The Effect of a Single Dose of Estradiol-17β on the Growth and the Succinic Dehydrogenase Activity of Rat Blastocysts during Delayed Implantation

Tohoku Journal of Agricultural Research
Vol. 28, No. 3, 4, December 1977
Printed in Japan

The Effect of a Single Dose of Estradiol-17β on the Growth and the Succinic Dehydrogenase Activity of Rat Blastocysts during Delayed Implantation

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(Received December 7, 1977)

Summary

Morphometric and histochemical studies of the effects of a single dose of estradiol-17β on the growth and the succinic dehydrogenase (SDH) activity of rat blastocysts during delayed implantation were undertaken.

The SDH activity increased with the development of blastocysts in estrogen-treated animals. On the contrary, neither increase of the enzyme activity nor the development of blastocysts was observed in non-estrogen-treated ones.

In order to evaluate the mechanism of development, growth and differentiation in mammalian eggs, it is necessary to understand metabolic pathways of various substrates and enzyme systems concerned in metabolism of blastocysts in the uterus.

It is well known that SDH occupies a pivotal position in the oxydative pathway of the tricarboxylic acid (TCA) cycle. So, in mammalian eggs, this enzyme has been investigated in mice (1, 2, 3), hamsters (4, 5, 6), and rabbits (7, 8). However, the SDH activity has not been demonstrated in rat blastocysts except the report of Christie (9).

Delayed implantation, which can be induced experimentally by bilateral ovariectomy followed by progesterone treatment in rats and mice, has been applied by many investigators as an experimental technique in the study of hormonal requirement for the maintenance of pregnancy. When a single dose of 1μg estrone is administered to the delayed implantation animals on the 8th day of pregnancy, the delayed blastocysts begin to undergo morphological changes within 12 hrs and implant in the uterus 24 to 30 hrs later (10).

Although the ovarian hormones influence the growth, development and differentiation of the rat blastocyst, little is known about the relationship between the SDH activity of blastocysts and the hormones, especially estrogen, in vivo. Accordingly, morphometric and histochemical studies of the effects of a single dose

of estradiol-17β on the growth and the SDH activity of blastocysts during delayed implantation as compared to those at various developmental stages before implantation in normal pregnant animals were made.

Materials and Methods

Adult virgin female rats of Wistar strain, weighing from 165 to 290 g, were maintained under constant condition of illumination (lights on at 7:00 a.m., off at 7:30 p.m.) and temterature (22±1°C). The females in proestrus were caged overnight with fertile males. The day of insemination was determined by the presence of spermatozoa in vaginal smears and designated day 1 of pregnancy.

Experiment I: Change of the SDH activity was observed in the developing blastocysts before implantation during normal pregnancy. Animals from each subgroup were killed at various times on day 5 and day 6 of normal pregnancy. The time of fertilization for all ova exmained was considered to be at 4:00 a.m. in the morning after proestrus (11).

Experiment II: Ovariectomy was performed on day 3 of pregnancy and followed by daily injection of 4 mg progesterone until day of autopsy. Artificial delayed implantation was induced by the method of Cochrane and Meyer (12). In order to observe the effect of estrogen on the growth, differentiation and the SDH

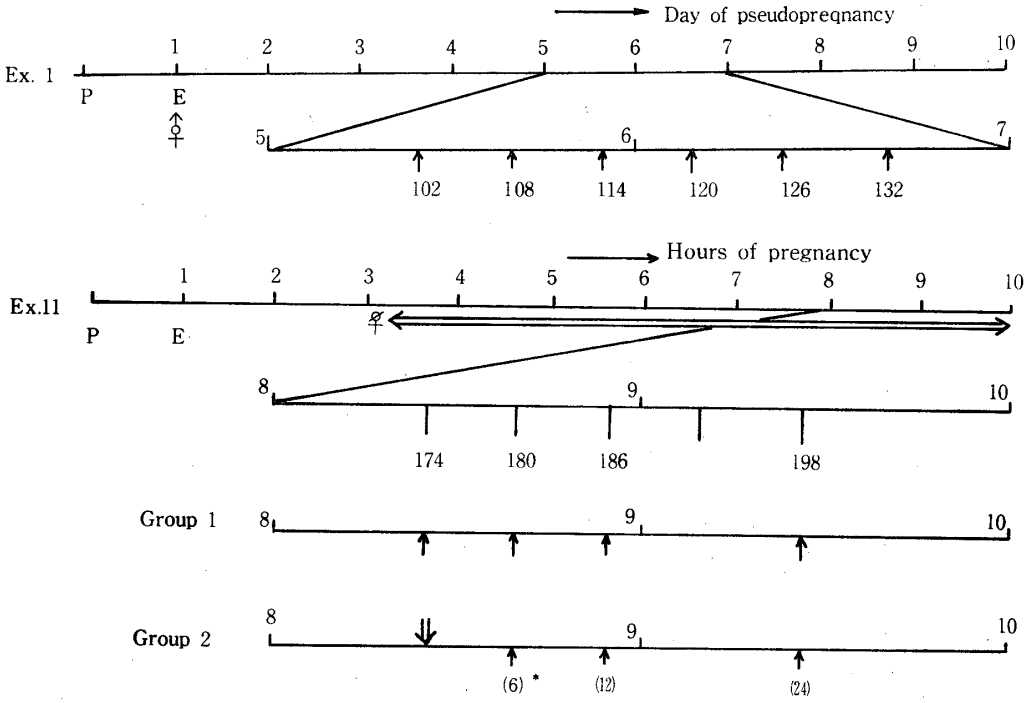

FIG 1 Design of experiment

Abbreviation

P: Proestrus ♂♀: Copulation ⇓: 0.1 μg Estradiol-17β administration
E: Estrus ♀: Ovariectomy ↑: Autopsy
⇔: Daily treatment of 4 mg progesterone Ex: Experiment
* Numbers in parentheses show hours after estradiol-17β administration.

activity of blastocysts during delayed implantation, animals of Group 2 were injected with 0.1 μg of estradiol-17β 172 hrs after fertilization, while the animals of Group 1 did not receive estrogen as shown in Fig. 1. Hormones were administered subcutaneously in 0.2 ml of sesame oil. The animals were killed at 0, 6, 12 and 24 hrs after estradiol-17β administration. Immediately after killing, blastocysts were recovered by flushing the uterine horns with 0.85% saline and were examined microscopically. The measurement of axis length and the calculation of the area of the blastocysts were performed by the method of Sugawara et al (13). Afterwards, using a micropipette under a dissecting microscope, the eggs were transferred into the fresh incubation medium prepared for the demonstration of SDH by the method of Barka & Anderson (14). Blastocysts were placed in a substrate solution containing 0.06 M sodium succinate, 0.2% nitro blue tetrazolium, 0.2 M phosphate buffer (pH 7.4) and Ringer's solution in a watch glass. For controls, the eggs were incubated in a medium without succinate. After incubation in the medium for 1 hr at 37 C, the blastocysts were examined by light microscope. The SDH activity of the blastocysts was expressed in four grades such as none, weak, moderate and strong.

Results

The results are summarized in Table 1, 2 and 3 and Fig. 2. Of 348 fertilized ova recovered, 340 developed to normal blastocysts and 8 remained in the morula stage. The former 340 ova were used for the measuring area of blastocysts. Owing to some loss of recovered ova during transferring into the incubation medium, 294 fertilized ova were served for demonstration of the SDH activity.

TABLE 1. *Rate of development to blastocyst, disappearance of zona pellucida and visualization in Experiment I and II.*

Ex.	Group	Hours of Pregnancy	Number of Rats	Number of Blastocysts	Number of Morula	Rate of development to blastocyst (%)	Disappearance of zona pellucida (%)	Visualization of blastocoele (%)
Experiment I	Normal pregnancy	102	4	26	6	76.2	0	90.0
		108	5	31		100	74.2	80.6
		114	6	28		100	100	53.6
		120	4	31		100	100	25.8
		126	4	17		100	100	41.2
		132	4	21		100	100	9.5
Experiment II	Group 1	174	5	21		100	100	71.4
		180	5	33	2	93.9	100	100
		186	6	26		100	100	81.2
		198	6	16		100	100	75.0
	Group 2	180	10	42		100	100	90.5
		186	5	19		100	100	78.9
		198	8	37		100	100	21.6

TABLE 2. *Intensity of SDH activity of normal pregnant rat blastocyst at various stages of development before implantation*

SDH activity	Hours of normal pregnancy											
	102		108		114		120		126		132	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
None	0	0	3	11.1	2	8.7	2	8.0	0	0	0	0
Weak	7	35.0	4	18.5	8	34.8	1	4.0	2	15.4	3	15.0
Moderate	13	65.0	16	59.3	10	43.5	16	64.0	6	46.2	6	30.0
Strong	0	0	4	18.5	3	13.0	6	24.0	5	38.5	11	55.0
Total number of blastocyst	20	100	27	100	23	100	25	100	13	100	20	100

TABLE 3. *Intensity of SDH activity in rat blastocysts at various stages after estradiol-17β administration during delayed implantation*

Group	SDH activity	Hours of pregnancy (Hrs after estradiol-17β administration)							
		174(0)		180(6)		186(12)		198(24)	
		No.	%	No.	%	No.	%	No.	%
Group I	None	1	5.0	4	18.2	4	20.0	1	6.3
	Weak	5	25.0	6	27.8	6	30.0	6	37.4
	Moderate	12	60.0	8	36.4	8	40.0	7	43.8
	Strong	2	10.0	4	18.2	2	10.0	2	12.5
	Total number of blastocysts	20	100	22	100	20	100	16	100
Group II	None	—	—	3	8.8	2	11.1	0	0
	Weak	—	—	7	20.6	2	11.1	4	11.1
	Moderate	—	—	9	26.5	2	11.1	4	11.1
	Strong	—	—	15	44.1	12	66.7	23	63.9
	Total number of blastocysts	—	—	34	100	18	100	36	100

As shown in Table 1, all ova from intact animals in Exp. 1 developed into blastocysts by 108 and 114 hrs after fertilization. The disappearance of zona pellucida at 108 and 114 hrs after fertilization was 74.2 and 100% respectively in normal pregnancy, and was 100% by 174 hrs during delayed implantation. The rate of visualization of blastocoele decreased from 90.0 to 9.5% between 102 and 132 hrs of pregnancy in proportion to the growth of trophoblast. Similar change occurred in Group 2 in Exp. 2, showing 71.4 and 21.6% in the rate at 0 and 24 hrs respectively after estrogen administration, whereas there was no change in Group 1 during delayed implantation.

Influence of estradiol-17β on the development and the growth of blastocysts is shown in Fig. 2. In both intact and estrogen-treated groups, the shape of blasto-

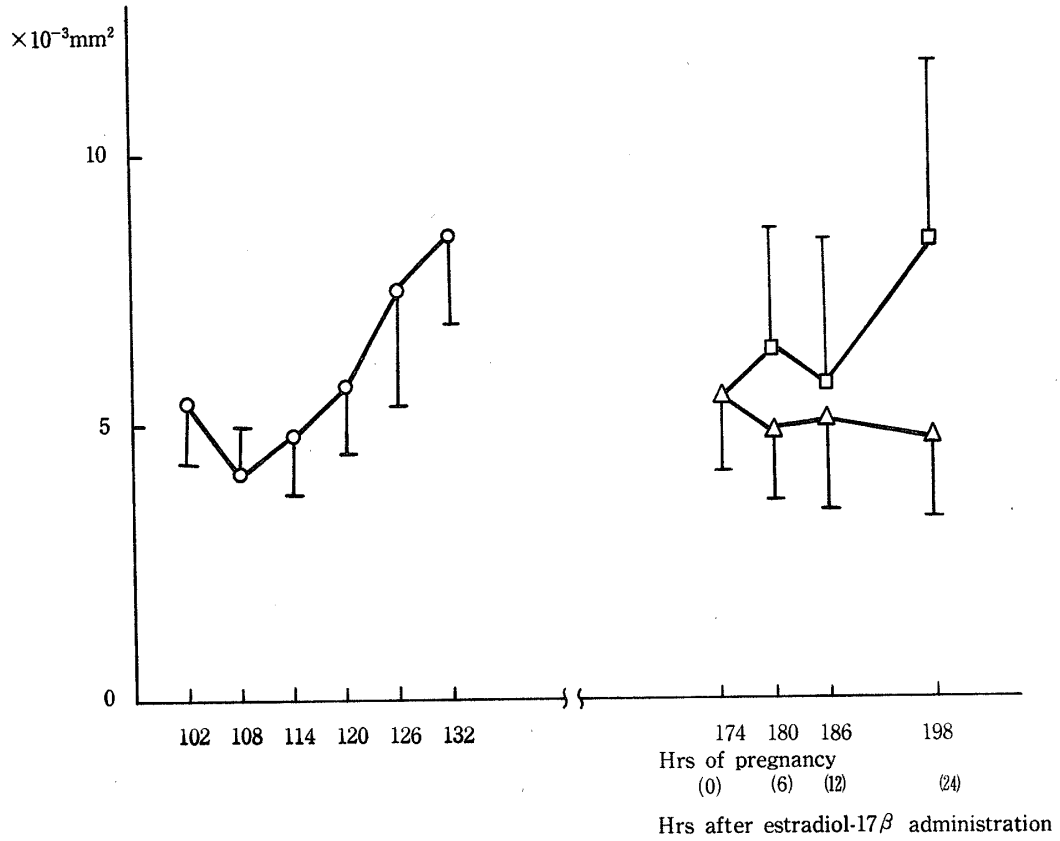

FIG. 2 The changes in the area of blastocyst after estradiol-17β treatment
□: Intact animals, △: Group 1, ○: Group 2
Vertical lines indicate standard deviation.

cysts was transformed from circular to elliptical because of the rapid elongation of the major axis length of the blastocyst.

The area of the blastocysts was reduced at 108 hrs ($P<0.01$) followed by rapid increase between 120 and 132 hrs in normal pregnancy, in proportion to the changes of the major and minor axis length of the blastocysts. Although the blastocysts from estrogen-treated animals (Group 2) grew markedly between 12 and 24 hrs after estrogen injection, those from non-estrogen-treated animals (Group 1) did not increase in area between 174 and 198 hrs of delayed implantation. Therefore, there was a signifiicant difference in the area of the blastocysts at 198 hrs of delayed implantation between Group 1 and Group 2.

Table 2 shows the change of the SDH activity of blastocysts at various stages before implantation in normal pregnancy. The SDH activity increased with the advance of blastocyst stage from 102 to 132 hrs after fertilization. To be exact, the "strong" enzyme activity of blastocysts was observed in 0% at 102 hrs, but increased to 55% at 132 hrs after fertilization. On the other hand, as shown in Table 3, it was 44% and 64% at 6 and 24 hrs respectively after estrogen administration in the estrogen-treated aminals (Group 2) and 10–18% at 174–198 hrs of

delayed implantation (Group 1).

Discussion

The effect of a single dose of 0.1 μg estradiol-17β on the development, growth and differentiation in rat blastocysts almost coincide with a result of our previous experiment (10) in which a single dose of 1 μg estrone was injected on the 8th day during delayed implatation. Therefore, 0.1 μg estradiol-17β may have almost the same effect as 1 μg estrone on the growth and differentiation of rat blastocysts, such as in the rate of development to blastocyst, disappearance of zona pellucida and visualization of blastocoele during delayed implantation. The complete disappearance of zona pellucida was observed in the blastocysts of normal pregnant animals 114 hrs after fertilization, which coincides with the previous report (10) and report by Dickmann and Noyes (15). Also, the gradual decrease in the visualization rate of blastocoele is due to the advance of trophoblast cells with the development of blastocyst, which coincides with previous observation (unpublished data).

The changes of the SDH activity in mammalian eggs have been demonstrated histochemically in the mouse (1, 2, 3) hamster (4, 5, 6) and rabbit (6), and biochemically in the rabbit (7, 8). However, so far as we are aware, the changes of the SDH activity in rat blastocysts has not been demonstrated histochemically except the report of Christie (9). In the present experiment, the change of the SDH activity was observed at various stages of the blastocyst in normal pregnancy and increased in accordance with the development of the blastocyst. When a single dose of estradiol-17β was administered on the 8th day of delayed implantation (Group 1), this enzyme activity increased from 10% at 0 hr to 64% at 24 hrs after estrogen injection in the percentage of blastocysts indicating strong enzyme activity, while no change of the enzyme activity in non-estrogen treated animals during delayed implantation (Group 1). This enzyme activity increased within 12 hrs after estrogen injection although the blastocysts did not increase in size.

It has been reproted that SDH activity is high in the lumenal, glandular epithelia and subepitheial layer of a rat uterus 12 hrs after a single dose of estrone injection (16). Judging from these results, it is likely that the estrogen activates the SDH in both rat uterus and blastocyst simultaneously. Blastocysts from both stages of 130 hrs in normal pregnancy and 24 hrs after estrogen treatment in delayed implantation seem to induce trophoblast cell attachment to the uterine wall, because of the rapid decrease of ova recovery from the uterus (unpublished data). It is interesting that the blastocysts in both groups are analogous with each other in area and in the SDH activity at those stages.

Oxygen consumption of rabbit eggs increases gradually from the 2-cell to the morula stage, but increases tremendously at the blastocyst stage (17, 18). This may also be the case for the rat eggs (19, 20, 21, 22). The activity of SDH in the rabbit blastocyst and trophoblast was significantly high on the 8th day of

pregnancy prior to implantation (8) which is consistent with Fridhandler's finding (23) that after the 6th day of pregnancy the rabbit blastocyst is capable of metabolizing glucose via the Embden-Meyerhof pathway and TCA cycle. It is also reported that the development of SDH in mammalian eggs (hamster and rabbit), its gradual increasing during cleavage, and its tremendous increase at the blastocyst stage have been clearly demonstrated (6). Furthermore, the glycogen and acid mucopolysacchardies in tubal eggs are considered to play an important role during cleavage (24, 25, 26).

Based on these observations and the results of the present study, morphological changes and the high respiratory rate of rat blastocysts may be due to the effect of estrogen influencing metabolic activity. It is probable that the tremendous increase of oxygen consumption at the blastocyst stage in rat eggs is closely associated with the increase of SDH activity and that the TCA cycle is activated by estrogen secretion from the ovary at this stage (27). The majority of incidences of pre-impalatation embyornic mortality occur at the stage of conversion of morula to blastocyst in the mouse (28) and rat (29). Therefore, it seems to be considerably important to elucidate the correlation between the enzyme system concerning carbohydrate metabolism and "estrogen surge" for ovo-implantation on the 3rd day of pregnancy (30).

Acknowledgement

We are very grateful to Professor J. Masaki for reading and criticizing the manuscript.

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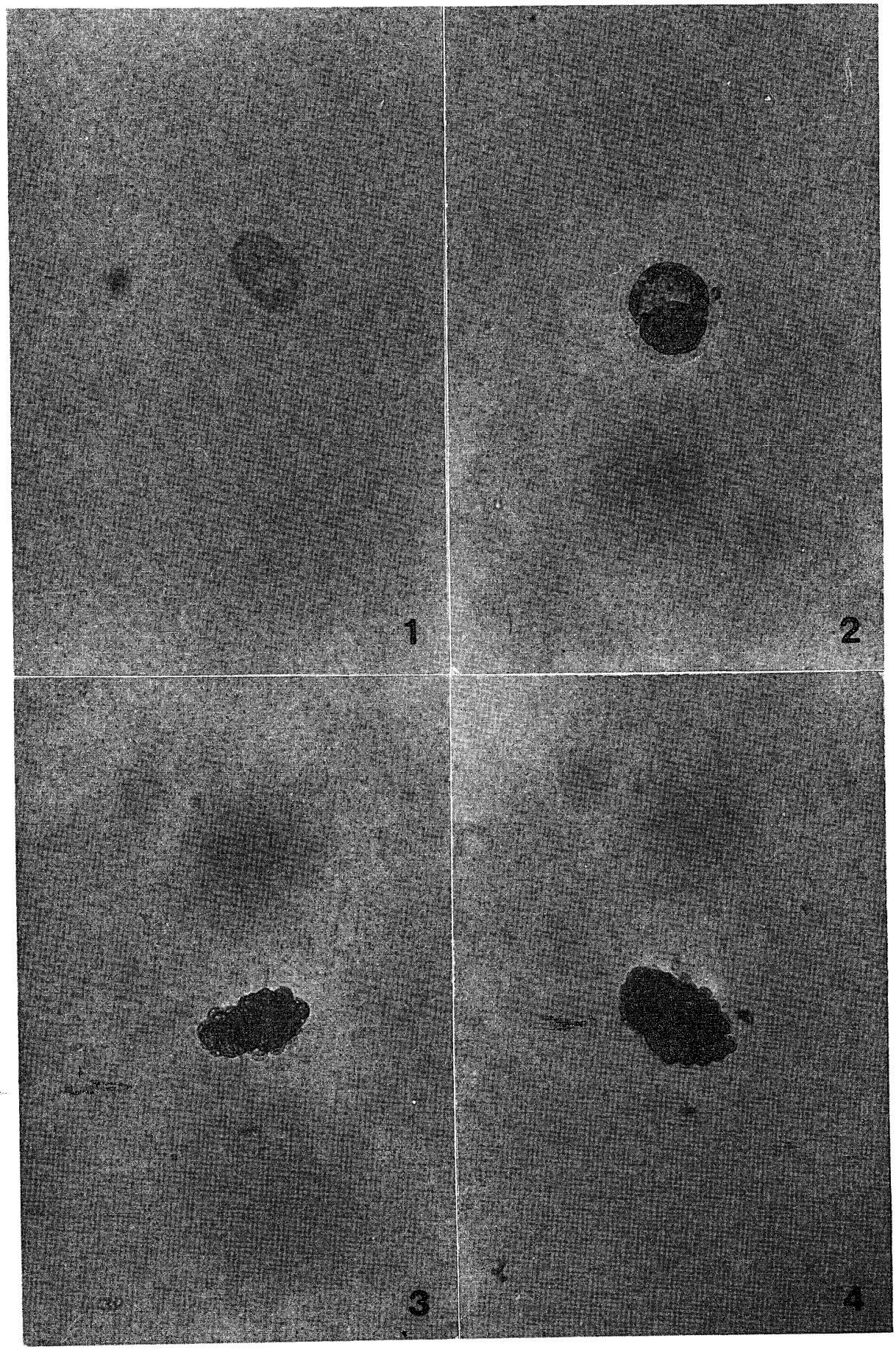
Explanations of Plate

1. Blastocyst recovered at 102 hrs after fertilization in normal pregnancy. SDH activity is none. × 100
2. Blastocyst recovered at 108 hrs after fertilization in nomral pregnancy. SDH activity is moderate. × 100
3. Blastocyst recovered at 126 hrs after fertilization in normal pregnancy. SDH activity is strong. × 100
4. Blastocyst recovered at 132 hrs after fertilization in normal pregnancy. SDH activity is strong. ×100
5. Blastocyst recovered at 174 hrs after fertilization in delayed implantation. SDH activity is weak. ×100
6. Blastocyst recovered at 180 hrs after fertilization (at 6 hrs after estradiol-17β administration) in delayed implantation. SDH activity is strong. ×100
7. Blastocyst recovered at 186 hrs after fertilization in delayed implantation. SDH activity is weak. ×100
8. Blastocyst recovered at 186 hrs after fertilization (at 12 hrs after estradiol-17β administration) in delayed implantation. SDH activity is strong. ×100
9. Blastocyst recovered at 198 hrs after fertilization in delayed implantation. SDH activity is weak. ×100
10. Blastocyst recovered at 198 hrs after fertilization (at 24 hrs after estradiol-17β administration) in delayed implantation. SDH activity is strong. ×100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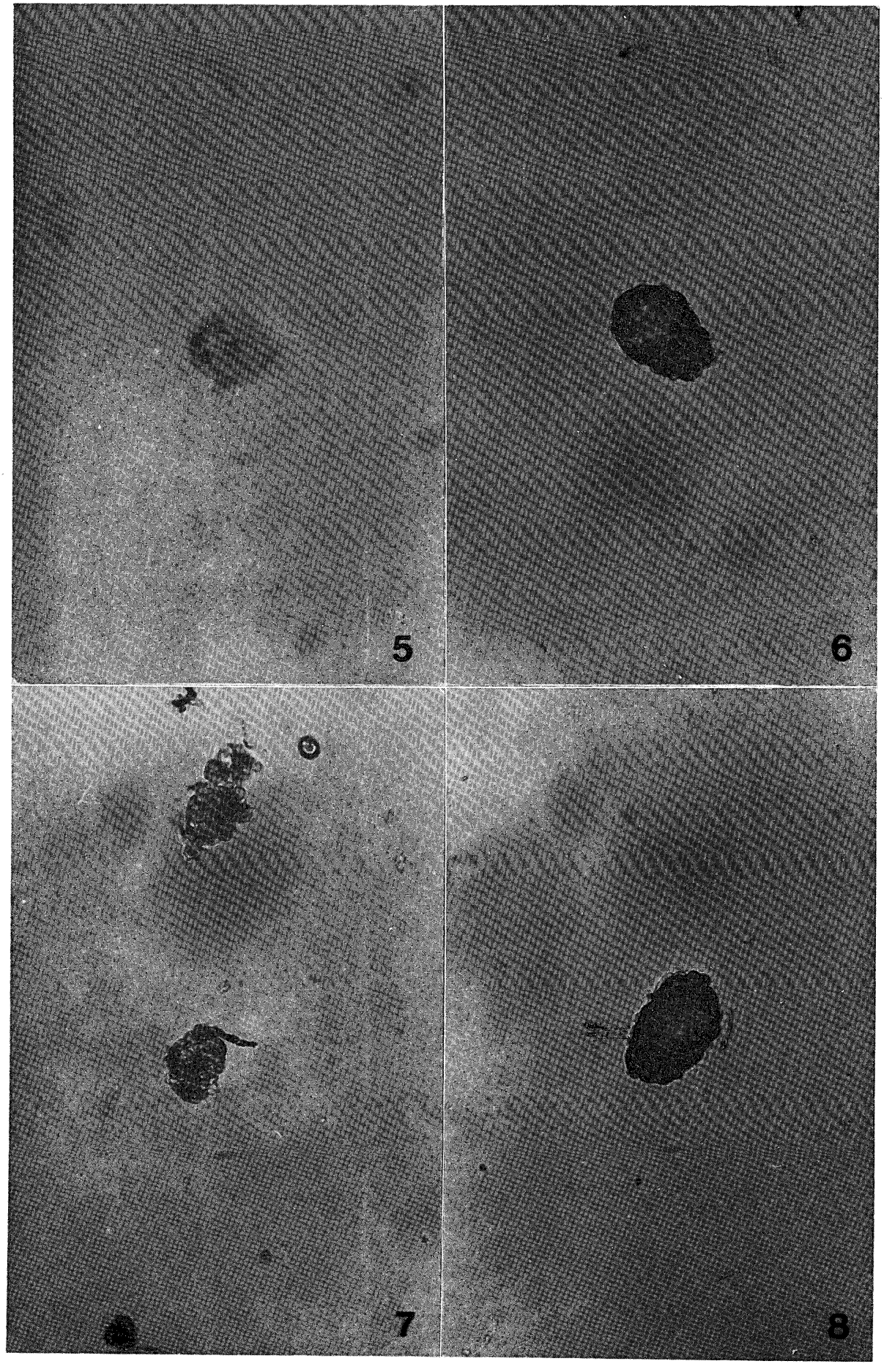
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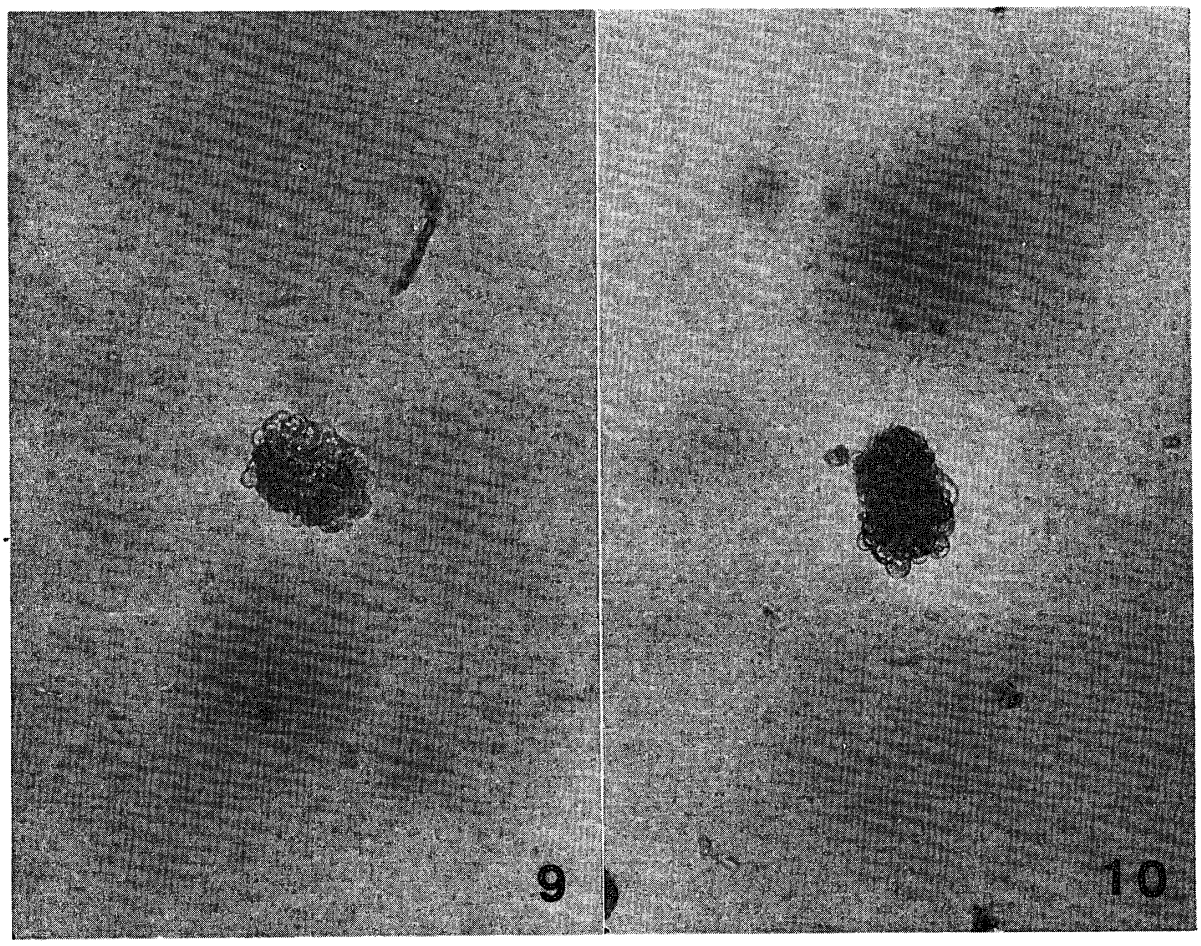
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