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BRIEF COMMUNICANION

EFFECT OF PHYTOSIN ON MOUSE EMBRYO SURVIVAL AFTER SHORT-TERM STORAGE

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Key words : phytosin, embryo, mouse

The short-term preservation of preimplantation mammalian embryos would be a valuable tool in routine embryo transfer procedures if embryo viability could be maintained long enough while waiting for recipients to reach the synchronous stage of their estrous cycles.[3] It is also an essential preliminary step in the long-term preservation of surplus embryos. Day 8 bovine embryos were successfully stored for more than 48 hours under refrigeration (4°C) using a modified Dulbecco's phosphate buffered saline (PBS) as the cold storage medium.[6] Likewise, rabbit embryos were successfully stored for a week or longer at 4°C using a modified PBS.[3] Studies on the short-term preservation of mammalian embryos had also been reported by several authors.[1,2,4,5,7,8]

Up to the present time, no study has been reported regarding the use of plant extracts such as phytosin to enhance the preservation or maintain the viability of preimplantation mammalian embryos during storage. Phytosin is a plant extract obtained from rice, wheat, or radish seeds and contains iron and neutral lipid compounds. Studies conducted by YAMASHITA[9] showed that this plant extract controls the flowering and vegetative growth of plants. Moreover, it suppresses bacterial and viral growth, and prevents the decomposition of animal tissues. In one of his studies, he demonstrated that the mouse tissues stored in the phytosin-supplemented water for about 14 years did not decompose in contrast to those tissues stored in the unsupplemented water which began to decompose after 1 week. Furthermore, when a portion of the tissues that had been preserved in the phytosin-supplemented water for a year was cultured in synthetic medium 858 for 24 hours, a 2.5 times increase in the

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cell numbers was observed.

This study was undertaken to examine the effect of phytosin on the survival of mouse embryos after short-term storage.

In the first experiment, the embryos were stored in either the phytosin-supplemented or unsupplemented physiological saline solution (PSS) at room (20–30°C) or refrigeration (4–5°C) temperature for up to 48 hours before they were cultured *in vitro* to determine their viability after storage.

Female ddY mice 4 weeks of age or older were superovulated by the intraperitoneal administration of 7.5 IU of PMSG followed by the same dose of hCG 48 hours later. The mice were mated after hCG injection and checked for the presence of vaginal plug the following morning. Embryos were flushed from the oviducts and uterus about 78 hours post hCG injection using PSS containing 5% calf serum (PSS + CS). The embryos were then washed with fresh flushing medium and evaluated for normal morphology. About 8–12 morphologically normal compacted morulae were randomly pipetted into 0.25ml plastic straws containing either PSS + CS or PSS + CS with phytosin (1 : 1000) as the storage medium. The plastic straws loaded with embryos were then placed in a small cardboard box covered with aluminum foil and stored at either room or refrigeration temperature for 24 and 48 hours. After storage, the embryos were washed with fresh culture medium [BMOC–3] and cultured *in vitro* by the microdroplet method in BMOC–3 at 37°C under 5% CO_2 and 95% air for 48 hours. Post-storage viability was assessed by the ability of the embryos to develop into the blastocyst stage.

In the second experiment, in lieu of PSS, PBS was used since it was reported to be a suitable storage medium for mammalian embryos using the test tube method.[3,6] In addition, the embryos were stored at 4–5°C based on the result of experiment 1.

Embryos were obtained in the same manner as described in experiment 1. PBS containing 10% CS (PBS + CS) was used as the flushing medium. About 20–25 morphologically normal compacted morulae were randomly selected and pipetted into 5 ml sterile test tubes containing 2 ml of either PBS + CS or PBS + CS with phytosin (1 : 1000). Each test tube was stoppered and placed in a 300 ml glass beaker filled with 200 ml distilled water at room temperature. The beaker was covered with aluminum foil to keep out light, and was then placed into an ordinary refrigerator for 24, 48, and 72 hours. After the end of the storage period, the embryos were pipetted into a petri dish, washed in BMOC–3, and cultured *in vitro* as described previously.

In both experiments, the results were analyzed using the x^2 test.

Table 1 shows the survival rate of mouse embryos stored in either the phytosin-supplemented or unsupplemented PSS at room or refrigeration temperature after 48 hours of *in vitro* culture.

After 24–hour storage at either room or refrigeration temperature, no difference

TABLE 1 *In vitro* survival of mouse embryos stored in phytosin-supplemented PSS for up to 48 hours at room (20–30°C) or refrigeration (4–5°C) temperature

Storage media	Percentage of embryos that developed to blastocyst after 48-hr culture in BMOC–3			
	Method of Storage			
	20–30°C		4–5°C	
	24 hr	48 hr	24 hr	48 hr
PSS + 5% CS	6/21 (29%)	0/41	22/26 (85%)	5/48 (10.5%)
PSS + 5% CS + Phytosin (1 : 1000)	8/28 (29%)	0/59	33/39 (85%)	10/45 (22%)

was observed between the survival rate of embryos stored in the phytosin-supplemented and unsupplemented PSS. However, after 48-hour storage at refrigeration temperature, the embryo survival rate was higher in the phytosin-supplemented PSS than in the unsupplemented PSS, but the difference was not statistically significant (22% vs 10.5%, respectively). No embryos survived after 48-hour storage at room temperature. The results also indicated that the viabilities of embryos stored at refrigeration temperature was higher than at room temperature.

The survival rate of mouse embryos stored in either the phytosin-supplemented or unsupplemented PBS at refrigeration temperature after 48 hours of *in vitro* culture is shown in Table 2. The embryos that were stored for 24 hours showed a survival rate of 82.5% and 87% in the unsupplemented and phytosin-supplemented PBS, respectively. Although embryo viability was higher in the phytosin-supplemented medium, there was no significant difference observed. Similarly, no significant difference was observed in the survival rate of the embryos that were stored for 48 hours in the unsupplemented and phytosin-supplemented PBS (69% vs 75.5%, respectively). The embryo survival rate in both groups of media decreased markedly after 72-hour storage. The results demonstrated that mouse embryos can be stored in PBS up to 48 hours at refrigeration temperature. No significant increase was observed in the survival rate of embryos with the addition of phytosin. PBS was found to be a more satisfactory medium for the short-term preservation of mouse embryos as compared to PSS.

REFERENCES

1) BIELANSKI, J. G., MANNS, J. G., SCHNEIDER, U. & MAPLETOFT, R. J. (1979) : Survival of mouse embryos after refrigeration and deep freezing. *Theriogenology*, **25**, 139

TABLE 2 *In vitro* survival of mouse embryos stored in phytosin-supplemented PBS for up to 72 hours at refrigeration temperature (4 – 5°C)

Storage media	Percentage of embryos that developed to blastocyst after 48-hr culture in BMOC–3		
	Length of Storage		
	24 hr	48 hr	72 hr
PBS + 10% CS	33/40 (82.5%)	29/42 (69%)	2/19 (10.5%)
PBS + 10% CS + Phytosin (1 : 1000)	33/38 (87%)	34/45 (75.5%)	2/19 (10.5%)

(abstr.)

2) BonDURANT, R. H., ANDERSON, G. B., BOLAND, M. P., CUPPS, P. T. & HUGHES, M. A. (1982) : Preliminary studies on bovine embryo survival following short-term storage at 4°C. *Ibid.*, **17**, 223–230
3) HUGHES, M. A. & ANDERSON, G. B. (1982) : Short-term storage of rabbit embryos at 4°C. *Ibid.*, **18**, 257–281
4) KANAGAWA, H. (1980) : One to two day preservations of bovine embryos. *Jpn. J. Vet. Res.*, **28**, 1–6
5) LADD, P. C., PLAIR, A. C., BRAIDLOW, B. K. & SNYDER, D. A. (1982) : Development of early mouse embryos and sheep embryos in serum-supplemented, phosphate-buffered saline. *Theriogenology*, **17**, 94 (abstr.)
6) LINDNER, G. M., ANDERSON, G. B., BonDURANT, R. H. & CUPPS, P. T. (1983) : Survival of bovine embryos stored at 4°C. *Ibid.*, **20**, 311–317
7) POOL, S. H., BLAKEWOOD, E. G., RORIE, R. W., McFARLAND, C. W. & GODKE, R. A. (1986) : Use of a household refrigerator for short-term storage of bovine embryos. *Ibid.*, **25**, 184 (abstr.)
8) TROUNSON, A. O., WILLADSEN S. M. & ROWSON, L. E. A. & NEWCOMB, R. (1976) : The storage of cow eggs at room temperature and at low temperatures. *J. Reprod. Fert.*, **46**, 173–178
9) YAMASHITA, S. (1985) : Principles of establishing living things. Dept. of Agricultural Chemistry, Faculty of Agriculture, Nagoya University, Nagoya, Japan (in Japanese)