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Survival of *in vitro*-produced cattle embryos from embryo transfer to weaning

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ABSTRACT

New reproductive technologies present both the beef and dairy industries of New Zealand with numerous opportunities. A key factor in the successful use of these technologies is the reproductive rate obtained from their use.

MOET (multiple ovulation and embryo transfer) is a fairly well established procedure to achieve pregnancy by embryo transfer. As an alternative, harvesting of oocytes, *in vitro* fertilization, culture, and subsequent embryo transfer (IVP-ET), also has potential. The technology is still relatively new and pregnancy rates of 30% have been reported. This is at least 10% lower than that achieved with MOET. As part of another project, approximately 400 recipient cows, in each of three years, were subject to ET using fresh IVP embryos. Pregnancy rates to calving, per embryo transferred, averaged 40, 44 and 33% in 1999, 2000 and 2001 respectively. Within years, average pregnancy rate varied from less than 20% to over 50% depending on recipient source ($P<0.05$), breed ($P<0.01$) and condition score ($P<0.05$). Once cows could be detected pregnant, at about 50 days after embryo transfer, they tended to stay pregnant through to calving. Neither embryo breed nor the type of oestrus-synchronizing device had a significant effect on pregnancy rate.

Keywords: cattle; embryo transfer; *in vitro* embryo; embryo survival; pregnancy; calving

INTRODUCTION

Reproductive technologies present both the beef and dairy industries of New Zealand with numerous opportunities. The technologies available include the following: artificial insemination, sperm sexing, control of the oestrous cycle, multiple ovulation and embryo transfer (MOET), *in vitro* production of embryos (IVP), cloning, and cloning to produce genetically modified animals (Thompson *et al.*, 1998).

Embryo technologies have the potential to substantially increase the rate of genetic progress in breeding schemes, since they allow higher selection intensity to be applied to female parents, especially in species like cattle, where the female reproductive rate is limiting. Other potential applications lie in the production of niche market animals or the multiplication of specific genetic lines, or individuals with novel genes, that require rapid multiplication from a very limited genetic source (Smeaton *et al.*, 2003). Key features for the successful use of embryo-based technologies are **firstly**, the cost of the embryo at the point where it is ready for transfer into the recipient female, and **secondly**, the pregnancy success rate from that transferred embryo.

Embryo production and subsequent transfer into recipient animals involves one of two pathways. The first pathway, MOET (multiple ovulation and embryo transfer), involves the collection of day-7 embryos from super-ovulated donor females, followed by their transfer into recipient animals (McDonald *et al.*, 1998). The second process involves IVP (*in vitro* production) of embryos (Thompson *et al.*, 1998) and the transfer of these into recipients. MOET was the earlier-developed of the two technologies and there is no doubt that it has, and will continue to have, a significant role in embryo transfer (ET) programmes (Smeaton *et al.*, 2003). However, its effective use in the bovine is limited by several problems and there has been no real progress in resolving these (Gordon, 1996; Galli, 2003).

ET technologies based on IVP embryos have been developed in the last 10-15 years (Vivanco, 2000; Gordon, 1996). The process involves transvaginal recovery (TVR) otherwise known as ovum pickup (OPU) from live donor females, or recovery of oocytes from the ovaries of animals after death or slaughter. The recovered oocytes are then matured *in vitro* in the laboratory (IVM) and subsequently fertilised (IVF). The fertilised embryos are cultured *in vitro* (IVC) for seven days, at which time embryos of suitable grade are selected and transferred into the recipient animal (Thompson, 1998; Vivanco, 2000; Gordon, 1996).

The efficiencies of the IVP process have been reviewed by Galli (2003) and Merton *et al.* (2003), and it is clear that the number of embryos produced per session from MOET is generally higher than from IVP, although the number of embryos produced per week tends to favour IVP (Merton *et al.*, 2003). The average calving rate after transfer of fresh IVP embryos has been reported to be about 30% with little reported variation (Peterson & Lee, 2003). Fresh IVP embryos generally have a pregnancy rate about 10-15% units higher than frozen IVP embryos but also perform poorer than MOET embryos (Hasler *et al.*, 1995). Higher embryonic, foetal and neo-natal mortality rates have also been observed after transfer of IVP embryos than for either AI or MOET embryos (Hasler, 1992).

It is clear that pregnancy and calving rates from IVP embryos need to be improved substantially before they can be widely used in seasonal, pasture-fed beef or dairy herds in New Zealand, where a high conception rate is required to maintain a calving interval of 365 days.

In this paper we report evidence of progress in embryo survival, compared to the above IVP results, and provide information on the level of variability that can be expected.

MATERIALS AND METHODS

The IVP embryos described in this project were

produced as part of another project investigating systems for the use of embryo-based technologies in New Zealand, such as described by Smeaton & Vivanco (2002). In each of three years (1999, 2000, 2001), ovaries were collected from Friesian cows (note exception below) at local abattoirs and transported to the laboratory in saline (approximately 30°C). Cumulus–oocyte complexes (COCs) were recovered by aspiration of follicles of 3-8 mm in diameter using an 18g needle under vacuum. COCs with evenly granulated cytoplasm and at least three layers of compact cumulus were selected for maturation. Selected COCs were washed in Hepes buffer TCM199 medium supplemented with 10% fetal bovine serum (FCS). They were then placed into a maturation medium of 50 µl drops (ten COCs per drop) of medium under oil and incubated for 24 hours at 39°C under humidified 5% CO_2 in air. TCM 199 was the base medium used for maturation supplemented with 10% FCS, 10 µg FSH, 1 µg LH, 1 µg oestradiol per ml and 100 µmol cysteamine.

After maturation the Friesian oocytes were inseminated with frozen-thawed spermatozoa from the desired sire (Simmental in 1999, Hereford in 2000, both breeds in 2001. In 2001, Wagyu oocytes were also provided, which were inseminated with Wagyu semen). The contents of a 0.25ml straw were layered upon a Percoll gradient (45%:90%) and motile spermatozoa were collected, washed in Hepes–buffered synthetic oviduct fluid medium (SOF) (Tervit *et al.*, 1972) and re-suspended to give a final concentration at insemination of 1 x 10^6 spermatozoa per ml. Insemination was performed in 50µl drops of modified SOF medium supplemented with 0.01 mmol heparin, 0.2 mmol penicillamine and 0.1 mmol hypotaurine under oil (approximately 5 oocytes per drop) over a 24-hour period under the same conditions as described for oocyte maturation.

After insemination presumptive zygotes were washed in Hepes-buffered SOF and most of the surrounding cumulus cells were removed by gentle pipetting. Zygotes were placed in 20µl drops of modified SOF medium (Thompson *et al.*, 2000) under oil and incubated under humidified 5% CO_2, 7% O_2, 88% N_2 at 39°C. Embryos were transferred to fresh media on day five of development. On day 7 of culture, grade 1 and 2 embryos (Stringfellow & Seidel, 1998) were selected for ET.

In each year, oestrus was synchronised in approximately 450 mixed-aged cows of either Hereford x Friesian (HxF), Friesian (F), Friesian x Jersey (FxJ) or Jersey (J) breeds. In the first year (1999), half the recipient cows were synchronised using Cue-Mate™, progesterone impregnated, devices. The remaining half were synchronised with CIDR™ devices. In subsequent years only Cue-Mate™ devices were used.

At day 7 after oestrus, all suitable cows (90 to 95% of those put up for synchronisation) were subject to non-surgical transfer of the above fresh embryos. Cows were rejected for ET only for failure to form a corpus luteum, disease or an unusually small pelvic area as determined by rectal palpation. One embryo was placed in each cow.

All cows were pregnancy tested at day 50-60 after synchronised oestrus in all years, and were re-tested again at approximately five months pregnant in the first two years but not the third year. At calving, cow and calf survival, calving difficulty, calf birth weight and cow and calf weights were recorded regularly. Condition score (CS) was also recorded in years 2 and 3 at mating, on a scale of 1 = emaciated to 10 = grossly fat (Scott & Smeaton, 1980). Only the pregnancy rate, calf survival and CS data are reported in this paper.

All the cows were aged three years or older at ET, except in 1999 when 22 two-year-old HxF heifers were included. The HxF cows were sourced from AgResearch herds at Whatawhata Research Centre, Ballantrae Research Centre and Flockhouse Research Centre. The FxJ, J and F cows were sourced from Waikato dairy farms, although those cows that calved successfully to ET were retained at Whatawhata Research Centre for further re-breeding in subsequent years. The cows from the dairy farms tended to be those animals rejected for use on that farm in the following season. A condition of purchase was that the animals be otherwise in good health. These cows were ET mated on the dairy farms and transferred to Whatawhata Research Centre in the autumn if they were determined to be pregnant to ET at that time.

The data were examined by analysis of variance (Genstat, 2000). All data presented are fitted estimates. First-order interactions were tested for all factors in all years but were never significant and so were omitted from the final models.

RESULTS

Results for each of the three years are shown in Figures 1, 2 and 3. They show that most embryo losses occurred between ET and pregnancy diagnosis at 50-60 days after synchronised oestrus. In the 1999 ET year, the 22 Whatawhata HxF two-year-old heifers performed very poorly with only 14% pregnant to ET by day 60 after synchronized oestrus. Their data are excluded from Figure 1. Both Figures 1 and 2 showed significant recipient breed effects ($P<0.05$) in favour of HxF versus J recipients. However, in the 2001 ET mating year (Figure 3), the J cows performed just as well as the HxF but the F and FxJ recipients did not ($P<0.05$).

Figure 3 indicates that farm source, or source within breed, can give very variable results ($P<0.05$), implying that J and HxF can both perform either well, or poorly,

FIGURE 1: Embryo survival (% of cows subject to ET in 1999) to weaning, by recipient breed (SEDs 6).

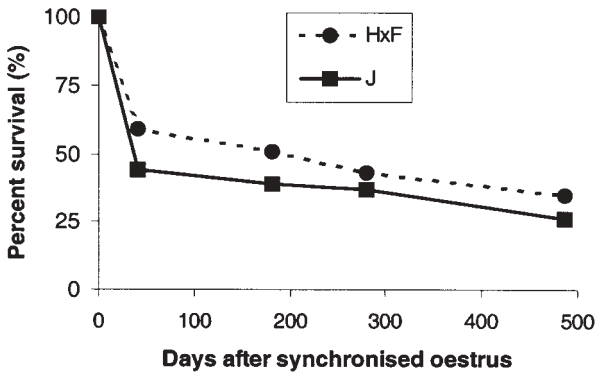


FIGURE 2: Embryo survival (% of cows subject to ET in 2000) to weaning, by recipient breed (SEDs 6).

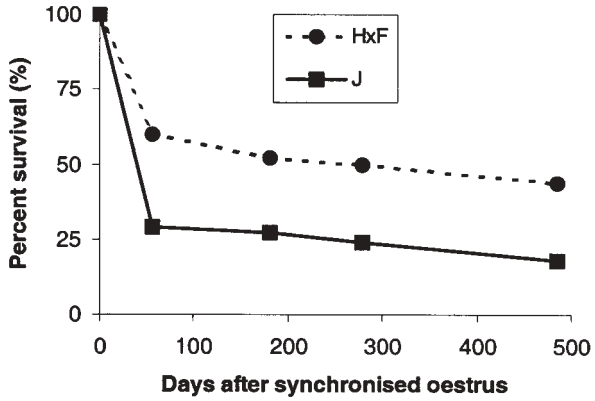
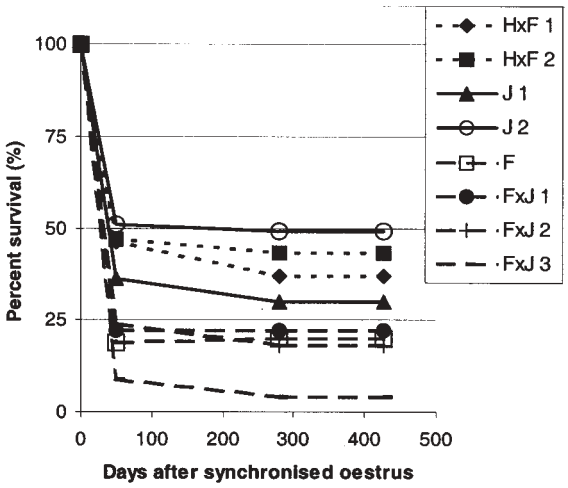


FIGURE 3: Embryo survival (% of cows subject to ET in 2001) to weaning, by recipient source, within breed (SEDs 12 to 14).



depending on their background source. This feature was observed in all three years with the J recipients appearing to have more variable pregnancy rates than the HxF cows, excluding the very poor two-year-old HxF heifer result described above.

In the third and only year that it was tested (Table 1), there were no significant effects of embryo breed on pregnancy or calving rates, but survival of the Wagyu calves was poor after calving (P<0.05).

Recipient CS in the second and third ET years appeared to be positively correlated with ET pregnancy rate (P<0.01). However, it was confounded with breed and was not significant (in the first two years) when the breed effects were removed. In the 2001 ET year, however, CS was positively related to calving rate within the J breed (P<0.05).

Oestrous synchronising device in the 1999 ET year, ET date within year and history of pregnancy to ET had no significant effects on calf output at any of the points tested (SEDs approximately five to six percentage units).

TABLE 1: Effect of embryo breed on embryo survival, to 50 days after oestrous, calving and weaning (for cows subject to embryo transfer in 2001): SEDs are approximately 7.

Embryo Breed	No. of cows ET mated	Embryo survival to: Day 50 after oestrous (%)	Calving (%)	Weaning (%)
HxF	151	35	32	29
SxF	104	39	38	36
Wagyu	123	37	34	23
	Significance	NS	NS	P<0.05

Note: SxF = Simmental x Friesian cross

DISCUSSION

Our results have demonstrated that average pregnancy rates of 40% to calving can be achieved for IVP embryos transferred fresh, although this average was not achieved in the third year. Some large groups of animals (each more than 150 cows) achieved calving rates of nearly 60%. The average figure was approximately 10 percentage units higher than rates typically reported from other laboratories around the world (Peterson & Lee, 2003). This improvement was likely due to enhancements to the media in which the embryos were cultured (Thompson *et al.*, 2000). The improved result compares with calving rates to a single mating of 55-60% reported to be the normal range for AI (Z. Z. Xu, pers. comm.). It is difficult to obtain truly comparable benchmark information to fairly compare ET, using IVP embryos, with AI. The best method would be to AI a random selection of the cows synchronised for ET. To our knowledge, this has not been done in the past, but clearly should be a priority design feature of any future work.

Despite the improved embryo survival outcome, we found that survival of the IVP embryo can be highly variable so that we still cannot predict the likely outcome of IVP-ET sessions with confidence. For example, recipient breed effects were sometimes, but not consistently, significant. In addition, and based on reproduction research in dairy cows (Holmes *et al.*, 1987; McGowan, 1981), we expected that CS might be a useful indicator of the likelihood of success, but we were not able to confidently use it as a predictor of pregnancy rate. Elsewhere (McMillan *et al.*, 1998), cows with a history of successful ET pregnancies in previous years have been reported to have a high chance of continuing with successful pregnancies, but this was not a significant feature of our results.

The large variation in embryo survival between mobs of animals was perhaps not unexpected given the results of McMillan *et al.* (1998). Wide variation in pregnancy rate also occurs with AI. Smith *et al.* (2002) reported conception rates to AI (determined at three weeks; equivalent to a "non-return rate") ranging from 39 to 99%.

Embryo breed appeared to have little effect on embryo survival to calving, although the Wagyu calves did not fare so well as the others after calving in the third year when comparison between embryo breeds was possible. McMillan *et al.* (1998) confirmed that differences in pregnancy outcomes are associated with differences in recipient competence and not solely embryo competence. Although some embryos were presumably causing failure of pregnancy in our study (35 –40% of failures; McMillan *et al.*, 1998), this failure rate appeared to be similar across embryo breeds, at least up to calving.

In conclusion, our results have confirmed significant improvements in pregnancy rates to IVP-ET, compared to other published data. In some cases, and with large

mobs, pregnancy rates to calving of 60% were achieved. However, there appears to be wide variation between mobs of animals for reasons that are not entirely clear so that the procedure remains risky.

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