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—Review—

Morphological and Histochemical Characteristics of Parthenogenetic Embryos

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Parthenogenesis is the phenomenon in which an ovum begins to develop by activation without sperm penetration. Artificial induction of this phenomenon is thought to be useful in clarifying the mechanisms of ovum activation and to investigate the role of sperm in embryo development [1].

Parthenogenesis of mammals was first studied in rabbits by Pincus in 1939 [2, 3]. He reported that a parthenogenetic embryo induced by heating could develop into a newborn, but no newborns were obtained from parthenogenetic rabbit embryos in similar studies conducted after Pincus although the embryos could develop to the blastocyst stage [4, 5].

In the 1970s, various methods were established for the activation of mouse ova, including electrical treatment [6], anesthetic treatment [7], hyaluronidase treatment [8] and heating [9]. Ova activated by any one of these methods were found to develop to the egg cylinder stage. Kaufman *et al.* [10] revealed that after that some diploid parthenogenetic mouse blastocysts derived from ova activated with a solution containing neither calcium nor magnesium developed to the forelimb bud stage. Kono *et al.* [11] recently reported that parthenogenetic mouse embryos develop to 13.5 days of pregnancy when ova with nuclear transplantation were treated with strontium, cultured and then transferred. So far no newborns have been obtained from parthenogenetic embryos. In this review, the author will introduce the results of morphological and histochemical studies, mainly ours, with regard to factors involved in the low potential of development in parthenogenetic embryos.

Numbers of Cells and Chromosomes

It is said that diploid parthenogenetic mouse blastocysts have about 20 inner-cell-mass cells [12] or about half of those observed in blastocysts developed from fertilized ova [1]. Haploid parthenogenetic blastocysts are known to have far fewer inner-cell-mass cells [1]. Such a small number of cells is thought to cause a developmental delay in parthenogenetic embryos, or to be related to high mortality in these embryos after implantation [1, 12].

We [13] also counted cells in parthenogenetic mouse blastocysts (Fig. 1) derived from ova, activated and diploidized by treatment with ethanol and cytochalasin B. Our results (Table 1) revealed that fertilized blastocysts had 47.2 cells on average, whereas parthenogenetic blastocysts had a significantly smaller number of cells, 32.1. Embryos are known to require a certain number of cells for their development [14]. The results of our experiments indicated that relatively large portions of parthenogenetic embryos could form blastocysts if they had about 30 cells [13].

The number of chromosomes has also been observed

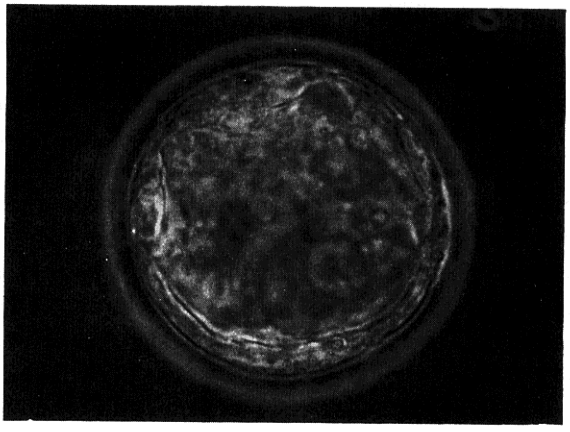

Fig. 1. A diploid parthenogenetic mouse blastocyst induced by treatment with ethanol and cytochalasin B. × 500.

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Table 1. Number of chromosomes in mouse blastocysts

Blastocysts	No. of blastocysts examined	No. of metaphase plates in a blastocyst	No. (%) of blastocysts showing			
			Diploidy	Hypodiploidy	Hyperdiploidy	Mosaic
Parthenogenone	20	3.35 ± 1.96*[a]	14 (70)[b]	3 (15)	0 (0)	3 (15)
Control (Fertilized)	20	7.40 ± 2.85[a]	19 (95)[b]	1 (5)	0 (0)	0 (0)

*Mean ± S.D. Values with the same superscripts in the same column are significantly different ([a]: P<0.001, [b]: P<0.05).

in haploid parthenogenetic mouse embryos induced by ethanol [15–17], electrical [18] or hypotonic [19–22] activation and in parthenogenetic mouse embryos of the LT/SV strain, which were spontaneously activated [23]. Anomalies in the numbers of chromosomes were detected in 15 to 28% of the embryos. As to diploid parthenogenetic mouse embryos induced by treatment with hyaluronidase [24] and those induced by treatment with ethanol and cytochalasin B [13], anomalies were found in 24% and 30%, respectively. The authors observed hypodiploidy and mosaic as chromosomal aberrations in parthenogenetic mouse blastocysts. Chromosomal anomalies are thought to occur in ethanol-treated embryos due to non-disjunction of chromosomes, resulting from a change in ovum cytoskeletal elements and incomplete formation of spindles at the second meiotic division [15, 17].

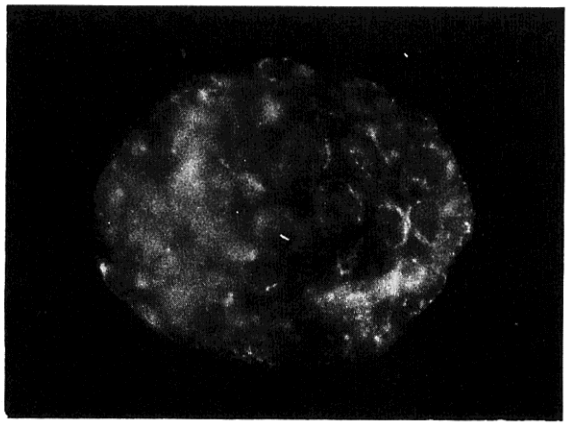

Fig. 2. Cortical granules in a diploid parthenogenetic mouse blastocyst. × 500.

Cortical Granules

In activated ova, cortical granules are known to be present without release because of incomplete cortical reaction as a result of the lack of stimulation by sperm penetration [25, 26]. It is therefore inferred that cortical granules remaining in ova may suppress the development of the ova due to their adverse effects on ova [1].

Nevertheless, the presence of cortical granules has been determined to have no adverse effects on the development of parthenogenetic embryos because cortical granules were observed electron microscopically or lectin-histochemically, even at a low density, in 8-cell mouse embryos induced by treatment with hyaluronidase and hypotonic solution [27], mouse blastocysts (Fig. 2) induced by treatment with ethanol and cytochalasin B [28], and in 12-cell porcine embryos induced by ethanol treatment [29].

Ultrastructure

It was reported that haploid parthenogenetic mouse embryos induced by treatment with hyaluronidase and hypotonic solution had nucleoli occupied by nucleolonemas to a large extent and many mitochondria with vacuolated cristae, but neither polysome nor rough-surfaced endoplasmic reticulum was observed in those embryos although a few free ribosomes were present [27]. In parthenogenetic mouse blastocysts, which developed spontaneously, a large number of mitochondria with vacuolated cristae were also observed [23]. It is therefore thought that these ultrastructural disorders may be involved in the suppression of the development of parthenogenetic embryos [23, 27].

The authors [30] also observed the following ultrastructural disorders in parthenogenetic mouse blastocysts induced by treatment with ethanol and cytochalasin B: most of the nucleolus was occupied by nucleolonema (Fig. 3). Many mitochondria with vacuolated cristae (Fig. 4) were present, and there were only a small number of Golgi apparatuses, ribosomes, multivesicular bodies, autophagic vacuoles, Myelin bodies, lysosome-like bodies and cytoplasmic folds on the cell surface. These ultrastructural findings in parthenogenetic blastocysts indicate that the capacity for energy production and protein synthesis, and for absorption and digestion

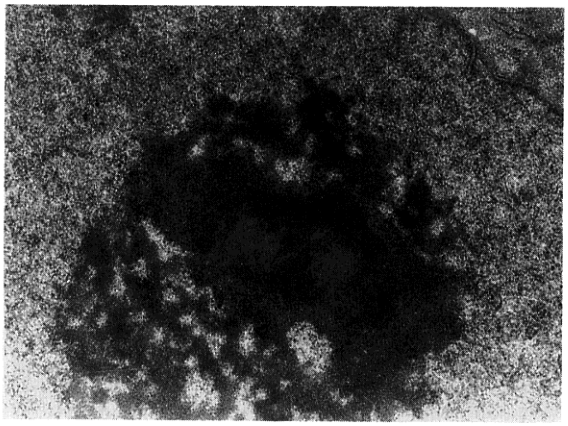

Fig. 3. A nucleolus in a trophoblast cell of a diploid parthenogenetic mouse blastocyst. × 16,000.

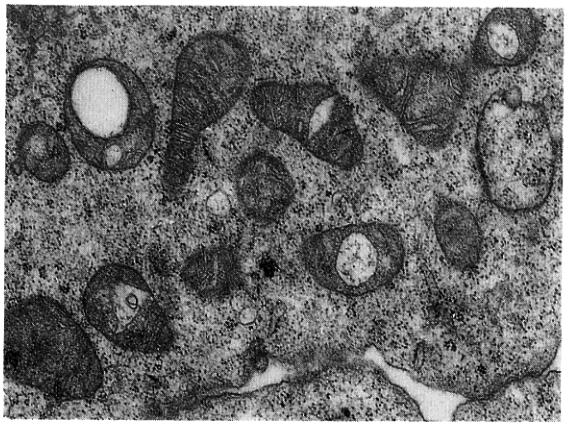

Fig. 4. Mitochondria in a trophoblast cell of a diploid parthenogenetic mouse blastocyst. × 32,000.

of nutrients are decreased.

Since the form of mitochondria is known to be affected by nuclear DNA [23], the difference seen between parthenogenetic and fertilized embryos in cristae of mitochondria is thought to be due to the lack or presence of a genetic linkage with sperm through fertilization [30].

Although Van Blerkom and Runner [31] reported that the form and number of nucleoli, mitochondria, ribosomes and endoplasmic reticula in parthenogenetic mouse blastocysts induced by electrical treatment or hyaluronidase treatment did not differ from those in blastocysts developed from fertilized ova, their findings are questionable [30].

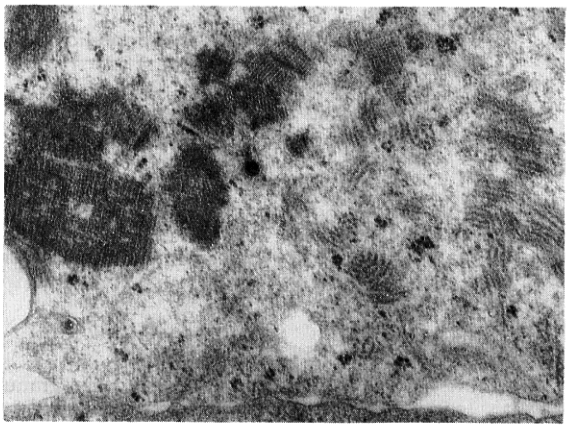

Fig. 5. Fibrous strands and crystalloids in a trophoblast cell of a parthenogenetic mouse blastocyst. × 35,000.

Amount of Cytoplasmic Inclusions

The amount of inclusions accumulated in the cytoplasm as an energy source for development has been observed only by the authors [32] using parthenogenetic mouse blastocysts induced by treatment with ethanol and cytochalasin B.

Proteins: It has been confirmed that the amount of fibrous strands (Fig. 5) is similar in parthenogenetic and fertilized blastocysts, whereas the amount of crystalloids (Fig. 5) is less in parthenogenetic blastocysts. A reason for a smaller amount of crystalloids in parthenogenetic blastocysts is thought to be that the amount of conversion from fibrous strands to crystalloids is decreased in those embryos because they contain only a few ribosomes and can synthesize only a small amount of protein, as mentioned previously [30].

Lipid droplets and glycogen granules: It has been ascertained that parthenogenetic blastocysts contain fewer Sudanophilic lipid droplets (Fig. 6) and a smaller number of periodic acid-Schiff reactive glycogen granules (Fig. 7), compared with fertilized blastocysts, as shown in Tables 2 and 3.

Generally, the speed of development of activated ova into blastocysts is comparable to that of fertilized ova when transferred [33, 34], but is slower when cultured [1, 24]. Such a developmental delay observed in parthenogenetic embryos *in vitro* is said to be a commom phenomenon, regardless of the method used to induce parthenogenesis [1, 12]. This phenomenon is thought to occur due to the insufficiency of cytoplasmic inclusions contained in parthenogenetic embryos as an energy source.

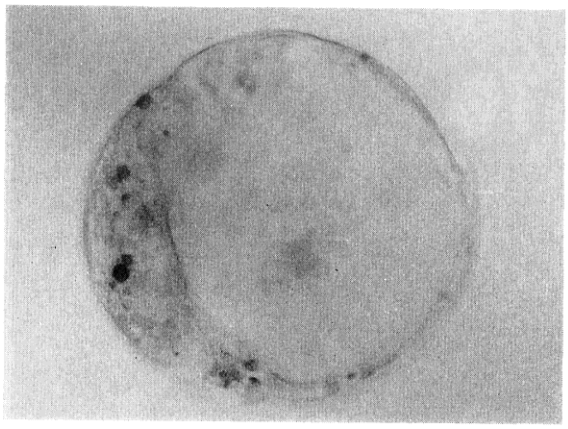

Fig. 6. Lipid droplets in a diploid parthenogenetic mouse blastocyst. × 500.

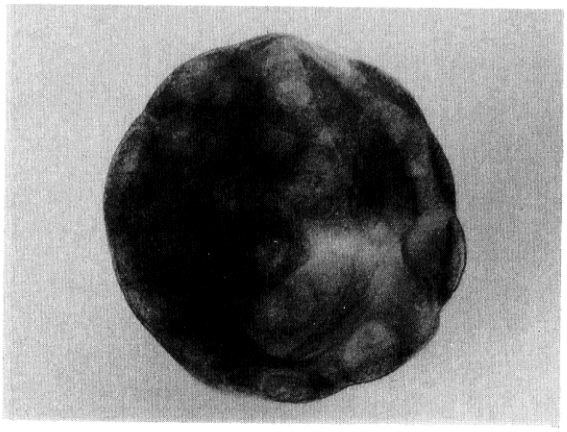

Fig. 7. Glycogen granules in a diploid parthenogenetic mouse blastocyst. × 500.

Table 2. Number of Sudanophilic lipid droplets of different sizes in mouse blastocysts

Blastocysts	No. of blastocysts examined	No. of lipid droplets		
		Small (<1 μm)	Medium (1–3 μm)	Large (≧4 μm)
Parthenogenone	30	86.7 ± 32.72*[a]	23.6 ± 9.29[a]	0.7 ± 1.04[b]
Control (Fertilized)	30	153.0 ± 37.85[a]	57.4 ± 20.57[a]	1.8 ± 1.71[b]

*Mean ± S.D. Values with the same superscripts in the same column are significantly different ([a]: P<0.001, [b]: P<0.01).

Table 3. Amount of glycogen granules in mouse blastocysts

Blastocysts	No. of blastocysts examined	No. (%) of blastocysts containing	
		Large number of glycogen granules	Moderate number of glycogen granules
Parthenogenone	30	20 (67)	10 (33)
Control (Fertilized)	30	25 (83)	5 (17)

Metabolic Activities

The authors [35, 36] have histochemically examined activities of various enzymes in parthenogenetic mouse blastocysts induced by treatment with ethanol and cytochalasin B, in order to investigate metabolic abilities. As shown in Tables 4 and 5, activities of Δ^5-3β-hydroxysteroid dehydrogenase with pregnenolone as the substrate, 17β-hydroxysteroid dehydrogenase with estradiol-17β as the substrate (Fig. 8), α-glycerophosphate dehydrogenase, $NADH_2$-dehydrogenase, acid phosphatase and adenylate cyclase (Fig. 9) have been determined to be significantly lower in parthenogenetic blastocysts than in fertilized blastocysts. These results indicate that the abilities of parthenogenetic blastocysts in digestion, interconversion between carbohydrates and lipids, and oxidation of NADH, and in metabolism of progesterone, estrogen and cyclic AMP are decreased [35, 36]. It has also been revealed that the metabolism of progesterone and estrogen, which is thought to decrease in parthenogenetic blastocysts, is increased by treatment with hCG (Table 6). The effect of hCG on the metabolism of progesterone and estrogen has been confirmed to be mediated by the adenylate cyclase-cyclic AMP system [36].

Estrogen is thought to involve substance uptake into

Table 4. Enzyme activities in mouse blastocysts

Blastocysts	Activities	Cytochrome oxidase	Alkaline phosphatase	Acid phosphatase	Adenosine tri-phosphatase	Esterase	Succinate dehydro-genase	Lactate dehydro-genase	α-Glycero-phosphate dehydro-genase	β-Hydroxy-butyrate dehydro-genase
Partheno-genone	Strong	21 (35)*	22 (54)	13 (33)[a]	22 (69)	0 (0)	14 (33)	26 (59)	11 (31)[a]	17 (55)
	Weak	39 (65)	19 (46)	27 (68)[b]	10 (31)	35 (100)	28 (67)	18 (41)	25 (70)[b]	14 (45)
Control (Fertilized)	Strong	28 (53)	25 (63)	23 (68)[a]	21 (70)	0 (0)	20 (56)	25 (60)	20 (63)[a]	23 (74)
	Weak	25 (47)	15 (38)	17 (33)[b]	9 (30)	38 (100)	16 (44)	15 (41)	12 (38)[b]	8 (26)

*Number of blastocysts with percentages in parentheses. Values with the same superscripts in the same column are significantly different ([a, b]: $P<0.05$).

Table 5. Enzyme activities in mouse blastocysts

Blastocysts	Activities	Δ^5-3β-HSD			17β-HSD		20α-HSD	20β-HSD	$NADH_2$-DH	$NADPH_2$-DH	Adenylate cyclase
		DHA	Pregnen-olone	17α-Hydroxy-pregnenolone	Estradiol-17β	Testos-terone	20α-Hydroxy-progesterone	20β-Hydroxy-progesterone	NADH	NADPH	AMP-PNP
Partheno-genone	Strong	22*	14[a]	0	5[a]	28	38	31	8[c]	15	13[e]
		(55.0)	(42.4)	(0.0)	(16.7)	(93.3)	(100.0)	(100.0)	(29.6)	(41.7)	(43.3)
	Weak	18	19[b]	30	25[b]	2	0	0	27[d]	21	17[f]
		(45.0)	(57.6)	(100.0)	(83.3)	(6.7)	(0.0)	(0.0)	(70.4)	(58.3)	(56.7)
Control (Fertilized)	Strong	22	32[a]	0	38[a]	29	30	30	23[c]	24	21[e]
		(57.9)	(100.0)	(0.0)	(95.0)	(92.9)	(100.0)	(100.0)	(69.7)	(63.2)	(70.0)
	Weak	16	0[b]	30	2[b]	2	0	0	10[d]	14	9[f]
		(42.1)	(0.0)	(100.0)	(5.0)	(7.1)	(0.0)	(0.0)	(30.3)	(36.8)	(30.0)

*Number of blastocysts with percentages in parentheses. Values with the same superscripts in the same column are significantly different ([a, b]: $P<0.001$; [c, d]: $P<0.01$; [e, f]: $P<0.05$).

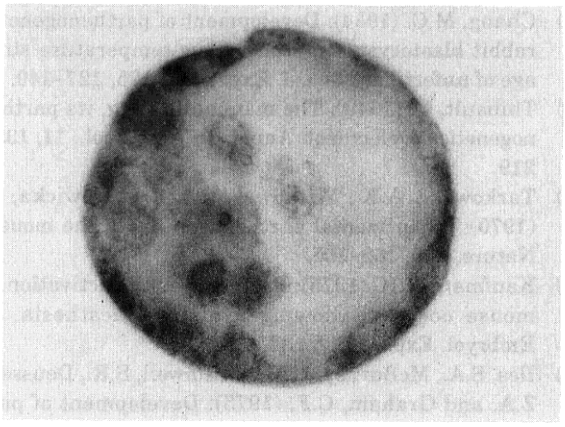

Fig. 8. An activity of 17β-hydroxysteroid dehydrogenase in a diploid parthenogenetic mouse blastocyst. × 500.

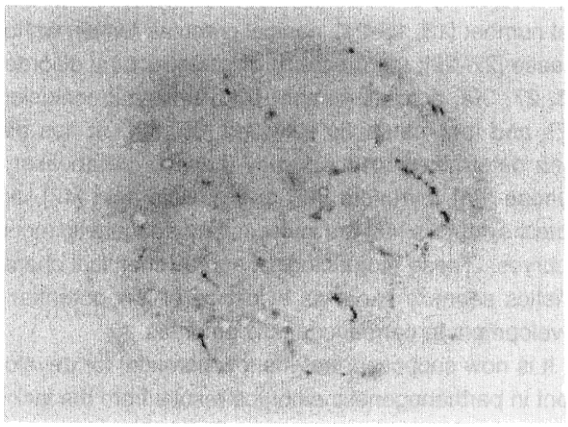

Fig. 9. An activity of adenylate cyclase in a diploid parthenogenetic mouse blastocyst. × 500.

Table 6. Effects of hCG on HSD activities in parthenogenetic mouse blastocysts

Amounts of hCG (i.u./ml)	Activities	Δ^5-3β-HSD	17β-HSD
		Pregnenolone	Estradiol-17β
0	Strong	14*[a] (42.4)	5[c] (16.7)
	Weak	19[b] (57.6)	25[d] (83.3)
100	Strong	58[a] (85.3)	34[c] (50.0)
	Weak	10[b] (14.7)	34[d] (50.0)

*Number of blastocysts with percentages in parentheses. Values with the same superscripts in the same column are significantly different ([a, b]: $P<0.001$; [c, d]: $P<0.05$).

blastocysts [37–39]. Estrogen synthesized in the embryos is also thought to play a role in the transformation of morula to blastocyst [40, 41] and maternal recognition of blastocysts [42–45]. These cellular functions must therefore decrease in parthenogenetic blastocysts, probably leading to low potential of development in parthenogenetic blastocysts [36].

Conclusion

To date, the following morphological and histochemical characteristics of parthenogenetic embryos, as compared with fertilized embryos, have been revealed: fewer cells and a slower rate of cell division [1, 12, 13]; a high frequency of chromosomal aberration from the normal number [13, 15–24]; cortical granules remain without release [27–29]; the presence of ultrastructural disorders [23, 27, 30]; a small number of cytoplasmic inclusions [32], and low metabolic activities [35, 36]. It has also been determined biochemically that the metabolism of glucose [46], pyruvate [46] and nucleic acid [47], and protein synthesis [48] are lower in parthenogenetic mouse embryos. These morphological and biochemical characteristics seem to serve as evidence of low potential of development in parthenogenetic embryos.

It is now supposed that the low potential for development in parthenogenetic embryos results from the lack of imprinting of paternal genomes [11, 49–54], but only insulin-like growth factor II (IGF II) [55] and IGF II receptor [56] have so far been detected in mouse as genomes that are expressed by imprinting and directly involve the development of embryos. It is therefore unclear whether low cellular functions seen in parthenogenetic embryos, as mentioned previously, are entirely attributable to the lack of imprinting of paternal genomes, but this issue is very important for the improvement of parthenogenetic embryos and should be further studied.

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