Peer-reviewed paper: Proc. 43rd Congress of the South African Society for Animal Science

Embryo transfer using cryopreserved Boer goat blastocysts

K.C. Lehloenya[1#] and J.P.C. Greyling[2]
[1] Department of Animal Science, Tshwane University of Technology, Private Bag X 680, Pretoria 0001, South Africa
[2] Department of Animal, Wildlife and Grassland Sciences, University of the Free State,
P.O. Box 339, Bloemfontein 9300, South Africa

Abstract

The aim of this trial was to evaluate the effect of embryo cryopreservation techniques on the survivability of embryos and fertility following transfer to Boer goat does. The oestrous cycles of 27 mature recipients Boer goat does were synchronised using controlled internal drug release dispensers (CIDR's) for 16 days. At CIDR removal, does were injected with 300 IU eCG. The recipient does were allocated to 3 groups (n = 9 per group), based on the technique of cryopreservation used for the embryos transferred. The *in vivo* produced embryos used were at blastocyst stage and surgically collected on day 6 following AI from Boer goat donors superovulated with pFSH. The first group received fresh embryos and served as the control, the second group of does received conventional slow frozen/thawed embryos and the third group received vitrified/thawed embryos. Two blastocysts were transferred per doe. A pregnancy rate of 85.7% (n = 6) was obtained following the transfer of fresh embryos and tended to be better than in does receiving slow frozen and vitrified embryos, (n = 4; 50.0% and n = 3; 37.5% does pregnant, respectively). The overall gestation period recorded for all does was 146.3 ± 3.0 d, with an overall litter size of 1.7 ± 0.5 being recorded. The kidding rate of the recipient does declined to 57.0% (4) and 25.0% (2) for fresh and conventional slow frozen groups, respectively. An embryo survival rate of 35.7% (n = 5) for fresh, 25.0% (n = 4) for conventional slow freezing and 31.3% (n = 5) for vitrification was recorded and was not affected by the number of CL's present on the respective ovaries at the time of transfer. There was a tendency for more females to be born than males (ratio 1 : 2, male : female) but this could not be related to the cryopreservation technique. Although the pregnancy rate following the transfer of fresh embryos was satisfactory, the embryo survival rate following the transfer of either fresh or cryopreserved embryos tended to be less acceptable. More research is warranted with larger numbers of animals, directed at improving the survivability of embryos following fresh and cryopreserved goat embryo transfer.

Keywords: Boer goat embryos, conventional slow freezing, vitrification,
[#]Corresponding author. E-mail: lehloenyakc@tut.ac.za

Introduction

Cryopreservation of embryos is an important aspect in the whole multiple ovulation and embryo transfer (MOET) programme, which could assist the storage, as well as helping in the improvement of transportation of genetic material across the globe. Goat embryos have generally been cryopreserved using the conventional slow freezing method with different cryoprotectants. Ethylene glycol is generally the preferred cryoprotectant used for slow freezing of goat embryos, due to higher embryo survival rate obtained compared to glycerol or dimethyl sulfoxide (DMSO). However, the embryo survival rate following the conventional slow freezing technique for goat embryos has generally been unsatisfactory (Li *et al*., 1990; Le Gal *et al*., 1993; Guignot *et al*., 2006). Currently vitrification of goat embryos is being attempted with relative success and with better results being reported when goat embryos are vitrified in a combination of ethylene glycol and DMSO. When comparing these two methods of embryo cryopreservation, contradictory results have been reported regarding the survival rate following embryo transfer, although only a few trials have been conducted on goats as such. In some trials similar embryo survival rates have been reported (El-Gayar & Holtz, 2001; Guignot *et al*., 2006). This trial evaluated the effect of embryo cryopreservation techniques on the survivability of embryos following transfer in Boer goat does.

Materials and Methods

Procedures used in this trial were approved by the ethical committee of the University of the Free State. The oestrous cycles of 27 multiparity Boer goat recipients used in this trial were synchronised using controlled internal drug release dispensers (CIDR®; Phamacica & Upjohn, Auckland, New Zealand) inserted intravaginally for a period of 16 days. At CIDR® removal, does were injected with 300 IU eCG (Fostim; Upjohn). All recipient does were teased from CIDR® withdrawal with the aid of vasectomised bucks. Does were then allocated to three groups (n = 9 per group), based on the technique of cryopreservation used for embryos transferred. The first group received fresh embryos (control); the second group received the conventional slow frozen-thawed embryos; while the last group received vitrified-thawed embryos. The *in vivo* produced embryos used were at the blastocyst when surgically collected on day six following AI - from the Boer goat donors superovulated with pFSH.

Blastocysts utilised for this study were obtained from eight Boer goat donors. Conventional slow freezing of embryos was conducted as described by Lewis (1992). Twenty blastocyst embryos were washed in Emcare® holding media and placed into a 1.5 M Emcare® ethylene glycol (ICPbio Ltd, Auckland, New Zealand) solution for a period of five minutes at room temperature to equilibrate. Thereafter, embryos were loaded in groups of two or four into 0.25 mL plastic straws (IMV®, L'Aigle, France). All straws were sealed and placed in a controlled freezer (Freeze Control, Model CL5500; Biogenics, Napa, CA) at -6 °C for one minute and the temperature then decreased to -35 °C, at a cooling rate of 0.5 °C/min and equilibrated for ten minutes. Frozen straws were plunged into liquid nitrogen (LN_2, -196 °C) and stored for two hours before thawing. Twenty blastocyst embryos vitrified were equilibrated in four steps at room temperature (Oberstein *et al*., 2001). Firstly, embryos were placed in a V_1 medium (Hepes + synthetic oviductal fluid [H-SOF]; Sigma, St Louis, MO, USA) and equilibrated for five minutes. Then embryos were placed into the same medium in another Petri dish (V_2) for five to 10 seconds The embryos were then transferred to a V_3 dish (H-SOF + 7.5% ethylene glycol (EG) + 7.5% dimethyl sulfoxide [DMSO]; Sigma, St Louis, MO, USA) for 2.5 seconds and lastly, placed in a V_4 solution (H-SOF + 16.5% EG + 16.5% DMSO +10 mg/ml Ficoll; Fisher Scientific, Ottawa, Ontario, Canada + 0.5 M sucrose) for eight seconds. The 20 embryos in a column of 3 µL V_4 solution were aspirated with a modified Pasteur pipette and released as a drop onto the CryoTips (Irvine Scientific) to form a glass bead in which the embryos were then transferred into half-sealed and pre-cooled straws. These straws were sealed and transferred into liquid nitrogen for at least two hours before thawing.

The conventional slow-frozen embryos were thawed using a sucrose solution in three steps (Lewis, 1992). Embryos from thawed straws were emptied into an empty Petri dish and the contents searched using a stereo microscope. Located embryos were then placed into a Petri dishes containing 0.75 M Emcare® ethylene glycol and 0.5 M Emcare® sucrose for five minutes and then moved to the second dish containing 0.5 M Emcare® sucrose and Emcare® holding medium for another five minutes and finally placed into a dish containing Emcare® holding medium for a period of five minutes at room temperature before transfer. For vitrified embryos, the thawing process was performed by introducing the CryoTip in a water-bath at 37 °C. Embryos were then placed into a thawing medium (H-SOF + 1 M sucrose) for two minutes and kept in a holding medium (H-SOF + amino acids), until transferred to recipient does. Laparoscopic embryo transfer was performed on day six after oestrus. Two embryos loaded into a Tom Cat catheter (AB Technology, Bioniche, USA) connected to a 1 mL syringe were transferred into the uterine horn of each recipient, ipsilateral to the functional CL. Pregnancy diagnosis was performed 40 days following embryo transfer with the aid of a sonographic scanner (Aloka, SSD 500, Tokyo, Japan), using a transrectal 5 MHz linear array probe. Data were analysed and compared using general linear model programme (SAS, 2003).

Results and Discussions

Only one doe from each group (11.1%) did not show any signs of overt oestrus following oestrous synchronisation. The overall response to synchronisation for all groups of 88.9% is comparable to the 97% reported previously in Boer goats during the breeding season (Motlomelo *et al*., 2002). The oestrous response is also in line with the response (80.0 to 94.4%) recorded when the synchronisation treatment is administered for a shorter period of time than in the present study (six to 11 days) (Fonseca *et al.*, 2005). The present results therefore, suggest that the progestagen (CIDR®) used in this study to be an efficient synchronising agent.

One doe from the fresh embryo transferred group became ill before pregnancy diagnosis, and this animal was removed from the trial. The pregnancy and embryo survival rates are set out in Table 1. More

recipients (six of the seven does receiving embryos) were diagnosed pregnant 40 days following embryo transfer in the fresh embryo group although differences were not significant due to the low number of recipient animals. The pregnancy rate of 85.7% following the transfer of fresh embryos was highly acceptable and comparable to a pregnancy rate of 79% recorded in dairy goats (Kiessling *et al*., 1986). The pregnancy rate obtained from this trial was higher, than the range of 27.0 to 60.4% quoted following laparoscopic, surgical and non-surgical transfer of goat embryos (Bessoudo *et al*., 1988; Li *et al*., 1990). These pregnancy rates obtained were also indicative of an acceptable procedure of embryo transfer used in this trial. The differences obtained could also be attributed to the stage of development of the embryos, as in this trial blastocysts were used. In most trials transferable embryos (morulae and blastocysts) were used (Bessoudo *et al*., 1988; Guignot *et al*., 2006), yet it has been observed that maximum survival of goat embryos is obtained at expanded, hatching and hatched blastocyst stages (Li *et al*., 1990). The number of embryos transferred per donor could also be a factor as in this trial only two embryos per recipient were transferred. It has been reported that the transfer of more than two embryos reduce the survival rate of the transferred embryos (Ishwar & Memon, 1996; El-Gayar & Holtz, 2005). The pregnancy rate following the transfer of conventional slow frozen-thawed embryos in this study was also in line with 58% obtained following the transfer of blastocysts cryoprotected with glycerol (El-Gayar & Holtz, 2001). It was however lower (50.0%) when compared to the 73% and 83% reported by Nowshari & Holtz (1995) and Guignot *et al*. (2006). Differences in pregnancy rates have been observed even when embryos at the same stage of development are preserved with similar cryoprotectants - demonstrating the unpredictability of the results following the transfer of slow frozen-thawed goat embryos. The pregnancy rate of (37.5%) following the transfer of vitrified-thawed embryos in the present study, was similar to the 39% and comparable to 52% reported following the transfer of standard vitrified and conventional standard vitrified goat embryos (Guignot *et al*., 2006). The pregnancy rate however, was lower when compared to the 100% obtained by El-Gayar & Holtz (2001) following the transfer of vitrified-thawed embryos. The higher pregnancy rate from the latter study may be due to the use of the open pulled straw (OPS) method of vitrification, which is currently reported to give better embryo survival results (Yuswiati & Holtz, 1990; Begin *et al*., 2003).

Table 1 Mean (± s.e.) pregnancy and embryo survival rate following the transfer of fresh, slow frozen and vitrified Boer goat embryo

Parameters	Embryos transferred		
	Fresh	Slow frozen	Vitrified
Number of embryos transferred	14	16	16
Number of recipients	7	8	8
Recipients pregnant (%)	6 (85.7 ± 1.3)	4 (50.0 ± 2.1)	3 (37.5 ± 2.2)
Mean gestation length (d)	147.5 ± 3.3	145.0 ± 2.8	146.3 ± 1.6
Recipients kidding (%)	4 (57.0 ± 2.0)	2 (25.0 ± 2.2)	3 (37.5 ± 2.1)
Embryo survival rate (%)	5 (35.7 ± 1.1)	4 (25.0 ± 1.0)	5 (31.3 ± 1.2)

No significant differences.

The number of recipient does kidding declined from six (87.5%) confirmed at pregnancy diagnosis (ultrasonography) to four (57.0%) does kidding following the transfer of fresh embryos. This observation may have been the culmination of many factors affecting the recipients or the embryos. From the recipient side, a low serum progesterone concentration from a single CL at transfer may reduce the number of does kidding (Armstrong *et al*., 1983). The decline in the recipients kidding also occurred following the transfer of frozen-thawed embryos in the present trial. Similar tendencies have been reported in goats (El-Gayar & Holtz, 2001). The kidding rate of 25.0% was however lower when compared to the 50%, 69%, 71% and 75% recorded following the transfer of conventional slow frozen-thawed goat embryos in the literature (El-Gayar & Holtz, 2001; Guignot *et al*., 2006). All does diagnosed pregnant kidded, in the group of does which

received the vitrified-thawed embryos – which could point to this method of embryo cryopreservation as being superior in terms of the viability of thawed embryos. Similar tendencies regarding pregnancy success following standard and the OPS vitrification methods have been reported in goats (Guignot *et al*., 2006). The percentage of does kidding (37.5%) in this study is still within the range of 22 to 48% reported previously in goats following the transfer of vitrified-thawed embryos (Yuswiati & Holtz, 1990; Guignot *et al*., 2006). The variation recorded in the literature regarding the success rates indicates that there is still much research needed to perfect the freezing and/or transfer techniques of embryos in goats.

The embryo survival rate following transfer of fresh, frozen-thawed and vitrified-thawed embryos in this study showed a non significant difference. The embryo survival of 37.5% with fresh embryos obtained in this study is in line with the 36.7% and 46.5% previously recorded following fresh goat embryo transfer in other trials (Udy, 1987; Bessoudo *et al*., 1988). The survival rate following transfer of conventional slow frozen-thawed embryos (25.0%), was lower and unsatisfactory but in line with previous findings using ethylene glycol and with other cryoprotectants (El-Gayar & Holtz, 2001; Guignot *et al*., 2006). The low survivability and viability of embryos following conventional slow freezing of goat embryos is often attributed to the sensitivity of the embryos to chilling and the formation of ice crystals during the freezing process (Niemann, 1991; Dobrinsky, 2002). The embryo survival rate following transfer of vitrified-thawed embryos (31.3%) was comparable to the 35 to 44% reported in other goat breeds (Branca *et al*., 2000; Guignot *et al*., 2006). The low embryo survival rate however following vitrification in this study may be attributed to the method used (cryoTips). A higher embryo survival rate of 64% has been recorded in the literature when the OPS method of vitrification was used (El-Gayar & Holtz, 2001). The mean gestation length (147.5 ± 3.3 d) following the transfer of fresh embryos was relatively shorter, when compared to the 151 d recorded in other goat breeds following embryo transfer but, this difference is insignificant (Yuswiati & Holtz, 1990). These observations indicate small differences in the gestation lengths of different goat breeds due to external factors, but all within an acceptable range.

Conclusions

The transfer of fresh goat blastocysts resulted in relatively higher pregnancy rates, while both cryopreservation methods (conventional slow freezing and vitrification) resulted in unsatisfactory low pregnancy rates. All recipients confirmed pregnant following pregnancy diagnosis using ultrasonography in the vitrification group kidded, while not all does from the fresh and slow frozen-thawed embryo groups kidded, thus, reducing the number of recipients kidding in both groups. The survival rate of embryos recorded following fresh, slow frozen-thawed and vitrified-thawed embryos was lower, although comparable to previous findings. It could be concluded that more research is warranted with larger numbers of animals, directed at improving the survivability of embryos following fresh and cryopreserved goat embryo transfer.

References

Armstrong, D.T., Pfitzner, A.P., Warnes, G.M. & Seamark, R.F., 1983. Superovulation treatments and embryo transfer in Angora goats. J. Reprod. Fertil. 67, 403-410.

Branca, A.M., Gallus, M., Dattena, M. & Cappai, P., 2000. Preliminary study of vitrification of goat embryos at different stages of development. In: Proc. 7th Int. Conf. Goats. Tours, France. p. 1032(Abstr.).

Begin, I., Bhatia, B., Baldassarre, H., Dinnyes, A. & Keefer, C.L., 2003. Cryopreservation of goat oocytes and in vivo derived 2 – to 4 – cell embryos using the cryoloop (CVL) and solid-surface vitrification (SSV) methods. Theriogenology 59, 1839-1850.

Bessoudo, E., Davis, L., Coonrod, S. & Kraemer, D.C., 1988. Commercial embryo transfer in Australian Angora. Theriogenology 29, 222 (Abstr.).

Dobrinsky, J.R., 2002. Advancements in cryopreservation of domestic animal embryos. Theriogenology 57, 285-302.

El-Gayar, M. & Holtz, W., 2001. Technical note: Vitrification of goat embryos by the open pulled-straw method. J. Anim. Sci. 79, 2436-2438.

El-Gayar, M. & Holtz, W., 2005. Transfer of sexed caprine blastocysts freshly collected or derived from cultured morulae. Small Rumin. Res. 57, 151-156.

Fonseca, J.F., Bruschi, J.H., Santos, I.C.C., Viana, J.H.M. & Magalhaes, A.C.M., 2005. Induction of estrus in non-lactating dairy goats with different estrous synchrony protocols. Anim. Reprod. Sci. 85, 117-124.

Guignot, F., Bouttier, A., Baril, G., Salvetti, P., Pignon, P., Beckers, J.F., Touze, J.L., Cognie, J., Traldi, A. S., Cognie, Y. & Mermillod, P., 2006. Improved vitrification method allowing direct transfer of goat embryos. Theriogenology 66, 1004-1011.

Ishwar, A.K. & Memon, M.A., 1996. Embryo transfer in sheep and goats: a review. Small Rumin. Res. 19, 35-43.

Kiessling, A.A., Hughes, W.H. & Blankevoort, M.R., 1986. Superovulation and embryo transfer in the dairy goat. JAVMA 188, 829-832.

Le Gal, F., Baril, G., Vallet, J. C. & Leboeuf, B., 1993. In vivo and in vitro survival of goat embryos after freezing with ethylene glycol or glycerol. Theriogenology 40, 771-777.

Lewis, I.M., 1992. Embryo freezing and thawing technologies. Embryo transfer and pregnancy diagnosis, Post graduate committee in veterinary science, University of Sydney, 125-133.

Li, R., Cameron, A.W.N., Batt, P.A. & Trounson, A.O., 1990. Maximum survival of frozen goat embryos is attained at the expanded, hatching and hatched blastocyst stages of development. Reprod. Fert. Dev. 2, 345-350.

Motlomelo, K.C., Greyling, J.P.C. & Schwalbach, L.M.J., 2002. Synchronisation of oestrus in goats: the use of different progestagen treatments. Small Rumin. Res. 45, 45-49.

Niemann, H., 1991. Cryopreservation of ova and embryos from livestock: current status and research needs. Theriogenology 35, 109-124.

Nowshari, M.A. & Holtz, W., 1995. In vitro culture of goat morulae to blastocysts before freezing. Theriogenology 44, 983-988.

Oberstein, N., O'Donovan, M.K., Bruemmer, J.E., Seidel, G.E., Carnevale, E.M. & Squires, E.L., 2001. Cryopreservation of equine embryos by open pulled straw, cryoloop, or conventional slow cooling methods. Theriogenology 55, 607-613.

SAS, 2003. Statistics Analysis System Institute Inc. Carry, NC 27513, USA.

Udy, G.B., 1987. Commercial splitting of goat embryos. Theriogenology 28, 837-845.

Yuswiati, E. & Holtz, W., 1990. Successful transfer of vitrified goat embryos. Theriogenology 34, 629-632.