

Factors affecting the *in vitro* production of bovine embryos in a commercial program

Fatores que afetam a produção *in vitro* de embriões bovinos em programa comercial

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Received: 01/22/2021 | Reviewed: 01/25/2021 | Accept: 02/01/2021 | Published: 02/09/2021

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Abstract

Variability in the production of bovine blastocysts per session of ultrasound-guided follicular aspiration (OPU) is still an obstacle in commercial production. Thus, the objective of the current work was to verify factors that influence *in vitro* embryo production (PIVE), using data from a commercial laboratory. Data from 2014 to 2016 were analyzed, referring to 799 OPU sessions of adult bovine females of the breeds: Nelore (n=83), Girolando (n=73), Brangus (n=49), HPB (n=20), and Senepol (n=10). The influence of five variables was analyzed: breed, genetic group, frequency of aspiration, type of semen (sexed/conventional), and seasonality (spring/summer vs. autumn/winter) on the rate of viable oocytes, cleavage, and blastocyst production (bl). The protocols for the *in vitro* production of embryos (IVP) followed the routine established by the partner laboratory of the present study. The statistical analysis of the data was performed using SAS, with the Chi-square test. The Girolando and Nelore breeds had a higher (p=0.0001) number of blastocysts/OPU session, as well as the *Bos indicus* blood group. Blastocyst production was higher (p=0.0059) with sexed semen compared to conventional semen (6.6 x 5.2 bl/OPU session); a higher frequency of aspirations also increased (p=0.005) the number of bl/OPU session (7.7 x 5.9). We conclude that the *in vitro* production of bovine embryos is influenced by the analyzed factors and knowledge of these variables could guide the commercial use of OPU-PIVE.

Keywords: Blastocyst; *Bos indicus*; Girolando; Ultrasound-guided follicular aspiration.

Resumo

A variabilidade na produção de blastocisto bovinos por sessão de aspiração folicular guiada por ultrassom (OPU) ainda é um entrave na produção comercial. Assim, objetivou-se verificar os fatores que influenciam a produção de embrião *in vitro* (PIVE), utilizando dados de um laboratório comercial. Foram analisados dados do ano de 2014 a 2016, referentes a 799 sessões de OPU de fêmeas bovinas adultas das raças: Nelore (n=83), Girolando (n=73), Brangus (n=49), HPB (n=20) e Senepol (n=10). Analisou-se a influência de cinco variáveis: raça, grupamento genético, frequência de aspiração, tipo de sêmen (sexado/convenção) e sazonalidade (primavera/verão vs outono/inverno) sobre a taxa de oócitos viáveis, clivagem e produção de blastocisto (bl). Os protocolos para a produção de embriões *in vitro* (PIV) seguiram a rotina estabelecida pelo laboratório parceiro do presente estudo. A análise estatística dos dados foi realizada utilizando o SAS, empregando-se o Teste Qui-quadrado. As raças Girolando e Nelore apresentaram maior

($p=0,0001$) número de blastocisto/sessão de OPU, assim como o grupamento sanguíneo *Bos indicus*. A produção de blastocisto foimaior ($p=0,0059$) com sêmen sexado em relação ao convencional ($6,6 \times 5,2$ bl/sessão OPU); a frequência maior de aspirações também elevou ($p=0,005$) o número de bl/sessão OPU ($7,7 \times 5,9$). Concluímos que a produção *in vitro* de embriões bovinos é influenciada pelos fatores analisados e conhecer estas variáveis pode nortear o emprego comercial da OPU-PIVE.

Palavras-chave: Blastocisto; *Bos indicus*; Girolando; Aspiração folicular guiada por ultrassom.

Resumen

La variabilidad en la producción de blastocistos bovinos por sesión de aspiración folicular guiada por ultrasonido (OPU) sigue siendo un obstáculo en la producción comercial. Así, el objetivo fue verificar los factores que influyen en la producción de embriones *in vitro* (PIVE), utilizando datos de un laboratorio comercial. Se analizaron datos del año 2014 al 2016, referidos a 799 sesiones de OPU de hembras bovinas adultas de las razas: Nelore ($n = 83$), Girolando ($n = 73$), Brangus ($n = 49$), HPB ($n = 20$) y Senepol ($n = 10$). Se analizó la influencia de cinco variables: raza, grupo genético, frecuencia de aspiración, tipo de semen (sexado / convencional) y estacionalidad (primavera / verano vs otoño / invierno) sobre la tasa de ovocitos viables, clivaje y producción de blastocistos (licenciado en Derecho). Los protocolos para la producción de embriones *in vitro* (PIV) siguieron la rutina establecida por el laboratorio socio del presente estudio. El análisis estadístico de los datos se realizó mediante el SAS, mediante la prueba de Chi-cuadrado. Las razas Girolando y Nelore tuvieron un mayor número ($p = 0,0001$) de sesiones de blastocistos / OPU, así como el grupo sanguíneo *Bos indicus*. La producción de blastocistos fue mayor ($p = 0,0059$) con semen sexado en comparación con el semen convencional ($6,6 \times 5,2$ bl / sesión de OPU); la mayor frecuencia de aspiraciones también aumentó ($p = 0,005$) el número de sesiones bl / OPU ($7,7 \times 5,9$). Concluimos que la producción *in vitro* de embriones bovinos está influenciada por los factores analizados y conocer estas variables puede orientar el uso comercial de OPU-PIVE.

Palabras clave: Blastocisto; *Bos indicus*; Girolando; Aspiración folicular guiada por ecografía.

1. Introduction

Commercial work with OPU-PIVE started in Brazil with the implementation of the first laboratory in 1998. These techniques were leveraged by beef exports, which have gained impetus since 2002, demanding greater productive efficiency from producers in order to meet world demand for meat. Due to the fact that the Nelore herd adapted extraordinarily to OPU-PIVE, its use quickly spread among ranchers, reaching more than 80% of the total embryos transferred (Viana, 2008). Since then, Brazil has been a leader in embryos produced *in vitro* (PIVE) (Perry, 2015).

When PIVE is used commercially, together with the follicular puncture technique guided by ultrasound (*ovum pick up* - OPU) to obtain oocytes, it is possible to maximize the productive potential of herds, increase the number of offspring, reduce the interval between generations, and accelerate animal genetic improvement (Varago et al., 2008), even allowing females with acquired reproductive pathologies to be used (Bueno and Beltran, 2008). Bovine IVP technology has contributed to accelerated genetic gain in dairy breeds, primarily because of sexed semen and genomics (Ferré et al., 2020a). Thus, these techniques present considerable advances and advantages compared to other breeding programs, enabling their large-scale application and the export of this model to several Latin American countries and other continents (Bols et al., 2012).

Despite the great potential and evolution of OPU-PIVE in recent years, these biotechniques present some limiting factors, with variability in results regarding blastocyst (10 to 40%) (Lonergan and Fair, 2008; Rizos et al., 2008) and pregnancy rates (30 to 50%) (Trigal et al., 2012; Holguín et al., 2013) being one of the most relevant, and which directly influences commercial success.

Methods of *in vitro* culture have improved significantly in the last 15 years (Blondin, 2015; Ferré et al., 2020b), and the general conditions of embryonic production are well established, however there is still variability in the results, which could be credited to the origin of the oocytes.

In commercial works the use of female oocyte donors of different breeds and origins, with different management systems, as well as different protocols and technicians, highlights the need for research that portrays the reality of commercial production in Brazil, contributing to the improvement or regularity of the final indices.

The objective of this analysis is to point out factors that affect the final results of blastocyst production in order to provide consistent data on the limitations found in the routine of a medium-sized commercial laboratory.

2. Methodology

Location

The current study was conducted using retrospective data provided by a commercial laboratory for in vitro embryo production, located in the municipality of Presidente Prudente, west of the State of São Paulo (Latitude 22°07'S and Longitude 51°23').

Data from 2014 to 2016 were used, referring to 235 bovine females, adults, oocyte donors, of the breeds: Nelore, Girolando, Brangus, Holstein, and Senepol from different locations. The animals were reared under different management conditions according to the routine established in each property and were aspirated at least 2 and at most 17 times. The vast majority of consultations were restricted to a 200 km radius covering the western region of the state of São Paulo, eastern Mato Grosso do Sul, and northwestern Paraná.

Cattle

Data were analyzed from 235 female donors of the breeds Nelore (n=83), Girolando (n=73), Brangus (n=49), Holstein (n=20), and Senepol (n=10), submitted to a total of 799 sessions of follicular aspiration guided by ultrasound (OPU).

Ovum pick-up (OPU)

Follicular aspiration was performed by a single trained technician. Briefly, each visible follicle > 2 mm in diameter was aspirated using a real-time B-mode ultrasound scanner (Mindray 2200; Mindray Bio-Medical Electronics, Shenzhen, China), a 7.5 MHz convex array transducer coupled to the intravaginal device (Watanabe Tecnologia Aplicada, São Paulo, Brazil), and a stainless steel guide. Follicular puncture was performed using a disposable 18 G hypodermic needle (Becton Dickinson, Curitiba, PR, Brazil) connected to a 50 mL tube (Corning, Acton, MA, USA) via a silicon tube (0.8 m long; 2 mm id). Aspiration was performed using a vacuum pump (WTA model BV-003, Watanabe Tecnologia Aplicada, São Paulo, Brazil) with a negative pressure between 60 and 80 mmHg. The OPU medium was DPBS (Nutricell – Nutrientes Celulares, São Paulo, Brazil), 0.05% sodium heparin (5000 IU/mL, Hemofol®, Cristália Produtos Químicos Farmacêuticos, São Paulo, Brazil), and 1% fetal bovine serum (Gibco®, Thermo Fisher Scientific, Massachusetts, United States).

OPUs were performed on the property of origin of each animal, where cumulus-oocyte complexes (COC's) were classified according to the presence of cumulus cells and the aspect of the ooplasm (Seneda et al., 2001). After selection, only atretic oocytes were discarded and the remainder were deposited in cryotubes containing maturation medium. The transport/maturation medium was TCM 199 (Gibco Life Technologies, Grand Island, NY, USA) supplemented with 25 mM HEPES (Sigma H-0763), 10% fetal calf serum (FCS), 50 µL/mL gentamycin sulfate (Schering-Plough, São Paulo, SP, Brazil), 0.5 145 µg/mL follicle-stimulating hormone (FSH) (Folltropin®, Bioniche Animal Health, Ontario, 146 Canada), 22 µg/mL pyruvate (Biochemical® 44094, Sigma-Aldrich, Saint Louis, United States), 50 µg/mL luteinizing hormone (LH) (Lutropin®, Bioniche Animal Health, Ontario, Canada), and 1 µg/mL estradiol (Sigma® E-2758, Sigma-Aldrich, Saint Louis, United States).

The cryotubes were aerated using a standard gas mixture (5% CO₂, 5% O₂, and 90% N₂) and sent to the laboratory in oocyte transport incubators (WTA®, Cravinhos, Brazil) at a controlled temperature (37° C).

In vitro embryo production

The in vitro procedures, IVM (in vitro maturation), IVF (in vitro fertilization), and IVC (in vitro cultivation), were performed according to Castilho et al. (2007) adapted by the analyzed laboratory.

Briefly, in the laboratory, the cryotubes were uncapped and placed in incubators with a 5% CO₂ atmosphere and a temperature of 38.5°C with maximum humidity. After 22 to 24 hours for IVM, in vitro fertilization (IVF) was performed with conventional or sexed semen after thawing in a water bath at 35°C for 30 seconds. The semen was washed by centrifugation through a 45% to 90% Percoll gradient at 200 × g for 9 minutes in order to separate mobile spermatozoa. Fecundation was performed in drops with 70 µL or 40 µL of TALP medium containing 20 oocytes for, respectively conventional or sexed semen. The TALP medium was supplemented: 10 µg/mL heparin (Sigma® H-3149, Sigma-Aldrich, Saint Louis, United States), 22 µg/mL pyruvate (Biochemical® 44094, Sigma-Aldrich, Saint Louis, United States), 159 µg/mL gentamicin (Sigma® G-1272, Sigma-Aldrich, Saint Louis, United States), bovine serum albumin (BSA; Sigma® A-3311, Sigma-Aldrich, Saint Louis, United States), PHE solution, 2 µM penicillin (Sigma® P-4875, Sigma-Aldrich, Saint Louis, United States), 1 µM hypotaurine (Sigma® 162 H-1384, Sigma-Aldrich, Saint Louis, United States), and 0.25 µM epinephrine (Sigma® E-4250, 163 Sigma-Aldrich, Saint Louis, United States). After centrifugation of the semen, the concentration of the sperm was adjusted to 1×10⁶ spermatozoa/mL, sperm was capacitated using heparin (30 µg/mL) and each fertilization drop received 3 – 4µL or 10 µL, respectively conventional or sexed semen.

After 15 to 24 hours of fertilization, potential zygotes were cultured in vitro in synthetic oviduct fluid (SOF) medium, supplemented with 5% FCS and 0.5% BSA in drops with 90 µL. The cleavage rate, that is, embryos with more than 2 cells, was evaluated 48 to 72 hours after fertilization and the embryonic development (blastocyst rate) on the seventh day. The blastocyst rate was based on the total number of matured oocytes. The culture medium was renewed on the cleavage day and on the 5th day (Day 0: IVF).

Analyzed variables

The influence of five variables was analyzed: breed and genetic group (*Bostaurustaurus*, *Bostaurusindicus* and cross) of the donor, frequency of aspiration of donor, type of semen (sexed vs. conventional), and seasonality (spring/summer vs. autumn/winter) on the rates of: viable oocytes, cleavage, blastocysts, and blastocyst production per OPU session.

The influence of the genetic group was analyzed by grouping donors according to breed: Bostaurus (Holstein and Senepol), Bosindicus (Nelore), Bostaurus x Bosindicus (Brangus and Girolando).

In order to verify the influence of the number of times the donor was aspirated, females aspirated only once were excluded, with the following ranges established: 2 to 8 sessions and 9 to 17 OPU sessions.

The seasonality effect was evaluated by dividing follicular aspiration procedures into two groups according to the dates on which they were performed; spring/summer group, females aspirated between September 22 and March 20 and autumn/winter group aspirated between March 21 and September 21.

Statistical analysis

The statistical analysis of the data was performed using the computer program Statistical Analysis System for Windows (SAS Inst., Inc., Cary, NC) using the chi-square test, with an adopted significance level of 5%.

3. Results

There was an effect of the donor's breed on the variables: viable oocytes, cleavage, and blastocyst production (Table 1).

The percentage of viable oocytes was higher in the Nelore and Brangus breeds (p<0.0001). However, the Nelore and Girolando breeds presented the highest (p<0.0001) blastocyst production per OPU session.

Table 1. Recovery rates, viable oocytes, cleavage, blastocysts, and number of blastocysts per OPU session, according to the breed of donors used in the commercial *in vitro* production of bovine embryos.

	Nelore (n=83)	Girolando (n=73)	Brangus (n=49)	Holstein (n=20)	Senepol (n=10)	p-value
Recovered oocytes	34.28 ^a (7268/212)	31.02 ^a (10764/347)	36.76 ^a (5440/148)	19.53 ^b (840/43)	28.86 ^{ab} (1414/49)	0.0051
% Viable Oocytes	74.34 ^a (5403/7268)	66.07 ^b (7112/10764)	75.44 ^a (4104/5440)	67.38 ^b (566/840)	60.75 ^c (859/1414)	0.0001
% Cleavage	77.61 ^b (4193/5403)	87.54 ^a (6226/7112)	57.38 ^d (2355/4104)	66.61 ^c (377/566)	56.23 ^d (483/859)	0.0001
% Blastocysts	30.74 ^a (1661/5403)	32.83 ^a (2335/7112)	17.23 ^c (707/4104)	21.55 ^b (122/566)	14.44 ^c (124/859)	0.0001
Blastocysts /OPU	7.83 ^a (1661/212)	6.73 ^a (2335/347)	4.78 ^b (707/148)	2.84 ^c (122/43)	2.53 ^c (124/49)	0.0001

Different letters on the same line differ by the 5% chi-square test.
Source: Authors.

The genetic group influenced the analyzed variables ($p < 0.0001$) (Table 2). When comparing donors from different genetic groups, the superiority of *Bos indicus* was observed, represented by the Nelore breed, followed by females from industrial crossbreeding (*Bos indicus* x *Bos taurus*), and lastly the *Bos taurus* sub-species with the lowest values.

Table 2. Viable oocyte rates, cleavage, blastocyst production, and number of blastocysts per OPU session according to the donor genetic group used in the commercial *in vitro* production of bovine embryos.

	<i>Bos indicus</i>	<i>Bos taurus</i> x <i>Bos indicus</i>	<i>BosTaurus</i>	p-value
% Viable Oocytes	74.34 ^a (5403/7268)	69.22 ^b (11216/16204)	63.22 ^c (1425/2254)	0.0001
% Cleavage	77.61 ^a (4193/5403)	76.51 ^a (8581/11216)	60.35 ^b (860/1425)	0.0001
% Blastocysts	30.74 ^a (1661/5403)	27.12 ^b (3042/11216)	17.26 ^c (246/1425)	0.0001
Blastocysts/OPU	7.83 ^a (1661/212)	6.15 ^b (3042/495)	2.67 ^c (246/92)	0.0001

Different letters on the line differ by the 5% chi-square test.
Source: Authors.

The rates of cleavage and blastocysts were higher ($p < 0.0001$) in donors aspirated more frequently (9 to 17 times), without, however, affecting the rate of viable oocytes (Table 3).

Table 3. Viable oocyte rates, cleavage, blastocyst production, and number of blastocysts per OPU session according to the frequency of ultrasound-guided follicular aspiration (OPU) of donors, regardless of breed, used in the commercial *in vitro* production of bovine embryos.

	2 to 8 OPU (n=590)	9 to 17 OPU (n=183)	p-value
% Viable Oocytes	70.08 (13180/18805)	70.36 (4829/6863)	0.6699
% Cleavage	74.50 ^b (9819/13180)	78.28 ^a (3780/4829)	0.0001
% Blastocysts	26.41 ^b (3481/13180)	29.49 ^a (1424/4829)	0.0001
Blastocysts/OPU	5.68 ^b (3481/613)	7.74 ^a (1424/184)	0.0005

Different letters on the line differ by the 5% chi-square test.
Source: Authors.

Cleavage and blastocyst rates were higher ($p < 0.0001$) using sexed semen compared to conventional semen (Table 4).

Table 4. Rates of cleavage, blastocyst, and blastocyst production per session of OPU according to the type of semen (sexed vs. conventional) used in the commercial *in vitro* production of bovine embryos.

	Sexed semen (n=555)	Conventional semen (n=218)	p-value
% Cleavage	81.61 ^a (10362/12697)	61.19 ^b (3272/5347)	0.0001
% Blastocysts	30.08 ^a (3819/12697)	21.13 ^b (1130/5347)	0.0001
Blastocysts/OPU	6.57 ^a (3819/581)	5.18 ^b (1130/218)	0.0059

Different letters on the line differ by the 5% chi-square test.
Source: Authors.

There was an influence of the season, as donors aspirated in the spring/summer seasons exhibited a higher number of viable oocytes in relation to autumn/winter. However, seasonality did not influence the rate of cleavage (0.5066) or embryonic production ($p = 0.403$) (Table 5).

Table 5. Viable oocyte rates, cleavage, blastocyst production, and number of blastocysts per OPU session according to the time of ultrasound-guided follicular aspiration (OPU) from donors used in commercial *in vitro* production of bovine embryos in Brazil.

	Spring/Summer (n=661)	Autumn/Winter (n=114)	p-value
% Viable Oocytes	70.56 ^a (15581/22081)	67.28 ^b (2463/3661)	0.0001
% Cleavage	75.70 (11794/15581)	74.70 (1840/2463)	0.2885
% Blastocysts	27.34 (4260/15581)	27.97 (689/2463)	0.5129
Blastocysts/OPU	6.23 (4260/684)	6.00 (689/115)	0.7217

Different letters on the line differ by the 5% chi-square test.
Source: Authors.

4. Discussion

In the present study, data from a commercial laboratory in Brazil were evaluated and through retrospective analysis it was possible to observe that breed and blood group have a strong influence on embryonic production. Regardless of breed or blood group, the higher frequency of aspirations increased the cleavage and blastocyst rates, as well as which, the use of sexed semen showed higher rates of both cleavage and blastocysts. However, the spring/summer season, although improving oocyte quality, did not influence blastocyst production.

Oocyte recovery was not influenced by the breeds worked with at the analyzed laboratory, and in HPB donors the recovery was greater than the results presented in the literature. Follicular development in Nelore females (Figueiredo et al., 1997) is similar to that observed in HPB (Bo et al., 2003), Girolando (Castilho et al., 2000), Angus, Brahman, and Senepol females (Alvarez et al., 2000), however, differences are observed mainly in follicular diameters, number of waves per cycle, as well as in the number of follicles recruited per wave. Alvarez et al. (2000) observed a greater amount of small follicles (3 to 5 mm) in the emergence of waves in Brahman (39 ± 4) compared to Senepol (33 ± 4) or Angus females (21 ± 4). Nelore females submitted to the same climatic and nutritional conditions as HPB cows exhibit a greater number of recruited follicles (42.7 vs 19.7) per follicular wave (Bastos et al., 2010).

This difference in the number of follicles recruited directly influences the application of OPU in terms of oocyte recovery and, consequently, embryonic production (Ferré et al., 2020b). In females of the Nelore breed, recovery is greater per session of OPU, around 30 to 38 total oocytes, when compared to means of from 6.5 to 16 COCs in the Holstein breed (Pontes et al., 2010; Ratto et al., 2011; Gimenes et al., 2015). Animals of the Girolando breed, due to crossbreeding and their different degrees of blood from the HPB breed (*Bostaurus*) with the Gir breed (*Bosindicus*), showed varying oocyte recovery according to the blood grade, from 20.4 to 31.4 oocytes per OPU session (Pontes et al., 2011).

The rate of viable oocytes, that is, the quality of the oocytes was influenced by breed and seasonality, however, this was not always a safe predictor for embryonic production. In donors of the Brangus and Holstein breeds the high rates of viable oocytes did not influence the production of blastocysts. However, the Senepol breed presented the lowest rate of viable oocytes, also resulting in low blastocyst production. On the other hand, seasonality strongly influenced the rate of viable oocytes but did not affect the rates of cleavage and blastocysts.

The oocytes that are selected for IVP are evaluated by morphological characteristics depending on the number of *cumulus* cells and homogeneity of the ooplasm (Seneda et al., 2001). This classification is related to oocyte competence for development up to the blastocyst stage, and according to Merton et al. (2003), oocyte quality is often defined as the ability to produce a blastocyst within an IVP system. In general, better quality oocytes generate higher rates of blastocysts (Merton et al., 2003), however, class 3 oocytes (mild signs of atresia) showed better rates when compared to classes 1 and 2 (Blondin and Sirard, 1995). Therefore, in order to obtain the largest number of embryos in commercial programs, normally only oocytes with a high level of degeneration are excluded from production (Pontes et al., 2011).

Although culture conditions can impact the embryo's early development potential, the intrinsic quality of the oocyte is the key factor that determines oocyte competence up to the blastocyst stage (Blondin & Sirard, 1995). Genetic improvement for milk production, along with changes in nutrition and management, has been associated with declining fertility in dairy cows (Snjiders et al., 2000). In studies conducted by the group of Prof. Baruselli, it was possible to observe that *B. taurus* females produce less blastocysts by OPU-PIVE when compared to *B. indicus* donors (Sales et al., 2015; Gimenes et al., 2015). However, in another study evaluating commercial data, embryo production (5.5 blastocysts) was higher in the Girolando breed, compared to the Gir (*Bosindicus*) and HPB breeds (*Bostaurus*, Pontes et al., 2011). However, oocyte competence does not depend only on intrinsic factors, but is also related to the type of protocol used in the laboratory, the semen, and method of obtaining oocytes

(Merton et al., 2003). Several authors have emphasized the improvement of oocyte quality in the supplementation of oocytes with antioxidant substances, to reduce oxidative stress (Oliveira et al., 2020).

In the current study, both the *in vitro* protocol and media, as well as the technique used, were the same and it is known that the type of media used in the cultivation of oocytes and embryos has an important influence on embryonic development (van Wagendonk-de Leeuw et al., 1998). Part of these variations may be due to the different capacities of bulls to produce embryos and a high male effect on the efficiency of a commercial IVP program (Palma & Sinowatz, 2004). In addition to the type of semen (conventional vs. sexed), which can markedly affect the efficiency of an *in vitro* fertilization program (Palma et al., 2008, Ferré et al., 2020b).

The type of semen used influenced the rate of cleavage and embryonic development *in vitro* and, to our surprise, sexed semen showed better rates when compared to conventional semen. Some researchers have reported that using sexed semen has no effects on blastocyst development rates (Carvalho et al., 2010; Lu and Seidel, 2004), while others report greater cleavage, but lower blastocyst rates (Blondin et al., 2009). Current *in vitro* procedures attempt to compensate for decreased embryonic development with the use of sexed semen (Rath et al., 2013), by increasing the concentration of sperm in the fertilization drop (Barceló-Fimbres et al., 2011). Increasing the concentration in the fertilization drop was one of the strategies used in the evaluated laboratory, in which sexed semen was most used for the Girolando breed. This is probably the reason for the better rates of embryonic development displayed in the current work. However, it is not only the low concentration of sperm per straw that is responsible for the low and variable results obtained with sexed semen. Morphological alterations in sexed spermatozooids resulting from the flow cytometry process may also be responsible for the impairment in embryonic development rates (Palma et al., 2008). Therefore, detecting bulls that show the best results in IVP with sexed semen is another strategy adopted by commercial laboratories to minimize the low production of blastocysts. Conventional or sexed semen from 3 different bulls were tested and in general the conventional semen produced more blastocysts, but this difference was inherent to the bull (Nascimento et al., 2015).

In the present study, there was variation in the intervals between OPU sessions, as it is not possible to maintain a specific routine in a commercial laboratory, which works according to demand. However, in the majority of cases, the same cows were aspirated at intervals of approximately 30 days. There was an increase in cleavage and blastocysts when statistically analyzing donors aspirated 9 to 17 times compared to those aspirated 2 to 8 times during the evaluated period. There is no physiological explanation for this increase, but we believe that this greater efficiency is related to the fact that the females that present the best results in PIVE are the ones that owners use most often in OPU and, consequently, they present the best results.

These biotechniques are extremely versatile and when well conducted result in extraordinary genetic gains for the meat or milk producer, however, the results still present variability, including the factors presented in the current work.

5. Conclusion

We conclude that the *in vitro* production of blastocysts in commercial programs is highly influenced by the breed and blood group of the donor, as well as the sexing of the semen and frequency of aspirations.

Knowledge of these factors can guide the use of OPU-PIVE in order to minimize variability in the results obtained commercially using these biotechniques.

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