Kafkas Univ Vet Fak Derg
25 (5): 627-632, 2019
DOI: 10.9775/kvfd.2018.21472

Kafkas Universitesi Veteriner Fakultesi Dergisi
ISSN: 1300-6045 e-ISSN: 1309-2251
Journal Home-Page: http://vetdergikafkas.org
Online Submission: http://submit.vetdergikafkas.org

Research Article

Effects of Presence or Absence of a Dominant Follicle Estimated by a Single Ultrasound Examination at the Time of Follicular Aspiration on Superovulatory Responses and Embryo Production in Lactating Simmental Cows [1]

Ümüt CİRİT [1,a] Mehmet Ferit ÖZMEN [1,b] Mehmet KÖSE [2,c] İbrahim KÜÇÜKASLAN [2,d]
Elif Merve ÇINAR [3] Hüseyin Gökhan KUTSAL [3]

[1] *This study was carried out within the scope of embryo transfer R & D study protocol for combined breed cattle production signed between TİGEM and Dicle University*

[1] Dicle University, Faculty of Veterinary Medicine, Department of Reproduction and Artificial Insemination, TR-21280 Diyarbakir - TURKEY
[2] Dicle University, Faculty of Veterinary Medicine, Department of Obstetrics and Gynecology, TR-21280 Diyarbakir - TURKEY
[3] TIGEM Ceylanpinar Directorate of Agricultural Enterprise, Ceylanpinar, TR-63570 Sanliurfa - TURKEY
[a] ORCID: 0000-0002-0187-2615; [b] ORCID: 0000-0002-5531-220X; [c] ORCID: 0000-0003-0070-8458; [d] ORCID: 0000-0002-3458-4409

Article Code: KVFD-2018-21472 Received: 05.12.2018 Accepted: 15.03.2019 Published Online: 15.03.2019

How to Cite This Article

Cirit Ü,A Özmen MF, Köse M, Küçükaslan İ, Çinar EM, Kutsal HG: Effects of presence or absence of a dominant follicle estimated by a single ultrasound examination at the time of follicular aspiration on superovulatory responses and embryo production in lactating Simmental cows. *Kafkas Univ Vet Fak Derg,* 25 (5): 627-632, 2019. DOI: 10.9775/kvfd.2018.21472

Abstract

The aim of the study was to evaluate effects of presence or absence of a dominant follicle (DF) estimated by a single ultrasound examination at the time of follicular aspiration (FA) on superstimulatory and superovulatory responses and embryo production in lactating Simmental cows. At random stages of the estrous cycle, the ovaries of cows (n=42) were examined by transrectal ultrasonography (US) and all follicles ≥3 mm were counted. Donors with <10 follicles 3-8 mm in diameter were considered to have a dominant follicle (group DF+; n=30), while donors ≥10 small follicles 3-8 mm were classified as having no dominant follicle (group DF–; n=12). Just after US examination, all cows were subjected to ultrasound-guided transvaginal aspiration of all follicles ≥5 mm and a progesterone-releasing device was placed in the vagina. Thirty-six h after FA, all cows were superstimulated with FSH, which was given as twice-daily injections over 6 days. Cows were pre-treated with a single dose of 400 IU of eCG 24 h before the start of FSH treatments. It was concluded from this study that the presence of a DF estimated by a single ultrasound examination at the time of FA effects negatively the superstimulatory and superovulatory responses, fertilization rate and embryo quality (P<0.05) but not the number of embryos collected. It was also concluded that estimation of a dominant follicle by a single ultrasound examination at the time of follicular aspiration based on the number of small follicles may be used the selection of potential donor cows and can significantly contribute to improvements in superstimulatory and superovulatory responses and embryo quality.

Keywords: *Superovulation, Cattle, Follicle aspiration, eCG, FSH*

Laktasyodaki Simental İneklerde Follikül Aspirasyonu Zamanında Tek Bir Ultrason Muayenesi İle Tahmin Edilen Dominant Follikül Varlığı veya Yokluğunun Süperovulatör Cevaplar ve Embriyo Üretimi Üzerindeki Etkileri

Öz

Bu çalışmanın amacı, laktasyondaki Simental ineklerde follikül aspirasyonu (FA) zamanında tek bir ultrason muayenesi ile saptanan dominant follikül (DF) varlığı veya yokluğunun süperstimülatör ve süperovulatör cevaplar ve embriyo üretimi üzerindeki etkilerinin değerlendirilmesiydi. Östrus siklusunun tesadüfi aşamalarında, ineklerin (n=42) ovaryumları ultrason (US) ile muayene edilerek ≥3 mm tüm folliküller sayıldı. Üç-8 mm çapında <10 küçük follikülü olan inekler DF'ü var olarak (DF+ grubu; n=30), ≥10 küçük follikülü olan inekler ise DF'ü yok olarak (DF– grubu; n=12) sınıflandırıldı. Ultrason muayenesinden hemen sonra tüm ineklerin ≥5 mm tüm folliküller aspire edildi ve vajina içine bir progesteron salıverici araç yerleştirildi. Tüm ineklere, follikül aspirasyonundan 36 saat sonrasından başlanarak 6 gün boyunca günde 2 kez FSH uygulandı. İneklere FSH uygulamasının başlamasından 24 saat önce 400 IU tek doz eCG ile ön tedavi uygulandı. Çalışmadan, FA günü tek bir ultrason muayenesi ile tahmin edilen DF varlığının süperstimülatör ve superovulatör yanıtları, fertilizasyon oranını ve embriyo kalitesini olumsuz etkilediği (P<0.05), fakat toplanan embriyo sayısını etkilemediği sonucuna varıldı. Çalışmadan ayrıca, FA günü yapılan tek bir ultrason muayenesinde küçük folliküllerin sayısına göre DF varlığının tahmin edilmesi işleminin potansiyel vericilerin seçiminde kullanılabileceği ve süperstimülatör ve superovulatör cevaplar ve embriyo kalitesinin arttırılmasında önemli katkıları olabileceği sonucu çıkarıldı.

Anahtar sözcükler: *Süperovulasyon, Sığır, Follikül aspirasyonu, eCG, FSH*

İletişim (Correspondence)
+90 412 2411000/8622
ucirit@dicle.edu.tr

INTRODUCTION

Major factors that influence the superstimulatory response in cattle are the status of the follicular wave (FW) at the start of the superovulatory program and the number of follicles ≥3 mm at the beginning of FSH treatments [1]. The ideal time to start FSH treatment in a program is at the emergence of the FW [2]. There are many methods to regulate the time of FW emergence, such as ablation of the dominant follicle (DF), treatment with a combination of estradiol and progesterone (P4) and GnRH treatment [2]. It has been stated that the most reliable method to synchronize wave emergence for superstimulation is follicle aspiration (FA) [2] and that is applied generally at 24, 36 or 48 h before the onset of superstimulation [3]. Aspiration of the DF results in a surge of FSH 1 day after aspiration, with a peak occurring 2 days later, causing the emergence of a new FW [1]. Although it is difficult to utilize in the field, the use of FA technology for *in vivo* embryo production is becoming increasingly common. However, it has been suggested that the synchronization of follicular wave emergence may not be the only requirement for successful superovulation. It was reported that superovulatory response was most dependent on the numbers of follicles entering the wave and a simple ultrasound examination at the start of superstimulatory treatments was highly predictive of the subsequent superovulatory response [4,5]. However, to our knowledge, there is no study investigating the effects of ovarian status at the time of FA on superstimulatory response and in vivo embryo production in cattle.

The goal of superstimulatory treatment is to induce the growth of multiple follicles to produce multiple competent oocytes capable of developing into transferable embryos [5,6]. However, the most significant limiting factor in the success of superovulation has been and continues to be the unpredictability, due to high between-individual variability, in the ovarian response to gonadotropin stimulation [7]. It has been stated that one-third of the donors treated do not answer to superovulation, another third yields an average of one to three embryos and only one-third actually superovulates giving a large number of embryos [8]. Therefore, selection of donors is very important for the success of superovulation. The presence of a DF before superstimulatory treatment decreases superovulatory response [4,9]. However, in order to predict superovulatory response by means of this relation, it is necessary to first monitor the ovaries of donor cattle for at least 4 consecutive days before the initiation of superstimulatory treatment, and confirm whether a follicle is functionally dominant or not. This is impractical under field conditions [9]. However, Bungartz and Niemann [4] clearly demonstrated that the presence or absence of a DF can be determined by a single ultrasound examination using the number of small follicles (3-8 mm in diameter). The researchers also reported that a cow with a DF and more than 10 small follicles have never been observed [4]. Therefore, it has been suggested that potentially good and poor responders to superovulation and the exclusion of poor responders from superovulatory treatment can be diagnosed by a single ultrasound examination at the start of superstimulatory treatments [4].

Knowing the effects of ovarian status at the time of FA on in vivo embryo production may allow eliminating of poor responders from superovulation program and contribute to increase superovulation success. Therefore the aim of the study was to evaluate effects of presence of a DF detected by a single ultrasound examination at the time of FA performed at 36 h before the start of FSH treatments on superstimulatory and superovulatory responses and embryo production in lactating Simmental cows.

MATERIAL and METHODS

All procedures were approved by the Animal Experiments Local Ethics Committee of Dicle University (Approval No: 92406). The study was completed in four replicates. This study was conducted at Ceylanpinar Directorate of Agricultural Enterprise under General Directorate of Agricultural Enterprises (TIGEM) (Şanlıurfa, Türkiye) from March to June 2018. Forty-two lactating Simmental cows housed in a free-stall facility, with a body condition score (BCS) of 3 to 4.5 (1-5 scale), 500 to 650 kg of body weight, milk production of 28.2±0.79 kg/day (mean±SEM) and >60 days in milk were used. The donor cows were maintained under the same nutritional, management and environmental conditions. All the cows underwent a gynecological examination before the commencement of the study.

Follicle Aspiration (FA) and Superovulation

At random stages of the estrous cycle, the ovaries of each cow were examined by transrectal ultrasonography (US, 8-MHz linear-array transducer; Esaote Pie Medical Aqulia, Türkiye) and all follicles ≥3 mm were counted. The criterion for the presence or absence of a dominant follicle (DF) on the day of FA was the number of small follicles 3-8 mm in diameter. Donors with <10 follicles 3-8 mm in diameter (on both ovaries) were considered to have a DF (group DF+; n= 30), while donors ≥10 small follicles 3-8 mm were classified as having no DF (group DF–; n= 12) [4]. Just after the US, cows were subjected to FA to synchronize the emergence of a new follicular wave [10,11]. Prior to FA, each cow received epidural anesthesia (5 to 7 mL of 2% lidocaine; Vilcain, Vilsan, Türkiye) to decrease peristalsis and discomfort [12]. All ovarian follicles ≥5 mm were aspirated *(Fig. 1)* with a 18-gauge disposable needle by using the ultrasound-guided transvaginal approach with a 7.5-MHz convex-array transducer (Nutricell/Esaote-Pie Medical, Campinas, Brazil) by an experienced researcher (ÜC). A progesterone-releasing device (1.38 g progesterone, CIDR; Zoetis Animal

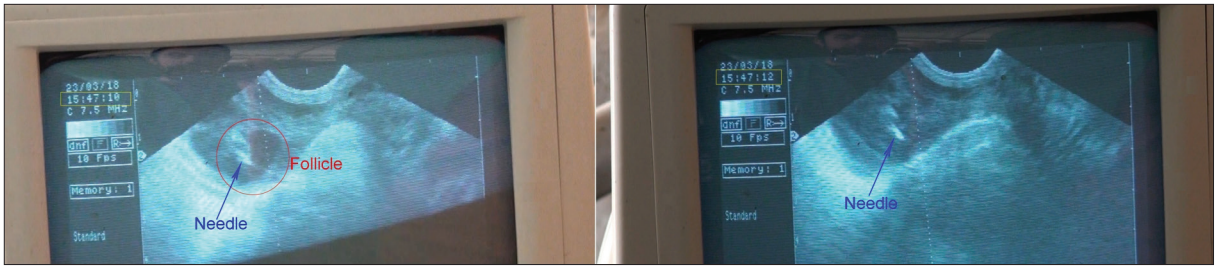

Fig 1. Ultrasound images of an ovary just before *(left image)* and after follicle aspiration *(right image)*

Table 1. *Schematic illustration of the superovulation protocol*

D-1.5	D-1	D0	D1	D4	D5	D6	D7	D8	D14
← CIDR →									
		← FSH →							
US	eCG			PGF (am)		hCG (pm)	TAI+PGF (am)	TAI (am)	US
FA				PGF (pm)		US (pm)	TAI (pm)		Flushing

D: *days;* ***FA:*** *follicle aspiration;* ***TAI:*** *timed artificial insemination;* ***PGF:*** *prostaglandin F2α;* ***US:*** *ultrasound*

Health, Türkiye) was placed in the vagina immediately after FA *(Table 1)*. Approximately 36 h (24 to 48 h) after FA, all cows were superstimulated with a total of 500 µg porcine FSH (pFSH, Stimufol; Reprobiol SPRL, Belgique), which was given as twice-daily injections over 6 days on a decreasing dose schedule (75, 65, 50, 50, 40, 40, 35, 35, 30, 30, 25 and 25 µg) [13]. Cows were pre-treated with a single dose of 400 IU of equine chorionic gonadotropin (eCG, Folligon, im; MSD Animal Health, Türkiye) 24 h before the start of FSH treatments. Cows received prostaglandin F2α (PGF, 25 mg, Dinolytic, im; Zoetis Animal Health, Türkiye) concomitant with the ninth and tenth FSH treatments. The CIDR was removed at the time of the last FSH treatment (36 h after the first PGF) and 24 h after CIDR removal, ovulations were induced with 1500 IU of human chorionic gonadotropin (hCG, Chorulon, im; MSD Animal Health, Türkiye). Timed artificial inseminations (TAI) were performed thrice at 12, 24 and 36 h after hCG treatment using previously tested frozen semen from two different bulls [13]. All cows were treated with PGF concurrent with the first TAI. The ovaries of each cow were examined by transrectal ultrasonography concomitant with hCG treatments to determine the number and size of follicles and positive response to superstimulatory treatment was considered when three or more ovulatory follicles ≥9 mm were found.

Ova/embryo Collection

Prior to flushing, each cow received epidural anesthesia (5 to 7 mL of 2% lidocaine; Vilcain, Vilsan, Türkiye). Ova/embryos were collected non-surgically 6.5 to 7 days after the first TAI using lactated Ringer's solution containing 1% calf serum (CS, N4762; Sigma-Aldrich, USA) and 125 mg/L of kanamycin (Kanovet, Vetaş, Türkiye) [14,15]. Each uterine horn was flushed with 800 to 1000 mL of the solution. A two-way Foley catheter and the interrupted-syringe technique were used for flushing. The aspirates were poured into embryo collection filters (EZ, 017726, IMV, Şark Kemikal, Türkiye). Recovered ova and/or embryos were evaluated for developmental stage and quality at x 50 magnification (Leica S8 APO) using the criteria of the International Embryo Technology Society (IETS) [16]. Embryos were defined as transferable (Grades 1, 2, and 3) and freezable (Grades 1 and 2) [17]. Ovaries were examined with transrectal ultrasonography (8-MHz linear-array transducer; Esaote Pie Medical Aqulia, Türkiye) to determine the presence and number of CL after embryo collection. Animals with 3 or more CL at the time of embryo collection were considered to have responded to the superovulatory treatment [18].

Statistical Analyses

Data were analyzed using a general linear model (GLM) procedure of SPSS. The initial statistical model included study groups as fixed effect and the time of FA (24 to 48 h) BCS, days in milk and milk yield as covariates. The time of FA, BCS, days in milk and milk yield were found insignificant effect as covariates. The non-significant covariates were not included in the final statistical model. The numbers of follicles, total ova/embryos, degenerate, transferable and freezable embryos, fertilized and unfertilized ova and CL were compared using independent samples t-test. Proportional data were compared using Chi-square test [19]. Results were expressed as mean±SEM and differences were accepted as statistically significant when $P<0.05$. Statistical analyses were performed using SPSS 10.0 for Windows (SPSS Inc., Chicago, IL, USA). In group DF+, abnormal vaginal discharge was determined in 4 cows during the period between the first TAI and embryo collection and these cows were excluded from the statistical analyses where superovulatory responses and embryo production were compared.

Table 2. *Mean (±SEM) number of follicles ≥ 9 mm and < 9 mm on the day of hCG treatment in DF+ and DF– groups*

Examined traits	Group DF+ (n= 30)	Group DF– (n= 12)	P value
Number of follicles ≥9 mm	15.1±1.30	23.1±3.20	<0.01
Number of follicles <9 mm	3.0±0.51	3.0±0.39	>0.05
Total number of follicles	18.1±1.50	26.1±3.00	<0.05
Percentage of superstimulatory response (%)	100	100	>0.05

Table 3. *Superovulatory responses and embryo production (mean±SEM) in DF+ and DF– groups*

Examined Traits	Group DF+ (n= 26)	Group DF– (n= 12)	P value
Number of CL on the day of embryo collection	19.4±1.60	26.8±3.31	<0.05
Percentage of superovulatory response (%)	100	100	>0.05
Recovery rate (%)*	57.7	49.4	<0.05
Total ova/embryos recovered	11.2±1.70	13.3±1.47	>0.05
Unfertilized ova	2.1±1.01	0.7±0.36	>0.05
Fertilized ova	9.0±1.27	12.6±1.29	=0.95
Percentage of fertilized ova (%)	81.0	95.0	<0.0001
Degenerate embryos	2.2±0.69	3.2±0.77	>0.05
Transferable embryos (Grades 1-3)	6.7±0.94	9.3±1.18	>0.05
Percentage of transferable embryos (%)	60.3	70.4	<0.05
Freezable embryos (Grades 1 and 2)	5.7±0.91	7.7±1.10	>0.05
Percentage of freezable embryos (%)	51.4	57.9	>0.05

** Total ova and embryos recovered/number of CL detected*

RESULTS

The mean (±SEM) number of ≥9 mm follicles on the day of hCG treatment ranged from 4 to 27 in group DF+ and from 12 to 50 in group DF–. The mean number of ≥9 mm follicles (P<0.01) and total numbers of follicles (P<0.05) on the day of hCG treatment were significantly higher in DF– group than in DF+ group *(Table 2)*.

The mean number of CL at the time of embryo collection were significantly higher in DF– group (26.8±3.31, ranged from 9 to 50) than in DF+ group (19.4±1.60, ranged from 6 to 39) (P<0.05, *Table 3)*.

However, the cows in DF– group had a decreased percentage of ova/embryo recovery than cows in DF+ group (P<0.05). The mean numbers of cows with ≥3 CL (superovulatory response), ova/embryos recovered, unfertilized ova, degenerate embryos, transferable embryos and freezable embryos did not differ between groups (P>0.05, *Table 3)*. However, the cows in DF– group had more increased percentages of fertilized ova (P<0.0001) and transferable embryo (P<0.05) than cows in DF+ group.

DICUSSION

One of the most important advantages of using embryo transfer is acceleration of the dissemination of desirable genetics by increasing the number of offspring obtained from donors with high genetic value [20-23]. It has been well known that the presence of a DF at the start of superstimulatory treatments represses the growing of subordinate follicles and affects the superovulatory response and embryo quality negatively [4]. Ultrasound-guided FA to eliminate the DF is usually performed at random stages of estrus, and the number of collected viable embryos increases when superstimulatory treatments are initiated 24-48 h after FA [3]. However, the effects of estrus cycle and/or follicular status during FA on superovulatory responses and embryo production are unknown. To the best of our knowledge, this is the first study investigating the effects of ovarian status at the time of FA on superstimulatory response and embryo production in cows. Since FA eliminates the DF, it may be expected that the superstimulatory and superovulatory response of cows with or without a DF will be similar. However, the cows estimated to have a DF at the time of FA had lower superstimulatory (mean number of ≥9 mm follicles on the day of hCG treatment; P<0.01) and superovulatory (number of CL at the time of embryo collection; P<0.05) responses than the cows without a DF. A possible explanation of these results may be related to the DF evaluation method. Animals with more than ten small follicles (3-8 mm) were evaluated as being without a DF [4]. This evaluation method allows us to have information about

both the presence of a DF and the number of small follicles in the ovaries. It has been reported that the main factor that affects the superovulatory response is the number of small follicles at the beginning of ovarian superstimulation with gonadotropins [5,24,25]. It was shown that cows with high antral follicle counts (≥3 mm) had significantly more ovulations and produced a greater number of transferable embryos than those with low antral follicle counts [5,24]. Another possible explanation of these results could be that although DF were eliminated with FA, the metabolic products that DF had previously given to the blood might have been continued to exert a repressive effect for a while longer on follicle development.

The fact that the cows in DF– group had an increased number of ≥9 mm follicle on the day of hCG treatment and CL at the time of embryo collection suggested that some of these 3-8 mm follicles present at the time of FA continued growing in response to FSH treatments and ovulated. Similarly, it has been suggested that some of these follicles that are 3-6 mm in diameter before superstimulatory treatment continue growing in response to exogenous gonadotropin, and eventually ovulate [9]. Therefore, it may be postulated that the larger the number of small follicles at the time of FA carrying out 36 h before the start of FSH treatments, the larger the number of follicles that will respond to exogenous gonadotropin and the resulting superovulatory response will be better.

Although the mean numbers of ≥9 mm follicles on the day of hCG treatment and CL at the time of embryo collection were significantly higher in DF– group than in DF+ group ($P<0.05$), the mean numbers of ova/embryos recovered, unfertilized ova, degenerate embryos, transferable embryos and freezable embryos did not differ between groups ($P>0.05$). The possible explanation of this can be that the cows in DF– group had a decreased percentage of ova/embryo recovery than cows in DF+ group ($P<0.05$). Similarly, it has been reported a negative correlation between the number of superovulated follicles and recovery rate [26]. One of the most remarkable results of our study was that the cows in DF– group had more increased percentages of fertilized ova ($P<0.0001$) and transferable embryo ($P<0.05$) than cows in DF+ group, while the mean numbers of ova/embryos recovered and transferable embryos were similar in both groups. These results suggest that the presence of a DF at the time of FA effects negatively the superstimulatory and superovulatory responses, fertilization rate and embryo quality but not the number of embryos collected.

Based on the American Embryo Transfer Association (AETA) survey report, the average number of viable embryos recovered from dairy donors was 5.7 (58% of the total ova/embryos recovered) and from beef donors was 6.9 (54%) in 2017 [27]. Previous studies, in which conventional 4 or 5-day programs were applied, reported that mean number of ova/embryos ranged from 10.2 to 20.5, number of transferable embryos from 4.0 to 12.8 and percent transferable from 39.2% to 77% in Simmental cows [28-34]. In a study analyzing the records of 1596 embryo collections from cows from 13 breeds, the mean number of total and transferable embryos and the rate of transferable embryos were reported as 13.1, 6.6 and 51%, respectively in Simmental cows [28]. However, except for one [29] of these studies, the lactation status (lactating or dry) of the Simmental cows is unclear. The mean numbers of transferable embryos in the present study (6.7 and 9.3 in DF+ and DF– groups, respectively) were comparable to those reported in the literature for Simmental cows [13,28-34]. Besides, the percentage of transferable embryos in DF– group (70.4%) were higher than those of most of the studies [29-33] and than general average reported for Simmental and beef cows [27,28].

One of the most striking results of our study was that fertilization rates were found to be high, especially in DF– group (81.0% and 95.0% in DF+ and DF– groups, respectively). Based on the AETA survey report, the average number of unfertilized ova recovered was 3.1 (29% of total ova/embryos) from dairy donors and 3.5 (27%) from beef donors [35]. Roussel et al.[36] examining nonsurgical embryo recoveries from 1116 beef and dairy cows from 15 different breeds over a 7-yr period reported that combining all embryo collections resulted in 31% unfertilized ova, 11% degenerate embryos and 58% transferable embryos. Similarly, previous studies reported that mean fertilization rates ranged from 65.6% to 79.4% in Simmental cows [29,30,34]. In the present study, in which a superovulation program lengthened to 7 days with eCG pre-treatment was used, cows were timely inseminated three times 12, 24 and 36 h after hCG injection and treated with PGF concurrently with the first TAI. It has been showed that treatment with PGF during the late growing phase of the DF of a wave can result in ovulation by a luteolysis-independent mechanism in prepubertal heifers [37]. Similarly, Ambrose et al.[38] reported that PGF treatment concurrent with AI significantly increased conception rate in dairy cows. The result of the study indicated that FA, lengthened superstimulation program, timings of P4 withdrawal and hCG treatment (P36LH60 strategy), three times AI and/or PGF treatment concurrent with the first TAI might have contributed to high fertilization rates either alone or combined. Besides these factors, it seems that the presence of a DF estimated by a single ultrasound examination at the time of FA also affects fertilization rates and, decreases fertilization rates significantly ($P<0.0001$).

It was concluded from this study that the presence of a DF estimated by a single ultrasound examination at the time of FA effects negatively the superstimulatory and superovulatory responses, fertilization rate and embryo quality but not the number of embryos collected. It was also concluded that estimation of a DF by a single ultrasound examination at the time of FA based on the

number of small follicles may be used the selection of potential donor cows and can significantly contribute to improvements in superstimulatory and superovulatory responses and embryo quality in Simmental cows. Further studies including different breeds are needed to determine the effect of ovarian status at the time of FA.

REFERENCES

1. Surjus RS, Prata AB, Borsato M, Mattos FCSZ, Martins da Silveira MC, Mourao GB, Pires AV, Wiltbank MC, Sartori R: *In vivo* embryo production in cows superovulated 1 or 2 days after ovum pick-up. *Reprod Fertil Dev*, 26 (4): 527-532, 2014. DOI: 10.1071/RD12398

2. Bó GA, Guerrero DC, Adams GP: Alternative approaches to setting up donor cows for superstimulation. *Theriogenology*, 69 (1): 81-87, 2008. DOI: 10.1016/j.theriogenology.2007.09.005

3. Lima WM, Vieira AD, Thaller Neto A, Mezzalira A, Matos RC, Gregory RM: Improved superovulatory response in beef cattle following ovarian follicular ablation using a simplified transvaginal device. *Anim Reprod Sci*, 100, 364-370, 2007. DOI: 10.1016/j.anireprosci.2006.10.023

4. Bungartz L, Niemann H: Assessment of the presence of a dominant follicle and selection of dairy cows suitable for superovulation by a single ultrasound examination. *J Reprod Fertil*, 101, 583-591, 1994. DOI: 10.1530/jrf.0.1010583

5. Singh J, Dominguez M, Jaiswal R, Adams GP: A simple ultrasound test to predict superovulatory response in cattle. *Theriogenology*, 62, 227-243, 2004. DOI: 10.1016/j.theriogenology.2003.09.020

6. İleri İK, Sayın T: Sığırlarda embriyo transfer çalışmaları. *J Fac Vet Univ Istanbul*, 12 (1): 23-35, 1986.

7. Mikkola M, Taponen J: Embryo yield in dairy cattle after superovulation with Folltropin or Pluset. *Theriogenology*, 88, 84-88, 2017. DOI: 10.1016/j.theriogenology.2016.09.052

8. Galli C, Duchi R, Crotti G, Turini P, Ponderato N, Colleoni S, Lagutina I, Lazzari G: Bovine embryo technologies. *Theriogenology*, 59 (2): 599-616, 2003. DOI: 10.1016/S0093-691X(02)01243-8

9. Kawamata M: Relationships between the number of small follicles prior to superovulatory treatment and superovulatory response in Holstein cows. *J Vet Med Sci*, 56 (5): 965-967, 1994. DOI: 10.1292/jvms.56.965

10. Dias FCF, Dadarwal D, Adams GP, Mrigank H, Mapletoft RJ, Singh J: Length of the follicular growing phase and oocyte competence in beef heifers. *Theriogenology*, 79 (8): 1177-1183, 2013. DOI: 10.1016/j.theriogenology.2013.02.016

11. Palomino JM, Cervantes MP, Mapletoft RJ, Woodbury MR, Adams GP: Effect of extending FSH treatment on superovulation and embryo production in wood bison *(Bison bison athabascae). Theriogenology*, 95, 18-23, 2017. DOI: 10.1016/j.theriogenology.2017.02.020

12. Pancarcı ŞM, Ari UC, Atakisi O, Güngör Ö, Çiğremiş Y, Bollwein H: Nitric oxide concentrations, estradiol-17β progesterone ratio in follicular fluid, and COC quality with respect to perifollicular blood flow in cows. *Anim Reprod Sci*, 130 (1-2): 9-15, 2012. DOI: 10.1016/j.anireprosci. 2011.12.013

13. Cirit Ü, Özmen MF, Küçükaslan İ, Köse M, Kutsal HG, Çınar EM: Effect of the interval from follicle aspiration to initiation of lengthened FSH treatment on follicular superstimulatory and superovulatory responses and embryo production in lactating simmental cows. *Theriogenology*, 128, 218-224, 2019. DOI: 10.1016/j.theriogenology.2019.02.008

14. Karaşahin T, Akyol N, Satılmış M, Kızıl SH, Bucak MN, Çoyan K: Investigation of conception rates achieved with the transfer of sexed and unsexed bovine embryos. *Turk J Vet Anim Sci*, 38, 253-256, 2014. DOI: 10.3906/vet-1310-22

15. Bülbül B, Kırbaş M, Köse M, Dursun Ş: Investigation of superovulation response in Brown Swiss cows after synchronization using progesterone and oestradiol valerate. *Kafkas Univ Vet Fak Derg*, 16 (3): 463-468, 2010. DOI: 10.9775/kvfd.2009.1024

16. Stringfellow DA, Givens MD: Manual of the International Embryo Transfer Society. 1-170, Illinois: Savoy, 2010.

17. García Guerra A, Tribulo A, Yapura J, Singh J, Mapletoft RJ: Lengthening the superstimulatory treatment protocol increases ovarian response and number of transferable embryos in beef cows. *Theriogenology*, 78 (2): 353-360, 2012. DOI: 10.1016/j.theriogenology.2012.02.010

18. Souza AH, Carvalho PD, Rozner AE, Vieira LM, Hackbart KS, Bender RW, Dresch AR, Verstegen JP, Shaver RD, Wiltbank MC: Relationship between circulating anti-Müllerian hormone (AMH) and superovulatory response of high-producing dairy cows. *J Dairy Sci*, 98 (1): 169-178, 2015. DOI: 10.3168/jds.2014-8182

19. Dias FCF, Costa E, Adams GP, Mapletoft RJ, Kastelic J, Dochi O, Singh J: Effect of duration of the growing phase of ovulatory follicles on oocyte competence in superstimulated cattle. *Reprod Fertil Dev*, 25 (3): 523-530, 2013. DOI: 10.1071/RD11284

20. Cevik M, Kocyigit A, Sen U, Kuran M: Can sequential human embryo culture media be used in bovine *in vitro* embryo culture. *Kafkas Univ Vet Fak Derg,* 20 (1): 149-153, 2014. DOI: 10.9775/kvfd.2013.9676

21. Enginler SÖ, Özdaş ÖB, Sandal Aİ, Arıcı R, Ertürk E, Baran A, Toydemir TFS, Tek Ç, Kılıçarslan MR, Ak K: The effect of cysteamine and oviductal cells in different culture media on the development of sheep embryos. *Kafkas Univ Vet FakDerg,* 22 (1): 139-145, 2016. DOI: 10.9775/kvfd.2015.14115

22. Evecen M, Demir K, Arıcı R, Yağcıoğlu S, Ersoy N, Coşkun N, Armutak E, Üvez A, Gürel Gürevin E, Eser A, Atalla H, Ak K, Pabuccuoğlu S, Birler S: Effects of ovary transport and storage temperature on in vitro maturation and cumulus cell apoptosis rates in cat oocytes. *Kafkas Univ Vet Fak Derg,* 24 (2): 301-306, 2018. DOI: 10.9775/kvfd.2017.18880

23. Akyol N, Ertem TB, Varışlı Ö: Investigation of the effects of storage period for frozen bull semen on *in vitro* embryo production. *Kafkas Univ Vet Fak Derg,* 25 (2): 257-262, 2019. DOI: 10.9775/kvfd.2018.20839

24. Ireland JJ, Ward F, Jimenez-Krassel F, Ireland JLH, Smith GW, Lonergan P, Evans ACO: Follicle numbers are highly repeatable within individual animals but are inversely correlated with FSH concentrations and the proportion of good-quality embryos after ovarian stimulation in cattle. *Hum Reprod*, 22 (6): 1687-1695, 2007. DOI: 10.1093/humrep/dem071

25. Kohram H, Moakhar HK: Relationship between follicular profiles prior to the initiation of superovulation and various types of superovulatory responses in cattle. *Bull Vet Inst Pulawy*, 54, 29-33, 2010.

26. Shaw DW, Farin PF, Washburn SP, Britt JH: Effect of retinol palmitate on ovulation rate and embryo quality in superovulated cattle. *Theriogenology,* 44 (1): 51-58, 1995. DOI: 10.1016/0093-691X(95)00147-Z

27. Demetrio D: AETA 2017 Survey Report. https://www.aeta.org/docs/ 2017_Stats.pdf; *Accessed:* 08.10.2018.

28. Donaldson LE: Cattle breed as a source of variation in embryo transfer. *Theriogenology*, 21 (6): 1013-1018, 1984. DOI: 10.1016/0093-691X(84)90396-0

29. Tríbulo A, Rogan D, Tríbulo H, Tríbulo R, Mapletoft RJ, Bó GA: Superovulation of beef cattle with a split-single intramuscular administration of Folltropin-V in two concentrations of hyaluronan. *Theriogenology*, 77 (8): 1679-1685, 2012. DOI: 10.1016/j.theriogenology. 2011.12.013

30. Kanitz W, Becker F, Schneider F, Kanitz E, Leiding C, Nohner HP, Pöhland R: Superovulation in cattle: Practical aspects of gonadotropin treatment and insemination. *Reprod Nutr Dev*, 42 (6): 587-599, 2002. DOI: 10.1051/rnd:2002045

31. Martens G, Nohner HP, Leiding C, Schneider F, Becker F, Nuernberg G, Kanitz W: Optimizing frequency of FSH application for superovulatory treatment in cattle. *Reprod Fertil Dev*, 17 (2): 313-314, 2005. DOI: 10.1071/RDv17n2Ab326

32. Carballo Guerrero D, Tríbulo A, Tríbulo R, Tríbulo H, Bó GA: Superovulatory response in beef cattle treated during the first follicular wave following synchronization of ovulation with a progestin device and GnRH. *Reprod Fertil Dev*, 21 (1): 242-243, 2009. DOI: 10.1071/RDv21n1Ab291

33. Bono G, Gabai G, Silvestrelli L, Comin A: Superovulatory and endocrinological responses of Simmental cows treated either with PMSG or hMG or in combination. *Theriogenology*, 35 (6): 1179-1190, 1991. DOI: 10.1016/0093-691X(91)90364-J

34. Breuel KF, Baker RD, Butcher RL, Townsend EC, Inskeep EK, Dailey RA, Lerner SP: Effects of breed, age of donor and dosage of follicle stimulating hormone on the superovulatory response of beef cows. *Theriogenology*, 36 (2): 241-255, 1991. DOI: 10.1016/0093-691X(91)90383-O

35. Stroud B: AETA 2009 Survey Report. https://www.aeta.org/docs/ 09statsreport.pdf; *Accessed:* 08.10.2018.

36. Roussel JD, Thibocieaux JK, Karihallo AK, Goodeaux LL: A seven-year study of embryo transfer donors. *PAS,* 7 (1): 8-12, 1991. DOI: 10.15232/S1080-7446(15)32169-0

37. Leonardi CEP, Pfeifer LFM, Rubina MIB, Singh J, Mapletoft RJ, Pessoa GA, Bainy AM, Silva CAM: Prostaglandin F2α promotes ovulation in prepubertal heifers. *Theriogenology*, 78 (7): 1578-1582, 2012. DOI: 10.1016/j.theriogenology.2012.06.030

38. Ambrose DJ, Gobikrushanth M, Zuidhof S, Kastelic JP: Low-dose natural prostaglandin F2α (dinoprost) at timed insemination improves conception rate in dairy cattle. *Theriogenology*, 83 (4): 529-534, 2015. DOI: 10.1016/j.theriogenology.2014.10.034