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THE EFFECT OF THE REDUCED DOSE OF GnRH ON CONCEPTION, OVULATION AND OVARIAN STRUCTURES IN OVSYNCH PROGRAM OF LACTATING DAIRY COWS

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ABSTRACT
This study was conducted to evaluate the efficacy of reducing GnRH dose on the formation of ovulation and conception, and sizes of the ovarian structures following an Ovsynch program in lactating cows. The cows were allocated randomly to two treatment groups (full dose; FD, n=20 and half dose; HD, n=20). Cows in the FD group were treated with 10.5 µg buserelin acetate on day 0, with 0.150 mg D- cloprostenol 7 d later and with 10.5 µg buserelin acetate 2 d later. Estrous cycles in HD group were synchronized using the same scheme as FD-treated cows, but the dose of buserelin acetate was reduced to 5.25 µg at both GnRH administration times. Ovarian structures were monitored by ultrasound with a 6-8 MHz linear trans-rectal probe on days 0, 7, 9, 10, and 11. Cows were inseminated at the 16-20 h after second GnRH administration. No significant differences were observed in the dominant or ovulatory follicle diameters in FD and HD groups. Ovulation incidence from second GnRH injection by the 24 hour after fixed-time AI did not differ between FD (85 %) and HD (90 %) groups. Also, the conception percentages did not differ statistically between the HD (50%) and FD (40 %) groups.

Keywords: Buserelin acetate, Ovsynch, Ovarian structures, Ovulation, Conception, Dairy cows.

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Contribution/ Originality

This study is one of very few studies which have investigated the effect of reducing doses of buserelin acetate on the follicular development, ovulation and conception rates in the Ovsynch program of dairy cows.

1. INTRODUCTION

The goal for a successful estrous synchronization program in lactating dairy cattle is the precise control of estrous, which will allow high fertility to a fixed-timed artificial insemination (FTAI) without the need for estrous detection [1]. Previous studies, performed with $PGF_{2\alpha}$ or progesterone based estrous synchronization programs in dairy cows, have shown that these programs are not adequate for FTAI without estrous detection [2, 3]. In 1995, a hormonal protocol was developed to synchronize ovulation (Ovsynch) in lactating dairy cows using GnRH and $PGF_{2\alpha}$. Ovsynch protocol may have a major impact on managing reproduction of lactating dairy cows, since it can permit FTAI without the need for estrous detection [4]. In the Ovsynch program, GnRH is given at a random stage of the estrous cycle, followed by $PGF_{2\alpha}$ on day 7 and second a dose of GnRH 48 h later [4, 5]. This program coordinates follicular recruitment, corpus luteum (CL) regression and time of ovulation also permits timed

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insemination 0 to 24 h after the last GnRH injection [1, 4, 6]. Although conception rates after detected estrous were greater than those after FTAI with Ovsynch, pregnancy rates were higher for the Ovsynch program because of estrous detection problems [7]. In addition, Pursley, et al. [5] suggested that even in dairy herds with good reproductive management, Ovsynch protocol can reduce days to conception. However, it has been suggested that the costs of labor and hormone expenses should be considered at deciding this form of reproductive program for routine use [8]. One of the most common objections to using Ovsynch is the overall cost of the hormones needed to synchronize ovulation. GnRH creates about 70 % of the total hormone costs in the Ovsynch protocols of dairy cows. It was reported that the reproductive performance in dairy cows was not effected when the GnRH dose (gonadorelin, fertirelin) was reduced to half (50 µg instead of 100 µg) in the Ovsynch protocols [9, 10]. In a study conducted by Ahmadzadeh, et al. [11] a reduced dose of GnRH (gonadorelin) did not affect ovulation time relative to the second GnRH injection and did not compromise the incidence of ovulation and luteal development used in Ovsynch protocol. Buserelin is a more potent GnRH agonist than both gonadorelin and fertilerin in the bovine for FSH and LH release [12, 13]. Therefore, it is generally used at 8-10 µg doses for synchronizing ovulation in the dairy cows [14-16]. However, reducing doses of buserelin in the Ovsynch program of dairy cows has not been tested yet. In this study, we have investigated the effect of decreasing the dose of buserelin acetate from 10.5 µg to 5.25 µg on sizes of the ovarian structures, ovulation and conception used in the Ovsynch program in lactating dairy cows.

2. MATERIALS AND METHODS

Present study was conducted at a commercial dairy cow farm (Ceyhan, Adana, Turkey). Animals were housed in a free stall barn, milked twice daily and fed with a total mixed ration ad libitum to meet the requirement for lactating cows. All cows were subjected to a gynecological examination by trans-rectal palpation and ultrasonography at the beginning of the study. Forty lactating Holstein cows (4-7 years of age) with normal reproduction at 70-110 days postpartum having body condition score of 2.5 – 3.0 were used in this trial. Cows were allocated randomly divided into full-dose (FD, n=20) and half-dose (HD, n=20) Ovsynch groups, according to status of luteal structures for minimized cyclic differences between groups. Status of luteal structures observed on ovaries in ultrasonic image at the time of first GnRH administration in the animals in treatments groups is presented in Table 1.

Table-1. Status of CL diameters at the time of first GnRH administration in treatment groups

Tratment groups	Diameter of CL (mm)		
	≥ 20 mm	11-19 mm	5-10 mm
FD Ovsynch (n=20)	8	8	4
HF Ovsynch (n=20)	7	8	5
Total	15	16	9

Cows in the FD group were treated with 10.5 µg buserelin acetate, which shows the same effect of 10 µg buserelin (Receptal, Intervet, Turkey) on day 0, with 0.150 mg D-cloprostenol (PGF 2α, Dalmazin, Vetaş, Turkey) on day 7, with 10.5 µg buserelin acetate on day 9. Cows in the HD group were synchronized by the same protocol and received 5.25 µg per buserelin acetate injection. Both GnRH and PGF2α were administered through intramuscular route, in the thigh muscle (semimembranosus) with 2 or 5 mL syringe with 22 gauge, 32 mm needle. Cows in both groups, 16-20 h after their second treatment using buserelin acetate, were inseminated by the same operator.

Ovarian structures (antral follicles >5 mm in diameter and corpus luteum) were monitored by ultrasound using scanner with 6-8 MHz linear trans-rectal probe (Falco Vet 100; Pie Medical; Holland) on days 0 (day of GnRH administration) 7, 9, 10, and 11. The equipment had an image freezer facility with electronic calipers for taking measurements. Presence of luteal structures at the time of first GnRH injection (day 0), diameter of corpus luteum at the time of $PGF_{2\alpha}$ injection (day 7), diameter of the largest follicle (Dominant follicle, DF) at the time of the second GnRH injection (day 9) and diameter of the largest follicle (Ovulatory follicle, OF) at the time of artificial insemination (day 10) were determined by ultrasound. Ovaries were also examined at 24 h (day 11) after artificial insemination to confirm ovulation. Luteal and follicular structures were defined in real time ultrasound on the basis of their diameter [17-19] and echogenic appearance [20, 21]. Ultrasound measurements of follicle and CL diameters were performed according to the technique described by Taylor and Rajamahendran [17]. Briefly, the ovaries were scanned in several planes to identify follicles ≥ 5 mm in diameter and to provide an image of the CL with its greatest cross sectional area. Desired images were frozen on the screen; measurements were taken using a built-in caliper system. All ultrasound examinations were performed by one operator.

Ovulations were determined by following the fate of follicles on the ovary at time of second GnRH injection, in artificial insemination (AI) time and at 24 h after FTAI. Ovulation was defined as the disappearance of any antral follicle ≥ 10 mm in diameter at the time of an ultrasound examination compared with the previous ultrasound examination [22]. Ovulation incidence was calculated as the number of cows that ovulated at least one follicle from the second GnRH injection to 24 hours after FTAI, expressed as a percentage of the total number of cows receiving Ovsynch protocol. Cows that ovulated at last one follicle between second GnRH injection and FTAI were classified as ovulating before FTAI. Cows that did not ovulate by 24 h after FTAI were classified as not ovulating during the treatment scheme. Diameter of ovulatory follicle in cows synchronized with full-dose or half dose GnRH were determined on the day second GnRH treatment (d 9) and FTAI (d 11) were provided. Conceptions were determined by ultrasound detection of embryonic fluid and fetus at 30 d post FTAI in all cows.

All statistical analyses were performed with the SPSS 22.0. The significance of differences in the average CL diameter at time of $PGF_{2\alpha}$ injection (d 7), dominant follicle diameter at time of second GnRH (d 9) injection and size of ovulatory follicle in the during FTAI (d 10) between the groups were analyzed by t-test. Number and percentage of cows ovulating after treatment with full dose or half dose of GnRH in the Ovsynch protocol were compared using the Fisher's exact Chi-square test. Conception incidence at 30 d post FTAI between treatment groups (FD or HD groups) were analyzed with Chi-square test.

3. RESULTS

Mean CL diameter (±SEM) at time of $PGF_{2\alpha}$ injection (d 7), dominant follicle diameter at time of second GnRH injection (d 9) and ovulatory follicle diameter during FTAI (d 10) in lactating Holstein cows treated with 10.5 µg (FD) or 5.25 µg (HD) of GnRH are shown in Table 2. All cows in treatment groups had a CL (≥ 10 mm) at time of $PGF_{2\alpha}$ injection, the mean of CL diameters (±SEM) were not different between FD and HD groups (P>0.05). No significant differences were observed between the means of dominant (16.8±0.7 mm vs. 17.7±0.8 mm) or ovulatory follicle (20.0±1.0 mm vs. 20.9±1.0 mm) diameters in FD and HD groups (P>0.05).

Table-2. The measurements of ovarian structures at time of $PGF_{2\alpha}$ injection (d 7), at time of second GnRH (d 9) injection and at AI (d 10) in treatment groups.

Treatment groups [A]	CL diameter (mm)	DF diameter (mm)	OF diameter (mm)
	at $PGF_{2\alpha}$ Injection	at second GnRH Injection	at time of AI
FD Ovsynch (n=20)	23.0 ± 1.6	16.8 ± 0.7	20.0 ± 1.0
HD Ovsynch (n=20)	20.4 ± 1.7	17.7 ± 0.8	20.9 ± 1.0

CL: Corpus luteum, DF: Dominant follicle, OF: Ovulatory follicle

[A] For each item, no statistical difference between treatment groups was detected using *t*- test. *P> 0.05*

Ovulation incidence of cows in the FD and HD groups from second GnRH injection to 24th hours after FTAI is presented in Table 3.

Table-3. Number and percentage of cows ovulating in full dose or half dose GnRH treatment groups

Treatment groups [A]	Ovulations before fixed-time AI[B], n (%)		Ovulations by 24th hours after fixed-time AI[C], n (%)	
	Yes	No	Yes	No
FD Ovsynch (n=20)	0	20 (100)	17 (85)	3 (15)
HD Ovsynch (n=20)	3 (15)	17 (85)	18 (90)	2 (10)

[A] For each item, no statistical difference between treatment groups was detected using the Fisher's exact Chi-square test. *P> 0.05*

[B] Time interval from second GnRH to fixed-time AI.

[C] Time interval from second GnRH injection to 24th hours after fixed-time AI.

Ovulation incidence from second GnRH injection by 24th hours after FTAI did not differ between FD (85 %) and HD (90 %) groups (P>0.05). Ovulation between second GnRH and FTAI was not observed in any of the cows in the FD group, however three cows (15 %) in the HD group showed ovulation within this time-interval. The percentages of non-ovulating cows within 24 h after FTAI were 15 % and 10 % for FD and HD groups, respectively and no statistically difference was found (P>0.05).

The percent of conception was 40 % (8/20) and 50 % (10/20) in FD and HD groups, respectively. The percent of conception was similar (P>0.05) between the treatment groups.

4. DISCUSSION AND CONCLUSION

Ovsynch is a useful protocol that control ovarian follicular and luteal functions also increases exactness of estrous synchronization in dairy cattle [5, 14]. GnRH is given at random stages of the estrous cycle to induce ovulation of the dominant follicle and synchronize follicle wave emergence. The morphological transformations of the CL present at the time of treatment are induced within a 6 d period after treatment with a GnRH agonist [14]. Seven days later, PGF is given to regress either the original and/or newly formed CL [1]. In this study, all cows of FD and HD groups had a CL (≥ 10 mm) at time of $PGF_{2\alpha}$ injection. Bülbül, et al. [23] reported cows of which the Ovsynch protocol began during oestrus, metoestrus, dioestrus or proestrus, 91.7 %, 100 %, 90 % and 100 % had lutein tissue on day 7, respectively. In addition, Pierson and Ginther [24] reported that the corpus luteum became visible approximately three days after ovulation and was identifiable throughout the rest of the interovulatory interval. Studies on CL diameter at time of $PGF_{2\alpha}$ injection (d 7) in the Ovsynch program in dairy cows are limited. However, Bülbül, et al. [23] reported that Ovsynch protocol began during oestrus, metoestrus, dioestrus or proestrus, mean corpus luteum diameters (mm ± S.E.M) on day 7 were 22.0 ± 2.20, 29.0 ± 2.33, 22.3 ± 2.74 and 20.6 ± 1.55, respectively. The mean (± SEM) corpus luteum diameters in the FD (23.0 ± 1.6 mm) and HD (20.4 ± 1.7 mm) groups were consistent with this report.

In the present study, average dominant follicle diameters (± SEM) at the time of second GnRH injection were 16.8 ± 0.7 mm and 17.7 ± 0.8 mm in the FD and HD groups, respectively. Cavestany, et al. [25] stated that the largest size of DF of the new wave was attained at the day of second GnRH and the diameter of DF on Day 9 was 16.2 ± 1.9 mm in cows treated with an Ovsynch protocol with medroxyprogesterone acetate. In a previous study [16] it was reported that DF diameter was 15.9 mm at 80 h after PGF injection of Ovsynch in lactating dairy cows. Bello, et al. [26] reported that the ovulatory follicle diameter was 15.7 ± 0.4 mm at the time of second GnRH injection of Ovsynch. In agreement with previous findings [16, 25, 26] the present study demonstrated that usage of half dose buserelin acetate for Ovsynch protocol in lactating cows did not have adverse effects on dominant follicle diameters.

In the current study, the mean ovulatory follicle diameters at the time of AI (d 10) were not different in FD (10.5 μg buserelin acetate) and HD (5.25 μg buserelin acetate) groups. Similarly, Ahmadzadeh, et al. [11] reported that the size of ovulatory follicle in lactating Holstein cows treated with a full (100 μg cystorelin) or half dose (50 μg cystorelin) of GnRH in the Ovsynch protocol did not differ between full and half dose groups. In earlier studies, the mean ovulatory follicle diameter at the time of FTAI at 7-day GnRH-based protocol in lactating dairy cows was determined to be between 15.3 and 20.7 mm [27-30].

In the present study, percentages of ovulation rate obtained in FD (85 %) and HD (90 %) groups are similar to the findings of Picard-Hagen, et al. [31]; Ahmadzadeh, et al. [11]; Yamada, et al. [10]; Cordoba and Fricke [32] and Fricke, et al. [9] who found ovulation rates ranging from 81.8 - 100 % in dairy cows treated with Ovsynch protocol using full and half dose GnRH. The aim of second GnRH injections in Ovsynch protocol is inducing the ovulations of dominant follicles [5, 10]. The results of this research shows that the reduced dose of buserelin acetate was as effective as full dose buserelin acetate for ovulation formation in Ovsynch protocol in lactating cows.

In the present study, conception percentages at 30 d post AI were similar in the FD (40 %) and HD (50 %) groups. Fricke, et al. [9] reported that conception rates were 41.0 % and 41.1 % at 28 post AI in the Ovsynch protocol using full and half dose GnRH (cystorelin: 100 or 50 µg). Cordoba and Fricke [32] reported that conception rate was 44.0 % on 32 d after AI in the Ovsynch protocol using half dose GnRH (cystorelin; 50 µg). Also, Yamada, et al. [10] reported that conception rates were 59.5 % and 61.1% in lactating dairy cows following synchronization of ovulation/FTAI using 100 or 50 µg fertirelin acetate. According to the results of the study reduced dose of buserelin acetate did not have any negative effect on conception rates. Also, this finding is consistent with results of previous studies [9, 10, 32].

These results indicate that a reduced dose of buserelin acetate used in Ovsynch protocol in lactating cows did not affect the corpus luteum diameters, dominant follicle diameters, ovulatory follicle diameters, ovulation and conception incidence. Therefore, we conclude that 5.25 µg buserelin acetate is as effective as full dose (10.5 µg) in the Ovsynch protocol of lactating dairy cows.

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