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HISTOCHEMICAL OBSERVATIONS OF LIPID DROPLETS IN MOUSE EMBRYOS

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Morphology of lipid droplets in 313 embryos of different developmental stages was examined. Embryos were obtained from spontaneously ovulated and superovulated mice of ddY strain. Sudan III was used to stain embryos so as to count the number of lipid droplets. Lipid droplets were classified into three groups according to the diameter: small ($\leq 1.0 \mu\text{m}$), medium (1.1-3.0 μm) and large (3.1-5.0 μm). From the 2-cell stage to the expanded blastocyst, lipid droplets were found to be abundant. Numerous small lipid droplets were observed in all developmental stages. The number of medium and large lipid droplets increased with the development of embryos. The numbers of medium lipid droplets in the embryos at the 2-cell stage, 8-cell stage and expanded blastocyst were 0.5 ± 0.4 , 11.2 ± 3.3 and 60.3 ± 3.2 , respectively. Large lipid droplets were not observed in the 2-cell and 4-cell stages, but were found to be abundant in the morula and blastocyst. A sudden and significant increase in the number of medium and large lipid droplets was observed from the 8-cell stage to the morula ($p < 0.01$). Similar increase of lipid droplets was also observed in morulae developed from 8-cell embryos in *in vitro* culture ($p < 0.01$). The number of lipid droplets in expanded blastocysts was not affected by the ovulation methods.

Key words: lipid droplet, mouse, embryo

INTRODUCTION

Mouse embryos contain many lipid droplets. These lipid droplets have been histochemically studied in many laboratories (DICKSON, 1969; ISHIDA, 1974; NIIMURA & ISHIDA, 1980). It was demonstrated that lipid droplets were sudanophilic and varied in size, and that they contained lipoids and neutral fats (NIIMURA & ISHIDA, 1980). By morphometric analysis under transmission electron microscope, it was reported that the volume density of lipid droplets in cytoplasm did not change from the 2-cell stage to the blastocyst (ČECH & SEDLÁČKOVÁ, 1983). With the exception of HENSLEIGH & WEITLAUF (1974), there are few reports on the lipid content of embryos. This is

probably due to its small amount in embryos. The above authors described that the lipid content of embryos did not change during the first four days of pregnancy. Recently, the number and size of lipid droplets in blastocysts were reported (SATO et al., 1980; NIIMURA et al., 1981). Also, it was found that changes occurred in the number of lipid droplets in cultured (SATO et al., 1980) and delayed implanting blastocysts (SANZEN et al., 1984), while none occurred in superovulated blastocysts (NIIMURA et al., 1981). These changes depend on the metabolism of the embryos (SATO et al., 1980). The present study was undertaken to examine the relationship between the developmental stages of embryos and the number of lipid droplets by making histochemical observations of lipid droplets.

MATERIALS AND METHODS

Mice and embryos

Embryos were obtained from spontaneously ovulated and superovulated mice of ddY strain. For the spontaneous ovulation, 2-month-old mature mice were used, while for the superovulation, 4-week-old immature mice were used. In this group, two levels of pregnant mare's serum gonadotrophin (PMSG) and human chorionic gonadotrophin (HCG) were given. In one group, an intraperitoneal injection of 5 IU of PMSG was followed by 5 IU of HCG 48 hours later. In the other group, 10 IU of PMSG and HCG were injected. Immediately after injection of HCG, mice were placed with fertile males and mating was confirmed by the presence of vaginal plugs.

The following developmental stages were collected at fixed time intervals after HCG injection: 2-cell (40 hours), 4-cell and 8-cell (66–70 hours), morula (76 hours) and expanded blastocyst (100 hours). The embryos were flushed from the oviduct and/or uterus with Dulbecco's phosphate buffered saline.

In order to observe the effect of *in vitro* culture on the number of lipid droplets, 8-cell embryos were cultured by BMOC-3 (BRINSTER, 1971) for 20 hours. Morulae that developed in this culture were stained for lipid droplets.

A total of 313 embryos was used in the present study.

Methods of histochemical observations of lipid droplets

Preparation of embryos in order to count the number of lipid droplets was done in the following manner. Firstly, embryos were fixed in 10% neutral formalin with 0.85% NaCl for 1 hour at room temperature. They were then rinsed with distilled water and stained with Sudan III for 30 minutes at 37°C. Whole mount preparations of the embryos were made. Lipid droplets were classified according to size into three groups: small ($\leq 1.0 \mu\text{m}$), medium (1.1–3.0 μm) and large (3.1–5.0 μm) according to SATO et al. (1980). The number of lipid droplets in an embryo was counted in all the embryos.

RESULTS

Lipid droplets in embryos were stained orange with Sudan III, which enabled the diameter and number to be measured and counted. However, small lipid droplets could not be counted because of their size.

Embryos at different developmental stages

The numbers of lipid droplets in the embryos of different developmental stages are shown in table 1. From the 2-cell to the expanded blastocyst, the embryos were observed to contain many lipid droplets (figs. 1-4). Small lipid droplets were observed in every stage, but the number seemed to decrease in the morula and the blastocyst. Medium lipid droplets were also observed in all stages with a significant increase in the number with the development of the embryos ($p < 0.01$). However, no significant increase was observed between the 4-cell and 8-cell stage, or between the morula and the blastocyst. Large lipid droplets were not observed in the 2-cell and 4-cell stage (figs. 1 & 2), but were found to be abundant in the morula and the blastocyst (figs. 3 & 4). There was an obvious significant increase in the medium and large lipid droplets from the 8-cell stage to the morula ($p < 0.01$). Among blastomeres of the 2-cell, 4-cell and 8-cell stage, no difference in the numbers of small lipid droplets were observed (fig. 1).

In vitro cultured morulae

As for the morulae that developed from the 8-cell stage in *in vitro* culture, similar increase of different sizes of lipid droplets was also observed. The numbers of lipid droplets in the morulae which developed *in vivo* and *in vitro* are shown in table 2. No significant difference in the numbers of medium lipid droplets were observed between

TABLE 1 *Number of lipid droplets in embryos of different developmental stages*

EMBRYO		NO. OF LIPID DROPLETS PER EMBRYO		
Developmental stage	No. of embryos examined	Small* ($\leq 1.0 \mu\text{m}$)	Medium ($1.1-3.0 \mu\text{m}$)	Large ($3.1-5.0 \mu\text{m}$)
2-cell	43	Abundant	0.5 ± 0.4^a	0^a
4-cell	20	Abundant	5.0 ± 1.3^b	0^a
8-cell	28	Abundant	11.2 ± 3.3^b	0.3 ± 0.2^a
Morula	26	Abundant	55.9 ± 3.8^c	4.3 ± 0.5^b
Expanded blastocyst	35	Abundant	60.3 ± 3.2^c	6.1 ± 0.7^b

Mean \pm SE

* The number of small lipid droplets could not be counted.

a, b, c Values in the same column with different superscripts were significant ($p < 0.01$).

TABLE 2 *Number of lipid droplets in morulae developed in vivo and in vitro*

MORULA	NO. OF MORULAE EXAMINED	NO. OF LIPID DROPLETS PER EMBRYO		
		Small ($\leq 1.0 \mu\text{m}$)	Medium (1.1–3.0 μm)	Large (3.1–5.0 μm)
<i>In vivo</i>	20	Abundant	53.5 \pm 3.0 ^a	4.6 \pm 0.6 ^a
<i>In vitro</i> *	40	Abundant	51.7 \pm 2.8 ^a	2.8 \pm 0.3 ^b

Mean \pm SE

* Morulae developed from 8-cell embryos in *in vitro* culture.

a, b Values in the same column with different superscripts were significant ($p < 0.01$).

these morulae. However, in the cultured morulae, the number of large lipid droplets was found to be significantly less as compared to those of the morulae that developed *in vivo* ($p < 0.01$).

Spontaneously ovulated and superovulated blastocysts

The numbers of medium lipid droplets of expanded blastocysts in the spontaneously ovulated mice and superovulated mice using 5 IU and 10 IU of PMSG were 62.9 \pm 3.3 ($n=31$), 61.0 \pm 2.7 ($n=30$) and 56.2 \pm 2.8 ($n=40$), respectively. The numbers of large lipid droplets in spontaneously ovulated mice and superovulated mice using 5 IU and 10 IU of PMSG were 6.8 \pm 0.6, 6.5 \pm 0.6 and 6.7 \pm 0.8, respectively. No significant difference was observed in the numbers of lipid droplets among these embryos.

DISCUSSION

The lipid content of an embryo is negligible, hence its measurement is difficult. The finding of lipid content has been reported in mouse ova (LOEWENSTEIN & COHEN, 1964) and in delayed implanting blastocysts (HENSLEIGH & WEITLAUF, 1974). The lipid content was measured by weighing the eggs before and after lipid extraction with chloroform and methanol. The total lipid content of a 1-cell embryo was 2.3 ng, and there was no significant increase in the content till the blastocyst (HENSLEIGH & WEITLAUF, 1974). In the present study, the size of the sudanophilic lipid droplets of the embryos in different developmental stages was measured according to SATO et al. (1980). The amount or content of lipid was not measured. It was observed that the number of the different sizes of droplets changed as the embryos developed from the 2-cell stage to the expanded blastocyst. There was a sudden and significant increase in the number of medium and large lipid droplets from the 8-cell stage to the morula. Concurrently, it was also observed that as the embryos developed, the number of small lipid droplets decreased conversely with the increase in the number of medium and large ones. Since HENSLEIGH & WEITLAUF (1974) reported that there was no change in the lipid content of preimplantation embryos with developmental stages, it is

suggested that the medium and large lipid droplets are formed from small ones. However, small lipid droplets were too small and numerous to count under a microscope.

Similar changes of lipid droplets were also observed in morulae cultured *in vitro*. However, the number of medium and large lipid droplets in cultured morulae was less than in the morulae developing *in vivo*. It was also reported that blastocysts cultured from the 2-cell stage contained more lipid droplets than the blastocysts developing *in vivo* (SATO et al., 1980). Therefore, it was suggested that these cultured morulae may be in the process of forming the large lipid droplets.

These morphological changes of lipid droplets were suggested to depend on the metabolism of the embryonic cells (SATO et al., 1980). However, embryo lipid metabolism has not been studied sufficiently. Histochemically, glucose-6-phosphate dehydrogenase and α -glycerophosphate dehydrogenase were demonstrated to be present in embryos (NIMURA et al., 1981). The metabolism of glucose in the embryos was suggested to be related to the changes of lipid droplets (SATO et al., 1980). The glycogen content also changes with the development of embryos (STEIN & BIGGERS, 1968; OZIAS & WEITLAUF, 1971; OZIAS & STEIN, 1973). The glycogen was shown to accumulate at the time of the first cleavage and to reach a maximum at the 8-cell stage (STEIN & BIGGERS, 1968). In the present study, a significant increase was observed in the number of medium and large lipid droplets from the 8-cell stage to the morula. This change was observed to occur before the divergence of the differentiated embryonic cells, which is microscopically observed at the blastocyst. Since medium and large lipid droplets were stable in number between the morula and the blastocyst, it was suggested that the changes of lipid droplets do not occur at the time of blastulation. Therefore, the morphological changes of lipid droplets probably reflect the change of the metabolism before the occurrence of differentiation in the embryonic cells.

The number of medium and large lipid droplets in expanded blastocysts was not affected by the ovulation methods; similar findings were also reported by NIMURA et al. (1981).

The results of the present study show that the morphology of lipid droplets in mouse embryos changes during the development. The relationship among the lipid content, the metabolism of lipid and the morphology of lipid droplets is still not clear; therefore, further biochemical studies should be conducted.

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EXPLANATION OF PLATE

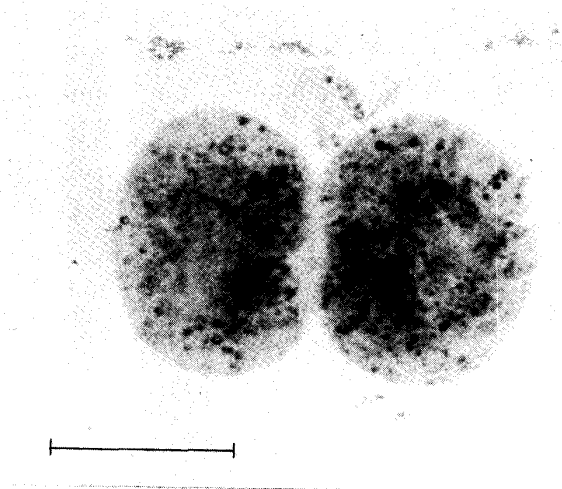
Embryos were stained with Sudan III.

Fig. 1 Lipid droplets in a 2-cell embryo Scale: 50 μ m

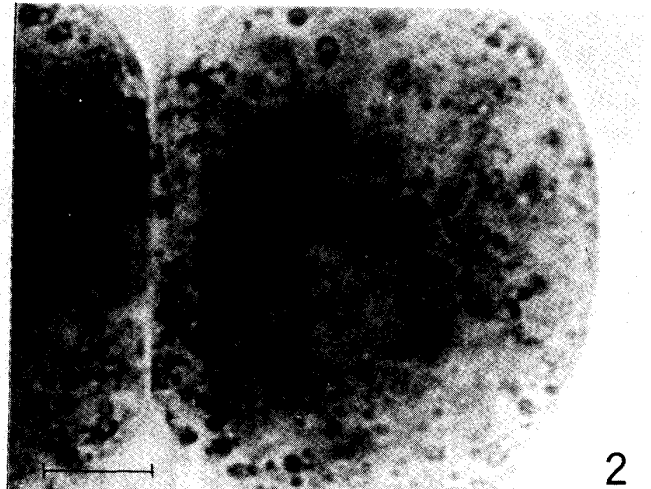
Fig. 2 High magnification of figure 1 Scale: 10 μ m

Fig. 3 Lipid droplets in an expanded blastocyst Scale: 50 μ m

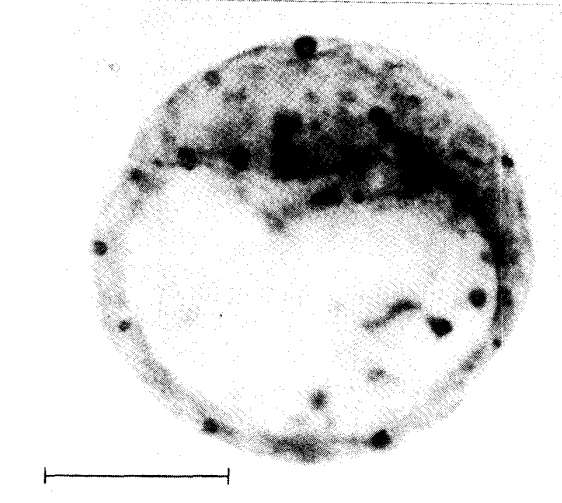
Fig. 4 High magnification of figure 3 Scale: 10 μ m



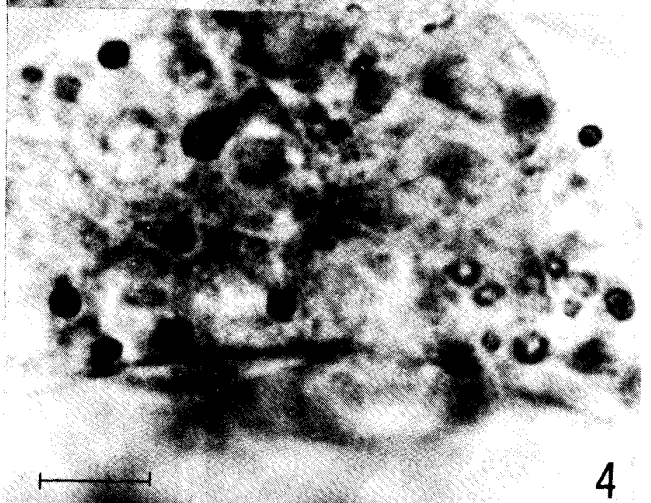
1



2



3



4