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Experiments on the Transplantation of Ova in Mice

Badania nad transplantacją jaj myszy

I. INTRODUCTION

Although research work on the transplantation of ova in mice has often been carried out, the results, in comparison with analogical experiments with rabbits, are still unsatisfactory (low degree of effectiveness of transplantation). It would be of real value to find out whether this is connected with the differences in the techniques applied, or with the differences in the suitability of the ova of these two species for manipulation in vitro.

The techniques hitherto applied for the transplantation of ova in mice have been based on the introduction of the oocytes under the ovarian capsule (Runner, 1951; Runner and Palm, 1953) and

the transplantation of morulae or blastocysts to the uterus (Fekete and Little, 1942; Fekete, 1947; Boot and Mühlbock, 1953; McLaren and Michie, 1956; Gates, 1956).

The possibility of transplanting 2-cell ova to the oviduct was announced in the work by Bittner and Little (1937), but from that time onwards this technique was not applied at all, neither did it later receive more detailed treatment.

Since, however, the technique of transplantation of 2-cell ova to the oviduct gives the best results in the case of the rabbit, and in addition makes it possible to apply this method to the solution of many other problems, it became evident that more detailed work on its application to mice, and comparison of the results with data so far obtained by the use of other methods, would be of essential value.

The second problem dealt with in this work is a repetition of the experiments made by Beatty (1951) on the transplantation of eggs without operation, that is, by introducing them into the uterus through the uterine cervix. This technique, although unusually interesting on account of its simplicity, has not so far been in general applied, presumably on account of its low degree of effectiveness.

II. MATERIAL AND METHODS

Albino, „grey", „beige" and brown and black mice, were used for the experiments. The brown and black mice were homozygous, and produced dark-coloured offspring. Albino mice when crossed with „grey" or „beige" individuals as a rule produced dark-coloured offspring in generation F_1. Eggs obtained from black or brown females after mating with males of the same strain, or from albino females mated with a „beige" or „grey" male (and vice versa), were used for transplantation. The recipients were albino females previously mated with a sterile (vasectomized) albino male, or „grey" or „beige" females after copulation with a vasectomized „beige" male. [1])

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[1]) The strains of mice used for the experiments were not accurately defined from the genetic point of view, and on this account the term „strain" is based entirely on the colour of the fur.

a. Technique of transplantation to the oviduct

The principal part of the experiments consisted in transplanting 2-cell ova. The recipients were as a rule females mated the previous night. The difference in the „phases" of pregnancy and pseudo-pregnancy in the donor and the recipient was therefore 1 day, and increased in the case of transplantation of 8-cell ova and blastocysts to 2 and 3 days respectively. The ova were washed out from the oviducts of the donors and transplanted in mouse serum diluted by an equal quantity of normal saline solution. The serum obtained from the blood of both males and females was kept in a refrigerator for a period not exceeding 3 days. This medium was not heated, but was of normal room temperature. The recipients were kept under an ether anaesthetic, and an incision made in the skin and muscular layer immediately above the ovary, which was then pulled out on to the exterior, and a small incision made in the ovarian capsule. A thin pipette was introduced through this incision into the infundibulum, and the ova blown in, with the smallest accompanying amount of fluid possible, to the lumen of the oviduct. The entire operation was carried out using a dissecting microscope. The time during which the ova were in vitro varied from 10—20 minutes (most often between the limits of 10—15 minutes). The experiments were carried out at various times during the first day after copulation.

b. Technique of transplantation through uterine cervix

The transplantation operation was carried out similarly to the experiments made by B e a t t y (1951), except that a suitably constructed pipette was substituted for the syringe. One end of the pipette was drawn out to form a micro-pipette (the edge of which had been melted to form a smooth surface), while the thick end was bent halfway along to form a right angle, and finished with a rubber tube which was kept in the mouth during the operation. The advantage of this pipette as compared with a syringe is that it makes control of the presence and position of ova possible during the operation, and is also easy to handle. Using this technique, 2-cell ova were transferred to the recipients on the second day, and blastocysts on the fourth day, post-coitum. In this series the „phase" of the pregnancy of the donor was synchronised with the „phase" of the pseudo-pregnancy of the recipient (employing the symbols

used in the work by McLaren and Michie (1956), these were the synchronous combinations 1½—1½ and 3½—3½). In a few cases blastocysts were transferred to females which had mated with normal males, selecting donors and recipients of different pigmentation. Locke's solution and a serum diluted by normal saline solution were used for recovery and transplantation of the ova.

III. RESULTS

a. Transplantation to oviduct

Before carrying out the basic series of experiments to determine the possibility of complete development of the transplanted eggs, two series of experiments were made, during which a check was made on the second, third and fourth day of the recipient's pregnancy. In the first series of experiments normal saline solution was used as the medium for recovering and transplanting the ova, and in the second, diluted serum. These experiments were aimed at determining the suitability for transplantation purposes of these two fluids, the effectiveness of the technique itself (what percentage of the transferred ova was to be found in the genital tract) and at checking the rate of development of the transplanted ova.

Five experiments were made using normal saline solution as a medium. In one case a check was made on the following (second) day, and in the remaining four cases, on the third day. On the second day the presence of 4-cell ova of normal appearance was confirmed, whereas on the third day all the ova were characterised by more or less advanced degenerative changes, and the stage of development did not in any of the cases exceed 16-cell morulae. These data revealed the complete unsuitability for transplantation purposes of a normal saline solution, even though the ova did not remain in it for longer than 15 minutes.

Data obtained in the second series (medium — serum-saline) showed that as a result of the operation, there was a preliminary check on the development of the transplanted ova (as in the first series), then development proceeded normally. At noon on the second day (actually this is the third day of development for the transplanted eggs), the ova have reached the 4-cell stage, although normally they should have reached the 8-cell stage. On this account the eggs have not yet attained the stage of well-formed blastocysts on the third

Table No. 1.

Result of transplantation operation on the development of recipient's own ova (autopsy on the third day post-coitum).

No.	Medium	Operated side	Non-operated side
1	saline	3 — fragmentation	1 — fragmentation 4 — 8-cell
2	saline	5 — fragmentation	4 — fragmentation
3	serum-saline	5 — fragmentation	5 — fragmentation
4	serum-saline	4 — fragmentation	6 — fragmentation
5	serum-saline	4 — 8-cell	not found
6	serum-saline	5 — fragmentation	4 — fragmentation

Table No. 2.

Results of transplantation of eggs to oviduct.

No.	Stage of development of transplanted eggs	Day of autopsy	No. of transplanted eggs	No. of embryos which developed from transplanted eggs
1	2-cell	15	5	3
2	2-cell	16	3	1
3	2-cell	17	3	3
4	2-cell	18	6	5
5	2-cell	19	6	6
6	2-cell	19	5	5
7	2-cell	birth	4	3
8	2-cell	birth	8	2
9	2-cell	birth	4	3
10	2-cell	birth	6	4
			Total 50	**35**
11	8-cell	19	4	4
12	blastocyst	19	8	3

day, but form a transit stage between morula-blastocyst, composed on an average of 30 cells. On the fourth day (fifth day of development of eggs) normally-shaped blastocysts are formed. As a supplementary note to these observations it is of interest to record that in one case the serum of a different species (*Microtus agrestis* L.) was used as a medium, and the normal development of the eggs up to the blastocyst stage was confirmed.

Check tests carried out in the pre-implantation period were intended, on the other hand, to determine what percentage of the eggs transferred to the oviduct remains in the genital tract, which is first and foremost a condition of the effectiveness of the entire technique. A check was made of 10 females into which 55 ova had been transplanted. When examination of the contents of the oviducts or uterus was carried out, 46 eggs were found, that is, 83.6% of the eggs transplanted. Two females from the first series and four females from the second were mated with normal males, and at the moment of transplantation ought to have had their own normally-developing eggs at the 2-pronuclei stage. In all these six cases a check was made on the third day when the possibility of differentiating between the recipient's own eggs and the transferred eggs still exists. The results given in Table 1 show that most often the operation had a harmful effect on the development of the recipient's own eggs, causing their fragmentation.

Table No. 3.

Distribution of embryos in both uterine horns in the first „normal" pregnancy after operation.

No.	Operated side	Non-operated side
1	2	10
2	0	7
3	1	4
4	4	6

The principal series of experiments was carried out on 10 females into which 50 2-cell eggs had been transplanted (Table 2). Autopsy was carried out between the 15th and 19th day of pregnancy on 6 females, and 4 females were left until they had littered. Of a to-

tal number of 50 transplanted eggs, 35 embryos (or young ones) developed, that is, 70%. The four females gave birth in the late hours of evening of night on the 19th day, that is, the normal time typical of the females reared and used by us.

Two attempts were also made to transplant eggs in a more advanced stage of development (8-cell ova and blastocysts) and positive results obtained (Table 2).

The four females which gave birth to young ones were mated, at the end of the nursing period, with normal males, then an autopsy made several days post-coitum. The distribution of the embryos in both horns (Table 3) shows that despite the incision made in the capsule during the transplantation operation, it is possible later on for the eggs to reach the oviduct and for normal development to take place on the side operated on.

b. Transplantation through the uterine cervix

Using the non-operative technique, 2-cell eggs were transplanted to the uterus on the second day post-coitum (Table 4), and blastocysts on the fourth day (Table 5).

Table No. 4.

Transplantation of 2-cell ova to the uterus by the non-operative method on second day of pseudo-pregnancy of recipient.

No.	Number of eggs transplanted	Day of autopsy	Results
1	5	7	—
2	6	7	—
3	10	7	—
4	7	5	—
5	6	4	—
6	7	4	—

Transplantation of 41 2-cell ova to 6 females gave no positive results. Checks made on the fifth and seventh day reveal complete absence of any trace of implantation, and on the fourth day, of any transplanted eggs in the uterus.

In the second series (Table 5) 154 blastocysts were transplanted to 30 recipients. Twenty-six of the females had mated with a sterile

Table No. 5.
Results of transplantation of blastocysts to uterus by the non-operative method.

No.	Day of transplantation	Character of the male used N – normal V – vasectomized	Medium	Day of autopsy or day new oestrus appeared (E)	Number of transplanted eggs	Number of implantation sites N – normal R – regressive	Occurence of vaginal bleeding. (Days of pregnancy)
1	4	V	Locke	12	3	—	—
2	4	V	,,	12-E	6	—	—
3	4	V	,,	12-E	2	—	—
4	4	V	,,	10-E	2	—	8
5	4	V	,,	12	4	1N, 3R	9, 10, 11, 12
6	4	V	,,	12	3	3R	10, 11, 12
7	4	V	,,	11-E	2	—	—
8	4	V	,,	11	3	1N	9, 10, 11
9	4	V	,,	12-E	5	—	—
10	4	V	,,	10-E	1	—	—
11	4	V	,,	12	7	1N, 4R	11, 12
12	4	V	,,	10	6	2R	9, 10
13	4	V	,,	9-E	7	—	—
14	4	N	,,	14	3	—	
15	4	V	,,	13	3	1N	12, 13
16	4	V	,,	11	5	2R	8, 9, 10
17	4	V	,,	11	3	1R	—
18	4	V	,,	16	10	—	not investigated
19	4	V	,,	15	9	—	,,
20	4	V	,,	7	9	5R	
21	4	V	,,	7	8	3N, 4R	
22	4	V	,,	19	4	—	not investigated
23	4	V	,,	19	8	—	,,
24	4	V	,,	19	9	4N, 1R	,,
25	4	V	serum-saline	14-E	4	—	—
26	4	N	,,	16	8	—	
27	3	V	,,	12-E	8	—	—
28	4	V	,,	13-E	5	—	11, 12
29	4	N	,,	15	2	—	
30	4	N	,,	19	5	2N	

male, and 4 with a normal male. From the seventh day of pregnancy vaginal smears were taken to determine the moment of vaginal bleeding, and possible appearance of new oestrus. Since it became clear as work proceeded that vaginal bleeding occurred very early in many of the females, immediately followed by the end of pregnancy and appearance of new oestrus, autopsy was carried out in a certain group of recipients between the seventh and 12th day of pregnancy. As check testing for the whole series was carried out in various days of pregnancy (from the 7th to the 19th), the results obtained do not on the one hand give a full picture of the extent of implantation (disappearance of regressive foetal chambers in later pregnancies), and on the other hand — as regards normally developing embryos — they are not final results.

The results are as follows after summing up: of the 154 transplanted blastocysts a total of 38 became implanted (24.6%) and of

Table No. 6.

Development of blastocyst transplanted by the non-operative method in the group of females in which implantation took place.

Total number of females	Females in which implantation took place		Number of transplanted eggs	Implantation sites		Normally developing embryos	
	no.	%		no.	%	no.	%
26	11	42,3	61	36	59,0	11	18,0

Table No. 7.

Development of blastycysts transplanted by the non-operative method depending on the number transferred. *)

No. of eggs transplanted	No. of females	Total no. of transplanted eggs	Implantation sites		Normally developing embryos		Females in which implantation took place	
			no.	%	no.	%	no.	%
1 — 5	13	42	12	28,5	3	7,1	6	46,1
6 — 10	10	79	24	30,3	8	10,1	5	50,0

*) Only cases in which females mated with vasectomized males and Locke's solution used as medium for transplantation were taken into consideration.

these only 13 were developing normally at the moment autopsy was carried out (8.4%).

Since in the majority of the recipients the transferred eggs never attained implantation, it is important to consider separately the group of females in which at least 1 blastocyst was implanted (Table 6). These data indicate that the total low effectiveness of this technique is due to the large number of completely unsuccessful experiments. In the group of recipients in which implantation occurred, 59% of the transferred blastocysts became implanted. The data contained in Table 7 may serve as support for this view, since they prove the absence of dependence on, or proportionality between the number of implantations and the number of blastocysts transplanted.

IV. DISCUSSION

The technique of transplantation of 2-cell eggs in mice direct to the oviduct was first applied in experiments by Little, but was not, however, discussed or worked out in detail by this author. In the work by Bittner and Little (1937) there is the following paragraph: „...Additional work by one of us is associated with this theory and gives supporting data. Fertilized ova from dilute brown (dba) females were removed within 24 hours after impregnation and were transplanted to the oviducts of C57 Black females. The dilute brown females obtained by this procedure were after being nursed by the C57 Black „mothers", used as breeders". From the text following this it is clear that many females were bred in this way. There is unfortunately no detailed description of the technique, nor data giving a picture of the effectiveness of the experiments carried out.

The technique described in the present work is in part similar to that used by Runner (1951) and Runner and Palm (1953). These authors, however, transplanted oocytes, surrounded by cumulus oophorus cells, introducing them not directly to the oviduct, but under the ovarian capsule. This operation must be carried out at the moment of occurrence of ovulation in the recipient.

Comparison of the results so far obtained from research work on the transplantation of mouse ova shows that the best results were obtained by transplanting blastocysts using the operation method (Gates, 1956) — 38.75% of developed embryos from the transplan-

ted ova (taking into consideration only those females in which at least one egg developed — approximately 50%). In other experiments where a similar method was used, the results vary between 20 and 30%. The total effectiveness of the technique used by Runner (1951) and Runner and Palm (1953) was expressed respectively by 20 and 14% of developed embryos.

The results of the experiments given in this work, although based on a relatively small amount of material, show clearly the high degree of effectiveness of the method used of transplantation to the oviduct. The results give 70% of developed embryos and are close to the results obtained during research work on ova-transplantation in rabbits, and demonstrate that there is no fundamental difference between the tolerance of the eggs of either species to a short period in vitro. As control tests of 10 females before implantation revealed the presence of 83.6% of the transplanted eggs in the genital tract, the ability of these eggs to develop normally is actually even greater.

In comparing the results of experiments using different methods of transplantation, it is necessary to consider whether the recipients were truly pregnant or pseudo-pregnant. In the first case the opportunity for development of the transplanted eggs is limited by the presence of the recipient's own eggs. In the second case the transplanted eggs have in principle every opportunity to develop if their number is not too great. However, the work of Boot and Mühlbock (1953) in which, amongst others, females mated with sterile males were used, and that of Fekete (1947) where pseudo-pregnant recipients only were used, show that implantation does not take place at all in a large number of females. This in effect brings about a considerable reduction in the total percentage of developed embryos. In the experiments described by the author, all the recipient females had been mated with sterile males, nevertheless normal pregnancy developed in all cases of transplantation to the oviduct.

The better results obtained by transplanting 2-cell ova to the oviduct, than by transplanting morulae and blastocysts to the uterus, may, amongst other causes, be connected with the fact that the shock caused to the eggs by the very act of transplantation falls in the first case at the beginning of development, and in the second directly precedes the moment of implantation. With regard to 2-blastomere eggs it has been found that this shock is expressed by a cer-

tain extent of arrested development directly after transplantation, as a result of which the formation of the blastocyst is delayed by 1/2 to 1 day. Since, however, by transplantation the ova „gain" one day, the initial disturbances are completely equalised at the moment of the transition of the eggs to the uterus. Eggs transplanted on the fourth day (i.e. 3 1/2 days old) have completed their pre-implantation development, but the result of the shock may be expressed by a weakening of the capacity for implantation of the blastocyst.

The above remarks are connected with the problem of synchronisation of the periods of pregnancy (or pseudo-pregnancy) of the donor and recipient at the moment of carrying out transplantation. Gates (1956) achieved his results with the synchronous combination 3 1/2—3 1/2, Boot and Mühlbock (1953) obtained their best results with the combination 3 1/2—2 1/2, and Fekete and Little (1942) cit. acc. to McLaren and Michie (1956) with 52—52 hours. McLaren and Michie carried out a synchronous combination (3 1/2—3 1/2; 2 1/2—2 1/2), and asynchronous (3 1/2—2 1/2; 2 1/2—3 1/2), and found the greatest effectiveness of transplantation obtainable with a combination 3 1/2—2 1/2. It is very probable that as in the case of 2-blastomere eggs, initial check in development also takes place when more advanced eggs are transplanted. [1])

In this connection every a priori defined combination, if it is to correspond to an actual situation, should be corrected by a period of 1/2—1 day (e.g. with the combination 2 1/2—3 1/2 the actual difference to the „disadvantage" of the transferred eggs is not 1, but 1 1/2—2 days).

If the technique of introducing ova to the uterus is employed, then transplantation of more advanced stages of development to the recipients in an earlier period of pregnancy (or pseudo-pregnancy) than the donors is possible only with the combination 3 1/2—2 1/2. Transplantation of the eggs to the uterus of the recipient on the second day after copulation does not really give any positive results (Fekete and Little, 1942; Beatty, 1951; experiments given in this work — Table 4). Negative results are probably accounted for (see below) by the fact that eggs introduced at this time to the

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[1]) Several unconnected observations confirming this viewpoint have already been made.

uterus cannot maintain themselves there and are discharged within a short time outside the genital system.

Fuller investigation of the possibilities of transplanting more advanced eggs to the recipients in earlier stages of pregnancy is rendered feasibly by transplantation to the oviducts. This is indicated by two successful experiments consisting in the transplantation of 8-cell eggs and blastocysts to the oviduct. Using the symbols employed by McLaren and Michie (1956) these are transplantations with the $2^1/_2$—$^1/_2$ and $3^1/_2$—$^1/_2$ combination. When formed blastocysts are introduced into the oviduct, or are formed there from transplanted 8-cell ova, they then undergo a check at this stage and reach the uterus in this form, where implantation takes place at the normal time. The absolute length of embryonic life of the transplanted eggs in these two cases is as follows: normal length of pregnancy +2 days and +3 days. We have here certain features in common with the phenomenon of prolongation of pregnancy in female mice still nursing their previous litter. The difference consists only in the fact that the blastocysts undergo the period of arrested development during their journey through the oviduct, and not in the uterus.

It is probable that an essential factor guaranteeing good results from the transplantation of ova to the oviduct is the use of serum as a medium for recovery and transplantation of the ova. While serum is in general use for all manipulations with the ova of the rabbit, it had not so far been used in experiments on ova-transplantation with mice. The reason for this is undoubtedly the difficulty in obtaining it in large quantities. During work it became plain, however, that an amount of serum could be obtained from the blood of one animal sufficient, after dilution by equal parts of normal saline solution, for 4—5 operations. The choice of medium in which the ova are to remain in vitro, for however short a period, undoubtedly is of great importance to the results of the transplantation. Gates and Runner (1952), using Locke's solution and semen diluter, became convinced of the superiority of the latter. If the fluids hitherto used were placed in the order of their suitability for ova-transplantation, the order would be as follows: serum, semen diluter, physiological saline solutions of varying constituents, normal saline solution (almost totally unsuitable).

Where all optimal conditions are present, a high degree of effectiveness in transplanting eggs to the oviduct depends to a great ex-

tent on the precision with which the actual operation of transplantation is carried out.

Experiments on the transplantation of blastocysts through the uterine cervix form a separate problem. The results given in this work are as unsatisfactory, by comparison with results yielded by other methods, as those obtained by B e a t t y (1951). A slightly larger amount of material and checks made at various periods during pregnancy make it possible to reach several important conclusions.

The first principal observation is the complete absence of proportionality between the numbers of transplanted blastocysts and the effect of the experiment. The percentage of blastocysts which implanted themselves, the percentage of normally developing embryos, and also the percentage of females in which implantation took place, is identical in the case of the group in which 1—5 eggs were transplanted and in the case of the group in which 6—10 eggs were transplanted. On the other hand in the group of females in which implantation of the transplanted blastocysts took place, the percentage of implantation was 59, which is a very high percentage. These data show that the low total effectiveness of the technique is chiefly caused by certain factors operating in individual cases on the whole group of the transferred eggs. Most probably as a result of some deficiency, which has so far proved impossible to detect, in the operation itself, the entire amount of the eggs transplanted are discharged from the uterus directly after transplantation. This view is to a certain extent confirmed by the observations made by B r i o n e s and B e a t t y (1954), who carried out the transplantation of eggs between two species — rat-mouse — using operative and non-operative techniques. They found that one day after the transplantations of the eggs through the uterine cervix, a far smaller percentage of eggs remained in the uterus than when they were introduced by the operation method.

Intensive mortality amongst the implanted embryos is a second factor reducing the final results. In a group of successful transplantations with 59% of implantation of blastocysts, the percentage of normally developing blastocysts was reduced to 18. This mortality would appear to be the result of the deleterious action of L o c k e's fluid, which was used in the experiments, on the blastocysts. Unfortunately the limited series of experiments carried out using serum does not permit of a decisive explanation of the causes of this phenomenon being obtained.

V. SUMMARY

1. Transplantations were carried out of mouse 2-cell and 8-cell ova, and of blastocysts, to the oviduct, and of blastocysts to the uterus through the uterine cervix. A detailed description is given of both techniques and also of the method of manipulating the ova.

2. Where normal saline solution was used for manipulation and for transplanting the eggs, these at first develop, but their development does not exceed the 16-cell morula stage.

3. When mouse serum diluted by equal parts of normal saline solution is used for this purpose, normal development of the ova is ensured.

4. It has been found that the transplantation operation is a shock to the ova, expressed in a slowing-down in the rate of cleavage directly after transplantation.

5. 50 2-cell ova were transplanted to 10 females, which were examined either during the period between the 15th and the 19th day of pregnancy, of after littering. 35 embryos developed from the transplanted ova, that is, 70%.

6. The possibility exists of transplanting 8-cell ova and blastocysts to the oviduct. The blastocysts, whether formed or in the process of formation in the oviduct, pass in this stage to the uterus, where they become implanted at the normal time for the recipient female.

7. The total length of embryonic life of the transplanted 2-cell and 8-cell ova, and blastocysts, is lengthened by 1, 2 and 3 days respectively.

8. Transplantation of blastocysts to the uterus through the uterine cervix is not an effective method (24,6% of the transplanted blastocysts become implanted, 8,4% develop normally). Presumably in the majority of cases the entire quantity of eggs transferred passes out from the uterus immediately after the operation. In the group of females in which implantation took place, 59% of the transplanted blastocysts became implanted. The number of embryos developing normally was again reduced as a result of the intensive foetal mortality after implantation.

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STRESZCZENIE

1. Przeprowadzono transplantacje jaj mysich 2-blastomerowych, 8-blastomerowych i blastocyst do lejka jajowodowego, oraz blastocyst do macicy przez szyjkę maciczną. Omówione zostały dokładnie obie techniki oraz sposób postępowania z wypłukanymi jajami.

2. Przy użyciu do wypłukania i przeszczepiania jaj zwykłego roztworu fizjologicznego, rozwój ich nie przekracza stadium 16-blastomerowej moruli.

3. Normalny rozwój jaj zapewnia surowica mysia rozcieńczona roztworem fizjologicznym w stosunku 1:1.

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[1]) Not in possession of original text.

4. Stwierdzono, że zabieg transplantacji stanowi szok dla jaja, wyrażający się w zwolnieniu tempa podziałów bezpośrednio po transplantacji. Postać całkowicie uformowanej blastocysty osiągana jest nie na 4-ty dzień a dopiero po 4—4½ dniach rozwoju.

5. 50 jaj 2-blastomerowych przeszczepiono do 10 samic, u których kontrolę przeprowadzono między 15 a 19 dniem ciąży lub po okoceniu. Z przeszczepionych jaj rozwinęło się 35 zarodków, co stanowi 70% (Tabela Nr. 2).

6. Istnieje możliwość transplantowania do lejka jajowodowego jaj 8-blastomerowych i blastocyst. Uformowane lub formujące się w jajowodzie blastocysty zostają w tym stadium przesunięte do macicy, gdzie implantują się w normalnym dla biorcy czasie.

7. Całkowita długość życia embrionalnego przeszczepionych jaj 2-blastomerowych, 8-blastomerowych i blastocyst zostaje przedłużona odpowiednio o 1, 2 i 3 dni.

8. Transplantowanie blastocyst do macicy przez szyjkę jest metodą mało efektywną (24,6% przeszczepionych blastocyst implantuje się, 8,4% rozwija się normalnie — Tabele Nr. 4 i 5). W większości przypadków następuje przypuszczalnie wypłynięcie z macicy całej porcji wprowadzonych jaj, bezpośrednio po zabiegu. W grupie samic, u których dochodzi do implantacji, implantuje się 59% przeszczepionych blastocyst (Tabela Nr. 6). Liczba normalnie rozwijających się zarodków zostaje wtórnie obniżona na skutek intensywnej śmiertelności płodów po implantacji.

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