Survival of embryos after transfer within multiple ovulation and embryo transfer (MOET) programme

Begum Fatema Zohara^{a*}, Azizunnesa^b, Md. Faruk Islam^a, Md. Golam Shahi Alam^c and Farida Yeasmin Bari^c

^a Department of Medicine, Surgery and Obstetrics, Hajee Mohammad Danesh Science and Technology University, Dinajpur 5200, Bangladesh

^b Department of Medicine and Surgery, Chittagong Veterinary and animal Sciences University, Chittagong 4202, Bangladesh.

^c Department of Surgery and Obstetrics, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh

* Corresponding author. Email: begumfatemafatema@ymail.com

Abstract

The experiment was designed to investigate factors affecting the survival of transferred embryos in indigenous recipient ewes (Wera breed) in Bangladesh. The factors were methods of transfer, number, stage and quality of the transferred embryos, recipient ewe's age and corpora lutea (CL) number of recipients. Transferring three embryos to recipient ewes had a significant effect on the lambing rate and embryo survival rate by both surgical and laparoscopic method. Triplet birth was observed only when recipients received three embryos by laparoscopic transfer. Laparoscopic transfer is safe, minimally invasive, and may be recommended for increased embryo survival. Stage of embryo development did not significantly affect the pregnancy rate but morula stage tended to give higher pregnancy rates (90.9%). **Significantly higher** pregnancy rate (77.3%) was observed when embryos of Grade 1 - 2 were transferred than Grade 3 - 4. There was no significant ($P>0.05$) effect of recipient ewe's age and **CL number** on pregnancy and embryo survival rates.

Keywords: *Ewes, Embryos, Transfer methods, Recipients, embryo survival, Pregnancy rates.*

1. Introduction

Factors specific to embryos and recipients may affect the survival of transferred embryos in cattle, sheep and goats. Among them, embryo transfer method, stage of embryo development, embryo quality, number of corpora lutea (CL), and age of the recipients have been reported to be significant (Armstrong and Evans, 1983; Thompson et al., 1995). Postoperative adhesions between oviduct, uterus and ovaries mean long intervals before reuse of a recipient: this has encouraged several groups to

32 develop a successful laparoscopic technique to transfer embryos to ewes (Lee et al., 2000; McKelvey
33 and Robinson, 1984). Comparison of surgical and laparoscopic transfer of embryos showed that the
34 laparoscopic method can achieve high pregnancy rates (Stefani et al., 1990; Silva et al., 2003). Studies
35 have been conducted in cattle and sheep to observe differences in embryo survival rate between
36 different stages of embryo development following transfer to synchronous recipients. Some studies
37 indicated higher survival rates for blastocysts compared to morulae (Armstrong and Evans, 1983;
38 Wright et al., 1981), while others found no difference in survival rate between these two stages (Shea,
39 1981; Thompson et al., 1995). At each stage of embryo development, embryo quality affects survival
40 rate (Breuel et al., 1991; Donaldson, 1985). Plasma progesterone (P4) concentration in recipients
41 around transfer could affect embryo survival rate. P4 concentration during the luteal phase is related to
42 the number of ovulations or corpora lutea in sheep (Trounson and Moore, 1974; Ashworth et al., 1989).
43 Survival of ovine embryos may be related to the age of recipients, since there is evidence for
44 considerable variation in P4 secretion and embryo survival over successive pregnancies in sheep
45 (Ashworth, et al. 1989). Studies of the effect of age of recipients on embryo survival rate after transfer
46 in sheep are limited. Most sheep in Bangladesh (*Ovis aries*) are indigenous and belong to the so-called
47 Wera breed (FAO, 1971). In Bangladesh there is no report on transfer of ovine embryos in a MOET
48 programme. This experiment was designed to investigate factors affecting the survival of transferred
49 embryos in indigenous recipient ewes. The factors were method of transfer, stage and quality of
50 transferred embryos, recipient's age and CL number of recipients.

51

52 **2. Materials and Methods**

53 *2.1. General materials and methods*

54 The study was carried out at the Department of Surgery and Obstetrics, Bangladesh Agricultural
55 University, Mymensingh, at N 24.73 latitude and E 90.44 longitude, and 9 m above sea level. The area
56 receives on average 174 mm of rainfall. Mean annual minimum and maximum temperatures are 16 and
57 29° C, respectively. The study was conducted between January 2011 and December 2014. Indigenous
58 ewes, 2 - 3 years old and weighing 15 to 19 kg with body condition score 2 to 3.5 were used. They
59 were maintained on natural grazing, with supplemented feeding (concentrate mixture composed of 25%
60 crushed maize, 50% wheat bran, 20% soybean meal, 1% fish meal, 2% DCP Powder, 1.5% salt and
61 0.5% vitamin- mineral premix) at 250 g/ head / day. Water and a mineral salt lick were provided *ad*
62 *libitum*.

63

64 2.2. *Synchronization and superovulation of donor and recipient ewes*

65 Donor and recipient ewes were synchronized by inserting intravaginal sponges impregnated with 30
66 mg FGA (Ova-Gest[®], Bioniche Animal Health Pty Ltd, Armidale NSW 2350, Australia) for 12 days.
67 To induce superovulation, donor ewes received pFSH 25 mg (Folltropin[®]-V, Bioniche Animal Health,
68 Belleville, Ontario, Canada) intra-muscularly twice daily from 48 hr prior to sponge withdrawal for
69 four consecutive days. Recipient ewes were treated with sub-superovulatory doses of PMSG (350 iu,
70 Folligon[®], Intervet, Boxmeer, The Netherlands) to stimulate folliculogenesis immediately after sponge
71 removal (Bari et al., 2003). This procedure ensured that recipients and donor ewes reached oestrus at
72 the same time.

73

74 2.3. *Oestrus detection and natural service*

75 The ewes were kept with teaser rams, and observed for signs of oestrus twice a day (0700 hrs and 1900
76 hrs) for 60 min in each observation from 12 to 66 hr following sponge removal. All donor ewes were
77 naturally mated 6 and 12 hr after the onset of oestrus.

78

79 2.4. *Recovery, handling, grading and transfer of embryos*

80 Embryos were collected from ewes on Day 5 or 6 after onset of oestrus by a semi-laparoscopic method
81 similar to that described by Naqvi et al. (2006). Embryo identification was carried out under stereo
82 microscope (MZ6, Leica Microsystems Ltd., Switzerland) on a thermal plate at 38°C. Embryos were
83 aspirated with a micropipette and placed in a small petri dish containing a holding medium enriched
84 with 20% serum. The stage of development and grade were recorded on a scale of 1 – 5, depending on
85 their morphological appearance (Bari et al. 2000). Embryos were transferred by surgical (González et
86 al., 1991) or laparoscopic (Bari et al., 2003) method immediately after collection. Pregnancy was
87 confirmed by non-return to oestrus and ultrasound scanning (DRAMINSKI ANIMAL profi portable
88 ultrasound scanner, Poland) 50 days after transfer.

89

90 2.5. *Experimental design*

91 Three experiments were conducted to investigate factors affecting the survival of transferred embryos
92 in indigenous ewes. In the first experiment the effects of embryo transfer methods on subsequent

93 survival rates were determined. This study involved 30 indigenous recipient ewes in which embryos
94 were transferred through surgical (n=15) and laparoscopic (n=15) procedure. The second experiment
95 determined the effect of stage and quality of the transferred embryos on survival rates. In this study 30
96 ewes received 72 embryos at different stages of development (early morula, morula, early blastocyst,
97 and blastocyst) and embryo qualities (Grade 1 - 2 and Grade 3 - 4) obtained from indigenous ewes in a
98 MOET programme. The third experiment determined the effects of recipient ewe's age and CL number
99 on embryo survival rates. This study used 30 recipient ewes with different corpora lutea (CL) numbers
100 (CL 1, CL 2 and CL 3+) and ages (1- 4 years).

101

102 *2.6. Statistical analysis*

103 The data were analysed using SPSS for Windows, Version 17.0 (SPSS 2008). Pregnancy, lambing,
104 embryo survival, lamb survival and abortion rates were compared by Chi-square analysis with Fisher's
105 Exact Test. Gestation length and lamb birth weight were compared by student t-test. Differences
106 between groups were considered significant at $P < 0.05$.

107

108 **3. Results**

109 *3.1. Effects of embryo transfer methods on survival rates*

110 Pregnancy rates (30 and 50 days), lambing, embryo survival, and lamb survival and abortion rates after
111 surgical and laparoscopic embryo transfer are presented in Table 1. Pregnancy rate was not
112 significantly ($P > 0.05$) different between methods at 30 and 50 days. However, pregnancy rate after
113 laparoscopic method tended to be higher than after surgical transfer. Thirty six embryos were
114 transferred to 15 recipients by surgical method, and 36 by laparoscopic method: the pregnancy rate at
115 30 days was 80.0% and 86.7%, respectively: at 50 days the rate was 66.7% and 86.7%, respectively.
116 Lambing rate, embryo survival rate at lambing and lamb survival rate tended to be higher after
117 laparoscopic method than surgical. There was no significant difference in gestation length after
118 surgical and laparoscopic procedure (145.2 ± 0.79 vs. 147.1 ± 2.82 days). The lamb birth weight was
119 1.2 ± 0.32 and 1.1 ± 0.29 kg after surgical and laparoscopic method, respectively. Significantly higher
120 abortion rate (33.3%) was observed after surgical embryo transfer, whereas there was no abortion after
121 laparoscopic method.

122

123 **Table 1**
124 Comparison of two transfer methods on subsequent success in MOET Programme
125

Variable	Methods of embryo transfer		P-value
	Surgical	Laparoscopic	
No. of recipients	15	15	-
No. of embryos	36	36	-
Pregnancy Rates at 30 days	80.0 (12/15)	86.7 (13/15)	1.00
Pregnancy Rates at 50 days	66.7 (10/15)	86.7 (13/15)	0.195
Gestation length (days)	145.2±0.79	147.1± 2.82	0.593
Embryo survival rate at lambing	30.6 (11/36)	52.8 (19/36)	0.094
Lambing rate	80.0 (8/10)	100.0 (13/13)	0.178
Lamb survival rate at weaning	66.7 (6/11)	81.3 (13/19)	0.630
Lamb birth weight (kg)	1.2±0.32	1.1± 0.29	0.848
% of Abortion	33.3 (4/12)	0.00 (0/13)	0.039

126

127

128

129 Effects of number of transferred embryos on survival and lambing rate in MOET programme are shown
 130 in Table 2. Transferring three embryos to recipient ewes significantly increased ($P<0.05$) the lambing
 131 rate and embryo survival rate by both surgical and laparoscopic methods compared with two embryos:
 132 laparoscopic embryo transfer showed higher lambing and survival rates. There was no significant
 133 difference in single and twin birth after transferring two or three embryos to the recipients. Triplets
 134 were observed only in ewes given three embryos by laparoscopy.

135 **Table 2**

136 Effects of number of embryos transferred on subsequent success in MOET Programme

137

Parameter	Transfer method				P-value
	Surgical		Laparoscopic		
Number of embryos per transfer	2	3	2	3	
Number of recipients	9	6	9	6	
Lambing rate	22.2 ^c (2/9)	100.0 ^a (6/6)	77.8 ^b (7/9)	100.0 ^a (6/6)	0.002
Embryo survival rate	11.1 ^b (2/18)	44.4 ^a (8/18)	50.0 ^a (9/18)	61.1 ^a (11/18)	0.016
Litter size					
Rate of single birth	100.0 (2/2)	66.7 (4/6)	71.4 (5/7)	33.3 (2/6)	0.304
Rate of twinning birth	0.00 (0/2)	33.3 (2/6)	28.6 (2/7)	50.0 (3/6)	0.610
Rate of triplet birth	-	-	-	16.7 (1/6)	-

138 ^{a, b} Values with different superscript within each row are significantly different (at least $P<0.05$).

139

140

141 *3.2. Effect of stage and quality of transferred embryos on survival rates in ewes*

142

143 Comparison of different stages of development and grades of embryo on pregnancy and survival rates

144 following transfer to indigenous ewes are shown in Table 3. Stage of embryo development did not
 145 affect pregnancy rate, but morulae tended to have a higher pregnancy rate (90.9%). The survival rate
 146 for morulae and blastocysts was not significantly different, but early morulae had a significantly lower
 147 survival rate than morulae and blastocysts ($P<0.05$). Significantly ($P<0.05$) higher pregnancy rate
 148 (77.3%) was observed when embryos of Grade 1 - 2 were transferred than Grade 3 – 4 (33%). Although
 149 the survival rate of different grades of embryo was not significantly different, there was a tendency for
 150 more Grade 1 - 2 embryos to survive.

151

152 **Table 3**

153 Comparison of different stages of development and grades of embryo on pregnancy and survival rates
 154 following transfer to indigenous ewes.

155

Variable	Number of embryos transferred	Pregnancy Rates	Embryo survival rates at lambing
Stage of development			
Early Morula	19	42.9	15.8 ^b
Morula	22	90.9	59.1 ^a
Early Blastocyst	17	50.0	29.4 ^{ab}
Blastocyst	14	83.3	64.3 ^a
P-value		0.094	0.007
Quality grade of embryos			
Grade 1-2	50	77.3 ^a	36.0
Grade 3-4	22	33.3 ^b	13.6
P-value		0.038	0.090

156

157 *3.2. Recipient ewe's age and CL number on survival rates in ewes*

158 The pregnancy and embryo survival rates at lambing following transfer to recipient ewes of different

ages and with different CL numbers are presented in Table 4. There was no significant effect of recipient ewe's age on pregnancy and embryo survival rates. Pregnancy rate tended to be higher in recipient ewes aged 2 or 3, but the embryo survival rate tended to be higher in recipient ewes of 3 and 4 years of age. There was no difference in pregnancy and embryo survival rates following transfer to recipients with different CL number. However, pregnancy rate and embryo survival rate tended to be higher in recipients with CL 3+ compared to CL 1 and 2.

Table 4

Comparison of different age and CL number of recipient ewes on subsequent pregnancy and survival of transferred embryos

Factors	Number of embryos transferred	Pregnancy Rates	Embryo survival rates at lambing
Age of Recipients			
1	22	60.0 (6/10)	36.4 (8/22)
2	15	80.0 (4/5)	26.7 (4/15)
3	20	80.0 (8/10)	55.0 (11/20)
4	15	60.0 (3/5)	46.7 (7/15)
P-value		0.699	0.353
CL number			
1	6	33.3 (2/6)	33.3(2/6)
2	30	75.0 (9/12)	33.3(10/30)
3+	36	76.9 (10/13)	61.1 (22/36)
P-value		0.133	0.062

4. Discussion

4.1. Effects of embryo transfer methods on embryo survival rate

The purpose of this study was to develop a simple and inexpensive method for transfer of embryos in sheep in Bangladesh. Though there were no significant differences between two transfer methods, pregnancy rates tended to be higher after laparoscopic compared with surgical transfer. Laparoscopic embryo transfer requires a small incision and exteriorization of the tip of a uterine horn (Stefani et al.,

1990; Flores-Foxworth et al., 1992; Ishwar and Memon, 1996; Cognié, 1999). The pregnancy rates were comparable with other studies (Stefani et al., 1990; Dattena et al., 2000; Cseh and Solti, 2001; Martinez et al., 2006). Pregnancy rates following surgical and laparoscopic transfer were 66.7 and 86.6% at 50 days, higher than reported by others (Dattena et al., 2000; Pant et al., 2003; Gibbons and Cueto, 2010). The differences could be due to season and recipient effects. The embryo survival rate ranged from 30.6 - 52.8%, within the range reported by others (Cseh and Seregi, 1993; Pant et al., 2003; Naqvi et al., 2007) but lower than some other observations involving transfer by laparoscopy (McMillan and Hall, 1994). The embryo survival rate was lower than expected: this may be due to the effects of infectious diseases and temperature stress in this sub-tropical region. Lambing and lamb survival rates were 100 and 81.3% following laparoscopic transfer, higher than the findings of others (Amiridis et al., 1999; Naqvi et al., 2007). The differences may be due to season, breed, age and condition of recipients, and stage and quality of embryos at transfer (Dixon et al., 2007; Dixon et al., 2008). The laparoscopic method causes less adhesion formation, and produced embryo survival rates as good as or better than the full surgical procedure. This result is in agreement with others (Walker et al., 1985; Stefani et al., 1990). Transfer method did not affect the gestation length or lamb birth weight, comparable to the findings of Naqvi et al. (2007). Significantly more abortions (33.3%) were observed after surgical embryo transfer: there was no abortion after laparoscopic embryo transfer. The increased abortion after surgical embryo transfer may be due to manipulation and post-operative adhesions (Bolet, 1986; Kleemann and Walker, 2005).

195

This study investigated the effect of the number of transferred embryos in indigenous sheep. Transferring three embryos rather than two increased the lambing rate and embryo survival rate after surgical and laparoscopic methods. Triplet birth was observed only when recipients received three embryos by laparoscopy. This may be due to an interaction between embryos (Nancarrow, 1994). Present results were in accordance with the findings of others (Mutiga, 1991).

201

202 *4.2. Effect of stage and quality of transferred embryos on survival rates in ewes*

The stage of development and quality of embryos has significant effects on survival rate after embryo transfer (Hasler, 1998; Lamb, 2005). Selection of the best embryos at the best stage of development for transfer improves the pregnancy rate. In the present study embryos were transferred to ewes in which oestrus was $\pm$ 4.0 hr synchronous with donor ewes. Pregnancy rate was not affected by stage of

207 development, while embryo survival rate was. Embryos in advanced stages resulted in higher
208 pregnancy rate. This result was in agreement with others (Hasler, 1998; Block et al., 2009). The
209 survival rate of morulae and blastocysts was comparable with other studies (Bari et al., 2003). The
210 absence of any difference in embryo survival rate between morulae and blastocysts indicates that the
211 recipient uterus is equally tolerant to morulae and blastocysts at ± 4.0 hr synchrony of oestrus between
212 donor and recipient ewes (Bari et al., 2003). On the other hand, some researchers reported that
213 blastocysts had higher survival rate than morulae in sheep (Armstrong and Evans, 1983). This
214 discrepancy could be due to transfer of more poor quality or defective morulae compared to blastocysts
215 in those experiments.

216

217 The early morulae represented very retarded embryos, which would have been much less synchronous
218 with recipients. This may have resulted in poorer survival compared to morulae and blastocysts. These
219 early embryos would also be more vulnerable to handling. Their lower survival rate agrees with the
220 literature, suggesting that very early embryos may survive better in recipient ewes that come into
221 oestrus after donors (Donaldson, 1985; Breuel et al., 1991; Bari et al., 2003). Although the literature
222 suggests that recipient ewes, through effects in their uterine environment, can alter the growth of
223 advanced and retarded embryos to match their status, advanced embryos are far better tolerated by the
224 recipient uterus than retarded embryos (Bari et al., 2003). Advanced embryos may be better able to
225 stimulate maternal recognition of pregnancy.

226

227 Pregnancy rate was significantly higher when Grade 1 - 2 embryos were transferred than Grade 3 - 4.
228 Embryo survival rate tended to be higher when Grade 1 - 2 embryos were transferred. All embryos
229 were transferred to recipients with a similar synchrony of oestrus to donors. The greater ability of high
230 grade embryos to alter the uterine environment for their development compared to poor grade embryos
231 is a possible explanation. The effect of grade was in agreement with others (Walker, 1989; Gutierrez-
232 Adan et al., 2004) but not consistent with Bari et al. (2003). This difference could be due to the
233 relatively small number of embryos in the present study.

234

235 *4.3 Recipient ewe's age and CL number on embryo survival rate in ewes*

236 Recipients 2 and 3 years old tended to show higher pregnancy rate than ewes aged 1 or 4 years. This

237 result was comparable to Bari et al. (2003) but in contrast to Wallace (1992). Inadequate CL function
238 may be a cause of low survival rate in ewe recipients. Studies in cattle indicated no better survival in
239 maiden heifers compared with those calved once, when maintained under identical management
240 (Broadbent et al., 1991). They suggested that maiden heifers are preferred as recipients, since they are
241 less likely to be under nutritional stress, or to have a history of health problems.

242

243 There was no significant difference in the pregnancy and embryo survival rates following transfer to
244 recipients with different CL number. However, pregnancy rates tended to be higher in recipients with
245 two or more CL compared to those with only one, comparable with Bari et al. (2003). There was no
246 significant effect of CL number on embryo survival rate in the present study, but the survival rate
247 tended to be higher with higher CL numbers. A certain amount of P4 is essential for embryo survival
248 (Remsen et al., 1982; Niemann et al., 1985; Northey et al., 1985). However, P4 concentration was not
249 measured in the present studies. Further studies should measure P4 concentration in recipients to study
250 the effects of CL number on embryo survival within a MOET programme.

251

252 **5. Conclusions**

253 Laparoscopic embryo transfer is safe and minimally invasive, and may increase embryo survival in
254 sheep. The grade of embryos and CL number of the recipients at transfer may affect the subsequent
255 pregnancy and survival rates of embryos.

256

257 **Acknowledgements**

258

259 The authors thank BAS-USDA Program in Agriculture and Life Sciences (BAS-USDA-PALS),
260 Agargaon, Dhaka-1207, Bangladesh, for financial support of this research work and Dr. W. R. Ward,
261 School of Veterinary Science, University of Liverpool, Leahurst, Neston, CH64 7TE, UK, for
262 reviewing the manuscript with linguistic correction.

263

264

265

266

267 **References**

- 268 Amiridis, G.S., Robertson, L., Reid, S., Boyd, J.S., O'Shaughnessy, P.J., Jef-fcoate, I.A., 1999. Plasma
269 estradiol FSH and LH concentration after dominant follicle aspiration in the cow.
270 Theriogenology 52, 995-1003.
- 271 Armstrong, D.T., Evans, G., 1983. Factors influencing success of embryo transfer in sheep and goats.
272 Theriogenology 19, 31-42.
- 273 Ashworth, C.J., Sales, D.I., Wilmut, I., 1989. Evidence of an association between the survival of
274 embryos and the periovulatory plasma progesterone concentration in the ewe. Journal of
275 Reproduction and Fert ility 87, 23-32.
- 276 Bari, F.Y., Khalid, M., Haresign, W., Murray, A., Merrell, B., 2000. Effect of mating system, flushing
277 procedure, progesterone dose and donor ewe age on the yield and quality of embryos within a
278 MOET programme in sheep. Theriogenology 53, 727-742.
- 279 Bari, F.Y., Khalid, M., Haresign, W., Murray, A., Merrell, B., 2003. Factors affecting the survival of
280 sheep embryos after transfer within a MOET program. Theriogenology 59, 1265-1275.
- 281 Block, J., Bonilla, L., Hansen, P.J., 2009. Effect of addition of hyaluronan to embryo culture medium
282 on survival of bovine embryos in vitro following vitrification and establishment of pregnancy
283 after transfer to recipients. Theriogenology 71, 1063-1071.
- 284 Bolet, G., 1986. Timing and extent of embryonic mortality in pigs, sheep and goats: Genetic variability.
285 In: J. M. Sreonan and M. G. Diskin (Ed.) Embryonic Mortality in Farm Animals. Martinus
286 Nijhoff for CEC. pp 12-43.
- 287 Breuel, K.F., Baker, R.D., Butcher, R.L., Townsend, E.C., Inskeep, E.K., Dailey, R.A., Lerner, S.P.,
288 1991. Effect of breed, age of donor and dosage of follicle stimulating hormone on the
289 superovulatory response of beef cows. Theriogenology 36, 241-225.
- 290 Broadbent, P.J., Stewart, M., F., D.D., 1991. Recipient management and embryo transfer.
291 Theriogenology 35, 125 - 139.
- 292 Cogni'e, Y., 1999. State of art in sheep and goat embryo transfer. Theriogenology 51, 105-116.
- 293 Cseh, S., Seregi, J., 1993. Practical experiences with sheep embryo transfer. Theriogenology 39, 207.
- 294 Cseh, S., Solti, L., 2001. Studies on factors affecting superovulation and embryo transfer in Hungarian
295 merino ewes. Acta Vet Hung 49, 431-441.

296 Dattena, M., Ptak, G., Loi, P., Cappai, P., 2000. Survival and viability of vitrified in vitro and in vivo
 297 produced ovine blastocysts. *Theriogenology* 53, 1511-1519.

298 Dixon, A.B., Knights, M., Winkler, J.L., Marsh, D.J., Pate, J.L., Wilson, M.E., Dailey, R.A., Seidel, G.,
 299 Inskeep, E.K., 2007. Patterns of late embryonic and fetal mortality and association with several
 300 factors in sheep. *J. Anim. Sci* 85, 1274-1284.

301 Dixon, S., Faghih Nasiri, F., Ledger, W.L., Lenton, E.A., Duenas, A., Sutcliffe, P., Chilcote, J., B. ,
 302 2008. Cost-effectiveness analysis of different embryo transfer strategies in England. *Fertility*
 303 *and assisted reproduction*, 758-766.

304 Donaldson, L.E., 1985. Recipients as a source of variation in cattle embryo transfer.
 305 *Theriogenology* 23, 188 (Abstr.).

306 Flores-Foxworth, G., McBride, B.M., Kr Hawk Aemer, D.C., Nuti, L.C., 1992. A comparison
 307 between laparoscopic and transcervical embryo collection and transfer in goats.
 308 *Theriogenology* 37, 213 (Abstract).

309 Gibbons, A., Cueto, M., 2010. Training manual for embryo transfer in sheep and goats. San Carlos de
 310 Bariloche: INTA EEA Bariloche, (Comunicación Técnica PA, n.559). 35.

311 González, R., García Vinent, J.C., Gibbons, A., Cueto, M.I., 1991. Laparoscopic embryo transfer in
 312 Merino sheep in Patagonia (Argentina). In XXIV World Veterinary Congress, Río de Janeiro,
 313 Brazil.

314 Gutierrez-Adan, A., Rizos, D., Fair, T., Moreira, P.N., Pintado, B., de la Fuente, J., Boland, M.P.,
 315 Lonergan, P., 2004. Effect of speed of development on mRNA expression pattern in early
 316 bovine embryos cultured in vivo or in vitro. *Molecular Reproduction and Development*
 317 68, 441-448.

318 Hasler, J.F., 1998. The current status of oocyte recovery, in vitro embryo production, and embryo
 319 transfer in domestic animals, with an emphasis on the bovine. *J. Anim. Sci* 76, 52-74.

320 Ishwar, A.K., Memon, M.A., 1996. Embryo transfer in sheep and goats: a review. *Small Ruminant Res*
 321 19, 35-46.

322 Kleemann, D.O., Walker, S.K., 2005b. Fertility in South Australian commercial Merino flocks: Sources
 323 of reproductive wastage. *Theriogenology* 63, 2075-2088.

324 Lamb, C., 2005. Factors affecting pregnancy rates in an IVF embryo transfer program. *Joint*

325 Proceedings of the AETA and the CETA, 31- 36.

326 Lee, C.S., Fanga, N.Z., Kooa, D.B., Leea, Y.S., Zhenga, G.D., Oha, K.B., Youna, W.S., Hana, Y.M.,
 327 Kima, S.J., Limd, J.H., Shind, S.T., Jine, S.W., Leea, K.S., Koof, J.H., Koof, J.S., Parkg, C.S.,
 328 K. S. Leea, Yoof, O.J., Lee , K.K., 2000. Embryo recovery and transfer for the production of
 329 transgenic goats from Korean native strain. (*Capra hircus aegargrus*). Small Rum. Res 37, 57-
 330 63.

331 Martinez, A.G., Valcarcel, A., Furnus, C.C., de Matos, D.G., Iorio, G., de las Heras, M.A., 2006.
 332 Cryopreservation of in vitro-produced ovine embryos. Small Rumin Res 63, 288-296.

333 McKelvey, W.A.C., Robinson, J.J., 1984. Normal lambs born following transfer of embryos by
 334 laparoscopy. Vet. Rec 115, 230.

335 McMillan, W.H., Hall, R.H., 1994. Laparoscopic transfer of ovine and cervine embryos using the
 336 transpic technique. Theriogenology 42, 137-140.

337 Mutiga, E.R., 1991. Embryo transfer from exotic to indigenous goats in Kenya. In: Disc and Innov.
 338 Acad. Sci. Publ. Vol.1 No.4, 39-43 (1989). Erick Onyango Odada; 1991. Vet. Research Commu
 339 15 315-317.

340 Nancarrow, C.D., 1994. Embryonic mortality in the ewe and doe, in: Zavy M.T., Geisert R. D. (eds.),
 341 Embryonic mortality in domestic species, CRC Press, London, UK. pp.79-98.

342 Naqvi, S.M.K., Joshi, A., Gulyani, R., Kumar, D., Kolte, A.P., Kumar, S., Maurya, V.P., Saha, S.,
 343 Mittal, J.P., Singh, V.K., 2006. Production of prolific microsheep by embryo transfer into large
 344 non-prolific sheep Veterinary Record 159, 522-526.

345 Naqvi, S.M.K., Joshi, A., Kumar, D., Gulyani, R., Maurya, V.P., Saha, S., Mittal, J.P., Sing, V.K., 2007.
 346 Developmental competence, birth and survival of lambs following transfer of twin or triple
 347 embryos of dwarf size prolific donor into large size non-prolific recipient sheep. Journal of Cell
 348 and Animal Biology 1, 082-086.

349 Niemann, H., Sacher, B., Elsaesser, F., 1985. Pregnancy rates relative to recipient plasma progesterone
 350 levels on the day of nonsurgical transfer of frozen/thawed bovine embryos. Theriogenology 23,
 351 631-639.

352 Northey, D.L., Barnes, F.L., Eyestone, W.H., First, N.L., 1985. Relationship of serum progesterone,
 353 luteinizing hormone and the incidence of pregnancy in bovine embryo transfer recipients.
 354 Theriogenology 23, 214 (Abstr.).

355 Pant, D., Choi, J.T., Luther, J.S., Navanukraw, C., Borowicz, p., Weigh, R.M., Kirsh, J.D., Kraft, K.C.,
 356 Redmer, D.A., Reynolds, L.P., Grazul- Bilska, A.T., 2003. Comparison of gravid uterine
 357 parameters in naturally bred ewes and ewes after transfer of in vitro produced embryos, and
 358 single, twin and triplet pregnancies. *Sheep Day Report* 44, 10-17.

359 Remsen, L.G., Rousell, J.D., Karihaloo, A.K., 1982. Pregnancy rates relating to plasma progesterone
 360 levels in recipient heifers at day of transfer. *Theriogenology* 18, 365-372.

361 Shea, B.F., 1981. Evaluating the bovine embryo. *Theriogenology* 15, 31.

362 Silva, J.C., Costa, L.L., Cidadao, R., Silva, J.R., 2003. Plasma progesterone profiles, ovulation rate,
 363 donor embryo yield and recipient embryo survival in native Saloia sheep in the fall and spring
 364 breeding seasons. *Theriogenology* 60, 521.-532.

365 SPSS, 2008. Statistical Package for Social Sciences Windows version 17.0. Chicago, Illinois, 323.

366 Stefani, J.S., Pahla, M.D.C., Christmann, L., Rosa, J.M., Silveira, M.C., Rodrigues, J.L., 1990.
 367 Laparoscopic versus surgical transfer of ovine embryos. *Theriogenology* 33, 330.

368 Thompson, J.G., Bell, A.C.S., McMillan, W.H., Peterson, A.J., Tervit, H.R., 1995. Donor and recipient
 369 ewe factors affecting in vitro development and post-transfer survival of cultured sheep
 370 embryos. *Anim. Reprod. Sci* 40, 269-279.

371 Trounson, A.O., Moore, N.W., 1974. Effect of progesterone and oestrogen on the survival and
 372 development of fertilised ova in the ovariectomised ewe. *Aust. J. Biol. Sci* 27, 511-517.

373 Walker, S., 1989. Collection, culture and pronuclear microinjection of sheep zygotes. Ph.D.
 374 Dissertation. University of Adelaide, South Australia.

375 Walker, S.K., Warnes, G.M., Quinn, P., Seamark, R.F., Smith, D.H., 1985. Laparoscopic technique for
 376 transfer of embryos in sheep. *Australian Veterinary Record* 62, 105-106.

377 Wallace, J.M., 1992. Artificial insemination and embryo transfer. In: *Progress in sheep and goat*
 378 *research*. Ed. Speedy, A. W., CAB International, U. K.

379 Wright, R.W., Jr., Bondioli, K., Grammer, J., Kuzan, F., Menino, A., Jr., 1981. FSH or FSH plus LH
 380 superovulation in ewes following estrus synchronization with medoxyprogesterone acetate
 381 pessaries. *J Anim Sci* 52, 115-118.