Effect of Administering Ovsynch Protocol Plus Postbreeding Infusion on First Service Pregnancy Outcome in Cows

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Abstract: The objectives were to determine the effect of ovsynch plus postbreeding infusion treatment combined with fixed time AI on pregnancy rate in healthy Holstein cows. A total of 36 cows ageing between three and eight were used. Cows were randomly assigned to one of three treatment groups. Cows (n = 12) in first group (control) received no treatment; they were managed as healthy cows and inseminated at the first estrus after the voluntary waiting period. Second group (n = 12) received ovsynch protocol. Ovsynch protocol included administration of GnRH (2.5 mL im, ReceptAlf, 0.004 mg Euserein mL⁻¹, Intervet) on day 1, administration of PGF2α analogue D-Cloprostenol (2 mL im, Dalmazin, 0.075 mg D-Cloprostenol mL⁻¹, Vetaj) on day 8 and second GnRH administration on day 10. Animals in this group were artificially inseminated 18 h following 2nd GnRH administration. Third group (n = 12) received ovsynch but with the addition of 500 mg of Gentamycin sulphate (10 mL added to 30 mL of saline iv, Gentavet, 50 mg Gentamycin sulphate mL, Vetaj) in 30 mL of saline by intrauterine infusion 2 h after artificial insemination. Pregnancy diagnosis was performed via rectal palpation 45 days post insemination. Pregnancy rate in the cows that received Ovsynch + Postbreeding infusion was higher (p<0.05) than that of cows synchronized with ovsynch. Based on the odds ratio, pregnancy rate for cows treated with Ovsynch + Postbreeding infusion increased 2.8 folds (Odds Ratio (OR): 2.80 (0.532-14.7355) 95% Confidence Interval (CI)) when was compared with control group and 6 folds (OR: 6.00 (1.0177-35.3753) 95% CI) when was compared with ovsynch group. The results of these studies indicate that acceptable pregnancy rates can be achieved in the recipients that have received the ovsynch plus postbreeding infusion protocol without the necessity of estrus detection.

Key words: Ovsynch, postbreeding infusion, timed artificial insemination, pregnancy rate, cow, Turkey

INTRODUCTION

Reproductive efficiency in dairy cattle tends to have lower than their counterparts from the last few decades ago (Weigel, 2006). First service conception rates to Artificial Insemination (AI) had fallen away from approximately 65-40% or lower (Butler, 1998). Although, the causes have not yet been fully described, the fertility of dairy cows is declining worldwide (Thatcher et al., 2006; Lucy, 2001). Low pregnancy rates to AI are multifactorial complex problems that are difficult to solve. High reproductive efficiency is necessary for a successful dairy operation and requires a calving interval that maximizes milk production within the herd (Balie, 1982). Good estrus detection, good insemination technique, quality semen and a healthy uterine environment are critical components of high reproductive efficiency (Yildiz, 2001, 2005). In animal production, the majority of reproductive disorders are associated with genital infections (Borsberry and Dobson, 1989; Kirkland et al., 1991). The consequences might be manifested as affected follicular growth impaired oocyte developmental capacity, disturbed ovulations, conception failures and early embryonic deaths or more obviously as abortions, stillbirths and foetal malformations (Grohn et al., 1990; Fray et al., 2000; Kafi et al., 2002). Development of Timed Artificial Insemination (TAI) programs allow for reduced emphasis on detection of estrus because all cows are inseminated at a specific time relative to hormone injection (Pursley et al., 1995).

Some investigator stated that the pregnancy rate to a TAI following with ovsynch was similar to AI after observed estrus (Stevenson et al., 1999; Britt and Gaska, 1998; Burke et al., 1996; Momcilovic et al., 1998). However, a lot of reports indicate that ovsynch followed by TAI performed 16-20 h after the 2nd GnRH treatment yields pregnancy rates of about 25% in cows with occurrence of synchronization of ovulation (Bartolome et al., 2000; Fricke and Wiltbank, 1999). The main objective of developments in mammalian reproductive technology has been to preserve fertility. However, despite progress in synchronizing estrus and
artificial insemination, the reproductive performance of dairy cows has not substantially improved (Lopez-Gatius et al., 2001). Therefore, methods for timed insemination of dairy cattle still need to be optimized. Uterine bacterial infections are important because they disrupt not only the function of the uterus but also the ovary and the higher control centers in the hypothalamus and pituitary causing sub fertility (Sheldon and Dobson, 2004). A method to reduce the effect of uterine disease on fertility is intrauterine treatment with antibiotics. The use of antibacterial intrauterine infusions within 24 h of a breeding has become an accepted treatment for known or suspected bacterial infections of the reproductive tract (Oxender and Seguin, 1976).

There is relatively little information available as to the efficacy of intrauterine antibacterial infusions in the prevention or treatment of infertility in dairy cows (Dohoo, 1984). The routine infusion of a penicillin, chloramphenical, sulfonamide mixture or gentamicin following the first service of normal lactating cows has also been shown to improve fertility (Oxender and Seguin, 1976; Yildiz, 2001). However, there have been no studies on the effectiveness of special ovysynx plus postbreeding infusion on conception rate in cow. Hence, the present study is conducted to study the effect of ovysynx plus postbreeding infusion treatment combined with fixed time AI on pregnancy rate in cows.

**MATERIALS AND METHODS**

The study was conducted on 3 commercial, family owned dairy farms in east Turkey (Elazig) from February-July 2009. All participating farmers were informed about relevant characteristics of the study and agreed with the study design (informed consent). Average herd size was 22 Holstein cows with a range from 13-29. For farm 1, 5 of a total of 13 lactating cows were allocated to this study for farm 2, 14 of a total of 24 lactating cows were allocated to this study for farm 3, 17 of a total of 29 lactating cows were allocated to this study. All the cows had no history of dystocia, dwarf syndrome, retained placenta, clinical mastitis, metritis, vaginal laceration or vaginitis in their last parturition.

The Body Condition Scores (BCS; point scale from 1-5) (Edwards et al., 1989) of the cows was 2.5-3.5. Between 40-45 days post partum all cows were examined by rectal palpation and vaginoscopy examination. They had not any touchable disorders in their reproductive tracts. The ages of the animal ranged between 3 and 5. All the cows were housed in free-stall barns with slotted concrete floors and cubicles. Average daily milk production for both farms was between 24-27 kg per cow during the study period. Lactating cows were milked twice daily. The voluntary waiting period varied between the herd from 45-60 days in Milk (DIM). Cows were randomly assigned to one of three treatment groups. Cows (n = 12) in first group (control) received no treatment; they were managed as healthy cows and inseminated at the first estrus after the voluntary waiting period. Second group (n = 12) received ovysynx protocol.

Ovysynx protocol included administration of GnRH (2.5 mL im, Receptafili, 0.004 mg Buserelin mL⁻¹, Intervet) on day 1, administration of PGF2α analogue D-Cloprostenol (2 mL im, Dalmazin, 0.075 mg D-Cloprostenol mL⁻¹, Vetas) on day 8 and second GnRH administration on day 10. Animals in this group were artificially inseminated 18 h following 2nd GnRH administration. Third group (n = 12) received ovysynx but with the addition of 500 mg of Gentamycin sulphate (10 mL added to 30 mL of saline in, Gentavet, 50 mg Gentamycin sulphate mL⁻¹, Vetas) in 30 mL of saline by intrauterine infusion 2 h after artificial insemination. Pregnancy diagnosis was performed via rectal palpation 45 days post insemination.

**Statistical analysis:** To assess the effectiveness of ovysynx plus postbreeding infusion regime in improving pregnancy rates in cows, the main outcome measure was pregnancy occurring. Odds Ratios (OR) and 95% Confidence Intervals (CI) were calculated for the effects of treatments. Data are presented as percentages and proportions with p-values for main effects. For analyses, a p<0.05 was regarded as statistically significant. Statistical analyses were performed using MedCalc Version 10.0 for Windows (MedCalc Software, Mariakerke, Belgium).

**RESULTS**

The results for the first service pregnancy rate following treatment regime are shown in Table 1. The pregnancy rate was 25.0% (3/12) for cows treated with the ovysynx protocol combined with fixed time AI. A pregnancy rate of 66.7% was observed with Ovysynx + Postbreeding infusion treatment combined with fixed time AI. In the control group that was inseminated after spontaneous estrus, 5 of 12 (41.7%) were confirmed pregnant. Numerically, the percent of cows that was pregnant was lower for cows treated with ovysynx protocol (25.0%) than for control group (41.7%). There was no meaningful difference (p>0.05) in pregnancy rate between Group I and II. Numerically, the percent of cows that was pregnant was higher for cows treated with Ovysynx + Postbreeding infusion (66.7%)
than for control group (41.7%). However, this difference was not statistically significant (p>0.05). Pregnancy rate in the cows that received Ovsynch + Postbreeding infusion was higher (p<0.05) than that of cows synchronized with ovsynch. Based on the odds ratio, pregnancy rate for cows treated with Ovsynch + Postbreeding infusion increased 2.8 folds (Odds Ratio (OR): 2.80 (0.532-14.7355) 95% Confidence Interval (CI)) when was compared with control group and 6 folds (OR: 6.00 (1.0177-35.3753) 95% CI) when was compared with ovsynch group. Pregnancy to first service was 6 times more likely (p<0.05) to occur in Ovsynch + Postbreeding infusion group than in ovsynch group.

**DISCUSSION**

The first service pregnant rate is a valuable tool for evaluation of fertility. A goal of 60-70% first service pregnant rate is a goal of well-managed herds. A variety of hormonal protocols have been developed to regulate the bovine estrous cycle without the necessity the estrus signs of individual cows (Refsdal, 2000). Ideal synchronization program should have conception rate similar to the natural rate (Vasconcelos et al., 1999). However, the conception rate after induction of estrus by using different hormone therapy regimens is around 30-45 that is much <60-70% of the natural conditions (Lopez-Gatius, 2000). In the present study, it was observed that ovsynch did not have any effect on increasing pregnancy rates, whereas it was obtained a satisfactory pregnancy rate by applying ovsynch plus postbreeding infusion protocol without the necessity of estrus detection into animals.

It was seemed to be no remarkable differences between ovsynch and control group for the percentage of pregnancy. In spite of this, the number of pregnant cows in the ovsynch group was numerically lower than the control group. The pregnancy rate of cows after synchronization with ovsynch was diminished by 16.7 percentage units compared with that of control cows. Overall, synchronization with ovsynch does not offer desired pregnancy rates with fixed time inseminations. The decreasing of the pregnancy rate of cows with ovsynch protocol was also similar to that reported previously (Fricke, 2002; Kasimaniekam et al., 2005b; Fallah Rad and Ajam, 2008; Alhimer et al., 2002; Lemaster et al., 2001; Moreira et al., 2000b). However, a higher percentage 48.9, 41 and 35% of conception rate of cows were reported by Klinkworth et al. (2001), Fricke et al. (1998) and Steveson et al. (1996), respectively. The exact causes of the decreased conception rate of cows after synchronization with ovsynch are unknown. The mechanism by which pregnancy rate is diminished involves several steps. The second GnRH injection of the ovsynch protocol may result in ovulation but the subsequent CL will produce less P4 and therefore reduce subsequent pregnancy rates (Moreira et al., 2000a).

In addition, the follicle may possibly have displayed dominance for an extended period by the time of the second GnRH injection. Follicles that display dominance for too long a period (>5 days) have decreased fertility (Austin et al., 1999). A similar situation exists with initiation of the first GnRH injection in the late luteal phase of the estrous cycle (13-17 days). Two-wave cycle cows will have a small, non-GnRH responsive dominant follicle that will not ovulate and form a new CL. Therefore, the cow spontaneously produces endogenous PGF2α from the uterus and regresses the previously existing CL about 4 days later. In this situation, the cow may already have expressed estrus and ovulated prematurely by the time of the exogenous PGF2α and subsequent GnRH administration. If the initial GnRH dose is given in the proestrus phase of the estrous cycle, the PGF2α injection may fail to completely regress the CL resulting in lower pregnancy rates following TAI (Moreira et al., 2000a).

It is clear that there is a need for improved knowledge of the factor the maintenance of gestation in the early fetal stage (Lopez-Gatius et al., 2002). The present study demonstrated the beneficial effect of a combined application of ovsynch and postbreeding infusion. Cows receiving ovsynch plus postbreeding infusion protocol had significantly increased pregnancy rate compared to ovsynch protocol. The pregnancy rate after first service of cows which received ovsynch in addition to intrauterine antibiotic therapy was improved 6 folds in comparison with ovsynch group and 2.8 folds in comparison with the controls. The results of these studies indicate that acceptable pregnancy rates can be achieved
in the recipients that have received the ovosynch plus postbreeding infusion protocol without the necessity of estrus detection. These findings agree with the results of the previous reports (Yildiz, 2001; Kutty, 2004; Singia et al., 1993; Awasthi and Kharche, 1987; Warriach et al., 2008) that Gentamicin application with or without GnRH was found to be effective on the the conception rate/pregnancy rate. On the other hand, Daniels et al. (1976) notified that gentamycin sulphate administered by intravenous infusion at the rate of 200 mg, 10 min following first service insemination in dairy cows did not enhance fertility.

Kasimaniyam et al. (2005a) demonstrated that treatment of cows diagnosed with subclinical endometritis with a single iu infusion of 500 mg of cephalosporin benzathine improved pregnancy per AI. Recent research has also indicated that subclinical endometritis is a prevalent problem, affecting 35-51% of cows at 40-60 DIM with substantial decreases in pregnancy rate in affected cows (Gilbert et al., 2004; Kasimaniyam et al., 2004). Gilbert et al. (2005) reported that the major contributor to impaired reproduction in cows with subclinical endometritis is to be a reduction in the first service conception rate and they showed that the prevalence of subclinical endometritis between herds varied from 37-74% of cows. Bacterial infections affect fertility by altering the uterine environment resulting in impairment of sperm transport, sperm death and hostile environment to the subsequent development and maintenance of the conceptus leading to their death (Dholakia et al., 1987; Rahman et al., 1996).

Inflammation and cytokine production by leukocytes in the uterus can disrupt endometrial function (Bondurant, 1999; Sheldon and Dobson, 2004) and impair embryo development (Soto et al., 2003). Furthermore, induction of inflammation in the absence of bacteria can lead to reduced embryo quality (Hill and Gilbert, 2008). The embryonic loss rate in lactating cows varies from 10-60.5% (Vasconcelos et al., 1997; Chebel et al., 2004; Sartori, 2004; Sartori et al., 2006). Therefore, antibiotics may improve fertility in cows with subclinical endometritis by reducing bacterial contamination of the uterus and concurrent inflammation (Galvao et al., 2009). The ovosynch plus postbreeding infusion protocol can improve the uterine environment of undesirable conditions for embryonic growth and alter the endocrinial status of cows and may cause an increase in the conception rate.

CONCLUSION

In this study, the synchronization with ovosynch did not offer desired pregnancy rates with fixed time insinations. The Ovsynch + Postbreeding infusion treatment combined with fixed-time AI increased hazard of pregnancy in healthy cows clinically. The results of these studies indicate that acceptable pregnancy rates can be achieved in the recipients that have received the Ovsynch plus postbreeding infusion protocol, without the necessity of estrus detection.

REFERENCES


