Maturation of Hamster's Follicular Ova in Culture

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Hamster의 濾胞卵子의 培養에 의한 成熟

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(Received Nov. 12, 1966)

摘　　要

본 실험은 4日間의 性週期가 뚜렷한 성체인 golden hamster로 부터 미성숙인 卵子를 摘出하여 化學的培養液 내에서 그의 성숙을 유도시키고 性週期에 따른 미성숙 卵子의 成熟率을 관찰하는 것을 주요 목적으로 하여 행하여졌다.

한 개의 卵巢로부터 5~6개의 미성숙 卵子를 摘出하여 5% bovine serum albumin이 섞인 TC Medium 199에 넣어서 CO_2-incubator를 이용하여 6時間 내지 24時間 동안 37°C를 유지해 가며, 培養液이 직접 空氣와 접촉하는 것을 막기 위해 유동성인 paraffin oil로 培養液을 덮고서 培養을 완수했다.

실험의 결과를 다음과 같이 요약할 수 있다.

1. Hamster의 卵子의 성숙분열을 유발시키는 데 가장 적합한 培養液은 BMOC, Eagle's Medium, Waymouth's Medium, 그리고 TC Medium 199의 네 가지 가운데 TC Medium 199이었다. 즉 이 培養液을 이용했을 때 卵子가 높은 成熟度를 보여 주었다.

2. 5% bovine serum albumin을 TC Medium 199에 섞어 주었을 때 미성숙 卵子가 성숙분열을 유기하는 率이 가장 높았다.

3. 發情期에 있는 卵巢로 부터 얻은 卵子가 가장 현저하게 높은 率로 성숙분열을 보여 주었다. 반대로 發情後期의 卵子는 배양 시작 후 단시간 내에 대부분이 퇴화하였으며 發情期에 가까와 갈수록 퇴화율이 줄어들었다. 이것은 濾胞가 성숙분열을 억제하는 물질을 생성하리라고 여겨지고 있긴 하지만, 동시에 이 濾胞는 또 한편 卵子의 排卵뒤에 까지도 生命力을 유지할 수 있는 能力을 發情期에 이르기까지의 期間 동안 卵子에게 부여하며, 卵子는 이 能力을 排卵前까지 축적하게 되며 이 때문에 濾胞로부터 유리되어 나온 發情期의 卵子가 長時間 그 生命力을 유지해 나가는 것이라고 推定된다.

4. Paraffin oil로 空氣를 차단하여서 배양한 卵子나 혹은 watch glass를 이용하여 空氣와 접촉시킨 卵子에서나 모두 비슷한 率로 성숙분열을 보여 주었다. 이로 보아 조작이 까다로운 paraffin oil을 이용하는 방법보다는 손쉽게 배양할 수 있는 watch glass의 方法이 오히려 유용하다고 할 수 있다.

INTRODUCTION

Since Pincus and Enzmann (1935, 1936) cultured follicular ova of rabbit for the first attempt to produce their maturation *in vitro*, Chang (1955) has made more extensive studies with rabbits, and Edwards (1962, 1965a and b) has concentrated his investigations on the production of maturation *in vitro* of ovarian oöcytes from several mammals

including human. The first two studies were conducted by means of use of media mainly originated from the plasma and the third was made using the chemically defined medium such as TC Medium 199. Although they used culture media originated from different sources one from the other, these works have shown several similar findings; firstly, the follicular ova which had been persisting as dictyate stage of their first meiosis for considerable period in the Graafian follicles resumed their ability to proceed maturation in the culture medium shortly after liberated from the follicles, secondly, cultured ova with polar body retained their fertilizability if they were transferred into the fallopian tubes even the proportion was not much as expected, thirdly, many of the cultured ova were blocked at Anaphase I and this configuration lasts longer until the ova become atresia, and lastly, the follicular ova from most of mammals need almost same intervals for the completion of the maturation *in vitro* as *in vivo*.

For the above studies on the cultivation of ova, they obtained the ova either from the ovaries of various mammals previously stimulated with gonadotrophic hormones or from the ovaries regardless of their estrous cycles which should play a great role on the sustaining of the ability for the maturation of ova in their own follicles. However, by this moment, as nobody has been trying to conduct the studies in connection with the potentiality for the maturation of the ova in different estrous cycles, the present study was planned mainly to confirm the relationships of them. That is, the purposes for the present investigations can be described as follows; firstly, whether the follicular ova are able to pertain a similar condition for the maturation *in vitro* even though they are originated from animals with different estrous cycles, secondly, which kinds of media for the ova cultivation give more sufficient results, and lastly, what results of the cultivation by two different methods, watch glass method and paraffin oil sealing method, are brought.

The present author, at this moment, should express his gratitudes to Dr. M. C. Chang by whose constant guidances the results could be made in the Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts, U.S.A., and to the Population Council, the Rockefeller University, New York, U.S.A. which gave him an opportunity to be a fellowship for two years since 1964.

MATERIALS AND METHODS

Adult golden hamsters, bred randomly in the Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts, U.S.A., showing their four-day regular estrous cycles were used in this research. The estrous cycles were defined as Day 1 (Metestrus), Day 2 (Diestrus), Day 3 (Proestrus) and Day 4 (Estrus). Each hamster was killed by capital dislocation and both sides of her ovaries were explanted into a few milliliters of sterilized saline containing heparin (1 unit per ml). The ovaries were washed two times in fresh saline with heparin so that excess blood adhering to the ovarian surface was removed, and their ovaries were transferred into the medium 199 supplemented with 5% v/v phosphate buffer salt solution and one unit of heparin, in which the ova were expelled from the larger follicles in the ovary by means of use of finely pointed needles under the stereomicroscope. Finally the freed ova were introduced to the drop of medium(approximately 0.05 ml), containing 100 units per ml of penicillin and 50 μg per ml of streptomycin, suspended in the paraffine oil (viscosity, 125/135) by means of a capillary pipette followed by the washing of the naked ova in the fresh medium 199 without heparin for two times. The paraffin oil for the sealing of culture medium was prepared by same process introduced by Brinster (1965). In order to compare the results of maturation of ova cultured by the paraffin oil sealing method with that by the opened method, watch glass method was employed for this investigation. The ova were introduced in the medium (approximately 0.1 ml) contained in the watch glass (diameter, 40 mm) placed on the moistened sponges in the covered culture dish (diameter, 60 mm). This application was referred to the method developed by Jensen *et al.* (1964) for organ culture, but in this experiment, the using of stainless steel mesh grids and lens papers were not considered. The ova in the medium either sealed or opened were incubated in a CO_2-incubator strictly controlled with temperature (37°C), gases phase (95% air and 5% CO_2) and moisture.

As the incubation was terminated, the ova were transferred into a few drops of saline in the test tube from the medium with a capillary pipette, and shaked the tube vigorously for the purpose of remove of excess cumulus cells whose assosiation with the ova is unfavorable at the staining and microscopy at the observation.

The ova were placed on the slide glass equipped with paraffin wax spots (Marston *et al.*, 1964) and covered by a cover slip. They were fixed in the acid-alcohol (one part of acetic acid and three parts of absolute methanol) for 4~12 hours and stained by 1% of aceto-orcein or by 1% of aceto-lacmoid under a dissecting microscope.

The present experiments were planned to compare the relationship between the kinds of media and the proportion of maturation of ova, between the concentration of bovine serum albumin (Pentex) and the induction of the maturation, between estrous cycles and the maturation and between the length for the incubation and their maturation.

For the comparison of media, were used BMOC (Brinster's Medium for Ova Culture) (Brinster; 1963), Eagle's Basal Medium, Waymouth's Medium and TC Medium 199. In order to see the most optimum concentration of the bovine serum albumin in the medium, 0%, 0.5%, 1% and 5% of the albumin were supplemented to the medium.

The experiments with the ova from the different estrous cycles and for the time interval for the incubation were accomplished by using TC Medium 199 supplemented with 5% of bovine serum albumin (BSA) which was established in this study as the best medium for the induction of maturation division of the hamster's follicular ova.

All glasswares and instruments used for the study were thoroughly sterilized by hot air sterilizer or by autoclave. Disposable plastic culture dishes (Falcon Plastic Co.) were preferably used for cultivation of ova by paraffin oil sealing method.

RESULTS

For the first experiment of the present study, the comparison of the rates of maturation of the follicular ova cultured in various media uniquely containing 0.5% of BSA was conducted. Five or six ova obtained from ovaries in Diestrus and Proestrus were incubated in each medium sealed by paraffin oil for 24 hours. As the table (Table 1) shows, metaphase figures could be seen in every medium even though the frequencies of maturation of ova were slightly different, but not reached the significant level. Among four kinds of media, TC Medium 199 with 0.5% BSA revealed the best results for resuming the maturation process, and on the other hand, BMOC, known as one of the most excellent media for the cultivation of the fertilized mouse ova, actually gave less favorable condition to the freed follicular ova, but still it induced the maturation division even the rates were the lowest among others.

Comparing the rates of sustainability of ova in BMOC with that in TC Medium 199, the difference between them are nearly significant ($X^2=2.74$). In other words, if the experiment would be conducted in great scale, it might be assumed that there could be the significant difference among them.

Table 1. Nuclear phases of cultured ova in the different media for 24 hours.

Media	No. of ova* cultured	Dictyate and Prophase	Metaphase I through Metaphase II	Degenerative ova
BMOC+0.5% BSA	42	6 (14.3)**	9 (21.4)	27 (64.3)
Eagle's Medium+0.5% BSA	36	8 (22.2)	8 (22.2)	20 (55.6)
Waymouth's Medium+0.5% BSA	36	7 (19.4)	7 (19.4)	22 (61.2)
TC Medium 199+0.5% BSA	47	11 (23.4)	14 (29.8)	22 (46.8)

* Ova were collected from the ovaries in Day 2 and Day 3 hamsters.

** shows percentage.

On Table 2, for the second experiment, the relationship between the proportions of maturation of the follicular ova and the concentration of BSA supplemented into TC Medium 199 was tabulated. Those ova cultured were collected from ovaries in Diestrus and Proestrus. Even though the frequencies of their maturation *in vitro* were not on the significant difference, it seemed that the best concentration would be contained 5% of BSA in medium for induction of the maturation of the follicular ova. The ova, however, cultured in plain medium revealed the lowest rate for the sustainability of living ova among others. Regarding with the sustainability of ova in medium containing plain and 5% BSA, the difference in rates was highly significant ($X^2=8.50$).

Table 2. Nuclear phases of cultured ova in the medium 199 supplemented with different concentration of bovine serum albumin for 24 hours.

Concentration of BSA	No. of ova* cultured	Dictyate and Prophase	Metaphase I through Metaphase II	Degenerative ova
0%	62	13 (21.0)**	12 (19.3)	37 (59.7)
0.5%	47	11 (23.4)	14 (29.8)	22 (46.8)
1.0%	41	9 (21.9)	11 (26.8)	21 (51.2)
5.0%	65	20 (30.8)	23 (35.4)	22 (43.8)

* Ova were collected from the ovaries in Day 2 and Day 3 hamsters.
** shows percentage.

The cultured ova from animals in different estous cycles resumed various phases from dictyate through Metaphase I with or without polar bodies. In order to compare the results of these cultured ova according to estrous cycles and the length of the cultivation, the summaries are tabulated in Table 3. From the table, it is clear that the ova from ovaries of different cycles show similar figures which ova in dictyate through all groups were decreased in number and the proportions of the maturation of ova from Metaphase I through Metaphase II showed increase as the duration of cultivation was extended. The rate of degenerative ova in culture was also the highest in Day 1 group, and in all groups of cultivation for 24 hours. Those results were concised in Tables 4 and 5.

Table 3. Nuclear phases of cultured ova according to estrous cycles and duration of cultivation.

Estrous Cycles	Duration cultured	Dictyate	Prophase	Metaphase I and Anaphase I	Metaphase II with polar body	Degenerative ova	Total
Day 1	Initial	37	3	2	—	4	46
	6 hours	3	—	3	—	14	20
	12 hours	7	1	11	—	18	37
	24 hours	6	—	2	5	30	43
	Total	53	4	18	5	66	146
Day 2	Intial	9	—	6	—	3	18
	6 hours	6	1	—	—	2	9
	12 hours	2	—	3	1	12	18
	24 hours	1	—	4	1	—	6
	Total	18	1	13	2	17	51
Day 3	Initial	14	—	1	—	—	15
	6 hours	11	2	5	2	—	20
	12 hours	7	3	2	2	4	18
	24 hours	3	1	1	—	10	15
	Total	35	6	9	4	14	68
Day 4	Initial	18	—	3	2	—	23
	6 hours	7	—	9	2	13	31
	12 hours	5	—	7	9	12	33
	24 hours	1	—	4	15	14	34
	Total	31	—	23	28	39	121
Total	Initial	78	3	12	2	7	102
	6 hours	27	3	17	4	29	80
	12 hours	21	4	23	12	46	106
	24 hours	11	1	11	21	54	98
	Total	137	11	63	39	136	386

On Table 4, showing the relationship between the proportion of maturation and the estrous cycles, only the ova from Day 4 (Estrus) ovary were resuming their maturation with great rate (47%), and on the other hand, the degeneration of ova was seen in Day 1 group in the highest rate (62%). The difference of frequencies of the maturation rates for every group is clearly on the significant level ($X^2=18.25$).

Table 4. Nuclear phases of cultivated ova according to estrous cycles.

Estrous cycle	No. of ova cultured	Nuclear phases		
		Dictyate and Prophase	Metaphase I to Metaphase II	Degenerative ova
Day 1	100	17 (17.0±5.6)*	21 (21.0±4.1)	62 (62.0±4.8)
Day 2	33	10 (30.3±8.0)	9 (27.3±7.7)	14 (42.4±8.6)
Day 3	53	27 (50.9±6.9)	12 (22.6±5.7)	14 (26.4±6.0)
Day 4	98	13 (13.3±3.4)	46 (46.9±5.0)	39 (39.8±4.9)

* Per cent ± Standard Error

On Table 5, the relation between the maturation of ova cultured and the duration of cultivation was shown. The ova in dictyate and prophase stages were seen in great numbers in the non-cultured group and only 14% of them were on the maturation while 7% demonstrated their ways on degeneration. The rate of ova in dictyate and prophase stages was gradually decreased, while both the rates of ova showing Metaphase I through Metaphase II chromosomes and of degenerative ova were gradually increased as the length of duration of cultivation was extended till 24 hours. The significant differences between groups are easily seen on the table ($X^2=14.85$).

Table 5. Nuclear phases of cultivated ova according to length of incubation.

Hours	No. of ova cultured	Nuclear phases		
		Dictyate and Prophase	Metaphase I to Metaphase II	Degenerative ova
Initial	102	81 (79.4±4.0)*	14 (13.7±3.4)	7 (6.9±2.5)
6	80	30 (37.5±5.4)	21 (26.3±4.9)	29 (36.4±5.4)
12	106	25 (23.6±4.1)	35 (33.0±4.6)	46 (43.4±4.8)
24	98	12 (12.2±3.3)	32 (32.7±4.7)	54 (55.1±5.0)

* Per cent ± Standard Error

Table 6 is the summary of the results of destinies of ova cultured in the medium sealed by paraffin oil and in the medium on the watch glass. The first method for culture, recommended for the cultivation of early embryos(2-cell stage through blastocysts) by Erinster, is designed to protect against direct contact to air while the latter, modified slightly from ordinary culture methods for cells and tissues in petri dish containing wet sponges, is permitted to contact directly to air. The results by both methods were so homogenous that the difference of rates of them was not significant.

Table 6. Comparison of maturation of ova cultured by paraffin sealing and watch glass methods for 24 hours.

Culture method	No. of ova cultured	Nuclear phases		
		Dictyate and Prophase	Metaphase I to Metaphase II	Degenerative ova
Paraffin oil sealing	161	40 (24.8±3.4)*	44 (27.3±3.5)	77 (47.8±3.9)
Watch glass	61	11 (18.0±4.9)	21 (34.4±6.0)	29 (47.5±6.4)

* Per cent ± Standard Error

Through the present experiments, five ova cultured for 12 hours or for 24 hours showed abnormalities in their shapes. One of them was a giant ovum resuming two Metaphase Is in different angles, two were binucleated, one had a bigger polar body and the last was binucleated with a polar body. The striking character could be seen in each polar body which retains a number of chromatin masses scattered inside as condensed dots.

DISCUSSION

It has been commonly known that the oöcytes in ovarian follicles of the most mammals are retained in dictyate of their first maturation division unless either they are stimulated by luteinizing hormone or they are in shortly before

or after releasing from the follicles (Brambell, 1956; Ingram, 1962). Edwards (1965a and b) has demonstrated the maturation *in vitro* of the ovarian oöcytes of human, monkey, mouse and other several mammals in the medium, and has shown that the major part of these cultured oöcytes resumed their maturation division and protruded their first or second polar bodies in the period of the cultivation terminated at 17th hour after the beginning of culture. He (1962) also suggested that such a resumption of the maturation process of freed ovum is mostly due to the liberation from the follicles which are assumed that they have certain kinds of inhibitory materials against the maturation division of ova *in vivo*. Actually, author, through the present studies, has to agree partially with Edwards' assumptions that there are factors to inhibit the further steps for maturation in follicles, because he could also find that ova freed from follicles revealed metaphase in the medium with higher rate.

Once Edwards (1962, 1965a and b) had cultured ovarian oöcytes with the medium 199 through his serial experiments with several animals. At this point, as the present author has tried to confirm the suitable medium for the culture of hamster's ovarian oöcytes, he compared the results of maturation of ova using several kinds of media such as BMOC, Eagle's Medium, Waymouth's Medium and TC Medium 199. Especially, particular attention was paid to the results of studies with BMOC because this medium known popularly as one of the most favourable media for the cultivation of fertilized ova since Brinster had obtained successful results with using it. But unlikely, the present data showed that this medium is unsuitable for the follicular ova even though some of them resumed their meiosis in the medium. Such an induction of the maturation of ova but with low rate should be due to only the fact that the ova were liberated from the follicles regardless of constituents of media. It has been known that such an induction of maturation division of ovarian oöcytes could be seen even in 0.85% of salt solution. According to the observations conducted by others, it is rather expectant to find meiosis in BMOC as well as in other effective media. Concluding this experiment with different media the present author should commend TC Medium 199 as one of the best media for culturing the ovarian oöcytes.

A part of the former workers such as Pincus and Enzmann (1935) and Chang (1955) prefered to use the salt solution with animal plasma for the essential nutrients for the metabolism and energy sources of follicular ova *in vitro*, and the other such as Edwards prepared the chemically defined media supplemented with 10% to 15% of mammalian serum for the cultivation of ova. For the present studies, the author supplied bovine serum albumin for the supplement of basic medium, which is made by crystallized and different from the original serum brought directly from animal blood. As Table 2 shows, the significant difference in rates of the maturation of ova was seen in the medium supplemented with the varied concentrations of BSA, and that the medium with 5% of BSA is a good enough for production of meiosis of the follicular ova could easily be assumed from the present results. Refering with the viscosity of the paraffin oil for sealing of the medium, the larger amount of albumin is not suitable because the medium with more concentrated BSA than 5% is not able to maintain its spherical shape in the oil. Therefore, even though it should be necessary to observe further the result of the medium with more concentrated BSA, it was unable to evaluate its efficacy in this research which was planned to culture ova by oil sealing method.

As Table 3 and Table 5 show, 14% of the ova fixed at immediately after liberation from the follicles had made their metaphase chromosomes. Almost all of these oöcytes were without surrounding of corona cells, whose existance is one of the important objects for evaluating the condition of oöcyte in connection with the atresia. Among them two ova from the estrous cycles, even showing their metaphase II chromosomes, would possibly be on degeneration. This assumption could be led by Edwards' opinion (1962) that the follicular ova without corona cells are generally on their ways to atresia even though they produce metaphase chromosomes. In the present studies in special regarding with the estrous cycles, it is most attractive that the greater proportions of the cultured ova from the estrous stage resumed meiosis and showed degenerative while only a few ova were remained in dictyate. On the other hand, the ova from metestrous through proestrous, the gradual increase of dictyate to 51% and decrease of degenerative ova from 62% were observed. Comparing two groups, estrous with metestrous through proestrous, quite a different views were of-

fered. The assumptions for these results could be developed as follow: firstly, the ova from the ovaries in metestrous easily loose their viability in the medium even supplemented with essential nutrients. Viewing Table 3, 70% of cultured ova in the metestrous group become degenerative in the medium within six hours after beginning of incubation. If ova liberated from diestrous ovary, the ability for the sustaining viability would increased, so the rate of degeneration actually decreased. Ova from proestrous ovary also could retain more viability than those from diestrous. The phenomenon of the increase of viable ova in the medium, as the table shows, is explainable along with the above assumptions. Secondly, that the prompt increase of degenerative ova in the experiment with estrous ovaries was taken place is probably due to the fact that some of nearly or previously matured ova in the follicles were possibly subjected to cultivate. That is, they are predicted to be degenerative soon after liberation from follicles. This means that ova from the ovaries in estrous cycle have already been accelerated for maturation by certain kinds of factors in the follicles which are even known as a factory producing antimeiogenic factors. The similar explanation could be permitted to the fact that the raise of the proportion of meiotic figures in ova from estrous ovaries as well. In other words, the oöcytes have accumulated the potentiality for the resumption of meiosis in their follicles up to the estrous cycle. Chang (1955), Edwards (1962, 1965 a and b) extended their own opinions in connection with the maturation of ova *in vivo*, claiming that the exogenous gonadotrophic hormone unlikely stimulates the maturation *in vivo* of ovarian oöcytes. According to the present investigation, however, the enough secretion of the pituitary hormone at the estrous stage should accelerate the further resumption of maturation and, consequently, such an accumulated potentiality for the resumption would emerge shortly after liberated or during the cultivation.

Refering tables (3 and 5), the gradual increase of the metaphase ova was seen in each group as the duration of the culture elapsed. And such a trend was prominent in ova from estrous ovaries. Up to 24 hours in culture, the dictyate ova from estrous ovaries abruptly decreased in number as the incubation period was elongated, and the other way, the ova showing maturation were raised in number. In other words, the great majority of the cultured ova produced their metaphase chromosomes in 12 to 24 hours. Once Edwards has observed the maturity *in vitro* of ovarian ova according to the period of culture with several mammals. By his experiments, the majority of the ovarian oöcytes of mouse accomplished their maturation in the medium in duration between 8 and 15 hours after incubation. And on the other hand, he examined the metaphase I chromosomes after 20～25 hours and their polar bodies after 45 hours in culture in the oöcytes from the monkey and human ovaries whose ovulation occur 24～48 hours after the injection of LH into female (Gemzell, 1961; Simpson and Wagenen, 1962). Thus the necessary times for the production of metaphase or extrusion of polar body are varied by animal species. However, it could be clear that the time for the production of maturation of the ovarian oöcytes in culture required almost same period as the ovulation induced by the injection of LH *in vivo* through those researches including the present one.

In this study, author has applied paraffin oil sealing method which was developed by Brinster for the cultivation of fertilized ova. This method has several advantages with regarding to the maintenance of unique pH value of the medium under the sealed oil and that considerably small volumes of medium is good enough to culture them. From the comparative studies of this method with the watch glass method without sealing of medium by oil, the insignificant difference was observed. That is, if the incubator would be equipped by the mechanics for supplying of 5% carbon dioxide and 95% of air, the cultivation of ovarian oöcytes in opened medium could be conducted in favourable condition as well as in sealed medium. Considering the technical complexities of preparation for the culture dishes with oil, the ordinary method such as using slide glass, test tube and watch glass allowing to contact to the direct air is rather recommendable.

CONCLUSION

The present investigations were designed in order to prove the problems regarding with the resumption of the maturation division in the follicular ova of golden hamsters in different estrous cycles in culture. Through the experiments, all of follicular ova were cultured in the medium 199 supplemented with 5% of BSA which was decided as

the most favourable medium for the hamster's ovarian oöcytes after the preliminary experiments for this investigation were conducted, and the paraffin oil sealing method was adopted for the culture except a part of experiments with watch glass for the comparison of the proportion of resumption of maturation to the former. The experiment showed that the majority of ova from metestrous ovary were degenerated in the medium within a six-hour period for incubation, while the high proportion of the ova from the estrous ovary were able to resume their maturation process up to 24 hours in incubator. These phenomena would be explainable as follows; the ova would accumulate their own viability which sustains the potentiality to resume meiosis in the growing follicles, known as the place to produce certain kinds of antimeiogenic factors, until the estrous cycle is returned. If this assumption is approvable, it is not difficult to manifest the reasons for the abrupt increase of degenerative ova from metestrous ovary in the time shortly after liberation from follicles, and for the gradual increase of meiotic ova up to 24 hours from estrous ovary.

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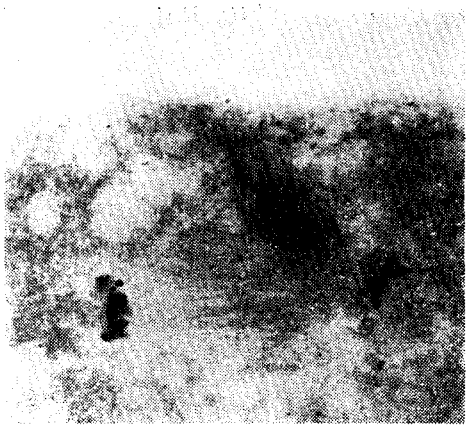

Fig. 1

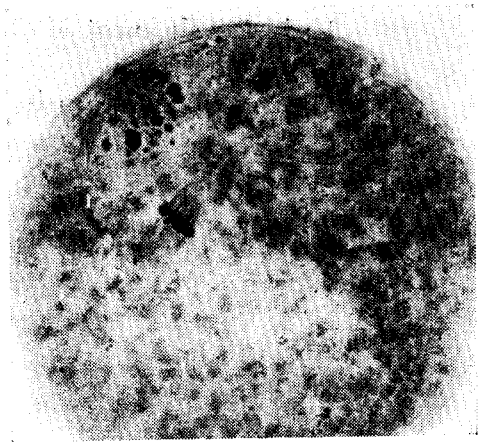

Fig. 2

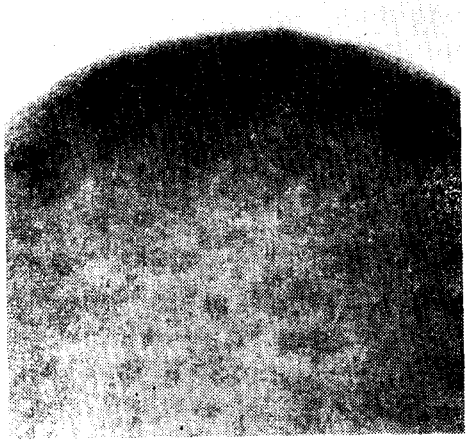

Fig. 3

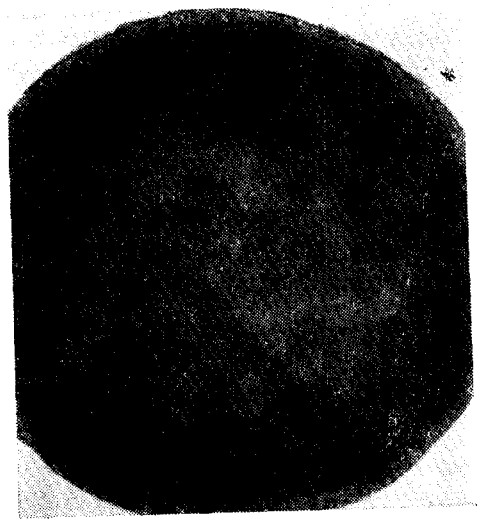

Fig. 4

Explanation of Figures

Fig. 1. Hamster's follicular ovum cultured for 12 hours in TC Medium 199 supplemented with 5% of BSA. Late anaphase. Spindle fibers are fairly seen between two poles. Ovum from metestrous ovary.

Fig. 2. Hamster's follicular ovum cultured for 24 hours in TC Medium 199 supplemented with 5% of BSA. Metaphase II chromosomes with spindle fibers. Polar body containing a number of chromatin masses is seen by the margin of upper right side of ovum. From estrous ovary.

Fig. 3. Hamster's follicular ovum cultured for 12 hours in TC Medium 199 supplemented with 5% of BSA. Double spindles are seen on the right and left hands near the margin of ovum. The ovum was particularly big as to be almost two times in diameter to other ordinary ones. From proestrous ovary.

Fig. 4. Hamster's follicular ovum cultured for 12 hours in TC Medium 199 supplemented with 5% of BSA. Binucleated ovum with polar body, one part of which is barely seen on the upper of right side of margin in cut. From proestrous ovary.