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ONE TO TWO DAY PRESERVATIONS OF BOVINE EMBRYOS

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Twenty morula stage bovine embryos were grouped into 2 categories and preserved: 8 morulae in room temperature (20–25°C) and 12 morulae in low temperature (4–5°C) for 1 to 2 days, then transferred to 8 recipient heifers. The recipient heifers were selected in the same stage of estrus as the donor. Five recipient heifers received 2 embryos, and 3 recipient heifers received 3–4 embryos. Three out of the 4 recipient heifers which received embryos preserved for 1 day at room and low temperatures became pregnant. However, there were no pregnancies observed in the 4 recipient heifers which received embryos preserved for 2 days, although the embryos looked normal morphologically.

INTRODUCTION

Following the independent reports describing the first successful freezing of mouse embryos by WHITTINGHAM et al. ('72) and WILMUT ('72), the embryos of small laboratory animals have been successfully frozen and thawed by many other researchers[1,6-14,19-24,26]. However, reports of bovine embryo freezing have been few and inconsistent[3-5,15-18,27-29,31,32]. Short term preservation of bovine embryos as a pre-stage for long term storage is an essential technique for embryo transfer.

In a previous paper the author described bovine ova culture in vitro in an incubator at 37°C (KANAGAWA, '79). This study was carried out in order to clarify the changes in bovine embryo preservation at room (20–25°C) and low (4–5°C) temperatures.

MATERIALS AND METHODS

Twenty fertilized embryos of the morula stage obtained on the 6th day after insemination were from 5 grade Holstein breed heifers. The heifers were subjected to superovulation by 2,000 I. U. of gonadotrophic hormone (Pregnant Mare's Serum, Ayerst Laboratories Inc.) administered intramuscularly during the postestral period on the 10th day after estrus. Forty-two hours after the gonadotrophin injection, 25 mg of prostaglandin (Prostin $F_{2}\alpha$, Upjohn Co.) were injected intramuscularly. Approximately 40 hours after the prostaglandin injection, the onset of standing estrus was observed. During estrus,

artificial insemination was performed twice at 12 hour intervals with two doses of frozen Holstein bull semen obtained from a local artificial insemination center.

Fertilized embryos were recovered from the uterine horn by using flank surgery under local anesthesia and by using the flushing method. The flushing utilized the tissue culture medium of TCM-199 (Grand Island Biological Co.) at 37°C on the 6th day after insemination. After recovery, the embryos were transferred into small, covered glass dishes (3 cm in diameter and 2 cm in height) with Brinster's medium of BMOC-3 (Grand Island Biological Co.). Two-4 embryos were kept in each dish with 2 ml of BMCO-3, and sterile vaseline was applied between the dish and cover to prevent evaporation and air flow. The dishes of the group at room temperature were kept on a laboratory bench without light at 20-25°C for 1 to 2 days. For the group at low temperature, the dishes were kept inside a refrigerator at 4-5°C for 1 to 2 days.

A summary of materials and results is shown in the table. After 1 to 2 days preservation, 20 embryos were transferred surgically from the flank into the uterine horn of 8 Hereford cross breed heifers under local anesthesia. The recipient heifers were selected to be in the same stage of estrus as the donor on the 6th day after estrus. Five recipient heifers received 2 embryos, 1 embryo in each uterine horn. Two recipient heifers received 3 embryos, 2 embryos in the ovulated side of the uterine horn and 1 embryo in the other side of the uterine horn. One recipient heifer received 4 embroys, 2 embryos in each uterine horn.

TABLE *Summary of materials and results*

TOTAL NO. OF EMBRYOS USED	NO. OF EMBRYOS	TEMPERATURE	NO. OF EMBRYOS	PRESERVATION PERIODS	NO. OF EMBRYOS TRANSFERRED TO EACH RECIPIENT	RESULTS
20	8	20-25°C	4	1 day	2	+
					2	−
			4	2 days	2	−
					2	−
	12	4-5°C	7	1 day	3	+
					4	+
			5	2 days	2	−
					3	−

Notes: + Pregnancy − No pregnancy

RESULTS

After 1 to 2 days of preservation at room (20-25°C) and low (4-5°C) temperatures, the embryos looked almost normal morphologically under microscopic examination. The embryos were the same size, shape and color as before preservation; there was no

damage to either the membrane or the blastomeres and no change in the granules on the membrane.

After transfer of the preserved embryos, the recipient heifers were tested for pregnancy by rectal palpation the 40-day examinations. Three out of the 4 recipient heifers which received embryos preserved for 1 day were diagnosed as being pregnant. However, none of the recipient heifers which received embryos preserved for 2 days became pregnant (table). The 3 pregnant recipients were sacrificed after 50 and 70 days in order to study whether or not twin or multiple pregnancies took place, as 2–4 embryos were transferred to each recipient heifer. However, all three were single pregnancies (figs. 3–4).

Discussion

Storage of mammalian embryos for limited periods of time is possible only if the embryo metabolism is greatly reduced without causing cell injury. In this study there were high pregnancy rates (3 out of 4 pregnancies) from embryos preserved in groups for 1 day at both room temperature (20–25°C) and low temperature (4–5°C). However, there were no pregnancies from embryos preserved in groups for 2 days at either temperature despite the fact that the embryos looked normal morphologically after 2 days preservation. There is a possibility that after 2 days of room and low temperature preservation, metabolic activity in the embryo is slowly carried out and a certain degree of development is continued. Therefore, there was doubt as to whether or not the synchronization of estrus in the recipient heifers with the same stage of estrus as the donor was correct or not.

The preservation system in the present study indicates that the morula stage of embryos can be preserved at least 24 hours at either room or low temperatures before transfer. This result may encourage further interest in this field. Additional studies are needed to investigate the many interrelated factors involved in the successful preservation of embryos in embryo transfer programs.

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Explanation of plates

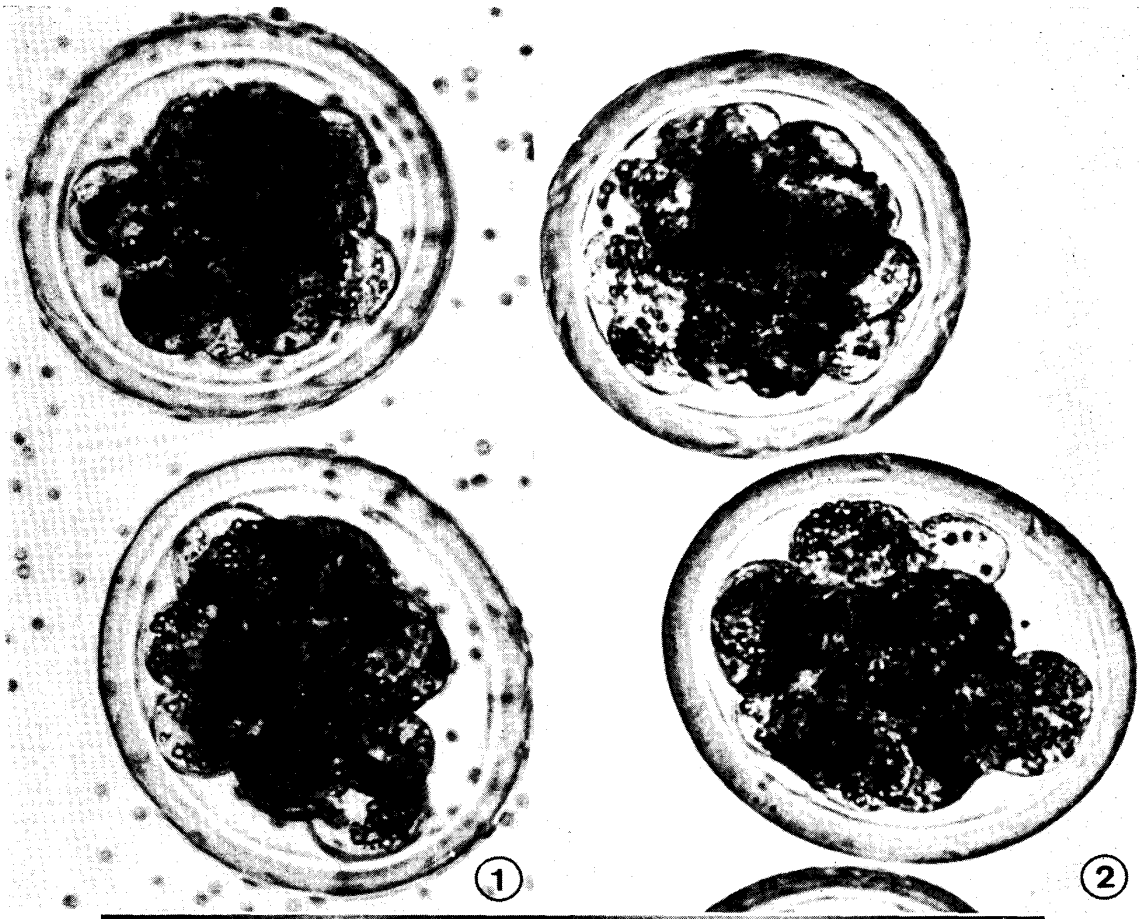
Plate I

Fig. 1 Two morula stage embryos just recovered from the uterus on the 6th day after insemination
Approx. ×100

Fig. 2 The same two embryos as in figure 1 after 1 day of preservation at 4–5°C
Approx. ×100

Fig. 3 The pregnant uteruses of two recipient heifers 50 days (left) and 70 days (right) after receiving 1 day preserved embryos

Fig. 4 The same as above, fetus and fetal membrane after being removed from the uterus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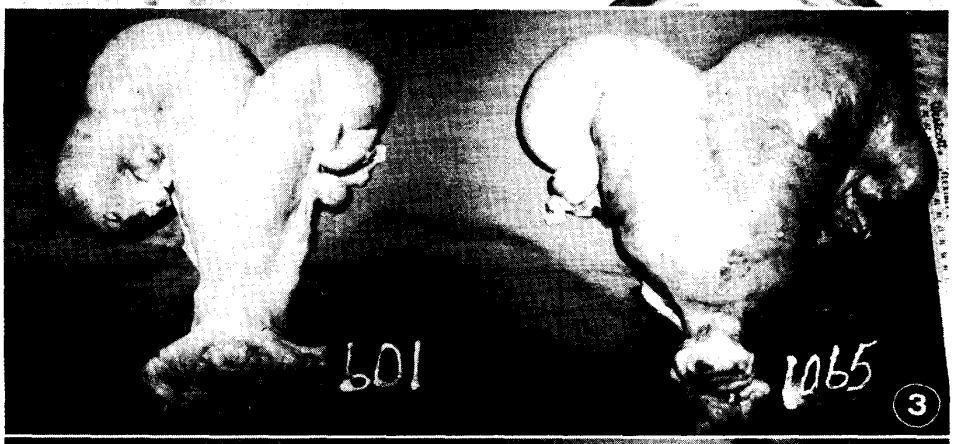
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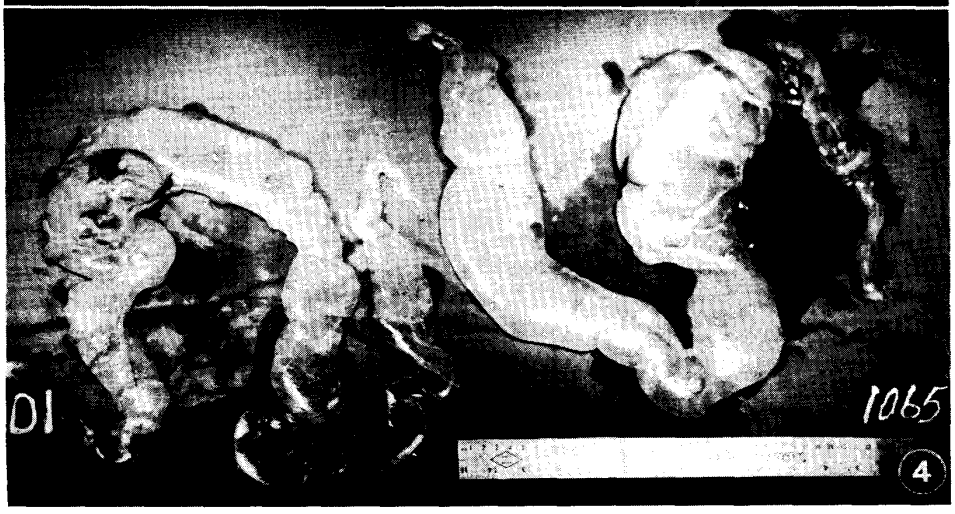
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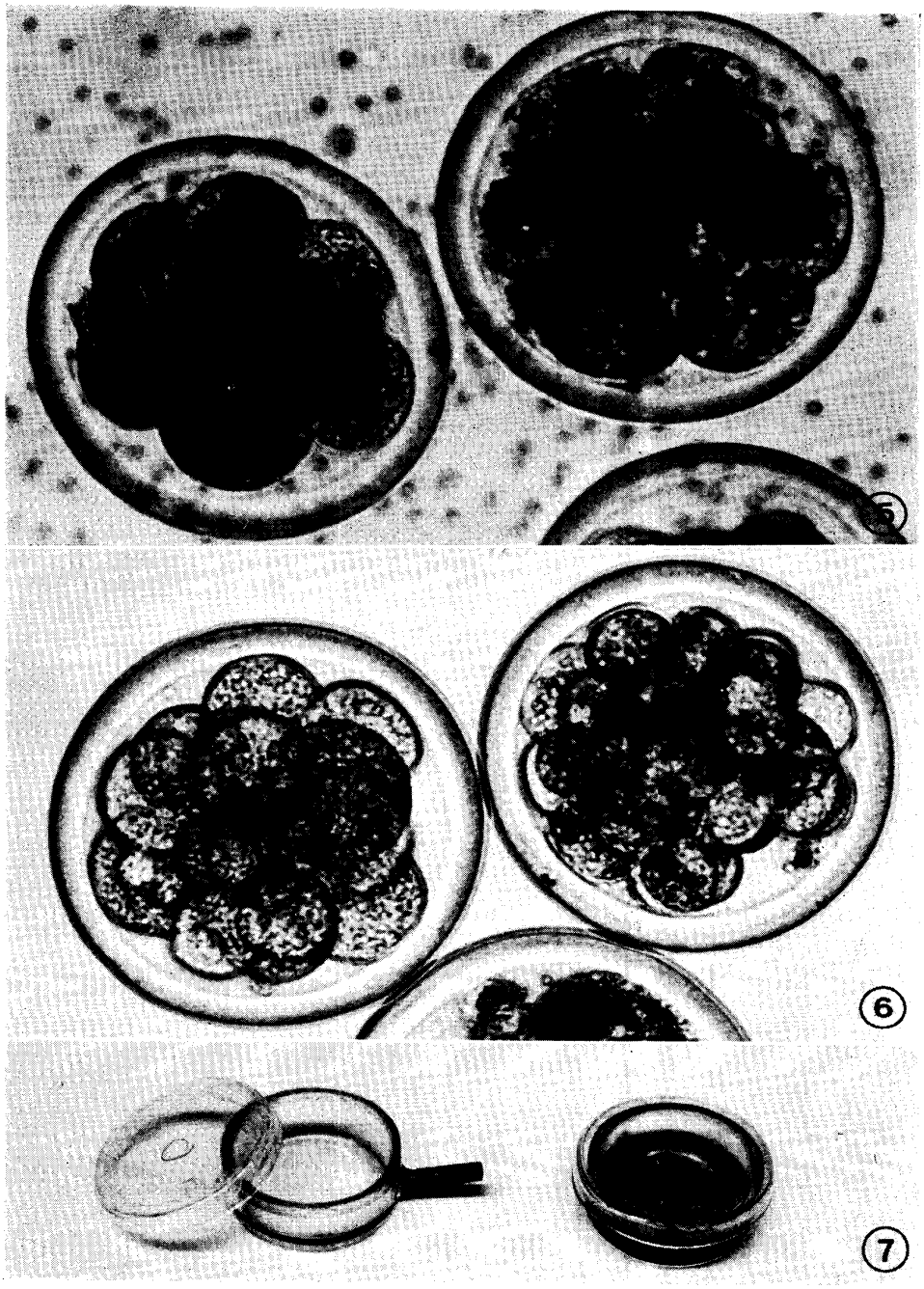
PLATE II

Fig. 5 Two morula stage embryos just after recovery on the 6th day after insemination
Approx. ×100

Fig. 6 The same two embryos as in figure 5 after 2 days of preservation at 4-5°C. Note no morphological changes
Approx. ×100

Fig. 7 Small glass dishes and their covers with the medium used for 1 to 2 day preservations of the embryos. A small amount of sterile vaseline was applied between the dish and cover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