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The Effect of Progesterone Based Ovsynch Protocol and GnRH Treatment after Artificial Insemination on Conception Rate in Repeat Breeder Cows

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Abstract: The aim of this study was to test the combined treatment protocol for improving fertility in repeat breeder cows by eliminating most of the hormonal etiological factors. Total 238 clinically healthy lactating dairy cows were included in the study; 175 dairy cows had more than three services (Repeat Breeder Cows) and 63 dairy cows had less than three services (Normal Cows). Ovsynch protocol was applied at random stages of the estrous cycle to all cows and exogenous progesterone (ear implant) was applied between GnRH and $PGF_{2\alpha}$ of Ovsynch. In addition, GnRH was administrated seven days after artificial insemination (GnRH–progesterone–7d–$PGF_{2\alpha}$–2d–GnRH–16h–AI–7d–GnRH). Response to the first GnRH of Ovsynch was greater (P<0.003) in repeat breeders (70.9%) than in normal cows (49.2%). However percentages of cows to responded to 2nd and 3rd GnRH were not different between groups (97.1%, 83.9% in repeat breeders and 98.4%, 87.1% in normal cows, respectively). Conception rates were similar at 31 and 62 d between groups (44.0%, 40.6% in repeat breeders and 47.6%, 42.9% in normal cows, respectively). In addition, embryonic loss was also similar in groups (7.8% in repeat breeders and 10.0% in normal cows). Thus, conception rate and response to hormonal treatments of the combined protocol except first GnRH were found to be similar in both repeat breeder and normal cows. So the combined protocol could be effective treatment for improving conception rate in repeat breeder cows.

Key Words: Repeat breeder cow, Ovsynch, synchronization.

Progesteron Tabanlı Ovsynch Protokolü ve Tohumlama Sonrası GnRH Uygulamasının Repeat Breeder İneklerde Gebelik Oranı Üzerine Etkisi

Özet: Bu çalışmanın amacı; repeat breeder ineklerde hormonal etiyolojik faktörleri elimine edecek, bir kombine tedavi protokolünü gebelik oranını artırmak için test etmekti. Klinik olarak sağlıklı, 175 inde üçten fazla (Repeat Breeder) ve 63 ünde üçten az suni tohumlaması (Normal) olan toplam 238 baş sağmal inek calışmaya dahil edildi. Tüm ineklere östrusun herhangi bir safhasında Ovsynch protokolü ve aynı zamanda Ovsynch`in GnRH ile $PGF_{2\alpha}$ arasına ekzojen progesteron (kulak implantı) uygulandı. Bunun yanında tüm ineklere suni tohumlamadan 7 gün sonra GnRH uygulaması yapıldı (GnRH-kulak implantı-7gün- $PGF_{2\alpha}$-2gün-GnRH-16saat-ST-7gün-GnRH). Ovsynch`in ilk GnRH`ına yanıt, repeat breeder ineklerde (%70.9) normal ineklere (%49.2) göre daha yüksek saptandı ($P$<0.003). Bununla birlikte ikinci ve üçüncü GnRH`a yanıt bakımından gruplar arasında bir fark tespit edilmedi (sırasıyla, repeat breeder ineklerde %97.1, %83.9 ve normal ineklerde %98.4, %87.1). Otuz birinci ve 62. gün gebelik oranı iki grup arasında benzer saptandı (sırasıyla, repeat breeder ineklerde %44.0,

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%40.6 ve normal ineklerde %47.6, %42.9). İlave olarak embriyonik kayıp da gruplar arasında farklı bulunmadı (repeat breeder ineklerde %7.8 ve normal ineklerde %10.0). Sonuç olarak gebelik oranı ve birinci GnRH hariç kombine tedavi protokolünün hormon uygulamalarına alınan yanıt gruplar arasında benzer bulundu. Kombine tedavi protokolunun repeat breeder ineklerde gebelik oranını artırmak için kullanılabileceği sonucuna varıldı.

Anahtar Kelimeler: Repeat breeder inek, Ovsynch, senkronizasyon.

Introduction

The Repeat Breeding is important cause of decreased fertility and results in economical loss in dairy industry due to more insemination cost, increased calving interval and increased culling rates. Incidence of repeat breeding in lactating dairy cows has been reported 10–25%[12,23]. Some of diverse causes of the repeat breeding are genetic, hormonal and nutritional imbalance, subclinical infection of uterus, and embryonic mortality[2,12,23]. Hormonal imbalance and its effects on reproduction in repeat breeder cows and heifers were well defined in several studies[3,10,14]. Hormonal imbalance can be classified as pre-ovulatory and post-ovulatory. Subluteal levels of progesterone during the pre-ovulatory period result in irregular, delayed or absent ovulation of dominant follicle[10,13]. In addition insufficient corpus luteum at post-ovulatory period leads to low progesterone level and delayed progesterone rising causing to embryonic loss[3,14]. In addition, repeat breeding increase by insufficient estrus detection and errors of timing insemination[23].

Several hormonal treatments have been used to improve the conception rate in repeat breeder cows, but with a limited success[12,13,19,22]. Timed artificial insemination (TAI) protocols such as Ovsynch (injection of GnRH 7 d before and 48 h after $PGF_{2\alpha}$, and timed AI 16-18h after second GnRH)[17] and progesterone/ progestagen based Ovsynch protocols have been used in normal and repeat breeder cows, but pregnancy rate was inconsistent among the studies[7,11,14,20]. However, pregnancy rate in lactating dairy cows following Ovsynch was affected response to each hormonal treatment[4,8,21]. In addition progesterone level after insemination has essential role in maintaining pregnancy[19]. In repeat breeder cows, it has been measured slower rise progesterone level than normal rise and lower progesterone level[1-3]. Therefore many researchers have been focused to increase progesterone level after AI by using exogenous GnRH or progesterone in dairy cows[1,20,22].

Thus, in this study we aimed to test the combined protocol for improve fertility in repeat breeder cows by eliminating most of the hormonal etiological factors. Progestagen administration during the Ovsynch can positive effects on follicular development and improve response to hormonal treatments of Ovsynch. In addition, Ovsynch protocol eliminates estrous detection and also ovulation errors. GnRH administration 7 days after AI can be increase progesterone level by developing second corpus luteum as additional endogenous progesterone source. Increased progesterone level can support embryonic survival in repeat breeder cows.

Materials and Methods

1. Cows, Housing and Management

This study was conducted on a commercial dairy herd, approximately 1000 lactating dairy cows in Bursa, Turkey. The lactating cows were housed in free stall facilities and grouped according to their milk production. All cows were milked three times a day and fed complete mixed rations based on NRC recommendations[15]. Mean milk production of the herd was 9.880 kg (305 d) per cow. Daily milk yield was collected by the ALPRO™ system (DeLaval, Sweden). Average milk production for each cow was recorded for 7 d before and after AI. The voluntary waiting period of this farm is 50-55 days in milk (DIM). Mainly reproductive management of the herd was AI following spontaneous estrus according to am/pm schedule and TAI protocols. All cows had given their body condition score using a 5-point (1=thin to 5=fat) scoring system[5].

2. Synchronization Protocol and Treatment

A total of 238 cows (175 Repeat Breeder and 63 Normal cows) were used in this study. Cows had more than three inseminations with no clinically abnormalities of the reproductive tract selected as repeat breeder and cows which had less than three inseminations with no clinically abnormalities of the reproductive tract selected as control (non-repeat breeder). Both repeat breeder and normal cows were received the same combined protocol; timed artificial insemination (TAI) protocol-the Ovsynch- added progestagen and GnRH administration seven

days after TAI. Cows, at random stages of the estrous cycle, received an ear implant containing progestagen (Norgestomet, 3 mg, Crestar®, Intervet, Turkey) and GnRH administration (Busereline acetate, 10 µg, i.m., Receptal®, Intervet) - on day 0 - and $PGF_{2\alpha}$ administration (Cloprostenol, 500 µg, i.m., Estrumate®, CEVA-DIF, Turkey) at the time of implant removal -on day 7-, and second GnRH injection - on day 9-. Then the cows artificially inseminated, at fixed time, 16-18 h after the second GnRH treatment (Busereline acetate, 10 µg, i.m., Receptal®), using frozen-thawed semen from bulls of proven fertility by farm veterinarians. All inseminated cows received third GnRH administration (Busereline acetate, 10 µg, i.m., Receptal®) seven days after AI.

3. Ultrasonographic Examinations

Cows' ovaries were examined on the day of first GnRH and ear implant administrations and 7 days later to determine ovulation response to first GnRH treatment by ultrasound (Honda HS 2000 equipped with a 5.0-7.5 MHz transducer, Honda®, Japan). Response to first GnRH was characterized by appearance of a new corpus luteum (CL) on ovaries. Maximum follicular size was measured at the time of AI. Cows were examined seven days after TAI to determine ovulation by disappearing of dominant follicle at time of AI and appearance of new CL. Response to the third GnRH administration was detected 7 days later third GnRH treatment.

Pregnancy diagnosis was performed 31 d post-insemination (Visualization of a fluid-filled uterine horn with embryonic vesicles) by ultrasonography. Pregnancy check was done post insemination 62 d (Presence of a fetus). Pregnancy loss was recorded when the second check founded negative. Conception rate was calculated as the number of cows diagnosed pregnant, divided by the number of cows receiving AI.

4. Statistical Analyses

Statistical analyses were conducted by using SAS (Version 9.2; SAS Institute, 2010). Data were evaluated using by PROC LOGISTIC, PROC GLM and PROC FREQ in SAS. PROC GLM procedure was performed in order to analyze the following; to compare milk production, DIM, body condition score (BCS), parity, number of services and follicle size at the time of AI between groups. Chi-square analysis using the PROC FREQ procedure was used to analyze the following: to compare of ovulatory response to first, second and third GnRH and conception rates (CR) at 31 and 62 d between groups. PROC LOGISTIC was used to analyze the effect of treatment, milk production, DIM, BCS, follicle size at the time of AI, response to first and third GnRH and breed on conception rate at 31 and 62 d.

Results

1. General Results

Mean lactation number of cows was similar between groups (2.21±0.11 in normal and 2.20±0.7 in repeat breeder cows). BCS was different between repeat breeder and normal cows (3.01±0.03 and 2.84±0.05, respectively, P=0.01). Milk production was different (P<0.001) between groups (29.5±0.63 kg/d in repeat breeders and 38.9±1.05 kg/d in normal cows). Number of services was greater (P<0.001) in repeat breeders (4.1±0.09) than normal cows (1.1±0.15) group and DIM was different between repeat breeders and normal cows, 269.5±6.4 and 132.2±10.8, respectively (P<0.001).

2. Response to Hormonal Treatments

Percentage of cows that ovulated in response to the first GnRH treatment was higher (P<0.003) in repeat breeders (70.9%, 124/175) than in normal cows (49.2%, 31/63) (Table 1). Response to second GnRH was not different between groups (97.1%, 170/175 in repeat breeders and 98.4%, 62/63 in normal cows). Similarly, ovulatory response to the third GnRH was similar between groups (83.9%, 141/168 in repeat breeders and 87.1%, 54/62 in normal cows) (Table-1). Presence of accessory CL did not differ between groups at the 31 d (40.4%, 57/141 in repeat breeders and 50.0%, 27/54 in normal cows) and 62 d pregnancy diagnosis (28.4%, 40/141 in repeat breeders and 42.6%, 23/54 in normal cows) (Table 1). Maximum follicular size at the time of AI did not differ between repeat breeders (16.13±0.18 mm) and normal cows (16.07±0.29 mm).

3. Treatment Effects on Conception Rate

Conception rate at 31 d was similar between groups with 44.0% (77/175) in repeat breeders and 47.6% (30/63) in normal cows. When 62 d CR were evaluated in groups, there was no difference between repeat breeders (40.6%; 71/175) and normal cows (42.9%;

27/63) groups (Table-2). Embryonic loss was also similar in groups (7.8%, 6/77 in repeat breeders and 10.0%, 3/30 in normal cows) (Table-2). In addition, other covariant factors including milk production, DIM, BCS, parity, breed, response to first, second and third GnRH administrations and follicle size at the time of AI did not effect on CR at 31 and 62 d.

Table-1. Ovulatory response to each hormonal treatment of combined protocol and accessory CL at 31 and 62 d pregnancy diagnosis in groups.

Tablo-1. Gruplarda kombine tedavi protokolünün hormon uygulamalarına alınan ovülatör cevaplar ve 31. ve 62. gün gebelikte aksesor CL oranları

	Repeat Breeder Cows	Normal Cows
Response to first GnRH	70.9% (124/175)[a]	49.2% (31/63)[b]
Response to second GnRH	97.1% (170/175)	98.4% (62/63)
Response to third GnRH	83.9% (141/168)	87.1% (54/62)
Presence of accessory CL at 31 d pregnancy	40.4% (57/141)	50.0% (27/54)
Presence of accessory CL at 62 d pregnancy	28.4% (40/141)	42.6% (23/54)

a,b; $P<0.003$

Table-2. Conception rate at 31 and 62 d post-insemination and embryonic loss in repeat breeder and normal cows.

Tablo-2. Repeat breeder ve normal ineklerde tohumlama sonrası 31. ve 62. gün gebelik ve embriyonik kayıp oranı.

	Repeat Breeder Cows	Normal Cows
31 d Pregnancy	44.0% (77/175)	47.6% (30/63)
62 d Pregnancy	40.6% (71/175)	42.9% (27/63)
Embryonic Loss	7.8% (6/77)	10.0% (3/30)

Discussion

The aim of this study was to increase conception rate in repeat breeder dairy cows by eliminate most of the hormonal etiologic factors in repeat breeder cows with administrated different combined treatment protocol used progesterone based Ovsynch and GnRH treatment after AI. In this respect, treatment with this protocol proved to be effective in repeat breeder cows (44%), as the resulting pregnancy rate was similar with that of the normal breeding cows (47%).

Several hormonal treatments have been used to improve conception rate in repeat breeder cows. These include GnRH administration at the time of AI and post insemination progesterone supplementation. Results were inconsistent among the studies[12,13,19,22]. Recently some studies indicated that TAI protocols especially progesterone based protocol had positively effect on conception rate in repeat breeder cows[11,14,20]. For instance, Kasimanickam et al.[11] reported that conception rate was 21% following treatment with the Ovsynch protocol in repeat breeder cows and 24% following PresynchOvsynch in normal dairy cows. Similarly, Kim et al.[14] showed that conception rate was 35% after CIDR based TAI protocol. Nevertheless some researchers indicated that progesterone based TAI protocol did not improve conception rate in normal and repeat breeder cows[7,20]. In this study we found that progesterone based TAI protocol increased conception rate.

Positive effect of progesterone based Ovsynch protocol on conception rate in repeat breeders probably originated from greater response to each hormonal administration. For instance, percentage of cows responded to first GnRH of Ovsynch was found to be 70% in repeat breeders and greater ($P<0.003$) than normal cows (47%). Early studies showed that response to the first GnRH of the Ovsynch was 50 to 70% in normal lactating dairy cows[8,21]. It's unclear why repeat breeder cows have greater ovulatory response to first GnRH of Ovsynch when compared to normal cows. It might be coincident or it can be explaining that repeat breeders cows are sensitive to GnRH treatments. For instance, Karche and Srivastava[13] showed that ovulatory response to GnRH increased dose depended in repeat breeder cows and pregnancy rate was found to be greater when GnRH dose increased. Moreover both groups in this study received exogenous progesterone between GnRH and $PGF_{2\alpha}$ of Ovsynch. Previous studies indicated that exogenous progesterone administration shortly after first GnRH of Ovsynch diminished response to GnRH and adversely effected on conception rate in lactating dairy cows[9,16]. Lower ovulatory response to first GnRH in normal cows can be

explaining adversely effect of exogenous progesterone administration following GnRH injection.

On the hand greater percentage of ovulatory response to first GnRH of Ovsynch (70%) and exogenous progesterone probably increased progesterone level at time of $PGF_{2\alpha}$ in repeat breeder cows. Previous studies indicated that increased serum progesterone during the period before $PGF_{2\alpha}$ injection improved fertility of lactating dairy cows[6,18]. It may be a key role for improving conception rate in repeat breeder cows following progesterone based TAI protocol.

Ovulatory response to second GnRH was found to be similar in both group (97% in repeat breeders and 98% in normal cows). Previous studies showed that response to second GnRH of Ovsynch without exogenous progesterone was detected between 75 and 95% in normal lactating dairy cows[4,8]. Greater response to second GnRH in both groups can be attributed to exogenous progesterone administration that could be inducing high progesterone at the time of $PGF_{2\alpha}$. High progesterone level at the time of $PGF_{2\alpha}$ had higher response to second GnRH of Ovsynch than cows with low progesterone[4,8]. In addition response to third GnRH was found to be similar between groups (83% in repeat breeders and 87% in normal cows). Accessory CL was numerically greater in normal cows when compared to repeat breeders at 31 d (50% vs. 40%) and 62 d (42% vs. 28%) pregnancy diagnosis, but a difference was insignificant. It's also unclear why accessory CL regressed in repeat breeder cows that had greater percentage accessory CL after 3rd GnRH treatment. Needs the further studies explain to this condition in repeat breeder cows.

Conclusion

The results of this study showed that, as regardless of the causative factor(s), the progesterone based Ovsynch protocol and GnRH after AI were effective in treating the repeat breeder cows. The conception rate reached after administration of this protocol was very close to the mean conception rate of normal breeding cows.

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