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Comparison of Reproductive Parameters, Plasma and Milk E2 and P4 Concentrations Following Application of Ovsynch-CIDR vs. Two Consecutive Injections of PGF2α, in Dairy Holstein Cows

A.H. Fallah Rad and G. Ajam
Faculty of Veterinary Medicine, Ferdowsi University of Mashhad,
P.O. Box 91775-1793, Mashhad, Iran

Abstract: In the present study, effects of Ovsynch-CIDR protocol was compared with the conventional method of two consecutive injections of PGF2α 14 days apart, on some reproductive indices and concentrations of E2 and P4 on day 1, 5 and 21 after insemination. All the cows assigned into the study were in their second parity onward and had no history of peri-parturient diseases including: retained placenta, dystocia, lameness, clinical mastitis and metritis. In the nPG group (n = 27), on day 40±5 postpartum, two doses of PG were injected 14 days apart and if after the second injection, cow was inseminated upon showing signs of estrus. In the OC group (n = 25), Ovsynch-CIDR protocol along with timed AI was applied. Milk and blood samples were obtained from all the cows on day 1, 5 and 21 post AI. E2 and P4 of the plasma and P4 of the milk samples were measured by ELISA and reproductive indices were calculated. In case of return to estrus, the program was repeated for up to three times. Conception Rate (CR) was lower in the nPG than OC but the difference was not significant ($p>0.05$). In the second and third AI, CR was higher in OC than nPG (71.42 and 85.71% vs. 23.8 and 61.9% in the OC and nPG, respectively, $p<0.05$). Number of AI/conception and overall CR were 3.13 and 40.3% and 2.23 and 55.71% for nPG and OC, respectively ($p<0.05$). Days Open (DO) and predicted calving intervals were 138 and 418 and 106 and 387 for nPG and OC, respectively ($p<0.05$). Milk P4 was higher on day 5 and 21 in the OC and plasma E2 was lower in OC on day 5 than nPG ($p<0.05$). Results show that ovulation synchronization by Ovsynch-CIDR, as compared with two consecutive PG injections reduced DO, increased CR in the 2nd and 3rd inseminations, increased overall CR and reduced number of inseminations per conception. It can not be claimed that results of this experiment might be similar in all the cows of the same herd or other herds because, cows assigned into this study were selected.

Key words: Ovsynch-CIDR, PGF2α, P4, E2, dairy Holstein

INTRODUCTION

Milk production of the dairy breeds had increase tremendously at the expense of decrease in reproductive performance (Lopez-Gatius *et al*., 2001), increase on the incidence of reproductive and metabolic diseases (Francos, 1998; Macmillan *et al*., 1996). From 1973-1995, First Service Conception Rate (FSCR) in the dairy cows has been declining at a rate of 0.4 and 1% per year in the US and UK, respectively (Grohn and Rajala-Schultz, 2000). The main reasons are: negative impacts of poor heat detection, early embryonic death, poor management and nutrition (Butler, 2001; Peters, 1996). In industrial dairy farming, heat detection rarely reaches a 50% rate. Moreover, after induction of estrus by using different hormone therapy regimens, CR is around 30-45% that is much <60-70% of the natural conditions (Lopez-Gatius, 2000).

With respect to the expansion of the dairy farms, difficulty of heat detection and also greater use of AI, estrus and ovulation synchronization methods are increasingly applied (Ambrose *et al*., 2008; Burke *et al*., 1999; Pursley *et al*., 1995; Xu and Burton, 2000). Application of some of these techniques along with Fixed Time AI (FTAI), had obviated estrus detection (Melendez *et al*., 2006; Rhodes *et al*., 2002). One of the main features of estrus synchronization is short standing heat. In the more sophisticated protocols for FTAI, ovulation is synchronized (Stevenson *et al*., 2006; Pursley *et al*., 1995, 1997), CR is lower but, most of the cows can be inseminated within a reasonable period after calving, while there is no need to see the estrus signs of individual cows (Refsdal, 2000). Ideal synchronization program should have CR similar to the natural rate (Silva *et al*., 2007; Stevenson *et al*., 2007; Vasconcelos *et al*., 1999). Goal of the present study was

Corresponding Author: A.H. Fallah Rad, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, P.O. Box 91775-1793, Mashhad, Iran

to compare effects of two different estrus synchronization protocols on the reproductive indices, plasma E2, P4 and milk P4 concentrations in dairy cows.

MATERIALS AND METHODS

Lactating Holstein dairy cows in a dairy farm in Mashhad, Iran, were randomly assigned into either nPG (n = 27) or OC (n = 25) groups. Average daily milk production of the cows in the barn was 38 kg with 4% milk fat with a VWP of 40±5 days. All the cows were in their second parity on, had no history of dystocia, downer syndrome, RFM, clinical mastitis, metritis, vaginal laceration or vaginitis in their last parturition. If metritis was seen after start of the experiment, the cow was replaced with a healthy one. Cows in the nPG group received two consecutive IM injections of 0.5 mg of Cloprostenol (Estrumate®, Schering-Plough, NL) 14 days apart. Upon observation of estrus after second injection of PG, cows were inseminated. If there were no signs of estrus within 14 days, a 3rd, 4th or 5th injection was given. Ovsynch CIDR protocol was applied to cows in the OC group as explained by Pursley *et al*. (1997). In case of return to estrus, the program was repeated for up to three times. In non-return cases, non pregnant cows, on day 45, were removed from the experiment.

On day 1, 5 and 21 post insemination, 8 mL of blood and 20 mL of milk were collected, blood plasma was separated and stored at -20°C until P4 and E2 were determined. CR from the first, second and third AI, DO, number of AI/conception and interval from the first AI to conception were calculated. ELISA kits (DRG Instruments, GmbH), were read by ELISA reader and Washer (Bio-Tek Instruments, USA). Milk P4 was measured by the method described by Waldmann *et al*. (2001).

Data were analyzed by SPSS software. For the analysis of non-normal data, non parametric tests were used. Concentrations of E2 and P4 in the two groups and within the groups were compared by independent and paired t-test, respectively. Levels of E2 and P4 in three different samplings were compared by repeated measures of GLM. For the correlation of plasma and milk P4 and also plasma E2 and P4 in each stage, Pearson correlation test was used. For the analysis of other factors, Mann-Whitney U-test was used. Values of p = 0.05 were considered significant.

RESULTS

Table 1 and 2 shows reproductive indices of the two groups and concentrations of E2 and P4 in the plasma and P4 of the milk, respectively. Significant differences are shown by different letters (a and b; $p<0.05$).

Table 1: Comparison of the reproductive indices of the two groups

Factors	nPG	OC
No. of cows	27	25
Pregnant cows	22 (81.48%)	21 (84%)
No. of previous pregnancies (Mean±SEM)	3.17±0.31	3.17±0.28
Corrected milk production (kg)	8093.34	8606.88
Calving to start of experiment (days, Mean±SEM)	41±1.08	41.82±0.97
No. of AI/conception (Mean±SEM)	3.13±0.26[a]	2.23±0.23[b]
1st service CR (%)	14.28	23.80
2nd service CR (%)	23.8[a]	71.42[b]
3rd service CR (%)	61.90[a]	85.71[b]
Total CR (Mean±SEM)	40.30±5.44[a]	55.71±5.93[b]

Table 2: Plasma E2 and P4 and milk P4 of the two groups (Mean±SEM)

Concentration of E2 (pg mL^{-1}) and/or P4 (ng mL^{-1})	nPG group	OC group
Plasma E2 on day 1	33.38±4.05	26.28±1.17
Plasma E2 on day 5	26.19±3.12[a]	36.28±2.41[b]
Plasma E2 on day 21	25.85±6.02	26.61±2.81
Plasma P4 on day 1	4.47±0.87	3.07±0.77
Plasma P4 on day 5	2.82±0.24	2.95±0.54
Plasma P4 on day 21	6.17±1.23	5.95±1.50
Milk P4 on day 5	15.37±2.36[a]	8.44±0.97[b]
Milk P4 on day 21	24.17±3.57[a]	15.89±2.15[b]

On day 21, correlation coefficient for CR and plasma P4 was 0.622 ($p<0.05$) and 0.723 for milk P4 ($p<0.05$). Plasma E2 on day 5 and 21 in nPG group was lower in the pregnant as compared to the non pregnant cows ($p<0.05$). On day 21, in the pregnant OC cows, P4 of the plasma was higher than non pregnant cows ($p<0.05$).

DISCUSSION

FSCR in both groups were <30% and was similar to the results of others. It has been shown that FSCR in the ovsynch groups were lower than the cows inseminated after showing estrus signs (Alnimer *et al*., 2002). In the herds with low estrus detection rate, use of estrus and/or ovulation synchronization along with FTAI may generally increase CR but, FSCR may be lower than 2nd or 3rd service (Xu *et al*., 2000b). In the beginning of the present study, only 16.66% of the cows had <1 ng mL^{-1} P4 in their plasma. Ovsynch protocols give the best results on day 5-9 of the cycle but, if it is applied late in the luteal phase, a large follicle is produced with lower CR (Cordoba and Fricke, 2001). Most of the cows with low FSCR might be in their mid luteal phase. Similar to results of others (Xu *et al*., 2000a), CR from the 2nd and 3rd insemination and total CR in the CO group in the present study were higher than the nPG group (Table 1). Effectiveness of Ovsynch protocol may be due to better control of follicular development and ovulation (Mialot *et al*., 1999).

When exogenous P4 is used in the synchronizing protocols, in the next cycle a durable follicle is produced and a larger CL with more P4 is produced naturally, therefore, CR may increase (Xu *et al*., 2000a). CR from the 2nd AI was higher in the present research which confirms the above concept. Xu *et al*. (2000a) stated that total CR was not different when Ovsynch protocol was compared with repeated injections of PG (38.9% against 37.8%) but, the experiment, total CR was higher in the OC than nPG, while number of AI per conception was lower.

By using Ovsynch protocol, Seguin (1997) could reduce DO from 121-98 days with no reduction in the CR. In the present experiment, mean DO and predicted calving interval was lower in the OC than nPG (106 and 387 days, for OC and 138 and 418 days for nPG, respectively).

There are plenty of plausible reasons for the lower CR in the PG group which include: heat detection mistakes, AI in un-appropriate time, interference of the synchronization protocol with the follicular development, incomplete regression of the CL (Drillich *et al*., 2000), short half life of the PG in some cows, long follicular phases after PG injections and cows with inactive cycles (Peters and Ball, 1995).

PG treatment is not followed by luteolysis in at least 10% of the cows and within 24-48 h P4 production returns back. If CL is not active, it doesn't respond to PG. Negative energy balance and heat stress (Alnimer *et al*., 2002), might be other reasons for low CR from the 1st insemination in both groups. On day 5 post insemination milk P4 was higher in OC than nPG probably due to the delay in ovulation, follicles and the subsequent CLs were larger and more P4 was produced (4 and 32). Highest CR was seen in cows with P4 concentrations between 3-9 ng mL^{-1} of plasma on day 5 post AI (Starbuck *et al*., 2001; Thatcher *et al*., 2001).

Mean plasma E2 on day 5 after AI in nPG group was distinctly higher than OC which shows higher number of pregnant cows in the nPG from the first AI. Some cows have high E2 even on day 5-6 after standing heat which might due to the delayed ovulation. This phenomenon had been noticed in cows treated with PGF2α (Humblot, 2001). When ovulation is close, second injection of GnRH in Ovsynch protocol helps surge of LH, therefore, hastens ovulation (Berber *et al*., 2002).

Mean milk fat P4 on day 21 in the OC was higher than nPG, showing higher CR in this group. Cows having <5 ng mL^{-1} plasma P4 levels and not showing signs of estrus had lower chance of pregnancy and may need up to 4 inseminations (Francos, 1998). In the present study, there was a significant correlation between P4 on day 20-23 post AI with FSCR which is similar to the results of others (Alvarez *et al*., 1989; Karagiannidis, 1990; Pieterse *et al*., 1990).

CONCLUSION

From the results of the present study it could be concluded that ovulation synchronization by using Ovsynch-CIDR protocol as compared with two consecutive injections of PGF2α, had decreased DO and CI, increased CR from the 2nd and 3rd insemination, increased total CR and number of inseminations/ pregnancy.

Cows assigned into Ovsynch-CIDR protocol had higher P4 on day 5 and 21 post insemination. In the present experiment cows were selected with specific conditions, therefore, these results may not be applicable to all cows of the herd or other herds.

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