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High numbers of antral follicles are positively associated with *in vitro* embryo production but not the conception rate for FTAI in Nelore cattle

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ABSTRACT

The objective was to compare the conception rates for FTAI and *in vitro* embryo production between Nelore cows with different antral follicle counts (AFC = number of follicles ≤3 mm in diameter in the ovaries). Nelore cows ($n = 547$) were subjected to ovulation synchronization. Randomly during the estrous cycle (D0), cows received an intravaginal device containing 1.9 g P4 (CIDR®) and 2 mg BE (Estrogin®), IM. When the device was removed (D8), the cows received 500 μg PGF2α (Ciosin®), 300 IU eCG (Novormon®) and 1 mg EC (ECP®), IM. All cows were inseminated 48 h after P4 device removal. Antral follicles ≥3 mm were counted using an intravaginal microconvex transducer (D0), and the cows were assigned to high (G-High, ≥25 follicles, $n = 183$), intermediate (G-Intermediate, 16–20 follicles, $n = 183$) or low AFC groups (G-Low, ≤10 follicles, $n = 181$). In another experiment, COCs were retrieved by OPU from Nelore cows ($n = 66$), which were assigned to groups according to oocyte production: G-High ($n = 22$, ≥40 oocytes), G-Intermediate ($n = 25$, 18–25 oocytes) or G-Low ($n = 19$, ≤7 oocytes). All COCs from the same cow were cultured individually (maximum of 25 COCs per drop) and then *in vitro* fertilized using thawed frozen sperm (2×10^8/dose) from a Nelore sire of known fertility. The data were analyzed using a Kruskal–Wallis and a Chi-square test ($P \leq 0.05$). There was no difference in the conception rates after FTAI between Nelore cows with high, intermediate or low AFC (51.9 *vs.* 48.6 *vs.* 58.6%). The number of viable embryos was 18.4 ± 6.7 (G-High), 6.1 ± 3.6 (G-Intermediate) and 0.6 ± 0.7 (G-Low; $P < 0.05$). Therefore, AFC had no influence on the conception rates for FTAI; however, Nelore cows with high oocyte production exhibited better *in vitro* embryo production.

1. Introduction

The large number of oocytes obtained from *Bos taurus indicus* donor cows has stimulated *in vitro* embryo production commerce in Brazil. In this context, Nelore cattle are reported to have high oocyte production, with reports of hundreds of oocytes collected from a single *ovum pick up* (OPU; Santos et al., 2005). In recent years, follicular aspiration procedure has been largely and successfully performed in cattle (Pontes et al., 2011; Sanches et al., 2013; Silva-Santos et al., 2014a) but high individual variability in the number of oocytes has influenced

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both embryo production and pregnancy (Pontes et al., 2011).

Recently, there has been an attempt to minimize the high individual variation responses to reproductive biotechnologies and identify predictive tools for the early selection of high-producer cattle. Variability in the number of antral follicle numbers/oocytes recovered by OPU in cattle appears to be a limiting factor for large scale *in vitro* embryo programs.

High variability in the number of preantral and antral follicles has been described in *Bos indicus* and *Bos taurus* cattle (Burns et al., 2005; Erickson, 1966; Silva-Santos et al., 2014a, 2014b), although the number of antral follicles ≥3 mm in diameter during follicular waves (antral follicle count, AFC) is repeatable (0.85–0.95) within individuals on both beef and dairy cattle (Burns et al., 2005; Ireland et al., 2007; Mossa et al., 2012; Silva-Santos et al., 2014a, 2014b). Therefore, ultrasonography has been used to identify cattle with high or low numbers of antral follicles during follicular waves (Singh et al., 2004).

Smaller ovaries, diminished ovarian reserves, lower responsiveness to superovulation and transferable embryos, lower concentrations of anti-Müllerian hormone (AMH) and other phenotypic characteristics associated with aging and fertility have been described in cattle with lower AFC (Evans et al., 2012; Ireland et al., 2007, 2011; Mossa et al., 2007; Singh et al., 2004).

Therefore, although the association between markers associated with low fertility has been reported in dairy cattle, few studies have reported the influence of AFC on conception rates resulting from artificial insemination in cattle (Mossa et al., 2012), and there has been no reports for Nelore cows. We hypothesized that Nelore cows with low or intermediate AFC had lower conception rates to fixed timed artificial insemination (FTAI) as well as lower *in vitro* embryo production compared to Nelore cows with high AFC. Therefore, two experiments were designed to compare (1) the conception rates after a hormonal protocol was administered for ovulation synchronization and (2) the *in vitro* embryo production between Nelore cows with high, intermediate or low AFC/oocyte production.

2. Materials and methods

2.1. Experiment I: Hormonal protocol for ovulation synchronization

2.1.1. Animals

Multiparous anestrus Nelore cows (*Bos taurus indicus*, $n = 701$) aged 72 ± 12 months and maintained in *Brachiaria brizantha* pasture supplemented with mineral salt *ad libitum* were submitted to a hormonal protocol for ovulation synchronization. The mean postpartum interval was 45 ± 15 days, the mean body weight was 450 ± 15 kg, and the average body condition score (BCS) was 3.0 ± 0.5 (scale 1–5; Lowman et al., 1976).

2.1.2. Hormonal protocol

The cows were inserted with a new intravaginal progesterone-releasing device containing 1.9 g of P4 (CIDR®, Zoetis, Brazil) and 2 mg of estradiol benzoate (EB, im, Estrogin®, Farmavet, Brazil) on day zero (D0). At device removal (D8), the cows were injected with 500 μg sodium cloprostenol (PGF2α, Ciosin®, Intervet-Schering Plough, Brazil), 300 IU equine chorionic gonadotropin (eCG, Novormon®, Syntex SA, Argentina), and 1 mg estradiol cipionate (EC, ECP®, Pfizer, Brazil), IM. After the device had been removed for 48–52 h, the cows were artificially inseminated using frozen-thawed semen from a single Aberdeen Angus sire with known fertility (Fig. 1). A pregnancy test was determined with a 5-linear transrectal array transducer (Aquila PRO, Pie Medical, Maastricht, Holanda), 45 days after FTAI.

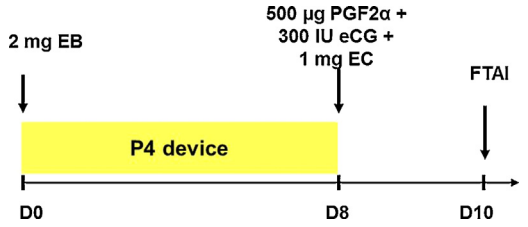

Fig. 1. The hormone protocol for ovulation synchronization in Nelore cows with high (G-High, ≥25 follicles), intermediate (G-Intermediate 16–20 follicles) or low AFC (G-Low, ≤10 follicles). EB: estradiol benzoate, eCG: equine chorionic gonadotropin, EC: estradiol cipionate, PGF2α: prostaglandin, P4: progesterone, FTAI: fixed time artificial insemination.

2.1.3. Antral follicular counts

When the progesterone device was inserted (D0), the ovaries of each animal were ultrasonographically monitored with a 7.5-convex intravaginal array transducer (Aquila PRO, Pie Medical, Maastricht, Holanda), and the antral follicles were counted as previously described (Burns et al., 2005; Ireland et al., 2008; Silva-Santos et al., 2014a, 2014b). Each ovary was systematically scanned from end-to-end and the AFC was determined for each animal, as well as the mean number of antral follicles and the standard deviation (SD) for the whole population ($n = 701$). After an ultrasound evaluation, the females were assigned to three groups according to the number of antral follicles ≥3 mm and the SD: females with a high (mean number of follicles of all the 701 cows plus 1 SD; G-High AFC, ≥25 follicles; $n = 183$), intermediate (25% of cows with AFC closest to the mean number of follicles of all the 701 cows; G-Intermediate AFC, 16–20 follicles, $n = 183$) or low AFC (mean number of follicles of all the 701 cows minus 1 SD; G-Low AFC, ≤10 follicles; $n = 181$) in all ultrasound scans.

2.2. Experiment II: In vitro embryo production

2.2.1. Animals

Multiparous Nelore cyclic cows ($n = 101$, *Bos taurus indicus*) aged 84 ± 12 months, mean postpartum interval was 45 ± 15 days, with a mean BCS of 3.5 ± 0.5 (scale 1–5; Lowman et al., 1976), mean body weight of 480 ± 20 kg, and maintained in *B. brizantha* pasture supplemented with mineral salt *ad libitum* were submitted to follicular aspiration without hormonal stimulation (Pontes et al., 2009, 2011). Each cow was aspirated once.

Before each procedure, feces were removed from the rectum, and the perineal area was cleaned with tap water

and disinfected with 70% ethanol. Prior to OPU, each cow received epidural anesthesia (7 mL of 2% lidocaine; Anestésico L, Pearson, São Paulo, SP, Brazil) to decrease peristalsis and discomfort. Follicular aspiration was performed by only one experienced operator. The number of antral follicles was estimated by the total number of oocytes recovered, because the average recovery rate of operator with vast experience is around 80%. Therefore, oocyte production can be considered as 80% AFC.

2.2.2. Ovum pick up and oocyte recovery

Previously described procedures were used for follicular aspiration by one experienced operator (Seneda et al., 2001). Briefly, each visible follicle was aspirated using a collection medium (phosphate buffer solution, PBS, Nutricell, Campinas, SP, Brazil) supplemented with 10,000 UI/L sodium heparin (Sigma H-3149). Immediately after recovery, COCs were classified and transported to a laboratory for *in vitro* fertilization (Pontes et al., 2011). The cows were assigned to groups according to the mean oocyte production of 101 cows and the SD: G-High (mean oocyte production of all the 101 cows plus 1 SD, $n = 22$, ≥40 oocytes), G-Intermediate (25% of cows with oocyte production closest to the mean oocyte production of all the 101 cows, $n = 25$, 18–25 oocytes) and G-Low AFC (mean oocyte production of all the 101 cows minus 1 SD $n = 19$, ≤7 oocytes). Oocytes from cows that were not assigned into groups were discarded.

2.2.3. In vitro embryo production

Immediately after recovery, the aspirated material was washed and filtered using a 75-μm filter (WTA Watanabe Tecnologia Animal, Cravinhos, SP, Brazil) and PBS. The COCs were classified according to the presence of *cumulus* cells and oocyte quality using the following criteria: good, more than three layers of *cumulus* cells; regular, at least one layer of cells; denuded, partially covered with *cumulus* cells or without *cumulus* cells; and atretic, dark *cumulus* oophorus and signs of cytoplasmic degeneration (Seneda et al., 2001). Both good and regular oocytes were considered viable and used in the procedure, whereas atretic oocytes were discarded.

The protocol used for *in vitro* embryo production was previously described (Pontes et al., 2011). The COCs in each category were separately cultured for 24 h in 100-μL drops of maturation medium under mineral oil (D'Altomare, Santo Amaro, SP, Brazil) at 39 °C and 5% CO2 in air (Gordon, 1994; Smith et al., 1996). The COCs were recovered from 66 cows on the same day, they were cultured at once and all the COCs of each cow were cultured separately (maximum of 25 COCs per drop). Thawed frozen sperm (2×10^8/dose) from a Nelore sire of known fertility (based on previous utilization for IVF) were used.

Presumptive zygotes had their *cumulus* cells removed by vortexing (120 s) and by gentle pipetting and were transferred to the 100-μL drops of culture medium of the embryos (SOFaa BSA—synthetic oviduct fluid supplemented with bovine serum albumin, containing 8 mg/mL BSA [free of fatty acid] and 1 mM glutamine) under identical temperature and gaseous atmosphere conditions used for IVF. The blastocyst rate was calculated based on the total viable oocytes aspirated. The embryos were evaluated until Day 7 (Day 0 = day of IVF) according to IETS criteria (Wright, 1998). Cleavage and blastocyst rates were assessed on Days 3 and 7 of culture. Embryos graded as I, II or III were classified as viable.

Table 1
Means ± SD antral follicle population and conception rates for FTAI of Nelore cows with high (G-High AFC, ≥25 follicles), intermediate (G-Intermediate, 16–20 follicles) or low AFC (G-Low AFC, ≤10 follicles).

	n	AFC	Conception rate (%)
G-Low AFC	181	7.8 ± 2.4[a]	58.5 (106/181)
G-Intermediate	183	18.6 ± 1.6[b]	48.6 (89/183)
G-High AFC	183	30.7 ± 6.5[c]	51.9 (95/183)
Total	547	19.6 ± 10.7	53.0 (290/547)

[a–b] Within a column, means without a common superscript differ ($P \leq 0.05$).

2.2.4. Statistical analysis

The results are presented as the means ± SD. All statistical analyses were performed using BioEstat 5.0 software (Ayres et al., 2007). The AFC was analyzed using a Kruskal–Wallis test. Conception rates after FTAI, cleavage and blastocyst rates, and the proportion of viable oocytes and embryos were compared using a Chi-square test. For all analyses, $P \leq 0.05$ was considered significant.

3. Results

3.1. Experiment I

The mean number of antral follicles (mean ± SD) was 19.6 ± 10.7 (range 2–50), and the average conception rate after FTAI range was 53% (290/547; Table 1). There was no difference in the conception rates for FTAI between Nelore cows in the high, intermediate or low AFC groups.

3.2. Experiment II

Cows with high oocyte production had a higher proportion of viable oocytes, blastocyst rate, and number of viable blastocysts per OPU compared to the intermediate and low AFC groups ($P < 0.05$; Table 2). The production of oocytes and viable embryos was ~10- and ~30-fold higher in Nelore cows with high oocyte production compared to cows in the low group. The high-oocyte production cows had also an impressive production compared to the intermediate group (~2.5 higher oocyte yield and ~10-fold higher viable embryo production).

4. Discussion

High success rates of *in vitro* produced embryos have been associated with the population of antral follicles and oocytes (Pontes et al., 2011; Silva-Santos et al., 2014a, 2014b; Singh et al., 2004). Furthermore, previous studies have associated lower numbers of antral follicles with reduced fertility in *B. taurus* dairy cattle (Ireland et al., 2007; Mossa et al., 2012). We therefore tested whether the number of antral follicles and oocytes in Nelore beef cattle with high, intermediate or low AFC/oocyte

Table 2
Means ± SD data for the IVF production of Nelore beef cattle with high (G-High, ≥40 oocytes), intermediate (G-Intermediate, 18–25 oocytes) or low oocyte production (G-Low, ≤7 oocytes) following *in vitro* (OPU/IVP) embryo production.

	G-High ≥ 40 follicles $n = 22$	G-Intermediate 18–25 follicles $n = 25$	G-Low ≤7 follicles $n = 19$
Total oocytes recovered	1109[a]	534[b]	101[c]
Oocytes/OPU (n)	50.4 ± 11.30[a]	21.4 ± 3.04[b]	5.3 ± 1.50[c]
Viable oocytes/OPU (n)	40.4 ± 10.6[a]	14.8 ± 3.02[b]	3.8 ± 1.08[c]
Viable oocytes (%)	80.07[a]	69.48[b]	71.29[b]
Cleavage rate (%)	78.96[a]	73.73[b]	70.65[ab]
Blastocyst rate (%)	41.97[a]	32.42[b]	13.04[c]
Mean viable blastocyst/OPU (n)	18.4 ± 6.71[a]	6.1 ± 3.57[b]	0.6 ± 0.68[c]
Vitrifiable embryos/OPU (n)	15.0 ± 7.05[a]	4.7 ± 3.09[b]	0.4 ± 0.68[c]
Proportion vitrifiable (%)	81.23[a]	77.12[b]	58.33[c]

[a–c] Within a row, means without a common superscript differ ($P \leq 0.05$).

production influenced the conception rate following FTAI and the proportion of embryos produced.

Although an association has been established between low AFCs and different markers associated with low fertility in dairy cattle, such as increased FSH secretion, diminished concentration of progesterone (Evans et al., 2012; Ireland et al., 2011), smaller ovaries (Ireland et al., 2008), reduced endometrial thickness (Jimenez-Krassel et al., 2009) and reduced superovulatory responsiveness (Ireland et al., 2007) compared to age-matched cows with high AFC, we did not observe a positive influence of AFC on the conception rate for FTAI in Nelore beef cows.

This study presents new data regarding AFC and fertility in cattle, and investigates Nelore beef cows (*B. indicus*) submitted to a hormonal protocol for FTAI for the first time. In the present study, there was no difference in the conception rate for FTAI between Nelore cows with high, intermediate or low AFC (Table 1). For Holstein-Friesian dairy cattle (*B. taurus*), cows with low AFC (<15 follicles) had lower pregnancy rates at the end of the breeding season (84 *vs.* 94%) and a longer interval between calving and conception (114 *vs.* 100 days) compared to cows with high AFC (>25 follicles; Mossa et al., 2012). Holstein and Jersey cows with low numbers of antral follicles (AFC < 20) also had lower conception rates for the first AI (45.2 *vs.* 66.5%) and lower calving rates (64.0 *vs.* 79.9%) than cows in the high AFC group (>30 follicles; Martinez et al., 2013). It is important to consider that these authors used conventional AI (with estrus observation), whereas the cows in the present study were submitted to a hormonal protocol for ovulation synchronization (FTAI). The hormones used in the protocol of FTAI could have mitigated our results. Although it was not evaluated in our study, we believe that the eCG could have influenced rates of ovulation and conception due to its reported effect on the growth of the preovulattory follicle (Bó et al., 2003; Cutaia et al., 2003).

In Section 3.2, the number of oocytes recovered was used as a parameter for classifying cows as high, intermediate or low-AFC donors. The stability in the number of antral follicles in the ovaries is of increasing importance because AFC affects both *in vivo* and *in vitro* embryo production procedures (Evans et al., 2012; Ireland et al., 2008; Mossa et al., 2007; Pontes et al., 2011; Silva-Santos et al., 2014a). In the present study, Nelore cows with high AFC/oocyte production had a higher proportion of viable oocytes compared to the intermediate or low AFC groups (Table 2). For Braford heifers with high AFC (≥40 follicles), the number of viable oocytes was higher compared to the low AFC group (≤10 follicles; 21.6 *vs.* 3.2), but conversely, there was no difference in the proportion of viable oocytes between groups with high and low AFC (58.9 *vs.* 55.2%; Silva-Santos et al., 2014a, 2014b). Oocyte quality has been associated with the population of antral follicles, and a diminished quality in oocytes from *B. taurus* cows with low AFC has been associated with a high amount of *cumulus* cell markers in cows with low AFC (Ireland et al., 2009). Nelore cows with high AFC had higher rates of blastocyst compared to cows with intermediate or low oocyte production (Table 2). Conversely, there was no difference in the blastocyst rates between the high and low AFC groups for Braford heifers (Silva-Santos et al., 2014a) or after the *in vitro* fertilization of oocytes from slaughtered ovaries (*B. taurus*; Ireland et al., 2007). These differences are possibly due to our study being performed with Nelore cattle (100% *B. indicus)* compared to previous studies conducted with *B. taurus* or *B. indicus* × *B. taurus*.

Nelore cows with high oocyte production had ~2.5–10-fold more oocytes and produced ~10–30-fold more viable embryos compared to the intermediate and low oocyte production group. Our results are consistent with the reported positive association between individual variations in oocyte production in Nelore cows with embryo production and pregnancy rates (Pontes et al., 2009, 2011). These authors observed higher embryo production and pregnancy rates (~6-fold greater) for Nelore cattle with high oocyte production (59 oocytes/OPU) compared to the low oocyte production group (10 oocytes/OPU; Pontes et al., 2011). However, this study was based on commercial data. It is the first time that Nelore cows have been evaluated for *in vitro* embryo production and pregnancy rates following OPU using semen from a single sire. Similarly, there were lower numbers of IVP embryos after oocyte fertilization from slaughtered ovaries in *B. taurus* cows with low (<15 follicles) *vs.* high (>25 follicles) antral follicular counts (Ireland et al., 2007).

We conclude that the population of antral follicles did not influence the conception rates for FTAI but influenced embryo production in Nelore beef cattle. The conception rates for FTAI did not differ between Nelore cows with high, intermediate or low AFC, but Nelore cows with high oocyte production had a higher proportion of viable oocytes and blastocyst rates compared to cows with intermediate or

low AFC. Therefore, the use of ultrasound to identify cattle with high, intermediate or low AFC would contribute to improvements in *in vitro* embryo production. However, some aspects remain to be elucidated in cattle with variable numbers of ovarian antral follicles with regard to breed (*B. taurus vs. B. indicus*), age (heifer *vs.* cow), quality of oocytes within follicles of various diameters, and the effect of selecting cattle with high AFC for animal breeding livestock.

Conflict of interest statement

The authors declare no conflict of interest.

Acknowledgments

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References

Ayres, M., Ayres Jr., M., Ayres, D.L., Santos, A.S., 2007. BioEstat 5.0. Statistical applications in biological and medical sciences. Sociedade Civil Mamirauá, CNPq, Brasília, pp. 59.

Bó, G.A., Baruselli, P.S., Martinez, M.F., 2003. Pattern and manipulation of follicular development in *Bos indicus* cattle. Anim. Reprod. Sci. 78, 307–326.

Burns, D.S., Jimenez-Krassel, F., Ireland, J.L.H., Knight, P.G., Ireland, J.J., 2005. Numbers of antral follicles during follicular waves in cattle: evidence for high variation among animals, very high repeatability in individuals, and an inverse association with serum follicle-stimulating hormone concentrations. Biol. Reprod. 73, 54–62.

Cutaia, L., Tribulo, R., Moreno, D., Bó, G.A., 2003. Pregnancy rates in lactating beef cows treated with progesterone releasing devices, estradiol benzoate, and equine chorionic gonadotrophin (eCG). Theriogenology 59, 216.

Erickson, B., 1966. Development and senescence of the postnatal bovine ovary. J. Anim. Sci. 25, 800–805.

Evans, A.C.O., Mossa, F., Walsh, S.W., Scheetz, D., Jimenez-Krassel, F., Ireland, J.L.H., Smith, G.W., Ireland, J.J., 2012. Effects of maternal environment during gestation on ovarian folliculogenesis and consequences for fertility in bovine offspring. Reprod. Domest. Anim. 47 (Suppl. 4), 31–37.

Gordon, I., 1994. Laboratory Production of Cattle Embryos. CAB. International. Cambridge University Press, London, UK.

Ireland, J.J., Folger, J., Bettegowda, A., Scheetz, D., Walsh, S., Mossa, F., Knight, P.G., Smith, G.W., Lonergan, P., Evans, A.C.O., 2009. Variation in the ovarian reserve is linked to alterations in intrafollicular estradiol production and ovarian biomarkers of follicular differentiation and oocyte quality in cattle. Biol. Reprod. 964, 954–964.

Ireland, J.J., Ward, F., Jimenez-Krassel, F., Ireland, J.L.H., Smith, G.W., Lonergan, P., Evans, A.C.O., 2007. Follicle numbers are highly repeatable within individual animals but are inversely correlated with FSH concentrations and the proportion of good-quality embryos after ovarian stimulation in cattle. Hum. Reprod. 22, 1687–1695.

Ireland, J.J.A., Smith, G.W.B., Scheetz, D.A., Folger, J.K.A., 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. Reprod. Fertil. Dev. 23, 1–14.

Ireland, J.L.H., Scheetz, D., Jimenez-Krassel, F., Themmen, A.P.N., Ward, F., Lonergan, P., Smith, G.W., Perez, G.I., Evans, A.C.O., Ireland, J.J., 2008. Antral follicle count reliably predicts number of morphologically healthy oocytes and follicles in ovaries of young adult cattle. Biol. Reprod. 79, 1219–1225.

Jimenez-Krassel, F., Folger, J.K., Ireland, J.L.H., Smith, G.W., Hou, X., Davis, J.S., Lonergan, P., Evans, A.C.O., Ireland, J.J., 2009. Evidence that high variation in ovarian reserves of healthy young adults has a negative impact on the corpus luteum and endometrium during estrous cycles in cattle. Biol. Reprod. 80, 1272–1281.

Lowman, B.G., Scott, N.A., Somerville, S.H., Revised edition 1976. Condition scoring of cattle. East Scotland Coll. Agric., 1–31 (Bulletin 6).

Martinez, M.F., Sanderson, N., Quirke, L., Lawrence, S., Bryant, A., Sara, E., Juengel, J., 2013. Antral follicular count (AFC) and fertility in New Zealand dairy cows. In: 46th SSR Annual Meeting, p. 23 (Abstract).

Mossa, F., Duffy, P., Naitana, S., Lonergan, P., Evans, A.C.O., 2007. Association between numbers of ovarian follicles in the first follicle wave and superovulatory response in ewes. Anim. Reprod. Sci. 100, 391–396.

Mossa, F., Walsh, S., Butler, S., Berry, D., Carter, F., Lonergan, P., Smith, G., Ireland, J., Evans, A.C.O., 2012. Low numbers of ovarian follicles >3 mm in diameter are associated with low fertility in dairy cows. J. Dairy Sci. 95, 2355–2361.

Pontes, J.H.F., Melo Sterza, F.A., Basso, A.C., Ferreira, C.R., Sanches, B.V., Rubin, K.C.P., Seneda, M.M., 2011. Ovum pick up, in vitro embryo production, and pregnancy rates from a large-scale commercial program using Nelore cattle (*Bos indicus*) donors. Theriogenology 75, 1640–1646.

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., Porcionato, 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.

Sanches, B.V., Marinho, L.S.R., Filho, B.D.O., Pontes, J.H.F., Basso, A.C., Meirinhos, M.L.G., Silva-Santos, K.C., Ferreira, C.R., Seneda, M.M., 2013. Cryosurvival and pregnancy rates after exposure of IVF-derived *Bos indicus* embryos to forskolin before vitrification. Theriogenology 80, 372–377.

Santos, R.G., Soto, M.A.B., Lourenço, R.X., Stranieri, P., Bishop, W., Accorsi, M.F., Watanabe, M.R., Dayan, A., Watanabe, Y.F., 2005. Aspiração folicular em Nelore. In Relato de caso de alto número de oócitos recuperados. Anais do Congresso Brasileiro de Reprodução Animal 16, 79.

Seneda, M.M., Esper, C.R., Garcia, J.M., Oliveira, J.A., Vantini, R., 2001. Relationship between follicle size and ultrasound-guided transvaginal oocyte recovery. Anim. Reprod. Sci. 67, 37–43.

Silva-Santos, K.C., Santos, G.M.G., Koetz Júnior, C., Morotti, F., Siloto, L.S., Marcantonio, T.N., Urbano, M.R., Oliveira, R.L., Lima, D.C., Seneda, M.M., 2014a. Antral follicle populations and embryo production – in vitro and in vivo – of *Bos indicus–taurus* donors from weaning to yearling ages. Reprod. Domest. Anim. 49, 228–232.

Silva-Santos, K.C., Siloto, L.S., Santos, G.M.G., Morotti, F., Marcantonio, T.N., Seneda, M.M., 2014b. Comparison of antral and preantral ovarian follicle populations between *Bos indicus* and *Bos indicus–taurus* cows with high or low antral follicles counts. Reprod. Domest. Anim. 49, 48–51.

Silva-Santos, K.C., Santos, G.M.G., Siloto, L.S., Hertel, M.F., Andrade, E.R., Rubin, M.I.B., Sturion, L., Melo-Sterza, F.A., Seneda, M.M., 2011. Estimate of the population of preantral follicles in the ovaries of *Bos taurus indicus* and *Bos taurus* taurus cattle. Theriogenology 76, 1051–1057.

Singh, J., Domínguez, M., Jaiswal, R., Adams, G.P., 2004. A simple ultrasound test to predict the superstimulatory response in cattle. Theriogenology 62, 227–243.

Smith, L.C., Oliveira-Angel, M., Groome, N., Bhatia, B., Price, C.A., 1996. Oocyte quality in small antral follicles in the presence or absence of a large dominant follicle in cattle. J. Reprod. Fertil. 106, 193–199.

Wright, J., 1998. Photomicrographic illustration of embryo codes. In: Stringfellow, D.A., Seidel, S.M. (Eds.), Manual of the International Embryo Transfer Society. 3rd ed. Savory, IL: International Embryo Transfer Society, pp. 167–170.