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RESEARCH ARTICLE

Investigation of Superovulation Response in Brown Swiss Cows After Synchronization Using Progesterone and Oestradiol Valerate [1]

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Summary

To evaluate the superovulatory response after synchronization using progesterone and oestradiol valerate, a total of 19 Brown Swiss cows were randomly allocated into two groups. Control cows (n=9) were superovulated with FSH (total: 400 mg), from d 10 to 13 (oestrus 0), using twice daily injections with decreasing doses (4, 4, 3, 3, 2, 2, 1, 1 ml). Treatment cows (n=10) were received ear implants containing 3 mg norgestomet for 9 days at random stage of the oestrus cycle, and treated intramuscularly with 3 mg norgestomet + 5 mg oestradiol valerate at the time of implant insertion. On day seven of the implant insertion, FSH was injected over a 4-d period described in the control group. D-cloprostenol was injected to cows in both groups with the 5th FSH injections. Animals in both groups were inseminated 12 and 24 h after the onset of oestrus. Seven days after the second insemination, the ova/embryos were collected. There was no significant difference between the groups for last FSH injection-oestrus intervals, the mean numbers of corpus luteum, total ova/embryos, grade 1, 2 and 3, transferable and degenerated embryos and unfertilized ova and recovery rate (P>0.05). The results confirmed that progesterone + oestradiol valerate injection at the time of insertion of an ear implant for 9 days, regardless of the stage of the oestrus cycle, can be used as a pretreatment for superstimulation started on day 7 of the implant insertion in Brown Swiss cows.

Keywords: *Cow, Oestradiol Valerate, Progesterone, Superovulation*

Progesteron ve Östradiol Valerat Kullanılarak Senkronize Edilen İsviçre Esmeri İneklerde Süperovulasyon Cevabının Araştırılması

Özet

Progesteron ve östradiol valerat kullanılarak yapılan senkronizasyondan sonra süperovulasyon cevabını araştırmak amacıyla 19 İsviçre Esmeri inek rastgele iki gruba ayrıldı. Kontrol grubu inekler (n=9) 10-13. günler arasında (östrüs= 0. gün) günde iki kez kas içi azalan dozlarda (4, 4, 3, 3, 2, 2, 1, 1 ml) FSH (toplam: 400 mg) enjeksiyonu ile süperovulasyona tabi tutuldu. Çalışma grubunda bulunan ineklere (n=10) ise östrüsün herhangi bir evresinde 3 mg norgestomet içeren kulak altı implantı 9 gün boyunca takıldı ve implant takıldığı gün kas içi yolla 3 mg norgestomet + 5 mg östradiol valerate enjeksiyonu yapıldı. İmplant takıldıktan sonraki 7. günden itibaren 4 gün boyunca FSH kontrol grubunda belirtildiği gibi enjekte edildi. Her iki gruptaki ineklere 5. FSH enjeksiyonu ile birlikte kas içi d-kloprostenol enjeksiyonu yapıldı. Her iki grupta da inekler östrüs başlangıcından 12 ve 24 saat sonra tohumlandılar. Ovum/embriyolar tohumlamadan 7 gün sonra toplandı. Gruplar arasında son FSH enjeksiyonu-östrüs aralığı, ortalama korpus luteum, toplam ovum/embriyo, kalite 1, 2 ve 3, transfer edilebilir ve dejenere embriyo ve fertilize olmamış ovum sayıları ve kazanım oranları açısından önemli bir fark bulunmadı (P>0.05). Elde edilen sonuçlara göre İsviçre Esmeri ineklerde süperstimülasyon öncesinde, östrüs siklusunun dönemine bakılmaksızın, 9 günlük kulak implantının uygulanma gününde progesteron+östradiol valerat enjeksiyonu ve 7. günde başlatılan süperovulasyon tedavisinin kullanılabileceği kanısına varıldı.

Anahtar sözcükler: *İnek, Östradiol Valerat, Progesteron, Süperovulasyon*

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INTRODUCTION

Embryo transfer (ET) technology has been used to accelerate genetically improving and to increase the number of valuable animals [1]. In this respect, the treatment of cattle to induce multiple ovulations (superovulation) has been the object of much research during the past thirty years [2]. Because the superovulation is the first step towards enabling the genetically superior cow to produce a greater number of calves, it has been a major consideration in the development of widely usage of ET technology [3].

In cattle, the growth of large antral follicles occurs in several distinct waves during the oestrus cycle [4]. Each wave is characterized by the simultaneous emergence of a cohort of follicles. One of the follicles becomes dominant and continues to develop to ovulatory size, while suppressing the growth of other follicles [5]. It is reported that the presence of dominant follicle at the time of gonadotrophin treatment adversely affects superovulatory response [6]. Transvaginal ultrasound-guided ablation of the dominant follicle before initiating gonadotrophin treatment is a new approach for increasing the superovulatory response [7]. Manipulation of the follicular wave is another new procedure used in the cattle ET industry for this purpose, therefore it is reported that there are few reports in the scientific literature on its use [8].

In superstimulatory treatments, gonadotrophin injections must be initiated at the expected time of the follicular wave emergence for the purpose of improve superovulatory response. Ear implants containing progesterone+oestradiol combinations at a random stage of oestrus cycle have been used over the past years to manipulate follicular wave emergence [9]. This combination consists of insertion of a progesterone implant and the injection of oestradiol+progesterone on the insertion day [10]. Oestradiol+progesterone injection suppresses FSH and LH release and is followed by atresia of large follicles at a variety of stages of follicular growth and dominance [11]. This would result in the synchronous development of a new crop of growing follicles that would respond more favorably to gonadotrophin treatments. Superstimulatory treatments initiated 4 days after treatment with 2.5 mg oestradiol benzoate or 5 mg or 2.5 mg oestradiol-17β + progesterone resulted in superovulatory responses comparable to those initiated 8-12 d after estrus [12]. However, because of the interval to emergence of a new follicular wave is quite variable in cows treated with different oestradiol preparations, iniation day of gonadotrophin treatment following these various preparations has a great importance on superovulation response [13]. In an experiment [14], superstimulation 5 days after treatment with 5 mg estradiol valerate and 3 mg norgestomet resulted in less synchronous emergence of a follicular wave and a lower superovulatory response.

Because of the absence of scientific literature investigating the superovulatory response of gonadotrophin injection started on day 7 of the progesterone + oestradiol valerate treatment in Brown Swiss cows, the objective of this study was to evaluate the manipulation of the oestrus cycle using progesterone and oestradiol valerate as a pretreatment for superstimulation started on day 7 of the implant insertion.

MATERIAL and METHODS

In February to December 2005, a total of 19 lactating Brown Swiss cows, aging 3-7 years were randomly allocated into two groups. The animals were selected taking the criterions listed as followed: **a)** no dystochia and retained fetal membranes in previous calving; **b)** no purulent discharge during vaginal examination; **c)** 50 to 90 d postpartum; **d)** have shown oestrus at least twice since calving; **e)** no AI or mating after previous calving. The cows were kept in similar conditions of handling and feeding and fed with a ration composed of corn silage, alfalfa hay and a concentrate-mineral mix, had ad libitum access to fresh water and housed in a free-stall confinement facility.

The cows in control group (n=9) were superovulated with FSH (Folltropin-V, total: 400 mg NIH-FSH-P1, Bioniche Animal Health Inc., Ontario, Canada), from day 10 to 13 (spontenoeus reference oestrus=day 0), using twice daily injections with decreasing doses (4, 4, 3, 3, 2, 2, 1, 1 ml) and injected 0.500 mg of D-cloprostenol (Estrumate, Sanofi Doğu İlaç, İstanbul, Türkiye) on day 12 with the 5th FSH injections.

In treatment group (n=10), cows were received ear implants (Crestar, Intervet, İstanbul, TURKEY) containing 3 mg norgestomet at random stage of the oestrus cycle. Ear implants were inserted to the ear of each cow subcutaneously for 9 days. Cows were treated intramuscularly with 3 mg norgestomet + 5 mg oestradiol valerate at the time of implant insertion. On day seven of the implant insertion, FSH was injected for 4 days as described in the control group. D-cloprostenol at the dosage of 0.500 mg was injected with the 5th FSH injections.

Following the last FSH injection, the cows in both groups were observed for oestrus signs (visual observation of standing heat, vaginal discharge) three times a day for 30 min each time. Oestrus was confirmed by rectal palpation of fluctuant dominant follicles and uterine tonus. Cows in both groups were artificially inseminated 12 and 24 h after the onset of oestrus. All cows were inseminated using frozen-thaved semen (20x10^6 spermatozoa/straw with about 60% progressively motility after thawing) from a single bull throughout the experiment.

Seven days after the second insemination, superovulatory response was assessed by rectal palpation and ultrasonography (ESAOTE Pie Medical, 100 Falco) with regard to the number of corpora lutea in the ovaries using a 7.5 MHz rectal linear-array transducer. The ovaries were scanned in several planes to identify all corpora lutea. All cows were flushed using 1000 ml ringer lactate (Ringesol®, Vilsan, Ankara, Türkiye) containing 1% fetal calf serum (N-4267, Sigma) and 0.1% kanamicyn sulfate (Kanovet, Vetaş, İstanbul, Türkiye) with a two-way disposable Foley catheter, inserted into the uterine horn through the cervix, and the ova/embryos were collected. The recovered lavage fluid was filtered through an 72 µm embryo filter (EMCON filter, Ref. No. 19010/8000, Minitüb, Abfüll- und Labortechnik GmbH & Co. KG, Tiefenbach, Germany). Searching and evaluation of embryos collected were carried out on petri dishes under a stereomicroscope at 20-fold magnification. The quality grading of the recovered embryos was done according to the morphological criteria of quality and viability determined by International Embryo Transfer Society (IETS) [15]. Embryos were classified as grade 1 (excellent or good), grade 2 (fair), grade 3 (poor) and degenerated embryos and unfertilized oocytes. Grade 1, 2 and 3 embryos were defined as transferable.

The Mann-Whitney nonparametric test was used to compare the mean numbers of corpus luteum, total ova and embryos, grade 1, grade 2 and grade 3, transferable and degenerated embryos and unfertilized ova between the groups. The last FSH injection-oestrus interval was compared using analysis of variance (One-way ANOVA). All analyses were carried out using a statistical analysis system configured for computer (MINITAB, Release 12.1, Minitab Inc). The differences were considered significant at P<0.05.

RESULTS

Oestrus was determined in all cows 12-44 h after the last injection of FSH in both groups. The onset of oestrus was determined in 1 and 8 cows in control group whereas 5 and 5 cows in treatment group 12 and 18 h after the last FSH injection, respectively. There was no significant difference between implant removal-oestrus intervals of control and treatment groups (17.3±0.67 and 15.0±1.0 h, respectively) (P>0.05).

The number of corpus luteum, total ova and embryos, transferable and degenerated embryos and unfertilized ova and recovery rate (%) were similar in control and treatment groups (P>0.05, *Table 1*).

Table 1. *Recovery rate (%) and the mean numbers of corpus luteum, total ova and embryos, transferable and degenerated embryos, unfertilized ova in control and treatment groups (±SEM)*

Tablo 1. *Kontrol ve çalışma gruplarında kazanım oranları (%) ve ortalama korpus luteum, toplam ovum ve embriyo, transfer edilebilir ve dejenere embriyo ve fertilize olmamış ovum sayıları (±SEM)*

Parameters	Control	Treatment
Recovery rate (%)	70	69
Numbers of:		
Corpus luteum	8.4±1.4	9.2±1.6
Total ova and embryos	5.9±1.7	6.3±1.3
Transferable embryos	4.7±1.6	4.5±1.0
Unfertilized ova	0.6±0.3	0.5±0.2
Degenerated embryos	0.7±0.2	1.3±0.6

The number of grade 1, 2 and 3 embryos were shown in *Table 2*. The differences were not significant among the groups (P>0.05).

Table 2. *The mean numbers of grade 1, 2 and 3 (transferable) embryos in control and treatment groups (±SEM)*

Tablo 2. *Kontrol ve çalışma gruplarında ortalama kalite 1, 2 ve 3 (transfer edilebilir) embriyo sayıları (±SEM)*

Groups	Grade 1	Grade 2	Grade 3
Control	2.9±1.3	1.1±0.4	0.7±0.2
Treatment	2.2±0.5	1.4±0.5	0.9±0.4

DISCUSSION

Initiating superstimulation during mid-cycle, especially on d 8-12, is a conventional protocol because of its greater superovulatory response which is reported by many researchers [12,16,17]. This protocol is based on the knowledge of that 8 to 12 d after oestrus would be the approximate time of emergence of the second follicular wave in two or three wave cycles [12]. According to the emergence of a new follicular wave, there would be a cohort of growing follicles around that time [5]. However, the day of the second follicular wave varies among cows, and between the cycles with two and three follicular wave [18]. This study evaluated the efficacy of progesterone + oestradiol valerate in synchronizing the oestrus cycle as a pretreatment for superstimulation in Brown Swiss cows.

To form a responsive, healthy, oestrogen-active dominant follicle at the end of treatment, the control of oestrus and ovulation is based on firstly that regulation of the life span of the CL and secondly that synchronization of follicle wave emergence [19]. The use of progestins in oestrus and ovulation synchronization programs has become widespread in the last years [20-22]. Progesterone has been shown to suppress follicular growth and thereby influence the onset of subsequent wave emergence. Recent reports have shown that oestradiol administered to progesterone-implanted cows results in follicle suppression and synchronous emergence of a new follicular wave around 4 days later, regardless of the phase of follicular development at the time of treatment [23,24]. In our experiment, all cows in both groups showed oestrus 12-44 h after the last FSH injection. The last FSH injection-oestrus interval was shorter than findings of Colazo et al.[13], who synchronized the cows with ear implants containing progesterone + oestradiol for 8 days prior to the superstimulation and injected gonadotrophin over a 4-d period initiated on day 5. The results obtained in this study confirmed that the protocol applied succeeded in synchronizing the oestrus cycle in the previous of superstimulation.

The number of CL in control and treatment groups was 8.4 and 9.2, respectively. Superovulatory response in this study was similar with the findings of some researchers [7,13,25,26] and lower than those of some others [27,28]. On the other hand, the number of transferable embryos that obtained per flush (4.7 and 4.5 in control and treatment groups, respectively) is comparable with many studies [29-31]. It is reported that, superovulatory response in cows and the number of transferable embryos that obtained per flush is affected by several factors such as gonadotrophic hormone, donor, nutrition, dominant follicle in the donor's ovary and etc. [3,32-35]. The differences in superovulatory response between the experiments can be explained by the factors which were cited above.

The recovery rates were 70% and 69% in control and treatment groups, respectively, in this study. Our recovery rate was similar with Mikkola et al.[3] and Barati et al.[36] who found this rate 68-72% whereas it had an inconsistency with the findings of Sartori et al.[35] (64%). The differences among the findings in various researches may be due to many factors such as recovery day and catheter type and positioning as reported by Kanagawa et al.[34].

Ovarian status at the time of gonadotrophic treatments has an influence on the superovulatory response of cattle [37]. It is shown that gonadotrophin treatments must be initiated at the expected time of the follicular wave emergence in order to optimize super-ovulatory response [16,38]. Hence, recognizing the importance of follicle wave dynamics and devising methods for the synchronization of follicular wave emergence have simplified the way in which superovulation is achieved [39]. Superovulatory response in our study was in consistent with Colazo et al.[13] and Mapletoft et al.[14] who initiated superstimulation in lactating Hollstein cows 5 days after treatment with 5 mg oestradiol valerate + progesterone. The main objective of this study was to evaluate the superovulatory response between superstimulation on d 8-12 after oestrus (conventional protocol) and super-stimulation after oestrus synchronization by using progesterone + oestradiol combination. There was no significant difference between groups in any criteria that is studied in this experiment. That is, superovulatory response did not differ between groups. The super-ovulation response in treatment group indicates that progesterone + oestradiol protocol not only synchronizes oestrus cycle but also synchronizes the follicular growth prior to the gonadotrophin treatment initiated on d 7 of the implant insertion for superstimulation.

The results obtained in this study confirmed that a progesterone + oestradiol treatment, regardless of the stage of the oestrus cycle, and gonadotrophin injection over a 4-d period initiated on day 7 of the implant insertion, was sufficient to synchronize oestrus cycle as a pretreatment for superstimulation to promote transferable embryos in cows. Applying similar procedures to the donors has made oestrus detection, and the need to wait for animals to "come into heat" unnecessary, facilitating the management of commercial embryo transfer programmes. Supersitmulation started 7 days after synchronization of follicular wave emergence by progesterone + oestradiol valerate treatment has resulted in comparable superovulatory response with conventional protocol which is started on d 8-12 after oestrus. The need for detecting oestrus or ovulation and waiting 8 to 12 days to initiate gonadotrophin treatments is eliminated with follicular wave synchronization by using progesterone + oestradiol. In conclusion, this study showed that exogenous control of oestrus cycle by using progesterone + oestradiol valerate combination at the time of insertion of an ear implant for 9 days, regardless of the stage of the oestrus cycle, and gonadotrophin injection over a 4-d period initiated on day 7 of the implant insertion, offers the advantage of initiating superstimulatory treatments at a time that is optimal for follicle recruitment in Brown Swiss cows.

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