

Evaluation of follicular growth and progesterone production in *Bos taurus* cows strategically treated with insulin

Avaliação do crescimento folicular e da produção de progesterona em vacas *Bos taurus* tratadas estrategicamente com insulina

Andressa Stein Maffi¹; Mityelle da Costa Chaves Rodrigues¹; Lucas Balinhas Farias¹; Joabel Tonello dos Santos²; Gabriela Bueno Luz¹; Lucas Jackson de Souza¹; Alice Benites¹; Carlos Eduardo Gomez Martin³; Marcio Nunes Corrêa⁴; Cassio Cassal Brauner^{4*}

Highlights:

Application of insulin dose in protocols of fixed-time artificial insemination and embryo transfer.

Isolated action of metabolic hormone at follicular dynamics in cattle.

The success of insulin application on reproductive parameters depends on the metabolic status.

Abstract

The goal of this study was to evaluate the effects of strategic insulin administration on follicular growth, progesterone production, and pregnancy rate in beef cattle. Two experiments were conducted, in experiment 1, 215 cows crossbreed cows (*Bos indicus* × *Bos taurus*) (Control Group, n = 109; Insulin Group, n = 106) were submitted to fixed-time artificial insemination (TAI) with application of 2 mg of estradiol benzoate and a vaginal device containing progesterone on day 0. On day 9, the device was removed and 0.5 mg of estradiol cypionate was administered with 12.5 mg of dinoprost and 0.25 UI kg⁻¹ of insulin for the treated animals. On day 11, artificial insemination (AI) was performed on all animals. On days 9 and 11 according to the protocol, was evaluated follicular growth and estrus manifestation, and 30 days after AI the pregnancy rate. In experiment 2, 85 cows were utilized, including Aberdeen Angus, Hereford, and crossbreeds (Control Group, n = 49 and Insulin Group, n = 36), and were submitted to the protocol for fixed-time embryo transfer (FTET), and progesterone concentrations were evaluated. On day 0, the cows received 2 mg of estradiol benzoate and a vaginal device containing progesterone. On day 5 of the protocol, 0.150 mg of cloprostenol was administered. On day 8, the vaginal device was removed and 20 mg of purified porcine pituitary extract and 0.25 UI kg⁻¹ of insulin was given to the animals in the treatment group, and on day 9, 1 mg of estradiol benzoate was administered. On day 17, an embryo transfer was performed and blood collection for evaluation of progesterone levels in animals submitted to FTET were collected. In experiment 1, the follicular growth rate and the pregnancy rate were similar between groups (P > 0.05) and in experiment 2, the production of P4 was not different between the animals that received the insulin application and the control group. The utilization of a single dose of insulin for beef cows did not affect the ovulatory follicular diameter, progesterone concentrations, or pregnancy rate.

Key words: Beef cattle. Follicular diameter. Progesterone. Reproduction.

¹ Discentes, Núcleo de Pesquisa, Ensino e Extensão em Pecuária, NUPEEC, Universidade Federal de Pelotas, UFPel, Pelotas, RS, Brasil. E-mail: adressamaffi@gmail.com; mitychaves@yahoo.com.br; lucasbalinhas@gmail.com; gabrielabluz.veterinaria@gmail.com; lucasjacksondesouza@hotmail.com; alicebenites@gmail.com

² Prof. Dr., Instituto Federal Farroupilha, IFFar, Frederico Westphalen, RS, Brasil. E-mail: joabelts@yahoo.com.br

³ Médico Veterinário, In Vitro Sul, Pelotas, RS, Brasil. E-mail: carlos@invitrosul.com.br

⁴ Profs. Drs., UFPel, Pelotas, RS, Brasil. E-mail: marcio.nunescorreia@gmail.com; cassiocb@gmail.com

* Author for correspondence

Resumo

O objetivo deste estudo foi avaliar o efeito da aplicação estratégica de insulina sobre o crescimento folicular, produção de progesterona e taxa de prenhez em bovinos de corte. Foram realizados 2 experimentos, no experimento 1, 215 vacas cruzas (*Bos indicus* x *Bos taurus*) (Grupo Controle = 109; Grupo Insulina = 106) foram submetidas a um protocolo que consistiu na aplicação de 2 mg de benzoato de estradiol e inserção de um dispositivo intravaginal de liberação lenta de progesterona (P4) sendo este o dia zero do protocolo. No dia 9, foi realizada a remoção do dispositivo de P4, a aplicação de 0,5 mg de cipionato de estradiol, e 12,5 mg de dinoprost. Os animais do grupo insulina receberam ainda a aplicação de 0,25 UI kg⁻¹ de insulina. No dia 11 foi realizada a inseminação artificial de todos os animais. Nos dias 9 e 11 de acordo com o protocolo, foi avaliado crescimento folicular e manifestação de estro, e 30 dias após IA foi avaliado a taxa de prenhez. O experimento 2 foi realizado com 85 vacas da raça Aberdeen Angus, Hereford e cruzas (Grupo Controle = 49; Grupo Insulina = 36) submetidas a um protocolo de Transferência de Embrião em Tempo Fixo (TETF). No dia 0 fez-se aplicação de 2 mg de benzoato de estradiol e a inserção de um dispositivo intravaginal de liberação lenta de progesterona. No 5º dia do protocolo foi aplicado 0,150 mg de d- Cloprostenol. No dia 8 fez-se a remoção do dispositivo de P4 e aplicação de 20 mg de foltropina de pituitária suína e de 0,25 UI kg⁻¹ de insulina nos animais tratados e no dia 9 foi aplicado 1 mg de benzoato de estradiol. No dia 17 procedeu-se com a transferência dos embriões e coleta de sangue para avaliação dos níveis de progesterona nos animais submetidos a TETF. No experimento 1 a taxa de crescimento folicular e a taxa de prenhez foram semelhantes entre os grupos ($P > 0,05$) e no experimento 2 a produção de P4 não foi diferente entre os animais que receberam a aplicação de insulina e o grupo controle. A utilização de uma única dose de insulina em vacas de corte não altera o diâmetro do folículo ovulatório, níveis de progesterona e taxa de prenhez.

Palavras-chave: Bovinos de corte. Diâmetro folicular. Progesterona. Reprodução.

Introduction

Reproductive capacity of cows is determined by the efficiency of the females return to cyclicity post-partum and development of pre-ovulatory follicles responsive to luteinizing hormone (LH) peaks, leading to ovulation and production of a corpus luteum that is able to secrete sufficient amounts of progesterone during a normal estrous cycle (SHORT et al., 1990; LUCY, 2003). The moment of return to cyclicity after parturition in beef cows is the determinant factor for general reproductive efficiency of the herd and the economic success of breeding systems (HESS et al., 2005). Physiologically postpartum is marked by a period of approximately 30 days of anestrus necessary for the reestablishment of processes necessary for the reestablishment of the physiological conditions of the reproductive system (SHORT et al., 1990). In the second week of postpartum, an increase in the follicle stimulating hormone (FSH) and the recruitment of a new follicular wave is observed

(YAVAS; WALTON, 2000); however, some factors, such as body corporal condition loss and suckling (WILLIAMS, 1990; LUCY, 2003) cause low systemic levels of insulin-like growth factor (IGF-I), glucose, and insulin. Changes in milk yield and hormonal circulation negatively influence the frequency of pulses of luteinizing hormone, compromising follicular growth (WILLIAMS, 1990; GRIMARD et al., 1995; HESS et al., 2005). Therefore, nutritionally compromised cows can remain acyclic beyond the 100 days post-partum period (SHORT et al., 1990; WILLIAMS, 1990).

To reduce the interval between parturition-conception hormonal protocols for fixed time artificial insemination (TAI) can be utilized, but the response to these hormonal treatments still exhibits large variation in results (BARUSELLI et al., 2004; COLAZO; AMBROSE, 2015). Follicles smaller than 11.5 mm give rise to small corpus luteum (CL), which produce lower levels of progesterone (VASCONCELOS et al., 2001), reducing the

conception rate (LAMB et al., 2001) and increasing the embryonic loss (PERRY et al., 2005). Pfeifer et al. (2009) related improvement in the pregnancy rate of female *Bos taurus* with follicles between 13 and 15 mm at TAI. As a strategy to increase the efficiency of TAI results, in these cases of low nutritional status in cows with calves, the application of equine chorionic gonadotrophin (eCG) showed positive results according to previous studies (BARUSELLI et al., 2004; ROSTAMI et al., 2011; PESSOA et al., 2016). In addition, a study conducted by our group showed that the association of insulin with eCG during the TAI protocol in beef cattle resulted in an improvement in the pregnancy rate in animals with a body corporal score (BCS) less than 2.5 (on a scale of 1 to 5), as compared with animals that received only eCG (SCHNEIDER et al., 2010). However, there are still few studies on the isolated action of metabolic hormones, such as insulin, which is an important marker of follicular dynamics in cattle (WEBB et al., 2004; HESS et al., 2005), and has the capacity to stimulate cell proliferation and follicular steroidogenesis (WETTEMANN; BOSSIS, 2000).

Insulin is responsible for the maintenance of peripheral glucose levels by the stimulation of glucose uptake, oxidation, and storage (DONATO et al., 2012). In the reproductive system, *in vitro* studies have demonstrated that this hormone increases the activity of the aromatase enzyme and the production of estradiol and progesterone in granulosa cells (BATHIA; PRICE, 2001; SPICER et al., 1993). It also stimulates the proliferation of granulosa, the increase of follicular diameter, and formation of antrum (PORETSKY et al., 1999; ITOH et al., 2002). Furthermore, *in vivo* studies have shown an increase in the number of CL, and higher estradiol and progesterone levels in goats (SUGUNA et al., 2009). In cattle, it was shown that insulin promotes an increase in the number of antral follicles (SIMPSON et al., 1994; GONG et al., 2002), and an increase in estradiol production (BUTLER et al., 2004).

Thus, the hypothesis of our study is that insulin application supports in the final development of the dominant follicle, an increase in progesterone production by the formed CL, and the consequent pregnancy rate. The goal of this study was to evaluate the effect of the application of an insulin dose during fixed-timed artificial insemination and embryo transfer protocol on follicular growth, progesterone production, and pregnancy rate in beef cows.

Material and Methods

Experiment 1

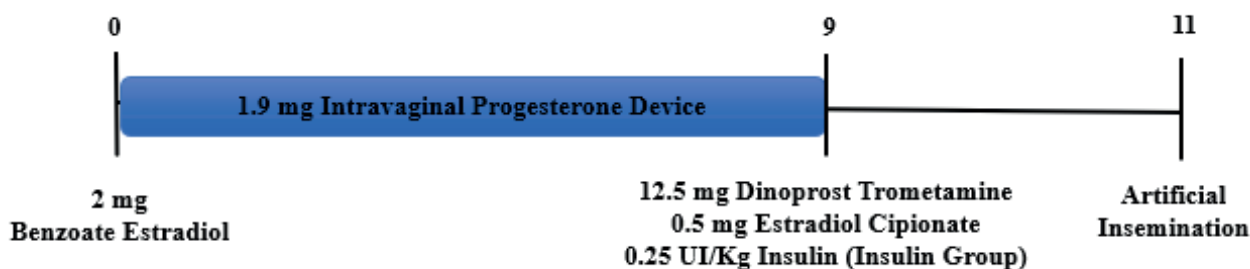
Study 1 was realized in two commercial farms of beef cattle in Pinheiro Machado - RS. We utilized 215 crossbred cows (Angus and Hereford), between 3 to 9 years old, approximately 40 and 90 days postpartum, BCS 2.9 ± 0.03 (Control Group: 2.9 ± 0.03 ; Insulin Group: 2.9 ± 0.03), on a scale where 1 = emaciated and 5 = obese (MORAES et al., 2007). All females were managed in their native field (*Paspalum notatum*), containing 93.2% dry matter, 9.9% ash, 7.32% crude protein, 28.8% fiber, 66.1% total digestible nutrients, 61.3% neutral detergent fiber, and 34.8% acidic detergent fiber, with an animal load of 315 Kg ha⁻¹ and free access to water and mineral salt.

All animals were submitted to a TAI protocol, which consisted of the application of 2 mg of estradiol benzoate, intramuscular (i.m.), (1 mg mL⁻¹, Gonadiol®-Zoetis, São Paulo, Brazil) and insertion of a P4 device (1.9 g of progesterone CIDR®-Zoetis, São Paulo, Brazil) on day 0. On the 9th day of the protocol, the P4 device was removed and 0.5 mg of estradiol cypionate was applied i.m. (2 mg mL⁻¹, E.C.P.-Zoetis, São Paulo, Brazil) and 12.5 mg of dinoprost, i.e., (5 mg mL⁻¹, Lutalyse®-Zoetis, São Paulo, Brazil). On day 11 of the protocol, artificial insemination was performed at a fixed time. The division of the animals was performed on the 9th day of the protocol, homogeneously, based on the

diameter of the dominant follicle and BCS, into two groups: the Control Group (n = 109) and Insulin Group (n = 106), with the latter receiving 0.25

UI kg⁻¹ of insulin, subcutaneously (Novolin N[®]-Novo Nordisk, Bagsvaerd, Denmark) according to Schneider et al. (2010) as described in Figure 1.

Figure 1. Description of the fixed-time artificial insemination protocol, performed on the animals in experiment 1.



Evaluation of signs of behavioral estrus and physical signs of estrus were recognized in all animals between days 9 and 11, based on tail paint. On day 11, artificial insemination was conducted, and the presence or absence of the tail paint was observed to note animals that did not demarcated with paint at the moment of the AI. The dominant follicle diameter assessment was performed on days 9 and 11 of the protocol using a Welld Wed-3000V Vet ultrasound, coupled to a linear probe 7.5 MHz inserted transrectally. On day 9, the follicular diameter was evaluated in all the animals for division into groups, and on day 11 it was performed only for 71 animals of property 1 to evaluate follicular growth. The calculation of follicular growth was performed by subtracting the follicular diameter measurement of day 11 from the follicular diameter measurement of day 9 of the protocol. The gestation diagnosis of all the animals was performed 30 days after the AI with a Welld Wed-3000V Vet ultrasound, coupled to a linear probe 7.5 MHz inserted transrectally.

Experiment 2

This study was performed on two beef farms, cows were used as embryo recipients, totaling 85

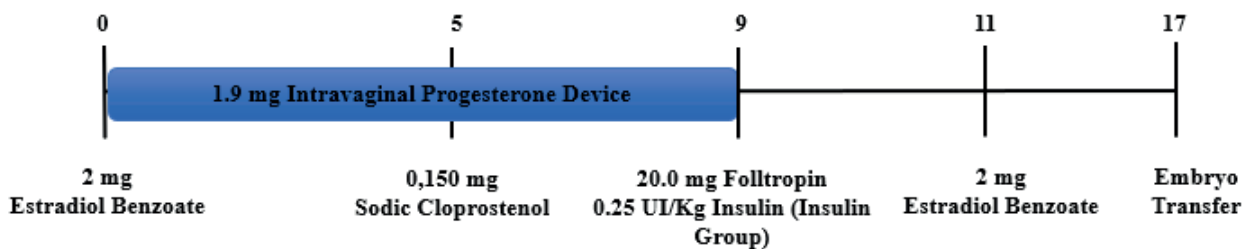
animals. Property 1 was located in the city of Santa Vitoria do Palmar-RS and property 2 was located in the city of Arroio Grande-RS. At farm 1, 48 cows receiving embryos of Hereford and crossbred (*Bos indicus* × *Bos taurus*), single, 3 years old, BCS 3.7 ± 0.07 (Control Group: 3.7 ± 0.07 ; Insulin Group: 3.8 ± 0.07) were managed on a cultivated pasture of ryegrass (*Lolium multiflorum*) and white clover (*Trifolium repens*). On farm 2, 37 cows received embryos of the Aberdeen Angus and crossbred (*Bos indicus* × *Bos taurus*). Embryos were evaluated using 4- to 6-year-old calves, BCS 4.1 ± 0.08 (Control Group: 4.1 ± 0.08 ; Insulin Group: 4.1 ± 0.08) managed in a cultivated pasture of ryegrass (*Lolium multiflorum*), oats (*Avena strigosa*), and mineral salt available *ad libitum*.

The synchronization protocol for fixed-time embryo transfer (FTET) in both properties consisted of the following steps: day 0 application of 2 mg estradiol benzoate (1 mg mL⁻¹, RIC-BE[®] -Tecnopac, São Paulo, Brazil) and insertion of a progesterone intravaginal device (P4) (1.9 g progesterone, CIDR[®] - Zoetis, São Paulo, Brazil). On the 5th day of the protocol, 0.150 mg of d-Cloprostenol (0.150 mg mL⁻¹, Prolise[®] - Tecnopac, São Paulo, Brazil) was applied. On the 8th day, the P4 device was removed and 20 mg of purified porcine pituitary extract was

applied (20 mg mL⁻¹, Folltropin® - Tecnopec, São Paulo, Brazil). The animals were randomly divided into groups: Insulin (n = 36) and Control (n = 49). On the same day, the Insulin Group animals received the application of 0.25 IU kg⁻¹ insulin (Novolin® N - Novo Nordisk, Bagsvaerd, Denmark). On the 9th

day of the protocol, 1 mg of estradiol benzoate (1 mg mL⁻¹, RIC-BE® - Tecnopec, São Paulo, Brazil) was applied and on the 17th day, the cows that responded to the protocol and presented a CL were classified as functional by the responsible veterinarian, as is described in Figure 2.

Figure 2. Description of the fixed-time embryo transfer (FTET) protocol, performed on the animals in experiment 2.



On day 17 of the FTET protocol, the veterinarian performed palpation of the ovaries to determine the presence and size of the CL. Animals that did not respond to the protocol or had small CLs (poorly delimited upon palpation) were discarded. The utilization rate was determined from the ratio between the total number of animals in the group and the number of animals eligible for the transfer. Each recipient received a low epidural anesthesia with 4 mL of 2% lidocaine hydrochloride (Bravet, Rio de Janeiro). The plastic sanitary shirt was used to prevent uterine contamination and removed when the applicator reached the first cervical ring. At the cranial portion of the horn ipsilateral to the CL, the embryo was deposited.

On the 17th day of the protocol, blood was collected in a subgroup of recipients that received the embryo (Control Group = 21; Insulin Group = 18). The collection was performed through the arteriovenous-coccygeal complex in a tube without anticoagulant. After collection, the tubes were centrifuged at 1000 g, and the serum was separated and allocated into properly identified Eppendorf tubes and stored at -20 °C until the analyzes were

performed. Progesterone levels were assessed by the chemiluminescence technique using a progesterone test kit from the Siemens Company using the Adivia Centaur device.

For statistical analysis of the data, treatment (Insulin and Control) was considered a fixed factor. BCS and progesterone levels were evaluated by analysis of variance (ANOVA). Follicular growth was assessed by repeated measures, whereas the number of embryo transfer animals (rate of use), estrus expression rate and pregnancy rate were analyzed by the chi-square test. In order to evaluate follicular growth according to body condition, animals categorized into BCS <2 and >3 and follicular growth were evaluated through repeated measures analysis. To evaluate the pregnancy rate according to the diameter of the follicle on day 9, segmentation was performed in follicles larger or smaller than 11 mm and evaluated using the chi-square analysis. All analyzes were performed using the NCSS program (2005) (Hintze, J. 2005). Results were presented as the mean ± standard error of the mean. $P \leq 0.05$ were considered significant.

Results and Discussion

From previous knowledge of the action of insulin on follicular development, it was expected that the animals treated with this hormone during the TAI and FTET protocol would respond positively with a greater follicular growth, a consequent increase in the follicular diameter and area of the CL, and thereby increase the production of progesterone and increase the pregnancy rate.

When we evaluated data from experiment 1, we observed that the daily follicle growth rate between the 9th and 11th day of the protocol was not different between the groups (Table 1, $P = 0.20$). In addition,

the daily follicular growth of all animals independent of the group was lower than the daily follicular growth rate reported in other studies (LUSSIER et al., 1987; RHODES et al., 1995; GINTHER, 2018). It is known that antral follicles may have a growth rate of up to 2 mm day⁻¹ (LUSSIER et al., 1987; GINTHER, 2018) with rates of 1.2 mm day⁻¹ in nutritionally challenged animals (PESSOA et al., 2016). When analyzing the diameter of the follicles on day 11 of the protocol in our study, some animals of both groups presented a reduction in this variable in relation to the first evaluation (D9), which caused a reduction in the mean follicular growth rate.

Table 1. Evaluation of follicular development, estrus presentation, and pregnancy rate of beef cows submitted to fixed-time artificial insemination protocol with strategic insulin application (Insulin Group) or not (Control Group).

Variables	Control Group	Insulin Group	P value
Diameter D9 mm (n = 71)	11.28 ± 0.23	11.45 ± 0.23	0.80
Diameter D11 mm (n = 71)	12.2 ± 0.23	11.80 ± 0.52	0.54
Growing D9-D11 mm (n = 71)	0.91 ± 0.32	0.33 ± 0.32	0.20
Estrus detection % (n=215)	82.6 (90/109)	69.8 (74/106)	0.02
Pregnancy rate % (n = 195)	42.3 (41/97)	35.7 (35/98)	0.64

The reduced follicular growth of the animals of both groups may also have occurred because of the intermediate BCS of the animals (3.0), because this parameter reflects energy balance and is related to systemic levels of IGF, insulin, GH, and neuropeptide Y, which alter the frequency of LH pulses (DICOSTANZO et al., 1999; WETTEMANN; BOSSIS, 2000; LEON et al., 2004) and affect the final follicular development and ovulation. To verify the BCS impact on the follicular growth of evaluated animals, we classified the animals as low (BCS ≤ 2.5) or high BCS (BCS ≥ 3.0), regardless of the group. From this evaluation, we found that animals with low body condition presented a decrease of 0.15 mm ± 0.44 mm, indicating possible atresia of the dominant follicle. However, animals with high BCS showed growth of 0.93 mm ± 0.28 mm ($P =$

0.04). In addition, the results of the present study were consistent with the results in the literature, which has been used to evaluate the effectiveness of TAI protocols in animals with BCS values of 3.0 or higher (HESS et al., 2005; BRIDGES et al., 2012).

The follicular diameter on the 11th day of the protocol did not differ ($P = 0.54$) between the groups (Table 1). However, there was an increase ($P ≤ 0.009$) in the diameter of the dominant follicle between days 9 and 11 of the protocol. The results of other studies, such as that of Simpson et al. (1994), observed a larger diameter follicles in *Bos indicus* cattle treated with insulin during superovulation protocol, but in this study 5 doses of insulin were applied twice a day. However, we chose to search for a single insulin application at a strategic moment to use the positive signaling

effect of this hormone; thus, enabling its practical application. Follicles smaller than 11.5 mm give rise to small CL, which produced lower progesterone levels (VASCONCELOS et al., 2001), reducing the conception rate (LAMB et al., 2001) and increasing the rates of embryonic losses (PERRY et al., 2005). Pfeifer et al. (2009) observed an improvement in the pregnancy rate in *Bos taurus* females that had follicles between 13 and 15 mm at the time of TAI. Our results indicated that both groups exceeded the desirable minimum preovulatory follicle threshold (Control Group: 12.20 ± 0.23 ; Insulin Group: 11.80 ± 0.52). However, the Insulin Group did not demonstrate the ability to increase follicle size at this time.

When we evaluated the estrus presentation of the 215 cows, we observed a higher percentage of estrus in the Control Group compared to that of the Insulin Group (Table 1, $P = 0.02$). The occurrence of estrus is directly related to the capacity of estradiol production by the dominant follicle, with larger diameter follicles presenting higher concentrations of estradiol (E2). We expected that cows treated with insulin to have higher expression of estrus because this hormone had a stimulating effect on the production of ovarian steroids and possibly increased ovarian responsiveness to LH and IGF-1 (BUTLER et al., 2004), however, this did not occur.

Regarding the pregnancy rate (Table 1.), there was also no difference between the treated and control groups ($P = 0.64$). However, in a previous study from our group, the application of insulin when associated with eCG promoted an improvement in the gestation rate of *Bos taurus* suckling cows with low BCS (2.5) (SCHNEIDER et al., 2010), but the isolated action of insulin was not evaluated. In our study, we could not segment the animals because of the low number of animals, but another variable that correlated with the occurrence of pregnancy was the follicular diameter (Figure 3). Because of this, when dividing animals within their group into animals with follicles greater than or equal to 11 mm at day

9 of the protocol and evaluating the pregnancy rate, we observed that the pregnancy rate of the control animals with follicles smaller than 11 was lower as compared to the control animals with follicles >11 mm ($P = 0.02$). There was no difference between the other groups of animals with follicles greater than 11 mm. In relation to the groups with follicles smaller than 11 mm, the animals that received the insulin application had a 15% increase in pregnancy rate ($P > 0.05$). We knew that because of the low number of animals in each group, it was possible to affirm this relationship, but we associated this response with the energetic stimulus that insulin promoted in these small follicles, acting as a metabolic signal, as reported in the study by Schneider et al. (2010).

In relation to experiment 2, the number of animals with adequate-sized CL that were used in embryo transfer was similar between the Control (77.1%; 37/48) and Insulin (78.9%; 28/36) groups ($P = 0.93$, Table 2). We believe that the high BCS of the animals in this study indicated that circulating levels of insulin, glucose, and IGF-I (LEON et al., 2004) were within the physiological levels, promoting adequate follicular growth independent of exogenous insulin supplementation.

The production of P4 was also similar ($P \geq 0.05$) between the Control Group (7.32 ± 0.63 ng mL⁻¹) and Insulin Group (7.56 ± 0.68 ng mL⁻¹) ($P = 0.80$; Table 2.), with levels higher than those reported in other studies (BATTOCCHIO et al., 1999; BORGES et al., 2003). There was a positive correlation between P4 plasma concentrations and CL quality (AMBROSE et al., 1999), with larger CL structures producing a greater amount of progesterone than small and medium ones (LEAL et al., 2009). The results of the use rate and the P4 levels of experiment 2, associated with the results found by Schneider et al. (2010), where the application of insulin associated with eCG in animals in good body condition that did not increase the pregnancy rate. This implies that a potential insulin effect is only in nutritionally challenged animals.

Figure 3. The pregnancy rate of beef cows submitted to an FTAI protocol with strategic insulin (Insulin Group) or not (Control Group), according to the follicular diameter (<11 mm or >11 mm).

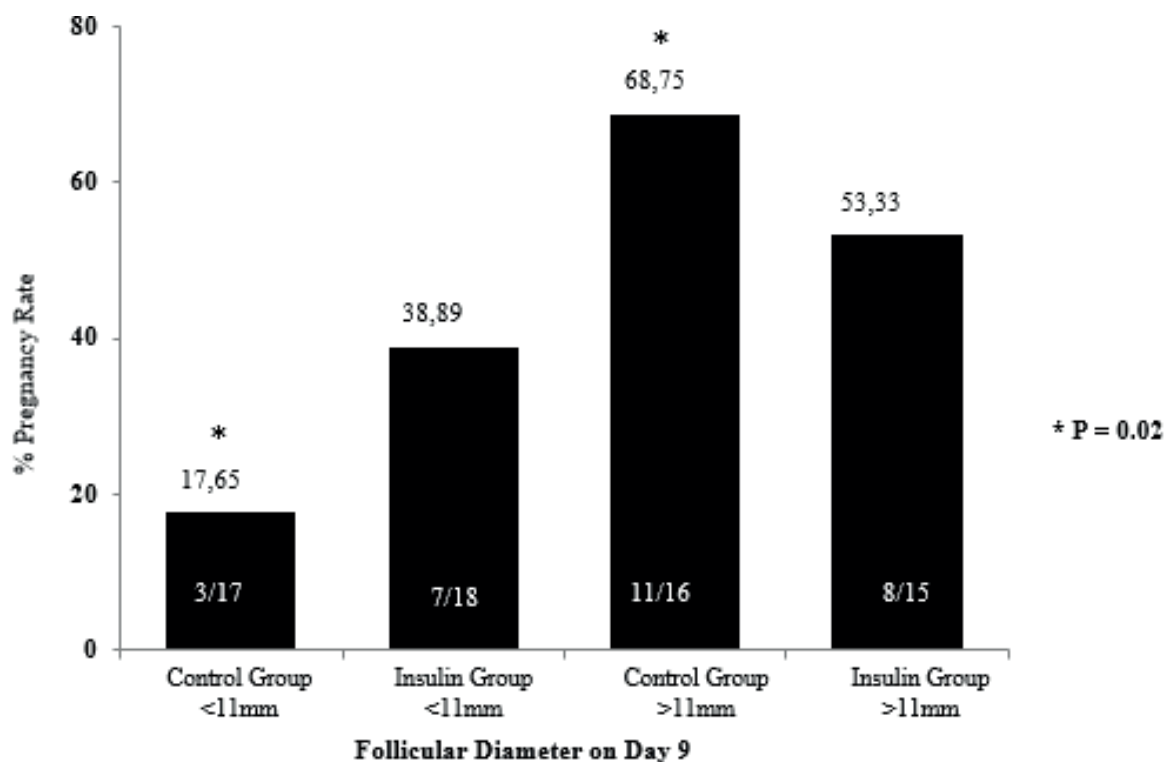


Table 2. Evaluation of embryo transfer and progesterone level of animals submitted to FTET, treated with strategic insulin (Insulin Group) or not (Control Group).

Variables	Control Group	Insulin Group	P value
Embryo transfer rate, % (n=84)	77.1% (37/48)	78.9 (28/36)	0.93
Progesterone levels, ng/ml (n= 39)	7.32 ± 0.63 (21)	7.56 ± 0.68 (18)	0.80

According to the results of experiments 1 and 2, we believe that there may be a positive performance of insulin when used in challenged animals; however, it must be confirmed with a larger number of animals. In addition, based on other works, this hormone seems to be dependent on a greater number of applications, taking into account its action time of only 24 h. In the study of Butler et al. (2004), by infusing insulin and glucose for 96 h, changes in estradiol and IGF-I levels occurred, but only after 30 h, indicating that the metabolic effects of

this hormone did not act instantaneously. Therefore, the application of a single dose of insulin in beef cows with adequate BCS did not increase ovulatory follicle diameter, pregnancy rate, or progesterone levels.

All the procedures performed were approved by the Ethics and Animal Experimentation Committee of the Federal University of Pelotas under number 2358.

References

- AMBROSE, J. D.; DROST, M.; MONSON, R. L.; RUTLEDGE, J. J.; LEIBFRIED-RUTLEDGE, M. L.; THATCHER, M. J.; KASSA, T.; BINELLI, M.; HANSEN, P. J.; CHENOWETH, P. J.; THATCHER, W. W. Efficacy of timed embryo transfer with fresh and frozen in vitro produced embryos to increase pregnancy rates in heat-stressed dairy cattle. *Journal of Dairy Science*, Champaign, v. 82, n. 11, p. 2369-2376, 1999. DOI: 10.3168/jds.S0022-0302(99)75487-1
- BARUSELLI, P. S.; REIS, E. L.; MARQUES, M. O.; NASSER, L. F.; BO, G. A. The use of hormonal treatments to improve reproductive performance of anestrus beef cattle in tropical climates. *Animal Reproduction Science*, Amsterdam, v. 82-83, p. 479-486, 2004. DOI: 10.1016/j.anireprosci.2004.04.025
- BHATIA, B.; PRICE, C. A. Insulin alters the effects of follicle stimulating hormone on aromatase in bovine granulosa cells in vitro. *Steroids*, v. 66, n. 6, p. 511-519, 2001. DOI: 10.1016/S0039-128X(00)00218-X
- BATTOCCHIO, M.; GABAI, G.; MOLLO, A.; VERONESI, M.; SOLDANO, F.; BONO, G.; CAIROLI, F. Agreement between ultrasonographic classification of the CL and plasma progesterone concentration in dairy cows. *Theriogenology*, Philadelphia, v. 51, n. 6, p. 1059-1069, 1999. DOI: 10.1016/S0093691X(99)80011-9
- BORGES, Á. M.; TORRES, C. A. A.; RUAS, J. R. M.; RIBEIRO, V. R. J.; CAROVALHO, G. R. D. Desenvolvimento luteal e concentrações plasmáticas de progesterona em vacas das raças Gir e Nelore. *Revista Brasileira de Zootecnia*, Viçosa, MG, v. 32, n. 2, p. 276-283, 2003. DOI: 10.1590/S1516-35982003000200005
- BRIDGES, G. A.; AHOLA, J. K.; BRAUNER, C.; CRUPPE, L. H.; CURRIN, J. C.; DAY, M. L.; GUNN, P. J.; JAEGER, J. R.; LAKE, S. L.; LAMB, G. C.; MARQUEZINI, G. H.; PEEL, R. K.; RADUNZ, A. E.; STEVENSON, J. S.; WHITTIER, W. D. Determination of the appropriate delivery of prostaglandin F₂alpha in the five-day CO-Synch + controlled intravaginal drug release protocol in suckled beef cows. *Journal of Dairy Science*, Champaign, v. 90, n. 13, p. 4814-4822, 2012. DOI: 10.2527/jas.2011-4880
- BUTLER, S. T.; PELTON, S. H.; BUTLER, W. R. Insulin increases 17 beta-estradiol production by the dominant follicle of the first postpartum follicle wave in dairy cows. *Reproduction, Cambridge*, v. 127, n. 5, p. 537-545, 2004. DOI: 10.1530/rep.1.00079
- COLAZO, M. G.; AMBROSE, D. J. Effect of initial GnRH and duration of progesterone insert treatment on the fertility of lactating dairy cows. *Reproduction in Domestic Animals*, Berlin, v. 50, n. 3, p. 497-504, 2015. DOI: 10.1111/rda.12518
- DICOSTANZO, A.; WILLIAMS, J. E.; KEISLER, D. H. Effects of short- or long-term infusions of acetate or propionate on luteinizing hormone, insulin, and metabolite concentrations in beef heifers. *Journal of Animal Science*, Oxford, v. 77, n. 11, p. 3050-3056, 1999. DOI: 10.2527/1999.77113050x
- DONATO, J. The central nervous system as a promising target to treat diabetes mellitus. *Current Topics in Medicinal Chemistry*, Doylestown, v. 12, n. 19, p. 2070-2081, 2012. DOI: 10.2174/1568026611212190005
- GINTHER, O. J. Variations in follicle-diameter deviation and a growth spurt in the dominant follicle at deviation in *Bos taurus* heifers. *Animal Reproduction Science*, Amsterdam, v. 188, p. 155-164, 2018. DOI: 10.1016/j.anireprosci.2017.11.020
- GONG, J. G.; LEE, W. J.; GARNSWORTHY, P. C.; WEBB, R. Effect of dietary-induced increases in circulating insulin concentrations during the early postpartum period on reproductive function in dairy cows. *Reproduction*, Bristol, v. 123, n. 3, p. 419-427, 2002. DOI: 10.1530/rep.0.1230419
- GRIMARD, B.; HUMBLLOT, P.; PONTER, A. A.; MIALOT, J. P.; SAUVANT, D.; THIBIER, M. Influence of postpartum energy restriction on energy status, plasma LH and oestradiol secretion and follicular development in suckled beef cows. *Journal of Reproduction and Fertility*, Cambridge, v. 104, n. 1, p. 173-179, 1995. DOI: 10.1530/jrf.0.1040173
- HESS, B. W.; LAKE, S. L.; SCHOLLJEGERDES, E. J.; WESTON, T. R.; NAYIGIHUGU, V.; MOLLE, J. D. C.; MOSS, G. Nutritional controls of beef cow reproduction. *Journal of Animal Science*, Oxford, v. 83, n. 13, p. 90-106, 2005. DOI: 10.2527/2005.8313_supplE90x
- HINTZE, J. PASS 2005. NCSS, LLC. Kaysville, Utah, 2005. USA. www.ncss.com.
- ITOH, T.; KACCHI, M.; ABE, H.; YUTAKA, S.; HIROYOSHI, H. Growth, antrum formation, and estradiol production of bovine preantral follicles cultured in a serum-free medium. *Biology of Reproduction*, Oxford, v. 67, n. 4, p. 1099-1105, 2002. DOI: 10.1095/biolreprod67.4.1099
- LAMB, G. C.; STEVENSON, J. S.; KESLER, D. J.; GARVERICK, H. A.; BROWN, D. R.; SALFEN, B. E. Inclusion of an intravaginal progesterone insert plus GnRH and prostaglandin F₂alpha for ovulation control in postpartum suckled beef cows. *Journal Animal Science*, Oxford, v. 79, n. 9, p. 2253-2259, 2001. DOI: 10.2527/2001.7992253x

- LEAL, L. D. S.; OBA, E.; FERNANDES, C. A. D. C.; SÁ FILHO, O. G. Avaliação do corpo lúteo, contratilidade uterina e concentrações plasmáticas de progesterona e estradiol em receptoras de embriões bovinos. *Ciência Animal Brasileira*, Goiânia, v. 10, n. 1, p. 174-183, 2009. DOI: 10.5216/cab.v10i1.448
- LEON, H. V.; HERNANDEZ-CERON, J.; KEISLERT, D. H.; GUTIERREZ, C. G. Plasma concentrations of leptin, insulin-like growth factor-I, and insulin in relation to changes in body condition score in heifers. *Journal of Animal Science*, Oxford, v. 82, n. 2, p. 445-451, 2004. DOI: 10.2527/2004.822445x
- LUCY, M. C. Mechanisms linking nutrition and reproduction in postpartum cows. *Reproduction Supplement*, Cambridge, v. 61, p. 415-427, 2003. DOI: 10.1530/biosciproc.5.031
- LUSSIER, J. G.; MATTON, P.; DUFOUR, J. J. Growth rates of follicles in the ovary of the cow. *Journal of Reproduction and Fertility*, Cambridge, v. 81, n. 2, p. 301-307, 1987. DOI: 10.1530/jrf.0.0810301
- MORAES, J. C. F.; JAUME, C. M.; SOUZA, C. J. H. D. Body condition score to predict the postpartum fertility of crossbred beef cows. *Pesquisa Agropecuária Brasileira*, Brasília, v. 42, n. 5, p. 741-746, 2007. DOI: 10.1590/S0100-204X2007000500018
- PERRY, G. A.; SMITH, M. F.; LUCY, M. C.; GREEN, J. A.; PARKS, T. E.; MACNEIL, M. D.; ROBERTS, A. J.; GEARY, T. W. Relationship between follicle size at insemination and pregnancy success. *Proceedings of the National Academy of Sciences*, Washington, v. 102, n. 14, p. 5268-5273, 2005. DOI: 10.1073/pnas.0501700102
- PESSOA, G. A.; MARTINI, A. P.; CARLOTO, G. W.; RODRIGUES, M. C. C.; CLARO JUNIOR, I.; BARUSELLI, P. S.; BRAUNER, C. C.; RUBIN, M. I. B.; CORREA, M. N.; LEIVAS, F. G.; SA FILHO, M. F. Different doses of equine chorionic gonadotropin on ovarian follicular growth and pregnancy rate of suckled *Bos taurus* beef cows subjected to timed artificial insemination protocol. *Theriogenology*, Philadelphia, v. 85, n. 5, p. 792-799, 2016. DOI: 10.1016/j.theriogenology.2015.09.057
- PFEIFER, L. F.; MAPLETOFT, R. J.; KASTELIC, J. P.; SMALL, J. A.; ADAMS, G. P.; DIONELLO, N. J.; SINGH, J. Effects of low versus physiologic plasma progesterone concentrations on ovarian follicular development and fertility in beef cattle. *Theriogenology*, Philadelphia, v. 72, n. 9, p. 1237-1250, 2009. DOI: 10.1016/j.theriogenology.2009.07.019
- PORETSKY, L.; CATALDO, N. A.; ROSENWAKS, Z.; GIUDICE, L. C. The insulin-related ovarian regulatory system in health and disease. *Endocrine Reviews*, Washington, v. 20, n. 4, p. 532-582, 1999. DOI: 10.1210/edrv.20.4.0374
- RHODES, F. M.; FITZPATRICK, L. A.; ENTWISTLE, K. W.; DE'ATH, G. Sequential changes in ovarian follicular dynamics in *Bos indicus* heifers before and after nutritional anoestrus. *Journal of Reproduction and Fertility*, Cambridge, v. 104, n. 1, p. 41-49, 1995. DOI: 10.1530/jrf.0.1040041
- ROSTAMI, B.; NIASARI-NASLAJI, A.; VOJGANI, M.; NIKJOU, D.; AMANLOU, H.; GERAMI, A. Effect of eCG on early resumption of ovarian activity in postpartum dairy cows. *Animal Reproduction Science*, Amsterdam, v. 128, n. 1-4, p. 100-106, 2011. DOI: 10.1016/j.anireprosci.2011.09.006
- SCHNEIDER, A.; PFEIFER, L. F. M.; SCHMITT, E.; BIANCHI, I.; VIEIRA, M. B.; XAVIER, E. G.; DEL PINO, F. A. B.; CORRÊA, M. N. The use of insulin to improve fertility of timed-inseminated postpartum suckled beef cows. *Pesquisa Agropecuária Brasileira*, Brasília, v. 45, n. 10, p. 1219-1221, 2010. DOI: 10.1590/S0100-204X2010001000023
- SHORT, R. E.; BELLOWS, R. A.; STAIGMILLER, R. B.; BERARDINELLI, J. G.; CUSTER, E. E. Physiological mechanisms controlling anestrus and infertility in postpartum beef cattle. *Journal of Animal Science*, Oxford, v. 68, n. 3, p. 799-816, 1990. DOI: 10.2527/1990.683799x
- SIMPSON, R. B.; CHASE JUNIOR, C. C.; SPICER, L. J.; VERNON, R. K.; HAMMOND, A. C.; RAE, D. O. Effect of exogenous insulin on plasma and follicular insulin-like growth factor I, insulin-like growth factor binding protein activity, follicular oestradiol and progesterone, and follicular growth in superovulated Angus and Brahman cows. *Journal of Reproduction and Fertility*, Cambridge, v. 102, n. 2, p. 483-492, 1994. DOI: 10.1530/jrf.0.1020483
- SPICER, L. J.; ALPIZAR, E.; ECHTERNKAMP, S. E. Effects of insulin, insulin-like growth factor I, and gonadotropin on bovine granulosa cell proliferation, progesterone production, estradiol production, and (or) insulin-like growth factor I production in vitro. *Journal of Animal Science*, Oxford, v. 71, n. 5, p. 1232-1241, 1993. DOI: 10.2527/1993.7151232x
- SUGUNA, K.; MEHROTRA, S.; AGARWAL, S. K.; HOQUEB, M.; SHANKER, U.; SINGH, S. K.; VARSHNEY, V. P. Effect of exogenous insulin administration on ovarian function, embryo/fetal development during pregnancy in goat. *Animal Reproduction Science*, Amsterdam, v. 111, n. 2-4, p. 202-213, 2009. DOI: 10.1016/j.anireprosci.2008.03.021

VASCONCELOS, J. L.; SARTORI, R.; OLIVEIRA, H. N.; GUENTHER, J. G.; WILTBANK, M. C. Reduction in size of the ovulatory follicle reduces subsequent luteal size and pregnancy rate. *Theriogenology*, Philadelphia, v. 56, n. 2, p. 307-314, 2001. DOI: 10.1016/S0093-691X(01)00565-9

WEBB, R.; GARNSWORTHY, P. C.; GONG, J. G.; ARMSTRONG, D. G. Control of follicular growth: local interactions and nutritional influences. *Journal of Animal Science*, Oxford, v. 82, p. E63-74, 2004. Special Supplement. DOI: 10.2527/2004.8213_supplE63x

WETTEMANN, R. P.; BOSSIS, I. Energy intake regulates ovarian function in beef cattle. *Journal of Animal Science*, Oxford, v. 77, p. 1-10, 2000. Supplement E. DOI: 10.2527/jas2000.77E-Suppl1c

WILLIAMS, G. L. Suckling as a regulator of postpartum rebreeding in cattle: a review. *Journal of Animal Science*, Oxford, v. 68, n. 3, p. 831-852, 1990. DOI: 10.2527/1990.683831x

YAVAS, Y.; WALTON, J. S. Postpartum acyclicity in suckled beef cows: a review. *Theriogenology*, Philadelphia, v. 54, n. 1, p. 25-55, 2000. DOI: 10.1016/S0093-691X(00)00323-X

