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Original Article

Embryo transfer of dorper breed to Mongolian sheep

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ABSTRACT The sheep can be reproduced by natural mating as well as applied reproductive biotechnology, embryo transfer (ET). However, this method in sheep is influenced by several factors such as season, photoperiod, latitude, temperature, nutrition, and breed. In addition, there is still less research on assisted reproductive technologies in small ruminants, compared to other livestock species such as cattle and pigs. Because there has been a need for an optimization and a continuous improvement of ET techniques in small ruminants. the main objective of this study was to evaluate the conception rate obtained after ET in Mongolian sheep (Dorper breed). After embryo recover, code 1 and 2 embryos (morula or blastocyst stage) for ET in the present study were 63% (63/100) and 24% (24/100), respectively. Then Each single embryo was transferred to a synchronized recipient who prepared by estrous synchronization protocol with fluorogestone acetate-cloprostenol sodium. The results demonstrated that an average conception rate and lambing rate was 35.6% (31/87) and 33.3% (29/87), respectively. Further study is still necessary, but these results indicated that single embryo of Mongolian sheep with the present protocol was enough to conducting ET when the genetically superior sheep were necessary to be expanded.

Keywords: embryo transfer, ovine, pregnancy, recipient, synchronization

INTRODUCTION

Many sheep breeds are seasonally polyestrous, and their sexual activities start when daily hours of sunlight decrease. There is not much difference in the follicle-stimulating hormone (FSH) concentration in ewes during the non-breeding and breeding seasons. However, the peak of luteinizing hormone (LH) level is much lower during the non-breeding season than in the breeding season. The progesterone concentration, in particular, is at almost undetectable levels during anoestorus period and is significantly different over the two different seasons. During the anestrous period, both ovaries and the LH pulse production system are inactive (Gordon, 1997). Reproductive activity in sheep is characterized by a seasonality influenced by several factors such as photoperiod, latitude,

temperature, nutrition, and breed.

The sheep can be reproduced by natural mating as well as applied reproductive biotechnology, embryo transfer (ET), in recent days. The first lamb followed by ET using a vitrified embryo was reported by Széll et al. (Széll et al., 1990). Afterward, in sheep and other species, vitrification of embryos was the focus of intense research, and several methodologies were developed; as a result, the numbers of transferred embryos were 406 in goats and 265 in sheep, and France was the nation where ET in these species was the most prevalently conducted (Schiewe et al., 1991; Ali and Shelton, 1993; Brown and Radcievic, 1999). However, there is still less research on assisted reproductive technologies in small ruminants, compared to other livestock species such as cattle and pigs.

But, during the last few years, there has been significant research in small ruminant embryo studies due to the rising importance of these animals in economically fast developing countries such as China and India, and also because of the growing interest in small ruminants, mainly goats, as animals to express recombinant proteins in milk (Paramio and Izquierdo, 2014). The first study demonstrating that sheep embryos could be recovered by nonsurgical embryo transfer (NSET) reported 42% (11/26) of success at almost 30 years ago (Coonrod et al., 1986).

Since ET in sheep is a well-known but insufficiently applied reproductive biotechnology. Therefore, there has been a need for an optimization and a continuous improvement of the techniques (Ciornei et al., 2022). Hence, the main objective of this study was to evaluate the conception rate obtained after ET in Mongolian sheep.

MATERIALS AND METHODS

Experimental animals

The study was carried out in October and November at a commercial sheep farm located in the Bayanjargalan sum of the Tuv aimag province in Mongolia. A total number of 100 clinically healthy Mongolian ewes (3-6-year-old, 5-6 months postpartum, weight of 40-50 kg, and a body condition score of 2.5-3.0) were used.

Embryo evaluation and thawing protocols

The 100 deep-frozen embryos of the Dorper breed were purchased from Mengniu Dairy LLC in China. The embryo was stored in liquid nitrogen tank. Take straw out of goblet and Hold the straw in air (18-23℃) for 3-5 second. Submerge into a 32℃ water bath for an additional 10 seconds. Remove the straw from water bath and Wipe the straw carefully. The straw was clipped and the inner trough with embryo could be removed and transferred into 37° Emcare holding solution (Pacificvet Australia).

Examination of the embryo was performed under a 20-80 magnification using a stereomicroscope. Criteria for embryo evaluation and classification were the same as for bovine embryos and as described in the IETS Manual (4th ed. 2010). The main difference in sheep embryos is that development occurs faster, and blastocyst stages are attained 0.5-1.0 days earlier than in bovine embryos. Embryo quality was evaluated under a numerical combination code based on the morphological integrity of embryos ranging from codes 1 to 4, from excellent and good to bad or degenerated embryos (5th ed. 2020). The excellent and good embryos (code 1 and code 2) were loaded into the embryo capillary in a small volume of holding medium between two air bubbles positioned on both sides of the embryo.

Synchronization protocol for recipient

All recipients and Mongolian ewes were synchronized in accordance with schedule in Fig. 1. The ewes received a 20 mg fluorogestone acetate (FGA; synthetic progestins) intravaginal sponge for 12 days. On day 12, when the intravaginal sponge was removed, ewes received 0.5 mL a synthetic analogue of prostaglandin F2 alpha (PGF2α; cloprostenol sodium, China) on day 12. Recipient ewes were examined 30 days after ET, for early pregnancy diagnosis (embryo survival).

Embryo transfer

Each single embryo was surgically transferred to Mongolian ewe recipients. The embryo was placed in the ipsilateral horn who showed the good quality corpus luteum (CL).

Furthermore, a local anesthetic is applied to the surgical

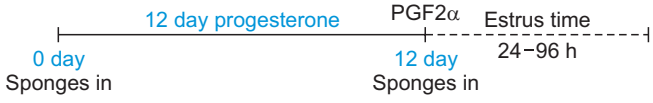

Fig. 1. The combination of drugs used to induce estrus and synchronize ovulation in recipient sheep.

Table 1. Evaluation and classification of sheep embryos

Embryo development	n	Embryo quality							
		Code 1		Code 2		Code 3		Code 4	
		n	%	n	%	n	%	n	%
Morula	32	22	69	6	19	2	6	2	6
Blastocyst	68	41	61	18	26	2	3	7	10
Total	100	63	–	24	–	4	–	9	–

Table 2. Results with embryo transfer recipients

Synchronization of Recipients (n)	In estrus		Received embryo		Conception rate		lambing rate	
	n	%	n	%	n	%	n	%
100	96	96	87	87	31	35.6	29	33.3

area (1 cc lidocaine 2%, Korea). A G-18 disposable, fresh, sterile, hypodermic needle was used to inject the anaesthetic in every recipient included in the study.

The pregnancy rate was confirmed the 30 ± 3 days after ET with transrectal ultrasonography (SonoVet R3, Korea).

RESULTS

The recovered embryos were coded according to the IETS recommended codification (Table 1). From the pool of recovered embryos, code 1 and 2 embryos (morula or blastocyst stage) for ET in the present study were 63% (63/100) and 24% (24/100), respectively; they were vitrificated until ET. The unviable and degenerated embryos as code 3-4 (13/100, 13%) were discarded.

In recipient ewes, estrous behavior appeared 24-96 h after the end of the FGA-cloprostenol treatment. This was considered an essential condition for the transfer of embryos to the recipient ewes. Each single embryo was transferred to a synchronized recipient. The proportion of sheep that received an embryo was 87% (87/100) (Table 2). All recipients had one or two CL on their ovaries, which guaranteed the ovulatory effect of the treatment applied. The transfer of 87 embryos resulted in an average conception rate of 35.6% (31/87), and the lambing rate (LR) was 33.3% (29/87). Because, in most of the world, fall mating with spring lambing is the predominant reproduction management system in the sheep, the management of recipient females in Mongolian farming system could serve successful results for ET program (Fig. 2).

DISCUSSION

Biotechnology of ET is applied to females of superior genetics and aims to increase the frequency of their genes by increasing their progeny. ET allows the transfer of embryos from donors to recipients with low genetic value or embryo freezing for later use. The development of ET in sheep has similarly progressed with other species during the past decades, especially in important breeds of sheep. However, small ruminant ET is a well-described but yet underexploited animal breeding technology, because the rate of an ET protocol in sheep depends on many factors such as photoperiod, latitude, temperature, nutrition, and breed.

In the estrous synchronization method, the function of the CL is simulated by the application of analogous progesterone compounds. The release of gonadotropins is inhibited by progesterone, and hence, ovulation is also inhibited until progesterone is removed. Applied to a group of receptors, it will synchronize estrous and ovulation. Following the hormonal protocol in the Mongolian sheep, the inhibition of mature follicle growth and synchronization of estrous were induced. We consider that the veterinary drugs that were administered to recipients (FGA and cloprostenol) provided a favorable response of 96% (96/100) as shown in Table 2.

The superovulation of Black Suffolk ewes may be affected by the seasonal changes. Generally, the ewe's ovulation rate is higher in May, whereas the viability rate of embryos is higher in September (Shi et al., 2015). Concomitantly, using fresh embryos, Rizzo et al., reported that the best yields were obtained when transferring two blastocysts to recipient ewes with more than two CLs (Rizzo et al., 2016). However, in our study, single embryo was only transferred into the recipient and the lambing rate was 33.3%; further study is still necessary, but it indicated that single embryo in Mongolian sheep was enough to conducting ET when the genetically superior sheeps were necessary to be expanded.

The ET timing and embryo stage were also critical for pregnancy rate, although modulated by the day of the estrous cycle of the recipient at ET. In sheep, results in recipient ewes after ET on Days 6-8 after estrous detection were 54.5% for pregnancy rates and 45.5% for lambing rates (Ledda et al., 1995). The pregnancy rate obtained af-

Fig. 2. Dorper lamb was born using embryo transfer.

ter ET at the morula stage on Day 7 was 40% (Vajta, 2000). The lower value of pregnancy rates (35.6%) in the present study than other reports might be explained by early timing for ET as on Day 6.

CONCLUSION

Both recipient synchronization and embryo transfer show acceptable and encouraging success in Mongolian sheep. We believe that increased number of studies and consequent progress in embryo transfer procedures in the animal will allow massive application of these procedures as has happened in the bovine species.

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