In vitro production of cattle embryos: Use in beef twinning programmes

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ABSTRACT

This study investigated the survival of *in vitro* derived embryos as single or twin pregnancies following their twin transfer to recipient cows. Abattoir-derived oocytes were matured, fertilised and then cultured for 6-7 days using our standard laboratory procedures. One hundred and forty good quality late morula and blastocysts were transferred non-surgically, in pairs, to recipient cows on day 7 of the oestrous cycle (day 0 = oestrus). Recipients were monitored by ultrasound for pregnancy up to Day 60 and embryo survival was determined. While 57 (81%) of recipients did not show oestrus by day 21, only 39 (56%) were pregnant on day 35 and 30 (77%) of the pregnancies were twins (embryo survival; 57%). Increasing stage of gestation was associated with a decrease in pregnancy rate, twin pregnancies and embryo survival (46%, 37% and 31%, respectively) on day 60. The results show that while high initial pregnancy rates can be established following the transfer of two *in vitro* derived embryos, embryo survival drops during early pregnancy. Current research efforts are investigating factors that may influence early embryo survival in cattle.

Keywords: Twinning rates; early pregnancy; IVP embryos.

INTRODUCTION

In recent years, methods have been established for the *in vitro* maturation, fertilisation and culture of abattoir-derived oocytes and pregnancies have resulted from the transfer of these *in vitro* produced (IVP) embryos (Goto *et al.*,1988; Lu *et al.*, 1990). Embryos derived by *in vitro* methods can be used for further procedures such as cloning, freezing studies and the transfer of embryos into recipients.

One such application could be for the production of twin calves in a beef production system, thereby increasing the efficiency of the system (Diskin and Hickey,1987). Previous studies, using embryos recovered from superovulated donor cows, have reported recipient pregnancy rates of around 75% with half of the pregnant recipients carrying twins (Sreenan and Diskin,1989). However early embryo mortality was noted. There have been few reports on twinning in cattle following the transfer of IVP embryos. Pregnancy rates of recipients can vary form 50% to 75% (Monson *et al.*, 1992; Reichenbach *et al.*, 1992) with twin pregnancy rates ranging from 35% to 60%. However, early foetal loss in recipients carrying IVP embryos is higher than for in vivo derived embryos (Reichenbach *et al.*,1992).

In a previous study (Pugh et al.,1993), we reported high rates of development to late morula and blastocyst stages of *in vitro* matured and fertilised bovine oocytes following culture in a modified serum- and cell- free medium (SOF: Tervit *et al.*, 1972) and confirmed their viability after transfer to recipients. This study investigated the ability of these IVP embryos to establish pregnancy to day 60 following their twin transfer to recipient cows. Embryo survival as single or twin pregnancies was determined and early foetal loss estimated.

MATERIALS AND METHODS

The techniques for the *in vitro* production of embryos have been reviewed previously (Tervit *et. al.*, 1990). Oocytes, recovered from the ovaries of slaughtered cattle, were matured for 24 hours at 39C in medium supplemented with foetal calf serum and hormones (oestradiol, FSH, LH). The matured oocytes were then co-incubated with percoll-separated, heparin-treated sperm (2 x 10^6/ml) for a further 24 hours. All oocytes were then cultured in 50ul drops of SOF containing amino acids and 8mg/ml Fatty acid free-BSA for 6 -7 days under 5% CO_2, 7% O_2 and 88% N_2 at 39C. The embryos were transferred to fresh drops every 48 hours throughout the culture period. Embryos that had developed to late morula and blastocyst stages were selected for transfer to synchronised recipients. Oestrus synchronisation of recipient cows was as previously reported (McMillan and Macmillan, 1989). Sevens days after oestrus, two embryos were transferred non surgically into the uterine horn ipsilateral to a palpable ovulation. Pregnancy and embryo survival were estimated by non-return rates and ultrasonography.

RESULTS

Five hundred oocytes were recovered from the ovaries of 60 cows (8 oocytes/pair of ovaries) and were matured and fertilised *in vitro*. Three hundred and seventy five (75%) cleaved at least once and one hundred and forty (28%) continued development to good quality late morulae and blastocysts (Lindner and Wright, 1983) during culture. These embryos were subsequently transferred to seventy synchronised recipients. There was a high non-return rate (57/70, 81%) on day 21. Subsequent pregnancy rates, measured ultrasonographically, are presented in Table 1. Day 25, ultrasonagraphy results may have been underestimated because of detection inaccuracies (McMillan, these proceedings), however subsequent examinations between days 35 and 60 showed a decline in pregnancy rate, embryo survival and percentage of pregnant animals carrying twins with increasing stage of gestation.

TABLE 1: Ultrasound determination in recipients of embryo survival following the transfer of two *in vitro* produced embryos.

Ultrasound Day	% Embryo Survival	Number(%) Recipients Pregnant	Number(%) Pregnant Recipients with Twins
25	35	32(46)	18(56)
35	57	39(56)	30(77)
45	41	40(56)	18(45)
60	31	32(46)	12(37)

DISCUSSION

Bovine late morulae and blastocysts, derived by the *in vitro* production system described, are capable of establishing early pregnancy following twin fresh transfer to recipients. Most recipients did not return to oestrous. However on day 25 less than half of the recipients were ultrasonographically determined as pregnant. Subsequent ultrasonographic determination on day 35 however, suggests that this result is low and that 60% or more of animals probably were pregnant, and hence a similar proportion of embryos were surviving on day 25. Errors in ultrasonography detection of pregnancy have been reported (McMillan, these proceedings) and are due to difficulty in visualising the small conceptus. Extended cycle length may occur for a number of reasons including endocrine or embryonic factors (King,1985) In the present study, 18 recipients that failed to return to oestrous on day 21 were non-pregnant on day 35, representing 25% of embryos transferred.. Reichenbach *et al.*(1992) described a similar level of an embryonic loss of 14% between days 21 and 35. In addition, embryo survival continued to drop up to day 90 in that study. Izaike *et al.*(1991) also reported early embryonic loss following the transfer of two or three in vivo derived embryos to recipients, indicating that there may be maternal factors influencing the establishment of twin pregnancy in cattle (Echternkamp and Gregory, 1987).

The survival of embryos as twin pregnancies was low in this study with less than half of the pregnant recipients carrying twins by day 45. Higher pregnancy distributions have been observed for cattle in other twinning programmes (reviewed by McMillan, 1994). Overall, in the studies, two thirds of the cows were pregnant with half of those carrying twins. Differences between the results in this study and those cited by McMillan (1994) may be attributed to embryo source or recipient type and management. This is supported by the observation that the survival of twin IVP embryos following fresh transfer to recipients was not significantly different to previous years where a single *in vivo* derived frozen embryo was transferred to an inseminated recipient (McMillan *et al.*, 1993).

This study shows that IVP embryos can be used as source of embryos for transfer to recipients in a beef twinning programme. Initial embryo survival is satisfactory but, following high embryo loss, twin pregnancy rates are low and more research is required to identify factors which may influence embryo survival.

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