Effect of glucose level in culture medium on survival of in vitro cultured sheep embryos following transfer to recipient ewes

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ABSTRACT

The total glucose concentration of a medium changes with the extent of serum supplementation, and this may have an effect on embryonic development in vitro. In this study, 1- and 2-cell sheep embryos (N=109) were cultured for approximately 120 hours at 39° C under humidified 5% CO_2, 5%O_2 and 90% N_2 in Synthetic Oviduct Fluid medium (SOF) supplemented with 20% human serum and a total glucose concentration of either 1.5 mM (modified-SOF) or 2.3 mM (unmodified-SOF). A slight effect was observed on the proportion of embryos developing to at least the expanded blastocyst stage (63% and 48% for modified-SOF and unmodified-SOF, respectively, P<0.1). Twenty-eight blastocysts from each treatment were selected and transferred to 28 synchronised, mature ewes (2 blastocysts per ewe). Pregnancy and number of foetuses were determined by ultrasonography 54 days after transfer. All ewes were pregnant and survival of embryos was 82% and 71% for modified-SOF and SOF, respectively. However, a high post-natal mortality rate was observed (37%), which was mostly attributed to a relatively high birthweight of dead lambs (6.4 ± 0.5 kg). Although only a slight advantage was demonstrated by adjusting the glucose concentration from 2.3 mM to 1.5 mM, the results from this study demonstrate the potential for high survival following transfer of sheep embryos cultured for 5 days.

Keywords Glucose, culture, sheep, embryos, transfer.

INTRODUCTION

Survival of sheep embryos following in vitro culture for 5 days is reportedly lower than after culture for 3 days (Gandolfi & Moor, 1987; Walker *et al.*, 1988). This applies to embryos which have been cultured in either a somatic cell-free system (Walker *et al.*, 1988) or co-cultured with oviduct epithelial cells (Gandolfi & Moor 1987). Recently we reported (Thompson *et al.*, 1991) a high incidence of development from the 1- and 2-cell stage to the expanded and hatched blastocyst stage when sheep embryos were cultured for 6 days in Synthetic Oviduct Fluid medium (SOF) (Tervit *et al.*, 1972) supplemented with 20% human serum at 39° C under humidified 5% CO_2, 5%O_2 and 90% N_2. This agrees with the previous results of Walker *et al.*, (1988, 1989). Furthermore, we have reported that glucose concentrations over 3.0 mM in serum-free medium were detrimental to embryonic development and suggested that concentrations around 1.0 to 1.5 mM are likely to be optimal for sheep embryo development (Thompson *et al.*, 1992). Given that the normal concentration of glucose in SOF medium is 1.5 mM, this probably needs no alteration under serum-free conditions. However, supplementation of SOF medium with 20% human serum is likely to increase the glucose concentration considerably, as normal serum glucose concentration is approximately 5 mM (Altman 1961). This study was conducted to assess the effect of adjusting the glucose concentration in serum supplemented SOF medium on embryonic development and survival following transfer.

MATERIALS AND METHODS

Collection of human serum and determination of glucose concentration

Human serum was prepared from 2 female donors (aged 21 and 34), pooled and heat-inactivated at 56° C for 30 minutes. Glucose concentration was determined with an enzyme-NADH fluorescence kit (Boehringer Mannheim, Germany), and found to be 4.2 mM. Thus, a 20% supplementation of medium with this serum raised the glucose level by approximately 0.8 mM.

Embryo collection and culture

In April 1991, 20 mature mixed-age Coopworth ewes were superovulated with 800 i.u. pregnant mare serum gonadotrophin (PMSG, "Consep", Heriot Agencies, Australia) and 0.48 units of ovine follicle stimulating hormone (FSH, "Ovagen", ICP, New Zealand) following synchronisation with sequential CIDR™ devices (Type G, Carter Holt Harvey, New Zealand), as described elsewhere (Thompson *et al.*, 1990). They were pen-mated at oestrus (day 0) with Suffolk and Dorset rams introduced on a rotational basis. One- and 2-cell embryos were collected on day 1.5 following oestrus by a retrograde flush of the oviduct following surgical exteriorisation. Embryos were collected in warm HEPES-buffered SOF supplemented with 3mg BSA/ml (Fraction V, Sigma, USA) (Thompson *et al.*, 1990)

One-cell (N = 99) and 2-cell embryos (N = 10) were randomly allocated to one of two treatments: a) SOF medium supplemented with 20% human serum (approximate total glucose concentration 2.3 mM), and; b) a modified-SOF medium with lowered glucose concentration, so that when supplemented with 20% human serum, total glucose concentration was 1.5 mM. All incubations were conducted in 0.5 ml volumes in 24-well plates (Nunc, Denmark) (3 - 5 embryos per well). Embryos were incubated undisturbed for approximately 120 h under humidified 5% CO_2, 5% O_2, 90% N_2, at 39° C as previously described (Thompson *et al.*, 1992). Our incubation system was designed to minimise disturbances in temperature, humidity and gas composition during the incubation period.

Embryos were visually assessed under a dissecting microscope at the cessation of culture. Blastocysts were identified and

pooled within treatment groups. Twenty-eight of the better quality blastocysts from each treatment were held for transfer to recipients. The remaining blastocysts were of good quality and considered suitable for transfer, but were used for other experimentation. Earlier cleavage stages were stained with Hoescht 33342 (Calbiochem, USA) to confirm that fertilisation and cleavage had occurred.

Embryo transfer and scanning

Thirty mature Coopworth ewes were synchronised with a CIDR™ device and given an intramuscular injection of 200 iu PMSG at device withdrawal. Onset of oestrus was synchronised to that of the donor ewes. Surgical embryo transfer was conducted approximately 6-7 days following onset of oestrus. Two ewes had not ovulated and were excluded. Two blastocysts from the same treatment group were loaded into a catheter ("Tom-cat", Monoject, USA) and transferred to the tip of the uterine horn ipsilateral to an ovary with at least one ovulation. Following transfer, each ewe received another CIDR™ device following transfer for 14 days.

All recipient ewes were scanned by ultrasound 54 days following transfer to confirm pregnancy status. All pregnant ewes were then allowed to lamb. Lambing data included date of birth, sex, phenotype (ie. Suffolk-cross or Dorset-cross) and birthweight.

TABLE 1 Embryo development (%) after 120 hours incubation in SOF medium with either 1.5 mM or 2.3 mM glucose.

		% Development				
Glucose (mM)	N	<16-cell	Morula	BL[a]	Expanded BL	Hatched BL
1.5	52	4	6	27	44	19
2.3	57	10	9	33	39	9

[[a] = Blastocyst.]

RESULTS

Embryo culture

The proportions of embryos developing to 16-cells or less, morulae, blastocysts (BL), expanded blastocysts and hatched blastocysts are presented in Table 1. Development to the expanded blastocyst stage and beyond was not significantly affected by glucose level, but tended ($P<0.1$) to be higher in the modified-SOF medium (33/52 or 63% developing in modified-SOF and 27/57 or 48% developing in SOF medium, respectively).

Embryo transfer

All 28 ewes were pregnant at the time of scanning. There was no difference in survival of embryos to term from culture in either SOF medium or modified-SOF medium [71% (20/28) vs 82% (23/28), respectively]. A high post-natal lambing mortality was observed (37%), which was associated with a high birthweight in the lambs which died (6.4 ± 0.5 kg vs. 4.6 ± 0.2 kg, $P<0.001$).

DISCUSSION

Our results demonstrate that a very high proportion of 1- and 2-cell embryos cultured for 5 days developed to foetuses following embryo transfer. The result is comparable to embryo survival (approximately 60%) in sheep multiple ovulation-embryo transfer programmes (Tervit 1989). In comparison, other studies have reported significant decreases in survival of sheep embryos following embryo culture, especially if they are cultured for more than 3 days (Gandolfi & Moor 1987; Walker *et al.*, 1988). Gandolfi & Moor (1987) reported that embryo survival of transferred embryos was 80% (development to day 13 conceptus) and 30% (development to term) following co-culture with oviduct cells for 3 days and 6 days, respectively. Walker *et al.*, (1988) have also reported that the proportion of Day 13 conceptuses recovered was reduced from 94% to 54% for embryos cultured in SOF medium with 20% human serum for 3 and 6 days, respectively. We have no real explanation as to why our embryo survival was so high, although we believe that our philosophy of leaving embryos undisturbed during culture may play an important role (Thompson *et al.*, 1991).

Reducing the glucose concentration from 2.3 mM to 1.5 mM had a slight positive, but not significant effect on embryonic development and no effect on subsequent survival. Our previous work has demonstrated that glucose concentrations of 3.0 mM or higher are detrimental to development in vitro in a serum-free culture system (Thompson *et al.*, 1992). Although the present study found little effect of glucose on embryo developmental capacity, we still advise caution when supplementing medium with serum as supplementation may inadvertently increase glucose concentrations to detrimental levels. We routinely determine the glucose concentration in our sera prior to use and adjust the concentration in our medium accordingly.

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