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Effect of Intravenous Infusion of Hypertonic Glucose on the Treatment of Cows with Postpartum Anestrus

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Abstract. The objective of this study was to evaluate the efficacy of intravenous infusion of hypertonic glucose for recycling of the cows with postpartum anestrus. One hundred true anestrus Holstein–Frisian cows with ≥ 60 days in milk (DIM) according to the ultrasound examinations were selected from a dairy herd located on the suburb of Tabriz. Anestrus cows randomly were allocated into two groups: A&B (n=50). In group A (Gluco–Select synch), cows received 500 mL hypertonic glucose for 3 consecutive days (–3 to 0) IV, 0.021 mg bucerelin acetate on the day 0, IM followed by 25 mg dinoprost tromethamine IM on the day 7. In group B (Select synch), the same protocol was carried out on the cows except that the glucose hypertonic solution replaced by normal saline as placebo. Thereafter all cows were detected for estrus signs for the next 5 days and inseminated based on *visual* signs. Twenty seven out of 50 cows (54 %) in group A and 8 out of 50 cows (16 %) in group B showed the estrus signs respectively. Conception rates were recorded as A=44.5 %, B=37.5 % for the first insemination and A=B=100 % for the second insemination of cows. The number of insemination per pregnancy was calculated as 1.55 and 1.62 for groups A and B respectively. In the case of estrus rate, the difference between two groups was statistically significant (P≤0/01), but in the cases of conception rate or the number of insemination per pregnancy rate the differences were not statistically significant.
Keyword: Anestrus, Cow, Glucose, Hypertonic, Postpartum.

Introduction

Postpartum anestrus is one of the most important reproductive problems in the dairy cattle industry worldwide including Iran [HADUSH *et al.*, 2013]. This period plays an important role in cattle reproduction [PETER *et al.*, 2009]. In dairy cattle, for economic reasons postpartum anestrus should not be longer than 65 days [OPSOMER *et al.*, 2000]. The metabolic disorders caused by lactation that includes low blood glucose and high NEFA and BHBA affects the time of ovarian cyclicity which in turn has important role in determining of calving interval period in the dairy herds.

During the recent years selection for high milk producing cows has led to a negative energy balance in postpartum period and decreases in reproductive performances. This feature can be lead to increased incidence of metabolic and reproductive disorders such as ketosis and postpartum anestrus [OPSOMER *et al.*, 1996; LUCY, 2001]. Metabolic disorders cause of reduced concentrations of reproductive hormones and nutrients such as progesterone (P4), estradiol (E2), insulin and glucose [SANGSRITAVONG *et al.*, 2002; BUTLER, 2005]. The decrease in blood glucose causes to an endocrine and metabolic disorder that affects estrous cyclicity, uterine health, and subsequent pregnancy [LUCY *et al.*, 2014]. Bicalho and collab. found that plasma glucose concentration to be associated with the postpartum uterine diseases, whereas BHBA and NEFA were not associated with the occurrence of any uterine disorder [BICALHO *et al.*, 2017].

Garverick and collab. showed that cows which became pregnant after first artificial insemination (AI) had greater blood concentrations of glucose when compared with cows that did not become pregnant [GARVERICK *et al.*, 2013].

Moreover they indicated that correlation between greater blood concentrations of glucose on day 3 postpartum and the first service conception rates were significant in dairy cows [GARVERICK *et al.*, 2013].

Glucose is a critical nutrient in the postpartum cow because it is an important component of cow's milk and also has a profound effect on the hypothalamo–hypophysial axis controlling ovarian cyclicity [LUCY *et al.*, 2014].

Many researchers reported that during postpartum anestrus the concentration of glucose in cow's circulation is in minimum level [GARVERICK *et al.*, 2013, CERNEA, *et al.*, 2015, SAMFIRA, *et al.*, 2015].

Therefore, cow's circulatory system tries to elevate blood glucose by some mechanisms. In addition to a large increase in hepatic gluconeogenesis in the postpartum period, it is hypothesized that in this period cows have a state of insulin resistance that redirects glucose to non–insulin–dependent tissues like mammary gland. Despite these pathways, cows after parturition usually have a low level of circulating glucose because of limited sources of available glucose.

It plays an important role in animal metabolism through its effect on the concentrations of insulin and IGF1 in the plasma [LUCY, 2008, FERENCZ, *et al.*, 2012, BUTU, *et al.*, 2014]. Insulin released in response to elevating concentrations of glucose in the circulation and increases the permeability of adipose tissue and muscle to nutrients. In response to insulin stimuli IGF1 is released into the plasma by the hepatocytes [BUTLER *et al.*, 2003].

When the concentration of glucose in the plasma is in the minimum level, insulin and IGF1 is not secreted in the normal level, and cows remain in a negative energy balance state during the peak of lactation.

Glucose deficiency is only corrected when cow produces less milk or receive extra glucose by IV infusion.

In this situation, cow enter a positive energy balance phase, blood insulin and IGF1 increase, and extra glucose absorbed by cow's adipose tissue and muscle. Changing from a negative energy balance state to a positive energy balance is an essential step for resumption of ovarian cyclicity [KAWASHIMA *et al.*, 2012].

According to above mentioned researches, we supposed that IV glucose infusion may increase of insulin and IGF1 secretion and reduces hepatic clearance of progesterone in anestrus cows. Progesterone is an essential hormone for ovarian activity and a successful pregnancy [SPENCER and BAZER, 2002; LOOPER *et al.*, 2003].

The aim of this study was to evaluate the effect of intravenous infusion of hypertonic glucose on the resumption of ovarian cyclicity and estrus onset in the cows with postpartum anestrus.

Material and methods

Hormonal drugs and solutions

Hypertonic glucose (Glucojet 50 mg glucose/mL, 500 mL/cow, manufactured by Zoofa–Parnian Co. Iran, bucerelin acetate (Vetocept, Each mL of drug contains 0.0042 mg buserelin acetate manufactured by Abu–Reihan Pharmaceutical Company, Iran), dinoprost tromethamine (Vetalyse, Each mL of drug contains 5 mg Dinoprost Tromethamine, manufactured by Abu–Reihan Pharmaceutical Company, Iran and normal saline (NaCl 0.9 %, 500 mL/cow, manufactured by Shahid Ghazi Pharmaceutical Co. Iran).

Location and Animals

This study was carried out in a dairy herd with 520 head Holstein–Friesian cows with mean milk production of 29.5 kg/day, located in the suburb of Tabriz, Iran (38° 07' N and 46º 29' E) from January 2014 to November 2016.

Temperature during the experiment ranged from 0 to 30° C with annual rainfall in this region ranges from 226 to 250 mm. Multiparous cows at least 60 days in milk (DIM) were included. All cows had the same management and nutrition (TMR) program and were fed by corn silage, hay, soybean and complementary minerals. They were housed in open shed barns, milked 3 times a day and estrus cows were inseminated artificially by an expert technician of farm.

Methods

One hundred anestrus cows with minimum 60 DIM and body condition score of 2.5–3 (based on a 1 to 5 score) were randomly assigned to Gluco–Select Synch (group A, n=50) or Saline–Select Synch (group B, n=50) based on the ear–

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tag numbers. According to clinical and ultrasound examinations selected cows had not functional ovaries at two consecutive examinations with 10 days interval (true anestrus).

Cows with health-related problems such as laminitis, mastitis, endometritis or cystic ovaries diseases were replaced by other anestrus animals. Cows in Gluco–Select Synch group (Treatment) received 500 mL hypertonic glucose from d –3 to d–0 IV, bucerelin acetate 0.021 mg on d–0 IM, followed by 25mg dinoprost tromethamine IM on d–7.

Cows in Sal–Select Synch group (Control), received the same hormones plus 500 mL normal saline IV as placebo. Glucose or saline were administered via subcutaneous abdominal vein of cows by a hypodermic needle, 18 gauges over a period of 1 h (on average 8.3 mL/min).

Needles were removed after infusion was finished. Thereafter all cows were detected for estrus signs (4 times a day: at 2, 8, 12 and18 o'clock) for the next 5 days and inseminated at observed estrus according to AM–PM rule by the technician of farm.

Data collection

The number of cows within two groups which responded to the treatment (%), the period of time between treatment and animal response (h), conception rates and the number of insemination per pregnancy were recorded.

Statistical Analysis

Independent sample t–test and Chi–square were used (by using SPSS software version 22, Inc., Chicago, IL, USA) for analysis of data in the present study. Statistical differences were considered significant when $P<0.05$ and trends are discussed when $P<0.01$.

Results and discussion

Of 50 cows in Gluco–Select Synch group (A), 27 cows (54 %) showed estrus signs and were inseminated by the farm inseminator, whereas only 8 cow (16 %) out of 50 cows in Sal–Select Synch (B) group displayed estrus behavior and were inseminated by the same person (Table 1 & Figure 1).

Table 1.

Rates of responsiveness and time required for onset of estrus in cows after treatments with hypertonic glucose or normal saline (hours)

Group	N	Overall time to response (h)	Number of cows responded
Gluco–Select Synch (A)	50	73.50±1.27	27 (54%)
Sal–Select Sync (B)	50	75.50±2.65	8 (16%)

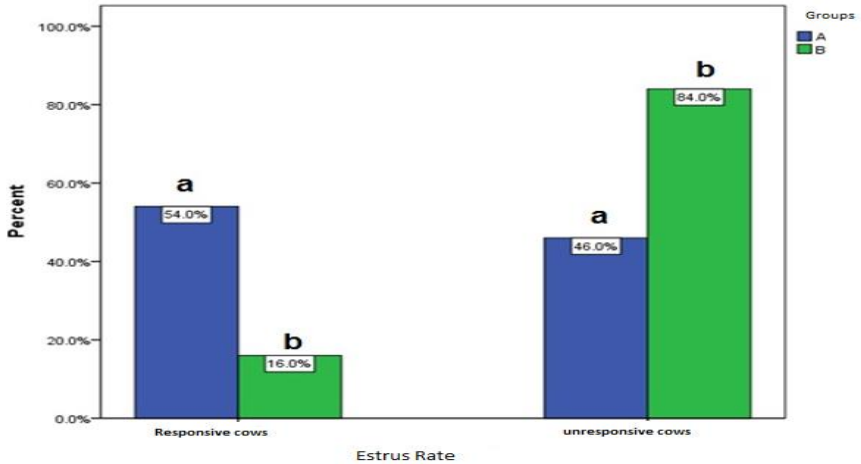

Figure 1. The rates of responsive or unresponsive cows in two groups. (Different letters indicate statistically significant difference between groups).

The difference between two groups was significant ($p \leq 0.01$).

Overall period between injections of $PGF_2\alpha$ to induction of estrus in cows was (73.50 ± 1.27) and (75.50 ± 2.65) hours in groups A and B respectively (Table 1).

No significant differences were found between the two groups for overall period from injection of PGF2α to onset of

estrus in cows (p≥0.05). Of 27 cows inseminated in Gluco–Select Synch (A) group 15 cows (55.6 %) and in group Sal–Select Sync (B), 5 out of 8 cows (62.5 %) returned to estrus and were inseminated again (Figure 2). No significant differences were found between the two groups for the rates of return or non–return to estrus of cows (NRR) (p≥0.05).

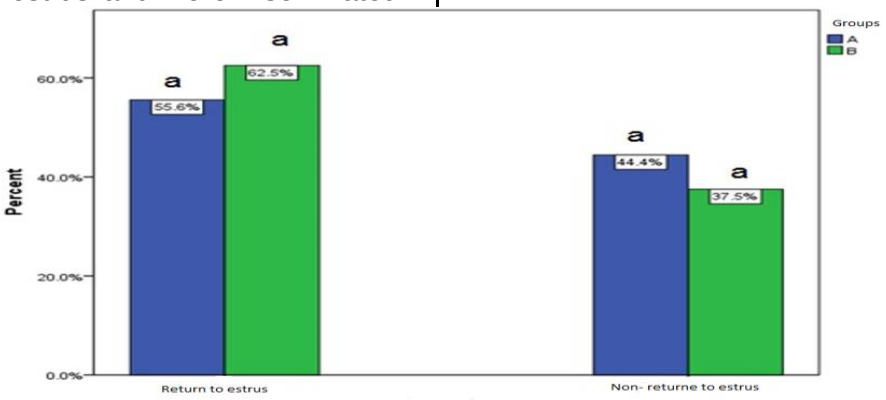

Figure 2. The non–return rate (NRR) or return rates of cows in two groups. (Groups that share the same letter are not significantly different)

After 30 days of second inseminations, pregnancy of cows examined by ultrasound and fortunately all of cows were pregnant. First service conception rate, second service conception rate and number of services per conception (NSC) were calculated (Table 2). No significant differences were observed between the two groups for above mentioned items (p≥0.05).

Table 2.

First service conception rates, second service conception rates and number of services per conception (NSC) in two groups: A (Treatment) and B (Control)

Group	First service conception rate	Second service conception rate	Number of services per conception (NSC)
Gluco–Select Synch (A)	44.4%	100%	1.55
Sal–Select Sync (B)	37.5%	100%	1.62

Postpartum anestrus is referred to all cows that not seen in heat 60 or more days after parturition and the term of true anestrus is used for cows which have no functional structures (such as follicle, corpus luteum or cysts) on their ovaries [OPSOMER *et al.*, 2004].

Extended postpartum anestrus period in dairy cattle leads to increased calving interval in herd that means decreased average milk production per cow per day, fewer calves born per year and less profitability for the herd.

Many researchers tried to treat postpartum anestrus cows by different methods including: CIDR insertion, Ovsynch protocol, progesterone injection, nutritional management or energy balances [McDOUGALL 2001; MOYOSHI *et al.*, 2001; OPSOMER *et al.*, 2000; RABIEE *et al.*, 2001; STAPLES *et al.*, 1998; MOZAFFARI *et al.*, 2014; BUTLER *et al.*, 2006] but very rare studies focused on intravenous infusion of hypertonic glucose for recycling of the anestrus cows [VIEIRA *et al.*, 2010; McCEUCSEV *et al.*, 1988; CAMPBELL *et al.*, 2010].

Some of authors reported that a great part of postpartum anestrus cows suffering from hypoglycemia and chronic ketosis which it has adverse effect on the hypothalamo–hypophysial axis controlling ovarian cyclicity [GARVERICK *et al.*, 2013].

Our results in agreement with the Garverick and Veena's findings that showed significant correlation between blood glucose, cholesterol and BUN with first postpartum heat [VEENA *et al.*, 2015], because infusion of hypertonic glucose caused the significant increase in the number of anestrus cows coming into heat. Also our results in agreement with Butler's research which indicated that feeding postpartum anestrus cows with

glycogenic substrates, such as propylene glycol had increased plasma concentrations of insulin and ovarian follicular growth rate [BUTLER *et al.*, 2006].

Insulin is a metabolic mediator between nutrition and cow's reproduction, and monitors fertility by affecting on reproductive hormones synthesis such as LH, estrogen and progesterone and follicular growth [DISKIN *et al.*, 2003; WEBB *et al.*, 2004]. Therefore, every nutrient which could make elevation in circulating insulin concentrations (such as glucose) will cause shortening in postpartum interval to first estrus and ovulation [LEMLEY *et al.*, 2008].

Our results in contrast with Vieira's research which suggested that the effects of IV glucose infusion on insulin concentration in ovariectomized and not lactating cows were dependent on cow body condition score (BCS) and nutritional balance [VIEIRA *et al.*, 2010].

This difference may come from the type of cows which in our experiment they were lactating and in Vieira's study non lactating cows used for experiment.

Overall time to estrus (Table 1), non–return rate (NRR) (Figure 2), and services per conception (Table 2) were not different between groups. Conception rates to first insemination were 37.5 % in the control group and 44.4 % in treated cows, but these were not significantly different (Table 2). These results in agreement with findings of Miyoshi research which indicated that after drenching cows with propylene glycol, plasma glucose and insulin increased but there was neither increase in conception rate nor decrease in services per conception rate of cows [MIYOSHI *et al.*, 2001].

The advantage of present study is that it allows you to recycle of anestrus cows without using external steroid hormones such as progesterone which is banned in milking cows in the United States and European Union countries and also there is no need to discard milk of cows subjected to treatment.

Conclusions

Beside of present study we performed some experiment on the primiparous cows and noticed that these animals also respond to IV infusion of glucose plus Select–Synch protocol as well as multiparous cows (unpublished data). Disadvantage of our study is that for intravenous infusion of hypertonic glucose there is need for an expert technician and also restraining of cows.

Therefore, we recommended this protocol for sporadic cases or for herds that they had expert and educated personnel for IV infusions and cows handling.

In conclusion our results indicated that IV infusion of glucose in combination with Select–Synch protocol is more effective than Select–Synch protocol alone for recycling of postpartum anestrus cows especially in the cases of true anestrus cows.

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