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Factors affecting the survival of sheep embryos after transfer within a MOET program

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Abstract

Multiple ovulation and embryo transfer (MOET) has the potential to increase the rate of genetic improvement in sheep. However, better realization of this potential requires maximum survival rates of transferred embryos of high genetic merit after transfer into recipient ewes. These studies were therefore conducted to investigate the effect of both embryonic and recipient ewe factors on the survival rate of transferred embryos. Survival rate was similar after transfer of morula or blastocyst stage embryos, and these were higher ($P < 0.05$) than for very early morulae and early morulae. Advanced embryos (Day 5 blastocyst) had an advantage ($P < 0.05$) in survival rate over retarded embryos (Day 6 morula). Grades 1 and 2 embryos survived significantly ($P < 0.05$) better than Grades 3 or 4 embryos. There was no difference in embryo survival rate following transfer to recipients with different numbers of corpora lutea. In general, age or parity of recipient ewes did not affect embryo survival rate, although a higher ($P < 0.05$) embryo survival rate was observed for yearling recipients. Buserelin (GnRH agonist) treatment of recipient ewes 5 or 6 days after transfer of embryos (Day 12 of the cycle) did not improve embryo survival rate. These results confirm that both embryonic and recipient factors can play an important role in the success of a MOET program in sheep.

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1. Introduction

Multiple ovulation and embryo transfer (MOET) has the potential to increase the rate of genetic gain through the female line. However, the full realization of this potential depends upon maximizing the number of progeny born from high merit females. While influenced by superovulatory responses and fertilization rates of donors in any MOET program, the number of progeny per donor is also directly related to the survival rate of transferred embryos. Factors specific to both embryos and recipients have been suggested to affect the survival of transferred embryos in cattle, sheep and goats. Among them, stage of embryo development, embryo quality, number of corpora lutea, and age and parity of the recipients have been reported to be of significance [1–7].

The effect of stage of embryo development on subsequent embryo survival has been investigated in both cattle and sheep. While some of these studies indicated higher survival rates for blastocysts compared to morulae [1–3,6,8,9], others failed to demonstrate any difference [5,10–12]. In the case of cattle embryos, uterine tolerance of recipients to advanced and retarded embryos appears to be very similar [13,14], but in the pig there is an advantage for advanced compared to retarded embryos [15].

Pregnancy rate in cattle has been reported to increase with an improvement in embryo quality [1,16]. However, this does not appear to be an especially precise association since in another study there was no significant difference in survival rate of Grades 1 and 2 embryos, or of Grades 3 and 4 embryos [17]. Similarly, in sheep there was no difference in survival rate between embryos classified as ‘good’ and ‘excellent’ at the time of transfer [4].

Progesterone plays a vital role in early embryo development, implantation and the establishment of pregnancy. Plasma progesterone concentrations in recipient animals are related to the number of ovulations or corpora lutea in sheep [18,19]. While embryo survival has been reported to increase with an increase in plasma progesterone concentrations in cattle [20,21] and the number of corpora lutea in goats [7], little information of this type exists for sheep. Since there is evidence for considerable variation in progesterone secretion over successive pregnancies in sheep [18], it is possible that age and parity of recipient ewes may also affect embryo survival.

One drawback with many previous studies designed to investigate factors affecting the survival of transferred embryos in sheep is that they were based on either small numbers of embryos transferred or small group sizes of recipients. However, our current MOET program [22,23] has provided a large database from which to undertake retrospective analyses, and in this paper we report on a number of factors that could potentially affect embryo survival in recipient ewes. Some of these are related to the embryos themselves (stage of development, embryo quality), while others are concerned with recipient ewe effects (recipient ewe age, number of corpora lutea, GnRH treatment).

2. Materials and methods

2.1. General materials and methods

This study used data obtained from a MOET program at two locations, ADAS Redesdale in northern England (Scottish Blackface ewes) and ADAS Pwllperian in Wales (Welsh

Mountain ewes). The MOET program involved 60 donor ewes and approximately 300 recipient ewes per site each year over a 5-year period, and treatment details for donor and recipient ewes remained constant over the duration of the study, with the exception of refinements to the flushing procedure itself [22].

Full details of the management and treatment of recipient ewes, and embryo collection, evaluation and transfer procedures have been described elsewhere [22,23]. Briefly, MOET was undertaken during the breeding season in mid-October (Welsh Mountain ewes) or mid-November (Scottish Blackface ewes). Estrus was synchronized in both donor and recipient ewes by the insertion of an intra-vaginal progestagen sponge (Chronogest[®] Intervet Laboratories plc., Cambridge, UK) left in situ for 12 days. From Day 10 after sponge insertion, donor ewes were treated twice daily with 1.25 ml of ovine FSH (Ovagen[®] Immunological Products Ltd., Auckland, New Zealand) over 4 days to induce super-ovulation. The sponges were removed at sixth FSH administration (1800 on Day 12). Recipient ewes were treated with 250–350 IU PMSG at the time of sponge removal (1400 on Day 12).

Donor ewes, when in estrus, were hand-mated with their respective rams (five ewes per ram). In addition, intra-uterine insemination with fresh semen from their respective rams was performed between 44 and 46 h after sponge removal. Embryos were recovered using a semi-laparoscopic procedure [22,23] on either Day 5 or 6 after insemination. Donor ewes were programmed in groups of 20, with the first 10 ewes to show estrus flushed on Day 5, and the remaining 10 donor ewes flushed on Day 6 of the cycle. The collection procedure involved laparoscopy to place a Foley catheter into the lumen at the base of the uterine horn, and then exteriorizing the tip of the uterine horn through a 1 cm mid-line incision to allow introduction of the flushing medium. All collected embryos were graded according to agreed IETS conventions [24].

Recipient ewes were synchronized in groups of 100, and were checked regularly for the onset of estrus with a vasectomised ram. The first 50 recipients to come into estrus were used for the Day 5 donors and the remaining 50 recipients for the Day 6 donors. Embryos were transferred laparoscopically as singletons, except where indicated, approximately 2 cm from the utero-tubal junction into recipient ewes of the same breed. Pregnancy rate of recipient ewes was assessed by ultrasound scanning 50 days after transfer. Past experience has shown that this method of estimating embryo survival rates is >99% accurate when recipients receive only a single embryo.

2.2. Effect of stage of embryo development and embryo quality, number of corpora lutea of recipients and age of recipients on embryo survival

Studies to investigate the effect of stage of embryo development and embryo quality, and the number of corpora lutea and age of recipient ewes on embryo survival were conducted using a total of 1513 embryos obtained only from Scottish Blackface ewes over a 5-year period. At the time of collection, embryos were categorized into six different stages of development (very early morula, early morula, morula, blastocyst, expanded blastocyst, hatched/collapsed blastocyst), and were also assigned one of four quality grades (Grades 1–4, based on IETS conventions) according to the compactness and uniformity of cell structure. The main aim within the breeding program was to transfer as close to five

embryos per donor ewe as possible to maintain as broad a genetic base as possible. While the better embryos within each flush were the ones selected for transfer, this still resulted in embryos of different quality grades being transferred (see [Table 3](#)). The number of corpora lutea of recipient ewes was assessed laparoscopically at the time of transfer, and all transfers were made into the uterine horn ipsilateral to the ovary with the greater number of ovulations.

2.3. *Effect of Buserelin[®] treatment of recipient ewes on embryo survival*

Studies to investigate the effect of GnRH agonist (Buserelin[®]) treatment of recipient ewes on embryo survival were conducted over a period of 2 years, and involved 633 Scottish Blackface and 586 Welsh Mountain recipient ewes. Ewes were randomly allocated to the treatment and control groups, ensuring an approximately equal distribution within each embryo quality category. All embryos were transferred as singletons in the Scottish Blackface ewes, but a small proportion of Welsh Mountain recipient ewes (41/586 ewes) received two embryos. The treated recipient ewes were treated with 1 ml (4 µg) GnRH agonist i.m. (Buserelin[®], Hoescht UK Ltd., Milton Keynes, UK) on 7 or 6 days after transfer of the embryos respectively for embryos transferred on Days 5 and 6 (equivalent to Day 12 of the cycle).

3. Statistical analyses

The effects of stage of embryo development and embryo quality at the time of transfer, recipient ewe age and corpora lutea number on the subsequent survival of transferred embryos were all analyzed using contingency Chi-squared analysis. In addition, the effect of Buserelin treatment of recipient ewes on embryo survival was also determined by Chi-squared analysis.

4. Results

4.1. *Effect of day of collection, stage of embryo development and embryo quality on embryo survival*

Of the total of 2080 embryos collected over the 5 years of the study, 1513 embryos were actually transferred. Although these represented the better quality embryos from each donor ewe, they still represented a range of stages of development and quality grades ([Tables 1–3](#)).

For embryos collected on either Day 5 or 6 of the cycle, the normal expectation was that they would be at the morula and blastocyst stages of development, respectively. The data in [Table 1](#) show that there was no significant overall effect of day of embryo collection on subsequent survival rates. However, advanced (blastocyst) embryos collected on Day 5 had a higher ($P < 0.05$) survival rate than retarded (morula) embryos collected on Day 6, with normal stage embryos (Day 5, morula; Day 6, blastocyst) having intermediate survival rates.

Table 1

Comparison of different stages of development of Scottish Blackface embryos on subsequent survival rate following transfer to recipient ewes

Stage of development	Embryos transferred (<i>n</i>)	Embryos survived (<i>n</i>)	Embryos survived (%)
Day 5 overall	790	591	74.8 ^a
Day 6 overall	723	518	71.7 ^a
Day 5 blastocyst	24	22	91.7 ^b
Day 5 morula	766	569	74.3 ^{ab}
Day 6 blastocyst	334	250	74.9 ^{ab}
Day 6 morula	389	268	68.9 ^a

Values with different superscripts (a, b) within a sub-column are significantly ($P < 0.05$ at least) different.

Table 2

Survival rate of Scottish Blackface embryos transferred into recipient ewes at different stages of development (pooled over day of collection)

Stage of development	Embryos transferred (<i>n</i>)	Embryos survived (<i>n</i>)	Embryos survived (%)
Very early morula	65	36	55.4 ^a
Early morula	388	271	69.8 ^{ab}
Morula	702	530	75.5 ^b
Blastocyst, expanded blastocyst and expanded-collapsed blastocyst	358	272	75.9 ^b

Values in the same column with different superscripts (a, b) are significantly ($P < 0.05$) different.

Table 2 provides greater detail of the association between stage of development and embryo survival, irrespective of the day of collection and transfer. While there was a progressive increase in survival rate with stage of embryo development, the overall survival rate for morulae and blastocysts was not significantly different. However, the survival rate of retarded embryos (very early morulae) was significantly lower than for normally developed embryos (morulae and blastocysts), with early morulae rates intermediate.

Embryo quality at transfer had a significant effect on subsequent survival rate, with a progressive reduction in survival as embryo quality declined (Table 3). While there was no difference in survival rate between Grades 1 and 2 embryos, the survival rates of Grades 3 and 4 embryos were significantly lower than those of Grade 1 or 2 embryos. Although the survival rate for Grade 4 embryos (37.5%) was much lower than that for Grade 3 embryos

Table 3

Survival rate of Scottish Blackface embryos of different quality grades after transfer to recipient ewes

Embryo grade	Embryos transferred (<i>n</i>)	Embryos survived (<i>n</i>)	Embryos survived (%)
Grade 1	825	624	75.6 ^a
Grade 2	550	406	73.8 ^a
Grade 3	114	70	61.4 ^b
Grade 4	24	9	37.5 ^b

Values with different superscripts (a, b) are significantly ($P < 0.05$ at least) different.

Table 4

Survival rate (%) of Scottish Blackface embryos transferred to recipient ewes of the same breed with different numbers of corpora lutea

Number corpora lutea	Grade 1	Grade 2	Grade 3	Grade 4	Mean
1	71.6 ^a (268)	74.7 ^a (154)	67.5 ^{ab} (40)	50.0 ^a (6)	72.0 ^a (468)
2	78.7 ^b (455)	71.9 ^a (302)	50.9 ^a (57)	33.3 ^a (12)	73.6 ^a (826)
>3	72.5 ^{ab} (102)	78.7 ^a (94)	82.3 ^b (17)	33.3 ^a (6)	74.9 ^a (219)

Numbers in parenthesis are the numbers of each grade of embryo transferred; values within each column with different superscripts (a, b) are significantly ($P < 0.05$) different.

(61.4%), this did not reach statistical significance, most probably because of the very small number of Grade 4 embryos transferred.

4.2. Effect of number of corpora lutea and recipient ewe age on embryo survival

There were no differences in overall embryo survival following transfer to recipient ewes with different numbers of corpora lutea (Table 4). While there were some significant differences when the number of corpora lutea were considered within the different embryo quality grades, these effects were not consistent.

The total numbers of embryos transferred and surviving following transfer to recipient ewes of different ages and parities are shown in Table 5. While the overall survival rate of embryos transferred into nulliparous (yearling) recipient ewes was significantly ($P < 0.05$) higher than for embryos transferred into parous (>2 years old) ewes, the difference was relatively small. Among the individual age categories there were some significant differences in survival rate of transferred embryos, but again these effects did not follow any defined pattern.

4.3. Effect of Buserelin treatment of recipient ewes on embryo survival

Table 6 shows the effect of Buserelin treatment of recipient ewes 7 or 6 days after transfer of embryos collected on Days 5 and 6, respectively (equivalent to Day 12 of the

Table 5

Survival rate of Scottish Blackface embryos transferred to recipient ewes of different parities and ages

Age (years) and parity of recipient ewes	Embryos transferred (n)	Embryos survived (n)	Embryos survived (%)
1	407	314	77.1 ^a
2	362	262	72.4 ^{ab}
3	318	237	74.5 ^{ab}
4	269	187	69.5 ^b
5	143	98	68.5 ^{ab}
6	14	11	78.5 ^a
Nulliparous (yearling)	407	314	77.1 ^a
Parous (2–6 years)	1106	795	71.9 ^b

Values with different superscripts (a, b) within a sub-column are significantly ($P < 0.05$) different.

Table 6

Survival rate (%) of Scottish Blackface and Welsh Mountain embryos after transfer into Buserelin-treated or untreated control recipient ewes of the same breed

Treatment	Grade 1	Grade 2	Grade 3	Grade 4	Overall
Buserelin	80.4 ^a (404)	74.0 ^a (158)	51.8 ^a (54)	35.3 ^a (17)	75.1 ^a (633)
Control	82.7 ^a (405)	71.9 ^a (153)	70.1 ^a (57)	50.0 ^a (10)	78.4 ^a (625)
Overall	81.5 ^b (809)	72.9 ^c (311)	61.2 ^c (111)	40.7 ^d (27)	

Numbers in parentheses are the numbers of embryos transferred; values with different superscripts (a–c), either within a column for individual treatments or along the row for overall effects of grade, are significantly ($P < 0.05$ at least) different.

cycle) on the subsequent survival of embryos. Chi-squared analysis indicated that there was no significant benefit of Buserelin[®] treatment on embryo survival. However, as for the Scottish Blackface ewes in Table 3, there was a significant ($P < 0.05$ at least) decline in embryo survival with decreasing embryo quality.

5. Discussion

In the present experiment, the method of selecting donor and recipient ewes for embryo collection and transfer on either Day 5 or 6 ensured a high degree of synchrony in the time of estrus between individual donors and their recipient ewes. Indeed, in recipient ewes to which embryos were transferred estrus occurred within ± 4 h of estrus in donor ewes. This may well have contributed to the high overall embryo survival rates achieved, with values similar to [25] or higher than in previous studies [26].

Irrespective of whether the embryos were collected and transferred on Day 5 or 6, their overall survival rates were similar (Table 1). This is in direct contrast to another study [27] that indicated a significant advantage of Day 6 and 7 embryos compared to Day 5 embryos. However, these authors suggested that embryo survival following transfer on Day 5 might be improved by deposition of embryos closer to the utero-tubal junction, a practice followed throughout in the current study.

The overall survival rate of embryos transferred as morulae and blastocysts (pooled over collection days) were also not different (Table 2). While these results agree with those of some studies in both sheep and cattle [15,16,28], they are at variance with others that indicated a higher survival rate for blastocysts compared with morulae [1,2,6]. How much of this difference between studies can be explained on the basis of transfer of inherently defective (retarded) morulae compared to blastocysts is not clear. Where collections occur only on Day 6, the time frequently used for many previous studies and in commercial practice, embryos would normally be expected to be at the blastocyst stage of development. Consequently, morulae collected from within a cohort of Day 6 blastocysts may already be showing signs of retarded or impaired development, and this might make them less likely to survive. However, if collections take place on Day 5, as was the case for half of the donor ewes in the present study, embryos would normally be expected to be at only the morula stage of development. These would not therefore be retarded in their development, and might be expected to have similar survival rates to blastocysts collected on Day 6. There is

some support for such a suggestion from within the more detailed assessment of stage of embryo development and its impact on subsequent survival within the current study. When pooled over day of collection, blastocysts and morulae had similar survival rates (Table 2). However, very early morulae (retarded relative to their cohort embryos) had a significantly poorer survival rate after transfer, with early morulae survival rates intermediate. Data from cattle studies [1,16] suggest that the retarded embryos might have survived better if they had been transferred to recipient ewes coming into estrus after donor ewes. The higher survival rates of advanced (Day 5 blastocysts) compared to retarded (Day 6 morulae) embryos in the present experiment are consistent with data previously reported for pigs [15]. Such factors may well reflect differences in the timing of the maternal recognition of pregnancy signal that is critical for inhibiting oxytocin receptor development and prostaglandin production [29] that are essential for the maintenance of the corpus luteum and therefore pregnancy.

Embryos of higher grades survived significantly better than those of poorer grades. Because all embryos were transferred to recipients with a similar degree of synchrony of estrus to donor ewes, the better survival rate of higher grade embryos must be attributed to their greater ability to adjust or alter the uterine environment in their favor. These results are consistent with those obtained in some cattle studies where higher pregnancy rates were observed with better quality embryos [1,16]. Although some other cattle studies indicated no relationship between embryo quality and subsequent survival [3,11], these involved small numbers of embryos, making the results less robust. Embryo quality is assessed morphologically and is also subjective. This may mean that differences between close grades are more difficult to demonstrate experimentally in comparison to those between more extreme grades. In the present study, there was no difference in survival rate between Grades 1 and 2 embryos (75.6 and 73.8%, respectively). However, as embryo quality declined further the differences became more notable. Grade 3 embryos had a significantly lower chance of survival (61.4%) than both Grades 1 and 2 embryos, with a more marked reduction in survival for Grade 4 embryos (37.5%), although it should be noted that the number of Grade 4 embryos transferred was relatively small. Overall, these results suggest that all embryos collected within a MOET program in sheep have a sufficiently high chance of survival to justify their transfer.

The number of corpora lutea in recipient ewes had no significant effect on the overall survival rate of transferred embryos. While some differences were apparent when comparing the effects of corpora lutea number by embryo grade, these were inconsistent and most probably reflect random effects. Had the effect been real, then it would be reasonable to expect that the differences would be more apparent with poorer grade embryos. However, this was not the case. Such results suggest that there is little point in modifying treatments of recipient ewes to increase ovulation rate in the belief that this will increase embryo survival. These results contrast with studies in the goat [7], where significantly higher embryo survival rates were observed in recipients with two and three or more corpora lutea compared to those with only one corpus luteum. It is difficult to explain such differences between species. However, it may reflect that fact that in the present study, the progesterone concentrations sustained by one corpus luteum were sufficient for growth, development and implantation of embryos. Such a suggestion is consistent with data from cattle which suggest that there is a minimum level of progesterone in recipients (5–8 ng/ml plasma) required for embryo survival [21,30,31].

There was a higher embryo survival rate following transfer to nulliparous (yearling) (77.1%) compared to parous ewes (71.9%), although the difference was not large. Differences in embryo survival among the various age categories of parous ewes were also small and inconsistent. In general, these results agree with data from cattle that reveal little or no difference in pregnancy rate of recipients with successive parity [32,33]. The small but significantly higher embryo survival rates in yearling recipients might be explained by the fact that they were mature enough to produce sufficient quantities of progesterone for embryo survival, while at the same time had no residual uterine problems from a previous pregnancy.

Buserelin treatment was given in an attempt to improve embryo survival rate. This has been shown to improve pregnancy rates in other situations in both sheep and cattle, although the precise mechanisms involved are not fully understood. Some have suggested that Buserelin enhances luteal function [34,35]. However, others support the concept that Buserelin interferes with the luteolytic mechanism [36], possibly by stimulating luteinization of large estrogenic follicles to delay the luteolytic process until the maternal recognition of pregnancy signal itself is sufficient to prevent luteolysis by blocking uterine oxytocin receptor development [29]. No such beneficial effect of Buserelin[®] was observed in the present experiment, even for the poor grade embryos. This suggests that the lower survival rate of poor quality embryos was mainly due to intrinsic defects within the embryo rather than resulting from any unfavorable uterine environment. These results are consistent with those of other studies [5,37] in which additional progesterone, administered through a CIDR device, did not improve embryo survival within a MOET program.

In conclusion, the results of the present study have identified a number of factors that may contribute to the success of a MOET program in sheep, but their effects are much smaller than frequently assumed.

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