Pertanika J. Trop. Agric. Sc. 41 (4): 1731-1740 (2018)

PERTANIKA JOURNALS

TROPICAL AGRICULTURAL SCIENCE

Journal homepage: http://www.pertanika.upm.edu.my/

Effect of Recipient Surgery Cycles on Survival Rate of Transferred Embryo in Goats

Md. Rokibur Rahman[1,*], Mohammad Mijanur Rahman[2], Wan Embong Wan Khadijah[3] and Ramli Bin Abdullah[3]

[1]School of Agriculture and Rural Development, Bangladesh Open University, 1705 Gazipur, Bangladesh
[2]Faculty of Agro Based Industry, Universiti Malaysia Kelantan, 17600 Jeli, Kelantan, Malaysia
[3]Faculty Bioresources and Food Industry, Universiti Sultan Zainal Abidin, Kampus Besut, 22200 Besut, Terengganu, Malaysia
[3]Faculty Bioresources and Food Industry, Universiti Sultan Zainal Abidin, Kampus Besut, 22200 Besut, Terengganu, Malaysia

ABSTRACT

An experiment was carried out to evaluate the effect of recipients physiological condition on pregnancy rate and subsequent kidding in does. Oestrus was synchronised by inserting controlled internal drug release device (CIDR, 0.33 g natural hormone progesterone; EAZI-BREED CIDR, Pharmacia & Upjtohn Limited, NZ) and an i.m. injection of 300 IU equine chorionic gonadotrophin (eCG) (Folligon®; Intervet International B.V., EU). A total of 31 Boer crossbred (Boer × local) recipient does were divided into two treatment groups namely intact group (15 does) and used group (63 does). Intact group may be defined as those goats that had not used for any surgery, while used group may be defined as those goats that had been used for surgery such as laparoscopic ovum pick up, embryo transfer. On Day 7 after CIDR removal, ovaries were checked for corpus luteum (CL) and recipient does with functional CL were only used as recipients. After flushing, recovered embryos were examined under a stereomicroscope and only transferrable embryos (morula and blastocyst) were transferred. Uterine horn was exteriorised over the surface of abdomen with the help of a grasping forceps and a surgical blade. One to 3 fresh embryos along with

ARTICLE INFO

Article history:
Received: 27 February 2018
Accepted: 30 August 2018
Published: 14 November 2018

E-mail addresses:
rokibur76@yahoo.com (Md. Rokibur Rahman)
mijanur.r@umk.edu.my (Mohammad Mijanur Rahman)
wkhadi@unisza.edu.my (Wan Embong Wan Khadijah)
ramliabd@unisza.edu.my (Ramli Bin Abdullah)
* Corresponding author

© Universiti Putra Malaysia Press

ISSN: 1511-3701
e-ISSN 2231-8542

a small amount of flushing medium were transferred into the lumen of the anterior part of the uterine horn. Once completed, the uterine horn was placed back into the peritoneal cavity and the incision was sutured. Pregnancy diagnosis was performed at around 30 to 35 days after embryo transfer by using a real-time ultrasound scanner. The average numbers of embryos transferred per recipient doe were 2.60 and 2.56 for intact and used groups, respectively. The percentage of pregnancy was 26.67 for intact group. No pregnancy was recorded in used group. Average embryo survival rate was calculated based on the number of kids born, which was 7.69. Average gestation length was 148 days. From the findings obtained in this research, it could be concluded that intact does were favourable as recipient in ET programme than repeated surgery does.

Keywords: CIDR, embryo survival rate, goats, oestrus, pregnancy diagnosis, recipient surgery cycle

INTRODUCTION

Assisted Reproductive Technologies (ARTs) such as artificial insemination (AI), oestrus synchronisation, superovulation and embryo transfer (ET) had been introduced to overcome reproductive inefficiencies in goats, and accelerate genetic gain (Nicholas, 1996). Using AI technology, benefit from male germplasm could be optimised via pure-breeding and cross-breeding, however, the female potential remained untouched. Superovulation is traditionally considered to be the main approach to utilise the superior female genetic potential effectively in order to produce high number of quality embryos from each donor at a rapid rate. Superovulation and ET is a systematic programme consists of management of donor and recipient does, oestrus synchronisation of donor and recipient does, superovulation of donor does, natural mating or AI, embryo collection by flushing oviduct, evaluation of collected embryos and subsequently transfer of the embryos to the oviduct of recipient does.

Pregnancy and kidding are the last stages as an acid test of *in vivo* development in which the success of any goat superovulation and ET programme depended on. Generally, high numbers of does were needed for transferring embryos in any superovulation programme. A lot of does used for different ARTs such as laparoscopic ovum pick up (LOPU), superovulation and ET research. Recycling as a recipient of that does used for multiple surgeries could supply the abundant number of recipients in the ET programme. Several researchers had studied the effects of different factors such as the quality of embryos (developmental stage and grade), number of embryos transferred, ovulation rate and progesterone level, ovulation and transfer site and the nutrition of the recipient does on the performance on survival rate of transferred embryo (Armstrong, Pfitzner, Warnes, & Seamark, 1983; Bari, Khalid, Haresign, Murray, & Merrell, 2003; Guignot et al., 2006; Ishwar & Memon, 1996; Mani, Watson & Mckelvey, 1994). However, there was no

data to substantiate whether any difference between intact (intact group may be defined as goats those had not used for any surgery) and repeated surgery recipient does with regard to ET performance. Therefore, the present study was designed to evaluate the effect of recipient surgery cycles on survival rate of transferred embryo in goats.

MATERIALS AND METHODS

A total of 31 Boer crossbred recipient does of 17 to 36 kg BW and 24 to 60 months of age from 2 physiological groups, such as intact recipient does (not used for any surgery before) and non-intact does (used for two times embryo flushing) were synchronised to evaluate the effect of recipient does physical condition on pregnancy rate and subsequent kidding. This experiment was carried out at the Institute of Biological Sciences Mini Farm (at a location of 2° 30′ N, 112° 30′ E), the University of Malaya, Malaysia. This location is 60 m above sea level and has an annual rainfall of 2600 mm. Recipient does of used group were prepared 3 months after any surgery. The experimental does were reared under an intensive management system and received fresh soya waste (20% dry matter) at a rate of 1 kg/head/d. The soya waste contained 27.9% crude protein (CP), 30.5% neutral detergent fibre (NDF) and 5.3% ash. Soya waste was offered to the animal once in the morning (8:00 am), while the Napier grass (*Pennisetum purpureum*) was offered in the morning (9:00 am) and afternoon (4:00 pm). All animals had free access to water and salt lick. All recipient does were synchronised by inserting a controlled internal drug release (CIDR) device for 14 days and an intramuscular (i.m.) injection of 300 IU eCG was carried out on Day 12 (Figure 1).

After CIDR removal, oestrus was observed 3 times in a day (morning at 0800-0900 hr, afternoon at 1300-1400 hr and evening 1900-2000 hr) by placing a buck of proven libido. Extra precaution was taken to prevent mating such as buck was kept in separate individual pen. On Day 7 after CIDR removal, embryo flushing was carried out according to Rahman, Rahman, Wan Khadijah and Abdullah (2014). One day before ET, the recipient does were scanned by using ultrasound scanner to confirm non-pregnant by the absence of pregnancy related structures such as placentomes and amniotic fluid (Figure 2). On the day of embryo flushing, recipient ovaries were checked for CL and does with functional CL were only used as recipients.

After flushing, recovered embryos were examined and graded according to their development stage and morphology under a stereomicroscope and only Grade I & II transferrable embryos (morula and blastocyst) were transferred. Uterine horn was exteriorised over the surface of abdomen with the help of a grasping forceps and a surgical blade. One to 3 fresh embryos along with a small amount of flushing medium (0.1-0.2 ml) were loaded in a 1 ml BD Unopette syringe (BD, 2771 Bristol circle, Oakville, ON L6H 6R5) and were transferred into the lumen of the anterior part of the uterine horn ipsilateral to the ovary

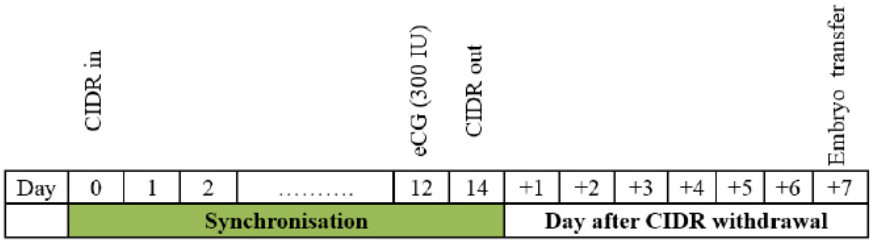

Figure 1. Schematic representation of the oestrus synchronisation protocol for recipient does

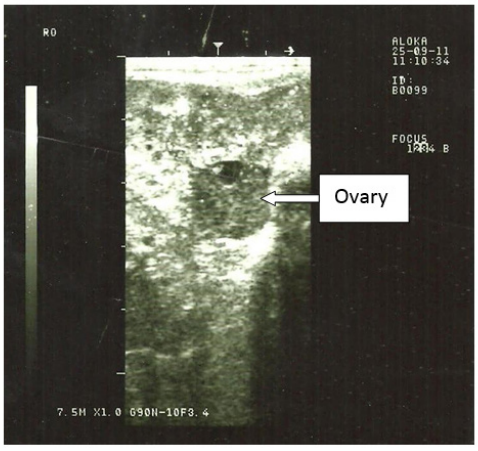

Figure 2. Typical ultrasound image of a recipient doe before embryo transfer

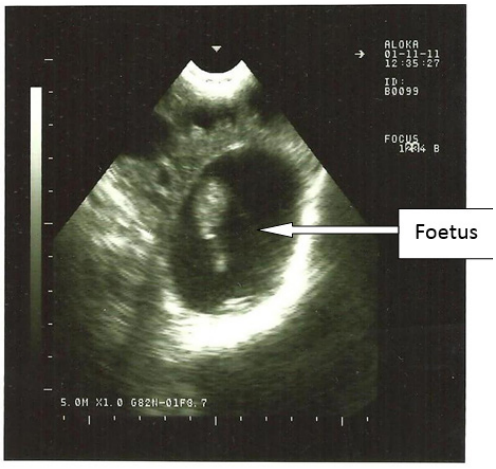

Figure 3. Typical ultrasound image of a pregnant recipient doe after 37 days of embryo transfer

containing a CL through a guided hole prior-punctured using a sterile paper clip. The procedure of embryo transfer was repeated after exteriorising the opposite uterine horn. Once completed, the uterine horn was placed back into the peritoneal cavity and the incision was sutured. Pregnancy diagnosis was performed at around 30 to 35 days after ET by using a real-time ultrasound scanner. Pregnancy rate, kidding and gestation period were calculated. Effect of recipient physical condition on pregnancy was analysed using χ^2 test and results were expressed as the mean ± SEM (standard error of the mean).

RESULTS

Pregnancy rate and subsequent kidding as affected by the physical condition of does i.e. intact does (have not been used for any surgery before) and used does (have been repeatedly used for different surgeries such as LOPU, embryo flushing or ET) are presented in Table 1. A total of 99 does (36 were intact does and 63 were used does) were synchronised, out of that, 31 does (15

Table 1

Effect of recipient surgery cycle on pregnancy rate and kidding

Parameters	Surgery cycle of recipient doe	
	Intact goat	Used goat for different surgery
Number of synchronised recipient does used for embryo transfer	15	16
Total number of transferred embryos	39	41
Average number of embryo transferred/recipient	2.60	2.56
Number of recipient in pregnant	4	0
Pregnancy rate of recipient (%)	26.67[a]	0[b]
Number of recipient delivered kid	2	-
Number of viable young kid	3	-
Embryo survival rate (%)	7.69	-
Average gestation length (day)	148.0±4.0	-

[a,b]Superscripts within rows with different letters differ significantly ($p<0.05$)

intact recipient does and 16 used recipient does) were used. Rest of the recipient was not used due to either have no CL or lack of transferrable embryo. Total 39 and 41 embryos (collected from 47 donors) were transferred to intact and used recipient, respectively. The average numbers of embryos transferred per recipient doe were 2.60 and 2.56 for intact and used groups, respectively. The percentage of pregnancy was 26.67 for intact group. No pregnancy was recorded in used group. In this experiment, only 4 recipient does of produced higher embryo survival rate than earlier stage of embryos in ruminants (Baril et al., 2001; Guignot et al., 2006). However, some earlier researchers found higher survival rates for blastocyst compared to morula in cow (Armstrong & Evans, 1983; Block, Bonilla, & Hansen, 2009; Donaldson, 1985; Hasler, McCauley, Lathrop, & Foote, 1987) and in doe (Li et al., 1990). On the contrary, some other earlier researchers reported that there was no effect of embryo developmental stage on pregnancy rate and subsequent kidding in doe (Gibbons, Cueto,

intact group were pregnant, and out of that 2 does were delivered and another 2 does died before the delivery due to diarrhoea. After ET, pregnancy was confirmed by transabdominal ultrasound scanning at 37 day after ET (Figure 3). The kidding was confirmed by the live born of kids. Average embryo survival rate was calculated based on the number of kids born, which was 7.69. Average gestation length was 148.00±4.00 d.

DISCUSSIONS

Pregnancy and kidding are the last stages which influence the success of any goat superovulation and ET programme. In this experiment, pregnancy rate following fresh embryo transfer was 27% for intact recipient group while no pregnancy was detected for repeated used recipient group. Present result on pregnancy was lower than those of other researchers who reported 79% pregnancy rate in dairy goat (Kiessling, Hughes, & Blankevoort, 1986), 58% (Hong et al., 2007) and 86% (Lehloenya & Greyling, 2010) pregnancy rate in Boer pure bred goat by transferring fresh embryos. On the contrary, present result on pregnancy was in agreement with some other researchers who reported a range of 27 to 60% pregnancy rate following laparoscopic, surgical and non-surgical ET in goat (Bessoudo, Davis, Coonrod, & Kraemer, 1988; Flores-Foxworth, McBride, Kraemer, & Nuti, 1992; Li, Cameron, Batt, & Trounson, 1990). Although only embryos of Grade 1 and II (good grades) were used in the ET program of this study, the reasons of inferior performance of present result were unknown. Many researchers stated that various factors affect on performance including quality of embryos (developmental stage and grade), number of embryos transferred, ovulation rate and progesterone level, ovulation and transfer site and the nutrition of the recipient does (Armstrong et al., 1983; Bari et al., 2003; Guignot et al., 2006; Ishwar & Memon, 1996; Mani et al., 1994).

In the present experiment, number of CL in recipient does was varied from 1 to 4, and the pregnant recipients had only 1 CL on either ovary. However, there were some intact recipients having 1 CL in this experiment did not conceive. Armstrong et al. (1983) reported that there were significant relationships between the number of CL and embryo survivability in recipients does which might be due to increased blood serum progesterone levels. On the contrary, White, Rizzoli and Cumming (1981) and Bari et al. (2003) concluded that recipient CL number in ewe had no effect on embryo survival rate. In normal circumstances, 1 CL is sufficient to secrete progesterone in order to maintain pregnancy in a doe. Therefore, the issue of number of CL affecting pregnancy still cannot be resolved.

In this experiment, morula and blastocyst stage embryos were transferred to the both recipient groups on Day 7 after CIDR removal (Day 6 after oestrus). Previous researchers suggested that morula and blastocysts stages embryos could be transferred into recipient goat on Day 6 after oestrus (Bessoudo et al., 1988; Guignot et al., 2006; Lehloenya & Greyling, 2010) and

& Bonnet, 2011), in ewe (Bari et al., 2003) and in cow (Breuel et al., 1991).

Number of transferred embryos might be a source of variations on pregnancy rate. In this experiment, the average number of embryo transferred per recipient doe was 2.60 and 2.56 for intact and multiple used recipient groups, respectively. Some researchers reported that transferring more than 2 embryos per recipient caused the low survival rate of the transferred embryos (Armstrong et al., 1983; El-Gayar & Holtz, 2005; Ishwar & Memon, 1996; Li et al., 1990). In addition, higher embryo survival rate in dairy goats was obtained after transfer of 2 embryos per recipient (Moore & Eppleston, 1979; Armstrong & Evans, 1983; Tervit, Gold, McKenzie, & Clarkson, 1983). Tervit, Gold and McKenzie (1986) reported that maximum pregnancy rate and kidding could be achieved by transferring 2 blastocysts in each recipient which might be due to the ability of doe to produce multiple kids per pregnancy. On the contrary, Melican and Gavin (2008) had transferred 1, 2 or 3 embryos per recipient during traditional and non-traditional breeding season, and concluded that there was no effect of number of transferred embryo on the pregnancy rate and kidding. They also mentioned that both pregnancy rate and kidding were increased by transferring single embryo to the recipient (Melican & Gavin, 2008).

Embryo survival rate by transferring fresh embryos were 7.69% in this experiment which was much lower than the 36, 37, 47 and 52% reported by earlier researchers by transferring fresh embryo in recipient (Bessoudo et al., 1988; Ishwar & Memon, 1996; Lehloenya & Greyling, 2010; Udy, 1987). The reason of inferior embryo survival rate was not identified, but it might be due to the maternal effect or interaction between transferred embryo and recipient does. All the live born kids were male in this current experiment. Some earlier researchers reported that the observed sex was higher for male kids than female kids. El-Gayar and Holtz (2001) reported that the sex ratio of male and female was 3.5:1 and 2.3:1 for vitrification and conventional freezing, respectively. Melican and Gavin (2008) also reported that the sex ratio of male and female was 2.3:1 and 1.8:1 for fresh embryo transfer during non-traditional and traditional breeding season, respectively. The reason for this result was unknown and more refined research in different aspects of intrinsic and extrinsic factors affecting pregnancy particularly involving male and female embryos/fetuses need to be conducted in future.

The average gestation length in current experiment was 148 days (Day 1 was day of mating and/or AI) which was similar with Lehloenya and Greyling (2010), who reported 148 days of gestation period for Boer goat after transferring fresh embryos. On the other hand, gestation length of doe in this experiment was relatively shorter than the previous finding of Yuswiati and Holtz (1990) who reported 151days of gestation period in Saanen breed following embryo transfer. These observations indicated that small differences in the gestation lengths of different goat breeds due to

external and internal factors such as breeds (gestation period was shorter in lighter breeds of goats) (Mellado, Amaro, Garcia, & Lara, 2000), season of kidding (Ruvuna, Cartwright, Blackburn, Okeyo, & Chema, 1988; Deshpande & Mehta, 1992), litter size (Deshpande & Mehta, 1992; Ruvuna et al., 1988), litter weight and parity (Mellado et al., 2000), but all within an acceptable range. In this experiment, recipient of the repeatedly used goat group was not found as pregnant which might be explained by resistance against gonadotrophin (Baril et al., 1993; Beckers et al., 1990; Remy et al., 1991), surgical trauma during surgery and formation of post-operative adhesions (Ishwar & Memon, 1996; Mckelvey, Robinson & Aitken, 1985; Pereira, Sohnrey, & Holtz, 1998; Suyadi & Holtz, 2000) or some other unknown factors yet to be studied. Moreover, the pregnancy rate and kidding rate from the intact group was rather low, thus there is a possibility that the outcome of this study may be also influenced by the technical skill of ET and animal husbandry and not only due to the repeated used of the recipient for other surgery that impacts their reproductive system.

CONCLUSION

From the findings obtained in this research, it could be concluded that intact does were favourable as recipient in ET programme than repeated surgery does but further detailed research on the latter to enhance their pregnancy efficiency is needed.

REFERENCES

Armstrong, D. T., & Evans, G. (1983). Factors influencing success of embryo transfer in sheep and goats. *Theriogenology, 19*(1), 31–42.

Armstrong, D. T., Pfitzner, A. P., Warnes, G. M., & Seamark, R. F. (1983). Superovulation treatments and embryo transfer in Angora goats. *Journal of Reproduction and Fertility 67*(2), 403–410.

Bari, F., Khalid, M., Haresign, W., Murray, A., & Merrell, B. (2003). Factors affecting the survival of sheep embryos after transfer within a MOET program. *Theriogenology, 59*(5-6), 1265–1275.

Baril, G., Chemineau, P., Cognie, Y., Guerin, Y., Leboeuf, B., Oregeur, P., & Vallet, J. C. (1993). Manuel de formation pour l'insémination artificielle chez les ovins et les caprins [Training manual for artificial insemination in sheep and goats]. *Production et Santé Animales, 83*, 186–188.

Baril, G., Traldi, A., Cognie, Y., Leboeuf, B., Beckers, J., & Mermillod, P. (2001). Successful direct transfer of vitrified sheep embryos. *Theriogenology, 56*(2), 299–305.

Beckers, J. F., Baril, G., Vallet, J. C., Chupin, D., Remy, B., & Saumande, J. (1990). Are porcine follicle stimulating hormone antibodies associated with decreased superovulatory response in goat? *Theriogenology, 33*(1), 192.

Bessoudo, E., Davis, L., Coonrod, S., & Kraemer, D. C. (1988). Commercial embryo transfer in Australian Angora goats. *Theriogenology, 29*(1), 222.

Block, J., Bonilla, L., & Hansen, P. J. (2009). Effect of addition of hyaluronan to embryo culture medium on survival of bovine embryos in vitro following vitrification and establishment of pregnancy after transfer to recipients. *Theriogenology, 71*(7), 1063–1071.

Breuel, K. F., Baker, R. D., Butcher, R. L., Townsend, E. C., Inskeep, E. K., Dailey, R. A., & Lerner, S. P. (1991). Effects of breed, age of donor and dosage of follicle stimulating hormone on the superovulatory response of beef cows. *Theriogenology, 36*(2), 241–255.

Deshpande, S. B., & Mehta, V. M. (1992). Effect of nongenetic factors on gestation length, weight of placenta and placental expulsion time in Surti and Marwari goats. *Indian Journal of Animal Sciences, 62*(2), 155–158.

Donaldson, L. E. (1985). Matching of embryo stages and grades with recipient oestrous synchrony in bovine embryo transfer. *Veterinary Record, 117*(19), 489–491.

El-Gayar, M., & Holtz, W. (2001). Technical note: Vitrification of goat embryos by the open pulled-straw method. *Journal of Animal Science, 79*(9), 2436–2438.

El-Gayar, M., & Holtz, W. (2005). Transfer of sexed caprine blastocysts freshly collected or derived from cultured morulae. *Small Ruminant Research, 57*(2-3), 151–156.

Flores-Foxworth, G., McBride, B. M., Kraemer, D. C., & Nuti, L. C. (1992). A comparison between laparoscopic and transcervical embryo collection and transfer in goats. *Theriogenology*, *37*(1), 213.

Gibbons, A., Cueto, M. I., & Bonnet, F. P. (2011). A simple vitrification technique for sheep and goat embryo cryopreservation. *Small Ruminant Research, 95*(1), 61–64.

Guignot, F., Bouttier, A., Baril, G., Salvetti, P., Pignon, P., Beckers, J. F., … Mermillod, P. (2006). Improved vitrification method allowing direct transfer of goat embryos. *Theriogenology, 66*(4), 1004–1011.

Hasler, J. F., McCauley, A. D., Lathrop, W. F., & Foote, R. H. (1987). Effect of donor-embryo-recipient interactions on pregnancy rate in a large scale bovine embryo transfer program. *Theriogenology, 27*(1), 139–168.

Hong, O. H., Tian, S. J., Zhu, S. E., Feng, J. Z., Yan, C. L., Zhao, X. M., … Zheng, S. M. (2007). Vitrification of Boer Goat morulae and early blastocysts by straw and open-pulled straw method. *Reproduction in Domestic Animals, 42*(1), 34–38.

Ishwar, A. K., & Memon, M. A. (1996). Embryo transfer in sheep and goats: A review. *Small Ruminant Research, 19*(1), 35–43.

Kiessling, A. A., Hughes, W. H., & Blankevoort, M. R. (1986). Superovulation and embryo transfer in the dairy goat. *Journal of the American Veterinary Medical Association, 188*(8), 829–832.

Lehloenya, K. C., & Greyling, J. P. C. (2010). Embryo transfer using cryopreserved Boer goat blastocysts. *South African Journal of Animal Science, 40*(5), 446–450.

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. *Reproduction, Fertility and Development, 2*(4), 345–350.

Mani, A. U., Watson E. D., & Mckelvey, W. A. C. (1994). The effects of subnutrition before or after embryo transfer on pregnancy rate and embryo survival in does. *Theriogenology, 41*(8), 1673–1678.

McKelvey, W., Robinson J., & Aitken, R. (1985). A simplified technique for the transfer of ovine embryos by laparoscopy. *Veterinary Record, 117*(19), 492-–494.

Melican, D., & Gavin, W. (2008). Repeat superovulation, non-surgical embryo recovery, and surgical embryo transfer in transgenic dairy goats. *Theriogenology, 69*(2), 197–203.

Mellado, M., Amaro, J. L., Garcia, J. E., & Lara, L. M. (2000). Factors affecting gestation length in goats and the effect of gestation period on kid survival. *Journal of Agricultural Science, 135*(1), 85–89.

Moore, N. W., & Eppleston, J. (1979). Embryo transfer in the Angora goat. *Australian Journal of Agricultural Research, 30*(5), 973–981.

Nicholas, F. (1996). Genetic improvement through reproductive technology. *Animal Reproduction Science, 42*(1-4), 205–214.

Pereira, R. J. T. A., Sohnrey, B., & Holtz, W. (1998). Nonsurgical embryo collection in goats treated with prostaglandin F2a and oxytocin. *Journal of Animal Science, 76*(2), 360–363.

Rahman, M. R., Rahman, M. M., Wan Khadijah, W. E., & Abdullah, R. B. (2014). Follicle Stimulating Hormone (FSH) dosage based on body weight enhances ovulatory responses and subsequent embryo production in goats. *Asian-Australasian Journal of Animal Sciences, 27*(9), 1270–1275.

Remy, B., Baril, G., Vallet, J. C., Dufour, R., Chouvet, C., Saumande, J., … Beckers, J. F. (1991). Are antibodies responsible for a decreased superovulatory response in goat which have been treated repeatedly with pFSH? *Theriogenology*, *36*(3), 389–399.

Ruvuna, F., Cartwright, T. C., Blackburn, H., Okeyo, M., & Chema, S. (1988). Gestation length, birth weight and growth rates of pure-bred indigenous goats and their crosses in Kenya. *Journal of Agricultural Science, 111*(2), 363–368.

Suyadi, B. S., & Holtz, W. (2000). Transcervical embryo collection in Boer goats. *Small Ruminant Research, 36*(2), 195–200.

Tervit, H. R., Gold P. G., & McKenzie, R. D. (1986). Development of an effective goat embryo transfer regime. *Proceedings of the New Zealand Society of Animal Production, 46*, 233–136.

Tervit, H. R., Gold, P. G., McKenzie, R. D., & Clarkson, D. T. (1983). Techniques and success of embryo transfer in Angora goats. *New Zealand Veterinary Journal, 31*(5), 67–70.

Udy, G. B. (1987). Commercial splitting of goat embryos. *Theriogenology, 28*(6), 837–845.

White, D. H., Rizzoli D. J., & Cumming, I. A. (1981). Embryo survival in relation to number and site of ovulations in the ewe. *Australian Journal of Experimental Agriculture, 21*(108), 32–38.

Yuswiati, E., & Holtz, W. (1990). Work in progress: Successful transfer of vitrified goat embryos. *Theriogenology, 34*(4), 629–632.