Morphology and Size of Embryos Recovered from Superovulated Cows

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ABSTRACT. A total of 691 normal embryos were recovered from 183 superovulated donor cows on the 5th and 6th days after the first insemination, and were examined for their morphology and size in relation to their developmental stage. There was no significant difference in the thickness of the zona pellucida, the diameter of the cell mass, and the overall diameter of the embryos among zygotes, 2-, 4-, 8- and 16-cell embryos, and morulae. In the blastocyst stage, however, the diameter of the cell mass and the overall embryo diameter were significantly greater and the zona pellucida was significantly thinner than in the earlier-stage embryos. The volume of the blastomere significantly decreased from zygote to morula in proportion to the increase in the number of blastomeres. The volume of the cell mass of 2-cell embryos was decreased by about 30% compared with that of zygotes and no increase in the volume of the cell mass was observed during the progression from 2-cell stage to morula. The diameter of the cell mass and the overall diameter of morulae recovered on the 6th day after the first insemination were significantly greater than those of morulae recovered on the 5th day.—**KEY WORDS:** cattle, embryo size, morphology, superovulation.

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Since the first description of bovine unfertilized and 2-cell embryos in 1931 [11], many studies on bovine embryos obtained from spontaneous ovulation have been reported [2, 5, 7, 10, 13, 16, 18, 20]. Following the development of bovine embryo transfer in the 1970's, several studies on the morphology of embryos recovered from superovulated donors were reported [6, 8, 9, 17]. However, there has been no report on the dimensions of bovine embryos obtained by superovulation. The aims of the present study were to classify embryos recovered from superovulated donor cows and to describe their morphological features according to the developmental stage.

MATERIALS AND METHODS

A total of 183 cows, consisting of 12 Holsteins, 47 Simmentals, 25 Limousins, 25 Charolais and 74 crossbreds, were used as the embryo donors. Superovulation was produced by an intramuscular injection of pregnant mare's serum gonadotrophin followed by the injection of prostaglandin F_{2α} 48 hr later. Donors were then artificially inseminated 2 to 3 times at 12 hr intervals during estrus. Embryos flushing was done surgically or nonsurgically from the donors on the 5th and 6th days after the first insemination.

A total of 691 normal embryos were obtained by the microscopic examination of the 774 embryos recovered. These normal embryos were classified according to their developmental stage. Embryos were photographed at 100 X magnification and at the same time a photograph with the embryo next to a micrometer was taken. The overall diameter of the embryo including the zona pellucida, the diameter of the cell mass and blastomere, the thickness of the zona pellucida and the width of the perivitelline

Fig. 1. Measurement sites of bovine embryos in relation to overall diameter, diameter of the cell mass, thickness of zona pellucida, width of perivitelline space and volume of blastomere.

$(F+f)/2$: Overall diameter

$(C+c)/2$: Diameter of the cell mass

$(A+E+a+e)/4$: Thickness of the zona pellucida

$(B+D+b+d)/4$: Width of the perivitelline space

$(\pi LS^2)/4/3$: Volume of the blastomere

(L: Major axis of the blastomere, S: Minor axis of the blastomere)

space were measured on the micrographs. The diameter was obtained as the mean of two measurements made perpendicular to each other (Fig. 1). Blastomere volume was calculated by the method of Tarcowski [19]. In this report, the morula was defined as a 32-cell embryo. Data were analyzed for statistical significance using Student's t-test.

RESULTS

The dimensions of embryos in relation to their developmental stage are shown in Table 1. For the thickness of the zona pellucida, the diameter of the cell mass, and the overall diameter of the embryo, no significant differences were observed among zygotes, 2-, 4-, 8-, and 16-cell embryos, and morulae. In the blastocyst stage, however, the diameter of the cell mass was significantly larger than in the earlier-stage embryos, while the zona pellucida was significantly thinner. The overall diameter of the blastocyst was significantly larger than those of the

Fig. 2. Volumes of the blastomeres and cell masses of embryos recovered from superovulated cows on the 5th and 6th days after the first insemination in relation to the developmental stage.

Table 1. Measurements of embryos recovered from superovulated cows on the 5th and 6th days after the first insemination in relation to the developmental stage

Embryo stage	No. of embryos	Measurements (Mean±S.E) μm			
		Thickness of zona pellucida	Width of perivitelline space	Diameter of cell mass	Overall diameter
Zygote	17	12.1±0.06 ^{a)}	10.0±1.12 ^{c)}	106.6±1.95 ^{f)}	150.7±1.46 ^{h)}
2-cell embryo	6	12.2±0.72 ^{a)}	13.3±1.37 ^{c)}	103.6±3.78 ^{f)}	154.6±4.16
4-cell embryo	8	12.1±0.27 ^{a)}	8.1±1.42	105.4±2.10 ^{f)}	145.7±1.85 ⁱ⁾
8-cell embryo	36	12.6±0.26 ^{a)}	6.0±0.46 ^{d)}	110.7±1.52 ^{f)}	147.8±1.42 ^{h)}
16-cell embryo	51	12.1±0.20 ^{a)}	6.9±0.59 ^{e)}	109.5±1.48 ^{f)}	147.5±1.06 ^{h)}
Morula	555	12.3±0.07 ^{a)}	8.1±0.19 ^{e)}	108.6±0.50 ^{f)}	149.3±0.46 ^{h)}
Blastocyst	18	8.7±0.57 ^{b)}	2.6±1.95 ^{e)}	142.0±4.32 ^{g)}	164.5±2.60 ^{j)}

Significant differences between a) and b), c) and e), f) and g), h) and j) at $P<0.01$. Significant differences between d) and e), i) and j) at $P<0.05$.

Table 2. Volume of the blastomere and the cell mass of embryos recovered from superovulated cows on the 5th and 6th days after the first insemination in relation to the developmental stage

Embryo stage	No. of blastomeres measured	Volume (×10 ⁶ μm ³)	
		Blastomere (Mean±S.E.)	Cell mass ^{a)}
Zygote	17	5.43±0.28 ^{c)}	5.43
2-cell embryo	12	1.91±0.18 ^{d)}	3.82
4-cell embryo	26	0.90±0.08 ^{e)}	3.60
8-cell embryo	111	0.47±0.02 ^{f)}	3.76
16-cell embryo	138	0.23±0.01 ^{g)}	3.68
Morula ^{b)}	76	0.13±0.01 ^{h)}	4.16

a) performed by multiplication of the mean volume of the blastomere by cell numbers.

b) defined as a 32-cell embryo.

Significant differences between c) and d), d) and e), e) and f), f) and g), g) and h) at $P<0.01$.

Table 3. Measurements of morula recovered from superovulated cows on the 5th and 6th days after the first insemination

Day of recovery	No. of embryos	Measurements (Mean±S.E) μm			
		Thickness of zona pellucida	Width of perivitelline space	Diameter of cell mass	Overall diameter
5	138	12.0±0.12	7.9±0.32	107.1±0.82 ^{a)}	146.8±0.69 ^{c)}
6	152	12.4±0.11	9.1±0.40	112.1±1.13 ^{b)}	155.1±0.45 ^{d)}

Significant differences between a) and b), c) and d) at $P<0.01$.

zygotes, 4-, 8-, and 16-cell embryos, morulae (zygotes, 8-, 16-cell embryos, morulae: $P<0.01$, 4-cell embryos: $P<0.05$).

The blastomere volume and the cell mass in relation to the developmental stage are shown in Table 2 and Fig. 2. Blastomere volume decreased significantly in proportion to the increase in the number of blastomeres ($P<0.01$). The mean volume of the cell mass (i.e., the mean blastomere volume multiplied by the number of cells in the embryos) was $5.43 \times 10^6 \mu\text{m}^3$ for zygotes, and this decreased during the early stages of embryogenesis up to the 16-cell stage, ranging from 3.60×10^6 to $3.82 \times 10^6 \mu\text{m}^3$.

The diameter of the cell mass and the overall diameter of morulae recovered on the 6th day after the first insemination were significantly larger than those of morulae recovered on the 5th day (Table 3).

DISCUSSION

The normal stage of bovine embryos recovered on the 5th and 6th days after estrus has been reported to be the morula [12, 14, 20]. In the present study, 80% (555) of the normal embryos recovered were in the morula stage. The earlier-stage embryos recovered seemed to be degenerating or to have been delayed in development by delayed ovulation [14].

The data obtained in the present study regarding embryo dimensions were similar to those in reports on embryos obtained by spontaneous ovulations [5, 7, 10, 11, 13, 16, 18]. There was no significant difference in the thickness of the zona pellucida and the overall diameter of the embryos from the zygote to the morula stage. This was in agreement with the findings of Olds *et al.* [16], who reported that the number of blastomeres recovered on the 2nd to 5th days after estrus appeared to have no effect on the thickness of the zona pellucida and its overall diameter. As the number of blastomeres increased,

the volume of each blastomere significantly decreased. In addition, no increase in the volume of the cell mass was observed during development from the 2-cell stage to the morula. Our results thus indicate that the first few cleavage divisions of the bovine embryo occur without any increase in the cell mass. Similar findings were reported by Anderson [1] and McLaren [15]. The volume of the cell mass in 2-cell to 16-cell embryos decreased by approximately 30% compared with that of the zygote. Similarly, about 20% reduction in the volume of the cell mass was reported by McLaren [15] and Bazer *et al.* [3].

The overall diameter and the diameter of the cell mass of the morulae increased between the 5th and 6th days. In the blastocyst stage, the thickness of the zona pellucida and the width of the perivitelline space decreased and the diameter of the cell mass and the overall diameter increased when compared with those of the morula. These results suggest that the thinning of the zona pellucida was due to expansion of the cell mass followed by stretching of the zona pellucida over the surface of the enlarged cell mass. These findings may represent the phenomenon that occurs prior to 'hatching' from the zona pellucida in the late blastocyst stage [1, 4]. The progressive observation of an embryogenesis is required for further research on the developmental morphology of the embryos.

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