ERCİYES ÜNİVERSİTESİ VETERİNER FAKÜLTESİ DERGİSİ
Journal of Faculty of Veterinary Medicine, Erciyes University

Araştırma Makalesi / Research Article
18(1), 41-47, 2021
DOI: 10.32707/ercivet.838794

The Effects of Pre-Superovulation GnRH and Short-Term Progesterone Administrations on the Quantity and Quality of Bovine Embryos*

Uğur KARA[1],Tayfur BEKYÜREK[2]

[1]Eastern Mediterranean Agricultural Research Institute, Adana-TURKEY
[2]Erciyes University, Faculty of Veterinary Medicine, Department of Obstetrics and Gynaecology, Kayseri-TURKEY

Corresponding author: Uğur KARA; E-mail :ugurvetkara@hotmail.com; ORCID:0000-0002-7977-6826
Atıf yapmak için: Kara U, Bekyürek T. The effects of pre-superovulation GnRH and short-term progesterone administrations on the quantity and quality of bovine embryos. Erciyes Univ Vet Fak Derg 2021; 18(1): 41-47

Summary: In this study we compared the quantity and quality of embryos recovered from Holstein donors superovulated after the induction of oestrus by human chorionic gonadotropin (hCG) and prostaglandin F_2-alfa ($PGF_2\alpha$), followed by gonadotropin releasing hormone (GnRH) and short-term progesterone treatment (a modified protocol) and Holstein donors superovulated with a conventional protocol. In research, 3-year-old Holstein cows (n=20) were assigned into two equal groups. In Group Ithe animals received two injections of cloprostenol 11 days apart, and follicle stimulating hormone (FSH)was injected twice daily for 4 days at decreasing doses starting from the 9th day after oestrus. Cloprostenol was co-administered with the 5th and 6th FSH injections. In Group II, the animals were treated with hCG at unknown stage of the oestrus cycle, and were given cloprostenol 7 days later. Buserelin was injected on the 7th day after oestrus, and PRID was inserted on9th day after oestrus and was removed on day 14 (6th FSH injection).In Group II, FSH was administered on days 12-15 of the cycle, in a way identical to that of Group I, including the two cloprostenol .All cows were artificially inseminated three times at 12-hour intervals after last FSH injection. Buserelin was injected concurrently with the second artificial insemination. Embryos were collected on the 7th day after the second insemination embryo quality and developmental stage were evaluated based on their morphology. In the present study, total CL (8.50±1.90 and 9.40±1.52), total ova and embryo (7.5±2.03 and 7.0±1.61), total embryo (6.00±2.00 and 6.10±1.47), transferable embryo (5.50±1.84 and 5.50±1.59), degenerate embryos (0.50±0.22 and 0.60±0.40) and unfertilized ova (1.60±0.97 and 0.90±0.31) numbers were determined per donor in Groups I and II respectively. There was no statistically significant difference between the two protocols in terms of embryo quality and quantity (P>0.05). Thus, it was concluded that the tested protocol could be used as an alternative to conventional one as it allows fixed-time embryo collection in multiple donors with no need for oestrus detection.
Keywords: Embryo, gonadotrop inreleasing hormone, human chorionic gonadotropin, progesterone, superovulation

Süperovulasyon Öncesi GnRH ve Kısa Süreli Progesteron Uygulamalarının Sığır Embriyolarının Sayısı ve Kalitesi Üzerine Etkisi

Özet: Bu çalışmada, insan koryonik gonadotropini (hCG) ve prostaglandin F_2-alfa (PGF2α) ile östruslarının indüklenmesinden sonra gonadotropin salgılatıcı hormon (GnRH) ve kısa süreli progesteron uygulamasını takiben süperovulasyon uygulanan Holştayn donörler (modifiye protokol) ile klasik süperovulasyon uygulanan Holştayn donörlerden elde edilen embriyoların kalitesi ve sayısı karşılaştırıldı. Araştırmada, üç yaşlı 20 adet Holştayn ırkı inek iki eşit gruba ayrıldı. Grup I' deki hayvanlara 11 gün ara ile iki defa kloprostenol enjeksiyonu yapıldı ve östrus sonrası takip eden siklusun 9. gününden itibaren, günde iki kez dört gün süresince azalan dozlarda FSH enjeksiyonları uygulandı. Beşinci ve 6. FSH enjeksiyonları ile birlikte kloprostenol uygulamaları yapıldı.Grup II' deki hayvanlara ise siklusun herhangi bir döneminde hCG uygulaması yapıldı. Bundan 7 gün sonra kloprostenol enjeksiyonu yapıldı. Östrus sonrası devam eden siklusun 7. günü buserelin enjeksiyonu yapıldı. PRID siklusun 9. günü yerleştirildi ve siklusun 14. günü çıkarıldı (6. FSH enjeksiyonu sırasında). Grup II'de FSH ve kloprostenol uygulamaları siklusun 12-15. günlerinde Grup I' deki gibi yapıldı. Tüm inekler son FSH enjeksiyonundan sonra 12 saat ara ile üç kez tohumlandı. İkinci suni tohumlama ile eş zamanlı olarak buserelin enjeksiyonu yapıldı. İkinci tohumlama sonrası 7. günde embriyolar toplandı, morfolojilerine bağlı kalite ve gelişim evreleri değerlendirildi. Bu çalışmada donör başına sırasıyla Grup I ve Grup II' de toplam CL (8.50±1.90 ve 9.40±1.52), toplam oosit ve embriyo (7.5±2.03 ve 7.0±1.61), toplam embriyo (6.00±2.00 ve 6.10±1.47), transfer edilebilir embriyo ı (5.50±1.84 ve 5.50±1.59), dejenere embriyo (0.50±0.22 ve 0.60±0.40) ve unfertilize oosit sayıları (1.60±0.97 ve 0.90±0.31) tespit edildi. Sonuç olarak, iki protokol arasında embriyo sayısı ve kalitesi açısından istatistiksel olarak fark bulunamadı (P >0,05). Böylece çok sayıda donöre östrus takibine gerek duyulmadan sabit zamanlı süperovulasyona olanak sağladığından dolayı klasik süperovulasyon uygulamalarına alternatif bir yöntem olabileceği sonucuna varılmıştır.
Anahtar kelimler: Embriyo, gonadotropin salgılatıcı hormon, insan koryonik gonadotropini, progesteron, süperovulasyon

Geliş Tarihi/Submission Date : 07.09.2020
Kabul Tarihi/Accepted Date : 07.12.2020
*This work is part of the doctorate thesis of the first author, which was financed under the "Project for the Improvement of the Anatolian Multi-coloured Cattle" (TAGEM/HAYSÜD/01-0902-03).

Introduction

The main aim of use of embryo transfer (ET) is the production of a maximum number of calves from high -yield donors. Thereby, four to fivefold of the total number of progeny produced by a female during her lifetime can be produced with ET within a year (Kanagawa et al.,1995; Sağırkaya, 2009).Despite a long history of bovine superovulation research, significant commercial applications did not start until the early 1970s. For some 20 years thereafter, superovulation represented the primary tool for the production of cattle embryos (Mikkola et al., 2020). One of the main problems encountered with conventional superovulation protocols is individual variability in donor responses and as a result, the varying number of transferable embryos not collected (Mapletoft et al., 2002).

The administration of follicle-stimulating hormone (FSH) at the time of the emergence of the follicular wave is reported to have a positive impact on the ovarian response (Adams et al., 1994). When applying conventional superovulation protocols, the optimal response for the recovery of a maximum number of embryos is generated with hormone administration at the time of the emergence of the second follicular wave and requires periodical examinations on ovarian between 8^{th} to 12^{th} days during oestrus cycles. Fixed-time superovulation, embryo collection and transfer procedures following the exogenous control of the emergence of the follicular wave can be performed to avoid such problems (Bó et al., 2004).Although follicular wave and ovulation control programmes are incapable of preventing the individual variability of superovulation responses, they enable the initiation of treatment at any time point throughout the sexual cycle (Alkemade et al, 1993; Mapletoft et al., 2002; Bó et al., 2008).Furthermore, as they involve ovulation synchronization protocols, these superovulation procedures also enable the fixed-time insemination of donors and thereby, rule out the obligation to detect oestrus (Baruselli et al., 2006;Bó et al., 2006).

Methods used for the synchronization of follicular wave emergence as part of superovulation protocols include oestradiol and progesterone administration, gonadotropin-releasing hormone (GnRH) administration and follicle ablation. While the most common method used for follicular wave synchronization is the administration of oestradiol, the use of this hormone is prohibited in many countries whole of the world. Therefore, alternative methods need to be developed for the superovulation of donors in countries that do not allow the use of oestradiol because of concerns about the effects of estrogenic substances in the food chain (Bó and Mapletoft, 2012).

This study was aimed to investigate the possibility of using fixed-time embryo collection and transfer for commercial production of bovine embryos without the need to oestrus detection. For this purpose, a conventional superovulation protocol compared to a modified protocol with hCG and $PGF_2\alpha$ administration followed by GnRH and short-term progesterone treatment as concern the quantity and quality of embryos. Thereby, it has been aimed.

Material and Methods

Animals

Twenty healthy, reproductively sound and regularly cycling 3-year-old Holstein cows, with at least one previous delivery were used. Cows were kept at the Research Farm of the Eastern Mediterranean Agricultural Research Institute (41.008857 latitude and 28.96747 longitude). The study was conducted in April 2016. The animals had a body condition score of 3-3.5 and weighed 500-550 kg and were at >100 days in milk. The average daily milk yield of the animals was 30.9±1.00 l in Group I and 32.0±1.12 lin Group II. The animals, which were assigned to two equal groups, were all fed on a total mixed ration (TMR) containing a combination of roughage (wheat straw, alfalfa hay and corn silage) and concentrate. Prior to the study, each animal underwent uterine and ovarian ultrasonographic examination for determine the possible uterine infections, status of genital tract and ovarium (5 MHz, Honda HS-101V, Japan). The experimental design of the study was approved by the Local Ethics Board for Animal Experiments of Erciyes University (Approval Number: 15/144).

Synchronization and superovulation

The superovulation protocols applied to the two study groups are presented in Figure 1 and Figure 2. The twenty cows included in the study were randomly assigned into two equal groups for superovulation treatment. The animals in Group I were intramuscularly (i.m.) injected twice with 500 µg of cloprostenol (Lutelen, Topkapı Pharmaceuticals and Premixes Industry and Trade Inc., Turkey), a $PGF_2\alpha$ analogue, 11 days apart, at unknown stage of the oestrous cycle. Oestrous detection was performed in 8-hour intervals starting 48h after the second cloprsostenol injection and the presence of clear mucous vaginal discharge was checked. Ovulation checks were performed by ultrasonographic examination 24 hours after the end of oestrus. A total amount of 400 mg FSH (Follitropin 700 IU, Bioniche Animal Health Canada Inc, Canada) was administered i.m., twice daily for 4 days at decreasing doses (Day 1 80/80 mg, Day 2 60/60 mg, Day 3 40/30 mg and Day 4 30/20 mg) starting from the 9^{th} day after oestrus .Two i.m. doses of 500 µg cloprostenol were co-administered with the 5^{th} and 6^{th} FSH injections (Fig. 1).

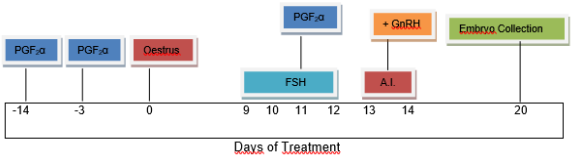

Figure 1. Superovulation protocol applied in Group I

The animals in Group II were given 3000 IU of hCG i.v. on day -10 (Chorulon 5x1500 IU+diluent, Intervet Veterinary Pharmaceuticals Marketing and Trade Inc., Turkey) and 7 days later (day -3) 500 µg of cloprostenol was injected i.m. to induce oestrus, the animals were monitored three times a day for oestrus detection, and the presence of clear mucous vaginal discharge was checked and observation 24 hours after the end of oestrus for ovulation. On day 7 after oestrus detection, a single i.m. dose of 10 µg of buserelin (Receptal, Veterinary Pharmaceuticals Marketing and Trade Inc., Turkey) was administered. On the 9thday of the oestrus cycle, a progesterone-releasing intravaginal device containing 1.55 g of progesterone (PRID DELTA®, Ceva Animal Health Joint Stock Company, Turkey) was inserted in the vagina, and a superovulation procedure similar to that applied in Group I, on days 12-15 of the induced cycle. The PRID was removed with 6th dose of FSH (Fig. 2).

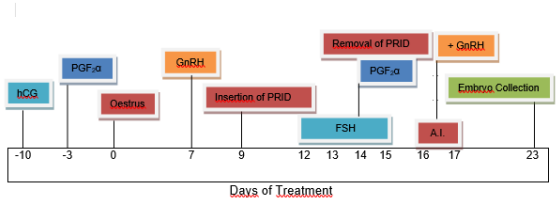

Figure 2. Superovulation protocol applied in Group II

In both treatment groups, each animal was artificially inseminated three times at 12 h-intervals, starting 12 hours after the last FSH injection. At the time of the second insemination, all animals included in the study were also administered 10 µg of buserelin.

Collection and evaluation of the embryos

Uterine lavage was performed seven days after the second insemination. Prior to uterine lavage, each animal underwent ultrasonographic examination (5 MHz, Honda HS-101V, Japan and 5 MHz, Honda HS - 2000VET, Japan) to determine the total numbers of corpora lutea (CL) and follicles (>10 mm) in the ovaries. Uterine lavage was performed by two experienced veterinary practitioners, whilst embryo evaluation was conducted by auxiliary staff with specific expertise in this field.

Uterine lavage was performed using 1000 ml of lactated Ringer's solution (Ringer-Fleks, Eczacıbaşı-Baxter Hospital Products Industry and Trade Inc., Ayazağa/Istanbul) containing 1% calf serum (Foetal Bovine Serum Sigma F 9665, Germany) and 0.1% kanamycin (Kanovet, Vetaş Veterinary Pharmaceuticals and Pesticides Joint Stock Company, Küçükçekmece/Istanbul) (Kanagawa et al., 1995).

Prior to the lavage procedure, epidural anaesthesia was administered with 4 ml of a local anaesthetic (L-Anestin, Alke Health Products Industry and Trade Inc., Turkey). Following uterine lavage, each donor was administered with 500 µg of cloprostenol i.m. and 500 mg of cephapirin benzathine (19 g, Metricure, MSD Animal Health, Turkey) by intrauterine route (Kara and Bekyürek, 2017).

The evaluation of embryo quality was conducted by auxiliary staff with specific expertise in this field, on the basis of morphological integrity according to the guidelines of the International Embryo Transfer Society (IETS) and were classified as Grade 1 and Grade 2 (Robertson and Nelson, 2010).

Statistical analyses

All analyses were performed using the SPSS 11.5 for Windows statistical software package. The numbers of embryos and ova obtained in the two study groups compared using the chi-square test. The Mann-Whitney U test was used to compare the treatment groups for the mean CL, mean ova and embryo, mean embryo, mean transferable embryo, Grade 1 embryo and Grade 2 mean embryo numbers. The recovery rates (Total Number of Ova/Embryos Recovered/Number of CL Detected x 100) of the two groups were compared with the t test. The findings obtained in this study are presented as mean values and percentages. All mean values are expressed as the mean ± standard error mean.

Results

All of the animals responded to the superovulation protocols applied, as confirmed by the presence of two or more corpora lutea on the day of uterine lavage. Embryos were recovered from all of the animals excluding one cow in Group I. A comparative presentation of the findings in the two study groups is given in Table 1 and Table 2.

No statistically significant difference existed between the treatment groups regarding CL, follicles (prior to the uterine lavage on the 7th day of the cycle, >10 mm follicles), ova and embryo, embryo, transferable embryo, Grade 1 and Grade 2 embryo numbers (P>0.05). The groups did not statistically differ for recovery rateeither (P>0.05).

Table 1. Numbers of total corpora lutea, follicles, ova/embryos, embryos, transferable embryos, degenerate embryos and unfertilized ova collected after superovulation of Holstein cows with a group I or group II protocol

Parameter (Total number)	Group I (n=10)	Group II (n=10)
Corpora Lutea	85	94
Follicles	18	23
Ova/Embryos	75	70
Embryos	60	61
Transferable Embryos	55	55
Degenerate Embryos	1	0
Unfertilized Ova	15	9

Table 2. Mean values for total corpora lutea, follicles, ova and embryos, embryos, transferable embryos, grade 1and 2 embryo numbers (Mean±SEM). No significant differences were observed between groups (P>0.05)

Parameter	Group I (n=10) X± SEM	Group II (n=10) X± SEM	P Value
Corpora Lutea Per Donor	8.50±1.90	9.40±1.52	0.544
Follicles Per Donor	1.80±0.81	2.40±1.63	0.107
Ova/Embryos Per Donor	7.50±2.03	7.00±1.61	0.847
Embryos Per Donor90	6.00±2.00	6.10±1.47	0.493
Transferable Embryos Per Donor	5.50±1.84	5.50±1.59	0.675
Grade 1 Embryos	4.40±1.50	4.90 ±1.37	0.731
Grade 2 Embryos	1.00±0.47	0.60±0.30	0.732
Degenerated Embryos Per Donor	0.50±0.22	0.60±0.40	0.720
Unfertilized Ova Per Donor	1.60±0.97	0.90±0.31	0.839
Recovery Rate (%)	88.23 %	74.46 %	0.257
Number and Percentage of Grade 1 Embryos	45 (63.4)	49 (76.6)	0.250
Number and Percentage of Grade 2 Embryos	10 (14.1)	6 (9.4)	0.250
Number and Percentage of Unfertilized Ova	16 (22.5)	9 (14.1)	0.250

X: Mean; SEM: Standard Error Mean

Discussion and Conclusion

For many years, practitioners have been initiating superstimulatory treatments during the midcycle (i.e., 8 to 12 days after oestrus) (Bó and Mapletoft, 2012). Superovulatory response has been shown to be higher when gonadotropin treatments are initiated precisely at the time of follicular wave emergence rather than 1 or 2 days later, making it necessary to synchronize the timing of follicular wave emergence in groups of animals (Nasser et al.,1993; Bó and Mapletoft, 2012).

In current study, we demonstrated the effects of pretreatment by GnRH and short-term progesterone treatment on fixed-time embryo collection and the quantity and quality of embryos in Holstein cows. It has been shown that following GnRH-induced ovulation (Macmillan and Thatcher,1991), anew follicular wave will emerge approximately 2 days later. However, follicular wave emergence occurs only when GnRH induces ovulation, if GnRH is administered when the dominant follicle is immature or postmature, ovulation may not occur and a new follicular wave will not emerge (Martinez et al., 1999). Ovulation rates after GnRH treatment at random stages of the oestrous cycle in cows have been reported to range from 44.3 % (Colazo et al., 2009) to 85 % (Pursley et al., 1995).Therefore, the animals were treated with hCG at unknown stage of the oestrus cycle to induce of dominant follicle ovulation and were given cloprostenol 7 days later to induce oestrus in Group II. On day 7 after oestrus detection, GnRH was administered to induce of dominant follicle ovulation of first follicular wave. On day 9 of the oestrus cycle, a progesterone-releasing intravaginal device was administered to control of second follicular wave emergence.

In the present study, no statistically significant difference was detected between the treatment groups for the mean CL, follicles, ova and embryos, embryos, transferable embryos, Grade 1 embryos, Grade 2embryos, degenerate embryos and unfertilized ova numbers (P>0.05). In the present study, the recovery rate of Group I was found to be higher than that of Group II (88.23% and 74.46%, respectively) (P>0.05). The mean CL of Group II was found to be higher than that of Group I (9.40±1.52 and 8.50±1.90, respectively) (P>0.05).The unfertilized ova of Group I was found to

be higher than that of Group II (1.60±0.97 and 0.90±0.31, respectively) (P>0.05). The obligation to continuously monitor oestrus and, in case of multiple donors, ensure that all donors enter oestrus at the same time is a disadvantage of conventional superovulation procedures (Bó and Mapletoft, 2012).In the present study, the similar results obtained in the two treatment groups suggest that fixed-time A.I. and embryo collection could be applied to multiple donors after superovulation with no need for oestrus detection.

In previous studies, better results have been reported regarding CL number ranging between 11.54±1.63 and 27.2±2.1 (Lovie et al., 1994; Baracaldo et al., 2000;Caccia et al., 2000; Köse et al., 2006;García et al., 2012), ova and embryos ranging between 8.5±1.3 and 17.4±9.9 mber (Baracaldo et al., 2000;Caccia et al., 2000; Andrade et al., 2003; Carballo et al., 2010;García et al., 2012), for embryos ranging between 7.2±1.4 and 10.1±1.2 (Baracaldo et al., 2000;Caccia et al., 2000;Carballo et al., 2010), and for transferable embryos5.85±1.48 and 9.8±8.1 (Baracaldo et al., 2000;Caccia et al., 2000; Andrade et al., 2003; Köse et al., 2006; Carballo et al., 2010).On the contrary other researchers have reported lower values ranging between 6.1±0.9 and 8.4±1.4 for CL (Kim et al., 2001; Bülbül et al., 2010),3.1±1.0 and 6.3±1.3 for ova and embryos (Lovie et al., 1994; Kim et al., 2001; Bülbül et al., 2010), 2.3±0.9 and 4.7±1.8 for embryos (Lovie et al., 1994), and 2.3±0.8 and 4.7±1.6 for transferable embryos (Lovie et al., 1994; Kim et al., 2001; Bülbül et al., 2010). Grade 1 embryos ranging from 1.9±0.3 to 4.7±0.37 (Goulding et al., 1990;Köse et al., 2006;Wock et al., 2008; Bülbül et al., 2010; Kara and Bekyürek, 2017), and Grade 2 embryos ranging from 1.1±0.4 to 4.5±0.41 (Goulding et al., 1990;Köse et al., 2006;Wock et al., 2008; Bülbül et al., 2010) have been reported.

The differences in the superovulatory responses (mean number of CL and the embryo yield per donor) reported in previous research could be related to differences in the superovulation protocols applied, in terms of the number protocol of injections, administration route and dose of FSH (Lovie et al., 1994; Caccia et al., 2000;Carballo et al., 2010;García et al., 2012), or could be a result of differences in the day or stage of the sexual cycle chosen for FSH administration (Goulding et al., 1990; Andrade et al., 2003; Wock et al., 2008; Bülbül et al., 2010; Kara and Bekyürek, 2017). Differences in the follicular waves during which FSH administration was initiated (Goulding et al., 1990; Carballo et al., 2010). It has been indicated that any asynchrony between the emergence of the follicular wave and the initiation of superovulation procedures significantly reduces the ovarian response (Nasser et al., 1993). This might be one of the reasons for the inferior results obtained in previous research in which conventional superovulation techniques were used (Nasser et al., 1993; Kim et al., 2001; Köse et al., 2006; Bülbül et al., 2010;Kaymaz, 2015).

Embryo recovery rates of 74.0% (Köse et al., 2006), 70% and 69% (Bülbül et al., 2010), and 83.5%, 41.5%, 47.6% and 51.5% (Taşdemir et al., 2016) have been reported in previous researchs. It is thought that, as also highlighted by Kanagawa et al. (1995), this difference may be related to several factors, such as the timing (day) of embryo collection, the type of catheter used, the positioning of the catheter during uterine lavage, and experience of the practitioner. Recovery rate found in this study (88.23% and 74.46% in Group I and Group II, respectively) was consistent with Köse et al. (2006) and Bülbül et al. (2010). However, the embryo recovery rates of Taşdemir et al. (2016) are lower than this study. Possible complications for embryo collection procedures and the performance of rectal palpation both due to the narrowness of the birth canal of Anatolian Black heifer that they studied might be the source of this difference, which is also highlighted by the researchers in the study of Taşdemir et al. (2016).

Use of GnRH at the onset of estrus increases the spontaneous LH peak, prevents delays in ovulation, and induces uniformly high postovulation progesterone concentrations (Kaim et al., 2003).In addition, in the study which administered LH for the induction of ovulation (Carballo et al., 2010), number of ova/ embryos, embryos and transferable embryos were higher than present study. But, this difference was predicted to be sourced from preferred superovulation protocol rather than administration of different hormones (GnRH/LH) for induction of ovulation.

Some researchers have indicated that the administration of a low dose of eCG (equine chorionic gonadotropin) prior to superovulation increases the number of embryos recovered (Caccia et al., 2000; Barros et al., 2008). This result obtained with the use of eCG has been attributed to the longer lasting FSH-like effect of this hormone, resulting in a greater number of follicles recruited into the follicular wave in the emergence of follicular wave (Saumande et al., 1978;Schams et al., 1978; Mikel-Jenson et al., 1982; Moor et al., 1984; Dieleman et al., 1993).

The assessment of the results obtained in the present study suggests that by means of superovulation after GnRH and progesterone administration, one of the problems encountered in commercial in vivo bovine embryo production, namely, the obligation to monitor oestrus, can be ruled out and multiple donors fixed-time embryo collection from multiple donors can be applied with no need for oestrus detection. Furthermore, it has also been ascertained that by con-

trolling the emergence of the second follicular wave, a satisfactory superovulator ovarian response can be achieved (Bo´ et al., 2004).

In conclusion pre-superovulation GnRH and short-term progesterone administration (modified protocol) and conventional superovulation protocols were proven equally effective in terms of the number and quality of the embryos recovered ($p>0.05$).However, periodic ultrasonographic examinations are required during 8^{th}-12^{th} days of eostrus cycle in conventional protocols, due to the requirement of concurrently applied superovulation protocol with the emergence of the second follicular wave for multiple donors (individual differences in days of emergence of follicular waves in donors).

As a result of the applied procedure of 1)GnRH and short-time progesterone treatment, 2) its synchronization with follicular wave, 3) superovulation protocol concurrently applied with the emergence of the second follicular wave may offer the advantage of initiating super stimulatory treatments at a time that is optimal for follicle recruitment in Holstein cows.

Acknowledgements

We would like to thank the Eastern Mediterranean Agricultural Research Institute and General Directorate of Agricultural Research and Policies for their contributions.

References

Adams GP, Nasser LF, Bó GA, Garcia A, Del Campo MR, Mapletoft RJ. Superovulatory response of ovarian follicles of wave 1 versus wave 2 in heifers. Theriogenology 1994; 42: 1103-13.

Alkemade S, Murphy B, Mapletoft R. Superovulation in the cow: Effects of biological activity of gonadotropins. Proceedings of the Twelfth Annual Meeting of the American Embryo Transfer Association, Portland, Maine. 1993: 1-19

Andrade JC, Oliveira MA, Lima PF, Guido SI, Bartolomeu CC, Teorio Filho F, Pina VM, Iunes-Souza TC, Paula NR, Freitas JC. The use of steroid hormones in superovulation of Neloro donors at different stages of oestrous cycle. Anim Reprod Sci 2003; 77: 117-25.

Baracaldo MI, Martinez M, Adams GP, Mapletoft RJ. Superovulatory response following transvaginal follicle ablation in cattle. Theriogenology 2000; 53: 1239-50.

Barros CM, Barcelos ACZ, Gouvea LM, Meneghel M, Barcelos DS, Barcelos LN, Trinca LA. Improvement of a superovulatory protocol in Nelore cows: Replacing the last two doses of pFSH by eCG. Reprod Fertil Dev 2008; 20:152.

Baruselli PS, Sá Filho M, Martins CM, Nasser LF, Nogueira MFG, Barros MC, Bó GA .Superovulation and embryo transfer in *Bos indicus* cattle. Theriogenology 2006; 65: 77-88.

Bó G, Baruselli P, Chesta P, Martins C. The timing of ovulation and insemination schedules in superstimulated cattle. Theriogenology 2006; 65: 89-101.

Bó GA, Moreno D, Cutaia L, Baruselli PS. Hormonal manipulation of the estrous cycle in bovine embryo donors and recipients. Acta Sci Vet 2004; 32: 1-22.

Bó GA, Mapletoft RJ. Recent advances in the control of follicular development and superovulation protocols in cattle. Twenty Eighth Annual Meeting AETE. September 7–8, Saint Malo-France.

Bó GA, Carballo Guerrero D, Adams GP. Alternative approaches to setting up donor cows for superovulation. Theriogenology 2008; 69: 81-7.

Bo´ GA, Adams GP, Pierson RA, Mapletoft RJ. Exogenous control of follicular wave emergence in cattle. Theriogenology 1995; 43: 31-40.

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 2010; 16 (3): 463-8.

Caccia M, Tríbulo R, Tríbulo H, Bó GA .Effect of eCG pretreatment on superovulatory response in CIDR-B treated beef cattle. Theriogenology 2000; 53: 495.

Carballo Guerrero D, Tríbulo A, Tríbulo R, Tríbulo H, Bó GA. Superovulatory response in beef donors treated during the first follicular wave or four days after progesterone and estradiol administration. Reprod Fertil Dev 2010; 22: 358.

Colazo MG, Gordon MB, Rajamahendran R, Mapletoft RJ, Ambrose DJ. Pregnancy rates to timed artificial insemination in dairy cows treated with gonadotropin releasing hormone or porcine luteinizing hormone. Theriogenology 2009; 72: 262-70.

Dieleman S, Bevers M, Vos P, de Loos F. PMSG/anti -PMSG in cattle: A simple and efficient superovulatory treatment. Theriogenology 1993; 39: 25-42.

García GA, Tribulo A, Yapura J, Singh J, Mapletoft RJ. Lengthening the super stimulatory treatment protocol increases ovarian response and number of transferable embryos in beef cows. Theriogenology 2012; 78(2): 353-60.

Goulding D, Williams DH, Duffy P, Boland MP, Roche JF. Superovulation in heifers given FSH initiated either at day 2 or day10 of the estrous cycle. Theriogenology 1990; 34: 767-78.

Kaim M, Bloch A, Wolfenson D, Braw-Tal R, Rosenberg M, Voet H, Forman Yl. Effects of GnRH administered to cows at the onset of estrus on timing of ovulation, endocrine responses, and conception. J Dairy Sci 2003; 86: 2012-21.

Kanagawa H, Shimohira I, Saitoh N. Manual of Bovine Embryo Transfer. National Livestock Breeding Center MAFF. JICA-Japan, 1995.

Kara U, Bekyürek T. The effect of double $PGF_2\alpha$ administrations applied to donor before oestrus with synchronized CIDR on the number and quality of obtained embryos during embryo transfer in cattle. J Health Sci 2017; 26 (3): 204-10.

Kaymaz M. Yardımcı üreme teknikleri. In: Semacan A, Kaymaz M, Fındık M, Rişvanlı A, Köker A, eds. Çiftlik Hayvanlarında Doğum ve Jinekoloji. İkinci Baskı. Malatya: Medipres, 2015; s. 695-811.

Kim HI, Son DS, Yeon H, Choi SH, Park SB, Ryu IS, Suh GH, Lee DW, Lee HJ, Yoon JT. Effect of dominant follicle removal before superstimulation on follicular growth, ovulation and embryo production in Holstein cows. Theriogenology 2001; 55: 937-45.

Köse M, Dursun Ş, Bülbül B, Kırbaş M. İsviçre Esmeri ineklerde FSH ile süperovulasyon ve embriyo transferi çalışmaları. Hay Araş Derg 2006; 16 (1): 1 -6.

Lovie M, García A, Hackett A, Mapletoft RJ. The effect of dose schedule and route of administration on superovulatory response to folltropin in Holstein cows. Theriogenology 1994; 41: 241.

Macmillan KL, Thatcher WW. Effect of an agonist of gonadotropin-releasing hormone on ovarian follicles in cattle. Biol Reprod 1991; 45: 883-89.

Mapletoft RJ, Steward KB, Adams GP. Recent advances in superovulation in cattle. Reprod Nutr Dev 2002; 42(6): 601-11.

Martinez MF, Adams GP, Bergfelt D, Kastelic JP, Mapletoft RJ. Effect of LH or GnRH on the dominant follicle of the first follicular wave in heifers. Anim Reprod Sci 1999; 57: 23-33.

Mikel-Jenson A, Greve T, Madej A, Edqvist L. Endocrine profiles and embryo quality in the PMSG-PGF2α-treated cow. Theriogenology 1982; 18: 33-4.

Mikkola M, Hasler JF, Taponen J. Factors affecting embryo production in superovulated *Bos taurus* cattle. Reprod Fertil Dev 2020; 32: 104-24.

Moor R, Kruip T, Green D. Intraovarian control of folliculogenesis: Limits to superovulation? Theriogenology 1984; 21: 103-16.

Nasser L, Adams GP, Bó GA, Mapletoft RJ. Ovarian superstimulatory response relative to follicular wave emergence in heifers. Theriogenology 1993; 40: 713-24.

Pursley JR, Mee MO, Wiltbank MC. Synchronization of ovulation in dairy cows using $PGF_2\alpha$ and GnRH. Theriogenology 1995; 44: 915-23.

Robertson I, Nelson RE. Certification and identification of embryos. Stringfellow DA, Givens MD, eds. In: Manual of The International Embryo Transfer Society. Forth Edition. Champaign: International Embryo Transfer Society 2010; 86-105.

Sağırkaya H. Sığırlarda embryo transfer uygulaması ve Türkiye açısından önemi. Uludağ Üniv Vet Fak Derg 2009; 28 (2):11-9.

Saumande J, Chupin D, Mariana J, Ortavant R, Mauleon P. Factors affecting the variability of ovulation rates after PMSG stimulation. Sreenan JM, ed. In: Control of Reproduction in the Cow. The Hague: Martinus Nijhoff, 1978; p.195-224.

Schams D, Menzer D, Schalenberger E, Hoffman B, Hahn J, Hahn R. Some studies on the pregnant mare serum gonadotropin (PMSG) and on endocrine responses after application for superovulation in cattle. Sreenan JM, ed. In: Control of Reproduction in the Cow. The Hague: Martinus Nijhoff, 1978; p.122-42.

Taşdemir U, Karaşahin T, Satılmış M, Kızıl SH, Kaymaz M. Various FSH administration on superovulatory response and embryo yield in Anatolian Black Heifers. Kocatepe Vet J 2016; 9(4):322-26.

Wock J, Lyle L, Hockett M. Effect of gonadotropin-releasing hormone compared with estradiol-17β at the beginning of a superstimulation protocol on superovulatory response and embryo quality. Reprod Fertil Dev 2008; 20: 228.