Development of an experimental herd for investigating maternal contributions to fertility in recipient cattle

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ABSTRACT

In order to develop an experimental resource to understand the biology of superior recipients, each of 155 contemporary yearling heifers received 2 *in vitro*-produced embryos on 6 occasions during a 30-month period. The number of foetuses present were determined at Day 60 of pregnancy, and pregnancies terminated. Heifers were ranked for their aggregate pregnancy performance, and the highest and lowest 25 were retained. Mean pregnancy rates of all recipients ranged from 0.20 - 0.67 depending on transfer occasion. The mean ± s.e. pregnancy rate of the High and Low groups were 0.77 ± 0.07 vs. 0.11 ± 0.06 respectively ($P<0.001$). No significant differences were observed between the two groups in oestrous cycle progesterone profiles or follicular dynamics. We conclude that repeated twin embryo transfer can be used to identify recipients of high and low potential fertility, and that uterine, rather than simple ovarian parameters, may be better correlates with high pregnancy rate.

Keyword: cattle fertility; recipients; embryo transfer; embryo survival.

INTRODUCTION

Considerable recent international research effort has focused on improving the potential for *in vitro*-produced (IVP) embryos to survive after transfer (e.g., Overstrom, 1996; Thompson, 1996). In contrast, little international effort is devoted to increasing the potential for recipients to support pregnancies to term, with the most significant recent research having mostly occurred over 10 years ago (e.g., King *et al*., 1985; Coleman *et al*., 1987; Hasler *et al*., 1987; Sreenan and Diskin, 1987; Broadbent *et al*., 1991). In spite of this research effort, limited progress has been made in defining suitable recipients, other than recognising that obvious pathology and more than about 36 - 48 hours asynchrony between recipient stage of the oestrous cycle and embryo stage of development, leads to low outcomes after embryo transfer (ET). In particular, none of the studies has investigated ovarian follicle dynamics as a potential biological correlate of superior recipient performance.

There are probably two key limitations in these studies that have contributed to the slow rate of progress. The first relates to using recipients, after only one round of ET, as subjects for investigating biological correlates of successful recipients. The consensus in the literature is that the subsequent success rate of recipients that were unsuccessful at their first ET attempt is no different to the overall success rate at the first ET attempt (e.g., Hasler *et al*., 1987; Coleman *et al*., 1987; Reichenbach *et al*., 1992). The second limitation has been the apparent lack of a suitable model for separating embryo and maternal contributions to pregnancy loss. As a consequence, it has been difficult to determine whether pregnancy failure for a group of recipients has been due to embryo and/or recipient factors.

This study attempts to overcome both of these limitations by providing recipients with 6 ET opportunities, and by applying a mathematical model that was initially developed to explain embryo fate in sheep (Restall and Griffiths, 1976), but has also been applied in the human IVP area (Walters *et al*., 1985) and to ET cattle (McMillan, 1996).

The aims of this study were to 1). describe the development of an experimental recipient cattle resource that has exhibited biologically significant divergent pregnancy rates, 2). determine if ovarian factors are associated with divergent pregnancy rate in recipient cattle.

MATERIALS AND METHODS

A herd of 155 yearling Hereford x Friesian heifers was purchased from several sources and randomly allocated within source and liveweight to two grazing mobs at the Ruakura Research Centre. At approximately 18-months of age, the heifers were used as recipients following the GENEMATE™ heifer oestrous synchrony protocol (Cliff *et al*, 1995). The two grazing mobs were programmed on adjacent days. Two IVP embryos, supplied by ArTech Ruakura, were simultaneously transferred into the same uterine horn of each recipient using standard non-surgical ET techniques at a mean of Day 7 (day of peak oestrous = Day 0). The apparent absence of ovulation as assessed during palpation, and to a lesser extent the difficulty with transfer, precluded some heifers from receiving embryos on all ET occasions. The pregnancy outcomes for each heifer (0, 1 or 2 foetuses present) were determined at about Day 60 using ultra-sonographic techniques. A luteolytic dose of prostaglandin was then administered to terminate pregnancies. Oestrous activity was then monitored over a 4 week period to ensure that most heifers exhibited two oestruses. Another round of GENEMATE™ synchrony was applied such that the subsequent ET took place at least 100 days after the previous ET. This was repeated on 6 occasions (ET 1 to ET 6). Observed embryo survival rate was calculated as the number of foetuses observed at Day

60 as a proportion of the number of embryos transferred. Observed pregnancy rate was calculated as the number of recipients with at least one foetus present at Day 60 as a proportion of the number of recipients undergoing ET.

Having completed ET 6 heifers were ranked from highest to lowest based on the number of times they had been pregnant at Day 60. The highest and lowest ranking 25 heifers were then retained as sub-herds for intensive studies to determine physiological correlates associated with superior pregnancy rates. A mathematical model (Walters *et al*., 1985) was then applied to the aggregate pregnancy outcome data (0, 1 or 2 foetuses present) for each of the sub-herds to determine whether the observed reproductive differences were due to embryo or recipient factors. The model estimates two parameters, *e* and *r*, which are measures of the probability of competent embryos and recipients present in a given ET programme (McMillan, 1996). Parameter *e* estimates potential embryo survival rate to Day 60, which is the survival rate expected of all embryos if they were transferred to only competent recipients. Parameter *r* estimates potential pregnancy rate at Day 60, which is the pregnancy rate expected if only single competent embryos are transferred to each recipient (McMillan, 1997). Potential embryo survival rate is usually higher than observed embryo survival rate, since it assumes that all transfers are into competent recipients. Similarly, potential pregnancy rate is usually higher than observed pregnancy rate, since it assumes that only competent embryos are transferred. In reality, about a third of all cattle embryos and recipients are estimated to be incompetent (McMillan, 1996).

In order to describe progesterone profiles and ovarian follicular dynamics during an oestrous cycle, daily blood samples were taken and indirect observations of the ovaries of the heifers in the two sub-herds were conducted using trans-rectal ultrasonography techniques (Burke *et al*., 1994).

RESULTS

The distribution of heifers with 0, 1 or 2 foetuses present and the observed and potential embryo survival rates on each transfer occasion are shown in Table 1. Overall embryo survival rate was 0.29, but varied considerably from 0.12 to 0.47 depending on ET occasion (P<0.001). Potential embryo survival rate also varied depending on ET occasion, ranging from 0.27 - 0.72 (Table 1) (P<0.001).

TABLE 1: Number of recipients with either 0, 1 or 2 foetuses present at Day 60, observed embryo survival rate and expected embryo survival rate during 6 ET occasions ([a] see text).

	Number of recipients with either 0,1 or 2 foetuses present at Day 60			Observed embryo survival rate	Potential embryo survival rate (*e*)[a]
	0	1	2		
ET 1	65	55	26	0.37	0.49
ET 2	64	35	46	0.44	0.72
ET 3	49	61	39	0.47	0.56
ET 4	95	35	11	0.20	0.39
ET 5	123	25	6	0.12	0.32
ET 6	106	38	7	0.17	0.27

Overall pregnancy rate was 0.43, but varied considerably from 0.20 to 0.67 (Table 2) depending on ET occasion (P<0.001). Pregnancy rate was not significantly different on the first three ET occasions (overall mean = 0.60), or last three occasions (overall mean = 0.26), but was higher during the first compared with the last three ET occasions (0.60 vs. 0.26, P<0.001). Potential pregnancy rate also varied depending on ET occasion, ranging from 0.37 - 0.83 (P<0.001).

TABLE 2: Observed and potential pregnancy rates during 6 ET occasions ([a] see text).

	Observed pregnancy rate	Potential pregnancy rate (*r*)[a]
ET 1	0.55	0.75
ET 2	0.56	0.60
ET 3	0.67	0.83
ET 4	0.32	0.52
ET 5	0.20	0.37
ET 6	0.30	0.64

Mean observed and potential pregnancy rate was different for the two extreme sub-herds (0.77 ± 0.03 vs. 0.11 ± 0.03, P<0.001, and 1.00 ± 0.08 vs. 0.15 ± 0.05, P<0.001, respectively.

No significant differences were observed between the two sub-herds in progesterone profiles. None of the following ovarian follicle dynamics variables were significantly different for the two sub-herds: number of dominant follicles per oestrous cycle, maximum size of the first two dominant follicles, day of emergence of the first two dominant follicles, mean corpus luteum size, maximum corpus luteum size, day of corpus luteum regression, interovulatory interval, and number of days the first two dominant follicles were either morphologically or functionally dominant.

DISCUSSION

There are two key findings in this study. Firstly, repeat contemporary ET has provided two groups of cattle that have consistently differed in their ability to initiate and maintain pregnancy for at least two months. Furthermore, twin ET has enabled the estimation of embryo and recipient contributions to pregnancy outcomes, through the application of a suitable mathematical model, and confirmed that differences in pregnancy outcomes are associated with differences in recipient competence and not solely embryo competence, as discussed later. Secondly, the preliminary findings suggest that the biological basis of superior recipient competence is not associated with simple ovarian parameters.

Improvement in recipient selection and successful management can produce significant savings in the cost of achieving a pregnancy (King *et al*., 1985; Broadbent *et al*., 1991). While it is recognised by both researchers and practitioners that the only truly successful recipient is a

pregnant one (e.g., Broadbent *et al*., 1991), the actual contribution of embryo incompetence to pregnancy failure has not been widely estimated. Based on the model used for estimating embryo and recipient contributions to pregnancy outcomes, and using the data from McMillan (1996) it is estimated that 35-40% of recipients exhibiting pregnancy failure by term do so due to incompetent embryos being transferred to competent recipients. An underlying premise in this study was that it is essential to eliminate this source of variation when finding an experimental model in which studies on the aetiology of superior recipient competence could be determined. While it may be argued that using Day 60 of pregnancy as an endpoint might not provide the same individuals as choosing term as an endpoint, other results indicate that recipients do not contribute to pregnancy loss beyond Day 60. Foetal factors alone account for all losses (McMillan *et al*., 1996).

Given the fact that embryos are an important source of pregnancy loss, it is not surprising that using the reproductive history of the recipient as a selection criterion produces variable results. Some researchers have reported no effects on survival of transferred embryos of the number of oestrous periods before transfer, the number of previous oestrous synchronisation treatments (Hasler *et al*., 1987), the number of abnormal oestrous cycles, prior incidence of cystic ovaries, or duration of behavioural oestrus before transfer (Coleman *et al*., 1987). Others have found that some oestrous synchronisation treatments can have adverse effects on pregnancy rates (Odde, 1989; Broadbent *et al*., 1991). Reichenbach *et al*., (1992) found a tendency for animals that failed twice to become pregnant to have lower pregnancy rates than animals that failed once or recipients that became pregnant with the first embryo transfer (39 vs. 46 vs. 55%, $P<0.1$)). However others (e.g., Hasler *et al*., 1987) have found that the use of heifers as recipients, a second or third time following failure to become pregnant after a previous transfer did not affect pregnancy rate.

The large variability apparent amongst ET occasions in observed and potential embryo survival as well as pregnancy rate was unexpected. It is difficult to determine, at least in this study, whether it was due to seasonal effects on recipient and embryo competence. However, it was clear that the difference in both observed and potential pregnancy rate between the two sub-herds remained large on all 6 ET occasions. This suggests that the difference in reproductive performance between the two sub-herds is independent of variation in mean herd performance.

Given the limitations associated with using recipients after only one or two rounds of ET for investigating biological correlates of successful recipients, and the apparent lack of a suitable model for separating embryo and maternal contributions to embryo survival, we propose that the two sub-herds that have been generated in the current study are a unique and valuable resource for future studies into the biology of recipient competence. Breeding from these sub-herds would be expected to generate differences in pregnancy rate of their daughters of the order of 0.03 to 0.04 (assumes differences of 0.66 in observed pregnancy rate and heritability of pregnancy rate of 0.10).

Our early findings that ovarian parameters did not vary amongst the two sub-herds is in support of most of the published literature where less robust animal models have been studied (King *et al*., 1985; Coleman *et al*., 1987; Hasler *et al*., 1987; Sreenan and Diskin, 1987; Broadbent *et al*., 1991). Current and future research plans will focus on uterine factors, as well as the relationship between ovarian and uterine factors, as the likely sources of difference between the two sub-herds. It is hoped that a diagnostic kit for identifying either superior and/or inferior recipients will be developed.

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