



Is the number of antral follicles an interesting selection criterium for fertility in cattle?

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Abstract

Recent studies indicate that the antral follicle population may be of paramount importance to improve reproductive performance in cows. There is already an agreement that the antral follicle count (AFC; follicles ≥ 3 mm in diameter) is a highly variable trait among animals, but with high repeatability in the same individual. Thus, females can be classified into low, intermediate or high AFC. Several studies in *Bos taurus* show a positive correlation between AFC and fertility parameters, such as increased quantity and quality of embryos, better pregnancy rates, higher progesterone levels, among others. However, there is still no consensus on AFC in *Bos indicus* females and *indicus-taurus*. This article aims to discuss the main aspects related to the population of antral follicles and its relation to the reproductive performance associated with the most common techniques in assisted reproduction (timed artificial insemination, *in vitro* embryo production, embryo transfer and superovulation).

Keywords: antral follicle count, embryo production, follicular dynamics, pregnancy rate, ultrasonography.

Introduction

High genetic quality animals can be multiplied efficiently using reproductive biotechnologies such as artificial insemination and embryo production. These biotechnologies are useful strategies and known worldwide for improving genetics and productivity of flocks over a short period (Mapletoft and Hasler, 2005; Boni, 2012; Hansen, 2014).

Recently, there have been an increasing interest in studies concerning antral follicle count (AFC) and its influence on the reproductive performance in cattle, as well as its applications in reproductive biotechnologies (Ireland *et al.*, 2011; Pontes *et al.*, 2011; Rico *et al.*, 2012; Silva-Santos *et al.*, 2014a, b). Such fact may result in immense repercussions on the current scenario of animal reproduction, considering the significant increase in the world's embryo production. Despite several favorable results about AFC in *Bos taurus*, many aspects of reproductive physiology remain unknown. Considering AFC in *Bos indicus* there are many points to be addressed, particularly the impact on

fertility when using *in vitro* embryo production (IVEP), timed artificial insemination (TAI) and timed embryo transfer (TET).

The high variability in the population of antral follicles is a hallmark in cattle (Burns *et al.*, 2005) with low, intermediate or high AFC (Santos *et al.*, 2012, 2013; Mendonça *et al.*, 2013). Despite the high variability among animals, there is a high repeatability of the number of follicles observed in the same individual through evaluations carried out during a period (Burns *et al.*, 2005; Ireland *et al.*, 2007, 2008, 2009).

This constancy in AFC in the same individual becomes a strategic resource for the possibility of classifying an animal by the AFC with a single ultrasound examination. For *taurus* animals, AFC is directly correlated with the size of the ovarian follicular reserve (Ireland *et al.*, 2011), which was not proven in *indicus* females, considering fetuses, heifers and cows (Silva-Santos *et al.*, 2011). However, other factors such as genetics (Walsh *et al.*, 2014), maternal environment, nutritional status and healthiness (Ireland *et al.*, 2011; Evans *et al.*, 2012) also appear to influence the AFC. For example, the nutritional status and the metabolic rate were mentioned as factors which affect the follicular growth, oocyte quality and secretion of reproductive hormones in cattle (Jimenez-Krassel *et al.*, 2009; Mossa *et al.*, 2010; Evans *et al.*, 2012).

The AFC may also influence the production of cattle embryos, both *in vivo* and *in vitro*, especially as the number of embryos produced by donor but also in process efficiency, with higher rates for high AFC animals (Ireland *et al.*, 2008; Santos *et al.*, 2014; Silva-Santos *et al.*, 2014a).

The importance of the AFC and its relationship with pregnancy rates must also be emphasized. Studies conducted with *taurus* females had higher pregnancy rates for high AFC females (Cushman *et al.*, 2009; Evans *et al.*, 2012; Mossa *et al.*, 2012). However, in recent studies with *indicus-taurus* and *indicus* animals, a better performance regarding pregnancy rates was not observed in high-AFC animals (Mendonça *et al.*, 2013; Santos *et al.*, 2014). Surprisingly, some data suggest a better performance regarding pregnancy rates for low AFC cows (Santos *et al.*, 2013).

In addition to ultrasound, the measurement of the concentration of anti-Mullerian Hormone (AMH)

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Received: May 27, 2015

Accepted: July 13, 2015



can also be used for selection of females according to the size of follicular population, since AMH has been considered as a factor highly correlated with the number of antral follicles and healthy oocytes (Hehenkamp *et al.*, 2006; Ireland *et al.*, 2011).

Considering the impact of reproductive biotechnology in genetic improvement and doubts as to the effect of antral follicle count on the reproductive performance in *indicus* animals, this review aims to discuss the main aspects of the AFC related to the number of antral follicles and its relationship with follicular dynamics, TAI, OPU/IVF and SOV.

Antral follicles count and its repeatability

The number of ovarian antral follicles is highly variable in different species (Burns *et al.*, 2005; Ireland *et al.*, 2007; Pontes *et al.*, 2011; Santos *et al.*, 2012), but in cattle, there is a high repeatability in the same individual (Burns *et al.*, 2005). Thus, the possibility of selecting *taurus* females by ultrasonography based on the AFC was demonstrated, due to the high degree of repeatability between individuals, regardless of race, age, breeding season, lactation or pregnancy conditions (Burns *et al.*, 2005). The same situation of AFC repeatability was demonstrated in *indicus* blooded animals; evaluating the same females for several months it was possible to identify the high correlation (0.90-0.92) in *taurus-indicus* weaned and yearling females (Santos *et al.*, 2012; Silva-Santos *et al.*, 2014a).

Therefore, a single routine ultrasound examination can identify females with low, intermediate or high AFC, according to findings also observed in Nelore females (Morotti *et al.*, 2015; DCV-CCA-UEL, Londrina, PR, Brazil; unpublished data).

Antral follicles count and embryo production

Considering the importance of the production of embryos for genetic improvement of livestock, it is noted that there is a great variability in the number of embryos produced by the donor, in both *in vitro* and *in vivo* methods (Pontes *et al.*, 2009, 2010, 2011). The high variability in oocyte recovery rate and superovulatory response are important factors that affect the success of the bovine embryo production (Taneja *et al.*, 2000; Ireland *et al.*, 2007, 2011; Silva-Santos *et al.*, 2014a). In this context, several studies with *taurus* animals and some studies with *indicus*-blooded animals have shown that a greater number of follicles is associated with quantitative benefits to the success of IVEP and SOV (Taneja *et al.*, 2000; Singh *et al.*, 2004; Ireland *et al.*, 2008; Silva-Santos *et al.*, 2014a). For *indicus-taurus* animals, the average number of embryos per collection was also higher for females with high compared to low AFC animals (6.9 ± 5.3 vs. 1.9 ± 2.1 ; $P < 0.05$). Therefore, this study reinforces the evident concept that there are quantitative benefits of embryo production according to the number of antral follicles (Table 1).

Table 1. Reproductive performance of *Bos taurus-indicus* females with high (G-High, follicles ≥ 40) or low (L-Low, ≤ 10 follicles) antral follicles count comparing the production of embryos *in vitro* (ovum pick up/*in vitro* production - OPU/IVP) and *in vivo* (superovulation (SOV)).

Variables	G-High (n = 20)	G-Low (n = 20)
Antral follicles (n)	47 \pm 6	9 \pm 3
Total of oocytes retrieved	738 ^a	116 ^b
Percentage of viable oocytes (%)	58.94 (435/738)	55.17 (64/116)
Cleavage rate (%)	61.25 (452/738)	56.03 (65/116)
Blastocyst rate (%)	16.53 (122/738)	9.48 (11/116)
Total embryos / OPU/IVP (n)	6.10 \pm 4.51 ^{aA} (122/20)	0.55 \pm 0.83 ^{bB} (11/20)
Total structures recovered / collected (n)	8.80 \pm 6.78 ^a (176/20)	2.25 \pm 2.63 ^b (45/20)
Total embryos / collected (n)	6.95 \pm 5.34 ^{aA} (139/20)	1.9 \pm 2.13 ^{bA} (38/20)
Frozen proportion (%)	78.42 ^a (109/139)	89.47 ^a (34/38)

The values followed by different superscript letters (^{a, b}) within the same line (G-G vs. high-low) or (^{A, B}) within the same column (the OPU/IVP vs. SOV) were significantly different ($P \leq 0.05$). Adapted from Silva-Santos *et al.* (2014a).

Similar results were obtained with *taurus* donors by Ireland *et al.* (2007), with low AFC females presenting a lower mean number of *in vitro*-produced

embryos compared with high AFC animals (1.3 vs. 4.9 embryos). In *in vivo* production of the embryos, low AFC donors (< 15 follicles) produced fewer structures



when compared with high AFC females (>25 follicles), with means of 3.8 vs. 5.4 embryos, respectively.

Contradictory results for *indicus* animals were found by our research team. In an experiment with Nelore donors (n = 66), they were separated into groups of high AFC (AFC > 40 follicles); medium AFC (18 to 25 follicles) or low AFC (AFC < 7 follicles), and the oocytes were fertilized with sperm from a single bull. In addition to the expected quantitative superiority, a qualitative superiority was observed according to the AFC. Cows with high, medium and low AFC had blastocyst rates of 42, 32 and 13%, respectively (P < 0.05; Santos *et al.*, 2015; DCV-CCA-UEL, Londrina, PR, Brazil; unpublished data). However, in a recently concluded study, this pattern was not confirmed. From a larger sample of Nelore cows (n = 356), oocytes were obtained from females of high (>92 follicles), medium (46-76 follicles) or low AFC (<31 follicles). All the oocytes were also fertilized with sperm from a single bull. There was no difference in cleavage and blastocyst rates: 40, 36 and 38% for high, medium and low AFC, respectively (Rosa *et al.*, 2015; DCV-CCA-UEL,

Londrina, PR, Brazil; unpublished data).

In addition to the results of embryo production and pregnancy rates, mRNA expression of genes associated with follicular and oocyte viability seem to show similar patterns in zebu females of high, medium or low AFC. There was no significant difference in the expression of genes related to steroidogenesis (CYP19 and STAR), cell proliferation and differentiation (TGFB1, LIFRa and BMPR2) and hormonal production or response to hormones (AMH, FSHr, PGR and PGRMC) in cumulus and granulosa cells of Nelore females (Rosa *et al.*, 2015; DCV-CCA-UEL, Londrina, PR, Brazil; unpublished data). The contradiction of such results in the same research team reinforces the complexity of this issue.

It is noted that AFC is not the only aspect to quantitatively interfere in embryo production, considering that donors respond to OPU/IVF or SOV according to variables not yet fully understood. In this context, Pontes *et al.* (2009) reported that some donors provided better results on *in vitro* or *in vivo* techniques, regardless of the number of follicles (Table 2).

Table 2. Variations in embryo production rates of six Nelore (*Bos indicus*) donors obtained by *in vitro* procedures (ovum pick up/*in vitro* production - OPU/IVF) or *in vivo* (superovulation and embryo collection - SOV).

Variables	Donor					
	I	II	III	IV	V	VI
Total procedures OPU/IVF	5	5	4	4	5	5
Mean oocytes per OPU	36.6	25.6	49	29.7	22.8	16
Mean viable oocytes per OPU	32.2	23.4	45.2	26	19.6	14.4
Mean embryos per OPU	15.6	10.4	24.1	10.3	6.8	3.8
Mean pregnancy per OPU	4.8	2.8	9.25	4.3	2.2	1
Total procedures SOV/collection	2	3	2	2	2	3
Mean embryos per SOV	10	4.3	6.5	2	12.5	5.3
Mean pregnancy per SOV	5.5	2	1	1.5	6.5	1.3

Adapted from Pontes *et al.* (2009).

Despite these individual variations according to the technique, there is a consensus that the quantitative advantages of the high AFC donor should be exploited. Thus, the variation in the number of follicles per donor is currently a very important aspect for the commercial programs of embryos production. A screening method

for selection of donors through an ultrasonographic pre-evaluation is commonly used. The selected donors are generally those with high AFC or a high number of oocytes, which are directly correlated variables. The impact on the final number of pregnancies varies widely, as shown in Table 3.

Table 3. Production of embryos and pregnancies according to the number of oocytes obtained by OPU/IVP (n = 656) from Nelore donors (n = 317). The values (mean ± SD) are presented per donor.

Donors according to oocyte production	N°. viable oocytes	N°. viable embryos	N°. pregnancy 30 days	N°. pregnancy 90 days
Elevated (n = 78)	47.06 ± 1.6 ^a	15.06 ± 0.86 ^a	5.62 ± 0.54 ^a	5.52 ± 0.81 ^a
High (n = 80)	24.95 ± 0.33 ^b	9.17 ± 0.63 ^b	3.63 ± 0.36 ^b	3.32 ± 0.33 ^b
Medium (n = 79)	15.57 ± 0.26 ^c	6.00 ± 0.39 ^c	2.10 ± 0.21 ^c	1.92 ± 0.20 ^b
Low (n = 80)	6.31 ± 0.38 ^d	2.42 ± 0.25 ^d	0.92 ± 0.13 ^d	0.85 ± 0.13 ^c

^{a-d} Within a column, the mean values with no common superscript differ significantly (P ≤ 0.05). Adapted from Pontes *et al.* (2011).



Despite the unquestionable quantitative advantages in number of embryos for the donor selection method for OPU/IVF based in the AFC, the impact of this criterion on other aspects of fertility and production of meat or milk is not well known for *indicus* animals. Thus, until more studies are performed, the choice of donor based on the number of follicles should be performed only after assessing the genetic merit for production traits of the donor.

Number of antral follicles and anti-Mullerian hormone

AMH is a hormone that belongs to the family of growth factor β , and is produced by granulosa cells from healthy growing follicles. Its expression is elevated in granulosa cells of small antral follicles and decreases during the follicular growth. The high concentrations of AMH are positively associated with follicular size of mice, women and cows' ovaries population. In the latter, the concentration of AMH is highly correlated with the number of antral follicles and healthy oocytes, thus can be considered a viable endocrine marker of AFC in cattle (Batista *et al.*, 2014).

Bovine females with high AFC (>25 follicles) have higher circulating AMH concentrations in comparison to the females with low AFC (<15 follicles; $P < 0.01$), with a high correlation between the average concentration of AMH and the mean AFC to *Bos taurus* cattle ($r = 0.88$, $P < 0.001$; Ireland *et al.*, 2008). When comparing *taurus* and *indicus* females, both beef and dairy cattle, AMH concentration was also positively correlated with AFC in *indicus* (Nelore, ranging from 0.56 to 0.68) and *taurus* (Holstein, ranging from 0.73-0.90). Regardless of the genetic group, females with high AFC showed higher AMH concentrations (0.57 ng/ml in *taurus* vs. 1.20 ng/ml in *indicus*) than females with low AFC (0.06 and 0.78 ng/ml, respectively). Furthermore, there is evidence that AFC in *indicus* (low and high, 28 and 48 follicles, respectively) is higher than in *taurus* (low and high, 13 and 34 follicles, respectively; Batista *et al.*, 2014).

Currently, AMH is recognized as a reliable indicator of ovarian response to superovulation. Therefore, the determination of AMH concentration in donor cows may help predict the follicular and ovulatory responses to gonadotropic treatment (Rico *et al.*, 2009).

The AMH concentrations in blood or plasma may be determined by an ELISA test. In a study with Holstein cows over a year, the concentrations of AMH were constant and strongly correlated with the AFC. Donors with AMH concentrations below 87 pg/ml showed less than 15 large follicles by estrous cycle and low efficiency in the production of embryos. Thus, the determination of the concentration of AMH in bovine plasma could routinely be considered in procedures of OPU/IVF and SOV for identification of animals with the best embryo production potential (Rico *et al.*, 2012).

Number of antral follicles and progesterone

The production of progesterone has been linked to physiological activity of the corpus luteum and to ovary and uterus functionality, with direct impact on embryonic development and pregnancy in cattle (Pohler *et al.*, 2012). Low progesterone concentrations are associated with high rates of embryonic mortality, less healthy oocytes and slower growth of the endometrium in these females (Diskin and Morris, 2008). Low AFC *taurus* females showed low concentrations of progesterone during their oestrus cycle, in comparison with high AFC females. The lower circulating concentrations of progesterone in cows with low AFC were mainly attributed to decreased function of the corpus luteum, possible changes in the responsiveness of luteal cells to LH, a potential reduction STAR protein in the corpus luteum, diminished responsiveness of granulosa and luteal cells to 25-hydroxycholesterol and the reduced ability of granulosa cells of dominant follicles to undergo luteinization in order to produce progesterone (Jimenez-Krassel *et al.*, 2009). It is not clear why an ovulated follicle that turned into a CL would produce less P4 when it is in a low AFC animal. Mainly considering recent studies in *Bos indicus* in which the situation seems to be the opposite (Seneda *et al.*, 2015; DCV-CCA-, Londrina, PR, Brazil; unpublished data). Considering this and other aspects, we believe in distinct pathways in *Bos taurus* and *Bos indicus* at least for some reproductive aspects. It has also been demonstrated that high AFC was positively associated with endometrial thickness. An increased endometrial thickness was associated with higher embryonic implantation rates (Basir *et al.*, 2002).

Antral follicle count and fertility parameters in beef heifers and cows

The low AFC in dairy *taurus* females (Holstein) was associated with several characteristics related to infertility, such as smaller ovaries (Ireland *et al.*, 2008), less chance of pregnancy at the end of the breeding season (Mossa *et al.*, 2012), reduced responsiveness to the SOV treatment, fewer viable embryos (Singh *et al.*, 2004; Ireland *et al.*, 2007), lower circulating concentrations of progesterone and AMH (Ireland *et al.*, 2011; Evans *et al.*, 2012) and reduced endometrial thickness (Jimenez-Krassel *et al.*, 2009). In this context, animals with high AFC were proved superior in all of the mentioned aspects. Thus, it is assumed and substantiated that in Holstein cows a linear correlation exists between high AFC and indicators for reproductive efficiency.

This context, however, has not been proven in Zebu animals. Recent studies have shown that reproductive traits of *indicus* (Nelore) and *taurus-indicus* females (Braford) did not show improved performance associated with high AFC (Santos *et al.*, 2012, 2013; Mendonça *et al.*, 2013; Morotti *et al.*, 2014;



Santos *et al.*, 2015, DCV-CCA-UEL, Londrina, PR, Brazil, unpublished data). A higher rate of follicular growth and larger follicular diameters have been described in *indicus-taurus* (Santos *et al.*, 2012) and *indicus* (Morotti *et al.*, 2014) females with low-AFC. Also, dominant follicles with larger diameters have been described in Nelore females with low AFC (Morotti *et al.*, 2015; DCV-CCA-UEL, Londrina, PR, Brazil; unpublished data).

Considering the AFC bimonthly basis in 137 *taurus-indicus* females, there was no difference in the average body weight from nine to 24 months, or in the diameter of the uterine horn (Santos *et al.*, 2012). In a subsequent experiment, 71 females from same herd were subjected to a TAI protocol to determine the values of certain variables associated with follicular dynamics. The data are presented in Table 4.

Table 4. Mean \pm SD values of follicular dynamics of *taurus-indicus* females (Braford) with different antral follicles counts (AFC; high, ≥ 40 follicles; medium, 17-23 follicles; and low, ≤ 10 follicles) after a protocol of ovulation synchronization.

Variables	High ≥ 40 follicles n = 24	Medium 17-23 follicles n = 24	Low ≤ 10 follicles n = 23
Antral follicle count on D5 (n)	47 \pm 9.9 ^a	24 \pm 9.9 ^b	9 \pm 3.9 ^c
Diameter of the largest follicle on D5 (cm)	0.66 \pm 0.3	0.70 \pm 0.2	0.80 \pm 0.2
Ovulatory follicle diameter (cm)	1.15 \pm 0.2 ^a	1.27 \pm 0.2 ^b	1.32 \pm 0.2 ^b
Interval between device removal and ovulation (h)	69.33 \pm 5.1	71.25 \pm 3.0	70.50 \pm 4.1
Ovulation rate (%)	75 (18/24)	67 (16/24)	70 (16/23)
CL diameter (cm)	1.93 \pm 0.3	1.97 \pm 0.3	2.04 \pm 0.3

^{a-c}Within a column, the mean values with no common superscript differ significantly ($P \leq 0.05$). Adapted from Santos *et al.* (2012).

Unlike the results reported for Holstein cows (Ireland *et al.*, 2011; Evans *et al.*, 2012), the data in Table 4 show that Braford females of low and medium AFC had ovulatory follicles with greater diameters.

Regarding pregnancy rates of *indicus* (Nelore) females, two studies have shown pregnancy data after TAI, one with postpartum cows (n = 691) and the other with heifers at 24 months of age (n = 208). On day 8 of the TAI protocol, the ovaries were evaluated by

ultrasonography and females were classified into groups of high, medium or low AFC. Pregnancy rates were evaluated by transrectal ultrasonography 30 days after TAI. The data, summarized in Table 5, demonstrate that low AFC cows were superior to medium AFC cows, while high AFC cows were similar to both. There were no differences for heifers. In short, it was not possible to establish the same relationship described for *taurus* females.

Table 5. Reproductive performance of Nelore (*Bos indicus*) females with high, medium and low antral follicle count (AFC) after a timed artificial insemination protocol.

Cows (n)	High ≥ 25 follicles (149)	Medium 11-24 follicles (400)	Low ≤ 10 follicles (142)	Total (691)
Antral follicles (n)	30.70 \pm 5.66 ^a	17.03 \pm 3.28 ^b	7.83 \pm 2.42 ^c	17.93 \pm 8.45 ^b
Pregnancy rate (%)	51.67 ^{ab}	48.00 ^b	60.50 ^a	51.49 ^{ab}
Heifers (n)	High ≥ 30 follicles (38)	Medium 13-29 follicles (143)	Low ≤ 12 follicles (27)	Total (208)
Antral follicles (n)	37.73 \pm 7.05 ^a	19.23 \pm 4.29 ^b	10.55 \pm 2.17 ^c	21.48 \pm 9.47 ^b
Pregnancy rate (%)	44.73	43.35	51.85	44.71

^{a-c}Within a column, the mean values with no common superscript differ significantly ($P \leq 0.05$). Data are presented as mean \pm SD. Adapted from Santos *et al.* (2013) and Mendonça *et al.* (2013).

The association between AFC and pregnancy rates was also analyzed in *indicus* recipients after the transfer of *in vitro*-produced embryos. Cyclical Nelore heifers (n = 281, ECC 3.0 \pm 0.5) underwent a classic

protocol for ovulation synchronization before TET. The heifers received the embryos 17 days after the beginning of the treatment. There was no difference in pregnancy rate according to AFC (Table 6).

Table 6. Antral follicle count and pregnancy rates of Nelore (*Bos indicus*) recipients after TET of *in vitro* embryo production.

Heifers (n)	High ≥25 follicles (38)	Medium 11 follicles (136)	Low ≤3 follicles (75)
Antral follicles (n)	25.8 ± 7.4 ^a	11.3 ± 2.9 ^b	3.8 ± 1.3 ^c
Pregnancy rate (%)	30.0	33.8	34.6

^{a-c}Within a column, the mean values with no common superscript differ significantly (P ≤ 0.05).

Antral follicle count, carcass phenotype and genetic characteristics of heritability

In a recent study, heritability and the impact of environmental effects during pregnancy on AFC was evaluated in cattle. In the Holstein breed, this parameter had heritability of 0.31 ± 0.14 and 0.25 ± 0.13 for cows and heifers, respectively. The AFC was negatively associated with genetic merit for milk fat. The authors concluded that the AFC in Holstein females is an inherited genetic trait moderately affected by age and lactation status, but not the mother's milk production level during pregnancy (Walsh *et al.*, 2014).

To *indicus-taurus* heifers (Braford, n = 270), the AFC was considered in a statistical model associated with phenotypic characteristics for selecting matrices. The variables considered were mean number of antral follicles, effect of contemporary group, age, birth weight gain at weaning, conformation at weaning, finishing at weaning precocity, musculature at weaning, weight gain from weaning to yearling, forming at yearling, precocity at yearling and musculature at yearling. For all parameters studied, the correlation values were very low (0.056 to 0.082; P > 0.05), pointing out that, for *taurus-indicus* animals, the antral follicle population has no association with the main selection criteria of matrices for beef herds (Morotti *et al.*, 2015; DCV-CCA-UEL, Londrina, PR, Brazil; unpublished data).

Final comments

After all comments above, our general conclusion is: so far, it is not possible to apply the *Bos taurus* AFC model for *Bos indicus* females. While the AFC seems to be a very clear criterium for reproductive selection in *taurus*, we have found contradictory results in *indicus* cattle. There are results signaling for a better reproductive efficiency of low AFC animals, others to the medium AFC females and even, in the same model of *taurus* cows, data suggesting that there is a greater reproductive efficiency in high AFC zebu females.

One of the main challenges in the analysis of articles refers to the large variation of the classification criteria of high, medium or low follicle count. There are values considered as low AFC in one article that are considered as high AFC in other study, using the same category and the same breed. This situation makes it

very hard to compare data.

Nevertheless, the inconstancy of the results shows the need to always prioritize genetic merit in the choice of a donor, and not AFC. Following this criterion is particularly important for oocyte donors. In *in vitro* embryo production, the quantitative advantage of number of structures tends to create a biased selection of donors with high AFC, because they make the entire IVEP chain more profitable. Considering the estimate that Brazil, as the world leader of this biotechnology, produces annually around 300,000 embryos *in vitro* (Viana *et al.*, 2012) it is necessary to consider that an immediate benefit in the number of embryos should not supercede a strict criterion of genetic merit, which is a more important aspect in short, medium and long term in the selection of animals.

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