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SOME ASPECTS OF BOVINE OVA CULTURE IN VITRO

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One hundred and thirty-three fertilized ova were grouped into three categories; normal, young and degenerated on the 5th day after insemination. A simple culture technique of a stoppered test tube with Brinster's medium and a gas mixture was applied. Thirty-eight out of 46 normal ova (82.6%) were developed into the blastocysts. The young and degenerated ova showed very poor development. Ten normal ova were kept for 2~4 hours at room temperature before culture and 56 normal ova were exposed under microscopic light for 30 seconds or 30 minutes before culture. Nine ova from the former group and 46 ova from the latter group were developed into the blastocysts, respectively.

INTRODUCTION

Since bovine embryo transfer has become commercial (SWENSSON & HEWETT, '74; ROWSON, '76; BETTERIDGE, '77; KANAGAWA, '77), short term preservation or culture of bovine ova from the time of recovery to transfer is essential for an embryo transfer program. A simple and reliable culture technique for utilization in the field is important. There are a few reports of in vitro culture of bovine ova, however, some required paraffin oil (MCKENZIE & KENNEY, '73; SEIDEL, '74; GORDON, '76; WRIGHT et al.,^{18,19} '76), a humidified atmosphere and a CO₂ gas incubator. In 1974, TERVIT et al. cultured bovine embryos in stoppered test tubes; and this report had been the most encouraging up to that time. Similar results were obtained with the stoppered test tube culture technique by SHEA et al. ('74), KANAGAWA et al. ('75) and KANAGAWA & BASRUR (unpublished data).

This study was carried out in order to adapt a simple culture technique for an embryo transfer program; factors such as temperature and artificial light before cultures were tested.

MATERIALS AND METHODS

One hundred and thirty-two fertilized ova, ranging from 8-cell to 32-cell stages, were obtained from 25 Holstein Beef cross bred heifers by uterine flushing surgically, under general anesthesia on the 5th day after onset of standing estrus. All heifers were subjected to superovulation by 2,000 I U of gonadotrophic hormone (Pregnant

Mare's Serum, Ayerst Laboratories, Inc), administered intramuscularly during a postestral period between days 9 and 14. The day of onset of standing estrus was considered as day 0. After 42 hours of the gonadotrophin injection, 25 mg of prostaglandin (Prostin F₂ alpha, Upjohn Company) was injected intramuscularly. The onset of standing estrus was observed approximately 40 hours after the prostaglandin injection. During estrus, artificial insemination was performed twice at 12 hour intervals with 2 doses of frozen semen which was obtained from a local artificial insemination center.

Fertilized ova were recovered from the oviduct and uterine horn by flushing with tissue culture medium (TCM-199, Grand Island Biological Company) at 37°C. After recovery, the ova were transferred into small glass dishes with Brinster's medium (BMOC-3, Grand Island Biological Company), and classified into 3 groups: normal, young and degenerated ova. One hundred and seventeen ova were classified as normal, ranging from 8-cell to 32-cell stages, without damage and degeneration. Six ova were classified as young, ranging from 2-cell to 4-cell stages. Ten ova were classified as degenerated ova with some degree of degeneration in the blastomeres.

Forty-six normal ova, 6 young ova and 10 degenerated ova were cultured in 5 ml glass test tubes with one ml of BMOC-3 (fig. 1). The gas (5 % of CO₂, 5 % of O₂ and 90 % of N₂) was applied for one minute just before the culture was put into the culture tube which was quickly shielded with a rubber stopper (fig. 2). Thereafter, the culture tube was kept in an incubator at 37°C.

Ova development in the culture was evaluated by phase contrast inverted microscopy at 24 hour intervals. After each observation of cultured ova a new medium and gas mixture were applied into the culture tube.

Seventy-one normal ova were treated at room temperature (25°C), with artificial light and transportation without any protection before the culture.

RESULTS

The result of the development of normal ova to the blastocyst stage is shown in table 1. Sixteen out of seventeen 8-cell ova were developed into the expanded blastocysts after 4 days incubation in vitro. Fourteen out of eighteen 16-cell ova after 3 days, and eight out of eleven 32-cell ova after 2 days, were developed into expanded blastocysts (figs. 3 & 4). As shown in table 1, 38 out of 46 normal ova (82.6 %) from three stages were developed into the expanded blastocysts after 2~4 days in vitro culture. The beginning of hatching occurred in some of the expanded blastocysts.

The culture result of 6 young ova from 2-cell to 4-cell stages and 10 degenerated ova of the 32-cell stage is shown in table 2. There were no developments from 5 young ova of the 2-cell and 3-cell stages. One 4-cell ovum was developed until 10-cell stage after 4 days in vitro; however, there was no further development and degeneration occurred (figs. 5~7). Only one out of ten degenerated ova was developed into a blas-

tocyst, and 9 ova were degenerated completely after 4 days in vitro.

The summary result of treatment before culture is shown in table 3. Treatment at room temperature (25°C) for 2 and 4 hours before culture caused 9 out of 10 normal ova to develop into expanded blastocysts. Fifty-six normal ova from three stages were kept under microscopic light using the maximum degree of the transformer without

TABLE 1 *Bovine ova culture in BMOC-3*

INITIAL STAGE	NO. OF OVA CULTURED	DAYS IN VITRO	DEVELOPED TO BLASTOCYST (%)
8-cell	17	4	16 (94.1)
16-cell	18	3	14 (77.8)
32-cell	11	2	8 (72.7)
TOTAL	46	2~4	38 (82.6)

TABLE 2 *Young and degenerated ova culture in BMOC-3*

INITIAL STAGE	NO. OF OVA CULTURED	DAYS IN VITRO	REMARKS
2-cell	1	4	No development
3-cell	4	4	No development
4-cell	1	4	10-cell, degeneration
32-cell (Deg.)	10	4	1 blastocyst

TABLE 3 *Treatment before culture in BMOC-3*

TREATMENT	TIME	INITIAL STAGE	NO. OF OVA CULTURED	DAYS IN VITRO	DEVELOPED TO BLASTOCYST
Room temp. 25°C	2 hr	8-cell	5	5	5
Room temp. 25°C	4 hr	16-cell	5	4	4
SUBTOTAL			10	4~5	9
Microscopic-light	30 sec.	8-cell	12	4	10
		16-cell	21	3	16
		32-cell	17	2	14
Microscopic-light	30 min.	16-cell	6	4	6
SUBTOTAL			56	2~4	46
Short transportation for 100 km		8-cell	5	5	4
TOTAL			71	2~5	59

any filter system for 30 seconds or 30 minutes before culture. Forty-six out of 56 ova (82.1 %) were developed into the expanded blastocysts after culture in vitro for 2~5 days. Five normal ova of the 8-cell stage were kept in a culture tube with one ml of BMOC-3 and placed in the chest pocket of a passenger in a car. The car was driven for approximately one hour for about 100 km before culture; using this method, 4 out of 5 ova developed into the expanded blastocysts.

DISCUSSION

Several studies have been carried out using the bovine ova culture in vitro micro-drop technique under paraffin oil (MCKENZIE & KENNEY, '73; WRIGHT et al.^{18,19}, '76); the covered glass dish technique (SEIDEL, '74; BOLAND et al., '75; SREENAN et al., '75; TROUNSON et al.¹⁷, '76); the medium covered with paraffin oil (SEIDEL, '74); the stoppered test tube with paraffin oil (GORDON, '76); and the one ml of medium under paraffin oil in test tube technique (RENARD et al., '76).

The results of this study show that bovine ova can be cultured in vitro into expanded blastocysts from the 8-cell stage in the BMOC-3 medium in a sealed tube with a gas mixture.

The quality of ova is an important factor in bovine embryo transfer. The results of this study of young ova and degenerated ova showed very poor results for development in vitro. Due to unknown factors, these young ova, ranging from 2-cell to 4-cell stages, stopped development on the 5th day after insemination in the donor's reproductive tract. KANAGAWA ('76) reported that no pregnancy was observed from the 2-cell to 7-cell ova transferred on the 5th or 6th day after insemination. Under normal conditions, SHEA et al. ('74) cultured early stages of recovered ova and only 6 % of those were fertilized. The one-cell stage developed beyond the 8-cell to 12-cell stages. BETTERIDGE ('77) stated that bovine embryos recovered in the early stages of cleavage can rarely be cultured beyond the 8-cell to 12-cell stages and he suspected that late 8-cell ova are easier to culture into blastocysts than are ova that have just reached the 8-cell stage.

KANAGAWA ('76) reported that few pregnancies occurred from the transfer of a certain degree of degenerated ova. The results of this study showed only one out of 10 degenerated 32-cell ova were developed into an expanded blastocyst. The only method that has been used to evaluate the quality of bovine ova after collection is morphological observation. Additional basic studies are needed so that ova judged viable can, in fact, produce a calf even to some degree of degeneration. Concerning the commercial basis of embryo transfer, it is difficult to discard a potentially valuable ova, and it is also an economical loss to transfer the non-potential ova.

No studies have been published specifically comparing room temperature and body temperature for storage of ova between recovery and transfer. The culture system in

the present study indicates that ova can be held for at least 4 hours at room temperature (25°C) before transfer.

Since the microscope is an essential tool for locating ova from the recovery fluid and for examining ova morphologically, a strong artificial light needs to be validated for ova handling. This study, however, shows that a high rate (82.1 %) of development was observed after exposure to the strong microscopic light, and that the rate of development was similar to normal ova cultures (82.6 %) without light exposure.

For practical embryo transfer, short transportation of recovered ova from one place to another is very useful. Only 5 ova were tested prior to this study, however, 4 ova were developed into the blastocysts. This result may encourage further development in this field.

Additional fundamental studies are needed to maintain and handle bovine ova in vitro for the improvement of an embryo transfer program. There is a lack of information on how the potential use of biochemical, biophysical and metabolic parameters may reflect the bovine ova.

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EXPLANATION OF PLATE

PLATE

- Fig. 1 A test tube with rubber stopper for culture in vitro
- Fig. 2 Gassing apparatus of culture tube before incubation
- Fig. 3 Six normal bovine ova at 32-cell stage before culture
Approx. $\times 120$
- Fig. 4 Same ova as in figure 3
Developed into late morula and early blastocyst after 3 days
in vitro
Approx. $\times 120$
- Fig. 5~7 A young ovum on 5th day after insemination was cultured
in vitro for 24 hours (fig. 5), 48 hours (fig. 6) and 96 hours
(fig. 7). One blastomere is large but has no cleavage. Some
cleavages from small blastomeres are observed.
Approx. $\times 200$

