Comparison of cattle embryo collection between Ringer's lactate solution and Dulbecco's phosphate-buffered saline

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ABSTRACT - The objective of this study was to determine the efficiency of Ringer's lactate solution (RL) and RL + 1% fetal bovine serum (FBS) and compare them with the efficiency of Dulbecco's phosphate-buffered saline (D-PBS). Twenty-two Wagyu female cattle were subjected to superovulation and were randomly distributed to form three groups: group 1 – uterine flushing with RL (n = 8), group 2 – uterine flushing with RL + 1% FBS (n = 7), and group 3 – uterine flushing with D-PBS (n = 7, control group). Cows received a CIDR® device containing 1.9 g of progesterone at random stages of the estrous cycle (day 0). Progesterone withdrawal occurred on day 8 in the morning. For heifers, 160 mg of porcine follicle-stimulating hormone (FSH-P) was used and for cows, 200 mg. Prostaglandin F2α was also injected on the eighth day of FSH-P administration. On day 9, in the morning, hCG was administered. Females were superovulated and inseminated twice in a fixed time for embryo transfer. On the 16th day, females were subjected to uterine flushing for embryo collection. We collected 76 embryos from 22 females subjected to superovulation, of which 52 were transferable and 24 had degenerated. The total of embryos collected was 23, 16, and 23 for groups 1, 2, and 3, respectively. The embryo recovery rates per group were 13.86±4.23, 15.39±4.61 and 27.16±13.33%, in groups 1, 2, and 3, respectively. The means for the total structures collected per female were 2.88±0.85, 3.00±1.23, and 4.57±1.72 in groups flushed with RL, RL + 1% FBS, and D-PBS, respectively. We conclude that Ringer's lactate solution and Ringer's lactate solution + 1% of FBS and Dulbecco's phosphate-buffered saline showed no significant differences in terms of embryo quality or quantity, suggesting that Ringer's lactate solution is an alternative for collecting embryos in cattle.

Keywords: corpus luteum, embryo quality, embryo recovery, female beef cattle, frozen embryos, superovulation

1. Introduction

Embryo transfer is a reproductive procedure that permits exploitation of females with high genetic value (McCue, 2011) so as to optimize animal breeding (Bülbül et al., 2010). Selk (2002) pointed out that embryo transfer generates a greater number of calves/year/female, accelerating the production of high-production beef and dairy cattle.

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Embryo collections using the transcervical method involve steps that increase costs (Selk, 2002). The steps described in the bovine embryo transfer method range from donor superovulation to recipient preparation with donor (Troxel, 2013). The approximate minimum cost per embryo transfer pregnancy is estimated at $ 250 (Troxel, 2013); needless to say, it is necessary to increase productivity and reduce costs (Viana et al., 2017).

One of the stages of embryo collection is the flushing procedure, consisting of washing the uterus seven days after the first artificial insemination (Troxel, 2013). This phase involves costs related to the solution used in embryo collection (Caiado et al., 2009). In cattle, it is traditional to use Dulbecco's phosphate-buffered saline (D-PBS) solution owing to the satisfactory responses obtained in this species (Curtis, 2015). Therefore, to improve the cost-benefit ratio, alternatives should be sought to lower costs and preserve embryos during the flushing procedure, as well as to achieve a good rate of embryos recovered per number of ovulations (Caiado et al., 2009).

Ringer's lactate solution (RL) is exclusively used in horses for embryo collection, and there is no interference with embryo quality (Imel et al., 1981; Iuliano et al., 1985; Dippert et al., 1992; Alvarenga et al., 1993). In Brown Swiss cows, for example, Bülbül et al. (2010) used RL ($ 1.30/liter) for embryo collection and achieved 69.5% embryo recovery and mean of 6.3 collected embryos and 4.6 viable embryos per female, without evaluating traditional solution (D-PBS: $ 13.44/liter).

Against this background, the objective of this study was to determine the efficacy of RL with or without 1% fetal bovine serum (FBS) for uterine flushing of female beef cattle to recover embryos, to evaluate the quality and quantity of the recovered embryos, and to compare the recovery efficacy with that of D-PBS.

2. Material and Methods

Research on animals was conducted according to the institutional committee on animal use under protocol nº 600570317.

2.1. Location

The study was conducted from January to February 2018 in Castelo Branco, northwestern Paraná (located at 23°25'31" south latitude, 51°57'19" longitude west of Greenwich, and 550 m altitude), with semi-humid climate (66% air humidity), classified as subtropical-temperate climate, with annual mean temperature of 22 °C, minimum of 10.3 °C and maximum of 33.6 °C, and mean annual rainfall of 1,500 mm. The lowest rainfall is from March to August and the highest is from September to February.

2.2. Animals

Twenty-two Wagyu females aged 2-7 years, 14 heifers, and eight cows were subjected to embryo collection. All females presented body condition scoring between 3.75±0.13 to 3.86±0.24 points, classified on a scale of 1 to 5 points, with 1 point for extremely thin females and 5 points for extremely fat females (Jílek et al., 2008).

Females were distributed in three groups in a completely random design. Each experimental group consisted of superovulated females. Seven days after the first artificial insemination, we flushed their uteruses with the following solutions: RL (n = 8), RL + 1% FBS (n = 7), and D-PBS (n = 7).

2.3. Feeding

The daily mean of dry matter intake was 2.5% of live weight, and females were kept on *Urochloa brizantha* pasture of approximately three hectares with a mixture of about 30% of *Cynodum dactylon*, where they remained during the experiment. The forage samples obtained to analyze the composition were taken from 1 m² of the area. Subsequently, dry matter was calculated as the difference of the natural matter and the matter obtained after drying in an oven at 75 °C. The calculated dry matter was 268 g.kg^{-1}.

The forages presented a mean of 60 g.kg^{-1} of crude protein, and analyses were performed using the Kjeldahl method (Kjeldahl, 1883). Analyses for neutral detergent fiber (699 g.kg^{-1}), acid detergent fiber (381 g.kg^{-1}), and lignin (44.9 g.kg^{-1}) were performed using the Van Soest method (Van Soest, 1967).

Females received mineral (MM Reproduction) consisting of 180 g calcium, 90 g phosphorus, 20 g magnesium, 30 g sulfur, 130 g sodium, 1,300 mg copper, 3,000 mg zinc, 30 mg cobalt, 70 mg iodine, 3,000 mg manganese, 20 mg selenium, 60 mg chromium, and 600 mg fluorine/kg of product (Phospec Animal Nutrition Industry and Com. Ltda – Maringá, PR, Brazil).

2.4. Superovulation protocol

Females received a CIDR® device containing 1.9 g of progesterone (InterAg, Hamilton, New Zealand) at random stage of the estrous cycle (D0), placed with specific speculum. On D0, 2 mL (2 mg) of estradiol benzoate (EB) were also administered intramuscularly (Sincrodiol – Ourofino Animal Health Ltda., SP, Brazil). The CIDR remained in the vagina until the eighth day (D8) of the protocol and was withdrawn in the morning. On the fifth day (D5) of progesterone treatment, the administration of porcine follicle stimulating hormone started (FSH-P, Folltropin, Bioniche, Belleville, Ontario, Canada, sold in Brazil by Farmagricola S/A, Mariporã, SP, Brazil), applying one dose in the morning and another in the afternoon, with an interval of 12 h between applications, totaling eight applications in four consecutive days. These intramuscular applications were completed on D8 of the superovulation protocol. The FSH-P dosages for each day are given below (Table 1).

In the morning of D8, 2 mL of prostaglandin F2α (PGF2α - D-cloprostenol) were administered intramuscularly (0.265 mg/mL - Ciosin, Ourofino Animal Health Ltda., SP, Brazil). On the ninth day (D9) of the protocol, in the morning, 2,500 IU of Human Chorionic Gonadotropin were administered (hCG - Intervet International – Netherlands, imported by Merck Sharp & Dohme Animal Health, Cruzeiro, SP, Brazil). In the afternoon of D9, the first fixed-time artificial insemination (FTAI) was performed, and in the morning of D10, the second FTAI (Baruselli et al., 2006), both made with good-quality frozen semen with 20 million sperm/dose, thawed at 35 °C for 30 s in a fertilized semen electronic defroster (Fertilize-Livestock Evolution, Vespaziano, MG, Brazil).

Seven days after the first artificial insemination (AI), the superovulatory response was evaluated by rectal palpation and ultrasonography (Mindray Bio-Medical DP 2200 Vet) to count corpora lutea. A 7.5-MHz linear rectal transducer was used for ultrasound. Females that presented at least three corpora lutea between the two ovaries (Monniaux et al., 1983; Center, 2015) were subjected to uterine flushing according to the groups, randomly.

On the sixteenth day (D16), in the morning, embryos were collected and those graded 1, 2, and 3 were frozen. Table 2 shows the female superovulation protocol.

Prior to uterine flushing procedures to collect embryos, females received low peri-coccygeal anesthesia of lidocaine hydrochloride local anesthetic (Lidovet – Vetmais Agropecuária, Campo Grande, MS, Brazil). The amount of anesthetic used for cows was 4 to 5 mL and 3.5 mL for heifers.

Table 1 - Intramuscular administration protocol (mg) of FSH-P for cows and heifers on days 5, 6, 7, and 8 of the superovulation protocol

Day	FSH-P			
	Cows		Heifers	
	Morning	Afternoon	Morning	Afternoon
5	40	40	30	30
6	30	30	30	20
7	20	20	20	20
8	10	10	10	10

FSH-P - porcine follicle stimulating hormone.

Table 2 - Protocol of female beef cattle superovulation and embryo collection

Time of the day	D1	D5	D6	D7	D8	D9	D10	D16
Morning	Implant P4 + 2.0 mg EB	FSH-P	FSH-P	FSH-P	FSH-P - implant withdrawal P4 + PGF2α	hCG	AI	Collection
Afternoon	-	FSH-P	FSH-P	FSH-P	FSH-P	AI	-	-

P4 - natural progesterone CIDR; EB - estradiol benzoate (Sincrodiol); FSH-P - porcine follicle-stimulating hormone (Folltropin); AI - artificial insemination without heat observation; PGF2α - prostaglandin F2 alpha (Ciosin); hCG - human chorionic gonadotropin (Chorulon).

2.5. Uterine flushing

In the transcervical route, an 18 and 20-port latex Foley catheter was inserted into the uterine body or the uterine horns, when the uterus was large, starting with the horn where the ovary contained the largest number of corpora lutea, using a total of 1,000 mL of solution for each collection in cows (Pascoe et al., 1985) and 750 mL for heifers. The uterine flushing was accompanied by transrectal uterine massage. The procedure lasted 20 to 30 min, including the time taken to fix the catheter in the uterus body or in uterine horns and injected slowly with approximately 10 mL of collection fluid to fill the inflatable cuff (Troxel, 2013).

Embryos were collected on a 70-μ filter (reference nº 19010/8000, Minitube of Brazil, RS; Bülbül et al., 2010), kept in about 30 mL of medium, and then transferred to square Petri dishes with grids for 30X magnification stereomicroscope analyses. Subsequently, the collected embryos were placed in D-PBS enriched with 0.4% bovine serum albumin (BSA; Biomate, Campinas, SP, Brazil), with the formulation described by Dulbecco and Vogt (1954).

In the maintenance solution, embryos were classified under 70X magnification, according to quality grade, considering morphology and age, as recommended by the International Embryo Transfer Society - IETS (Stringfellow and Seidel, 1998). Embryos were classified as grade 1 (excellent and good), grade 2 (fair), grade 3 (poor), or grade 4 (degenerated, underdeveloped, empty pellucid zone, and/or unfertilized oocytes). The percentage of unviable structures was calculated by dividing the unviable structures of each uterine flushing experimental group by the total number of embryos obtained and multiplied by 100.

2.6. Embryo freezing

For freezing, viable embryos (grades 1-3) were loaded into 0.25-mL straws with 1.5 M ethylene glycol freezing medium (Biomate, Campinas, SP, Brazil). The straws were loaded using straight embryo oocytes micropipettes (Biocom Animal reproduction products, Uberaba, MG, Brazil), in the following sequence: a portion of freezing medium, one air bubble, the embryo with the freezing medium plus one air bubble, and finally filling the straw with freezing medium and stopping with a plastic plug (Nutricell, Campinas, SP, Brazil), labelled with donor identification code, date, and quality of the embryo.

To freeze the embryos, the Biocom (Programmable Embryo Freezer Dominium KSE) was used with a capacity to freeze 37 straws of 0.25 mL (Biocom Animal reproduction products, Uberaba, MG, Brazil).

The freezing curve started at 10 °C and reduced to −7 °C, with a drop of one degree per minute. At this temperature, the straws remained for 5 min, then seeding was performed, a procedure that consisted of touching the air bubbles with a nitrogen-frozen forceps to initiate the freezing process. Thereafter, the temperature was reduced by 0.5 °C/min to −35 °C, remaining at this temperature for 10 min. Frozen embryos were stored in a cryogenic cylinder with liquid nitrogen at −196 °C, where they awaited transfer to recipients.

2.7. Statistical analysis

Means of the analyzed variables were obtained per female, which was considered as the experimental unit. Data were analyzed using the GENMOD procedure of SAS (Statistical Analysis System, version 6.0), and the means were compared using contrast and t tests ($P<0.05$).

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The correlations between the number of total embryos and the number of follicles analyzed prior to the superovulation protocol of 3 mm or more were determined using Pearson's correlation coefficient tests. Analyses with P<0.05 were considered significant.

3. Results

We collected 76 embryos from 22 animals subjected to superovulation, of which 52 were transferable, 24 had degenerated, and six were unfertilized structures. Among the transferable embryos, 75% were classified as grade 1 or 2 and 25% were grade 3, according to the Stringfellow and Seidel (1998) criteria.

The numbers of total embryos collected per group were as follows: 23 in the group with uterine flushing with RL, 21 in the group with uterine flushing with RL + 1% FBS, and 32 embryos in the group with uterine flushing with D-PBS. The numbers of transferable embryos were 13, 19, and 20 in the RL, RL + 1% FBS, and D-PBS groups, respectively.

Mean results of live weight (P = 0.552), body condition scoring (P = 0.992), number of viable embryos (P = 0.212), frozen embryos (P = 0.085), and number of total embryos (P = 0.169) obtained per female of a single superovulation showed no differences with the means used for uterine flushing (Table 3). However, there were fewer ovarian follicles in females with uterine flushing with D-PBS, evaluated before starting the superovulation protocol (P = 0.033) (Table 3).

The number of corpora lutea was lower in the group of females that had uterine flushing with RL + 1% FBS (P = 0.007). The percentage of unviable structures was 43.47% in the group in which the uterine flushing was done with RL, 9.52% in those flushed with RL + 1% FBS, and 37.50% in those collected with D-PBS. The lowest percentage was observed in the RL + 1% FBS group (P = 0.016) (Table 3). The embryo recovery rate (P<0.0001) was higher with the medium containing D-PBS, with no difference between RL and RL + 1% FBS.

Table 3 - Performance and breeding parameters of Wagyu females superovulated with FSH-P and collected with alternative medium

Variable	Solution[1]			P-value
	RL	RL + 1% FBS	D-PBS	
	Females			
Number of animals	8	7	7	-
Live weight (kg)	436.88±28.27a	472.29±31.02a	507.00±69.00a	0.552
BCS (1 to 5 points)	3.75±0.13a	3.86±0.09a	3.86±0.24a	0.992
NPF (≥ 3 mm)	26.63±2.03a	19.43±2.34ab	17.71±2.12b	0.033
NCL (day of collection)	23.00±2.44a	16.43±4.33b	22.71±3.00a	0.007
	Embryos			
Recovery rate (%)	13.86±4.23b	15.39±4.61b	27.16±13.33a	<0.0001
Viable embryos	1.63±0.71a	2.71±1.25a	2.86±1.39a	0.212
Unviable structures	1.25±0.49a	0.29±0.18b	1.71±1.25a	0.016
Frozen embryos	1.00±0.76a	2.00±0.95a	2.43±1.41a	0.085
Total embryos	2.88±0.85a	3.00±1.23a	4.57±1.72a	0.169

BCS - body condition scoring; NPF - number of pre-superovulation follicles; NCL - number of corpora lutea on the day of collection.
[1] RL - Ringer's lactate solution; RL+1% fetal bovine serum; D-PBS - Dulbecco's phosphate-buffered saline.
a,b - Means in a line with different letters are significantly different by the t test (P≦0.05).

4. Discussion

The groups of animals presented similar mean weight, ranging from 436.88±28.27 to 507±69.00 kg, higher than the 400 kg found by An et al. (2016), who found similar results of body condition scoring as in the present study, which ranged from 3.75±0.13 to 3.86±0.24 points. The numbers of ovarian

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follicles evaluated by ultrasound before the beginning of the superovulation protocol were lower in the group with uterine flushing with D-PBS. The evaluation was performed without considering the estrous cycle; however, it was performed immediately before superovulation to analyze a possible correlation of embryo recovery. There was no significant correlation between the number of ovarian follicles analyzed in the pre-superovulation and the mean number of total embryos/female (r^2 = 0.2334; P = 0.296). This result may be related to the blood levels of the anti-Müllerian hormone, discussed by Rico et al. (2012) and Sevgi et al. (2019). Rico et al. (2012) collected blood samples one day before the first FSH dose administration in a protocol with progesterone and observed that the anti-Müllerian hormone did not vary during the estrous cycle. However, Sevgi et al. (2019) analyzed anti-Müllerian hormone in the 60th day after superovulation with a progesterone protocol, without considering the estrous cycle phase. The authors analyzed the follicular responses to treatments with follicle stimulating gonadotropins. Batista et al. (2014), working with *Bos taurus* (Holstein) heifers, found that, in those with low (13.4) and high (34.3) numbers of ovarian follicles, the anti-Müllerian hormone values ranged from 0.06 to 0.57 mg/mL in the plasma, respectively. In *Bos indicus* heifers (Nellore), the authors found a plasma concentration of the hormone of 0.78 mg/mL in those with 28.4 ovarian follicles and 1.20 mg/mL in those with 48.1 ovarian follicles. These authors worked with Holstein and Nellore; when the females had their ovarian follicular waves synchronized, serum samples were collected at 0, 60, and 120 days to measure anti-Müllerian hormone levels. The estrous cycle period was not considered.

In our experiment, hormone levels were not analyzed; however, it may be possible to infer that serum levels of anti-Müllerian hormone were similar in all animal groups, since, according to Rico et al. (2012) and Sevgi et al. (2019), higher concentrations of this hormone favor the production of embryos. The numbers of viable embryos in the animals of this study did not differ among the groups, with values similar to those observed by Sekizawa et al. (2012), Takahashi et al. (2013), and Facioli et al. (2020), when conducting research on the same breed of animals used in this study.

A recent study reported slightly larger total and viable embryos/female (An et al., 2016); nevertheless, these are considered lower than those observed in the European and Nellore breeds (Baruselli et al., 2006; Viana et al., 2017).

The number of unviable structures (degenerated, underdeveloped, empty pellucid zone, and/or unfertilized/female) observed in our study ranged from 0.29±0.18 to 1.71±1.25, similar to those observed by An et al. (2016) and Mapletoft et al. (2002). An et al. (2016) observed a variation ranging from 0.6 in Wagyu cattle to 1.0/female of degenerated structures using Folltropin. Mapletoft et al. (2002) showed degenerate structure variations ranging from 0.6 to 1.5/female. However, in the present study, a smaller number of unviable structures/female were observed in the group that had uterine flushing with RL + 1% FBS, the same as in those where uterine flushing was performed with RL and D-PBS (Table 3). We can discern no scientific explanation for this finding. By contrast, we used the 1% FBS to enrich the RL medium as did Alvarenga et al. (1993) in mares. Fetal bovine serum contains nutritional factors, growth factors, chelators of heavy metals (Abe and Hoshi, 2003), and vitamins (Abdel-Wahab et al., 2018). It also possesses potent antioxidant activity (Mahmoud and Nawito, 2003), many hormones, minerals, trace elements, lipids, and detoxifying factors (Gstraunthaler, 2003). Fetal bovine serum has properties that are beneficial for the embryos during the flushing procedure (Abe and Hoshi, 2003). In addition to the benefits of FBS on embryo collection, it can also be assumed that embryo production could be related to animal physiology and individuality (Monniaux et al., 2010). The major problem in commercial embryo production is the superovulatory response following treatment with gonadotropins such as FSH.

Frozen female embryos classified in grades 1 to 3 (Stringfellow and Seidel, 1998) showed no differences (P = 0.085) between the evaluated groups (Table 3), and information on the pregnancy rate is not yet available. However, performing superovulation on Wagyu females with Folltropin and freezing embryos with 1.5 M ethylene glycol solution, An et al. (2016) obtained 35.2% of pregnancy by thawing embryos and transferring them to recipients. The same authors obtained five viable frozen embryos/female

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from 24 donors, a response that differs from that found in the present study, in which there was a mean of 2.46 frozen embryos/female. Barros and Bényei (2000) reported that, when performing uterine flushing of Holstein-Frisian donors superovulated with FSH-P (Pluset, Serono, Rome, Italy), D-PBS, and zwitterionic medium, the means observed were of 43.15 and 36.15% of pregnancy using thawed embryos of grades 1 and 2, respectively. Barros and Bényei (2000) obtained 8.4±6.8 total structures when flushing the uterus of bovine females with zwitterionic medium and 7.8±6.3 with D-PBS.

The total number of embryos was 2.88±0.85 in females in which uterine flushing was done with RL, 3.00±1.23 in those with RL + 1% FBS, and 4.57±1.72 in those in which D-PBS was used (Table 3), resulting in a mean of 3.48 embryos/female, lower than the results obtained by An et al. (2016) of 6.2 embryos/donor, and Facioli et al. (2020) of 4.54 embryo/donor. Research from other countries studying Wagyu breed showed reduced number of embryos obtained/female, ranging from 0.20±0.03 total embryos and 0.10±0.02 viable embryos (Sekizawa et al., 2012) and total structures of 10.00±7.6 and 4.5±3.9 of viable structures (Takahashi et al., 2013).

The low embryo production (recovery rate) might be due to the Japanese black cattle breed (Wagyu) that has improved metabolism that contributes to higher marbling scores compared with other breeds. However, this may affect other aspects of the animal's physiology, including hormone secretion and their reproductive performance, including their response to the synchronization protocols and embryo production (Facioli et al., 2020).

Results aimed at finding less expensive alternatives to D-PBS are important for Wagyu embryo transfer. Our data indicated with 95% confidence that it is possible to perform uterine flushing with RL with or without FBS, because statistically, the number of total, viable, and frozen embryos obtained in this experiment did not differ among flushing procedures. It was also the case that the group in which females had uterine flushing with RL enriched with 1% FBS had the lowest number of unviable structures among the experimental groups.

The recovery rate was higher in the females flushed with D-PBS than in those flushed with RL or RL + 1% FBS. This may have been due to the individuality of the animals as commented by Monniaux et al. (1983). According to Alvarenga et al. (1993), there was no influence of RL or D-PBS on embryo collection in mares with respect the recovery rates or pregnancies, even when these media were supplemented with 1% FBS.

It is worth noting that the media for embryo collection should have a pH varying from 7.2 to 7.6 and osmolarity around 300 mOsm (Wright Jr. and Bondioli, 1981; Contu et al., 2002). All media utilized in our experiment contained 6 g/L of NaCl in the RL and 8 g/L of NaCl in the D-PBS. According to Wright Jr. and Bondioli (1981), generally, it is suggested that the principal function of NaCl is to regulate the osmolarity of the medium, maintaining it around 300 mOsm. However, it appears that the effect of osmolarity of the media on embryonic development is minimal (Wright Jr. and Bondioli, 1981). Astiazaran and Longoria (1988) studied collection and embryo transfer in rats and cows using Hartmann's physiological solution, which is easier to obtain than D-PBS; according to these authors, the composition of Hartmann's medium is very similar to that of RL (Astiazaran and Longoria, 1988). According to Ayebale et al. (2017), RL is an isotonic balanced salt solution very similar to the ionic composition of the blood.

5. Conclusions

Ringer's lactate solution or Ringer's lactate solution + 1% fetal bovine serum are as efficient as Dulbecco's phosphate-buffered saline solution with respect to the quality and quantity of embryos. Therefore, both solutions could be used as alternatives for embryo collection.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: G.V. Moraes, R.C. Braganholo and F.L.B. Cavalieri. Data curation: G.V. Moraes, R.C. Braganholo and F.L.B. Cavalieri. Formal analysis: E. Gasparino. Funding acquisition: L.P. Rigolon. Investigation: G.V. Moraes and R.C. Braganholo. Methodology: G.V. Moraes, R.C. Braganholo, F.L.B. Cavalieri, L.P. Rigolon and E. Gasparino. Project administration: G.V. Moraes. Resources: L.P. Rigolon. Supervision: G.V. Moraes and L.P. Rigolon. Visualization: G.V. Moraes, R.C. Braganholo and T.C. Santos. Writing-original draft: G.V. Moraes, R.C. Braganholo and T.C. Santos. Writing-review & editing: G.V. Moraes, R.C. Braganholo and T.C. Santos.

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