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Effect of the Strategic Supplementation for Producing Embryos of Native Hartón del Valle Heifers

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Abstract: Nutritional imbalances have been confirmed in other breeds, related to energy, proteins and minerals and this affects embryonic development, production of quality. In this regard, the objective of this research was to evaluate the effect of strategic supplementation for the production of embryos in native Hartón del Valle heifers. For that purpose, 18 pure heifers were used, fed with a diet containing 0.708 kg of crude protein and 73.58 kg of ME Mjul. The economic evaluation was carried out using the partial budgeting technique. Treatments consisted of T1: Without supplementation, T2: Supplementation, 42 days before protocol, T3: 42 days of supplementation, 26 days before and 16 days during protocol. Variables evaluated were number of structures, transferable embryos, number of Non-Transferable Embryos (NTE), number of corpora lutea, follicles and embryonic quality. Non parametric analysis of variance was performed, according to a completely randomized design and comparison of proportions (chi-square). When analyzing the proportion of NTE Vs. the total, it was lower ($p<0.05$) in supplemented treatments, regardless of the period in which supplementation was carried out. Supplementation, in both phases of the protocol, increased the proportions of early morulae and blastocysts ($p<0.05$). In the economic analysis, profits increased by 2.05 and 1.53 times in favor of the supplementation. Supplementation to Hartón del Valle heifers, before and during superovulation, favored the biological response of collected structures, number of follicles and corpora lutea. Similarly, the proportion of transferable embryos, early morulae and quality one blast cells increased, in addition to achieving a higher cost/benefit relationship.

Keywords: *Piptocoma discolor*, Putumayo, Blastocyst, Superovulation

Introduction

In the technological development of cattle rearing field, there is a search for optimizing the conservation of genetic resources (Prenteci and Anzar, 2011). This is also applied to assisted reproduction. In this regard, embryo production is one of the methods for boosting livestock production in the world mundo (Borge *et al*., 2019). In native Hartón del Valle cattle, this method is a tool for germplasm conservation, used in Colombia, with institutions such as the Colombian Corporation for Agricultural Research (AGROSAVIA) and private farms (Martínez, 2019). Native breeds are considered to be in danger of extinction. However, variability of embryonic response depends on different factors such as environmental, technical, genetic and nutritional management. The latter takes relevance due to the effect of nutrients on embryonic development (Buerkle, 2007).

In some cases, nutritional balance of donors is inappropriate due to specific conditions of soils (Staal *et al*., 2020). In the Colombian Amazon, the problem increases because soil is unsuitable for livestock activity, which makes soil-plant-animal relation to be limited for some nutrients nutrientes (Suárez *et al*., 2018; Mathios Flores *et al*., 2019; Staal *et al*., 2020), especially when it is necessary to fulfill the minimum requirements

of bovines to obtain favorable results, in terms of oocyte quantity and embryo quality (Calderón *et al*., 2017).

This response is given by the development of antral follicles, mediated by the Follicle-Stimulating Hormone (FSH) action, as one of the important factors that affect embryo production in cattle (Ibtisham *et al*., 2018). Antral follicle development is correlated with multiple ovulation. A nutritional flushing could increase maturation of primary follicles, due to the increase of Insulin Growth Factor (IGF1) insulina (Larson and White, 2016). These factors increase the sensitivity of granulosa cells and stimulate receptors for Luteinizing Hormone (LH). However, a high amount of insulin in blood compromises embryo viability and quality (Rossetto *et al*., 2016).

In this context, the Amazonian piedmont includes a wide variety of species, suitable for animal feeding animal (Moniruzzaman and Miyano, 2010; Sotelo *et al*., 2017, Riascos-Vallejos *et al*., 2020a). This fact allows to state that there are forage resources of high quality in the Amazonian area, with potential for animal feeding. Supplements based on these species, with proper contents of protein, energy, minerals and degradability, compensate animal requirements because livestock production systems, in this region, base their feeding on native grasses (Riascos-Vallejos *et al*., 2020b; Cardona-Iglesias *et al*., 2016).

A diet that meets all nutritional requirements, in different periods, could have a positive effect on embryo production. Therefore, the objective of this research was to evaluate the effect of a strategic supplementation for embryo production of Hartón del Valle heifers.

Materials and Methods

Location

This research was conducted in Villa Lucero farm, at 0°35'25.6"N 76°32'05.3"W, in Putumayo department, Republic of Colombia, at 256 m.a.s.l. There is 25.3°C as mean temperature, 85% of relative humidity and 3,355 mm of average annual rainfall (IDEAM, 2017), belonging to a life area of tropical humid forest (Holdridge, 1987; Landínez-Torres, 2017).

Animals and Reproductive Diagnosis

For this study, 18 Hartón del Valle heifers were selected, with 34±2 months of age and body weight of 340±20 kg, were selected. Prior to the beginning of experimentation, these animals underwent a general physical examination, including the evaluation of morphology and integrity of their reproductive tract, according to Schneider methodology (Schneider *et al*., 2012). A portable ultrasound equipment (Econ Control Medical Ref. IMAGO), with a 7.5 MHz linear transducer, was used, to confirm the absence of pregnancy, assess ovarian condition and verify the absence of cysts, adhesions and other pathologies. Furthermore, the absence of fibrosis and malformations was confirmed by passing through the cervix with a catheter.

Feeding

Heifers were fed a diet containing 0.690 kg of crude protein and 79.53 kg of Metabolizable Energy (ME Mjul kg DM^{-1}). They received a supplement containing *Piptocoma discolor* (28, 29), corn, soybean cake, corn bran, molasses, palm oil and mineral premix (Table 1).

A supplement of 2 kg was offered per day in the period of 7:00 bm-9:00 am, then they went to grazing *Brachiaria decumbens* was used as forage source (Ruíz and Menchaca, 1990). Food balance calculation was performed according to nutritional requirements (NRC, 2001). It was carried out with CALRAC® computer program (version 1.0).

Proximal chemical analyzes (Table 1) were calculated, according to procedures and recommendations established by (AOAC, 2016), as follows: Humidity content (Method 930.04), crude protein by Kjeldahl method (N*6.25) (method 955.04), ashes (calcination at 6,000) (method 930.05), ether extract (method 962.09) and crude fiber (method 920.39). Gross Energy (GE) was determined with a calorimetric pump. Digestibility of Dry Matter (DM) was reached according to (Ariza-Nieto *et al*., 2018) methodology and *in situ* ruminal degradability by (Chen *et al*., 1990; Mehrez and Orskov, 1997).

Superovulation Protocol and Embryo Collection

P36/L60 superovulation protocol, developed by (Baruselli *et al*., 2006; Moraes *et al*., 2007), was used. This protocol started on day 0 with the placement of an intravaginal device (CIDR® Pfizer Lab), filled with 0.5 g of natural progesterone, plus an Intramuscular (IM) injection of estradiol benzoate at a dose of 2.5 mg (EB; Ric-Be, Syntex SA, Argentina), 600 IU of FSHp and 600 IU of LHp (Pluset® Calier Lab, Spain) in four days, with decreasing applications at 12 h intervals, two doses of cloprostenol sodium (Estrumate® Schering- Plough Lab), each dose of 750 mcg, one in the morning and one in the afternoon on day 6 and the device was removed on day 7. After five days of the beginning of Superovulation (SO), estrus was detected and animals were inseminated at 12, 24 and 36 h post estrus, with a commercial semen of proven fertility (belonging to the same breed). On day 8, 0.25 mg of a synthetic GnRH gonadorelin analog (Fertagyl® Intervet Lab) was applied. On day 15 of treatment, embryo collection was carried out by uterine lavage with a two-way catheter. On this same day, the biological material obtained in filters was evaluated and an intramuscular injection of 750 mcg of cloprostenol sodium (Estrumate® Schering-Plough Lab) was administered (Fig. 1).

Table 1: Composition of experimental diets (DM)

	Chemical composition (%)		
Ingredients	Supplement	*Piptocoma discolor*	*Brachiaria decumbens*
Dry matter, %	89.81	28.86	27.99
Ashes %	3.80	8.17	7.31
Ether extract, %	6.98	3.95	1.95
Crude protein, %	11.78	21.51	6.33
Crude Fiber, %	4.38	8.63	33.97
ME Mjul $kgDM^{-1}$	11.76	10.26	6.63
DM digestibility		74.71	
DM degradability	81.58		53.92
Ingredient		Quantity (%)	
Piptocoma discolor		20.00	
Corn meal		43.00	
Soy bean cake		0.20	
Corn bran		30.30	
Palm oil		0.50	
Molasses		5.00	
Microminerals [a]		1.00	

[a]Micromineral premix content: Magnesium 10%, zinc 10%, iron 10%, copper 2%, iodine 0.12%, selenium 0.06% and cobalt 0.02%. DM: Dry Matter

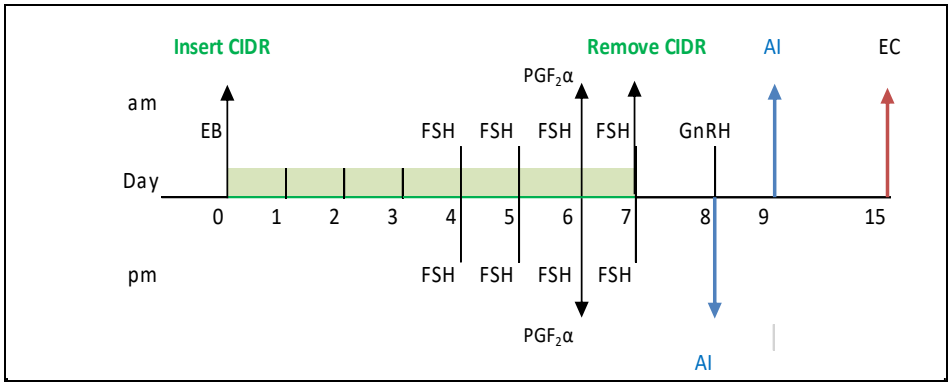

Fig. 1: Superovulation protocol and treatments

Embryo Collection

Embryos were collected seven days after heat was detected. Donors were placed in a restrain cage, where dorsal coccygeal and perivulvar areas were cleaned and disinfected, applying between 5 and 8 mL of 2% lidocaine epidurally. A two-way Foley catheter was vaginally inserted, adjusting it in the horn curvature, to perform uterine infusion-collections. An amount of 1.5 L of phosphate buffer solution was used, PBS with penicillin (100 mg mL^{-1}) and streptomycin (100 mg mL^{-1}) (ViGro™ Complete Flush Solution®) (Dulbecco and Vogt, 1954).

Embryo Search and Evaluation

On embryo collection day (day 15) and on insemination day (day 8), the number of follicles and corpora lutea were determined by rectal palpation, respectively. Once the lavage of each donor was completed, filter content was placed in a 100×100 mm square Petri dish with a gridded background and put in a reflected light stereo microscope with a magnification range from 6.7 to 45 X, for searching embryos. A 35-mm Petri dish was prepared with a maintenance solution (Syngro tm Holding. Bioniche®) to place collected embryos. Selection and classification of collected structures (embryos and oocytes) were performed following the procedure indicated by the International Embryo Transfer Society (IETS) (Stringfellow and Seidel, 1998).

Embryos were classified according to their development stage, on a scale from 1 (one cell stage) to 9 (hatched blastocyst stage) and according to their quality as 1 (excellent), 2 (good), 3 (fair) and 4 (degenerate) (Phillips and Jahnke, 2016). For cryopreservation, morula and blastocyst stage embryos with qualities 1 and 2 were placed in a freezing medium with 10% ethylene glycol and 0.3 M sucrose and placed into French straws (Baruselli *et al*., 2015).

Variables, Treatments and Periods

Evaluated response variables were Number of Structures (NS), Transferable Embryos (TE), number of Non-Transferable Embryos (NTE), number of Corpora Lutea (CL), number of Follicles (F) and extraction percentage (% E) = (Number of embryos/CL) * 100 and embryonic quality. Treatments consisted of T1: Without Supplementation (WS), T2: Supplementation, 42 days Before Protocol (SBP), T3: 42 days of Supplementation, 26 days Before and 16 days During Protocol (SBDP).

Economic Analysis

The evaluation was carried out using the partial budgeting technique, which allows to measure direct economic impact of the technological alternative that is intended to be implemented through the relationship between costs and incomes generated by it, compared to the technology that is to be replaced or improved (Mendieta, 1996).

Statistical Analysis

Three groups of animals were used, six replicates each, which were selected from a group of 30 animals. For the variables number of follicles, number of collected embryos and corpora lutea, a non-parametric (Kruskal and Wallis, 1952) analysis of variance was used, according to a completely randomized design. For the comparison of mean ranges, (Conover, 1999) test was applied for $p<0.05$. For variables TE, NTE and quality of the stages in early morula, morula and blastocysts, a Chi-square comparison proportion analysis (Font *et al*., 2007) was performed and (Duncan, 1995) test was applied for $p<0.05$, in the necessary cases. The statistical program InfoStat (Di Rienzo *et al*., 2012) was used for processing information.

Results

Number of Follicles and Corpus Luteum

Table 2 shows results of the effect of supplementation period. No differences were detected among treatments under evaluation. Biological responses of follicles and corpora lutea were in the range of 6.17 to 8.33 and 5.5 to 7.17, respectively. These results were similar to those reported by (Acosta *et al*., 2016), who determined the effects of methionine and choline supplementation during prepartum and postpartum periods in embryos before their implantation in Holstein cows.

Results regarding number of structures demonstrated that there were no differences among supplementation periods, with means from 2.0 to 3.0 ($p = 0.6456$) structure treatment^{-1}. This agrees with reports of (Estrada, 2018), who studied estimated means of structures for seven breeds of native cattle, which ranged between 0.3 and 3.9 viable embryos.

Regarding percentage of extraction, there are no statistical differences among supplementation periods, with means between 38.63 and 42.22 ($p = 0.5779$). In this sense, response capacity to nutritional programs increases when animal requirements of these programs are fulfilled, depending on soil-plant-animal relationship (Silva *et al*., 2016).

Table 2: Response of native Hartón del Valle heifers to supplementation

Treatment Variables	Without supplementation	Supplementation before the period	Supplementation before and during the period	Sign.
Follicles	7.92 (6.33)	7.50 (6.17)	13.08 (8.33)	p = 0.1220
	SD = 1.97	SD = 2.23	SD =1.75	
Structures	11.08 (3.00)	8.33 (2.00)	9.08 (2.50)	p = 0.6456
	SD = 1.26	SD = 2.00	SD = 2.74	
CL	7.58 (5.50)	8.00 (5.67)	12.92 (7.17)	p = 0.1460
	SD = 1.64	SD = 2.07	SD = 1.17	
% Extraction	11.33 (58.33)	8.75 (42.22)	8.42 (38.63)	p = 0.5779
	SD = 26.67	SD = 46.51	SD = 42.56	

() General means SD: Standard Deviation

Table 3: Embryo production in native Hartón del Valle heifers

	Transferable embryos		Non-transferable embryos		Total structures	
Treatment	No.	%	No.	%	No.	%
WS	10	31.25	8	61.54[a]	18	40.00
SBP	9	28.12	3	23.08[b]	12	26.67
SBDP	13	40.63	2	15.38[b]	15	33.33
SE and Signif.	±8.33 p>0.05		±13.07 p<0.05		±7.03 p>0.05	
Total	32	100	13	100	45	100

[a.b.c]: Different letters per column indicate significant differences for $p<0.05$; WS: Without Supplementation, SBP: Supplementation Before the Period, SBDP: Supplementation Before and During the Period

Transferable Embryos

Proportion of transferable embryos, non-transferable embryos and total structures, obtained by the collection in each treatment (Table 3), indicate that TE percentage per treatment, regarding total TE, showed no difference among them, ranging between 28.13 and 40.63%. However, NTE proportion, with respect to total, was lower ($p<0.05$) in supplemented treatments, regardless of the period in which it was carried out.

The aforementioned could be favored by supplementation, which provided an energy balance from transfer up to recovery of embryos in cattle, as proposed by (Shorten *et al*., 2018). In addition, it is stated that cows with higher energy balance have lower embryo losses (Obeidat *et al*., 2019).

Figure 2 shows results of the excellent quality of collected structures. It demonstrates that, when supplementing in both protocol phases, early morulae and blastocyst proportions were increased ($p<0.05$).

While in morulae, there were no differences among studied treatments. Snider *et al*. (2019) used a registered nutritional supplement and the results indicated an improvement of embryo quality and a decrease of non-transferable embryos, with a supplementation period of 49 days, before embryo collection.

Results of the current research were consistent with those reported by (Sales *et al*., 2015), who studied *Bos Taurus* and *Bos indicus* donor cows, fed a maintenance diet (maintenance energy and 1.7 higher than maintenance), 21 days before the beginning of superovulation protocol. *Bos indicus* cows, with a higher energy diet, had better oocyte quality and superior number of viable oocytes.

Economic Evaluation

Table 4 shows the economic evaluation. Expenses for superovulation inputs represent between 72.0 and 86.1% of total expenses. Supplementation expenses represented 17% of total expenses in the treatments in which supplementation was use in any of the stages.

Profits increased by 1.53 and 2.05 times when comparing supplementation treatments in both stages and non-supplementation.

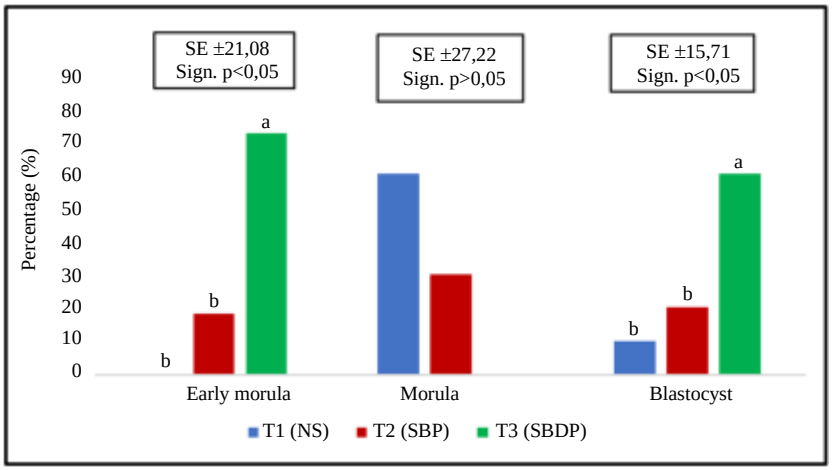

Fig. 2: Excellent embryonic quality per treatment; [a,b]: Different letters indicate significant differences for $p<0.05$

Table 4: Economic analysis of embryo production, according to supplementation periods with 20% of *Piptocoma discolor*

	Treatments		
Indicators	T1	T2	T3
Expenditure			
Supplement intake (kg heifer^{-1})	0	29,40	29,40
Total expenses of food ($)	6.5	6.5	6.5
Total input expenses ($heifer^{-1})	124,50	124,50	124,50
Labor expenses ($heifer^{-1})	12.50	12.50	12.50
Total cost ($heifer^{-1})	143.58	172,98	172,98
Total transferable embryo heifer^{-1}	1.67	1.5	2.17
Total cost ($ embryo^{-1})	85.98	115.32	79.71
Income			
Transferable embryo sale income ($)	334.0	300.0	434.0
Profit ($)	190.42	127,02	261.02
Cost/benefit relationship	2.33	1.73	2.51

This favored the increase of cost/benefit relationship in 7.72 and 45.09%, after comparing the same treatments.

Discussion

Regarding the number of structures (González *et al*., 2017), found that the measured ovulatory response in heifers and found an average of 2.0±1.1 embryos per animal. Supplementation favors the response to number of follicles (Occhio *et al*., 2019), which could be related to changes in dry matter intake or during the supplementation period (Kumar and Laxmi, 2015) and this may propitiate an early embryonic development (Meléndez and Bartolomé, 2017).

These results were consistent with those reported by Molina-Coto *et al*. (2018). In that study, 300 mL of Propylene Glycol (PPG) were orally provided per day at the beginning of superovulation protocol. It was inferred that, despite PPG was a gluconeogenic supplement and ovaries were glucodependent, PPG supplementation did not affect the amount of collected embryos and follicles (Pérez-Clariget *et al*., 2017; Ribeiro, 2018). Results of the experiment suggested that other factors unrelated to the effect of supplementation, affected animal response to superovulation and embryo collection treatments (Ruiz *et al*., 2017).

Another factor was breed, which affects embryonic development during *in vitro* production, with the use of Swedish red breed as oocyte donors, there were differences with red Holstein regarding percentage of blastocysts (Ballesteros *et al*., 2017). Results of different studies showed that crosses and Scandinavian red breeds had better fertility than pure Holstein breed (Heins *et al*., 2006; Aguilar *et al*., 2018). This performance in native cattle could increase circulating glucose concentrations (Leane *et al*., 2018), which are positively associated with ovulation rates and *in vivo* follicular growth and, therefore, with a greater number of transferable embryos (Guanga *et al*., 2020; Laskowski *et al*., 2016).

For Chagas *et al.* (2007) sated that one of the reasons no significant differences were obtained in PPG supplementation, despite a different biological response, was that donors maintained an adequate base nutrition and body condition during the experiment. This limited the response to any supplement. However, improvement was achieved when it was provided to animals with low body condition. These studies were consistent with Hurley and Doane, (1989), who found that superovulated cows with an optimal feeding scheme, supplemented with selenium, showed 100% of fertilized structures, compared to 41% of fertilized egg cells in non-supplemented cows.

Likewise, Abuelo *et al*. (2015) evaluated the response of Angus heifers to superovulation protocols, which were fed mineral mixtures from organic and inorganic sources for 23 days with respect to a non-supplemented group (C). Although similar amounts of TE were collected per treated heifer, animals of the group of organic sources averaged 0.44 NTE per female treated at the time of collection, which were lower values than those found in the current experiment. Published information indicates that populations of native cattle have, as an average, lower number of transferable embryos than those obtained from other breeds (Mikkola *et al*., 2020; Robinson *et al*., 2019).

At the beginning of supplementation, an energy deficit compromises the high levels of urea, which can lead to changes that may produce infertility. This could alter the protein-energy relationship and be the cause of poor embryonic development (Crociati *et al*., 2017). This could explain the decrease of the number of non-transferable embryos with the use of supplementation (Summers *et al*., 2018; Kruse *et al*., 2016).

However, supplementation effects on embryo obtaining were contradictory. Thus, Hackbart *et al*., (2017) reported that supplementation with PPG reduced total ovulations and fertilization rate. This flexibility in the results of this experiment suggests that response of cows to multiovulation was affected by multiple factors, including paternal effect (Rani *et al*., 2018; Mikkola and Taponen, 2017; Chinchilla-Vargas *et al*., 2018). This fact makes difficult to evaluate animal response to a supplement. The supply of 2.0 kg MF^{-1} of animal $supplement^{-1}$ day^{-1}, with an effect on embryonic development, could improve physiological conditions of the animal (Perez *et al*., 2017; Gallego *et al*., 2017; Moscoso-Piedra and Cabrera, 2019; Kasimanickam *et al*., 2020).

Contrary to the findings of this study, for Serrano-Pérez (2020) found that undernourishment did not affect pregnancy in the first week of gestation, as well progesterone levels (Stephenson *et al*., 2018; Noya *et al*., 2020). Sartori *et al*. (2016) showed that Gyr cows had a decrease in blastocyst rate (46.9 Vs. 25.7%) during dry period, in which a diet of 1.7 over the maintenance was offered, for more than 60 days. However, these authors concluded that blastocyst rate was the same in non-lactating Holstein cows before or after being overfed for more than 60 days.

Although oocyte intrinsic quality determines developmental competence, that is, oocyte proportion that becomes blastocysts, environmental conditions have a major impact on their quality (Cuevas *et al*., 2018; Tahuk *et al*., 2018). In fact, management conditions influence on the moment of embryonic development, as the first division, beginning of a superior genomic activation and blastocyst quality (Zullo *et al*., 2016; Gerger *et al*., 2017; Byrne *et al*., 2018).

On the other hand, the number of embryos in this study were low, compared to higher means in other breeds, possibly due to factors such as age and physiological status. Kenny *et al*. (2018); Krause *et al*., (2017) reported improvements in oocyte competition,

fertilization and formation rates of blastocysts with the aging of animals.

According to Nieddu *et al*., (2015) it is probable that, with a severe nutritional restriction, with 0.6 of energy requirements for maintenance during the first 100 days of gestation, the number of antral follicles decreases in undernourished mothers.

Results of a 42-day supplementation with treatment 3 were similar to those of Gardinal *et al.* (2018). These authors evaluated different durations of Whole raw Soybeans (WS) supplementation during prepartum period in embryo quality of transition cows. All groups receiving WS during prepartum period showed the highest value in embryonic quality. As stated by Santos *et al.* (2016), a gluconeogenic and protein source helps the complete metabolism of glucose that favors reproduction. In turn, blastocyst morphology and its quality are related to glucose consumption. Thus, blastocyst low quality is associated with a lower glucose consumption and the high amount of energy in the diet increases insulin receptors and excesses affect the development of blastocysts (Gobikrushanth *et al*., 2018).

Studies conducted on humans used C13 fatty acids in hours after culture (when the embryo has 8 cells or more). They improved embryonic quality, which was more important in the final development stages because it stimulates protein kinase C, essential for cell differentiation and growth (Gardner *et al*., 2011).

Melatonin supplementation reduced apoptosis, increased the proportion of Inner Cell Mass (ICM) to total cells and improved the development of bovine embryos generated *via* Somatic Cell Nuclear Transfer (SCNT) both *in vitro* and *in vivo*. Moreover, global H3 acetyl lysine 9 (H3K9ac) level was significantly elevated in the melatonin-treated SCNT group, indicating that the addition of melatonin may affect nuclear reprogramming, resulting in improved blastocyst quality (Yun-Wei *et al*., 2018). However, Yun-Wei *et al*., (2018) stated that flushing only during the period prior to insemination is insufficient and that it should be maintained for at least 21 days after insemination for a positive effect on fertility through ovarian activation (López-Gatius *et al*., 2017).

Results of the current study agree with Gamarra *et al.* (2018), who observed a strong positive relationship among circulating levels of Anti-Müllerian Hormone (AMH) and *in vivo* embryo production after superovulation in cattle. In this regard, nutritional factors, which can be balanced by supplementation, seem to influence plasma AMH levels (Souza *et al*., 2015; Baruselli *et al*., 2011), which positively influences on the number of excellent quality embryos in embryo transfer programs.

Values referred in the current study could be related to the amounts of amino acids due to the effect of the microbial protein, according to studies of Chavatte-Palmer *et al*. (2018). For authors like Stokes *et al*., (2017), total protein concentration in the follicular fluid was an indicator of the quality of the oocyte, expression, presentation and concentration of different proteins, which varied with follicle size. Furthermore, fluid composition influenced on oocyte quality. Several studies agree that factors associated with good embryonic quality require simple carbohydrates, pyruvate, ATP, microminerals, antioxidants and vitamins (López-Velázquez *et al*., 2019; Cajas *et al*., 2019; Torres Osorio *et al*., 2019; Abdelatty *et al*., 2018; Agarwal *et al*., 2018).

Regarding the economic the costs of embryo transfer technique, it was only suitable for registered pure herds or for genofund conservation (Naranjo-Chacón *et al*., 2016).

Nevertheless the costs of this study are higher than those reported by Castaño (2016) with a conventional embryo transfer protocol and without supplementation with $ 50.85 per embryo. These costs are relatively low, since *Bos taurus* breed was used, in which the average number of embryos was higher. This increased cost/benefit relationship by achieving a better embryonic response by hormonal methods (Baracaldo Camargo, 2018).

It was recently determined that orally administered propylene glycol to Holstein heifers increased the number of follicles and blastocyst quality of heifers having higher plasma Anti-Müllerian Hormone (AMH) levels. Therefore, measuring AMH before applying FSH programs to cows will allow professionals to increase the number of produced embryos and thus, reduce costs per produced embryo (Sánchez-Castro, 2017).

Conclusion

Supplementation to Hartón del Valle heifers, before and during superovulation, favored the biological response of collected structures, number of follicles and corpora lutea. Similarly, the proportion of transferable embryos, early morulae and quality one blastocytes increased, in addition to achieving a higher cost/benefit relationship.

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Author Contributions

All authors equally contributed in this work.

Conflict of Interest

The authors have no personal financial or nonfinancial competing interest in the product.

Data Availability Statement

Datasets generated for this study are available on request to the corresponding author.

Contribution to Field Declaration

In many species, local genetic resources are indiscriminately crossed with foreign breeds, leading to a reduction or extinction of locally adapted breeds that are promising to adjust to climate change. Native animals demonstrate their ability to survive despite very precarious and inhospitable conditions, such as those found at the time of their introduction by conquistadors. In addition, it is demonstrated that small existing native groups of animals have a high diversity degree, which indicates a great genetic potential for selection and crossing to produce crossbred animals for their inclusion in the milk and meat production chain. The conservation of genetic resources of native breeds, through embryo collection and diet improvement of supplementation with forages from the Amazonian piedmont, is theoretically important for having updated information regarding the use of local resources for bovine feeding and ovarian activation, in order to increase the production of quality embryos and improve gestation rates.

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