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Research Article

Effect of recombinant bovine Somatotropin (rbST) and propylene glycol (PG) on the quantity and quality of embryos in early lactation Brown Swiss dairy cows

Efeito da somatotropina bovina recombinante (rbST) e do propilenoglicol (PG) na quantidade e na qualidade dos embriões de vacas leiteiras da raça suíça castanha no início da lactação

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ABSTRACT

This study aimed to evaluate the effects of recombinant bovine somatotropin (rbST) and propylene glycol (PG) on embryo production in early-lactating dairy cows. Thirty Brown Swiss cows were randomly assigned to three groups: control (n=10), PG (n=10), and rbST (n=10). All animals underwent a superovulation protocol. On Day 0, cows in the PG and rbST groups received 200 ml of PG (RUMINIL®, Montana) and 500 mg of rbST (BOOSTIN-S®, Battilana), respectively. Additionally, on Day 0, all cows were administered an intravaginal progesterone-releasing device (1200 mg DISPOCEL®, Von Franken), 1 mg of estradiol benzoate, and 40 mg of cloprostenol intramuscularly. On Day 4, they received 350 mg of FSH (Folltropin® - Vetoquinol). On Day 7, the device was removed, and 40 mg of cloprostenol was administered. On Day 9, artificial insemination was performed. On Day 16, embryos were collected and evaluated. ANOVA results indicated that cows in the PG group exhibited a higher number of recovered structures, recovered embryos, and unfertilized structures compared to the control and rbST groups. The rbST group showed the highest number of viable embryos (quality 1 and 2). The PG group also had the highest number of morulas and blastocysts, while the rbST group presented the highest number of early blastocysts. Additionally, rbST administration resulted in a 19.3% and 18.3% increase in serum glucose concentrations (mg/dL) ($p < 0.05$) and milk production ($p < 0.05$), respectively compared to both the PG (16.7%, 12.5%) and control groups (10.6%, 3.4%). The inclusion of PG in the superovulation protocol enhanced the number of embryos, whereas rbST improved embryo quality and developmental stage.

KEYWORDS: Embryonic development. Cows. rbST. Fertility. Perú.

RESUMO

Este estudo teve como objetivo avaliar os efeitos da somatotropina bovina recombinante (rbST) e do propilenoglicol (PG) na produção de embriões em vacas leiteiras em lactação precoce. Trinta vacas da raça suíça parda foram distribuídas aleatoriamente por três grupos: controle (n=10), PG (n=10) e rbST (n=10). Todos os animais foram submetidos a um protocolo de superovulação. No Dia 0, as vacas dos grupos PG e rbST receberam 200 ml de PG (RUMINIL®, Montana) e 500 mg de rbST (BOOSTIN-S®, Battilana), respectivamente. Além disso, no Dia 0, todas as vacas receberam um dispositivo intravaginal de liberação de progesterona (1200 mg DISPOCEL®, Von Franken), 1 mg de benzoato de estradiol e 40 mg de cloprostenol por via intramuscular. No dia 4, receberam 350 mg de FSH (Folltropin® - Vetoquinol). No 7º dia, foi retirado o dispositivo e administrado 40 mg de cloprostenol. No dia 9, foi efectuada a inseminação artificial. No 16º dia, os embriões foram recolhidos e avaliados. Os resultados da ANOVA indicaram que as vacas do grupo PG apresentaram um maior número de estruturas recuperadas, embriões recuperados e estruturas não fertilizadas em comparação com os grupos de controle e rbST. O grupo rbST apresentou o maior número de embriões viáveis (qualidade 1 e 2). O grupo PG também apresentou o maior número de mórulas e blastocistos, enquanto o grupo rbST apresentou o maior número de blastocistos precoces. Além disso, a administração de rbST resultou num aumento de 19,3% e 18,3% das concentrações de glicose no soro (mg/dL) ($p < 0,05$) e da produção de leite ($p < 0,05$), respetivamente, em comparação com os grupos PG (16,7%, 12,5%) e de controlo (10,6%, 3,4%). A inclusão de PG no protocolo de superovulação aumentou o número de embriões, enquanto a rbST melhorou a qualidade e o estádio de desenvolvimento dos embriões.

PALAVRAS-CHAVE: Desenvolvimento embrionário. Vacas. rbST. Fertilidade. Perú.

INTRODUCTION

In recent years, dairy production in cows has more than doubled due to a combination of genetic improvement, nutrition, and effective management of these animals (BRITO *et al.* 2021). However, this high productivity has been accompanied by a general decrease in reproductive efficiency, with infertility being one of the main causes of economic loss in this sector (BELLO *et al.* 2012, DEKA *et al.* 2021).

ROELOFS (2023) indicates that fertility problems can occur in three phases: i) from childbirth to the first insemination; ii) from the first insemination to pregnancy; and iii) from pregnancy to delivery, with the greatest issues observed in the first two phases. In the first phase, failure to exhibit estrus may be related to the presence of ovarian cysts (which hinder sperm-egg contact) or inactive ovaries, often due to thermal stress and negative energy balance (NEB). NEB during the early postpartum period reduces conception rates and increases the incidence of early embryonic mortality (LUCY 2019b). Additionally, inactive ovaries may be linked to uterine inflammation (metritis) caused by bacteria (LIMA 2020). In the second phase, non-fertilization or early embryonic death may occur due to the aging of the egg or sperm caused by thermal stress, which deteriorates the quality of eggs and ovarian follicles (SILVA *et al.* 2023). The third phase is characterized by infectious diseases, fungi, or bacteria, although these are less common (SILVA *et al.* 2023). Therefore, recognizing fertility problems is crucial for timely treatment or prevention.

To address these issues, various strategies have been implemented, including nutritional and data management, genetic selection, control of infectious diseases, reproductive management, estrous/ovulation synchronization, application of biotechnologies, and ovulation induction protocols (ALBORNOZ *et al.* 2015, CROWE *et al.* 2018, SALES *et al.* 2019).

In Peru, cattle reproduction is conducted through embryo transfer biotechnology aimed at genetic improvement but has reported highly variable results (DURAND *et al.* 2022). The success of this biotechnology depends on nutritional status, nutrient flow, and superovulation protocols. During the postpartum period, the duration and intensity of negative energy balance (NEB) adversely affect reproductive performance (BUTLER & SMITH 1989). The production of estradiol (E2) and its growth in the first follicle are crucial for successful ovulation (MACMILLAN *et al.* 2018). Its deterioration is attributed to reduced pulses of luteinizing hormone, decreased circulating insulin, and the concentration of insulin-like growth factor I (IGF-I) (GINTHER *et al.* 2001). During the early postpartum period, increases in growth hormone (GH) and reductions in insulin concentrations facilitate milk production and the mobilization of adipose tissue. In metabolism, IGF-I together with NEB reduces the expression of the hepatic GH receptor, resulting in decreased serum IGF-I concentrations (RHOADS *et al.* 2008). Thus, cows that ovulate the first dominant follicle postpartum have higher IGF-I concentrations compared to cows with non-ovulatory follicles (RINCÓN *et al.* 2019). Growth hormone receptors (GHR) and IGF-1 are prevalent in the liver but are also present in reproductive tissues, performing functions in embryonic development and positively affecting steroidogenesis. Strategies have been evaluated to reduce the effects of NEB and increase serum IGF-I, enabling earlier postpartum ovulation and improving reproductive performance in dairy cows.

Recombinant bovine somatotropin (rbST) is applied during the postpartum period to enhance lactation persistence and milk synthesis efficiency (RAUX *et al.* 2022). During the peripartum period, rbST increases glucose and serum IGF-I concentrations and decreases beta-hydroxybutyrate and non-esterified fatty acids (FEIJÓ *et al.* 2015). rbST can act on cattle reproductive tissues through several mechanisms. It acts directly on the ovary or uterus and can also have indirect effects through IGF-I. The effects of IGF-I are widespread because IGF-I receptors are found throughout the body in various tissues and reproductive tracts, including the follicles, corpus luteum (CL), oviduct, and uterus (ACOSTA *et al.* 2017). The bovine embryo also has IGF-I receptors and is synthesized in the theca cells of the ovary, participating in follicular growth, development, and maturation (COSTA *et al.* 2014). Additionally, cows receiving rbST at insemination increase IGF-I levels in the blood during early embryonic development. Higher concentrations of IGF-I at this stage may act on the uterus or embryo to enhance embryonic development before the critical period of maternal recognition. Larger or healthier embryos in cows supplemented with rbST are typically more viable and more likely to result in pregnancy (CONSENTINI *et al.* 2021).

Propylene glycol (PG) is a glycogenic precursor used to increase the molar percentage of propionate in the rumen of dairy cattle during the postpartum period. After oral administration, a small portion of PG is metabolized to propionate, while most is converted to glucose by the liver (ZHANG *et al.* 2020).

Some studies have reported that dietary supplementation with PG increases the *in vitro* production of high-quality embryos in heifers with restricted feeding, and also increases IGF-I concentrations, gene expression for IGF-I in oocytes and cumulus cells, and selected genes for blastocysts (DEMETRIO *et al.* 2020, FERNÁNDEZ *et al.* 2020). PG also reduces plasma concentrations of non-esterified fatty acids (NEFA) and beta-hydroxybutyrate (β-HB) in early lactating cows, promoting tissue and follicle growth (BJERRE-HARPØTH *et al.* 2015). Some metabolic factors respond to glucose concentrations, including insulin, which indicates the activation of growth factors in ovarian structures such as follicles and the corpus luteum, leading to a better response to superovulation (KAEWLAMUN *et al.* 2020). Furthermore, PG may improve reproduction, although more studies are needed to confirm this. In this context, the present study aims to evaluate the effects of rbST and propylene glycol administration on embryo production in terms of quality and quantity in early lactating Brown Swiss dairy cows.

MATERIAL AND METHODS

The procedures applied in this work were previously approved by the Ethics Committee of the Faculty of Zootechnics of the National University of Central Peru.

Animals and treatments

The study was conducted between January and June 2019, involving thirty newly lactating dairy cows (90 to 110 days of lactation) of the Brown Swiss breed. The cows showed a body condition score (BCS) between 2.75 to 3.5 (on a scale from 1 to 5) and were randomly assigned to three groups: control (C, n=10), propylene glycol (PG, n=10), and recombinant bovine somatotropin (rbST, n=10). The rbST group received a subcutaneous injection of somatotropin (500 mg/cow, BOOSTIN-S®, Battilana, SP, Brazil) which was administered on day 0 of the superovulation protocol. The PG group was given 200 mL of propylene glycol (RUMINIL®, Montana, Lima, Peru) between days 0 to 16 of the superovulation protocol. The control group only followed the protocol (Figure 1). During the experimental field the climatology showed variation with rain events (13 mm to 33 mm) from January to May, and June without rain. The temperature in the experimental field ranged from 11 to 13 ºC. This experimental center reports about 50 births a year.

Superovulation protocol

This protocol was developed in the experimental center "El Mantaro" of the Faculty of Zootechnics – National University of Central Peru. All animals (semi-intensive systems) were included in this protocol. On day 0, the cows received an intravaginal progesterone delivery device (1.2 g, DISPOCEL, Von Franken, NJ, USA), 1 mg of estradiol benzoate, ® and 40 mg of cloprostenol intramuscularly. On day 4 they were given 350 mg of FSH (Folltropin® - Vetoquinol, SP, Brasil), divided into 8 decreasing doses. On day 7 the DISPOCEL® device was removed and 40 mg of cloprostenol IM was administered. On day 9, artificial insemination (AI) was performed (12-24 h after estrus). On day 16, the embryos were collected and evaluated. The entire process of the superovulation protocol is presented in Figure 1.

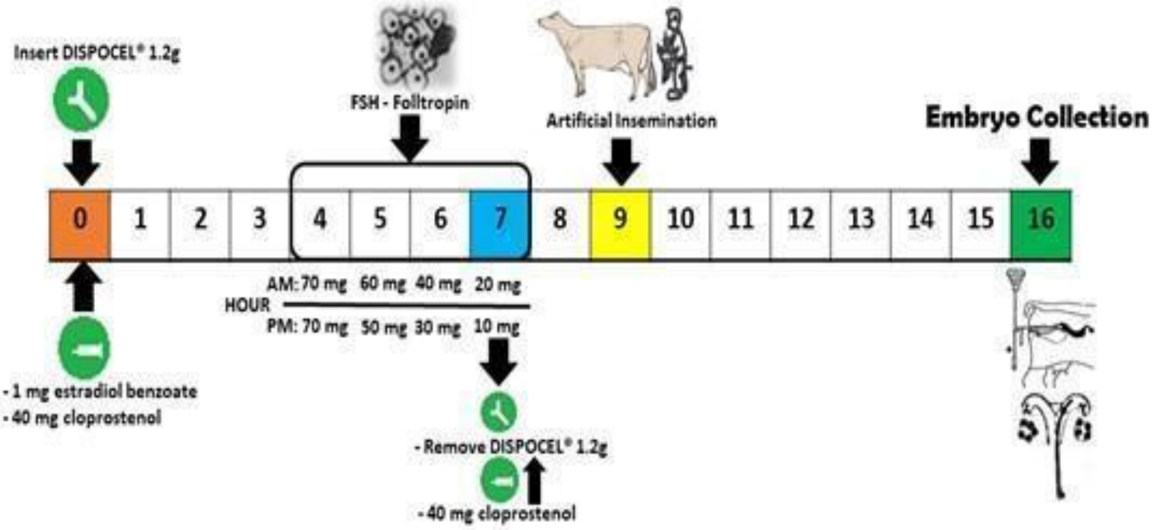

Figure 1. Timeline of specific superovulation protocol events developed in the three groups.

Bovine embryo evaluation

The contents of the wash were taken to the laboratory and passed through a filter 100 µ in diameter to separate the embryos from blood and other cellular debris. The medium used to count and classify the embryos was PBS (phosphate buffered saline) + 1% SFB (fetal bovine serum) (QUISPE *et al.* 2018). The embryos were counted and visualized using a stereo microscope (Boeco, Model BS-80, Germany) at 40X on a 35 x 10 mm plate containing the medium mentioned above.

The responses evaluated according to the quality and quantity of embryos in vivo were the total of recovered structures, total of recovered embryos, total viable embryos (quality 1 and 2), and total unfertilized/degenerated structures. Likewise, the effect of recombinant bovine somatotropin (rbST) and propylene glycol (PG) on the stage of embryo development was also evaluated (BÓ & MAPLETOFT 2013, IETS 2011)

Glucose concentration and milk production collection

For serum glucose analysis (mg/dL), blood samples were collected two hours after a meal (post prandial) from the jugular vein and subcutaneous abdominal vein every other day from day 0 to 16. The glucose concentration collected was analyzed in triplicate using a handheld glucose meter (Accu-Chek Aviva Plus, Roche, Lima, Peru).

Milk production (L/day) was recorded in each cow of the 2 daily milk shifts from day 0 to 15 of the superovulation protocol.

Statistical analysis

The response and effect variables were obtained from the superovulation protocol (VSO). These results were analyzed through descriptive statistics, and the differences between treatments (media) (control, rbST, and PG) were done by applying the analysis of variance ANOVA and subsequent Tukey test (Kruskall-Wallis), with the level of significance was 5%. All statistical treatment was performed using the free software R (R TEAM CORE 2019), version 4.2.1.

RESULTS

Superovulation Response

Table 1 presents the results of the superovulation protocol applied to lactating Brown Swiss cows, evaluating the effect of recombinant bovine somatotropin (rbST) and propylene glycol (PG) compared to a control group (C). A total of 341 structures, 228 embryos, 171 viable embryos, and 113 unfertilized or degenerated structures were recovered (Table 1).

The PG treatment group showed a higher mean number of recovered structures (11.8 ± 3.1; $p = 0.0002$) and recovered embryos (7.9 ± 3.6) compared to the C and rbST groups. No differences were observed between the C and rbST groups regarding these measures ($p > 0.05$).

For viable embryos (quality 1 and 2), the rbST group had the highest mean (6.0 ± 2.8), followed by PG (4.7 ± 2.9) and C (3.6 ± 2.0). Differences were noted between rbST and the other two groups ($p < 0.05$), but not between the control and PG groups.

The number of unfertilized/degenerated structures was lower in the rbST group (1.7 ± 1.5) compared to the PG (3.9 ± 2.0) and C (3.6 ± 2.0) groups. Differences were found between rbST and the other groups ($p < 0.05$).

Table 1. Effect of recombinant bovine somatotropin (rbST) and propylene glycol (PG) on superovulation response in embryos produced in vivo in lactating Brown Swiss cows.

Variables	N	C (n = 10)	PG (n = 10)	rbST (n =10)
Recovered Structures	341	10.2 ± 2.0b	11.8 ± 3.1a	9.3 ± 3.0b
Embryos recovered	228	6.6 ± 2.4b	7.9 ± 3.6a	7.1 ± 3.2b
Viable embryos (quality 1 and 2)	171	4.4 ± 2.0b	4.7 ± 2.9b	6.0 ± 2.8a
Unfertilized/degenerated structures	113	3.6 ± 2.0a	3.9 ± 2.0a	1.7 ± 1.5b
According to state of development				
Morula	116	4.1 ± 1.6a	4.8 ± 2.1a	2.0 ± 1.6b
Early blastocyst	73	1.2 ± 1.4a	1.9 ± 1.1a	4.1 ± 1.1b
Blastocyst	35	1.1 ± 1.2a	1.3 ± 1.0a	1.0 ± 0.4a

* [a,b]Different letters within rows indicate differences between treatments ($p<0.05$); (mean ± standard deviation).

Embryonic Development

Embryonic development, detailed in Table 1, showed the recovery of 116 morulas, 73 early blastocysts, and 35 blastocysts. No embryos in different developmental stages were found in this study.

The PG group had higher mean number of morulas (4.8 ± 2.1; p =0.002) compared to the rbST group (2.0 ±1.6; p =0.0001), without difference between the C group (4.1 ± 1.6; p =0.06) and PG. In contrast, the rbST group had a lower mean number of morulas, compared to the C and PG groups ($p < 0.05$).

The number of early blastocysts was highest in the rbST group (4.1 ± 1.1), followed by PG (1.9 ± 1.1) and C (1.2 ± 1.4). Differences were observed between rbST and the other two groups ($p < 0.05$). The number of blastocysts was similar across all treatments: C (1.1 ± 1.2), PG (1.3 ± 1.0), and rbST (1.0 ± 0.4), ($p > 0.05$).

Glucose, serum, and milk production analysis

Typically, serum glucose concentrations are associated with the administration of recombinant bovine somatotropin (rbST) and the administration of propylene glycol, which is why these two parameters were evaluated.

The mean value of the serum glucose measurements (mg/dL) made to each group (C, PG, and rbST) from day 0 to day 16, is shown in Figure 2. Figure 2 shows an increase and slight reduction (basically in the PG and rbST groups) of serum glucose between the days measured for the three groups. For example, day 2 showed an increase in glucose of 0.9% in group C, 3.2% in group PG and 11% in group PG. Likewise, the maximum increase in glucose was 19.3% (day 14) observed in the rbST group, followed by 16.7% (day 14) in the PG group, and 10.6% (day 8) in group C. The mean serum glucose measured throughout the reporting period was 62.4 ± 3.9 mg/dL for the rbST group, followed by the PG group with 58.9 ± 2.6 mg/dL and 55.7 ± 2.4 mg/dL for group C.

Analysis of variance (ANOVA) was conducted for serum glucose concentrations, and it was found that the rbST group showed higher glucose levels (62.4 ± 3.9 mg/dL) compared to the PG group (58.9 ± 2.6 mg/dL) and the control group (55.7 ± 2.4 mg/dL). The statistical analysis revealed differences ($p < 0.05$) in glucose concentrations between the rbST group and the other two groups, while no differences between PG and the control group were found ($p > 0.05$).

The effects of administration with recombinant bovine somatotropin (rbST) and propylene glycol on milk production (L/day) from day 0 to day 15 are presented in Figure 3. In general, an increase in daily milk production can be observed in the PG group (12.5%, day 9) and rbST (18.3%, day 6), with higher yields in the latter group.

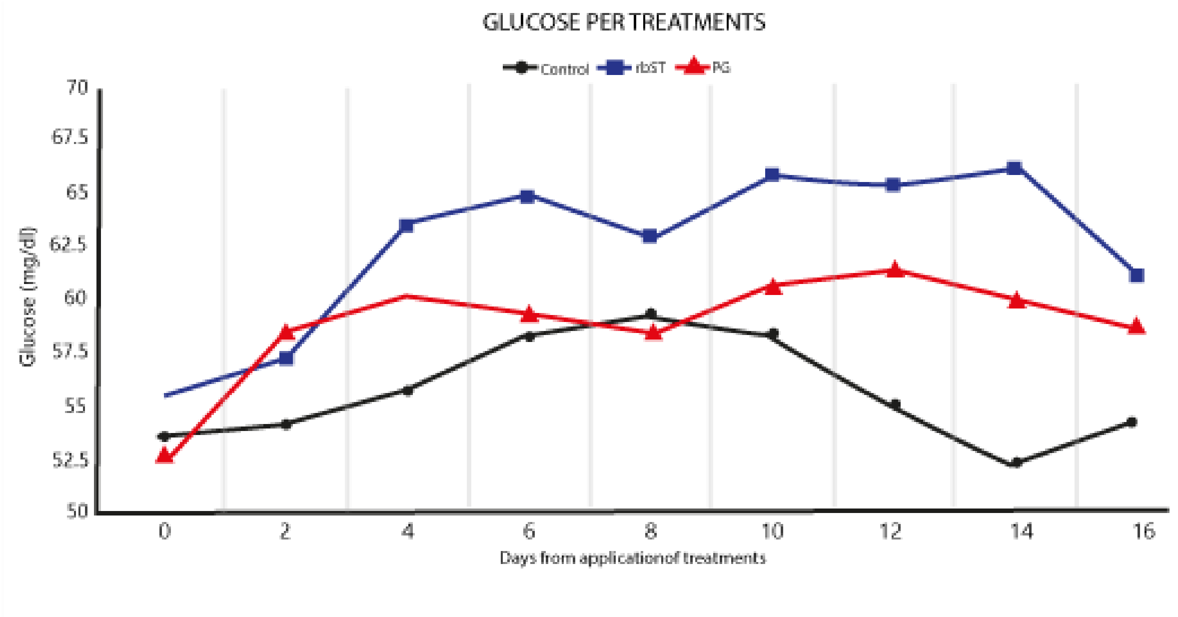

Figure 2. Glucose concentrations (mg/dL) from day 0 to 16 of the superovulation protocol for the recombinant bovine somatotropin (rbST), propylene glycol (PG), and control (C) groups.

The daily milk yield of the control group (C) remained almost constant on most days sampled, with a maximum increase of 3.4% (day 12). There were no differences ($p>0.05$) between group PG and group C, but there was a difference ($p<0.05$) between these two groups and group rbST. The highest milk production throughout the period was obtained in the rbST group (15.6 ± 1.0 L/day), followed by the PG group (14.4 ± 0.5 L/day), and finally by the group C (14.0 ± 0.7 L/day).

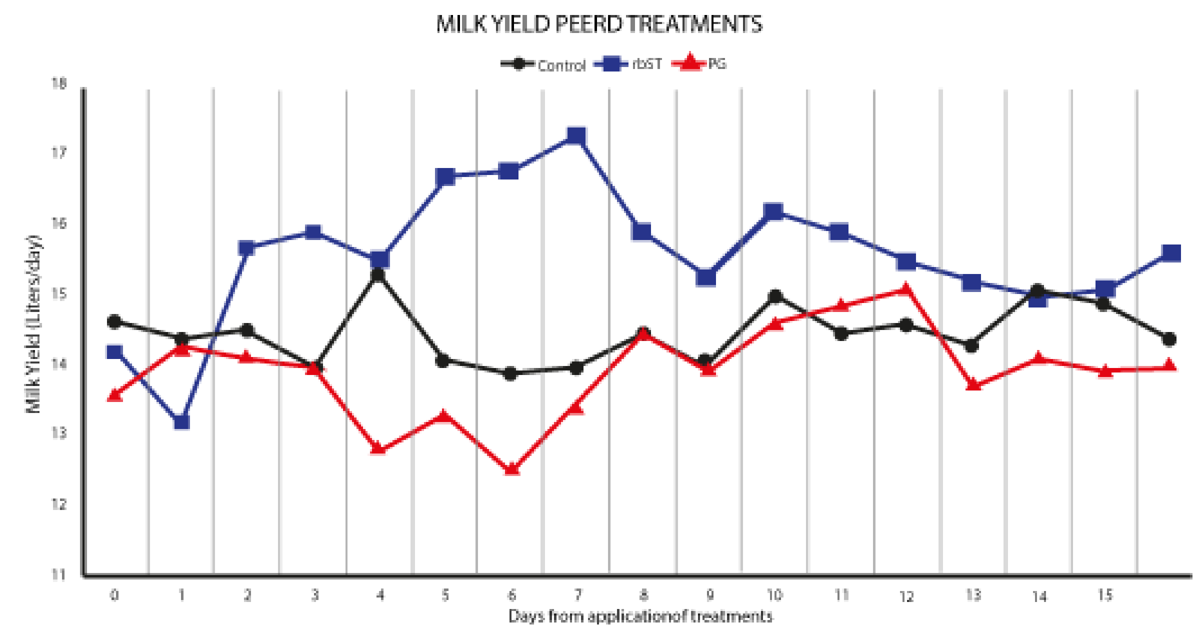

Figure 3. Milk production (liters/day) from day 0 to 15 of the superovulation protocol for the recombinant bovine somatotropin (rbST), propylene glycol (PG), and control groups.

Analysis of variance (ANOVA) was conducted to compare milk production among the rbST, PG, and control (C) groups. The results showed that the rbST group had higher milk production (15.6 ± 1.0 L/day) compared to the PG group (14.4 ± 0.5 L/day) and the control group (14.0 ± 0.7 L/day). The ANOVA revealed differences ($p < 0.05$) between the rbST group and the other two groups, with rbST showing higher performance. No differences ($p > 0.05$) were found between the PG and control groups.

DISCUSSION

Postpartum reproductive events are controlled by various hormones and metabolites, including insulin-like growth factor I (IGF-I) (LUCY 2019a). This peptide is synthesized in organs of reproductive importance, such as the hypothalamus, ovaries, oviducts, and uterus. However, most of the IGF-I that is measured in the blood is produced by the liver (RHOADS *et al.* 2007). Recombinant bovine somatotropin (rbST) and propylene glycol increase this metabolite, but in different pathways, and may hold the key to understanding the effects on reproduction. Despite advances in science, there is still a lack of information that allows us to understand these processes.

An important finding of this study was that rbST improves the quality of embryos and PG increases the number of embryos. A possible explanation may be related to three important metabolites: IGF-I, insulin, and glucose, and how these metabolites affect gonadotropic hormones (FSH and LH) during the recruitment and maturation of follicles and subsequent embryonic development.

For instance, HAN *et al.* (2019) and AFRADIASBAGHARANI *et al.* (2022), evidenced the ability of the ovary to produce IGF-I in theca cells and the granulosa cell membrane, likewise, a higher expression of ARNm for IFG-1 was observed in the initial luteal phase. In addition, it was observed that it participate in follicular growth, development, and maturation, thus playing an important role in gonadotropin-induced folliculogenesis, ovarian steroidogenesis, corpus luteum (CL) function, in the activity of the pituitary gland and hypothalamus, and acting synergistically with gonadotropins, due to the ability of IGF-I to increase the number of receptors for FSH and LH. This would explain embryonic development in cows under the administration of PG and rbST because there is an increase in IGF-I, and consequently a greater number of FSH receptors. As found, the doses applied in the superovulation protocol showed a better effect of the PG and rbST groups than the control group, where an additional effect on the quality of the embryos was also observed.

However, SPICER & AAD (2007) found in a study no expression of mRNA for IGF-I in active and inactive estrogen-dominant follicles of pre and postpartum cows, concluding that IGF-I from follicular fluid is derived from the liver. This finding allows assuming that when rbST is administered, the metabolites that are generated as glucose and IGF-I are mainly derived from milk production and reproductive functions, and consequently, embryo production would decrease. The mechanism of IGF-1 inhibition is activated mainly by insulin, FSH, and cortisol (YAKAR *et al.* 2018), thus, it is likely that increased insulin production under PG administration influences embryo quality by inhibiting IGF-1. Thus, it is assumed that the application of rbST and PG at the beginning of the superovulation protocol activated the FSH receptors because of the increase in IGF-1 and this allowed the increase in embryo production.

The results of this study show that rbST improves embryo quality, and this may be because the bovine embryo has receptors for IGF-I. Insulin-like growth factor I is a growth factor that causes cells to grow and develop (JOUSAN & HANSEN 2007). However, in this study the number of embryos in cows under the administration of rbST was not greater than the PG group, and it is probably due to the dose used and the time of application; a single dose was used at the beginning of the protocol that lasted 16 days.

A study developed by RINCÓN *et al.* (2019) mentions that a single dose of rbST had no long-term effects on the antral follicle population and serum and follicular concentrations of E2/P4 in non-lactating dairy cows. The available literature on PG supplementation in embryo donor cows or heifers is limited. An interesting aspect is that PG increases the number of embryos compared to other treatments, which can be explained by the relationship between the metabolites generated by the administration of PG and anti-Müllerian hormone. In this sense, GAMARRA *et al.* (2015), found that administration of PG increased follicle count and blastocyst quality above the control group. During follicular growth, PG exerts epigenetic regulatory effects on gene expression in blastocyst-stage embryos (GAMARRA *et al.* 2018). SOUZA *et al.* (2008), in goats, conclude that propylene glycol supplementation did not improve the quality of the collected structures, because the number of quality 1 and 2 embryos did not increase. Other factors that probably influenced this result is that the cows were never part of an embryo production program and that the donors maintained basic nutrition and normal body condition during the experimental period (HANSEN 2023).

The modification of the nutrient metabolism of recombinant bovine somatotropin (rbST) is achieved by direct effects on endogenous somatotropin receptors and by receptors located in hepatocytes and adipose tissue, which stimulate the synthesis and secretion of a biological product (COSTA *et al.* 2020). The mediator (IGF-I) produces a redistribution of nutrients, allowing the increase of glucose, and the basal rate of gluconeogenesis, reducing the ability of insulin to inhibit gluconeogenesis and glucose oxidation (SOTY *et al.* 2017). The above described allows an increase in circulating glucose for use mainly in milk synthesis, which would explain a higher concentration of glucose and milk production found in this study.

Oral administration of propylene glycol (PG) decreases the molar ratio of acetate to propionate in the rumen because some PG is metabolized to propionate, while the remaining PG is absorbed directly from the rumen without alteration and enters gluconeogenesis through pyruvate (LIU *et al.* 2009). Therefore, metabolites, hormones, feed intake, and milk production are affected, preventing excessive fat mobilization and imbalances in carbohydrate and fat metabolism and, thus, reducing the risk of ketosis (SUNDRUM 2015).

Blood glucose concentration is considered a biomarker for the health and reproduction of postpartum dairy cows (LOPES *et al.* 2019). The increase in glucose and insulin concentrations is a metabolic signal for growth factors such as IGF-I (RHOADS *et al.* 2004). In this work, the administration of rbST and PG increased serum glucose concentrations and milk production (Figures 2 and 3). In addition, there were differences between the groups, rbST had higher glucose concentrations than the PG group and control group, but although the PG group had higher glucose concentrations than the control group, no differences were found between these groups.

Authors reported an increase in glucose because of the administration of PG, as well as insulin, not measured in our study (CHUNG *et al.* 2009, GAMARRA *et al.* 2015, LIU *et al.* 2009). For instance, NIELSEN & INGVARTSEN (2004), conclude that oral administration of PG increases insulin by 200–400% within 30 minutes after administration, indicating that PG is absorbed rapidly. Biochemically, insulin allows glucose oxidation, which probably reduces glucose concentrations in cows treated with PG (Figure 2). This mechanism does not occur with rbST (higher glucose concentrations) because it prevents glucose oxidation, which allows glucose homeostasis at the metabolic level, which supplies more circulating glucose for the synthesis of milk or other organs that require it (KAMINSKI *et al.* 2019). This probably also explains why milk production followed the same pattern between the groups, where rbST administration also worked better (Figure 3).

Previous studies showed similar results regarding milk production with PG administration, noting that PG tends to increase milk production but has no effect on milk composition (GARCÍA *et al.* 2011, MIKUŁA et al. 2018, ZHANG *et al.* 2020). Another likely factor was that most of the cows used in the study were not on BEN, their body condition was between 2.75 to 3.2, and the lactation period was between 90 to 110 days after calving. It is also likely that these factors also influence the increase in milk production in cows with rbST administration because the positive effects of rbST are from the ninth week of lactation, but in general, the best response occurs between 100 days after calving and 15 days before the end of lactation (TARAZÓN-HERRERA *et al.* 2009), whereas when administered during early lactation in the period between delivery and peak lactation, the response is minimal.

CONCLUSION

Compared to the control group (C), propylene glycol (PG) was found to increase the number of embryos in early lactating Brown Swiss cows undergoing a superovulation protocol. However, recombinant bovine somatotropin (rbST) improved embryo quality and had a more favorable effect on embryonic development stages. Specifically, the rbST group exhibited higher milk production and glucose levels, while the PG group showed increased embryo numbers, but did not differ significantly from the control group in terms of glucose and milk production. We conclude that the best treatment in practical terms of milk production is the rbST, while to increase embryo number it would be the PG treatment.

NOTES

AUTHOR CONTRIBUTIONS

Conceptualization: I.U.P., C.Q.E., and F.A.V.; Methodology: I.U.P., C.Q.E., and E.A.G.; Formal analysis: I.U.P., C.Q.E., and F.A.V.; Software: C.Q.E., E.A.G., and A.H.d.I.C.; Validation: C.Q.E., E.A.G., and A.H.d.I.C.; Investigation: E.A.G., F.A.V., and A.H.d.I.C.; Resources: E.A.G., F.A.V., and A.H.d.I.C.; Data curation: E.A.G., F.A.V., and A.H.d.I.C.; Writing—original draft: I.U.P., C.Q.E., and E.A.G.; Writing—review & editing: F.A.V., A.H.d.I.C., and I.U.P.; Visualization: C.Q.E., E.A.G., and A.H.d.I.C.; Supervision: I.U.P., F.A.V., and A.H.d.I.C.; Project administration: I.U.P., F.A.V., and A.H.d.I.C.; Funding acquisition: I.U.P., C.Q.E., and F.A.V. All authors have read and agreed to the published version of the manuscript.

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INSTITUTIONAL REVIEW BOARD STATEMENT

Animal procedures were conducted in accordance with the ethical guidelines of the Universidad Nacional del Centro del Perú.

INFORMED CONSENT STATEMENT

Not applicable because this study did not involve humans.

DATA AVAILABILITY STATEMENT

The datasets generated and analyzed during this study are available from the corresponding author upon reasonable request.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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