

***In vitro* Culture, Storage and Transfer of Goat Embryos**

R. J. Bilton and N. W. Moore

Department of Animal Husbandry, University of Sydney, Camden, N.S.W. 2570.

Abstract

Goat embryos, collected 5 and 7 days after mating, were cultured *in vitro* at 37°C for 2 days, or stored at 5°C for 1 or 2 days and then cultured for 2 days, or stored in liquid nitrogen (-196°C) for 2-4 weeks and then cultured for 1 day. After culture some of the embryos were transferred to recipient does.

Culture and storage was carried out in Dulbecco phosphate buffer enriched with 25% goat serum. 1M glycerol or 2M dimethylsulphoxide (DMSO) was added to the media used for frozen storage.

Thirteen of 15 embryos cultured without prior storage showed apparently normal development in culture. Ten of the 13 were transferred and five kids were born.

Twenty of 38 embryos stored at 5°C developed in culture and six kids were born following the transfer of 17 embryos. Duration of storage at 5°C had no marked effect upon subsequent development.

Six of 48 frozen stored embryos developed in culture. All six were transferred and three kids were born.

Introduction

Numerous applications of methods for the long-term storage of embryos can be foreseen, particularly in the transport of genetic material between countries and in the preservation of genetic material for future use and study. The successful low-temperature preservation of spermatozoa of a number of species has been well documented, but attempts to store embryos in a similar manner have met with variable, and frequently little success. Reliable methods for the frozen storage of mouse embryos have been reported from several laboratories (Whittingham *et al.* 1972; Wilmut 1972) and on transfer to recipient females the proportion of frozen-thawed embryos developing to normal young has been similar to that for unfrozen embryos (Whittingham 1974). In other species (rabbit, sheep and cow) successes have been claimed, but when frozen-thawed embryos have been transferred to recipient females a very low proportion, generally less than 10%, developed into viable young (Wilmut and Rowson 1973; Bank and Maurer 1974; Whittingham and Adams 1974; Willadsen *et al.* 1974).

In an extensive study on the development of embryos of several species of farm animals, carried out in this laboratory, attempts were made to develop effective methods for *in vitro* culture and for low-temperature storage of goat embryos.

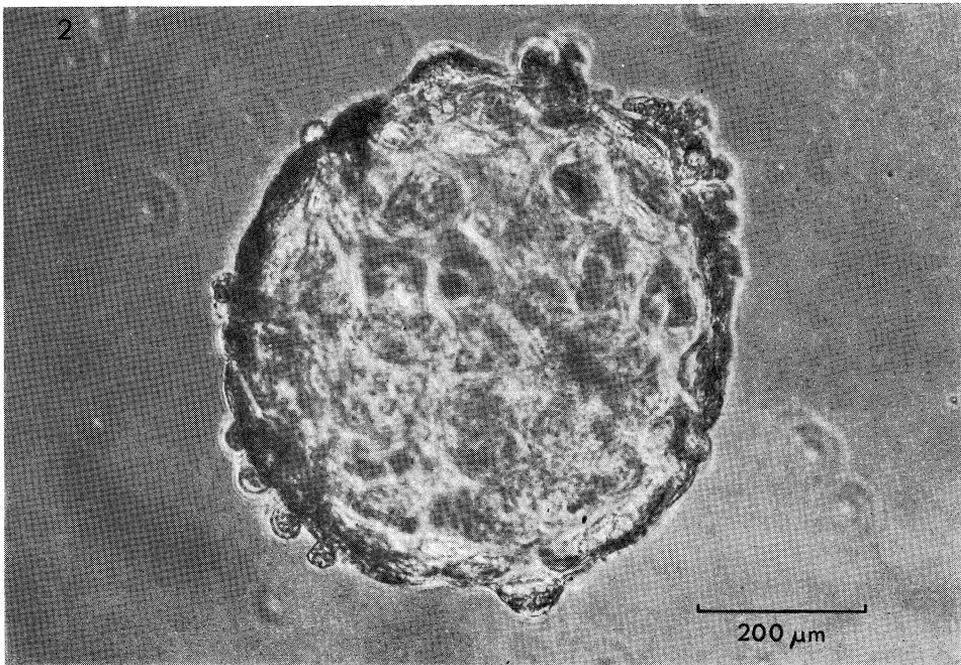
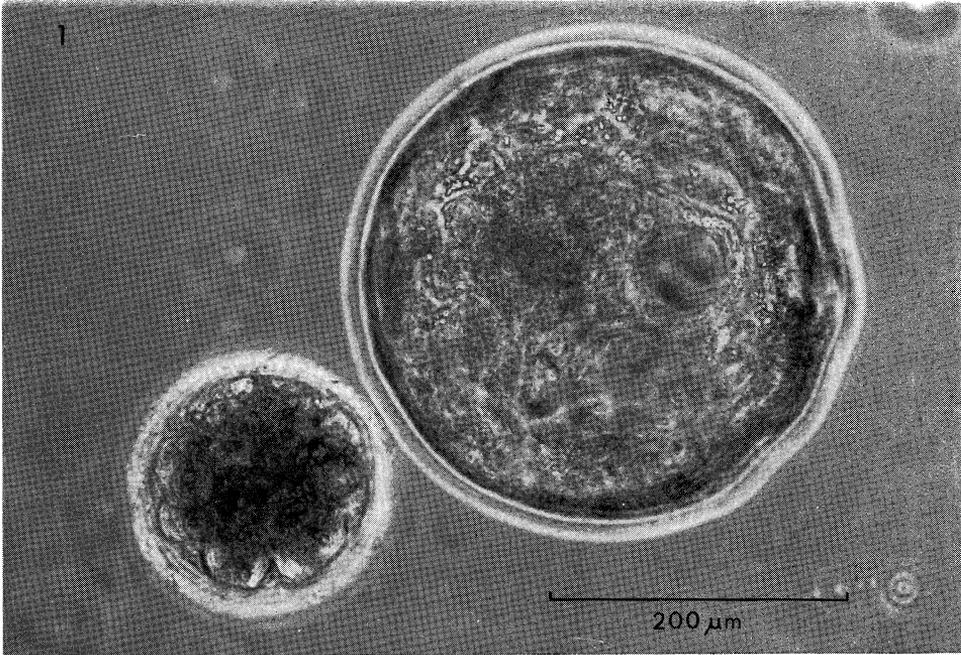


Fig. 1. Two day-5 goat embryos after storage at 5°C for 2 days and culture *in vitro* for 2 days. Left, no development during culture, remained as a morula. Right, development from morula to expanded blastocyst during culture.

Fig. 2. Day-7 embryo that developed to an expanded and hatched blastocyst during 1 day's culture after storage at -196°C for 4 weeks.

Materials and Methods

Embryos collected from donor does 5–7 days after mating were cultured *in vitro* at 37°C for 2 days, or stored at 5°C for 1 or 2 days and then cultured for 2 days, or stored in liquid nitrogen (–196°C) for up to 4 weeks and then cultured for 1 day. Following culture the embryos were examined both as fresh and orcein-stained preparations or were transferred to recipient females.

Donor and recipient does were feral animals of mixed breeding which had been run under field conditions for 2–3 years prior to the study. Bucks were of the Angora breed. Embryos collected 5 days after mating were morulae of some 20 or more cells, whilst those collected at 7 days were expanded or hatched blastocysts. Embryos were stored and cultured in Pyrex glass tubes (7.5 by 1.0 mm) containing 1–2 ml Dulbecco phosphate buffer (NaCl 8.0, KCl 0.2, Na₂HPO₄ 1.15, KH₂PO₄ 0.2, CaCl₂ 0.1, MgCl₂ 0.1 g/l) enriched with 25% heterologous goat serum (DB+25% S). During culture the tubes were held at 37°C under air.

In an initial experiment 15 embryos collected 5 days after mating were cultured for 2 days. Subsequently, 38 day-5 embryos were cooled to 5°C at a rate of 1.0°C/min, retained at 5°C for 1 or 2 days, warmed to 37°C (0.6°C/min) and then cultured for 2 days.

In a further experiment, 48 day-7 embryos were cooled, stored in liquid nitrogen for 2–4 weeks, warmed and then cultured for 1 day. Two to five embryos were placed in tubes containing 0.3 ml DB+25% S and a further 0.3 ml DB+25% S containing 2M glycerol or 4M dimethylsulphoxide (DMSO) was added over a period of 15 min to give final concentrations of 1M glycerol or 2M DMSO. Glycerol was added at 37°C and the embryos were immediately cooled to 6°C (1.0°C/min) and held at 6°C for 40 min. DMSO was added at 6°C after cooling from 37°C at a rate of 1.0°C/min. After the addition of DMSO the tubes were held at 6°C for 20 min. After holding at 6°C the tubes were transferred to a dry ice–ethanol bath and cooled to –60°C at rates of 0.2 or 1.4°C/min. All tubes were seeded with ice crystals at –2.5°C. Once the tubes had reached –60°C they were then transferred to liquid nitrogen.

To thaw the embryos after storage the tubes were warmed to 0°C at rates of between 1.4 and 8.0°C/min (measured over the range of –50 to 0°C). They were then taken to 37°C at a rate of 0.6°C/min and sufficient DB+25% S was slowly added over a period of 15 min to give final concentrations of glycerol and DMSO one-quarter of those in the frozen media. The embryos were then washed three times in fresh DB+25% S and cultured for 1 day.

A number of embryos were transferred after culture to recipient does. Where embryos were cultured without prior storage, transfers were made to recipients which had been first detected in oestrus by vasectomized bucks, within 24 h of their respective donors. Those that had been stored and then cultured were transferred to recipients whose 'post-oestrus age' was within 24 h of the 'assumed age' of the embryos. It was assumed that embryos did not develop during storage and that development in culture proceeded at about the same rate as *in vivo*.

Results

Culture

Of the 15 embryos cultured without prior storage, 13 developed during 2 days' culture to be expanded or expanded and hatched blastocysts. Ten of the 13 were transferred to nine recipients of which five subsequently produced kids, each producing one kid.

Storage at 5°C

Eight of 16 and 12 of 22 embryos stored for 1 and 2 days, respectively, showed apparently normal development to expanded or expanded and hatched blastocysts during 2 days culture (Fig. 1). The remaining 18 showed no, or only limited, development.

Seventeen embryos were transferred to 11 recipients of which six kidded. Five of the six which produced kids received one embryo that had shown apparently normal development during culture; the remaining doe received one embryo that

had, and one that had not developed during culture and she produced one kid. No recipient which received embryos that had failed to show normal development produced a kid.

Storage at -196°C

Six of the 48 frozen-thawed embryos showed further expansion during culture (Fig. 2). All six had been cooled and warmed at the slow rates. Five of the six had been frozen in media containing 1M glycerol (Table 1). They were transferred to six recipients of which three subsequently produced kids. The remaining 42 embryos failed to show any appreciable development during culture and the cells of many of those that did not develop in culture were darkened and dispersed.

Table 1. Development of frozen stored (-196°C) goat embryos following *in vitro* culture and transfer to recipient does

Cryoprotectant	Cooling rate 0 to -60°C ($^{\circ}\text{C}/\text{min}$)	Warming rate -50 to 0°C ($^{\circ}\text{C}/\text{min}$)	Number of embryos			Kids born
			Stored	Developed in culture	Trans- ferred ^A	
2M DMSO	0.2	1.4	5	0	0	—
	0.2	2.2	7	1	1	0
	1.4	4.6	5	0	0	—
	1.4	8.0	6	0	0	—
Total			23	1	1	0
1M Glycerol	0.2	1.4	5	2	2	2
	0.2	2.2	8	3	3	1
	1.4	4.6	6	0	0	—
	1.4	8.0	6	0	0	—
Total			25	5	5	3

^A Embryos that developed in culture transferred singly to six recipients.

Discussion

It is apparent that goat embryos, like those of the sheep (Moore and Bilton 1973) and cow (R. J. Bilton and N. W. Moore, unpublished data), can be successfully stored for several days at temperatures around 5°C . Equally apparent is the value of *in vitro* culture for the rapid assessment of the viability of embryos following storage. Following storage at 5°C , culture and transfer, five kids developed from six embryos that showed apparently normal development in culture, while no kids resulted from nine embryos that did not show continued development in culture. The remaining kid was born to a recipient which received one embryo that had, and one that had not, shown continued development.

Limited success was achieved with frozen storage, but again a reasonable proportion (three of six) of frozen-thawed embryos that showed continued development in culture developed into kids in recipients. The results of the frozen storage study suggest that glycerol, under our experimental conditions, might be a more effective cryoprotectant than is DMSO. This is in contrast to the mouse in which the proportion of embryos which survived freezing in media containing 1.0–1.25M DMSO was about twice that of those frozen in similar media containing 1M glycerol (Whittingham

et al. 1972). However, the procedures used in the present study may not have favoured the use of DMSO. DMSO was added to the media at 6°C. With sheep embryos the addition of DMSO at temperatures greater than 6°C has been found to be less deleterious to embryo survival (R. J. Bilton and N. W. Moore, unpublished data).

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References

- Bank, H., and Maurer, R. R. (1974). Survival of frozen rabbit embryos. *Exp. Cell Res.* **89**, 188–96.
- Moore, N. W., and Bilton, R. J. (1973). The storage of fertilized sheep ova at 5°C. *Aust. J. Biol. Sci.* **26**, 1421–7.
- Whittingham, D. G. (1974). The viability of frozen-thawed mouse blastocysts. *J. Reprod. Fertil.* **37**, 159–62.
- Whittingham, D. G., and Adams, C. E. (1974). Low temperature preservation of rabbit embryos. *Cryobiology* **11**, 560–1.
- Whittingham, D. G., Leibo, S. P., and Mazur, P. (1972). Survival of mouse embryos frozen to –196°C and –269°C. *Science* **178**, 411–14.
- Willadsen, S. M., Polge, C., Rowson, L. E. A., and Moor, R. M. (1974). Preservation of sheep embryos in liquid nitrogen. *Cryobiology* **11**, 560.
- Wilmot, I. (1972). The effect of cooling rate, warming rate, cryoprotective agent and state of development on survival of mouse embryos during freezing and thawing. *Life Sci.* **11**, 1071–9.
- Wilmot, I., and Rowson, L. E. A. (1973). Experiments on the low-temperature preservation of cow embryos. *Vet. Rec.* **92**, 686–90.

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