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RESEARCH ARTICLE

Comparison of Pregnancy Rates after Timed Artificial Insemination in Ovsynch, Heatsynch and CIDR-Based Synchronization Protocol in Dairy Cows [1]

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Summary

The objective of this study was to compare the effects of three different estrus synchronization protocols on the pregnancy rates of lactating dairy cows. Group 1 (Ovsynch protocol; n=13) animals received 20 µg GnRH on day 0, 500 µg PGF2α on day 7 and 20 µg GnRH on day 9; Group 2 (Heatsynch protocol; n=15) received 20 µg GnRH on day 0, 500 µg PGF2α on day 7 and an injection of 1 ml estradiol cypionate i.m. on day 8; Group 3 (CIDR protocol; n=15) an intravaginal CIDR insertion on day 0, inject with 500 µg PGF2α on day 6, and removed CIDR on day 7. All animals were fixed-time artificial inseminated (FTAI) on day 10. Pregnancy diagnosis was performed on all animals via transrectal ultrasonography at 35±3 days following FTAI. Estrus activity observed in the Heatsynch group animals was higher than that in the Ovsynch and CIDR-based groups (P<0.05). There were significant differences among groups for vaginal electrical resistance in days 7 and 10 (P<0.05), but there was no significant difference among groups on first and second service pregnancy rates (P>0.05). In conclusion, these three synchronization protocols providing satisfactory pregnancy rates in lactating dairy cows can be equally usable.

Keywords: *Estrus synchronization, Ovsynch, Heatsynch, CIDR, Dairy cow*

Sütçü İneklerde Ovsynch, Heatsynch ve CIDR Senkronizasyon Protokollerinde Uygulanan Sabit Zamanlı Suni Tohumlama Gebelik Oranlarının Karşılaştırılması

Özet

Çalışmanın amacı, laktasyondaki sütçü ineklerin gebelik oranları üzerinde üç farklı östrus senkronizasyon protokolünün etkilerini değerlendirmektir. Grup 1'deki (Ovsynch protokolü; n=13) hayvanlara 0. günde 20 µg GnRH, 7. günde 500 µg PGF2α ve 9. günde 20 µg GnRH verildi; Grup 2'deki (Heatsynch protokolü; n=15) hayvanlara, 0. günde 20 µg GnRH, 7. günde 500 µg PGF2α ve 8. günde 1 ml estradiol cypionate i.m. yapıldı; Grup 3'teki (CIDR protokolü; n=15) hayvanlara ise 0. günde intravaginal olarak CIDR yerleştirildi, 6. günde 500 µg PGF2α ve 7. günde CIDR vaginadan uzaklaştırıldı. Uygulamaya alınan hayvanların tamamına 10. günde suni tohumlama yapıldı. Gebelik tanısı suni tohumlamadan sonraki 35±3 günlerde transrektal ultrasonografi ile yapıldı. Heatsynch grubunda östrus aktivitesi Ovsynch ve CIDR gruplarına oranla daha önemli derecede yüksek gözlendi (P<0.05). 7. ve 10. günlerde yapılan vaginal elektriksel direnç ölçümlerinde her 3 grup arasında önemli farklılıklar bulundu (P<0.05). Ovsynch, Heatsynch ve CIDR grupları arasında 1. ve 2. suni tohumlamalardan elde edilen gebelik oranları açısından farklılık bulunmadı (P>0.05). Sonuç olarak, laktasyondaki ineklerde tatmin edici gebelik oranları veren bu 3 senkronizasyon protokolü eşdeğerli olarak kullanılabilir.

Anahtar sözcükler: *Östrus senkronizasyonu, Ovsynch, Heatsynch, CIDR, Sütçü inek*

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INTRODUCTION

A major limiting factor for reproductive performance on many dairy farms is sensitive and specific estrus detection. Estrus detection rates in dairy herds are often low (<50%) and even when observation follows $PGF_{2\alpha}$-induced luteolysis for estrus synchronization, low rates of estrus detection persist. Recent studies have shown that it is possible to manipulate the follicular and luteal dynamics precisely, eliminating the need for estrus detection for AI and for embryo transfer [1,2].

Gonadotrophin-releasing hormone (GnRH) has been used to cause follicle wave emergence in some cattle synchronization protocols, that is, either in combination with $PGF_{2\alpha}$ [3] or as part of a progesterone regimen [4]. Ovsynch is a program developed to synchronize ovulation for timed breeding. Several studies during the past few years have compared the Ovsynch protocol with other synchronization protocols such as double $PGF_{2\alpha}$ [3,4-7], progesterone based protocols, Select Synch [5,6], Heatsynch [1,8] and natural breeding [9]. The Heatsynch protocol involves replacing the second GnRH in the Ovsynch protocol with estradiol cypionate (ECP) which is injected 24 h after the $PGF_{2\alpha}$, followed by FTAI 48 h after ECP [8,10,11]. Treatments successfully used to synchronize wave emergence have included GnRH, estradiol-17β, and ECP [8,12]. Estradiol valerate [13] and estradiol cypionate [14] at doses of 5 and 1 mg, respectively, resulted in longer and more variable intervals to follicular wave emergence than E-17β.

The controlled internal drug release (CIDR) device has recently been approved in Canada and the United States for synchronization of estrus in beef cattle and dairy heifers [15,16]. The CIDR device is well suited to various approaches used to synchronize ovarian follicular development and ovulation [2,13,14,17].

The objective of this study was to evaluate the effect of three estrus synchronization protocols (Heatsynch, Ovsynch and CIDR-based programs) on the reproductive efficiency of lactating dairy cows.

MATERIAL and METHODS

This study was conducted on a private dairy farm located in Samsun province (northern Turkey) during the periods from February to June.

Animals

A total of 43 healthy Holstein cows at 40-45 days postpartum were selected and used in this study. Cows were examined for health disorders and palpated per rectum for ovarian activity before synchronization. In addition, animals were scored for their body condition (Scale 0-5 units; 1 = emaciated, 5 = obese) [18]. Cows having body condition scores greater than 2.5 have been selected and included in the study. All animals were randomly distributed to the treatment groups *(Table 2)*.

Estrus was detected by the presence of a clear mucous vaginal discharge, decreased feed intake and milk yield and by uterine tone and a large ovarian follicle detected by rectal examination. Additionally, vaginal electrical resistance (ohms) was recorded by heat detector (Draminski Estrus Detector, Poland; 10 units = 1 ohm) on days 7 and 10. Non pregnant cows diagnosed by ultrasonography on day 35±3 after the first artificial insemination (AI) have been reinseminated in the following estrus. The number of reinseminated animals was 3 for group 1 and 2, and 7 for group 3.

Experimental procedure

Group 1 (Ovsynch protocol): Cows in Ovsynch group received 20 µg GnRH (buserelin acetate; Receptal, Intervet International, Germany) intramuscularly (IM) on day 0 (start of the protocol); followed by 500 µg $PGF_{2\alpha}$ (cloprostenol; Juramate, Jurox Pty, Limited, Australia) IM on day 7, and 20 µg GnRH (buserelin acetate) IM on day 9. Fixed-time AI took place on the morning of day 10, approximately 20 h after the second GnRH treatment.

Group 2 (Heatsynch protocol) were treated similarly except that an injection of 1 ml (1 mg/ml) of estradiol cypionate in sesame oil IM (ECP; Sigma Chemical Co, St. Louis, MO, USA) on day 8, 24 h after the $PGF_{2\alpha}$ (Juramate, 500 µg) was substituted for the last GnRH in the Ovsynch protocol. All cows were inseminated using FTAI 48 h after ECP administration.

Group 3 (CIDR-based protocol): Cows in this treatment group received an intravaginal CIDR containing 1.9 g of progesterone (EAZI-BREED CIDR™, InterAg, Hamilton, New Zealand) for 7 days, starting on day 0. Animals were injected with $PGF_{2\alpha}$ (Juramate, 500 µg) IM, the CIDR were removed on day 7 and on day 10 (72 h after CIDR removal) FTAI was done.

Pregnancy Diagnosis

Pregnancy was diagnosed by visualization of an embryo with transrectal ultrasonography at 35±3 d after FTAI (Pie Medical 100 Falco Vet Model 5.0-7.5 MHz probe, The Netherlands).

Statistical Analysis

Data on vaginal electrical resistance were analyzed by standard GLM procedure. All descriptive statistics in

data were analyzed parametrically with SAS [19]. Proportional data e.g., percentage of cows in estrus, first and second AI pregnancy rates were analyzed by GENMOD procedure with Binomial distribution and log link function. SAS [19] statistical software in GENMOD (Generalized Linear Models) with the probability that 0 (zero) is failure (non-pregnant) and that 1 is pregnant. A probability level of P<0.05 was considered significant.

RESULTS

The vaginal electrical resistance readings on days 7 ($PGF_{2\alpha}$ injection) and 10 (FTAI) are shown in *Table 1*. In 14 cows in the Ovsynch and Heatsynch groups, electrical resistance was lower at day 10 than day 7 (P<0.05), while in the CIDR group, electrical resistance was higher on day 10 than day 7 (P<0.05).

Table 1. *Variations in vaginal electrical resistance (ohms) on 7 and 10 days*
Tablo 1. *Yedi ve 10. günlerde vaginal elektriksel direnç (ohms) değişimleri*

Treatments	No. of animals	Day 7	Day 10
Group 1	4	23.5±0.3 ^{ab}	22.0±1.6 ^{ab}
Group 2	15	29.9±2.1 ^{a}	25.1±1.1 ^{a}
Group 3	11	21.0±1.1 ^{b}	21.5±0.9 ^{b}

Variation analyses between groups at days 7 and 10 were performed and F values were found 0.0116 and 0.0691, respectively

a,b: *Values within a row, which do not share a common superscript, are significantly different (P<0.05)*

Estrus activity and first and second AI pregnancy rates for all synchronization protocols are presented in *Table 2*. Estrus activity observed in the Heatsynch group was statistically higher than that in Ovsynch and CIDR-based groups (P<0.05). Pregnancy rates after first AI for Heatsynch, Ovsynch and CIDR-based groups were 80.00% (12/15), 76.92% (10/13) and 53.33% (8/15), respectively *(Table 2).* In the case of the second AI, pregnancy rates of Heatsynch, Ovsynch and CIDR-based groups were found 100% (3/3), 100% (3/3), and 71.43% (5/7), respectively *(Table 2).* No significant difference was observed for pregnancy rates after first and second AI among and within groups (P>0.05).

Table 2. *Estrus and pregnancy ratios with 3 different synchronization protocols in lactating dairy cows*
Tablo 2. *Sütçü ineklerde üç farklı senkronizasyon protokolü ile elde edilen östrus ve gebelik oranları*

Treatment (no of animals)	First AI period		Second AI period
	Estrus ratio (%)	Pregnancy ratio (%)	
Group 1 (n=13)	6/13 (46.2)	10/13 (76.9)	3/3 (100)
Group 2 (n=15)	14/15 (93.3)	12/15 (80.0)	3/3 (100)
Group 3 (n=15)	10/15 (66.7)	8/15 (53.3)	5/7 (71.4)

Means within rows followed by different superscripts are not significantly different (P>0.05)

DISCUSSION

This study was designed to determine if pregnancy rate following fixed-time AI would differ among three different synchronization protocols in lactating dairy cows.

Fertility in lactating dairy cows is clearly lower than is desired. Pregnancy rates of lactating dairy cows per AI have decreased from 66% in 1951, to about 50% in 1975, to about 40% currently [9]. Some factors that limit fertility of lactating dairy cows include negative energy balance [7], toxic concentrations of urea and nitrogen [9], heat stress and other stresses [20], and vitamin and mineral deficiencies [9]. A wide range of reproductive technologies are available to producers to program reproductive management with the use of ovulation synchronization and timed insemination protocols. Users of these technologies need to understand which of these programs are more practical, convenient and effective. The Ovsynch/timed artificial insemination protocol is a recent advance in reproductive biology that has been recommended to improve reproduction of lactating dairy cows [4,12].

According to Ahmadi et al.[21], the density of blood vessels and the oedema level were higher in the CIDR-treated cows than those in the other synchronization protocols. Probably, the mechanical effect of the CIDR on the vagina caused migration of blood neutrophils to the vagina and reproductive tract, hence, a high percentage of neutrophils in the cervical mucus. Therefore, this study, an increased vaginal electrical resistance level at 10 days in CIDR group may be due to the mechanical effect of the CIDR on the vagina.

Electrical resistance of reproductive tissues and their secretions has been measured and used as a means of estrus detection and for timing of insemination. The electrical resistance of reproductive tract secretions is highest during the luteal phase and declines during the follicular phase of the estrus cycles. The lowest resistance readings occur coincident with the LH surge, a few hours after the estrogen peak and onset of estrus [21,22]. In this study, we found that vaginal electrical resistance measurements in Ovsynch and Heatsynch groups were lower at day 10 than during 7th day (P<0.05), while vaginal electrical resistance level in CIDR group was found higher at day 10 than day 7 (P<0.05). These results confirm the earlier findings of Meena et al.[22] in lactating dairy cows and Taşal et al.[23] and Ahmadi et al.[21] in dairy cows. Changes in electrical resistance of vaginal mucous membrane with dropping in estrus and rising in dioestrus during the sexual cycle have been well documented in cattle [22,23]. In this study, minimum electrical resistance values for cows were measured during the estrus period *(Table 1)*.

Estrus activity after ECP was somewhat consistent with other reports in replacement dairy heifers (41%) and lactating dairy cows (76%) [8]. In our study, estrus response rate in Ovsynch and Heatsynch protocols (46.15% (6/13) and 93.33% (14/15), respectively) was higher than those reported by Kasimanickam et al.[10] and Pancarci et al.[8].

In cattle, synchronization of stage of the estrous cycle with Ovsynch was associated with pregnancy rates of between 30 and 40% [3,11,20,23], although pregnancy rates of around 20% have also been reported by Cartmill et al.[6]. The overall pregnancy rate after synchronization with Ovsynch (36%) was higher that after synchronization with PRID (Progesterone Releasing Intravaginal Device) (28%), although the difference was not significant Kasimanickam et al.[24]. Previous studies indicated the pregnancy rate following Ovsynch program varied from 27 to 39% [3,25,26]. Although, in this study, the pregnancy rates (76.9%) for the Ovsynch group cows was higher than those reported by Ahmadi et al.[21], Bartolome et al.[1], Kasimanickam et al.[10], and Rabie et al.[12], and similar to those of Alnimer et al.[20].

Substituting ECP for GnRH in an Ovsynch-like protocol (known as Heatsynch) induced estrus, preovulatory LH (luteinizing hormone) surge, ovulation, and normal corpus luteum development in dairy heifers, and induced estrus and ovulation in lactating dairy cows [4,5,8]. In the study by Pancarcı et al.[8], the mean interval to onset of estrus was 29.0±1.8 h after ECP administration (range, 11.0-46.6 h) and the mean interval to ovulation was 55.4±2.7 h after ECP administration (range, 13.6-82.6 h). The optimal time recommended by Pancarcı et al.[8] study for insemination is 48 h after ECP. In this study, the pregnancy rate for the Heatsynch group cows (80.00%) was higher than those reported by Bartolome et al.[1], and Pancarci et al.[8].

According to Kim et al.[15], treatment with GnRH in a CIDR-based FTAI protocol induced synchronized follicular wave emergence, a large preovulatory follicle and synchronous ovulation following a second injection of GnRH. This protocol resulted in an acceptable pregnancy rate following FTAI in lactating Holstein cows. The CIDR+$PGF_{2\alpha}$ treatment was effective in both acyclic and cyclic cattle enrolled in these studies. A 7-d administration of the CIDR with an injection of $PGF_{2\alpha}$ on day 6 of insertion was an effective method for estrus synchronization in cattle [16]. In our study, the first service pregnancy rate (cow: 53.3%) for the CIDR-based synchronization program was higher than those reported by Chebel et al.[27] and Lucy et al.[16], and similar to those of Cavalieri et al.[28], Kim et al.[15], and Peeler et al.[17].

In summary, the present study showed that acceptable pregnancy rate in lactating dairy cows had been obtained by using Ovsynch, Heatsynch and CIDR-based synchronization protocols. Furthermore, vaginal electrical resistance can effectively be used as an additional parameter for the detection of estrus and determination of the optimum time of insemination in cows.

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