Journal of Animal and Veterinary Advances 9 (9): 1345-1350, 2010
ISSN: 1680-5593

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

Atilla Yildiz
Department of Dairy Animal Breeding, Sivrice Vocational Collage,
University of Firat, Turkey

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 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, Receptalfi, 0.004 mg Buserelin mL^{-1}, Intervet) on day1, administration of PGF2α analogse D-Cloprostenol (2 mL im, Dalmazin, 0.075 mg D-Cloprostenol mL^{-1}, Vetaş) 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 iu, Gentavet, 50 mg Gentamycin sulphate mL, Vetaş) 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 (Bailie, 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 hyothalamus 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 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 onsynch plus postbreeding infusion on conception rate in cow. Hence, the present study is conducted to study the effect of ovsynch 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, downer syndrome, retained placenta, clinical mastitis, metritis, vaginal laceration or vaginitis in their last parturition.

The Body Condition Scores (BCS; point scale from 1-5) (Edmonson *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 vaginoscopic 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 herds 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 ovsynch protocol.

Ovsynch protocol included administration of GnRH (2.5 mL im, Receptalfi, 0.004 mg Buserelin mL^{-1}, Intervet) on day 1, administration of PGF2α analogse D-Cloprostenol (2 mL im, Dalmazin, 0.075 mg D-Cloprostenol mL^{-1}, Vetaş) 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 iu, Gentavet, 50 mg Gentamycin sulphate mL^{-1}, Vetaş) 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 ovsynch plus postbreeding infusion regime in improving pregnancy rates in cows, the main outcome measure was pregnancy occuring. 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 ovsynch protocol combined with fixed time AI. A pregnancy rate of 66.7% was observed with Ovsynch + 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 ovsynch 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 Ovsynch + Postbreeding infusion (66.7%)

Table 1: First service pregnancy rates following different treatment regime in cows

Groups	Cows (n)	Pregnancy rate in first service (%, n/n)	Odds ratio	95% confidence interval	p-values
I (Control)	12	41.7 (5/7)	Referent		
II (Ovsynch)	12	25.0 (3/12)	0.47	0.082-2.6564	0.3904
III (Ovsynch + Postbreeding infusion)	12	66.7 (8/12)	2.80	0.532-14.7355	0.2243
			Referent (0vsynch)		
			6.0	1.0177-35.3753	0.0478

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). İdeal 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 pregnant rate by applying ovsynch plus postbreeding infusion protocol without the necessity of estrus detection into animals.

It was seemed to be no remarkable differents 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; Kasimanickam *et al*., 2005b; Fallah Rad and Ajam, 2008; Alnimer *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 Klindworth *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 GnRHadministration. 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 ovsynch 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 intrauterine infusion at the rate of 200 mg, 10 min following first service insemination in dairy cows did not enhance fertility.

Kasimanickam *et al.* (2005a) demonstrated that treatment of cows diagnosed with subclinical endometritis with a single iu infusion of 500 mg of cephapirin 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; Kasimanickam *et al*., 2004). Gilbert *et al.* (2005) reported that the major contributor to impaired reproduction in cows with subclinical endometritis 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 ovsynch plus postbreeding infusion protocol can improve the uterine environment of undesirable conditions for embryonic growth and alter the endocrinal status of cows and may cause an increase in the conception rate.

CONCLUSION

In this study, the synchronization with ovsynch did not offer desired pregnancy rates with fixed time inseminations. 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

Alnimer, M., G. De Rosa, F. Grasso, F. Napolitano and A. Bordi, 2002. Effect of climate on the response to three oestrous synchronization techniques in lactating dairy cows. Anim. Reprod. Sci., 71: 157-168.

Austin, E.J., M. Mihm, M.P. Ryan, D.H. Williams and J.F. Roche, 1999. Effect of duration of dominance on the ovulatory follicle on onset of estrus and fertility in heifers. J. Anim. Sci., 77: 2219-2226.

Awasthi, M.K. and K.G. Kharche, 1987. Coparative efficacy of antibacterial and non-hormonal therapies in repeat breeder crossbred cows. Indian Vet. J., 64: 887-889.

Bailie, J.H., 1982. Management and economic effects of different level of oestrus detection in the dairy herd. Vet. Rec., 110: 218-221.

Bartolome, J., L. Archbald, P. Morresey, J. Hernandez and T. Tran *et al*., 2000. Comparison of synchronization of ovulation and induction of estrus as therapeutic strategies for bovine ovarian cysts in the dairy cow. Theriogenology, 53: 815-825.

Bondurant, R.H., 1999. Inflammation in the bovine female reproductive tract. J. Anim. Sci., 77: 101-110.

Borsberry, S. and H. Dobson, 1989. Periparturient diseases and their effect on reproductive performance in five dairy herds. Vet. Rec., 124: 217-219.

Britt, J.S. and J. Gaska, 1998. Comparison of two estrus synchronization programs in a large, confinement-housed dairy herd. J. Am. Vet. Med. Assoc., 212: 210-212.

Burke, J.M., R.L. De la Sota, C.A. Risco, C.R. Staples, E.J.P. Schmitt and W.W. Thatcher, 1996. Evaluation of timed insemination using a gonadotropin-releasing hormone agonist in lactating dairy cows. J. Dairy Sci., 79: 1385-1393.

Butler, W.R., 1998. Effect of protein nutrition on ovarian and uterine physiology in dairy cattle. J. Dairy Sci., 81: 2533-2539.

Chebel, R.C., J.E.P. Santos, J.P. Renolds, R.L. Cerri, S.O. Juchem and M. Overton, 2004. Factors affecting conception rate after insemination and pregnancy loss in lactating dairy cows. Anim. Reprod. Sci., 84: 239-255.

Daniels, W.H., D.A. Morrow, B.W. Pickett and L. Ball, 1976. Effects of intrauterine infusion of gentamycin sulfate on bovine fertility. Theriogenology, 6: 61-68.

Dholakia, P.M., N.M. Shah, J.H. Purohit and H.N. Kher, 1987. Bacteriological study on non-specific genital infection and its antibiotic spectra in repeat breeders. Indian Vet. J., 64: 637-640.

Dohoo, I.R., 1984. A retrospective evaluation of postbreeding infusions in dairy cattle. Can. J. Comp. Med., 48: 6-9.

Edmonson, A.J., I.J. Lean, L.D. Weaver, T. Farver and G. Webster, 1989. A body condition scoring chart for Holstein dairy cows. J. Dairy Sci., 72: 68-78.

Fallah, R.A.H. and G. Ajam, 2008. Aplication of ovsynch-CIDR vs 2 consecutive injections of PGF 2a, 14 days apart in dairy Holstein cows and comparison of reproductive parameters, plasma and milk E2 and P4 concentraitons. J. Anim. Vet. Adv., 7: 312-315.

Fray, M.D., D.J. Paton and S. Alenius, 2000. The effects of bovine viral diarrhoea virus on cattle reproduction in relation to disease control. Anim. Reprod. Sci., 60-61: 615-627.

Fricke, P.M. and M.C. Wiltbank, 1999. Effect of milk production on the incidence of double ovulation in dairy cows. Theriogenology, 52: 1133-1143.

Fricke, P.M., 2002. Scanning the future-ultrasonography as a reproductive management tool for dairy cattle. J. Dairy Sci., 85: 1918-1926.

Fricke, P.M., J.N. Guenther and M.C. Wiltbank, 1998. Efficacy of decreasind the dose of GnRH used in a protocol for synshronizationof ovulation and timed AI in lactating dairy cow. Theriogenology, 50: 1275-1284.

Galvao, K.N., L.F. Greco, J.M. Vilela, M.F. Sá Filho and J.E.P. Santos, 2009. Effect of intrauterine infusion of *Ceftiofur* on uterine health and fertility in dairy cows. J. Dairy Sci., 92: 1532-1542.

Gilbert, R.O., S.T. Shin, C.L. Guard, H.N. Erb and M. Frajblat, 2005. Prevalence of endometritis and its effects on reproductive performance of dairy cows. Theriogenology, 64: 1879-1888.

Gilbert, R.O., S.T. Shin, M. Frajblat, C.L. Guard, H.N. Erb and H. Roman, 2004. The incidence of endometritis and its effect on reproductive performance of dairy cows. Proceedings of the 12th International Conference Production Diseases Livestock, E. Lansing, MI.

Grohn, Y.T., H.E. Erb, C.E. McCulloch and H.S. Saloniemi, 1990. Epidemiology of reproductive disorders in dairy cattle: Association among host characteristics, disease and production. Prev. Vet. Med., 8: 25-39.

Hill, J. and R. Gilbert, 2008. Reduced quality of bovine embryos cultured in media conditioned by exposure to an inflamed endometrium. Aust. Vet. J., 86: 312-316.

Kafi, M., M.R. McGowan and P.D. Kirkland, 2002. *In vitro* maturation and fertilisation of bovine oocytes and *in vitro* culture of presumptive zygotes in the presence of bovine pestivirus. Anim. Reprod. Sci., 71: 169-179.

Kasimanickam, R., T.F. Duffield, R.A. Foster, C.J. Gartley, K.E. Leslie, J.S. Walton and W.H. Johnson, 2005a. The effect of a single administration of cephapirin or cloprostenol on the reproductive performance of dairy cows with subclinical endometritis. Theriogenology, 63: 818-830.

Kasimanickam, R., J.M. Cornwell and R.L. Nebel, 2005b. Fertility following fixed-time AI or insemination at observed estrus in Ovsynch and Heatsynch programs in lactating dairy cows. Theriogenology, 63: 2550-2559.

Kasimanickam, R., T.F. Duffield, R.A. Foster, C.J. Gartley, K.E. Leslie, J.S. Walton and W.H. Johnson, 2004. Endometrial cytology and ultrasonography for the detection of subclinical endometritis in postpartum dairy cows. Theriogenology, 62: 9-23.

Kirkland, P.D., S.G. Richards, J.T. Rothwell and D.F. Stanley, 1991. Replication of bovine viral diarrhoea virus in the bovine reproductive tract and excretion of virus in semen during acute and chronic infections. Vet. Rec., 128: 587-590.

Klindworth, H.P., M. Hoedemaker, D. Burfeindt and T. Heilkenbrinker, 2001. Ovulationssynchronisation (Ovsynch) in hochleistenden Milchviehherden. I. Fruchtbarkeitsparameter, body condition score und plasma progesterone konzentration. Dtsch. Tierärztl. Wschr., 108: 11-19.

Kutty, C.I., 2004. Effect of intrauterine infusion of gentamicin in repeat breeding cattle. Indian J. Anim. Reprod., 4: 123-127.

Lemaster, J.W., J.V. Yelich, J.R. Kempfer, J.K. Fullenwider, C.L. Barnett, M.D. Fanning and J.F. Selph, 2001. Effectiveness of GnRH plus prostaglandin $F_{2\alpha}$ for estrus synchronization in cattle of Bos indicus breeding. J. Anim. Sci., 79: 309-316.

Lopez-Gatius, F., 2000. Reproductive performance of lactating dairy cows treated with cloprostenol, hCG and estradiol benzoate synchronization of estrous followed by timed artificial insemination. Theriogenology, 54: 551-558.

Lopez-Gatius, F., P. Santolaria, J. Yaniz, J. Rullant and M. Lopez-Bejar, 2001. Persistent ovarian follicles in dairy cows: A therapeutic approach. Theriogenology, 56: 649-659.

Lopez-Gatius, F., P. Santolaria, J. Yaniz, J. Rutllant and M. Lopez-Bejar, 2002. Factors affecting pregnancy loss from gestation day 38-90 in lactating dairy cows from a single herd. Theriogenology, 57: 1251-1261.

Lucy, M.C., 2001. Reproductive loss in high-producing dairy cattle: Where will it end? J. Dairy Sci., 84: 1277-1293.

Momcilovic, D., L.F. Archibald, A. Walters, T. Tran, D. Kelbert, C. Risco and W.W. Thatcher, 1998. Reproductive performance of lactating dairy cows treated with gonadotropin-releasing hormone (GnRH) and/or prostaglandin $F_{2\alpha}$ ($PG_{f2\alpha}$) for synchronization of estrus and ovulation. Theriogenology, 50: 1131-1139.

Moreira, F., C.A. Risco, M.F.A. Pires, J.D. Ambrose, M. Drost and W.W. Thatcher, 2000a. Use of bovine somatotropin in lactating dairy cows receiving timed insemination. J. Dairy Sci., 83: 1237-1247.

Moreira, F., R.L. de La Sota, T. Diaz and W.W. Thatcher, 2000b. Effect of day of the estrous cycle at the initiation of a timed artificial insemination protocol on reproductive responses in dairy heifers. J. Anim. Sci., 78: 1568-1576.

Oxender, W.D. and B.E. Seguin, 1976. Bovine intrauterine therapy. J. Am. Vet. Med. Assoc., 168: 217-219.

Pursley, J.R., M.O. Mee and M.C. Wiltbank, 1995. Synchronization of ovulation in dairy cows using $PG_{f2\alpha}$ and GnRH. Theriogenology, 44: 915-923.

Rahman, A., A. Rahman, H. Rahman and M.U. Ahmed, 1996. Anaestrus and repeat breeding problems in indigenous cattle in Bangladesh. Trop. Anim. Health Prod., 7: 605-609.

Refsdal, A.O., 2000. To treat or not to treat: A proper use of hormones and antibiotics. Anim. Reprod. Sci., 60: 109-119.

Sartori, R., 2004. Fertilizacao e morte embrioneria em bovinos. Acta Scientiae Veterinariae, 32: 35-50.

Sartori, R., A. Gumen, J.N. Guenther, A.H. Souza, D.Z. Caraviello and M.C. Wiltbank, 2006. Comparison of artificial insemination versus embryo transfer in lactating dairy cows. Theriogenology, 65: 1311-1321.

Sheldon, I.M. and H. Dobson, 2004. Postpartum uterine health in cattle. Anim. Reprod. Sci., 82-83: 295-306.

Singia, V.K., G. Singh, P.N. Dwivedi and R.D. Sharma, 1993. *In vivo* evaluation of drug sensitivity pattern of bacterial isolates in repeat-breeder cows. Indian J. Anim. Sci., 63: 425-426.

Soto, P., R.P. Natzke and P.J. Hansen, 2003. Actions of tumor necrosis factor-a on oocyte maturation and embryonic development in cattle. Am. J. Reprod. Immunol., 50: 380-388.

Stevenson, J.S., Y. Kobayashi and K.E. Thompson, 1999. Reproductive performance of dairy cows in various programmed breeding systems including Ovsynch and combinations of gonadotropin releasing hormone and prostaglandin $F_{2\alpha}$. J. Dairy Sci., 82: 506-515.

Steveson, J.J., Y. Konayashi, M.P. Shipka and K.C. Rauchhotz, 1996. Altering conception of dairy cattle by gonadotropin releasing hormone preceding luteolysis induced by prostaglandin $F_{2\alpha}$. J. Dairy Sci., 79: 402-410.

Thatcher, W.W., T.R. Bilby, J.A. Bartolome, F. Silvestre, C.R. Staples and J.E.P. Santos, 2006. Strategies for improving fertility in the modern dairy cow. Theriogenology, 65: 30-44.

Vasconcelos, J.L.M., R.W. Silcox, G.J.M. Rosa, J.R. Pursley and M.C. Wiltbanksa, 1999. Synchronization rate, size of the ovulatory follicle and pregnancy rate after synchronization of ovulation beginning on different days of the estrous cycle in lactating dairy cows. Theriogenology, 52: 1067-1078.

Vasconcelos, J.L.M., R.W. Silcox, J.A. Lacerda, J.R. Pursley and M.C. Wiltbank, 1997. Pregnancy rate, pregnancy loss and response to heat stress after AI at 2 different times from ovulation in dairy cows. Biol. Reprod., 56: 140-140.

Warriach, H.M., N. Ahmad, G. Ahmad, M.S. Khan, M. Rabbani and I. Ahmad, 2008. Effect of antibiotic treatment on pregnancy rate of repeat breeder dairy cross bred cows with sub-clinical uterine infection. Pak. Vet. J., 28: 40-42.

Weigel, K.A., 2006. Prospects for improving reproductive performance through genetic selection. Anim. Reprod. Sci., 96: 323-330.

Yildiz, A., 2001. Effects of administration of fertirelin acetate and gentamycin sulphate after artificial insemination on pregnancy rate of cows. Turk. J. Vet. Anim. Sci., 25: 1001-1006.

Yildiz, A., 2005. Effect of oxytocin on conception rate in cows. F.U. Saglik Bil. Derg., 19: 75-78.