Animal Reproduction Science 193 (2018) 165–170

Contents lists available at ScienceDirect

Animal Reproduction Science

journal homepage: www.elsevier.com/locate/anireprosci

Likelihood of pregnancy after the transfer of embryos derived from follicle aspiration and *in vitro* embryo production sessions with different relative efficiencies

Luiz Fernando Feres[a,c], Luiz Gustavo Bruno Siqueira[b], Miller Pereira Palhao[c], Lívia Loiola dos Santos[d], Felipe Zandonadi Brandao[a], Joao Henrique Moreira Viana[c,e,*]

[a] Universidade Federal Fluminense, Niteroi, RJ, 24230-340, Brazil
[b] Embrapa Gado de Leite, Juiz de Fora, MG, 36038-330 Brazil
[c] Universidade Jose do Rosario Vellano, Alfenas, MG, 37130-000, Brazil
[d] Universidade Federal de Minas Gerais, Belo Horizonte, MG, 31270-901, Brazil
[e] Embrapa Cenargen, PqEB, Av. W5 N, Brasilia, DF, 70770-917 Brazil

ARTICLE INFO

Keywords:
Blastocyst rate
Cleavage
Gir breed
Oocyte quality
Pregnancy rate

ABSTRACT

The aim of the present study was to evaluate the likelihood of pregnancy of *in vitro*-produced (IVP) embryos from batches with distinct relative efficiencies. Data were retrospectively analyzed from 605 transvaginal ultrasonic-guided follicle aspiration sessions (OPU) followed by *in vitro* embryo production (IVEP) and 2456 fresh embryo transfers (ET), performed between 2008 and 2012 in individuals of the Gir (dairy *Bos indicus*) breed. The OPU and IVEP were performed using standard procedures by a single group of technicians at the same laboratory facility. Records were stratified into quartiles (I to IV) according to the total of cumulus-oocytes complexes (COC) produced per donor, or in percentile ranges (0%–25%, 26%–50%, 51%–75%, and 76%–100%) for endpoints related to COC quality or efficiency of embryo production. Pregnancy per embryo transfer (P/ET) was compared among quartiles or ranges using the chi-squared test. Donors producing a greater number of total COC (quartile I) also had more viable and grade I COC, and a greater number of embryos than donors ranked in quartiles II, III or IV, respectively ($P < 0.0001$). Nevertheless, P/ET did not differ ($P > 0.05$) among embryos produced by donors ranked in Quartiles I to IV. Similarly, there was no difference ($P > 0.05$) in P/ET for embryos derived from OPU sessions with a relatively greater or lesser percentage of viable or Grade I COC. Cleavage and blastocyst rates within each IVEP batch had no effect ($P > 0.05$) on P/ET. In conclusion, data suggest that there is no relationship among oocyte yield after OPU, or efficiency of IVEP, and the likelihood of pregnancy after ET of fresh IVP embryos.

1. Introduction

The use of *in vitro* technologies for embryo production in cattle has been increasing in both beef and dairy industries worldwide. *In vitro* embryo production (IVEP) was initially considered merely a complement to conventional superovulation programs (Faber et al., 2003). In the past decade, however, IVEP became the technique of choice and there was a greater number of embryos by IVEP

* Corresponding author at: Embrapa Cenargen" to "Embrapa Recursos Geneticos e Biotecnologia.
E-mail address: henrique.viana@embrapa.br (J.H.M. Viana).

https://doi.org/10.1016/j.anireprosci.2018.04.065
Received 9 February 2018; Received in revised form 6 April 2018; Accepted 11 April 2018
Available online 15 April 2018
0378-4320/

compared to *in vivo*-derived counterparts in South America, and there was a similar trend in other regions of the world such as in North America (Perry, 2017).

The progressive growth in the adoption of IVEP was supported by improvements in procedures for collection of cumulus-oocyte complexes (COC) by transvaginal ultrasonic-guided follicle aspiration (OPU - Galli et al., 2001), together with advances in systems and protocols for *in vitro* oocyte maturation, fertilization, and embryo culture (Lonergan, 2007; Lonergan and Fair, 2008). Consequently, the costs of IVP decreased substantially and this technology became an alternative for large-scale embryo transfer (ET) programs (Pontes et al., 2010; Morotti et al., 2014). Nonetheless, pregnancy rates of *in vitro* produced (IVP) embryos remains less than those when *in vivo* counterparts are used for ET (Pontes et al., 2009; Ferraz et al., 2016). To overcome this limitation, it is important to identify potential risk factors associated with the likelihood of pregnancy after transfer of IVP embryos.

In this regard, morphological classification is still the most used criterion for embryo selection and it has been useful to predict the chances of pregnancy for IVP embryos (Chebel et al., 2008). Particularly for IVP embryos, however, morphological classification is generally less informative, mostly due to differences in the developmental kinetics and morulae compaction (Holm et al., 2002). Additionally, most commercial IVEP companies adopt the standard operating procedure of transferring only embryos at the blastocyst or expanded blastocyst stages, a developmental stage when morphological defects such as cell extrusion are not easily detected. A number of microscopic, ultra-structural and functional parameters have been used to make inferences about embryo quality in scientific studies (Ott et al., 2002; Neuber et al., 2002; Rekik et al., 2011; Hoelker et al., 2013; Salzano et al., 2014), but such analyses generally impair further development or compromise embryo viability. Conversely, non-invasive approaches to assess embryo quality such as measurement of embryo metabolism, kinetics of development, or cleavage symmetry are either complex, expensive, or time-consuming (Perkel et al., 2015) and thus not very useful in large-scale ET programs.

Both the number and quality of COC recovered by OPU affect the number of embryos produced per donor (Pontes et al., 2010, Watanabe et al., 2017). It is still not clear, however, whether COC number and quality affect the likelihood of pregnancy after ET. In the present study, it was hypothesized that 1) transfer of embryos derived from OPU sessions with a greater proportion of viable or good quality COC result in improved pregnancy rates; and 2) transfer of embryos derived from IVEP batches where there were greater cleavage and blastocyst rates would be more likely to result in a pregnancy than those transferred that were from IVEP batches with lesser development. Thus, the objective of the present study was to evaluate the likelihood of pregnancy of IVP embryos where there was transfer of embryos from batches derived from OPU and IVEP sessions with distinct outcomes.

2. Materials and methods

In the present study, data were analyzed from 702 OPU and subsequent IVEP and ET, performed between May 2008 and February 2012 at a single commercial farm, located in the state of Minas Gerais, Brazil (21°31′55″ S and 42°38′35″ W). The regional climate is Aw according to Köppen's system (Peel et al. 2007), with an average precipitation of 1307 mm. All COC donors and semen were from the same dairy *Bos indicus* breed (Gir). Donors (n = 90) were maintained in similar housing, nutritional, and sanitary conditions throughout the study period. Procedures for OPU and IVEP were performed by the same groups of technicians and there were standard operating protocols used that were routine for a single commercial laboratory. The use of data from OPU, IVEP, and ET in the present study was previously approved by the farms' owner.

Immediately after OPU, recovered COC were visualized using a stereo microscope and classified for quality by categorization into Grades I–V. The morphological features used to define each grade, such as number of cumulus cells layers surrounding the zona pellucida and the aspect of the cytoplasm, correspond to those described elsewhere (Viana et al., 2004). Viable COC (Grades I, II, and III) were transferred into cryotubes with 400 μL of a TCM-199 bicarbonate solution (Gibco Life Technologies, Grand Island, EUA) supplemented with 10% fetal calf serum (FCS, Vitrocell, Campinas, Brazil), 50 IU/mL human chorionic gonadotrophin (hCG, Sigma C-1063, Sigma Chemical, St. Louis, MO, USA), 0.5 μg/mL FSH (Folltropin, Vetrepharm, Belleville, ON, Canada), 1 μg/mL estradiol (estradiol-17b, Sigma E-8875), 2.2 μg/mL pyruvate (Sigma P-4562), and 70 μg/mL amikacin (Sigma A-2324). This medium was covered with 300 μL of mineral oil (Sigma M-8410) and the empty space left in the cryotube was filled with a gas mixture of 5% CO_2 and 90% N_2.

Tubes with COC were transported to the laboratory at 38.5 °C, in a portable incubator (Minitube, Tiefenbach, Germany). *In vitro* maturation, fertilization, and embryo culture were performed according to the standards and protocols of the commercial IVEP Company. Briefly, COC were matured *in vitro* for 24 h (transportation period included) in the same cryotube and were placed in a medium (TCM-199 bicarbonate solution, Gibco Life Technologies) for transportation, at 38.5 °C and 5% CO_2. For *in vitro* fertilization, sex-sorted semen from Gir sires (n = 12) were used. Straws were thawed in a water bath at 35 to 37 °C for 30 s followed by semen centrifugation at 200 x g for 30 s to pass through a gradient with two Percoll (Sigma -P1644) columns (45% and 90%). Sperm concentration was adjusted to a final concentration of 1.2^{56} viable spermatozoa per mL. Sperm and COC were co-incubated in modified Tyrode's medium (TALP) supplemented with 10 μg/mL heparin (Sigma H-3149) and penicillamine, hypotaurine and epinephrine (PHE, 10 μL/mL) for 18 h at 39 °C and 5% CO_2 in a humidified atmosphere. Presumptive zygotes were then cultured in 50 μL drops of modified synthetic oviduct fluid (SOF) covered with mineral oil, at 38.5 °C in humidified atmosphere of 5% CO_2, 5% O_2 and 90% N_2 for 6 days. Cleavage was evaluated on Day 3 (72 h post-insemination, hpi) and medium renewal occurred. Formation of blastocysts was determined on Day 7 (168 hpi), when embryos were evaluated and classified for developmental stage and morphological quality according to the International Embryo Transfer Society standards (Stringfellow and Givens 2010). Only Grade I blastocysts or expanded blastocysts were selected for transfer, and loaded in 0.25 mL straws (IMV, São Paulo, Brazil).

Crossbred Zebu x Holstein (F1) recipients were estrous synchronized using a timed embryo transfer (TET) protocol as follows: Day 0, insertion of an intravaginal progesterone (P4)-releasing device (0.558 g P4, Cronipres, Biogenesis-Bagó, Buenos Aires, Argentina)

and injection of 2 mg estradiol benzoate im (Bioestrogen, Biogenesis-Bagó); Day 8, P4 device withdrawal, injection of 300 IU of eCG im (Novormon, MSD Saúde Animal, São Paulo, Brazil), 150 μg sodium cloprostenol (Croniben, Biogenesis-Bagó) and 1 mg estradiol cypionate (ECP, Zoetis, Guarulhos, Brazil). The IVP embryos (n = 2456) were transferred non-surgically on Day 17 of the estrous synchronization protocol, to eligible recipients (presence of a corpus luteum; CL), into the uterine horn ipsilateral to the ovary bearing a CL. Pregnancy diagnosis was performed 23 days after ET, by ultrasonography, to visualize the fetus and fetal heartbeat.

2.1. Statistical analysis

Because pregnancy outcomes of frozen-thawed IVP embryos are still unreliable and inconsistent, it was arbitrarily decided to use only data from ET of fresh IVP embryos transfer. Records, therefore, from 97 OPU-IVEP sessions which indicated that there was not production of embryos or embryos were produced but not transferred fresh due to an insufficient number of recipients were excluded from analyses.

From the remaining 605 OPU sessions, there was not information from 16 sessions about oocyte quality and, therefore, records were used only for analysis of endpoints related to IVEP and pregnancy outcomes. Data were ranked into quartiles according to the total number of oocytes recovered per donor, per OPU session. Alternatively, data were grouped based on the percentage of viable or Grade I COC recovered per OPU, or based on the cleavage or blastocyst rates (percentile ranges of 0%–25%, 26%–50%, 51%–75%, and 76%–100%). Pregnancy per embryo transfer (P/ET) were compared among these ranges for each endpoint. Due to the small number of events for % of grade I COC in the range 76%–100%, and for % of viable COC and cleavage rate in the range 0%–25%, data were pooled with the next category (51%–75% and 26%–50%, respectively).

Data were also stratified according to the sire used for *in vitro* fertilization, and according to season. The rainy season encompasses October to March, and was characterized by an average monthly precipitation of 183.8 mm (or 84.5% of year rainfall), while during the dry season (April to September) monthly precipitation was 33.7 mm.

Endpoints related to the number and quality of COCs and IVEP efficiency were analyzed among quartiles by analysis of variance, using The SAS MIXED procedure (9.3 Version; SAS Institute Inc., Cary, NC, USA). If a significant effect of quartile was detected, differences among means were determined using the Tukey's post hoc test. Pregnancy rate (P/ET) and the proportion of events for each bull and for each season were analyzed using the chi-squared test for differences among the percentile range groups and among quartiles. Results are shown as mean ± SEM or percentages. A P-value of 0.05 indicated statistical significance.

3. Results

From the 589 OPU sessions with data of oocyte quality, a total of 14,633 COC were recovered (mean 24.8 ± 0.6 per donor/OPU) and 57.8% (8453) were classified as viable (mean 14.4 ± 0.4), with 1887 (12.9%; 3.2 ± 0.1) Grade I; 2598 (17.8%; 4.4 ± 0.1) Grade II; and 3968 (27.1%; 6.7 ± 0.2) Grade III. The overall mean cleavage and blastocyst rates of the 605 OPU sessions were 89.8% and 43.4%, respectively. A total of 2456 fresh IVP embryos were transferred, resulting in 760 pregnancies (P/ET = 30.9%).

Data for endpoints related to COC recovery and IVEP within quartiles are included in Table 1. Donors with a greater number of total COC were the ones producing a greater number of viable and Grade I COC and, subsequently, a greater number of embryos ($P < 0.0001$). There was not a difference ($P > 0.05$) in the percentage of viable COC among quartiles. Donors in Quartile I, however, produced a lesser percentage of Grade I COC and there was a negative relationship between total COC recovery and blastocyst rate among quartiles. The P/ET were similar among quartiles (> 0.05) even though there were differences observed for other endpoints.

Table 1
Cumulus-oocyte complexes (COC) quality, development potential, and subsequent pregnancy outcomes (from 2391 embryo transfers) in donors ranked in quartiles (I to IV) according to their total number of COC recovered (Quartile I = greatest number of COC; Quartile IV = least number of COC).

	Quartile			
Endpoint	I	II	III	IV
COC				
Mean	44.8 ± 1.1^{a}	27.0 ± 0.2^{b}	18.0 ± 0.2^{c}	9.6 ± 0.3^{d}
Viable	25.5 ± 0.7^{a}	15.7 ± 0.3^{b}	10.5 ± 0.2^{c}	5.8 ± 0.2^{d}
Viable (%)	56.8^{A}	58.3^{A}	58.3^{A}	59.8^{A}
Grade I	4.8 ± 0.4^{a}	3.9 ± 0.3^{b}	2.6 ± 0.2^{c}	1.6 ± 0.1^{d}
Grade I (%)	18.8^{A}	24.5^{B}	25.0^{B}	27.1^{B}
Development to blastocyst				
Mean	9.0 ± 0.4^{a}	6.9 ± 0.3^{b}	5.0 ± 0.2^{c}	3.3 ± 0.1^{d}
Blastocyst rate (%)	36.8^{A}	43.5^{B}	48.5^{C}	62.2^{D}
Pregnancy outcome				
%, (n)	30.9^{a} (280/905)	29.3^{a} (186/635)	31.5^{a} (152/483)	30.5^{a} (112/367)

^{a,b,c,d}Within a row, means with different superscripts differ ($P < 0.0001$).
^{A,B,C,D}Within a row, means with different superscripts differ ($P < 0.05$).

Table 2
Pregnancy outcomes after the transfer of 2391 *in vitro* produced (IVP) embryos derived from batches divided according to the percentage of viable or grade I COC recovered.

Endpoint	Percentile range	P/ET	n
Viable COC			
	76%–100%	25.2[a]	34/135
	51%–75%	31.5[a]	557/1,767
	0%–50%*	28.4[a]	139/489
COC Grade I			
	51%–100%**	29.4[a]	62/211
	26%–50%	29.5[a]	220/525
	0%–25%	31.2[a]	448/987

*Due to the small number of events between 0% and 25%, data were pooled with 26%–50%.
**Due to the lesser number of events between 76% and 100%, data were pooled 51%–75%.
No orthogonal contrasts between percentile ranges was significant for P/ET ($P > 0.05$).

In addition, P/ET did not differ ($P > 0.05$) when data was stratified based on relative percentage of different variables related to COC quality: % viable or % Grade I embryos (Table 2). Similarly, P/ET did not differ ($P > 0.05$) among percentile ranges of variables of IVEP efficiency (cleavage and blastocyst rates) as shown in Table 3.

Blastocyst rate differed ($P < 0.01$, range 23.1%–46.4%) among bulls, but not pregnancy rate ($P > 0.05$). There was no bias in the distribution of bulls within groups, i.e., there was no difference in the proportion of OPU-IVEP sessions using each bull for each quartile or percentile range ($P > 0.05$), except for one bull that was used only twice and contributed to only two ET. There was no effect of season on blastocyst rate (38.6% compared with 38.8% for the dry and rainy seasons, respectively; $P > 0.05$), but pregnancy rate was less during the dry season (28.65 compared with 32.3%, $P > 0.05$). The proportion of OPU-IVEP sessions performed during each season were equally distributed among quartiles or percentile ranges ($P > 0.05$).

4. Discussion

In the present study, the relationship was examined between number and quality of recovered COC, and IVEP rates, with subsequent pregnancy outcomes. The hypotheses for the present study was not supported by the results in that use of COC collected during OPU sessions where there was a relatively greater number or proportion of good quality COC that were recovered, or transfer of embryos from IVEP batches where there was greater cleavage and blastocyst rates did not lead to enhanced pregnancy outcomes. In the present study, there was not any clear relationship between the results of procedures commonly used for producing IVP embryos and a successful pregnancy outcome.

A number of factors affecting IVEP were reviewed by Lonergan and Fair (2008). The majority of the studies on IVEP, however, did not evaluate subsequent pregnancy rates, mainly due to the great costs of this type of experiment, limited availability of recipients, or limitations in the experimental design. In fact, because pregnancy rate is a binomial variable statistical differences can only be detected with a large number of observations, particularly if the expected magnitude of the difference in pregnancy rates between experimental groups is not great. Commonly, large numbers of observations can only be achieved in commercial embryo transfer programs (Pontes et al., 2010; Morotti et al., 2014), which led to analysis of retrospective data from a commercial IVP operation.

Additionally, retrospective studies have inherent flaws and, therefore, an effort was made in the present study to account for a number of possible sources of variation in data analysis, such as breed of sires and dams (Pontes et al., 2010), herd (Sartori et al., 2016), nutrition (Sales et al., 2015), heat stress (Torres-Júnior et al., 2008), protocols for estrous synchronization or stimulation of follicular development with FSH (da Silva et al., 2017), and culture conditions (Lonergan et al., 2006). To minimize possible bias related to these factors in the current study, data were used from a single farm, i.e., sires and dams were from a single breed, donor

Table 3
Pregnancy outcome after the transfer of 2456 embryos derived from *in vitro* embryo production (IVEP) batches grouped according to cleavage or blastocyst rates.

Endpoint	Percentile range	P/ET	*n*
Cleavage rate			
	76%–100%	30.96[a]	589/1,908
	51%–75%	30.7[a]	141/459
	0%–50%*	33.7[a]	30/89
Blastocyst rate			
	76%–100%	27.9[a]	97/348
	51%–75%	34.5[a]	266/770
	26%–50%	29.9[a]	326/1,091
	0%–25%	28.7[a]	71/247

*Due to the small number of events between 0% and 25%, data were pooled with 26%–50%.
No orthogonal contrasts between percentile ranges was significant for P/ET ($P > 0.05$).

and recipient management was standardized and similar throughout the period of data collection, and OPU-IVEP were performed by the same groups of technicians and laboratory. Thus, possible differences in pregnancy rates were expected to be related mainly to intrinsic characteristics of the embryos produced.

For example, follicular population has been associated with fertility and pregnancy success after artificial insemination (Mossa et al., 2012). Furthermore, there is a significant variation in the ovarian reserve of follicles in cattle population, either in *Bos taurus* (Ireland et al., 2011) and *Bos indicus* (Monteiro et al., 2017), which is probably due to genetic components (Merton et al., 2009; Perez et al., 2016, 2017). Thus, it seems reasonable to hypothesize that pregnancy rates would be greater after ET of embryos derived from donors with greater COC yield after OPU, an indirect indicator of a greater ovarian follicular populations. The fact that pregnancy outcome was similar among quartiles, however, is consistent with the lack of difference in the proportion of viable COC per quartile. In fact, it was observed that there was a negative relationship between total number of COC and blastocyst rate, suggesting that a donor phenotype with a greater follicular population and, thus, greater embryo production, is related neither to IVEP efficiency nor to the likelihood of pregnancy following ET.

It is noteworthy that donors enrolled in the current study did not undergo synchronization of stage of follicular wave development or stimulation with FSH prior to OPU, which occurred at random stages of the estrous cycle. Thus, it is recognized that some donors may have had a small number and quality of recovered COC due to the presence of an active dominant follicle, as demonstrated by a previous study (Hendriksen et al., 2004). Surprisingly, development to the blastocyst stage was not associated with neither the number of COC recovered nor the proportion of viable COC. Nevertheless, it is worthy of consideration that in Zebu breeds the dominant follicle generally has a smaller maximum diameter and persists for a shorter period of time compared to *Bos taurus* breeds (Sartori and Barros, 2011). The greater turnover rate of dominant follicles may have decreased the potential negative impact of the presence of a functional dominant follicle upon the number of recovered COC per OPU (Viana et al., 2004), so that differences in follicular population were possibly due primarily to intrinsic distinct features among donors.

Based on the rationale for the present study, it was also expected that endpoints of IVEP efficiency, such as cleavage and blastocyst rates would be correlated with subsequent pregnancy rates. The relationship between COC quality and IVEP outcomes is well documented (Lonergan et al., 1994; Sirard et al., 2006; Lonergan and Fair 2008; Saini et al., 2015). The IVEP batches with lesser cleavage and blastocyst rates, therefore, conceivably reflect a lesser COC quality, which could be either due to intrinsic cellular aspects or environmental effects. One could thus speculate whether such variables (relatively lesser and greater cleavage and blastocyst rates) would directly affect pregnancy success. Results of the present study, however, indicated that Grade I blastocysts have similar developmental potential after ET into recipients, regardless of the relative success rates of the IVEP batch. Based on these results, it is inferred that once an IVP embryo reaches the blastocyst stage, it was able to advance through some of the most important stages of early development, for example embryonic genome activation and the first cell fate decisions to start differentiation (Vigneault et al., 2004). Thus, development to the blastocyst stage represents a critical period for the embryo, whereas later likelihood of pregnancy establishment will be similar, regardless of the IVEP efficiency to produce blastocysts (blastocyst rate).

In summary, the present results support the existing criteria used by commercial IVEP companies to select transferable embryos, i.e., development to the blastocyst stage. This stage is widely adopted as a standard developmental stage for evaluation, selection, and transfer of IVP embryos, but mostly because of the well-known inconsistencies in the evaluation at earlier stages (Holm et al., 2002). Conversely, results of the present study indicate that the overall IVEP batch relative efficiency is not a useful predictor of subsequent pregnancy outcome, at least for ET of embryos at a similar developmental stage and quality.

Acknowledgements

The authors thank Fapemig CVZ APQ 03430/17 for financial support and the farm '*Fazendas do Basa* for giving us access to their database. Luiz F. Feres received a scholarship from CAPES, Brazil.

References

Chebel, R.C., Demétrio, D.G., Metzger, J., 2008. Factors affecting success of embryo collection and transfer in large dairy herds. Theriogenology 69, 98–106.

da Silva, J.C.B., Ferreira, R.M., Maturana Filho, M., Naves, J.R., Santin, T., Pugliesi, G., Madureira, E.H., 2017. Use of FSH in two different regimens for ovarian superstimulation prior to ovum pick up and in vitro embryo production in Holstein cows. Theriogenology 90, 65–73. http://dx.doi.org/10.1016/j.theriogenology.2016.11.016.

Faber, D.C., Molina, J.A., Ohlrichs, C.L., Vander Zwaag, D.F., Ferré, L.B., 2003. Commercialization of animal biotechnology. Theriogenology 59, 125–138.

Ferraz, P.A., Burnley, C., Karanja, J., Viera-Neto, A., Santos, J.E., Chebel, R.C., Galvão, K.N., 2016. Factors affecting the success of a large embryo transfer program in Holstein cattle in a commercial herd in the southeast region of the United States. Theriogenology 86, 1834–1841. http://dx.doi.org/10.1016/j.theriogenology.2016.05.032.

Galli, C., Crotti, G., Notari, C., Turini, P., Duchi, R., Lazzari, G., 2001. Embryo production by ovum pick up from live donors. Theriogenology 55, 1341–1357.

Hendriksen, P.J., Steenweg, W.N., Harkema, J.C., Merton, J.S., Bevers, M.M., Vos, P.L., Dieleman, S.J., 2004. Effect of different stages of the follicular wave on in vitro developmental competence of bovine oocytes. Theriogenology 61, 909–920.

Hoelker, M., Held, E., Salilew-Wondim, D., Schellander, K., Tesfaye, D., 2013. Molecular signatures of bovine embryo developmental competence. Reprod. Fertil. Dev. 26, 22–36. http://dx.doi.org/10.1071/RD13255.

Holm, P., Booth, P.J., Callesen, H., 2002. Kinetics of early in vitro development of bovine in vivo- and in vitro-derived zygotes produced and/or cultured in chemically defined or serum-containing media. Reproduction 123, 553–565.

Ireland, J.J., Smith, G.W., Scheetz, D., Jimenez-Krassel, F., Folger, J.K., Ireland, J.L., Mossa, F., Lonergan, P., Evans, A.C., 2011. Does size matter in females? An overview of the impact of the high variation in the ovarian reserve on ovarian function and fertility, utility of anti-Müllerian hormone as a diagnostic marker for fertility and causes of variation in the ovarian reserve in cattle. Reprod. Fertil. Dev. 23, 1–14. http://dx.doi.org/10.1071/RD10226.

Lonergan, P., Monaghan, P., Rizos, D., Boland, M.P., Gordon, I., 1994. Effect of follicle size on bovine oocyte quality and developmental competence following oocyte maturation, fertilization and culture in vitro. Mol. Reprod. Dev. 37, 48–53.

Lonergan, P., Fair, T., Corcoran, D., Evans, A.C., 2006. Effect of culture environment on gene expression and developmental characteristics in IVF-derived embryos. Theriogenology 65, 137–152.
Lonergan, P., 2007. State-of-the-art embryo technologies in cattle. Soc. Reprod. Fertil. Suppl. 64, 315–325.
Lonergan, P., Fair, T., 2008. In vitro-produced bovine embryos: dealing with the warts. Theriogenology 69, 17–22.
Merton, J.S., Ask, B., Onkundi, D.C., Mullaart, E., Colenbrander, B., Nielen, M., 2009. Genetic parameters for oocyte number and embryo production within a bovine ovum pick-up-in vitro production embryo-production program. Theriogenology 72, 885–893. http://dx.doi.org/10.1016/j.theriogenology.2009.06.003.
Monteiro, F.M., Batista, E.O.S., Vieira, L.M., Bayeux, B.M., Accorsi, M., Campanholi, S.P., Dias, E.A.R., Souza, A.H., Baruselli, P.S., 2017. Beef donor cows with high number of retrieved COC produce more in vitro embryos compared with cows with low number of COC after repeated ovum pick-up sessions. Theriogenology 90, 54–58. http://dx.doi.org/10.1016/j.theriogenology.2016.11.002.
Morotti, F., Sanches, B.V., Pontes, J.H., Basso, A.C., Siqueira, E.R., Lisboa, L.A., Seneda, M.M., 2014. Pregnancy rate and birth rate of calves from a large-scale IVF program using reverse-sorted semen in Bos indicus, Bos indicus-taurus, and Bos taurus cattle. Theriogenology 81, 696–701. http://dx.doi.org/10.1016/j.theriogenology.2013.12.002.
Mossa, F., Walsh, S.W., Butler, S.T., Berry, D.P., Carter, F., Lonergan, P., Smith, G.W., Ireland, J.J., Evans, A.C., 2012. Low numbers of ovarian follicles 3 mm in diameter are associated with low fertility in dairy cows. J. Dairy. Sci. 95, 2355–2361. http://dx.doi.org/10.3168/jds.2011-4325.
Neuber, E., Luetjens, C.M., Chan, A.W., Schatten, G.P., 2002. Analysis of DNA fragmentation of in vitro cultured bovine blastocysts using TUNEL. Theriogenology 57, 2193–2202.
Ott, M., Schernthaner, W., Sinowatz, F., Wolf, E., 2002. Effects of bovine serum albumin and estrous cow serum on development and ultrastructure of in vitro-produced porcine embryos. Anat. Histol. Embryol. 31, 151–157.
Peel, M.C., Finlayson, B.L., McMahon, T.A., 2007. Updated world map of the Köppen-Geiger climate classification. Hydrol. Earth Syst. Sci. 4, 439–473.
Perez, B.C., Peixoto, M.G., Bruneli, F.T., Ramos, P.V., Balieiro, J.C., 2016. Genetic analysis of oocyte and embryo production traits in guzerá breed donors and their associations with age at first calving. Genet. Mol. Res. 16 (15). http://dx.doi.org/10.4238/gmr.15027583.
Perez, B.C., Balieiro, J.C.C., Ventura, R.V., Bruneli, F.A.T., Peixoto, M.G.C.D., 2017. Inbreeding effects on in vitro embryo production traits in guzerá cattle. Animal 17, 1–8. http://dx.doi.org/10.1017/S1751731117000854.
Perkel, K.J., Tscherner, A., Merrill, C., Lamarre, J., Madan, P., 2015. The ART of selecting the best embryo: a review of early embryonic mortality and bovine embryo viability assessment methods. Mol. Reprod. Dev. 82, 822–838. http://dx.doi.org/10.1002/mrd.22525.
Perry, G., 2017. 2016 statistics of embryo collection and transfer in domestic farm animals. Embryo Transf. Newsl. 35, 08–23.
Pontes, J.H.F., Nonato Junior, I., Sanches, B.V., Ereno Junior, J.C., Uvo, S., Barreiros, T.R.R., Oliveira, J.A., Hasler, J.F., Seneda, M.M., 2009. Comparison of embryo yield and pregnancy rate between *in vivo* and *in vitro* methods in the same nelore (*Bos indicus*) donor cows. Theriogenology 71, 690–697.
Pontes, J.H.F., Silva, K.C.F., Basso, A.C., Rigo, A.G., Ferreira, C.R., Santos, G.M.G., Sanches, B.V., Porcinato, J.P.F., Vieira, P.H.S., Faifer, F.S., Sterza, F.A.M., Schenk, J.L., Seneda, M.M., 2010. Large-scale *in vitro* embryo production and pregnancy rates from *Bos taurus, Bos indicus*, and *indicus-taurus* dairy cows using sexed sperm. Theriogenology 74, 1349–1355.
Rekik, W., Dufort, I., Sirard, M.A., 2011. Analysis of the gene expression pattern of bovine blastocysts at three stages of development. Mol. Reprod. Dev. 78, 226–240. http://dx.doi.org/10.1002/mrd.21286.
Saini, N., Singh, M.K., Shah, S.M., Singh, K.P., Kaushik, R., Manik, R.S., Singla, S.K., Palta, P., Chauhan, M.S., 2015. Developmental competence of different quality bovine oocytes retrieved through ovum pick-up following in vitro maturation and fertilization. Animal 9, 1979–1985. http://dx.doi.org/10.1017/S1751731115001226.
Sales, J.N., Iguma, L.T., Batista, R.I., Quintão, C.C., Gama, M.A., Freitas, C., Pereira, M.M., Camargo, L.S., Viana, J.H., Souza, J.C., Baruselli, P.S., 2015. Effects of a high-energy diet on oocyte quality and in vitro embryo production in Bos indicus and Bos taurus cows. J. Dairy Sci. 98, 3086–3099. http://dx.doi.org/10.3168/jds.2014-8858.
Salzano, A., Albero, G., Zullo, G., Neglia, G., Abdel-Wahab, A., Bifulco, G., Zicarelli, L., Gasparrini, B., 2014. Effect of resveratrol supplementation during culture on the quality and cryotolerance of bovine in vitro produced embryos. Anim. Reprod. Sci. 151, 91–96. http://dx.doi.org/10.1016/j.anireprosci.2014.09.018.
Sartori, R., Barros, C.M., 2011. Reproductive cycles in Bos indicus cattle. Anim. Reprod. Sci. 124, 244–250. http://dx.doi.org/10.1016/j.anireprosci.2011.02.006.
Sartori, R., Prata, A.B., Figueiredo, A.C.S., Sanches, B.V., Pontes, G.C.S., Viana, J.H.M., Pontes, J.H., Vasconcelos, J.L.M., Pereira, M.H.C., Dode, M.A.N., Monteiro Jr, P.L.J., Baruselli, P.S., 2016. Update and overview on assisted reproductive technologies (ARTs) in Brazil. Anim. Reprod. 13, 300–312.
Sirard, M.A., Richard, F., Blondin, P., Robert, C., 2006. Contribution of the oocyte to embryo quality. Theriogenology 65, 126–136.
Stringfellow, D.A., Givens, M.D., 2010. Manual of the International Embryo Transfer Society (IETS), 4th ed. IETS, Champaign, IL.
Torres-Júnior, J.R., Pires, M.A., Sá, W.F., Ferreira, A.M., Viana, J.H., Camargo, L.S., Ramos, A.A., Folhadella, I.M., Polisseni, J., de Freitas, C., Clemente, C.A., de Sá Filho, M.F., Paula-Lopes, F.F., Baruselli, P.S., 2008. Effect of maternal heat-stress on follicular growth and oocyte competence in Bos indicus cattle. Theriogenology 69, 155–166.
Viana, J.H.M., Camargo, L.S.A., Ferreira, A.M., Sá, W.F., Fernandes, C.A.C., Marques Júnior, A.P., 2004. Short intervals between ultrasonographically-guided follicle aspiration improve oocyte quality but do not prevent establishment of dominant follicles in the gir breed (*Bos indicus*) of cattle. Anim. Reprod. Sci. 84, 1–12.
Vigneault, C., Mcgraw, S., Massicotte, L., Sirard, M.A., 2004. Transcription factor expression patterns in bovine in vitro-derived embryos prior to maternal-zygotic transition. Biol. Reprod. 70, 1701–1709.
Watanabe, Y.F., Souza, A.H., Mingoti, R.D., Ferreira, R.M., Batista, E.O.S., Dayan, A., Watanabe, O., Meirelles, F.V., Nogueira, M.F.G., Ferraz, J.B.S., Baruselli, P.S., 2017. Number of oocytes retrieved per donor during OPU and its relationship with *in vitro* embryo production and field fertility following embryo transfer. Anim. Reprod. 14, 635–644. http://dx.doi.org/10.21451/1984-3143-AR1008.