since a large percentage of ova matured *in vivo* were capable of fertilization (90.1%). Deficiencies in oocytes matured *in vitro* could either result from the initial selection of the oocytes from the ovary or could develop as a consequence of suboptimal culture conditions. The present study did demonstrate that the inclusion of 15% serum in the maturation medium significantly improved the ability of oocytes to be fertilized and thus suggests that at least part, if not all, the deficiencies associated with oocytes matured *in vitro* results from inadequate culture conditions. One possible explanation for the beneficial effect of serum is that the increased adherence of the cumulus cells to the oocytes induced by serum increased the efficiency of transport into the oocyte. It is known that maternal serum molecules are transferred into preovulatory mouse oocytes, and it has been suggested that the follicle cells are involved in this transfer (see Glass, 1969). The serum molecules accumulate during maturation and then disappear after maturation prior to follicle cell dispersal, suggesting that this material functions at fertilization (Glass, 1963).

The only reported attempts to demonstrate the postimplantation development of oocytes matured *in vitro* have failed. None of 88 rabbit oocytes matured *in vitro* (Chang, 1955b) and 0 out of 432 mouse oocytes matured *in vitro* (Donahue, 1968a) developed into viable fetuses. The development of fetuses from oocytes matured and

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fertilized *in vitro* in the present study demonstrates that the culture conditions for oocyte maturation are adequate to support post-implantation development in at least some of the oocytes. It now remains to improve the percentage of harvested oocytes which can be fertilized *in vitro* and survive to term when reintroduced into a mother.

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