Effects of GnRH, $PGF_{2\alpha}$ and oxytocin treatments on conception rate at the time of artificial insemination in lactating dairy cows

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ABSTRACT: In several studies, hormones such as gonadotropin-releasing hormone (GnRH), prostaglandins and oxytocin were used to increase pregnancy rate by inducing ovulation and improving the sperm transport in the female reproductive tract in lactating dairy cattle. The objective of this study was to compare the effects of GnRH, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and oxytocin treatments at the time of artificial insemination (AI) after spontaneous oestrus on the conception rate (CR) of lactating dairy cows. Oestrus was detected by visual observations by experienced personnel. All cows (n = 430, 308 Holstein-Frisian and 122 Swedish-Red dairy cows) were inseminated based on the am/pm rule by veterinarians of the farm. After AI, cows were alternately assigned to one of the four treatment groups: (1) GnRH (n = 113); (2) $PGF_{2\alpha}$ (n = 106); (3) oxytocin (n = 106) and (4) non-treated control (n = 105). Pregnancy diagnosis was performed 28–34 and 58–64 days post-insemination by transrectal ultrasonography. Conception rates on days 28–34 and 58–64 were not different among GnRH (46.0%; 52/113 and 44.3%; 50/113), $PGF_{2\alpha}$ (37.7%; 40/106 and 35.9%; 38/106) and control (49.5%; 52/105 and 47.6%; 50/105) groups. However, conception rates were lower (P = 0.02) in oxytocin (31.1%; 33/106 and 30.2%; 32/106) than in GnRH and control groups on days 28–34 and 58–64. Other covariant factors, such as milk production, days in milk (DIM), breed, parity, service number did not affect the conception rate. Thus, there were no beneficial effects of treatments with GnRH and $PGF_{2\alpha}$ at the time of AI, and oxytocin had an adverse effect on CR in lactating dairy cows in this study.

Keywords: cow; spontaneous oestrus; GnRH; $PGF_{2\alpha}$; oxytocin; pregnancy

There are many factors affecting fertility in lactating dairy cows including high milk production, postpartum disorders, and low body condition score (Vacek et al., 2007; Jílek et al., 2008). Many attempts have been done to increase fertility in lactating dairy cows. Gonadotropin-releasing hormone (GnRH) and its analogues administered at the time of AI are the most common treatments in management programmes for herds, recommended by veterinary practitioners (Stevenson et al., 1984; Chenault, 1990; Stevenson et al., 1990; Morgan and Lean, 1993). Improvement of the conception following GnRH treatment has been attributed to the prevention of an ovulation failure or a reduced variation in the interval between the onset of oestrus and ovulation by means of the induced preovulatory luteinizing hormone (LH) surge (Mee et al., 1993; Kaim et al., 2003). However the results are controversial after

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GnRH treatment of lactating cows. Some researchers found out that CR was improved (Kaim et al., 2003; Lee et al., 1983; Nakao et al., 1983; Lopez-Gatius et al., 2006) while others reported that no effect on pregnancy rate was obtained (Stevenson et al., 1984; Lee et al., 1985; Chenault, 1990; Mee et al., 1990; Perry and Perry, 2009).

$PGF_{2\alpha}$ and its analogues are widely used as a luteolytic agent for the treatment of uterine conditions in dairy cattle reproduction. In addition, these usage areas of the endogenous $PGF_{2\alpha}$ have been shown as an essential part of ovulation process (Armstrong, 1981; Algire et al., 1992; Neglia et al., 2008), and has been known that the increase of uterine and oviductal contractility (Hawk, 1983) affects the sperm transport. There are few studies focused on the effect of $PGF_{2\alpha}$ administration at the time of AI on pregnancy (Lopez-Gatius et al., 2004; Neglia et al., 2008).

Oxytocin was used to increase conception rate (CR) by improving the sperm transport in the female reproductive tract of several species (Hays et al., 1958; Hawk 1987; Sayre and Lewis 1997; King et al., 2004; Yildiz, 2005). Clitoral massage which probably releases oxytocin following artificial insemination increased pregnancy in beef cows but not in beef heifers or dairy cows (Randel et al., 1975; Cooper et al., 1984). The administration of oxytocin following AI also increased CR in lactating dairy cows (Yildiz, 2005) but in another study it had hardly any effect on pregnancy in cows (Hays et al., 1958).

Thus, the objective of this field study was to compare the effects of GnRH, $PGF_{2\alpha}$, and oxytocin treatments on CR at the time of AI after spontaneous oestrus in lactating dairy cows.

MATERIAL AND METHODS

Cows, housing and management

This study was performed on a commercial dairy herd with approximately 1000 lactating dairy cows in Bursa, Turkey. The herd composition was 2/3 of purebred Holstein-Friesian dairy cows and 1/3 of purebred Swedish Red dairy cows. Holstein-Friesian and Swedish Red cows were inseminated by the semen of fertile bulls of their own breed. The cows were housed in free stall facilities and grouped according to their milk production, they were milked three times a day and fed complete mixed rations based on NRC recommendations (National Research Council 2001). Average milk production of the herd was 9.880 kg (305 days) per cow. Daily milk yield was collected by the ALPRO® system (DeLaval, Stockholm, Sweden). Average milk production of each cow was recorded from day 7 before AI to day 7 after AI. The Animal Care Committee of Lalahan Livestock Central Research Institute approved all animal procedures.

Treatments and examinations

A total of 430 cows were used in this study, 308 of them were Holstein-Friesian and 122 were Swedish Red dairy cows. Visual detection of oestrus was done by experienced personnel three times a day for 20 min at least. Artificial insemination (AI) was performed based on the am/pm rule by veterinarians after detection of standing oestrus. Cows were alternately assigned to one of the four treatments after AI: (1) GnRH (n = 113) (busereline acetate, 10 µg, intramuscular, Receptal®, Intervet, Turkey); (2) $PGF_{2\alpha}$ (n = 106) (cloprostenol, 500 µg, intramuscular, Estrumate®, CEVA-DIF, Turkey); (3) oxytocin (n = 106) (oxytocin, 50 IU, intramuscular, Oksitosin®, Vetaş, Turkey); and (4) non-treated control (n = 105).

Pregnancy diagnosis was performed 28–34 and 58–64 days post-insemination by transrectal ultrasonography using a portable B-mode ultrasound scanner (Honda HS 2000 equipped with a 5.0 to 7.5 MHz transducer; Honda, Japan). Visualization of a fluid-filled uterine horn with embryonic vesicles (days 28–34) and the presence of a foetus (days 58–64) were used as positive indications of pregnancy. Pregnancy rate was calculated from the number of cows diagnosed pregnant in 28 to 34 days post-insemination divided by the number of inseminated cows.

Statistical analyses

All statistical procedures were performed using the computational software of SAS (Release SAS®9.2, SAS Institute, Cary). For statistical analyses, the breed of lactating cows was coded as 1 (Holstein-Friesian) or 2 (Swedish-Red). Conception on days 28–34 and 58–64 after insemination was coded as 0 (not pregnant) or 1 (pregnant). Primiparous and multiparous cows were coded as 1 or 2, respectively. Service number of cows was coded as 0 (first service of AI) or 1 (more than 1 service).

Table 1. Conception rates after treatments in lactating dairy cows, Holstein-Friesian, and Swedish-Red dairy cows

	Lactation days	Conception rate (%)			
		GnRH	PGF2α	oxytocin	control
Lactating dairy cows	28–34	46.0 (52/113)[a]	37.7 (40/106)[a,b]	31.1 (33/106)[b]	49.5 (52/105)[a]
	58–64	44.3 (50/113)[a]	35.9 (38/106)[a,b]	30.2 (32/106)[b]	47.6 (50/105)[a]
Holstein-Friesian	28–34	46.8 (34/78)	33.3 (26/78)	29.3 (22/75)	46.8 (36/77)
	58–64	41.0 (32/78)	31.0 (24/78)	35.4 (21/75)	44.2 (34/77)
Swedish Red	28–34	51.4 (18/35)	50.0 (14/28)	35.5 (11/31)	57.1 (16/28)
	58–64	51.4 (18/35)	46.4 (13/28)	35.5 (11/31)	57.1 (16/28)

[a,b]$P = 0.02$

Chi-square analysis using the PROC FREQ procedure was used to compare the conception rate (28–34 and 58–64 days) among the treatment groups and pregnancy rate between breeds. A logistic procedure was used to analyse the effect of treatment, milk production, days in milk (DIM), parity, breed and service number on pregnancy, and the interactions between milk production and treatment.

RESULTS

Conception rates (days 28–34) were found to be similar among the GnRH (46.0%; 52/113), $PGF_{2\alpha}$ (37.7%; 40/106) and control groups (49.5%; 52/105). However, oxytocin treatment (31.1%; 33/106) significantly decreased ($P = 0.02$) the conception rate in lactating dairy cows compared to the control and GnRH groups (Table 1). In addition, conception rates on days 58–64 were also similar among the groups (44.3%; 50/113 in GnRH, 35.9%; 38/106 in $PGF_{2\alpha}$ and 47.6%; 50/105 in control) and oxytocin group had a lower ($P = 0.02$) conception rate (30.2%; 32/106) when compared to the control and GnRH groups.

Conception rates for Holstein-Friesian and Swedish Red cows after treatments are shown in Table 2. There was no breed effect on conception rate. Interactions among milk production, treatment and conception rate were found to be insignificant. The effect of treatments was not different when cows were evaluated according to their parity (Table 3). Other covariant factors, such as DIM and service number, had no effect on conception rate.

DISCUSSION

High milk production, several health disorders, retained placenta, mastitis, and low body condition score decreased fertility in dairy cows (Vacek et al., 2007; Jílek et al., 2008). Many attempts including oestrus synchronization and timed artificial insemination protocols have been made to increase dairy cow fertility. So this study was aimed to improve fertility in lactating dairy cows by using GnRH, $PGF_{2\alpha}$ and oxytocin at the time of AI.

In several previous studies, GnRH improved pregnancy rate by 7–21% in lactating dairy cows (Lee et al., 1983; Nakao et al., 1983; Stevenson et al., 1990; Kaim et al., 2003; Lopez-Gatius et al., 2006). On the other hand, in some studies the pregnancy rate was not affected by GnRH treatment follow-

Table 2. Conception rates after treatments according parity in lactating dairy cows

	Conception rate (%)			
	GnRH	$PGF_{2\alpha}$	Oxytocin	control
Primiparous				
28–34-days	45.7 (16/35)	30.6 (11/36)	32.4 (12/37)	54.2 (13/24)
58–64 days	42.8 (15/35)	30.6 (11/36)	32.4 (12/37)	50.0 (12/24)
Multiparous				
28–34 days	46.1 (36/78)	41.4 (29/70)	30.4 (21/69)	48.1 (39/81)
58–64 days	44.9 (35/78)	38.5 (27/70)	28.9 (20/69)	46.9 (38/81)

ing AI and was similar to our findings (Stevenson et al., 1984; Lee et al., 1985; Chenault 1990; Mee et al., 1990; Perry and Perry 2009). Variability in pregnancy rate among the different studies might be associated with the potency of GnRH on gonadotropin release (Souza et al., 2009) or the timing of GnRH (Mee et al., 1990) and AI relative to the onset of oestrus (Stevenson et al., 1984; Mee et al., 1990). Earlier studies showed that the timing of GnRH injection according to the onset of oestrus affected gonadotropin release. Although exogenous GnRH at the onset of oestrus increased the preovulatory LH surge (Mee et al., 1990; Kaim et al., 2003; Perry and Perry, 2008), conception rate was increased in one study (Kaim et al., 2003) but not in others (Mee et al., 1990; Perry and Perry, 2009). However, the administration of GnRH at the time of AI, approximately 12 h after the initiation of standing oestrus, need not result in a greater surge of LH (Lee et al., 1985; Lucy and Stevenson 1986; Mee et al., 1990). In addition, the insufficient LH surge did not have any ovulatory effect (Lee et al., 1985; Lucy and Stevenson, 1986; Kaim et al., 2003) and did not improve pregnancy (Lee et al., 1985). Thus our result and previous studies indicated that the administration of GnRH at the time of AI did not affect pregnancy rate.

In the present study we demonstrated that the administration of $PGF_{2\alpha}$ at the time of AI following spontaneous oestrus did not have any beneficial effect. Lopez-Gatius et al. (2004) showed that $PGF_{2\alpha}$ administered at the time of fixed timed insemination had no effect on conception rate in cows with acceptable reproductive performance, $PGF_{2\alpha}$ used at the time of AI in cows in which stress factors and in repeat breeders' conception rate increased. So the results of this study indicated that $PGF_{2\alpha}$ administrations at the time of AI after spontaneous oestrus did not affect CR.

In cattle only a few studies showing the effect of oxytocin on pregnancy rate at the time of AI were published (Hays et al., 1958; Yildiz 2005). In an early study, Hays et al. (1958) showed that oxytocin treatment did not affect pregnancy rate in cows. However, in a recent study, pregnancy rate was increased in lactating dairy cows after oxytocin administration just before AI, but the number of cows (n = 17) that were included in the study was very low (Yildiz, 2005). In the present study, the administration of oxytocin at the time of AI decreased CR in lactating dairy cows. This could be due to changes in uterine contractility and possibly to the impairment of sperm transport in the reproductive tract of cows in contrast with other species (Hawk 1983; Sayre and Lewis, 1997; King et al., 2004).

As a conclusion, our results suggest that the administration of GnRH, oxytocin and $PGF_{2\alpha}$ at the time of AI does not increase CR in lactating dairy cows. Moreover, oxytocin treatment at the time of AI decreases CR in lactating dairy cows showing spontaneous oestrus and therefore it is not recommended for these animals.

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