TÜBİTAK

Turkish Journal of Veterinary and Animal Sciences

http://journals.tubitak.gov.tr/veterinary/

Research Article

Turk J Vet Anim Sci
(2014) 38: 157-160

doi:10.3906/vet-1304-43

The results of consecutive superovulations in cows by induction with various exogenous progesterone routes

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Received: 18.04.2013 • **Accepted:** 24.10.2013 • **Published Online:** 28.02.2014 • **Printed:** 28.03.2014

Abstract: The aim of this study was to determine the possibility of yielding more transferable embryos from cows superovulated with/without exogenous progesterone (ear implant and intravaginal device). Two experiments were conducted in Holstein Friesian cows to evaluate the effect of exogenous progesterone on the yield of corpus luteum (CL), total embryo and ova, and transferable embryos before superovulation. A superovulation program using 2 different progesterone resources was applied (G1 = intravaginal device, G2 = ear implant). Superovulation and uterus irrigations were done at 28-day intervals in the treatment groups. Cows in the control group were superovulated at 60-day intervals without application of exogenous progesterone. The mean numbers of CLs and transferable embryos for the G1, G2, and control groups were, respectively 4.82 ± 0.29, 6.71 ± 0.29, and 7.81 ± 0.31 for CLs and 0.86 ± 0.35, 3.50 ± 0.35, and 1.53 ± 0.39 for transferable embryos. It was shown that exogenous manipulation of the estrus cycle by progesterone could be applied without superovulation intervals in Holstein cows. It may also be postulated that the ear implant was more effective than the intravaginal device as a progesterone source in the superovulation program.

Key words: Cattle, exogenous progesterone, consecutive superovulation

1. Introduction

Superovulation responses in cows are affected by many factors, such as individual response, endocrinological and ovarian status, breed, age, season, number of stimulations, type of gonadotropin used, and feed intake (1–3). The intensive control of ovarian function is necessary for successful superovulation (4). The aim of superovulation is to suppress atresia in an excessive number of follicles, thus enabling them to get ready for ovulation. Furthermore, the presence of a dominant follicle before superovulation can have negative effect on superovulatory response (5). Superovulatory response is declined in cows with a dominant follicle of larger than 5 mm as compared with cows without a dominant follicle at the beginning of the superovulatory treatment (6,7). Superovulations should be applied with intervals of 2–4 months to obtain transferable embryos for repeated superovulation programs (8,9). Information on the results of consecutive stimulations is limited and a significant variation in embryo production is found (1,10). The physiological and chemical factors involved in successful superovulation have not been completely understood yet. Superovulation programs can be repeatedly done at 60-day intervals with a slight decrease in embryo production over time (11). The effectiveness of superovulation programs depends on getting a large number of transferable embryos with a low cost per stimulation.

The aim of this study was to show the possibility of getting more transferable embryos from cows superovulated with exogenous progesterone than from cows superovulated without exogenous progesterone.

2. Materials and methods

A total of 17 lactating Holstein Friesian cows between 3 and 6 years old were randomly allocated into 3 groups as donors. Donors were selected from at least 60 days after postpartum and had regular estrus cycles with no dystocia or purulent discharge. All cows were stimulated with follicle-stimulating hormone (FSH; Folltropin V total = 400 mg NIH-FSH-P, Bioniche Animal Health Inc.,

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Ontario, Canada). Injections of FSH were given twice daily (morning and evening) for 4 days with decreasing doses (4 and 4, 3 and 3, 2 and 2, and 1 and 1 mL). In the control group (n = 5), the cows were stimulated after 11 days from spontaneous estrus at day 0. Repeated superovulations were then done at 60-day intervals. In the treatment groups, cows had either an intravaginal device (PRID, Sanofi Doğu İlaç, İstanbul, Turkey) containing 1.55 g of progesterone plus 10 mg of estradiol benzoate capsule as group 1 (G1; n = 6) or ear implants (Crestar, Intervet, İstanbul, Turkey) containing 3 mg of norgestomet acetate plus 5 mg of estradiol valerate injection as group 2 (G2; n = 6). Progesterone applications were initiated at a random stage of the estrus cycle for all treatment groups and were stopped on the eighth day of the superovulation application scheme.

All of the cows were observed 3 times a day for estrus signs for at least 15 min each time following the last FSH injection. Estrus was determined by rectal palpation from the presence of preovulatory follicles and uterine tonus. The cows were artificially inseminated in all groups with same frozen bull semen during the experiment. Seven days after the last insemination, superovulation responses were evaluated by rectal palpation and ultrasonography (ESOATE, Maastricht, Aquila, the Netherlands) with regard to the number of CLs in the ovaries. The uteri were irrigated by 1000 mL of ringer lactate solution (Ringesol, Vilsan, Ankara, Turkey) containing 1% fetal calf sera (N-4267, Sigma) and 0.1% kanamycin sulfate (Kanovet, Vetaş, İstanbul, Turkey) by 2-way silicone catheter. The catheter was inserted into the uterine horn and embryos were collected by routine irrigation method. Recovered embryos were graded according to the morphological criteria determined by the International Embryo Transfer Society under stereomicroscope and were classified as grade 1 (excellent or good), grade 2 (fair), grade 3 (poor), and degenerated and unfertilized oocytes (UFO). Grades 1 and 2 were defined as transferable embryos for this study.

Progesterone implant and intravaginal devices were applied at 28-day intervals as shown in the Figure. The criteria for each stimulation were based on the number of CLs, large follicles, ova, and embryos. There was no extra feeding program for the cows during the experiment.

Repeated measures were used to analyze the data. Statistical analyses were done using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC, USA). Tukey's pairwise comparisons were performed to separate the differences among treatment groups.

3. Results

The numbers of irrigations of the uterus were 36, 36, and 30 for G1, G2, and the control group, respectively. The recovery rates were 57.5%, 58.0%, and 83.3% in G1, G2, and the control group, respectively, when the present number of large follicles was disregarded.

All donor ovaries were examined by ultrasonography after the embryo collection process. Least squares means of ovarian responses and significance levels of Tukey's pairwise comparison between the treatment means are shown in the Table. The means of large follicles and degenerated embryos were similar in the control and treatment groups. The CL means between the control group and G1 and between G1 and G2 were statistically significant ($P < 0.05$). Total ova and embryo mean differences were significant between the control group and G1 and G2 ($P < 0.05$). Mean differences of transferable embryos were significant between G2 and the other groups ($P < 0.05$). The only unfertilized ova mean difference was between the control group and G2 ($P < 0.05$) (Table).

4. Discussion

The use of exogenous progesterone in estrus synchronization programs has become widespread in the last years (12,13). However, exogenous progesterone application for such programs may have negative effects on high quality embryo yield (1). Goulding et al. (14) emphasized that superovulation can alter the number of recovered embryo quality by exogenous progesterone treatment. There are many factors involved in the superovulatory process in cows. However, it is possible to control the estrus cycle precisely using reproductive hormone preparation, and there is large variation in

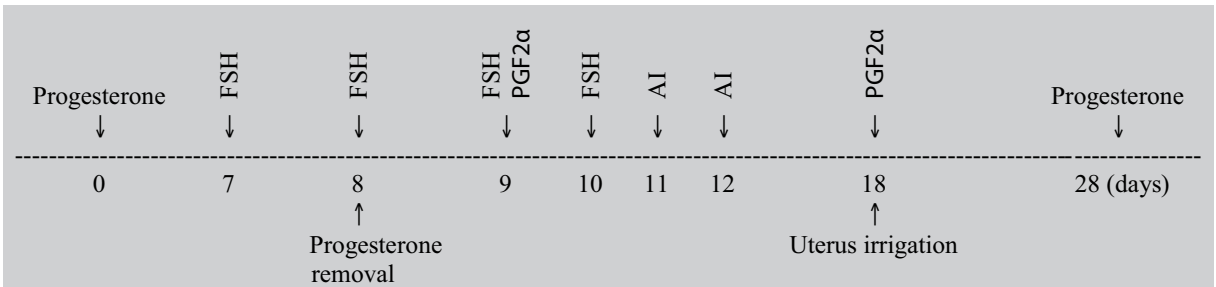

Figure. Superovulation application scheme. AI: Artificial insemination.

Table. Least squares means ± SEM of ovarian responses.

Number of:	G1	G2	Control
Corpora lutea*	4.8s2 ± 0.29[b]	6.71 ± 0.29[a]	7.81 ± 0.31[a]
Large follicles	0.89 ± 0.12	0.50 ± 0.12	0.60 ± 0.14
Total ova and embryos*	3.39 ± 0.44[b]	4.22 ± 0.44[b]	7.00 ± 0.48[a]
Transferable embryos*	0.86 ± 0.35[b]	3.50 ± 0.35[a]	1.53 ± 0.39[b]
Unfertilized ova*	0.25 ± 0.09[ab]	0.05 ± 0.09[b]	0.57 ± 0.09[a]
Degenerated embryos	0.75 ± 0.14	0.44 ± 0.14	0.66 ± 0.16

*: Means within same rows with different superscripts differ significantly ($P < 0.05$).

the yield of transferable embryos among donors (15). For these reasons, this discussion will be focused on the relationship between exogenous progesterone application and recovered number of transferable embryos for the superovulation programs in this study.

The stage of the cycle at which the progesterone was inserted had significant effects on the results. When the progesterone was inserted during the early luteal phase, there was a reduction in the ovulation rate and increase in the number of large follicles compared with insertion at the middle or late luteal phase. Therefore, superovulatory response can be adversely affected if the treatments are not initiated precisely at wave emergence. Follicular waves and terms of the ovarian cycle were disregarded for treatments groups but the superovulation program was started at the middle cycle as the common practice for the control group (4,16,17).

The differences in findings in recovery rates among the various studies might have been due to many factors such as technician experiences, recovery time, and catheter type positioning, as reported by Kanagawa et al. (18). The differences in the recovery rates of the groups of the present study could have also depended on these factors mentioned above. However, the recovery rates found in this study might be satisfactory as compared to some other studies (15,19). Some researchers reported that they had collected about 10.1 embryos, of which 4.5 or 5.0 were transferable embryos, using FSH for stimulation of thousands of donors (1,20). The number of transferable embryos in the present study was lower than those of the above reports but similar to the findings of Martínez et al. (13). The means of degenerated embryos were similar in the control and treatment groups. The means of total ova and embryos for the control group were superior to those of other groups ($P < 0.05$). The mean of unfertilized ova was greater in the control group as compared to G2, while the mean of transferable embryos in G2 was superior to those of the other groups ($P < 0.05$) in the present study.

Bo et al. (21) acquired 12.7 CLs, 9.4 ova and embryos, and 3.7 transferable embryos using progesterone + estradiol benzoate combined with a superovulation program in their studies. The values achieved in the present study were higher than those of Sartori et al. (22). Bülbül et al. (23) found a mean of 4.5 transferable embryos in Brown Swiss cows after superovulation with Crestar. The classification of the number of transferable embryos covered in this study was just grade 1 and grade 2, but their transferable embryo classifications were grade 1, grade 2, and grade 3. This result was higher than that of the present study. This situation may be due to differences in donor breed and nutritional intake.

Hasler et al. (24) reported that the number of ova was not affected through 10 consecutive superovulations but fertilization rate and the number of embryos decreased when cows were superovulated 1 to 10 times. Mori (25) reported that superovulation responses were not affected if there were 60 days between repeated superovulations. The present study's findings on repeated stimulation were similar to the findings of some other studies (24,25).

There was no significant difference between the mean number of CLs in the control group and G2, and the superovulation response of G1 was lower than those of the other groups in the present study. Superovulatory response in this study was similar to the findings of some other researchers (6,20,23). The number of CLs tended to be decreased in some other researchers' results (15,26). These lower responses might have been dependent on the individual differences and lack of an additional feeding program to promote superovulation for donors during the present experiment. Santos et al. (27) reported that nutrition of the donor cow influences oocyte and embryo quality. Nutritional controls of reproduction are complex and mediated by a range of endocrine and metabolic signals that influence follicle, oocyte, and embryo production. Therefore, diets should be formulated to optimize the supply of nutrients to meet the needs of different tissues

according to the physiological status of the animal (16). Moreover, Kojima et al. (28) reported the importance of cattle herd management under good feeding and breeding conditions for superovulatory responses. The reason for low superovulatory responses in this study might have been that there was no extra feeding of donor cows during the experiment as advised by Kanagawa et al. (18) for donor cows.

In conclusion, application of exogenous progesterone before superstimulation for synchronization of ovarian status eliminates difficulties of estrus detection, especially for commercial embryo transfer programs. In this study, it was shown that exogenous control of the estrus cycle by progesterone could be applied without superovulation intervals in Holstein cows. The use of ear implant was found to be more effective than the intravaginal device as a progesterone administration route.

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