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Impact of Drenching of Propylene Glycol during the early postpartum period on the response of Primiparous Holstein cows to ovsynch

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ABSTRACT

The specific objective was to determine the possible influences of drenching Propylene Glycol (PrGl) at the early postpartum period on circulating levels of progesterone during ovsynch protocol and risk of pregnancy establishment in primiparous Holstein cows. Animals (n=45) were classified into a control group which did not receive any dietary supplementation (n=20) and PrGl group (n=25) where individual cows received once daily 250 mg PrGl drench starting from day of calving till day 30 postpartum. On day 60 postpartum, individual cows of both groups were submitted to ovsynch program and were timely inseminated. Serum samples as well as ovarian examination using ultrasonography were carried out on two occasions, on day seven of ovsynch at PGF2 alpha and at timed artificial insemination (TAI). Serum samples were assayed for progesterone. Results showed that PrGl drenching did not affect daily milk production, BCS of cows at synchronization as well as pregnancy rate after ovsynch (40 vs. 35% in PrGl and control cows, respectively). Regardless of treatment, cows with low progesterone at TAI had higher PR/TAI. When circulating progesterone levels were divided into quartiles, cows within the first quartile at TAI had nearly five folds greater PR/TAI, when compared to those within the forth quartile (70 vs. 14.5%, respectively). Further, treated and control cows had nearly similar size of CL at PGF2 alpha and pre-ovulatory follicle (POF) size at TAI. The size of CL at PGF2 alpha did not vary between pregnant and non-pregnant cows, regardless of treatment, however, pregnant cows had significantly higher POF size (1.58±0.25 cm) than control cows (1.39±0.27 cm). In conclusion, drenching of PrGl during the first 30 days postpartum for primiparous dairy cows did not affect their response to ovsynch. In addition, higher sizes of POF as well as lower levels of circulating progesterone at TAI were associated with greater PR/TAI.

Keywords: Propylene glycol, Ovsynch, Pregnancy, Progesterone, CL size, Pre-ovulatory follicle

Introduction

Primiparous cows approximately account for 30% of dairy cattle herds as documented by Ozturk *et al.* (2010). After parturition, primiparous dairy cows are known to suffer greater metabolic stress than multiparous herd-mates due to the acute onset of a sudden high milk production coupled with the still-growing nature of cows during their first lactation (Meikle *et al.*, 2004). Thus, it is clear that health as well as reproductive problems, mainly negative energy balance (NEB) due to excessive condition score loss and anestrum, are inevitable in primiparous cows (Ozturk *et al.*, 2010). The effect of postpartum NEB on reproductive cyclicity has been previously reported and cows in NEB are more prone to delayed cyclicity and lower pregnancy rates (Beam and Butler, 1999).

The strategic use of gluconeogenic precursors as propylene glycol (PrGl) to minimize the severity of postpartum NEB in dairy cattle has been studied in several research experiments with conflicting results. Oral PrGl decreased the incidence of displaced abomasum and increased the risk of conception to the first service in cows with subclinical ketosis (McArt *et al.*, 2015). In addition, other studies reported that oral drenching of PrGl increased conception in cows (McArt *et al.* 2015; Slobodanka *et al.*, 2012). On the other hand, Hoedemaker *et al.* (2004) reported that supplementation of concentrate with 10% PrGl did not affect the fertility parameters in dairy cows. Similarly, Yildiz and Erizir (2016)

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concluded that drenching with 250 mL of PrGl per day during the first two weeks postpartum did not improve the onset of luteal activity postpartum, in spite of a tendency to shorter interval from calving to first service in PrGl-treated cows.

To the best of our knowledge, no available literature discussed the effects of oral drenching of PrGl on hormonal and ovarian status of primiparous Holstein cows in response to ovsynch carried out 60 days postpartum. Thus, the present work was designed to compare circulating progesterone levels as well as ovarian structures together with pregnancy rate per timed artificial insemination (PR/TAI) in primiparous Holstein cows supplemented or not with PrGl during the first month after calving.

Material and Methods

1. Animals, housing and management

The study was carried out on primiparous Holstein cows (n=45) which were housed in a free stall barn, milked three times daily using an automatic parlor and fed a totally mixed ration formulated in line with the nutritional research council recommendations for dairy cattle (NRC, 2001). Cows received regular vaccination programs and were apparently clinically healthy.

2. Reproductive program and synchronization

After calving, cows were monitored for normal placental drop. Fifteen days later, individual cows received 12 micro-gram buserelin I/M. On day 60 postpartum, cows were submitted to ovsynch protocol (12 micro-gram buserelin I/M on day 60, 750 micro-gram cloprostenol on day 67, 12 micro-gram buserelin on day 69 and TAI 16 hours later).

3. Ultrasonography

The ultrasound examinations (Sonoscape, 5-7 MHz, linear probe) were carried out in treated and control cows to determine size of CL at PGF2 alpha injection on days seven of ovsynch and to measure the size of POF on day ten at TAI.

Pregnancy diagnosis was carried out on day 35 post-insemination using ultrasound and positive signs confirming pregnancy were presence of embryo surrounded by anechoic amniotic fluid. Ultrasound exams were carried out according to Kasimanickam *et al.* (2018).

4. Sampling and assays

Coccygeal venous blood samples were obtained from individual cows on day seven of ovsynch (day of cloprostenol injection) and at TAI. The obtained samples were cooled overnight and were centrifuged at 3500 rpm to separate serum. Each serum sample was identified using individual cow's code and day of collection. Then, serum samples were kept frozen at -80 ^{o}C till analysis of progesterone. Analysis of serum progesterone was carried out using a radio-immunoassay (RIA) kit following manufacturer instructions. The sensitivity of the assay was 0.1 ng/ml.

5. Data collection

Data pertaining to milk production and BCS were retrieved from the automatic farm software program.

Statistical analysis

Data were tested for normality using Sharpino Wilk test (Sharpino and Wilk, 1965). Data proved normally distributed where the p value of the test was greater than 0.05. Thus, we used the student's T test to compare means between the two groups (PrGl and control), meanwhile, Chi square analysis was used to compare different proportions as PR/TAI. Altogether, a p value less than 0.05 was considered significant. The statistical analysis was done by SPSS program version 17 (SPSS, 2007).

Results

Data displayed in table 1 show that PR/TAI after ovsynch did not vary significantly between PrGl drenched (40%) and control cows (35%), despite higher pregnancy rate in drenched cows.

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As presented in table 2, lower circulating progesterone concentrations at TAI were associated with higher PR/TAI, regardless of treatment. This is evident, when comparing PR/TAI between cows in the first quartile (70%), and PR/TAI achieved by cows in the fourth quartile (14.9%).

As regards to utrasonographic findings, the present study revealed that variation of CL size at PGF2 alpha and size of POF at TAI did not differ significantly between PrGl drenched and control cows as illustrated in table 3.

Table 4 clarifies the relationship between the utrasonographic measurements and PR/TAI, regardless of treatment. Based on these findings, there were similar sizes of CL at PGF2 alpha in both pregnant (1.51±0.35 cm) and non-pregnant cows (1.51±0.52 cm), whereas, the size of POF at TAI differed significantly and was higher in pregnant cows than non-pregnant counterparts (1.58±0.25 vs. 1.39±0.27 cm in pregnant and non-pregnant cows, respectively).

Table 1: Pregnancy rate in control and propylene glycol treated cows after application of Ovsynch protocol

	Control group			Propylene glycol treated group		
	Number	**Pregnant**	**PR/TAI**	**Number**	**Pregnant**	**PR/TAI**
	20	7	35.00	25	10	40.00
Chi squared				0.12		
P value				0.73		

PR/TAI: Pregnancy rate per timed artificial insemination

Table 2: Quartiles of circulating progesterone (ng/ml) at timed artificial insemination and corresponding pregnancy rate per timed artificial insemination following ovsynch program in primiparous Holstein cows

Circulating progesterone at timed artificial insemination				
Quartiles of progesterone	Q1	Q2	Q3	Q4
Value (ng/ml)	0.20	0.60	1.7	7.40
Number of cows inseminated	10	17	11	7
Number of cows Pregnant	7	7	3	1
PR/TAI (%)	70.00[a]	41.18[a]	27.27[ab]	14.29[b]

Q: Quartile; **PR/TAI:** Pregnancy rate per timed artificial insemination

Table 3: Ultrasonographic measurements of CL and pre-ovulatory follicle in primiparous Holstein cows synchronized with ovsynch with or without propylene glycol drenching

	Size of CL at PGF (cm)			Size of POF at TAI (cm)		
	Control	**Prop Gl**	**Overall**	**Control**	**Prop Gl**	**Overall**
Mean	1.54	1.49	1.51	1.43	1.48	1.46
SD	0.51	0.42	0.46	0.33	0.41	0.38
SEM	0.11	0.08	0.07	0.07	0.08	0.06

PGF: Prostaglandin F2 alpha; **POF:** Pre-ovulatory follicle; **TAI:** Timed artificial insemination; **SD:** Standard deviation; **SEM:** Standard error of mean; Prop Gl: Propylene glycol

Table 4: Ultrasonographic measurements of pregnant and non-pregnant primiparous Holstein cows synchronized with ovsynch

Item	Pregnant	Non-pregnant	Chi squared	P value
CL size at PGF (cm)	1.51±0.35	1.51 ±0.52	0.004	0.97
POF size at TAI (cm)	1.58±0.25	1.39±0.27	2.35	0.02

Data expressed as mean±SD

PGF: Prostaglandin F2 alpha; POF: Pre-ovulatory follicle; TAI: Timed artificial insemination.

Discussion

Success of the implementation of estrus and ovulation synchronization programs among dairy cows depends on achievement of satisfactory PR/TAI. To achieve acceptable pregnancy figures in timely inseminated cows, all aspects related to synchronization protocol such as timely dosing of hormonal injections, careful application of the insemination technique, considerations regarding condition score and energy balance status of submitted cows.

In the current study, supplementation of PrGl to primiparous early lactation dairy cows during the first 30 days postpartum did not influence their BCS significantly on day 60 at the beginning of ovsynch. In addition, the dietary manipulation did not cause a significant improvement in PR/TAI to ovsynch protocol, despite higher figures achieved by PrGl-drenched cows. As mentioned earlier in the introductory section in that study, no available literature exists regarding effects of PrGl drenching during the first month after calving on the response of primiparous Holstein cows to ovsynch. In our study we hypothesize that PrGl as an energetic source might reduce the severity of postpartum NEB and condition loss in treated cows and consequently a better response would be expected in these cows. However, findings did not prove this hypothesis. Albeit, numerically higher PR/TAI were recorded for drenched cows (40 vs. 35%). Positive effects of PrGl drench on pregnancy in dairy cows have been previously reported (Myioshi *et al.*, 2001; Hidalgo *et al.*, 2004). Nevertheless, research work deciding absence of any effects of PrGl on fertility and pregnancy in cows should be considered (El-Kasrawy *et al.*, 2020). The discrepancy might be due to differences between studies in the dose of PrGl, the method of application, the duration of supplementation, existence of another supplement as glycerol as reported by El-Kasrawy *et al.* (2020).

Findings from the present study revealed that lower progesterone concentrations at TAI were associated with higher PR/TAI in primiparous Holstein cows. These results coincide with the results obtained by Patricia *et al.* (2016) who mentioned that dairy cows with circulating progesterone less than 0.5 ng/ml at insemination achieved greater pregnancy rates than cows presenting concentrations higher than 0.5 ng/ml. Further, Ghanem *et al.* (2006) argued that increased progesterone concentration at the time of artificial insemination coupled with a delayed rise of progesterone post-insemination might lead to decrease in fertility of dairy cows. The lower PR/TAI in cows with high progesterone may be due to the negative impacts of higher progesterone levels on synchrony of peri-ovulatory endocrine events leading to abnormal fertilization and impaired early embryonic development (Patricia *et al.*, 2016).

In this study, drenching of PrGl to primiparous Holstein cows for one month postpartum did not affect CL size at day seven of ovsynch and POF size at TAI as determined by ultrasonography. Moallem *et al.* (2007) found that PrGl drench did not influence the pattern of development of ovarian follicles, however, they studied follicular dynamics during the early postpartum period. In spite of that, drenching of PrGl to embryo recipient dairy cows increased CL general quality and progesterone production as reported by Hidalgo *et al.* (2004).

Regardless of treatment, the CL size at PGF2 alpha was not associated with variation of PR/ET following ovsynch, nevertheless, size of POF at TAI was significantly higher in pregnant cows than in non-pregnant cows. Several studies describe the relationship between CL size, progesterone production and risk of pregnancy in dairy cattle. Hidalgo *et al.* (2004) reported higher progesterone with increased CL quality. Meanwhile, McArt *et al.* (2010) found that neither CL size, nor serum progesterone levels were associated with pregnancy rate for first or second service after ovsynch protocol. The positive association observed between PR/TAI and size of POF in synchronized heifers in this study matches the results obtained by Perry *et al.* (2005). Those authors observed that GnRH-induced ovulation of follicles less than 11 mm in diameter resulted in decreased pregnancy and increased late embryo mortality. The same authors attributed this finding to reduced estradiol production by the follicle on day of TAI, slower rise and lower rate of progesterone secretion after ovulation.

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