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## BRIEF COMMUNICATION

# HISTOCHEMICAL OBSERVATIONS OF LIPID DROPLETS AND GLYCOGEN IN MOUSE EGGS WITH ABNORMAL DEVELOPMENT

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Lipid droplets and glycogen in mouse embryos have been histochemically studied in many laboratories.<sup>2,4,7,8,9,10,11)</sup> The number of lipid droplets in embryos was found to change with the advance of developmental stages.<sup>4,8)</sup> Also, a difference in the number of lipid droplets and the amount of glycogen was reported in delayed implanting blastocysts as compared to blastocysts that were obtained from normally pregnant mice on Day 4 of pregnancy.<sup>9)</sup> However, in abnormal eggs, no histochemical studies on lipid droplets and glycogen have been carried out. In this study, observation of lipid droplets and glycogen was carried out in eggs with abnormal development, namely, aged 1-cell eggs, embryos with delayed development and fragmented eggs.

Eggs were obtained from 4-week-old immature mice of ddY strain. Mice were superovulated by an injection of pregnant mare's serum gonadotrophin and human chorionic gonadotrophin (HCG) as described previously.<sup>4)</sup> Day of HCG injection was designated as Day 0 of pregnancy.

Unfertilized 1-cell eggs that were obtained from Day 2 to Day 4 from females placed with males were considered as aged unfertilized 1-cell eggs. As the control, unfertilized 1-cell eggs were collected on Day 1 from mice not placed with males. Two-cell and 4-cell embryos that developed normally were collected on Days 2 and 3, respectively. Two-cell embryos recovered on Days 3 and 4 and 4-cell embryos recovered on Day 4 were classified as embryos with delayed development. Fragmented eggs were also collected on Days 3 and 4.

For observation of lipid droplets, eggs were stained with Sudan III as described previously.<sup>4)</sup> Sudanophilic lipid droplets were classified according to their diameter into three groups: small ( $\leq 1.0 \mu\text{m}$ ), medium (1.1-3.0  $\mu\text{m}$ ) and large (3.1-5.0  $\mu\text{m}$ ) by

the method of SATO et al. (1980). The size, number and distribution of lipid droplets in each egg were examined.

For observation of glycogen, eggs were fixed in either 10% neutral formalin or Bouin-Allen's fluid for 24 hours. These eggs were embedded in 1.2% agar and then in paraffin. The paraffin blocks were sectioned serially at  $5\mu\text{m}$ . The sections were stained by the periodic acid-Schiff (PAS) method. The glycogen was identified by means of the salivary test.<sup>5)</sup>

Ninety-eight unfertilized 1-cell eggs obtained from Day 1 to Day 4 had numerous small lipid droplets. No medium or large lipid droplets were found. In unfertilized 1-cell eggs recovered on Day 1, lipid droplets were diffused throughout the cytoplasm (fig. 1). However, in aged unfertilized 1-cell eggs, clustering of lipid droplets was observed (fig. 2). Glycogen was observed in 20 unfertilized 1-cell eggs obtained from Day 1 to Day 4 (fig. 3). The amount of glycogen did not seem to differ between aged eggs and the control.

The number of lipid droplets in the 2-cell and 4-cell embryos with normal and delayed development is shown in the table. Both types of embryos had numerous small lipid droplets and no large ones (fig. 4). In the embryos with normal development, a significant increase in the number of medium lipid droplets was observed with development from the 2-cell to 4-cell stage ( $p < 0.01$ ). However, no significant difference in the number of lipid droplets was found between the 2-cell and 4-cell embryos with delayed development. Clustering of lipid droplets was observed only in the embryos with delayed development. Twenty-three 2-cell and 4-cell embryos contained glycogen (figs. 5 & 6). These embryos contained a larger amount of glycogen than unfertilized 1-cell eggs. The amount of glycogen did not seem to differ between normal and abnormal embryos.

In fragmented eggs, small lipid droplets were observed; however, the number of small lipid droplets varied with fragments (fig. 7). The number of medium lipid droplets was  $0.1 \pm 0.1$  (Mean  $\pm$  SE,  $n=62$ ). No large lipid droplets were detected. Clustering of lipid droplets was also observed in these eggs. Nineteen fragmented eggs contained glycogen; however, the amount of glycogen varied with the fragments (fig. 8).

Clustering of lipid droplets was observed in abnormal eggs. Similarly, clustering of cytoplasmic organelles was reported in rhesus monkey abnormal eggs<sup>3)</sup> and rabbit aged eggs.<sup>6)</sup> Clustering of cytoplasmic organelles seemed to be a common feature in early degeneration of ova and blastomeres.<sup>3)</sup> It was suggested that the separation of cytoplasmic ground substance from the cytoplasmic organelles marked the beginning of the fragmentation of eggs.<sup>1)</sup>

The number of medium lipid droplets in the normal embryos increased from the 2-cell to the 4-cell stage. However, the number of medium lipid droplets was found to be the same between the 2-cell and the 4-cell embryos with delayed development.

This observation suggests an abnormality in the lipid metabolism in these embryos.

In the aged eggs and in the embryos with delayed development, no difference in the amount of glycogen observed as compared to the control; similar findings were also reported in the embryos that did not develop in *in vitro* culture.<sup>11)</sup>

In the embryos at early cleavage stages, it was found that the number of lipid droplets did not differ among the blastomeres.<sup>4)</sup> However, in fragmented eggs, the number of lipid droplets varied with the fragments. The amount of glycogen also varied with the fragments. These results agreed with the report that the cytoplasmic components in the fragments were varied.<sup>6)</sup>

In the present study, although the total amount of lipid and glycogen were not determined, it was shown that the size, number and distribution of lipid droplets were affected by developmental abnormality of eggs, and that the distribution of glycogen was affected by the fragmentation of eggs.

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TABLE *Number of lipid droplets in embryos with normal and delayed development*

TYPE OF EMBRYOS	NO. OF EMBRYOS EXAMINED	NO. OF LIPID DROPLETS PER EMBRYO	
		Small ( $\leq 1.0 \mu\text{m}$ )	Medium (1.1–3.0 $\mu\text{m}$ )
Normal			
2-cell	50	Abundant	$0.5 \pm 0.4^a$
4-cell	30	Abundant	$4.7 \pm 1.2^b$
Delayed development			
2-cell	23	Abundant	$0.7 \pm 0.4^a$
4-cell	25	Abundant	$0.3 \pm 0.1^a$

Mean  $\pm$  SE

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

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

#### EXPLANATION OF PLATE

Scale indicates 25  $\mu\text{m}$ .

Fig. 1 Lipid droplets in an unfertilized 1-cell egg.

Fig. 2 Clustering of lipid droplets in an aged unfertilized 1-cell egg.

Fig. 3 Glycogen in an aged unfertilized 1-cell egg.

Fig. 4 Lipid droplets in a 2-cell embryo with delayed development.

Fig. 5 Glycogen in a 2-cell embryo with delayed development.

Fig. 6 Glycogen in a 4-cell embryo with delayed development.

Fig. 7 Lipid droplets in a fragmented egg.

Fig. 8 Glycogen in a fragmented egg.

