

The Comparison of the Pregnancy Rates Obtained after the Ovsynch and Double Dose PGF_{2α} + GnRH Applications in Lactating Dairy Cows

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Abstract: This study was carried out to compare the pregnancy rates in timed artificial inseminations after Ovsynch and PGF_{2α} + GnRH applications on lactating dairy cows. The research was conducted in a commercial dairy farm on 84 lactating Holstein cows which the ages were ranging between 3-5 years old and between 80-120 days postpartum. The cows were randomly assigned to two groups as Ovsynch (n = 42) and Prosta Glandin (PG) (n = 42). The cows in Ovsynch group recieved 10.5 µg⁻¹ GnRH at day 0, 500 µg⁻¹ PGF_{2α} at day 7 and 10.5 µg⁻¹ GnRH at day 9 intramuscularly. The cows in PG group recieved 500 µg⁻¹ PGF_{2α} two times with an interval of 14 days and 10.5 µg⁻¹ GnRH intramuscularly 48 h after the second PGF_{2α} injection. Artificial inseminations were applied to the cows in both of the groups between 16-20 h after the GnRH injections. The pregnancy diagnosis was performed 30 days after the artificial inseminations by ultrasonography. The pregnancy rates were determined as 35.7% (15/42) in Ovsynch group and 47.6% (20/42) in PG group. Although, the pregnancy rate were higher in PG group than the Ovsynch group the difference was not statistically important (p>0.05). It was concluded that the programme consisted of 14 days interval double dose PGF_{2α} injections, GnRH applications 48 h following the second PGF_{2α} injection and timed artificial insemination 16-20 h after the GnRH injection could be an alternative to the Ovsynch programme in timed artificial inseminations.

Key words: Ovsynch, prostaglandin, timed artificial insemination, pregnancy, dairy, cow, Turkey

INTRODUCTION

Fertility is an important factor for production and profitability in dairy herds. Having a calf per year is the main target in cow breeding. For achieving this target, the estrous should be correctly detected and the cows should become pregnant before 85 days postpartum. Studies suggest that although, the cows has regular estrous cycles the rate of correct estrous detection in cows are <50%, so this causes the extension of calving interval (Larson and Ball, 1992; Jainudeen and Hafez, 2000; Kim *et al.*, 2005). By using the systematic reproductive programmes fertility could be improved. The systematic reproductive programmes enables to fix-time insemination by synchronizing the estrous or ovulations in large numbers of cows at desired periods without detecting estrous (Kim *et al.*, 2005; Nak *et al.*, 2005). The advantages of the fixed-time artificial insemination programmes combined with ovulation and estrous synchronization are reported as abating the estrous

detection problem, decreasing the days open till conception and maintaining the conception rates in economic limits (Lean *et al.*, 2003; Perry *et al.*, 2004). Although, the problem for undetected estrous is solved by fixed-time inseminations, it increases the risk of inseminating cows, which are not in estrous (Tenhagen *et al.*, 2000). Other problems associated with the Ovsynch protocol are the interval variation between the GnRH injection and ovulation (Patterson *et al.*, 2003), the loss of functional dominance of the follicle, less ovulatory capacity of the dominant follicle and the cows in standing estrous prior to prostaglandin injections (Vasconcelos *et al.*, 1999; Perry *et al.*, 2004).

The Ovsynch protocol includes the GnRH administration at a random stage of the estrous cycle. GnRH provokes the LH release also ovulates or luteinizes the the dominant follicle and starts a new follicular wave in 2-3 days. The new luteal tissue is regressed by the PGF_{2α} which is applied at day 7. The GnRH injected at day 9 provokes the ovulation of the dominant follicle formed

in the new follicular wave (Vasconcelos *et al.*, 1999). The ovulations in the Ovsynch procedure occur 24-32 h following the second GnRH administrations (Pursley *et al.*, 1997a; Stevenson *et al.*, 1999).

The PGF_{2α} administrations cause the regression of the functional Corpus Luteum (CL). Owing to the regression of CL the blood progesterone concentration rapidly decreases and a significant rise in the estrogen causes an increase in the LH surge and ovulation (King *et al.*, 1982; Diskin *et al.*, 2002; Yaniz *et al.*, 2004). While one dose regimen of the PGF_{2α} is used in the luteal phase (6-16 days) of the cycle, 11-14 days apart injection is advised to be used in the unknown stage of the cycle in large herds (Yaniz *et al.*, 2004; Momcilovic *et al.*, 1998; Cavalieri *et al.*, 2006). After the last application of the PGF_{2α}, the estrous initiation varies 39-80 h in the heifers, 48-76 h in cows (Le Blanc *et al.*, 1998). Cavalieri *et al.* (2006) states that this large variation in estrous initiation time requires a labour and time for artificial inseminations. Le Blanc *et al.* (1998) reports that GnRH injections following the PGF_{2α} applications ensures providence in labour and time for artificial inseminations.

This research was carried out to compare the pregnancy rates in timed artificial inseminations after Ovsynch and double dose PGF_{2α} + GnRH applications on lactating dairy cows.

MATERIALS AND METHODS

This study was carried out at a commercial dairy cow farm in Hatay in Turkey. The research material consisted of 84 holstein cows, which the ages ranged between 3-5 and were between the intervals of 80-120 days postpartum. All cows were housed in free stall barns with free access to feed and water.

The cows were milked twice a day and the daily milk yield of the trial group cows varied between 20-35 litres/cow during the experiments.

The cows were randomly assigned to two groups as Ovsynch and Prosta Glandin (PG). The cows in Ovsynch group (n = 42) recieved 10.5 µg GnRH (Buserelin acetate, Receptal, Intervet, Turkey) at day 0, 500 µg PGF_{2α} (Cloprostenol, Joramut, Ege-Vet, Turkey) at day 7 and 10.5 µg GnRH at day 9 intramuscularly. The cows in PG group (n = 42) recieved 500 µg PGF_{2α} two times with an 14 days interval and 10 µg GnRH intramuscularly 48 h after the second PGF_{2α} injection.

Rectal palpation was carried out in the cows both in Ovsynch and PG group before the PGF_{2α} injections to control the luteal structures. According to the rectal palpation results fully formed and regressed corpus luteums were recorded. Estrous detection was performed

3 times a day for 45 min at the interval of PGF_{2α}-GnRH injection period and the cows which showed estrous was recorded. Timed artificial insemination was applied to the cows in both of the groups 16-20 h after the GnRH injections.

The pregnancy diagnosis was carried out 30 days after the artificial inseminations by a real time B-mode 6-8 MHz rectal probe ultrasound equipment (Pie medical falco Scanner 100 LDC, VET). The X² test in SPSS 14.00 programme was used in the comparison of the pregnancy rates obtained in the Ovsynch and PG groups.

RESULTS AND DISCUSSION

The rectal ovarian findings at the time of PGF_{2α} injection and the estrous detection results at the interval of PGF_{2α}-GnRH injection period are summarized at Table 1. The artificial inseminations and conception rates are shown at Table 2.

Prior to the PGF_{2α} injection, while 4 regressed and 38 fully formed corpus luteum were detected in the Ovsynch group, all CL were fully formed in the PG group. A total of 6 cows, (14.3%), together with the 4 cows having regressed CL showed estrous at the interval of PGF_{2α}-GnRH injection period in the Ovsynch group. No estrous symptom were detected in the PG group at the interval of PGF_{2α}-GnRH injection period.

The pregnancy rates were 35.7% (15/42) and 47.6% (20/42) in the Ovsynch and PG groups respectively. The difference in the pregnancy rates among the groups were not statistically significant (p>0.05).

Systematic fertility programmes are widely used in cattle production without the need for estrous detection. Two effective protocols including GnRH-PGF_{2α}-GnRH combination (Ovsynch) and a series of PGF_{2α} applications followed by a timed artificial insemination are preferred (Nebel and Jobst, 1998).

Table 1: Rectal palpation before the PGF_{2α} administration and estrous detection results at the interval of PGF_{2α}-GnRH injection period in the ovsynch and PG groups

Groups	Rectal palpation ^a		Estrous detection ^b	
	Fully formed CL (n)	Regressed CL (n)	Yes (%)	No (%)
Ovsynch	38	4	14.3	85.7
PG	42	0	0	100

^aRectal palpation prior to PGF_{2α} injection; ^bEstrous detection at the interval of PGF_{2α}-GnRH injection period

Table 2: The artificial insemination and conception rates in the Ovsynch and PG groups

Groups	Insemination rates (%)	Conception rates (%)
Ovsynch	100 (42/42)	35.7 (15/42)
Prostaglandin	100 (42/42)	47.6 (20/42)

Recently, it is outlined that the ovulation synchronization by the Ovsynch protocol resulted in a better conception rates than the PGF_{2α} based estrous synchronization protocols (Pursley *et al.*, 1997a, b). However, the reason for the variation in the response of the cows and heifers in Ovsynch protocol is informed to be arising from the time variations of the second GnRH injection and ovulation interval (Patterson *et al.*, 2003). Important problems in Ovsynch protocol are suggested as the loss of functional dominance in the dominant follicle prior to second GnRH treatment and a follicle in the newly emerging follicular wave which had not yet acquired ovulatory capacity. Also the 15% of the cows which are in standing estrous prior to or at the prostaglandin injection time is pointed out to be another disadvantage in Ovsynch procedure (Vasconcelos *et al.*, 1999; Perry *et al.*, 2004). The probability of stimulating ovulation during Ovsynch programme in lactating cows is mentioned to be 85% and the synchronization of ovulation is reported as 87-100% (Pursley *et al.*, 1997a). Santos *et al.* (2004) stated that asynchrony was determined in 13-16% of the cows in Ovsynch programme. In the present study prior to the PGF_{2α} injection, 4 regressed corpus luteum were detected, together with the 4 cows having regressed CL, 6 cows (14.3%) were standing estrous at the interval of PGF_{2α}-GnRH injection period in the Ovsynch group. However, no estrous symptom were detected in the PG group at the interval of PGF_{2α}-GnRH injection period. If the Ovsynch program has been initiated in the late phase of the oestrous cycle (such as day 15) at the time of prostaglandin injection (day 7), the CL has already been regressed and the cow may even be in standing estrous (Moreira *et al.*, 2000). This result indicates that double dose PGF_{2α} + GnRH programme may extinguish the disadvantages of the Ovsynch procedure mentioned above.

The conception rates in the Ovsynch studies carried out on lactating cows varied from 28.3-42.4% (Moreira *et al.*, 2000; Cartmill *et al.*, 2001; Williams *et al.*, 2002; Tenhagen *et al.*, 2004). The conception rate obtained in Ovsynch group in this study is 35.7% and this is in the ranges of the authors stated.

Less *et al.* (1992) stated that when randomly cyclic cows were subjected to the double 14 days prostaglandin protocol at least 67% of the cows had a corpus luteum at the time of treatment onset on day 7-20 of their estrous cycle which underwent luteolysis either spontaneously (cows on cycle day 18-20) or in response to PGF_{2α} treatment (cows on cycle day 7-17). In the study, the rectal palpation prior to the second prostaglandin administration showed that all of the cows had mature CL

in PG group. Murugavel *et al.* (2003) reported that reproductive performance in dairy cattle was also improved following double dose 14 days interval PGF_{2α} treatment without assessing ovarian status compared with a single dose protocol based on detecting a corpus luteum by rectal palpation or by milk progesterone enzyme immunoassay.

The conception rates in the synchronization programmes with the PGF_{2α} and the analogues varied between the ranges of 37-60% (Drillich *et al.*, 2000). This variation is due to the the interval of prostaglandin administration and ovulation period depending on the development stage of the preovulatory follicle on the ovary (Mialot *et al.*, 1999; Yaniz *et al.*, 2004). Saumande and Humblot (2005) pointed out that the length of interval between the onset of estrous and ovulation may result in lower fertilization rates. Starbuck *et al.* (2006) declared that the interval from luteolysis to ovulation shows the greatest variability as 72 h. In this study the cows in the PG group was synchronized with an 14 days interval prostaglandin application. Additionally, GnRH was administered 48 h after the last prostaglandin application to minimize the variability between the luteolysis and the ovulation.

The conception rates following double dose 14 days interval PGF_{2α} treatment was reported as 34% (Kurykin *et al.*, 2006), 37.3% (Williams *et al.*, 2002). Young (1989) obtained 51% conception rate after two artificial inseminations 72 and 96 h following the last prostaglandin administration in the study which the prostaglandin was applied 14 days apart. Although, one artificial insemination, the 47.6% conception rate in the PG group determined in the study was higher than the results obtained by the Tenhagen *et al.* (2000) and Kurykin *et al.* (2006) and similar to the results of Young (1989). This situation may be related to the GnRH application 48 h following the last prostaglandin administration. Cartmill *et al.* (2001) obtained a 27% conception rate in lactating cows in which the prostaglandin was applied twice with an 12 days apart, GnRH administration 48 h after the last prostaglandin application and artificial insemination 16-20 h following the GnRH administration. Stevenson *et al.* (1999) obtained a 24.6% conception rate in lactating cows in which the prostaglandin was applied twice with an 14 days apart GnRH administration 33 h after the last prostaglandin application and artificial insemination 16-18 h following the GnRH administration. The 47.6% conception rate obtained in PG group was higher than these results. Differences in conception rates could be due to the PG injection interval and GnRH administration time. Stevenson *et al.* (1999) reported that two injections of PGF_{2α} given 14 days apart produced

pregnancy rates equal to that produced by the Ovsynch protocol. In this study, the conception rates obtained in Ovsynch and PG groups were 35.7 and 47.6%, respectively. Although, a rise is seen in the conception rate of PG group no statistical difference was achieved ($p>0.05$).

CONCLUSION

The programme consisted of 14 days interval double dose PGF_{2α} injections, GnRH applications 48 h following the second PGF_{2α} injection and timed artificial insemination 16-20 h after the GnRH injection may be an alternative to the Ovsynch programme in timed artificial insemination programmes.

REFERENCES

- Cartmill, J.A., S.Z. El-Zarkouny, B.A. Hensley, G.C. Lamb and J.S. Stevenson, 2001. Stage of cycle, incidence and timing of ovulation and pregnancy rate in dairy cattle after three timed breeding protocols. *J. Dairy Sci.*, 84: 1051-1059.
- Cavalieri, J., G. Hepworth, L.A. Fitzpatrick, R.W. Shephard and K.L. Macmillan, 2006. Manipulation and control of the estrous cycle in pasture-based dairy cows. *Theriogenology*, 65: 45-64.
- Diskin, M.G., E.J. Austin and J.F. Roche, 2002. Exogenous hormonal manipulation of ovarian activity in cattle. *Domestic Anim. Endocrinol.*, 23: 211-228.
- Drillich, M., B.A. Tenhagen and W. Heuvelink, 2000. Effect of one spontaneous estrus cycle (after synchronization with PGF₂) on reproductive performance in dairy cows. *Theriogenology*, 54: 1389-1394.
- Jainudeen, M.R. and E.S.E. Hafez, 2000. Cattle and Buffalo. In: *Reproduction in Farm Animals*, Hafez, B. and E.S.E. Hafez (Eds.). Lippincott Williams and Wilkins, Maryland, USA.
- Kim, U.H., G.H. Suh, H.W. Nam, H.G. Kang and I.H. Kim, 2005. Follicular wave emergence, luteal function and synchrony of ovulation following GnRH or estradiol benzoate in CIDR-treated, lactating Holstein cows. *Theriogenology*, 63: 260-268.
- King, M.E., G.H. Kiracofe and J.J. Stevenson, 1982. Effect of stage of the oestrus cycle on interval to oestrus after PGF_{2α} in beef cattle. *Theriogenology*, 18: 191-200.
- Kurykin, J., U. Jaakma, A. Waldmann, M. Jalakas, M. Aidnik, L. Majas and P. Padrik, 2006. Low semen dose intracornual insemination of cows at fixed time after PGF_{2α} treatment or at spontaneous estrus. *Anim. Reprod. Sci.*, 95: 116-124.
- Larson, L.L. and P.J.H. Ball, 1992. Regulation of estrous cycles in dairy cattle: A review. *Theriogenology*, 38: 255-267.
- Le Blanc, S.J., K.E. Leslie, H.J. Ceelen, D.F. Kelton and G.P. Keefe, 1998. Measures of estrus detection and pregnancy in dairy cows after administration of gonadotropin-releasing hormone within an estrus synchronization program based on prostaglandin F_{2α}. *J. Dairy Sci.*, 81: 375-381.
- Lean, I.J., J.A. Porte, A.R. Rabiee, W.F. Morgan, W.P. Tranter, N. Moss and R.J. Rheinberger, 2003. Comparison of effects of GnRH and prostaglandin in combination and prostaglandin on conception rates and time to conception in dairy cows. *Aust. Vet. J.*, 81: 488-493.
- Less, M.C., J.D. Savio, L. Badinga, R.L. de La Sota and W.W. Thatcher, 1992. Factors that affect ovarian follicular dynamics in cattle. *J. Anim. Sci.*, 70: 3615-3626.
- Mialot, J.P., G. Laumonnier, C. Ponsart, H. Fauxpoint, E. Barassin, A.A. Ponter and F. Deletang, 1999. Postpartum subestrus in dairy cows: Comparison of treatment with prostaglandin F₂ or GnRH + prostaglandin F₂+GnRH. *Theriogenology*, 52: 901-911.
- 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_{2α} (PGF_{2α}) for synchronization of estrus and ovulation. *Theriogenology*, 50: 1131-1139.
- Moreira, F., R.L. de La Sota, T. Diaz and W.W. Thatcher, 2000. 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.
- Murugavel, K., J.L. Yaniz, P. Santolaria, M. Lopez-Bejar and F. Lopez-Gatius, 2003. Prostaglandin based estrus synchronization in postpartum dairy cows: An update. *Int. J. Applied Res. Vet. Med.*, 1: 51-65.
- Nak, Y., D. Nak and E. Karakas, 2005. Comparison of effects on pregnancy rates of ovsynch and cosynch + ovsynch administrations in cyclic or noncyclic anestrous cows. *Uludağ Univ. J. Fac. Vet. Med.* 24: 41-46.
- Nebel, R.L. and S.M. Jobst, 1998. Evaluation of systematic breeding programs for lactating dairy cows: A review. *J. Dairy Sci.*, 81: 1169-1174.
- Patterson, D.J., F.N. Kojima and M.F. Smith, 2003. A review of methods to synchronize estrus in replacement beef heifers and postpartum cows. *J. Anim. Sci.*, 81: 166-177.

- Perry, G.A., M.F. Smith, A.J. Roberts, M.D. Macneil and T.W. Geary, 2004. Effect of ovulatory follicle size on pregnancy rates and fetal mortality in beef heifers. *J. Anim. Sci. Suppl.*, 82: 101-101.
- Pursley, J.R., M.C. Wiltbank, J.S. Stevenson, J.S. Ottobre, H.A. Garverick and L.L. Anderson, 1997b. Pregnancy rates per artificial insemination for cows and heifers inseminated at a synchronized ovulation or synchronized estrus. *J. Dairy Sci.*, 80: 295-300.
- Pursley, J.R., M.R. Kosorok and M.C. Wiltbank, 1997a. Reproductive management of lactating dairy cows using synchronization of ovulation. *J. Dairy Sci.*, 80: 301-306.
- Santos, J.E.P., W.W. Thatcher, R.C. Chebel, R.L.A. Cerri and K.N. Galvao, 2004. The effect of embryonic death rates in cattle on the efficacy of estrus synchronization programs. *Anim. Reprod. Sci.*, 83: 513-535.
- Saumande, J. and P. Humblot, 2005. The variability in the interval between estrus and ovulation in cattle and its determinants. *Anim. Reprod. Sci.*, 85: 171-182.
- Starbuck, G.R., C.G. Gutierrez, A.R. Peters and G.E. Mann, 2006. Timing of follicular phase events and the postovulatory progesterone rise following synchronisation of oestrus in cows. *Vet. J.*, 172: 103-108.
- 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.
- Tenhagen, B.A., M. Drillich and W. Heuwieser, 2000. Synchronization of lactating dairy cows with prostaglandin $F_{2\alpha}$ insemination on observed oestrus versus timed artificial insemination. *J. Vet. Med.*, 47: 577-584.
- Tenhagen, B.A., M. Drillich, R. Surholt and W. Heuwieser, 2004. Comparison of timed AI after synchronized ovulation to AI at estrus: Reproductive and economic considerations. *J. Dairy Sci.*, 87: 85-94.
- Vasconcelos, J.L.M., R.W. Silcox, G.J.M. Rosa, J.R. Pursley and M.C. Wiltbanks, 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.
- Williams, S.W., R.L. Stanko, M. Amstalden and G.L. Williams, 2002. Comparison of three approaches for synchronization of ovulation for timed artificial insemination in *Bos indicus*-influenced cattle managed on the Texas gulf coast. *J. Anim. Sci.*, 80: 1173-1178.
- Yaniz, J.K., K. Murugavel and F. Lopez-Gatius, 2004. Recent developments in estrous synchronization of postpartum dairy cows with and without ovarian disorders. *Reprod. Domestic Anim.*, 39: 89-93.
- Young, I.M., 1989. Dinoprost 14-day oestrus synchronization schedule for dairy cows. *Vet. Rec.*, 124: 587-588.