Livestock Studies, 62 (2): 86-91
http://doi.org/10.46897/livestockstudies.1209138

LIVESTOCK STUDIES

RESEARCH PAPER

Superovulatin Peformance and Embryo Recovery in South Anatolian Red Cows

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Article History
Received: 27 September 2022
Accepted: 22 Noevmber 2022
First Online: 24 November 2022

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Keywords
Embryo Transfer
South Anatolian Red
Superovulation

Abstract
South Anatolian Red (SAR) cattle which breeds from Mersin to Şanlıurfa with centered Kilis in the region of South Anatolia, is one of native breeds of Türkiye. The aim of this study is to evaluate the superovulator response and embryo recovery rates after the superovulation protocol applied to the SAR cattle. For this purpose, 10 donors were selected from the conservation herd in the institute. FSH was performed to donors in decreasing doses twice daily over a 4 day period. Before uterine flushing, the ovaries were examined by ultrasound and the structures on them were recorded in order to determine the superovulation response. Each cornu uteri was flushed with foley catheters using a 3-way Y catheter. In the evaluation of embryo recovery, embryos were classified as transferable and non-transferable embryos. The response to superovulation was found as average 7.8 corpus luteum and 2.8 anovulatory follicule for 10 donors. After uterine flushing, 5 UFOs, 3 non-transferable embryos and 2 transferable embryos were obtained from 10 animals. Although the superovulation response was good, the reason for the low embryo recovery rate may be due to the low reproductive performance in SAR cattle. It has been concluded that the hormonal imbalance of these aggressive animals and the difficulties that occur during uterine flushing affect embryo recovery. It was thought that more studies should be done, different superovulation protocols should be tried and OPU technic should be evaluated in order to increase the rate of transferable embryos from SAR cattle. In addition, it was concluded that different techniques should be tried while performing uterine flushing procedures.

Introduction

Animal-originated foods have been one of the fundamental nutritient sources for humankind for ages. Moreover, this condition will maintain as long as human being exists. As a result of the studies, although the productivity increase in the production of animal origin foodstuffs an increase is achieved, some adverse circumstances may also occur. At the beginning of these negativities is the decrease in the sustainable production of local genetic resources. Therefore, the importance of protecting local genetic resources, the essential elements of diversity, increases constantly.

The South Anatolian Red (SAR) breed, which has the best performance in milk yield among the domestic breeds in Türkiye, differs from other breeds in terms of adaptation to warmer and harsher environmental conditions.

This breed is also privileged by having resistance to diseases, especially blood parasites. Formerly, it was reported that the SAR breed was spread over a wide region in the southern region of Türkiye, from Içel in the west to Sanlıurfa and even Hakkari in the east (Göncü, 2005). In 2018 Animal Information System records, the population size of SAR in Türkiye was reported to be smaller than 5500 heads (HBS,2018 October records).

SARs are very noble and elegant-looking cattle. SAR breed has a short neck, an erect head, and prominent and high withers. Moreover, the body is narrow and relatively short. Body color in SAR cattle is generally in combinations ranging from light yellow to dark chestnut red (Yarkın, 1961; Pekel et al., 1990). In SARs, the mean birth weight was variation from 19 to 27 kg. It is reported that an adult's live weight average is 314 kg, but it can

vary between 250 and 424 kg depending on feeding and maintenance conditions (Yarkın, 1961).

Embryo transfer is the process of transferring the embryo or embryos collected from the genital tract of the female donor to one or more synchronized females (Kanagawa et al., 1995; Sağırkaya, 2009). In other words, the transferring of embryos obtained through selected cows and bulls, whose genetic capacity and yield levels have been determined, to carrier cows (Kaymaz, 2015).

One of the most significant techniques to provide rapid genetic progress in dairy cattle and to increase the number of female and male animals with high genetic merits in the herd is embryo transfer applications (Akyol, 2001; Pabuçcuoğlu, 2013, Seidel and Seidel, 1991; Tekeli, 2010). In addition, embryo transfer is one of the leading modern techniques used to increase the success of animal breeding in the most effective way (Bülbül and Dursun, 2005). While only one calf can be obtained from a cow per year, the number of offspring obtained during a year can be increased five times by embryo transfer (Seidel and Seidel, 1991; Tekeli, 2010). The most critical aim of the embryo transfer technique, which consists of a series of biological processes, is to obtain more offspring from high-quality animals, which are healthy and with high genetic capacity, by obtaining high-quality oocytes and embryos (Santos et al., 2008).

The embryo transfer technique is treated by fresh transfer or transfer of frozen embryos that do not have a detrimental effect on embryo viability. The embryo transfer process has become much more accessible through one-step freezing methods using cryoprotectants with high molecular weight (such as sucrose) that do not enter the cell or low molecular weight (Ethylene glycol) that can enter the cell (Palasz and Mapletoft 1996, Massip 2001).

This study aimed to evaluate the success of the superovulation protocol and the embryo recovery rates in SAR cattle. One of the most important reasons for this study is that there has been no previous study on this subject with the SAR local cattle breed. For this purpose, this study will make a great contribution to crossbreeding studies of SAR cattle through the determination of superovulation performance. Thus, crossbreeding studies will be accelerated not only by artificial insemination but also by the embryo transfer method.

Materials and Methods

Ethics Committee approval was obtained for the present study from Adana Veterinary Control Institute Experimental Animals Local Ethics Committee with the decision dated 23.09.2019 and numbered 2019-7/2428.

Donor Selection

The SAR cattle to be used as the donors were selected from the conservated SAR population in the Dogankent Campus of the Eastern Mediterranean Agricultural Research Institute. Ten donors were selected from these cattle in the institute. Before donors were selected, genital organs such as ovaries, uterus and cervix were examined by transrectal ultrasonography using portable the ultrasound (5 MHz, Honda, HS-1001V, Japan). Furthermore, it was determined that they have not any pathological disorders, abnormal uterine structure, and pathological discharge.

Superovulation Protocole

PGF2α (Estrumate, Vet Pharma, Germany) was treated in the cows determined as donors. In 48 hours after PGF2α administrations, animals exhibiting estrus behaviors were detected GnRH (Receptal, Intervet, Germany) was treated in those animals between the 7th and 12th days of the estrus cycle for regression of the dominant follicle, and an ultrasound examination was performed 36-48 hours later after GnRH adminitration. Then, an intravaginal device that releases Progesterone (PRID Delta, 1.55g, Ceva, France) was inserted into the appropriate donors. FSH (Stimufol®, Reprobiol SPRL, Belgium) was received in decreasing doses (100-100 µg, 75-75 µg, 50-50 µg, 25-25 µg) at 12 hours intervals on the 5th day after the PRID was inserted. PGF2α was applied to ensure the regression the corpus luteum at the same time with the 5th and 7th FSH applications. Bulls were utilized to inseminate the cows 12 hours after the last FSH treatment. The superovulation protocol used in the present study was developed taking into account the methods previously described in the literatures (Mapletoft et al., 2002; Merton et al., 2003; Hasler 2004; Bó et al., 2002 and Thangavelu et al., 2007).

Superovulation Success Determination

On the 7th day after the last FSH treatment, uterus flushing was performed in the donors. Before uterine flushing, the number of the corpus luteum (CL) and non-ovulatory (AF) follicles on the ovary was recorded by transrectal ultrasonography using portable ultrasound with a 7.5-MHz linear probe (HS2000, Honda, Japan).

Uterus Flushing

The uterus flushing in donors was performed by using 1000 ml of lactated Ringer's solution (Ringer VIP, Polifarma, Türkiye) containing 0.1% Kanamycin (Kanovet, Deva, Türkiye) and 1% fetal calf serum (FCS) (Sigma, USA). 500 ml of the flushing solution was used for each cornu uteri. After the balloon of the Foley catheter was positioned in the horns of the uteri, each horn of the uteri was filled with 50-100 ml of the solution utilizing a 3-prong Y-catheter and recovered to a sterilized bottle. The fluid, which was given into the cornu uteri and recovered, was collected in sterile bottles. The obtained uterine flushing liquid was examined at the embryo laboratory to find the embryos and evaluate them according to their quality and stages.

Search and Evaluation of the Embryos

The liquid collected in the sterile bottle was filtered by using filters (EMCON filter) (Agtech Zona Filter, Radiated, CAT. #D03, USA) with pores of 70 µm wide for filtration. After washing the filters through holding solution (TCM-199 + 200 mM L-glutamine + 10 mg/ml gentamicin + 20% FCS), the liquid in the filter was taken into 3 Petri dishes (Agtech Square Search Dish, VWR, CAT#D09A, USA). Embryo scans were performed in Petri dishes using heated stereo microscopes (Leica, S8APO, Japan). The embryos were classified according to their quality and developmental stages regarding the evaluation criteria of IETS (Kanagawa et al., 1995; Silva et al., 2009).

Statistical Analysis

SAS software program (SAS Institute Inc., Cary, NC, USA) was utilized for the descriptive statistics in the study.

Results and Discussion

This study was carried out to evaluate the superovulation response and embryo retrieval rate of SAR cattle by FSH hormone treatment twice a day in decreasing doses. Obtained embryos were classified according to the criteria reported by the International Embryo Transfer Society. In terms of response to superovulation in SAR cattle, the mean number of the corpus luteum (CL) per donor was 7.8, and the mean number of novulatory follicle (AF) per animal was 2.8 (Table 1). After the flushing of 10 animals, five (5) unfertilized oocytes (UFO), three (3) degenerated, and two (two) suitable embryos were obtained in the first flushings (Table 2). It was seen that although the superovulation response is quite successful, the success of the embryo recovery rate is low.

Table 1. Response to Superovulation Protocoles

Cow No	Right Ovary		Left Ovary		Total	
	CL	An. Fol.	CL	An. Fol.	CL	An. Fol.
1	2	3	3	1	5	4
2	4	0	4	0	8	0
3	3	1	3	1	6	2
4	3	2	6	2	9	4
5	7	0	3	1	10	1
6	3	2	5	2	8	4
7	3	3	3	2	6	5
8	5	2	1	1	6	3
9	5	1	5	0	10	1
10	3	2	3	2	6	4
TOTAL	**38**	**16**	**36**	**12**	**74**	**28**
MEAN	**3.8**	**1.6**	**3.6**	**1.2**	**7.4**	**2.8**

*CL : Corpus Luteum; An. Fol. : Anovulatory Follicle

Tablo 2. Embryo Recovery after Superovulation

Cow No	Oocyte	Embryo		Total
		Tranferable	Nontransferable	
1	1			1
2			1	1
3				
4			1	1
5		2	1	3
6	2			2
7				
8				
9				
10	2			2
TOTAL	**5**	**2**	**3**	**10**
MEAN	**0.5**	**0.2**	**0.3**	**1**

Considering that the aggressive behavior of these animals can cause hormonal imbalances as well as difficulties in the flushing processes, it was concluded that the aggressive characteristics of SAR cattle may play a role in the low embryo recovery rate. It was thought that more studies that including different superovulation protocols and ovum pick-up (OPU) techniques are needed to increase the transferable embryo recovery rate from SAR animals. In addition, it was concluded that different techniques should be studied for uterine flushing procedures.

Performing a successful superovulation protocol is not only enough for in vivo embryo production. Nutrition, management, and productivity are the other important factors affecting the estrus, superovulation response, and embryo recovery rate (Mapletoft & Bó, 2016). The donors used in this study were from the conservated SAR herd in the institute. Therefore, it was evaluated and fed only for survival rates rather than yield traits.

Tasdemir et al. (2012) reported that the rate of transferable embryo rates for both applications was low in Turkish Native Black cattle, in which they applied FSH in 2 different ways. In the study conducted with Turkish Native Black, the embryo recovery rate was higher than in our study, while the response to superovulation was higher in SAR cattle than those in Turkish Native Black. It is thought that this result may be related to the differences in response to superovulation and ovulation rates of the superstimulated follicles between the breeds.

On the other hand, superovulation success was higher in SAR cattle in this study compared with the study reporting the responses to different superovulation protocols with Turkish Native Black Heifers (Satılmış et al., 2017). Moreover, although the embryo recovery rate was low for both studies, the embryo recovery rate was higher than in our study. It is thought that the difference in superovulation success may be due to the difference in the animal material (heifer-cow) used in the two studies. In addition, it should not be disregarded that the different breeds used in the studies may also have an effect on the results.

It has been reported that excessive stress causes a decrease in the superovulation response or a change in LH increase before ovulation (Bo et al. 2010).. In this study, it should be also considered that manipulations, injections and twice-daily FSH hormone treatment to these animals, which are usualy unmanageable, cause high stress in the aggressive and vicious SAR breed and may have affected the results.

Similarly, superovulation success and embryo recovery rates were evaluated in a study with Tianzhu White Yak cattle, a native breed of China. In the study, the mean number of CLs was 4.75, the number of follicles was 1.13, the number of transferable embryos was 2.50, and the number of non-transferable embryos was 1.38 (Yu et al. 2007). Both in our study and in the of Yu et al.'s (2007) research,, the embryo recovery rate is excessively lower than the world average. On the other hand, it was found that superovulation success was higher in SAR cattle than in Yak cattle. Yu et al. (2007) reported that although they obtained embryos lower in number than the world average, they had a solid understanding of Yak's reproductive physiology through more than 20 years of study, which they claimed as a success. Therefore, this study with SAR cattle is the first study in the literature, and it is an important research area that needs to be studied for many years to obtain more successful results.

It is known that 8 to 12 days after estrus (7 to 11 days after ovulation) second follicular wave starts in animals with two or three follicular waves. It was reported that the second follicular wave day differs between two-wave and three-wave cycles (1 or 2 days before in three-wave cycles) (Bo et al. 2002; Ginter et al. 1989). In this context, it has been clearly shown that superovulation success is higher when super stimulatory treatments are applied as soon as the wave appears (Adams et al. 1994; Bo et al. 2002; Nasser et al. 1993). Initiating the superovulation protocol when a new follicular wave occurs is only suitable for 20% of the estrous cycle (4 or 5 days), while the remaining 80% of the estrus period is not appropriate for an optimal superovulation response. Waiting until the middle phase of the estrus and monitoring oestrus (Bo et al., 2002) are necessary to start the superovulation protocol.

Conclusion

Reproductive characteristics in SAR cattle in this study have not been fully revealed. Moreover, the number of follicular waves in SAR cattle has not been determined yet. Thus, these may have affected the results of the study. For this reason, it was concluded that fully revealing of estrus cycle in the SAR cattle and after the reproductive physiology studies, trying to apply different superovulation protocols suitable for the reproductive characteristics of SAR breed would yield better results.

Acknowledgments

This study is a part of the TAGEM project named' Superovulation Performance and Embryo Freezing in South Anatolian Red Cows' (TAGEM/HAYSSUD/B/20/A4/P1/1639). We would like to thank the Directorate of Eastern Mediterranean Agricultural Research Institute and TAGEM for the opportunities they provided for the study.

Author contributions

All authors contributed equally to this study.

Conflicts of interest

The authors declares that they have no known competing financial or non-financial, professional, or personal conflicts in this paper.

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