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RESEARCH ARTICLE

The Effects of Ovsynch, Ovsynch with Progestin and Progestin plus Double TAI on Pregnancy Rates in Unobserved Oestrus Dairy Cows and Heifers

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Summary

The objective of this study was to determine whether progestin (norgestomet) supplementation and double-timed artificial insemination (TAI) during the Ovsynch protocol enhances fertility in dairy cows and heifers when the state of oestrus was not determined. In group I (Ovsynch), cows (269), heifers (152) were treated with GnRH on day 0, PGF2α on day 7, GnRH on day 9. TAI was performed approximately 16 h after the second GnRH. In group II (Ovsynch, norgestomet), cows (268) and heifers (152) were treated as in group I, but the implant was received at the time of first GnRH. All implants were removed at the time of the PGF2α. Cows (268) and heifers (150) in group III were treated as in Group II, but a double TAI was performed. First TAI was administrated at the time of the second GnRH and the second TAI was administered approximately 18-20 h later. Cows (270) and heifers (153) detected as being in natural oestrus were used as untreated control animals. The use of the norgestomet implant at the same time as the Ovsynch protocol did not increase pregnancy rates in cows and heifers that were classified as cyclic or noncyclic before treatments began. Total pregnancy rates were significantly greater for group III heifers than for group I, II, control heifers and group III cows.

Keywords: *Heifer, Cow, Unobserved oestrus, Ovsynch, Norgestomet, Double TAI, Pregnancy*

Kızgınlıkları Gözlenemeyen Sütçü İnek ve Düvelerde, Ovsynch, Ovysnch - Progestin, Ovsynch - Progestin - Çift Sun'i Tohumlama Uygulamalarının Gebelik Oranları Üzerine Etkileri

Özet

Bu çalışmanın amacı, östrusları belirlenememiş inek ve düvelerde ovsynch protokolüne progestin (norgestomet) ve çift sun'i tohumlama uygulamalarının eklenmesinin fertiliteyi arttırıp artırmadığını belirlemekti. Grup I (ovsynch): İnek (269) ve düvelere (152), GnRH (0. gün), PGF2α (7. gün), GnRH (9. gün) uygulandı ve ikinci GnRH uygulamasından yaklaşık 16 sat sonra sun'i tohumlama yapıldı. Grup II (Ovsynch, norgestomet): İnek (268) ve düvelere (152) grup I' deki uygulamalara ek olarak ilk GnRH ile birlikte kulak implantı (norgestomet) takıldı ve implantlar PGF2α uygulanırken çıkarıldı. Grup III: İnek (268) ve düvelere (150) GrupII' deki uygulamalara ek olarak,ilki ikinci GnRH uygulaması sırasında ve ikinciside yaklaşık 18-20 saat sonra olmak üzere çift sun'i tohumlama uygulandı. Doğal östrus gösterip tohumlanan 270 inek ve 153 düve kontrol grubu olarak değerlendirildi. Ovsynch protokolüne eklenen norgestomet içeren implantların, tedaviler başlamadan önce siklik ve nonsiklik olduğu belirlenen inek ve düvelerde gebelik oranlarını arttırmadığı tespit edildi. Grup III'deki düvelerin toplam gebelik oranının grup I, II, kontrol gruplarındaki düvelere ve III'cü gruptaki ineklere göre belirgin şekilde yüksek olduğu belirlendi.

Anahtar sözcükler: *Düve, İnek, Kızgınlıkların gözlenememesi, Ovsynch, Norgestomet, Çift sun'i tohumlama, Gebelik*

INTRODUCTION

Reproductive performance is a key factor in maximising profits at dairy farms [1]. The major factors limiting reproductive performance on many dairy farms are prolonged post-partum anoestrus intervals related to anovulatory anoestrus [2], a failure of the cows to display oestrus [3] and poor oestrus detection practices (i.e., the inability to detect

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oestrus in a timely and accurate manner) [1,4]. Postpartum suboestrus is defined by the absence of an observed oestrus at the time when a cow with normal oestrus cycles would be inseminated. Approximately 50% of all oestrus behaviours is not detected during the postpartum period [5]. Heifers in such herds typically graze separate from lactating cows and, unlike lactating dairy cows that are brought in for milking at least twice a day, special efforts have to be made to access these heifers for oestrus detection and AI [6]. In lactating dairy cows, reproductive management at 50 day postpartum consists of oestrus detection using the a.m./p.m. insemination schedule and the occasional use of $PGF_{2\alpha}$, $PGF_{2\alpha}$ administration after responsive Cl is detected or two $PGF_{2\alpha}$ injections given at 11 or 14-day interval, or progesterone in cows that were not observed to be in oestrus at 60 d postpartum [7,8].

The GnRH - $PGF_{2\alpha}$ - GnRH protocol was used with the aim of synchronising cows to eliminate oestrus detection and to achieve timed AI [9]. This protocol has two different application methods: cosynch [1,10] and Ovsynch [11]. Ovsynch involves the administration of GnRH, followed by $PGF_{2\alpha}$ 7 day later and a second treatment of GnRH 36 to 48 h after the administration of $PGF_{2\alpha}$. In the Ovsynch protocol, cows and heifers are not observed for oestrus but are inseminated at a specific time (12-20 h) following the second GnRH administration [1,12]. The first administration of GnRH is given at a random stage of the oestrus cycle, causing either luteinisation or ovulation of the largest follicle in cycling or anoestrus cows [1,9,13,14]. The administration of $PGF_{2\alpha}$ causes to regress of the corpus luteum (CL) or the luteinised follicle induced by the GnRH. A new dominant follicle forms and is available for ovulation by the time of the second GnRH administration, which is given 36 to 48 h after the $PGF_{2\alpha}$ treatment [1,9]. Pregnancy rates were consistently improved with the GnRH - $PGF_{2\alpha}$ - GnRH protocol and double TAI in dairy heifers in which oestrus was unobserved in comparison to heifers treated with only cosynch in dairy herds with poor oestrus detection [15]. There was a positive correlation between serum P4 before AI and the subsequent conception rate, indicating that progesterone is important to fertility [16,17]. In cows synchronised with PG, the conception rate was positively related to plasma progesterone concentrations preceding the second PG injection [17]. Studies with P4 (CIDR) alone have not only improved the synchronisation of oestrus, but also initiated oestrus and ovulation in a percentage of prepubertal heifers [18] and anoestrus cows [18-20]. The insertion of PRID without the oestradiol capsule during the luteal phase has been found to increase conception in cows with low plasma progesterone and decrease conception in cows with high progesterone [17]. Comparisons of Ovsynch and Ovsynch plus CIDR treatments for lactating dairy cows have indicated that the addition of progesterone to the Ovsynch protocol improved pregnancy rates [21].

This study aims to determine whether exogenous progestin supplementation and double TAI during an Ovsynch protocol enhances fertility in lactating dairy cows (60 day postpartum) and heifers (16-18 m old) that are not observed and that have an undetermined oestrus state.

MATERIAL and METHODS

The study was began in September 2007 and ended in December 2009.

Location

This study was conducted on three commercial dairy farms (A, B and C) located in Yenişehir (latitude 40° 13´ N, longitude 29° 30´ E, altitude 240 m above sea level) in the province of Bursa in southeast Marmara, Turkey.

Animals and Trials

The cows were carefully selected to be unobserved oestrus, as defined by the absence of detectable oestrus from calving. The methods of oestrus detection used in this study included a computerized system which used increased walking activity and an accompanying decrease in milk production (Alpro, DeLaval, Seweden), as well as daily visual observation of the cows. A specific individual was as assigned the duty of observation cows for oestrus during each 8-h shift. Thus, the cows were continuously observed for oestrus during an 8-h period of time for 24 h. All cows on farms were housed in free-stall barns; heifers were housed in open barns with shade and a yard with a concrete floor. Cows were grouped by production level and stage of lactation. All animals were fed a total mixed ration (TMR) (consisting of alfalfa, straw, maize silage and concentrates) twice daily with food according to their production level and water provided ad libitum. Lactating cows were milked twice per day at intervals of approximately 12 h. The average milk production per cow on farms A, B and C was 23.3, 24.2, 26.4 kg/day during the study, respectively.

Before the treatments, on day -11, the ovaries of all cows and heifers were examined by both rectal palpation (RP) and transrectal ultrasonography (US) (Terason Portable Ultrasonography System; Teratech Corporation,Burlington, Massachusetts, USA) using a 5-7.5 MHz transducer (5LV) to determine CL formation. A second examination was performed 11 day later (on day 0) to determine the presence or absence of a CL in animals in which a CL was not confirmed on the ovaries at the first examination. In either or both of the examination days (on day -11 and on day 0), cows and heifers that had palpable and observable CL were considered to be cyclic. On both of the examination days, cows and heifers that did not have CL were defined as acyclic. At the beginning of the study, cows with body condition score (BCS) <1.5 were excluded from the trial. Because they were considered as too thin and not suitable for synchronization program (for ovulation induction). Also

acyclic cows with ovarian follicles <10 mm in diameter on the day of start of treatment were not included in this study. Because follicles were considered as not satisfactory size for either luteinisation or ovulation induction with first GnRH administration

In this study, 805 lactating Holstein cows (postpartum 60 day) and 454 Holstein heifers (16-18 months old) that were not determined to be in oestrus were used as study subjects. Cows and heifers were assigned as far as possible equally to each of three treatment groups and treatment groups were balanced for BCS, cyclic and acyclic animals. In this study, 270 lactating Holstein cows and 153 Holstein heifers that were detected as being in natural oestrus were used as controls.

Group I (Ovsynch; N=421): Cows (n=269) and heifers (n=152) were given an IM injection of synthetic GnRH (10 mg of Buserelin, Receptal®, Intervet International, B.V. Boxmeer, Netherland) on d 0. Seven days after the GnRH injection, all cows and heifers were given an IM injection of $PGF_{2\alpha}$ analogue (25 mg of Dinoprost tromethamine, Dinolytic®, Pharmacia, Rijksweg 12, 2870, Puurs, Belgium). A second injection of GnRH (10 mg) was administered 48 h after the $PGF_{2\alpha}$ injection and all cows and heifers were bred by timed AI (TAI) approximately 16 h after the second GnRH injection.

Group II (Ovsynch plus Norgestomet Ear Implant; N=420): Cows (n=268) and heifers (n=152) were treated as in group I, but an ear implant containing 3 mg norgestomet (Crestar®, Intervet International, B.V. Boxmeer, Netherland) was inserted at the time of the first GnRH injection (on day 0). Ear implants were removed at the time of the $PGF_{2\alpha}$ injection (on day 7).

Group III (Ovsynch plus Norgestomet Ear Implant; N=418): Cows (n=268) and heifers (n=150) were treated as in group II, but double TAI was performed. The first TAI was administrated at the time of the second GnRH injection, and the second TAI was administered approximately 18-20 h later.

Control (N=423): Cows (n=270) and heifers (n=153) that were determined to be in natural oestrus during the study were used as controls. All control cows and heifers received AI according to the a.m - p.m. schedule.

In all animals, pregnancy was diagnosed by US approximately 45 d following AI.

Statistical Analysis

In all trials, data were compared using SPSS Statistics 17.0 for Windows (SPSS Inc., Illinois, USA). Pregnancy rates are shown as percentages. Effects of treatments on pregnancy rates were performed using Chi-square procedures. Differences were considered to be statistically significant at α=0.05, 0.01, 0.001.

RESULTS

The effects of treatments on total pregnancy rates were similar for all treatments and controls. Nevertheless, the total pregnancy rate for group III (48.08%) was numerically higher than that of group I (42.3%) or group II (41.6%). Similarly, the total pregnancy rate for cows was comparable for all treatment groups. On the other hand, heifers treated with Ovsynch with a norgestomet ear implant and double TAI (Group III) (60.7%) had higher total pregnancy rates than those treated with Ovsynch (Group I) (41.4%) or Ovsynch with a norgestomet ear implant (Group II) (44.07%) and the control (47.05) (P<0.01). Total pregnancy rates for heifers treated with Ovsynch with a norgestomet ear implant and double TAI (Group III) (60.7%) were greater than that of group III cows (42.9%) (P<0.001). Pregnancy rates for all groups were similar in cycling cows. However, the pregnancy rate for the Ovsynch group (42.18%) was higher than those of group II (29.6%) and group III (31.8%) in noncycling cows, though the differences were not significant. The pregnancy rate for group III (65.6 %) was higher than that for group II (47.3%) in cycling heifers (P<0.01). The pregnancy rate in noncycling heifers treated with Ovsynch, a norgestomet ear implant and double TAI (Group III) (53.3%) was higher than that for heifers treated with Ovsynch (P<0.01) (26.3%). Pregnancy rates in both cycling (65.5%) and noncycling (53.3%) heifers in group III were higher than those observed in group III cycling (46.5) and noncycling (31.8%) cows (P<0.01; P<0.015).

DISCUSSION

Pregnancy rates of our study were compared for unobserved oestrus lactating dairy cows and heifers after three synchronisation treatments involving an Ovsynch protocol with or without ear implant containing norgestomet, and an Ovsynch protocol with norgestomet and double TAI.

Pregnancy rates obtained from this study for cows treated by Ovsynch plus progestin (40.29%) were similar to those observed for Ovsynch cows (42.75%). In a previous study [22], pregnancy rates in dairy cows at 40 to 46 d after TAI were greater (P<0.01) after treatment with Ovsynch plus CIDR (45.1%) than Ovsynch alone (20.9%). Stevenson et al.[21] reported that overall pregnancy rates for lactating dairy cows treated Ovsynch plus CIDR were 10 and 5% higher than those treated with Ovsynch at d 28 and 56 after TAI, respectively. However, the differences between pregnancy rates obtained by Ovsynch and Ovsynch plus CIDR at the day 56 after TAI were not statistically significant. Pregnancy rates of cows treated with Ovsynch plus PRID were 11% higher (44.7%) than those of cows treated with Ovsynch alone (33.7%), though the differences in pregnancy rates were not significant [23]. In present study, pregnancy rates for heifers treated with Ovsynch plus

Table 1. *Pregnancy rates in unobserved oestrus cows and heifers treated with Ovsynch (Group I), Ovsynch with a norgestomet ear implant (Group II) and Ovsynch with a norgestomet ear implant and double TAI (Group III)*

Tablo 1. *Ovsynch (Grup I), Ovsynch - norgestmet içeren kulak implantı (Grup II), Ovsynch - norgestmet içeren kulak implant - çift sun'i tohumlama (Grup III) uygulanan kızgınlıkları gözlenemeyen inek ve düvelerde gebelik oranları*

Items		Treatment Group and Pregnancy Rates			
		Ovsynch (Gr I)	Ovsynch Plus A Norgestomet Ear Implant (Gr II)	Double TAI (Gr III)	Control
Cows	Cycling	42.9 (205/88)	43.8 (203/89)	46.5 (202/94)	
	Noncycling	42.18 (64/27)	29.6 (65/19)	31.8 (66/21)	
	Total	42.75 (269/115)	40.29 (268/108)	42.9 (268/115)	45.5 (270/123)
Heifers	Cycling	51.6^{ab} (91/47)	47.3^{b} (91/43)	$^{*}65.6^{a}$ (90/59)	
	Noncycling	26.3^{b} (61/16)	39.4^{ab} (61/24)	$^{**}53.3^{a}$ (60/32)	
	Total	41.4^{b} (152/63)	44.07^{b} (152/67)	$^{***}60.7^{a}$ (150/91)	47.05^{b} (153/72)
Total	Cycling	45.6 (296/135)	44.9 (294/132)	52.4 (292/148)	
	Noncycling	34.4 (125/43)	34.1 (126/43)	42.1 (126/53)	
	Total	42.3 (421/178)	41.6 (420/175)	48.08 (418/201)	46.1 (423/195)

* Means within column between cycling cows and heifers are significantly different (P<0.01)
** Means within column between noncycling cows and heifers are significantly different (P<0.015)
*** Means within column between total cows and heifers are significantly different (P<0.001)
^{ab} Means within row with dissimilar superscript letters are significantly different (P<0.01)
^{ab} Means within row with dissimilar superscript letters are significantly different (P<0.001)

progestin (44.07%) were higher than those of Ovsynch heifers (41.4%). However, the differences in pregnancy rates obtained by Ovsynch and Ovsynch plus progestin after TAI were not significant. Martinez et al.[24] reported that the addition of progestin to the cosynch or Ovsynch regimen resulted in significantly improved pregnancy rates in beef heifers but not in beef cows.

El-Zarkouny et al.[22] reported that anoestrus dairy cows treated with Ovsynch plus CIDR had a higher pregnancy rate (64%) than anoestrus cows treated Ovsynch alone (27%). However, cycling cows receiving Ovsynch plus CIDR had a pregnancy rate similar to that of cycling cows receiving Ovsynch alone. It has been reported that pregnancy outcomes showed larger increases when cows were treated with Ovsynch plus CIDR than with Ovsynch alone because more anoestrus cows conceived [25]. Our pregnancy results for cyclic cows are consistent with the results from previous studies. However, on the contrary, in the present study, pregnancy rates for noncycling cows treated with Ovsynch plus progestin were lower than those observed in cow treated with Ovsynch. However, differences between pregnancy rates were not significant. Stevenson et al.[21] reported that conception rates for cycling cows with active CL and noncycling cows in which active CL were induced before $PGF_{2\alpha}$ injection did not have improved responses to the additional P4 provided by the CIDR insert. In contrast, regardless of the pretreatment cycling status, noncycling cows without an active induced CL and cycling cows without an active CL at the time of $PGF_{2\alpha}$ injection had higher conception rates when treated with Ovsynch plus CIDR than Ovsynch cows with a similar luteal status [21]. However, in present study, we only considered the pretreatment cyclic status, but we did not determine the effects of presence of corpus luteum at the time $PGF_{2}\alpha$ injection.

In a previous study [26], the pregnancy rate for CIDR-based co-synch protocols in beef heifers was found to be 53.1%. The cyclicity of heifers before treatments ranged from 78 to 100% at eight different locations. In present study, the total pregnancy rate for heifers treated with Ovsynch plus norgestomet was 44.4%, but the cyclic heifer's rate was 47.3%. Pregnancy rates for cycling beef heifers and pre-peripubertal beef heifers treated with co-synch plus CIDR were 47% and 48%, respectively [27]. Pregnancy rates of this study for cyclic and noncyclic heifers treated with Ovsynch plus norgestomet were 47.3%, 39.4%, respectively and difference between pregnancy rates were not significant. Pregnancy rates for cycling heifers did not differ between Ovsynch and Ovsynch plus norgestomet treatments. Supplementation with a norgestomet implant

for 7 day concurrent with the Ovsynch protocol did not substantially improve pregnancy rates in comparison to Ovsynch alone in noncycling heifers. Literature comparing reproductive parameters for Ovsynch and Ovsynch plus progesterone or progestagen treatments in heifers could not be found.

A comparison of Ovsynch and Ovsynch plus norgestomet implant treatments for lactating dairy cows and dairy heifers indicated that the addition of norgestomet implant to the Ovsynch protocol did not improve pregnancy rates after TAI in this study. The use of the norgestomet implant concurrent with the Ovsynch protocol did not increase pregnancy rates in cows and heifers that were classified as cyclic and noncyclic before applying the norgestomet implant concurrent with the Ovsynch protocol.

Total pregnancy rates were significantly higher for heifers treated with Ovsynch plus norgestomet implants plus double TAI than for Ovsynch heifers and Ovsynch plus norgestomet implant heifers. Moreover, double TAI, which was administrated twice (the first TAI was administered at the time of the second GnRH injection and the second TAI was administered approximately 18-20 h later), significantly improved pregnancy rates for heifers in group III in comparison to pregnancy rates in similarly treated cows and control heifers. In our previous study [15], dairy herds with poor oestrus detection and dairy heifers in which oestrus was unobserved were treated with GnRH - $PGF_{2\alpha}$ - GnRH and double TAI and were found to achieve significantly greater pregnancy rates than dairy heifers in which only the cosynch procedure was used. In another study [11], ovulations occurred between 26 and 32 h after the second injection of GnRH in dairy cows treated using the Ovsynch protocol. The majority of follicles in cows ovulated approximately 28 h after the second GnRH injection. Almost all ovulations in heifers occurred between 26 and 30 h after the second GnRH injection. Ovulation in heifers treated with Ovsynch occurred more frequently than Ovsynch cows around 26 h after the second GnRH administration. Demiral et al.[28] demonstrated that ovulations in cows and heifers using Ovsynch were distributed between 0 and 42 h after AI. While ovulations were detected between 0-6 h, no ovulations were detected at 6-12, 12-18 and 18-24 h intervals in cows. Ovulations in Ovsynch heifers were started earlier and distributed over a long time interval than in Ovsynch cows [11,28]. In this study, the addition of norgestomet implants to the Ovsynch protocol did not improve pregnancy rates after TAI in cows and heifers. However, pregnancy rates were significantly improved in heifers using Ovsynch plus norgestomet with a double TAI protocol in comparison to Ovsynch heifers, Ovsynch plus norgestomet heifers and cows treated with Ovsynch plus norgestomet double TAI. The fertile lifespan of sperm and ovum dictates synchronous insemination and ovulation to achieve high conception rate. Ovulations occur at various times after the onset of oestrus. Sperm longevity in the female reproductive tract appears to be related to the length of oestrus. Regardless of the timing of ovulation, high conception rates result if sperm are present in the oviduct shortly before ovulation. If insemination occurs too early, it reduces conception rates, which results from the loss of sperm viability and the number of sperm at the site of fertilisation. However, the loss of ovum viability can result from insemination after ovulation [29]. In the present study, improved pregnancy rates with double TAI for ovsnch plus norgestomet protocol in heifers may result from the genital tract being exposed to higher spermatozoa concentrations for a longer period of time with double TAI than those of single TAI. Thus, fertilisation can be improved by synchronising the fertile lifespan of sperm with ovulations, which are distributed over a long time interval and occur earlier in heifers than in cows.

As a result, comparisons of Ovsynch and Ovsynch plus norgestomet implant treatments for lactating dairy cows and dairy heifers indicated that the addition of norgestomet implants to Ovsynch protocols did not improve pregnancy rates after TAI. The use of the norgestomet implant concurrent with the Ovsynch protocol did not increase pregnancy rates in cows and heifers that were classified as cyclic and noncyclic before applying the norgestomet implant concurrent with the Ovsynch protocol. Total pregnancy rates were significantly higher for heifers treated with Ovsynch plus norgestomet implant plus double TAI than for heifers treated with Ovsynch or Ovsynch plus norgestomet implant. Moreover, double TAI (the first TAI was administered at the time of the second GnRH injection and the second TAI was administered approximately 18-20 h later) significantly improved pregnancy rates for heifers in group III in comparison to similarly treated cows and control heifers.

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