ISSN 0370-2731/99 Proceedings of the New Zealand Society of Animal Production 59: 226-228

A methodology for dealing with uncertain outcomes when applying embryo technologies

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ABSTRACT

High variability in number of embryos collected per donor has implications when programming recipients for 'fresh' embryo transfer because decisions on the number of recipients required need to be made prior to collecting embryos. This paper describes a methodology for dealing with these uncertainties. The distribution of number of transferable quality embryos per donor was determined and conformed to a Poisson distribution. The distribution of the proportion of recipients that are suitable for transfer was assumed to be binomial. The model determined recipient numbers under 4 contrasting risk scenarios, and compared these with a simple arithmetic approach. The simple arithmetic approach is likely to expose programmes to high levels of risk. The proposed methodology enables practitioners to programme the number of recipients that is consistent with *any* level of risk they are prepared to take in having surplus embryos or recipients. The model could be extended to provide economic or utility maximisation endpoints.

Keywords: modelling uncertainty; reproductive technologies; recipients; embryo technologies; oestrous synchronisation.

INTRODUCTION

Reproductive technologies have the potential to significantly improve the rates of genetic gain achieved in farmed species (e.g., Nicholas and Smith, 1983; Lohuis, 1995). Although multiple ovulation and embryo transfer (MOET) has been an important embryo technology for exploiting female genetics (Lohuis, 1995), in vitro produced (IVP) embryos, at least in cattle, have developed to the stage where they are having an increasing impact in breeding programmes. The development of ovum pick-up techniques for directly harvesting immature ova from cattle (Kruip *et al*., 1994) and sheep ovaries (Kuhholzer *et al*., 1997), in conjunction with in vitro techniques for producing embryos, has been commercialised in several countries (Hasler *et al*., 1995; Bousquet *et al*., 1999).

One of the practical issues in applying advanced embryo technologies in ruminants is the high variability in number of embryos collected per donor. This has important implications when programming recipients for 'fresh' embryo transfer since decisions on the number of recipients required needs to be made *prior* to collection of embryos. This is less of a planning issue where embryos can be successfully frozen and thawed because the number of embryos for transfer is known in advance. However, because many MOET programmes require 'fresh' transfer, and because robust freeze-thawing techniques are yet to be developed for IVP embryos, programming recipients for 'fresh' embryo transfer will continue to be a practical issue. If too many recipients are available, unnecessary expense is incurred. On the other hand, too few recipients results in additional expense through embryos being either discarded, frozen or transferred as multiples.

The purpose of this paper is to describe a methodology for dealing with uncertain outcomes when planning the logistics associated with the supply of embryos and recipients. The proposed methodology enables practitioners to programme the number of recipients that is consistent with any level of risk they are prepared to take in having surplus embryos or recipients. The particular cases examined explore the provision of recipient cattle to match the expected supply of fresh embryos derived using ovum pick-up and in vitro techniques.

MATERIAL AND METHODS

The distribution of number of transferable quality embryos per donor was determined following the ultrasound guided trans-vaginal recovery of ovarian oocytes using an ovum pick-up technique (Kruip *et al*., 1994) in the spring of 1997 with 63 Friesian donor cows. These lactating cows were in a number of herds in the Waikato and Taranaki regions, and were subjected to several rounds of ovum pick-up. Oocytes were processed using standard in vitro procedures after transfer back to the ARTech central laboratory at Ruakura. The identity of each donor's ova and embryos was maintained throughout these procedures.

The distribution of the proportion of recipients that were suitable for transfer after oestrous synchrony treatment was assumed to be binomial. According to industry experience and the published literature (e.g., Broadbent *et al*., 1993), between 70 and 90% of recipients commencing a synchrony programme are assessed as suitable to receive embryos. The current analysis assumes that 80% of recipients are suitable.

The particular risk associated with donors addressed in this paper is that of having *surplus* embryos (i.e., more embryos available for transfer than suitable recipients available to receive them). With recipients, the particular risk addressed is that of having *insufficient* recipients (i.e., fewer recipients than embryos). There are several reasons why it is difficult to determine how New Zealand cattle embryo transfer practitioners assess risk. First, in their 'fresh' transfer MOET programmes they nearly always use embryo freezing as a tactic to deal with the risk of producing surplus embryos. Second, they occasionally transfer two rather than one embryo into recipients. Third, they have little or no experience as yet with IVP embryos that must be transferred fresh. Fourth, in their centralised embryo transfer stations, they generally have access to a large pool of re-

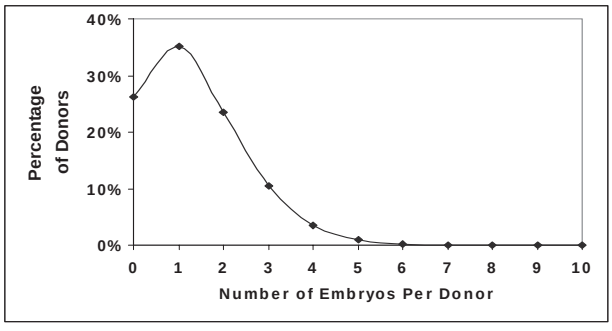
cipients and programme surplus numbers. For these reasons, the assessments of risk in the following scenarios are somewhat arbitrary.

Two broad scenarios with contrasting risk for donors and recipients were considered. The first scenario was considered very low risk (Low Risk) since it took into account nearly all (i.e., >99.5%) of the expected outcomes from donors and recipients. For the Low Risk donor scenario, there was less than a 0.5% chance that more than a given number of embryos were produced. This number was derived from the Poisson distribution, and varied depending on the numbers of donors used. For the Low Risk recipient scenario, there was less than a 0.5% chance that the given number of suitable recipients was insufficient to receive the given number of embryos estimated in the donor scenario. This number of recipients was derived from the binomial distribution. The second scenario examined was considered more risky (High Risk) in that it took into account only 80% of the expected outcomes. High and Low Risk donor and recipients combinations were examined. The upper and lower boundaries for the number of embryos produced in the Low and High-Risk donor scenarios were determined from the Poisson distribution. The number of recipients required estimated using this methodology was compared with the number determined using the following simple arithmetic approach: Number of recipients = [(mean embryos per donor x number donors)/proportion of suitable recipients) x multiplication factor]. The multiplication factor was used to recognise the fact that sometimes more embryos and/or recipients would occur compared with the mean.

RESULTS

The mean number of transferable quality embryos produced per donor at each ovum pick-up session was 1.34. About 25% of the donors did not produce any transferable quality embryos with very few (~1%) producing 5 or more. These data conformed to the Poisson distribution as illustrated in Figure 1.

Figure 1: Poisson distribution of expected percentage of donors with 0-10 transferable embryos produced per donor session (mean = 1.34 embryos/donor/session).



The upper and lower boundaries for the number of embryos produced per session for Low and High-Risk scenarios when 5, 10, 15 or 20 donors were programmed is shown in Table 1. As expected, there is a larger difference in these expected numbers in the Low compared with the High-Risk scenario, because more of the expected outcomes are included within the area of risk. Furthermore, this difference increased as the number of donors increased.

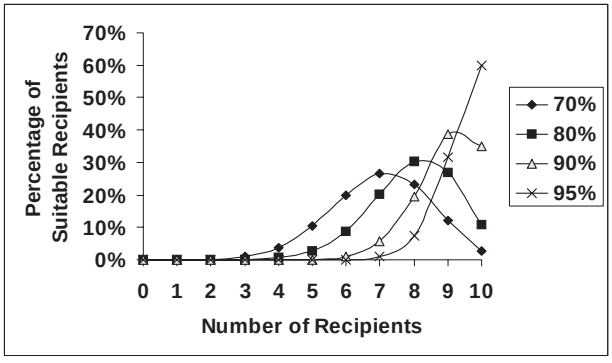
Table 1: Upper and lower boundaries for the number of embryos produced in the Low and High Risk donor scenarios[1] when using either 5, 10, 15 or 20 donors per session

Scenario[1]	Total No. of Donors per Session			
	5	10	15	20
Low Risk (Lower)	0	4	9	13
Low Risk (Upper)	14	24	33	41
High Risk (Lower)	3	9	15	21
High Risk (Upper)	9	16	24	31

[1]See text for explanation

The assumption of binomial variation in the proportion of recipients that are suitable for use as recipients has several implications for managing risk. Some of these implications can be deduced from Figure 2, where the probability of from 0-10 out of 10 recipients being suitable is illustrated. First, with at least 70% expected to be suitable, it is very unlikely that fewer than 3 out of 10 recipients will be suitable. Second, the maximum chance of achieving exactly the expected number of recipients ranges from about 25% (when 70% are expected to be suitable) to about 60% (when 95% are expected). Third, the chance of all 10 recipients being suitable is very sensitive to the expected proportion of suitable recipients, but does not exceed 60%.

Figure 2: Binomial distribution of expected percentage of recipients assessed as suitable when recipients have either a 70, 80, 90 and 95% chance of being suitable (N=10 recipients).



The effect of high and low risk donor and recipient combinations on the number of recipients required is illustrated in Table 2. The main effect of moving from the low-

Table 2: Number of recipients required to commence oestrous synchrony programme for scenarios involving high and low risks of donors producing surplus embryos or insufficient suitable recipients.

Donor Risk[1]	Recipient Risk[2]	Number of Donors in Programme			
		5	10	15	20
Low	Low	26	40	52	63
Low	High	20	34	45	55
High	Low	18	28	40	49
High	High	13	23	34	43

[1]Risk of donors producing surplus embryos; [2]Risk of insufficient suitable recipients

est risk (Low-Low) to the highest risk (High-High) combination was to reduce the number of recipients required to start synchrony treatment by 30-50%, being higher when fewer donors were used. For intermediate combinations, the reduction was 15-30%.

With the simple arithmetic approach and using a multiplicative factor of 1.0, the numbers of recipients required (Table 3) were 20-40% lower than estimated in the High-High combination in Table 2. Interpolation of the data in Table 3 suggests that a multiplicative factor of about 1.3 resulted in similar recipient numbers to those in the High-High combination. A multiplicative factor of about 2 predicted similar recipient numbers as the Low-Low combination, at least when 15-20 donors were used. A multiplicative factor of about 2.5 was required when 5-10 donors were used.

Table 3: Estimate of number of recipients[1] to commence programming based on: a mean of 1.34 embryos/donor; 80% of recipients being suitable for transfer and from 5-20 donors

Multiplicative[1]	Number of Donors in Programme			
	5	10	15	20
1.0	8	17	25	34
1.2	10	20	30	40
1.4	12	23	35	47
1.6	13	27	40	54
1.8	15	30	45	60
2.0	17	34	50	67

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DISCUSSION

The main finding in this study is that the proposed methodology allows practitioners to design recipient oestrous synchrony programmes that can accommodate varying levels of risk that they are prepared to take in any given donor programme. Furthermore, it has been demonstrated that using a simple arithmetic approach may expose programmes to unacceptably high levels of risk of surplus embryos, especially if a standard multiplicative factor is used. The main risks in a programme requiring the transfer of fresh embryos is that surplus embryos may have to be either frozen-thawed, discarded or transferred as multiples. On the other hand, it is well recognised that surplus recipients also incur unnecessary costs. Although not attempted in this paper, the model may be extended to determine an economic optimum number of recipients to include in any programme. This optimum could be for a given level of risk, or have weightings against risk (i.e., utility maximisation).

The embryo production rate in the current study is lower than that potentially possible under laboratory conditions for harvesting (Hagemman *et al*., 1999). However, it is similar to that achieved in practice under field conditions (e.g., Bols *et al*., 1998). Although the model used in this analysis assumed a Poisson distribution for embryo availability, other distributions and means could be used when appropriate. For example, recent field trails have shown a mean of 4-5 embryos per donor session, although the distribution was not reported (Bousquet *et al*, 1999). In some instances, it may be appropriate to use simulation analyses to determine risks.

The application of this basic methodology to other aspects of applied reproductive technology has been reported elsewhere (Vivanco and McMillan, 1999). In that study a stochastic model was used to predict the likelihood of achieving 3 surviving bulls calves in an ovum pick-up and IVP system. The model predicted that with 14 sessions per donor, at least 75% of donor cows would produce 3 surviving bull calves.

In conclusion, we have presented a methodology that enables practitioners to determine the number of recipients required in an embryo transfer programme. Furthermore, the methodology allows an assessment of the risks of too few or too many embryos or recipients. We have demonstrated that simple arithmetic methods using the same multiplicative factor are likely to expose programmes to considerable risk, but that a series of scaling factors could be used to accommodate varying donor numbers and levels of risk.

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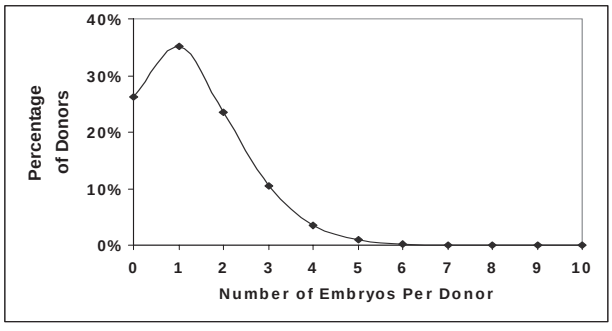

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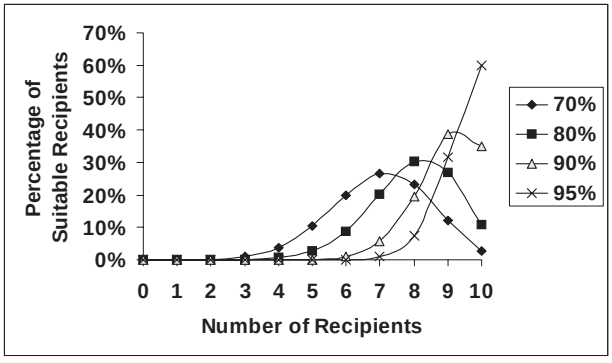

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