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Influence of propylene glycol on conception rate in cows

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ABSTRACT

The aim of the study was to evaluate effect of propylene glycol (PG) drenched during the first 7 days after fixed-time artificial insemination (FTAI) on plasma progesterone (P_4) and insulin levels, and on pregnancy rate in Holstein cows in summer. Ovsynch program was performed in 40 healthy Holstein cows. Cows were distributed randomly into two groups as propylene glycol (group PG) (20) and control group (group C) (20). Group PG and group C were daily administered with 500 ml of PG and water , respectively for 7 days from day 0 to 7 post insemination. Pregnancy diagnosis was carried out 60 days after insemination via rectal examination. Cows exposed to moderate heat stress due to temperature-humidity index (74.4 ± 1.9 mean THI). Conception rate for cows in group PG increased 2.9 fold when was compared with group C. On day 7, the insulin levels were higher in group PG than group C. It was concluded that PG administered once daily from day 0 to 7 after FTAI increased insulin and P_4 concentrations and the conception rate in Holstein cows. The rise in insulin and P_4 levels depending on the drenched PC are likely related with the increases of conception rate.

Key words: Fertility, Heat stress, Insulin, Progesterone, Propylene glycol

Fertility in lactating cows is decreased during the hot seasons of the year. Summer-calved cows had greater number of services per conception and lower conception rates than cows calved in other seasons (Ghavi Hossein-Zadeh *et al.* 2013). The lactating cow at an air temperature of 30°C is at risk of infertility because of heat stress (Peter 2007). Heat stress has two major effects on reproduction. First, heat stressed cows demonstrate estrus less highly than other cows. Secondly, conception rate is decreased during heat stress (Hansen 2007). Thatcher (1974) showed that conception rates are depressed at environmental temperatures above the critical temperature of 21 °C. Heat stress affects the development and the function of the corpus luteum and causes a decrease in P_4 levels (Ullah *et al*. 1996). Therefore, the FTAI and various hormonal protocols to improve the use of artificial insemination are widely used in the dairy farms to prevent the negative effects on reproductive performance of high temperature environment. However, the ability of these strategies to improve the fertility is limited. Use of FTAI protocols can eliminate problems of estrus detection that heat stress caused. But this was not adequate to reinstate herd pregnancy rates to a level seen in cool weather due to the serious outcomes of heat stress for embryogenesis (Hansen and Are´chiga 1999). Hence, alternative techniques to FTAI protocols that improve pregnancy rates for a systematic breeding system should be developed. Leroy *et al*. (2006) showed that cleavage rate and blastocyst development were severely reduced *in vitro* in a low glucose environment vs. a physiologically normal glucose environment. The use of glycogenic supplements during the estrous cycle increased the synthesis of P4. Oral drenching of PG raises insulin levels which affect the developing corpus luteum directly. (Miyoshi *et al*. 2001). Insulin is required for maintenance of P_4 synthesis, which eases lipoprotein using in bovine luteal cells (Poff *et al*. 1998). Luteal function and conception rate improves in cows treated with insulin subcutaneously (Selvaraju *et al*. 2002). Propylene glycol is a substance used in prevention and treatment of ketosis in dairy cows (Rakkwamsuk *et al.* 2010). There are studies where oral drenching of PG has an increased conception rate in cows (McArt *et al*. 2012, Slobodanka *et al*. 2012). However, other groups have reported no differences in the success of pregnancy at first artificial insemination for cows drenched with PG (Chagas *et al*. 2007, Miyoshi *et al*. 2001). Hidalgo *et al.* (2004), using PG for embryo transfer, found that the group of cows treated had higher 60–day pregnancy rates than the control group. Propylene glycol improved *in vitro* embryonic development rate (Ponsart *et al.* 2014). However, the results of using PG are controversial for the success of conceiving and need more investigation. Thus, it can be speculated that PG drenched in the first seven days post insemination are useful for the establishment of pregnancy by increasing plasma P_4 and insulin concentrations in dairy cows during the hot season. Therefore, the present investigation was undertaken to evaluate the effect of PG

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drenched during the first seven days after FTAI on plasma P_4 and insulin levels, and on conception rate in Holstein cows during summer.

MATERIALS AND METHODS

Study area, animals and treatments: The study was conducted on 40 healthy Holstein cows at a private dairy farm located in Elazig province of Turkey in summer season. Elazig region is between the latitude of 38º 40'N and longitude of 39º 13'E, at an altitude of 1,093 m. Ambient temperature (AT) (°C) and relative humidity (RH) were obtained from reports prepared at Turkish State Meteorological Service. The mean temperature was 29.4 ± 0.5°C with in the period of the experiment. The relative humidity is 29.7 ± 2.3%. The temperature and humidity index (THI) was obtained using the formula: THI=(0.8×AT+(RH%/100)×(AT"14.4)+46.4) (Thom 1959). Cows with detectable puerperal complications following calving were not included in the experiment. The body condition score (point scale from 1 to 5) were 2.5–3.0 units. The mean body weight was 517.0 ± 34.57 kg. The parity of cows ranged between 2 and 3. During the experiment, all the animals were kept under the similar feeding and managemental conditions. Average daily milk production for the farm was between 20 to 23 kg/cow during the study period. Lactating cows were milked twice daily. All cows were examined by rectal palpation and vaginoscopic examination at week 6 post-partum. There were no clinically detectable disorders in their reproductive tracts. On 45th day of lactation, Ovsynch protocol was done with the administration of GnRH (Busereline acetate). The administration of PGF2α analogue D- Cloprostenol (25 mg, Dinoprost tromethamine) was done on day 8 after first administration and second GnRH administration was on day 10 after first administration. Cows were artificially inseminated after 18h following second GnRH administration. At insemination (day 0), cows were distributed randomly into two groups as group PG and control group (group C). The randomization was stratified by parity, body weight and BCS. Propylene glycol (group PG) and water (group C) were supplied via drench in a volume of 500 ml once daily from day 0 to 7 post insemination. The diagnosis of pregnancy was done on 60th day after insemination via rectal examination.

Sample collection and biochemical assays: In all animals, blood samples to be assayed for P4 and insulin were obtained from the jugular vein using heparinized vacutainer tubes just before the application on day 0 (oestrus) and one hours after the administration of PG or water on day 7. Samples were later centrifuged at 1,500 × g for 15 min for plasma separation, which was then stored at –20 °C until being analyzed. Insulin concentrations were measured by RIA in solid phase, using the insulin kit, having a 0.05 ng/ml sensibility and a 9.5% intra assay variation coefficient. Plasma P4 concentration was determined using a commercial solid-phase RIA kit. The sensitivity of the assay was 0.1 ng/ml and the intra assay CV was 5.6%.

Statistical analysis: The conception rates of groups were compared with the Odds ratio (OR). The reference category had an OR = 1. An adjusted OR > 1.0 indicates that the probability of the increased conception rate because of the treatment was, compared with cows in the reference category. Insulin and P_4 concentrations were compared using a *t*-test to determine the effects of treatment. Results were considered statistically and declared significant at $P < 0.05$.

RESULTS AND DISCUSSION

Mean values of THI were equal to 74.4 ± 1.9. Cows in the study exposed to moderate heat stress according to the temperature-humidity index (74.4 ± 1.9 mean THI).

Plasma concentrations of insulin and P4 on Day 0 and Day 7 are presented in Table 1. On day 7, the insulin levels were higher in group PG ($P < 0.05$) than group C. The insulin levels on Day 7 for cows in group PG were statistically higher compared to ones on day 0 (before treatment) ($P < 0.05$). In the current study, we obtained that drenching of PG increased insulin plasma concentrations, which is in accordance with other previous studies that reported an increase in the concentrations of insulin following administration of PG (Christensen *et al*. 1997, Miyoshi *et al*. 2001). Propylene glycol may stimulate insulin secretion directly (Studer *et al*. 1993). Likewise, it is possible that the PG stimulates pancreatic insulin secretion indirectly by PG metabolizes to propionate (Webb *et al*. 1999).

The average P4 concentration was higher ($P<0.05$) in group PG when compared with group C on Day 7 after insemination. Plasma P_4 concentrations increased significantly on day 7 after PG drenching. This was similar to the findings of Miyoshi *et al*. (2001) who found a significant rise in milk P_4. Elevation of insulin by PG drenching affects the developing corpus luteum directly (Miyoshi *et al*. 2001). Insulin is a requisite to maintain P_4 synthesis and ease lipoprotein using in bovine luteal cells (Poff *et al.* 1998). Cows treated with insulin subcutaneously display rises in P_4 concentrations (Selvaraju *et al*. 2002). Likewise, insulin-like growth factor-I (IGF-I) stimulates P_4 production by cultured bovine luteal cells (Chakravofty *et al.* 1993). Also, drenching of PG is known to rise IGF-I

Table 1. Mean levels and standard deviations (±SD) of plasma progesterone (P_4) and insulin concentrations for cows in group C and group PG

Parameters	Days	Group C n=20	Group PG n=20
Progesteron	0	0.67 ± 0.19^{A}	0.69 ± 0.17^{A}
(ng/mL)	7	2.89 ± 0.37^{Ba}	3.38 ± 0.41^{Bb}
Insulin	0	15.69 ± 0.43	15.63 ± 0.51^{A}
(ng/mL)	7	14.78 ± 0.37^{a}	19.87 ± 0.59^{Bb}

^{a,b}Values with different superscript letters within a row differ significantly at $P < 0.05$. ^{A,B}Values with different superscript letters within a column differ significantly at $P < 0.05$.

Table 2. The conception rates of cows in group C and group PG

Groups	n	Conception rate (%,n/n)	Odds ratio	95% Confidence interval	P values
Group C	20	30.0 (6)	Referent		
Group PG	20	55.0 (11)	2.9	0.78 to 10.47	P =0.11

(Grummer *et al*. 1994). Change in insulin is closely associated to IGF-I levels (O'Callaghan and Boland 1999). In a direct way, IGF-I stimulates corpus luteum growth and steroidogenesis (Alvarez *et al*. 2000). That's why, P_4 levels could raise in reply to insulin and IGF-I, whose levels also would be more elevated after a PG drenching.

Mean conception rate at first insemination was 55.0% (11/20) in group PG and 30.0% (6/20) in group C (Table 2). Cows in group PG had 2.9 times more conception rate than control ones. Supplementation of PG from day 0 to day 7 improved conception rate at a rate of 25.0%. The present finding is in accordance with León *et al*. (2010) who also recorded higher conception rate in Holstein cows cured with a glycogenic diet (glycerol). Hidalgo *et al*. (2004) observed that the group of cows treated using mono propylene glycol for embryo transfer had higher 60-day conception rates than group C. The mechanism through which PG act on conception rate has been completely understood; nevertheless, it could be stated by the effects of insulin and P_4 on early embryo development (Block *et al*. 2011). Due to the fact that adding insulin to a culture media promotes embryo cell mitosis and the ratio of embryos reaching the blastocyst stage (Augustin *et al*. 2003). Therefore, concentrations of tau interferon (IFN-t) were higher in cows fed regimes prepared to create elevated insulin levels (Mann *et al.* 2003). Likewise, the increased P_4 levels during the early stages of pregnancy may contribute to a progress of embryonic development causing to a raised level of IFN-t production and a related rise in conception rate (Morris and Diskin 2008). The inadequate IFN-t synthesis is one of the reasons of conception failure. (Diskin and Morris 2008). Therefore, PG improves embryo capacity to prompt maternal recognition of pregnancy, which influences embryo health and ability to establish pregnancy and results in an increase in conception rate. In addition, P_4 has an important role in regulating changes in the uterine environment for supporting early embryonic growth and development. Early P_4 stimulation changes endometrial secretions and proceeds to development of conceptus (Butler 2003).

In conclusion, the results of the present study indicate that drenching of PG at a dose rate of 500 ml/day from days 0 to 7 after insemination increases insulin and P_4 concentrations and the conception rate in Holstein cows. The rise in insulin and P_4 concentrations after drenching PG leads to increase in the conception rate. This application can provide a contribution to improve low conception rates due to moderate heat stress occurred especially in the summer.

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