DOI: 10.21451/1984-3143-AR994

Proceedings of the 31st Annual Meeting of the Brazilian Embryo Technology Society (SBTE); Cabo de Santo Agostinho, PE, Brazil, August 17th to 19th, 2017.

Antral follicle count in cattle: advantages, challenges, and controversy

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Abstract

The antral follicle count (AFC) represents the number of follicles visualized by ultrasonography in the ovaries. Antral follicle count tends to be variable among cows but with high repeatability within the same individual. In the last decade, AFC has been considered a biological marker of fertility with many positive aspects of reproductive efficiency for those animals with high AFC. For instance, a larger number and better-quality embryos, better pregnancy rates, increased concentrations of circulating progesterone, in addition to other characteristics linked to fertility, are all responses observed in individuals with high compared with those with low AFC in *Bos taurus* cattle. However, the positive association between AFC and fertility did not follow the same pattern for *Bos indicus*. Recent articles showed no connection between fertility and number of antral follicles or better reproductive efficiency for high AFC group in Zebu cows. Thus, the aim of this review is to discuss the various data concerning AFC between *indicus* and *taurus* cattle. Additionally, we consider AFC to be a possible tool to improve cattle performance in reproductive biotechnology.

Keywords: antral follicle count, *Bos indicus*, *Bos taurus*, embryo production, fertility.

Introduction

Assisted reproductive technologies, such as artificial insemination and embryo production, represent crucial tools to improve genetic merit in cattle production (Mapletoft and Hasler, 2005; Hansen, 2014; Bó *et al*., 2016). Recently, reports in the literature have documented improved reproductive parameters and response to reproductive biotechnologies in cattle with increased antral follicle count (Ireland *et al*., 2011; Rico *et al*., 2012; Silva-Santos *et al*., 2014a). These first reports described benefits of high AFC in *Bos taurus*, and the evaluation of antral follicles by ultrasound was considered the most practical strategy to classify a cow for reproductive purposes. Despite the considerable variability in AFC among cows, the number of antral follicles observed in the same animal is highly repeatable over several evaluations (Burns *et al.*, 2005; Morotti *et al*., 2017). This reproducibility of AFC in the same individual becomes a strategic resource for 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 when examining 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, nutritional status and general metabolism were mentioned as factors that affect follicular growth, oocyte quality, and secretion of reproductive hormones in cattle (Jimenez-Krassel *et al*., 2009; Mossa *et al*., 2010; Evans *et al*., 2012).

Considering *Bos taurus* cattle, the AFC has been directly linked to the female reproductive performance. Several reports suggest that high AFC is related to the total number of follicles that are morphologically healthy (Ireland *et al*., 2008, 2011). Additionally, the number of oocytes and blastocysts (Guerreiro *et al*., 2014), as well as the concentration of progesterone, were greater for cows with more antral follicles (Jimenez-Krassel *et al*., 2009). Moreover, females with high AFC showed a greater number of embryos produced by the donors in *Bos taurus* (Ireland *et al*., 2008), crossbred *indicus-taurus* (Silva-Santos *et al*., 2014a) and *Bos indicus* (Santos *et al*., 2016). Additionally, cows with high AFC presented greater pregnancy rates compared with those with low AFC (Evans *et al*., 2012; Mossa *et al*., 2012).

In contrast, other researchers reported controversial data regarding AFC and parameters of fertility (Santos *et al*., 2013). Recent studies with timed artificial insemination (TAI) in *indicus* and crosses of *indicus-taurus* cattle described no positive correlation between AFC and pregnancy rates (Mendonça *et al*., 2013; Santos *et al*., 2014). Interestingly, certain results suggest better pregnancy rates for low AFC cows (Santos *et al*., 2013).

Considering the application of AFC as a valuable tool to assist cattle performance and reproductive biotechnology, this review aims to discuss the following: i) the relationship between the number of antral follicles and physiological parameters; ii) the challenges to applying AFC in the field, and iii) the different data for AFC between several research teams.

Repeatability of AFC and anti-müllerian hormone

Several studies performed in *taurus* (Burns *et al*., 2005; Ireland *et al*., 2008), *indicus* (Santos *et al*., 2012; Silva-Santos *et al*., 2014b) and *indicus-taurus* crosses (Silva-Santos *et al*., 2014a) reported AFC to be

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Received: 27 June, 2017
Accepted: 7 July, 2017

a parameter that was highly variable among cows but with a very consistent repeatability within the same animal. Based on this characteristic, the females can be classified as low, intermediate, or high AFC according to the number of antral follicles visualized by ultrasonography (Ireland *et a*l., 2008; Guerreiro *et al*., 2014; Silva-Santos *et al*., 2014a). An important aspect related to AFC is its high correlation with concentrations of the anti-müllerian hormone (AMH).

The AMH belongs to the β-growth factor superfamily and is synthesized from granulosa cells of antral and preantral follicles (Cate *et al*., 1986). The concentration of this hormone in blood is highly correlated with the AFC. Thus, AMH can be considered an endocrine marker of the AFC in *Bos indicus* and *Bos taurus* females (Batista *et al*., 2014; Morotti *et al*., 2015). The significant advantage of this property is the possibility of using AFC instead of AMH dosage, as a single ultrasound evaluation is less expensive and easier than determining the AMH concentration. In *taurus* animals, a high correlation ($r = 0.88$, $P < 0.001$) was observed between the AMH concentration and the ovarian AFC. The females classified as high AFC showed greater concentrations of AMH when compared to cows with low AFC (Ireland *et al*., 2008). The same pattern was observed in *Bos indicus,* with high AFC cows presenting greater AMH concentration than cows with low AFC (Batista *et al*., 2014). In this context, the AMH is recognized as a reliable indicator of ovarian activity and ability to respond to a super-stimulation protocol and ovum pick-up/*in vitro* production (IVP; Rico *et al*., 2009; Baruselli *et al*., 2016).

Concentrations of progesterone and AFC

The production of progesterone by the corpus luteum (CL) is necessary for maintaining the appropriate uterine environment, enabling embryonic development and maintaining pregnancy in domestic animals (Bazer *et al*., 2010; Pohler *et al*., 2012). Consequently, low progesterone concentration is associated with reproductive problems, such as embryonic mortality and slower development of the endometrium in cattle (Inskeep, 2004; Diskin and Morris, 2008).

Interestingly, the number of follicles in the ovary was associated with concentrations of progesterone, as described by Martinez *et al*. (2016), corroborating the data of previous studies (Ireland *et al*., 2011; Evans *et al*., 2012). All these authors reported greater concentrations of plasma progesterone in *taurus* cows with high AFC during diestrus and pregnancy compared with low-AFC females. The reduced concentration of progesterone in plasma of low-AFC females was associated with decreased capacity of luteal and granulosal cells to produce progesterone, and reduced abundance of STAR and mRNA for STAR and LH receptor in CL (Jimenez-Krassel *et al*., 2009).

Antral follicle count and efficiency in embryo production

Increased AFC has been related to a larger number of IVP embryos for both *Bos taurus* and *Bos indicus* cows (Taneja *et al*., 2000; Singh *et al*., 2004; Silva-Santos *et al*., 2014a). Considering *Bos taurus* cattle from Europe, the group classified with low AFC presented smaller number of embryo compared with cows classified in the high AFC group (Ireland *et al*., 2007). In the same way, *indicus* cows with high AFC also presented a greater number of embryos than those considered to have low AFC as shown in Table 1.

Table 1. Production of embryos and pregnancies according to the number of oocytes obtained by OPU/IVP (n = 656) from Nelore donors (n = 317). Values are presented per donor (mean ± SD).

Donors according to oocyte production	N°. viable oocytes	N°. viable embryos	Blastocyst rate, %	N°. pregnancy 30 days	N°. pregnancy 90 days
Elevated (n = 78)	47.06 ± 1.6[a]	15.06 ± 0.86[a]	32.00	5.62 ± 0.54[a]	5.52 ± 0.81[a]
High (n = 80)	24.95 ± 0.33[b]	9.17 ± 0.63[b]	36.75	3.63 ± 0.36[b]	3.32 ± 0.33[b]
Intermediate (n = 79)	15.57 ± 0.26[c]	6.00 ± 0.39[c]	38.54	2.10 ± 0.21[c]	1.92 ± 0.20[b]
Low (n = 80)	6.31 ± 0.38[d]	2.42 ± 0.25[d]	38.35	0.92 ± 0.13[d]	0.85 ± 0.13[c]

[a-d]Within a column, mean values with uncommon superscripts differ significantly ($P \leq 0.05$). Adapted from Pontes *et al*. (2011).

Considering the efficiency of embryonic production, and not only the total number of ova/embryos, the relationship between IVP and AFC in *indicus* donors is controversial. Several studies with *indicus* showed the same pattern of that obtained from *taurus* donors. For example, Nelore cows (n = 66) classified into high (>40 follicles), intermediate (18 to 25 follicles) or low AFC (<7 follicles) groups produced 42, 32 and 13% ($P < 0.05$) of total blastocyst rates, respectively (Santos et *al*., 2016). Considering *indicus-taurus* heifers classified in high (≥40 follicles) or low (≤10 follicles) AFC groups, the results showed a greater number of embryos produced in females with high AFC compared with those with low AFC (6.9 ± 5.3 *vs.* 1.9 ± 2.1; Silva-Santos *et al*., 2014a). However, Monteiro *et al*. (2017) did not find any advantage of a high AFC when considering IVP of embryos. Those authors classified *Bos indicus* females into high (>15) or low (<15) cumulus-oophorous complex (COC) counts, and data were collected through 12 consecutive OPU/IVP sessions. Differences in COC and number of blastocysts were observed, but production of blastocyst per COC did not differ according to category of COC (Tab. 1).

Similar results were obtained by another team. The authors studied a large number of Nelore donors (n = 356), which were classified into high (>92 follicles), intermediate (46 to 76 follicles), or low AFC groups (<31

follicles). *In vitro* embryo production did not differ among the three groups: 40, 36 and 38%, respectively (Rosa *et al*., 2015). Thus, although several studies involving Zebu animals demonstrate a positive correlation between embryo production and high AFC, this aspect is not entirely clear in *Bos indicus* cattle (Tab. 2). To date, only a quantitative advantage for high AFC is evident, but proportion of blastocyst obtained per COC is not supported by *indicus* donors with more antral follicles.

To date, it is not possible to clarify the controversial data regarding AFC and IVP. We have several considerations to comment regarding the contradicting results between *Bos taurus* and *Bos indicus*. First, we should consider the physiological differences involving reproductive patterns between *taurus* and *indicus* cattle. Several aspects have been reported on that (e.g., number of follicular waves, metabolism of hormones, and diameter of ovulatory follicle). The influence of AFC on IVP may be another physiological difference related to breeds of European origin compared with Zebu cattle. Another question involved in the different data of AFC and IVP is the classification of animals into high, intermediate, or low AFC groups. The literature in this area is heterogenous and there is no standard to establish the follicular groups. In summary, the full understanding of how AFC could influence IVP is not understood to date. We believe that further studies will certainly help to improve our knowledge on the subject.

Table 2. Cumulative production of blastocysts by Nelore donors (*Bos indicus*) according to the category (high, n = 18 or low, n = 18) of COC recovered (LSM ± SEM).

Ovum pickup program	Cumulative blastocyst production per donor			P value
	Low COCs	High COCs	Overall	
After 3 months	10.6 ± 1.6	25.6 ± 3.9	18.1 ± 2.4	0.001
After 6 months	22.2 ± 2.7	47.7 ± 6.8	34.9 ± 4.2	0.0004
After 30 months	43.6 ± 5.2	85.6 ± 11.4	64.6 ± 7.1	0.001

Adapted from Monteiro *et al*. (2017).

Fertility parameters and AFC in cows

The hypothesis that fertility is influenced by the number of follicles and oocytes in ovaries is an old idea (Hunter, 1787; Erickson, 1966). Over the years, several studies have described a high AFC as being positively correlated with female fertility. Many advantageous features that affect fertility have been related to the number of follicles in the ovary (Ireland *et al*., 2008), such as plasma progesterone concentration, (Jimenez-Krassel *et al*., 2009; Martinez *et al*., 2016), shorter interval between calvings (Mossa *et al*., 2012), and CL function and endometrial thickness (Jimenez-Krassel *et al*., 2009). However, it is important to emphasize that the positive correlation between AFC and cow fertility was mainly described in *Bos taurus* cattle (Mossa *et al*., 2012, 2013; Walsh *et al*., 2014; Jimenez-Krassel *et al*., 2015; McNeel and Cushman, 2015; Santos *et al*., 2016).

However, even for *taurus* cows, the association between AFC and fertility parameters may be controversial. When considering New Zealand *taurus* lactating dairy cows, it was not possible to verify any association between AFC and proportion of cows pregnant after artificial insemination, as shown in Table 3 (Martinez *et al*., 2016), but future studies may contribute to improving the accuracy of this information.

Table 3. Reproductive responses in *Bos taurus* New Zealand lactating dairy cows subjected to artificial insemination according to antral follicle count.

Item[1]	High ≥ 30 follicles n = 104	Intermediate 21-29 follicles n = 137	Low ≤ 20 follicles n = 200
Days to pregnancy (LSM ± SEM)	82.4 ± 1.6[a]	85.2 ±1.6[ab]	87.3 ±1.2[b]
Number of AI per pregnancy (LSM ± SEM)	1.2 ± 0.1	1.3 ± 0.1	1.3 ± 0.1
Estrus-detected cows (%)	50.9	48.9	40.9
Cows with a CL at scanning (%)	91.5[a]	84.7[ab]	82.4[b]
Pregnant to first AI (%)	54.6	49.4	48.5
Pregnant after 6 weeks of AI (%)	70.0	70.4	61.2
Pregnant overall (%)	87.1	79.4	81.0

[a,b]Different superscripts within the same row indicate significant effect ($P < 0.05$). [1]AI = artificial insemination; CL = corpus luteum. Adapted from Martinez *et al*. (2016).

When considering studies with *indicus* (Nelore) and *indicus-taurus* (Braford), the connection between of AFC and fertility seems to be even more complex. The evaluation of follicular dynamics of Nelore cows with low AFC (<15 follicles) demonstrated greater follicular diameter at TAI (13.4 *vs*. 12.2 mm, $P \leq 0.05$) and increased pregnancy to insemination (62 *vs*. 50%) than cows with a high AFC (>45 follicles; Morotti *et al*.,

2017; unpublished data). An increased rate of follicular growth and larger follicular diameters were previously described in *indicus-taurus* (Santos *et al*., 2012) and *indicus* cows with low AFC (Morotti *et al*., 2014). A larger ovulatory follicle diameter has been clearly associated with better pregnancy per insemination (Sá Filho *et al*., 2010; Pfeifer *et al*., 2012), and this aspect reinforces the hypothesis that cows with low AFC may present increased pregnancy rates.

Relationship between AFC and phenotypic and genotypic production characteristics

A recent study was performed to verify the influence of the phenotypic and genotypic characteristics of the genetic improvement program on AFC *indicus-taurus* heifers. This study revealed only one parameter, the visual score for finishing precocity at weaning, that had a very small negative correlation with AFC, and all other parameters evaluated for genetic merit for beef cattle showed no correlation with AFC (Morotti *et al*., 2017).

Another recent study conducted in crossbred beef heifers (*Bos taurus,* n = 95) confirmed that AFC is associated with calving day. Heifers giving birth early in the calving season presented more antral follicles than heifers giving birth later in the calving season (McNeel and Cushman, 2015). Therefore, the AFC can be used as an ovarian phenotype for the reproductive tract in commercial production.

For dairy cattle, it was demonstrated that AFC is a reproductive characteristic with moderate heritability that is affected by age and lactation status but not correlated with milk production or other characteristics associated with the genetic merit (Walsh *et al*., 2014).

When considering beef and dairy cattle, the AFC seems to not be related to parameters of production, either for meat or milk. In this way, the number of antral follicles could be used as secondary criteria for considering the use of reproductive biotechnologies. The first aspect always must be the genetic merit of the dam. Next, an analysis of AFC may be considered depending on the technique to be used.

Possible mechanisms involving AFC and fertility

The physiological mechanisms regarding number of follicles and fertility parameters are not currently understood. To date, most of the published literature has described primarily applied results. In other words, most published articles focused on rates of pregnancy, embryo production, follicular growth, and similar responses; however, a thorough description of the molecular mechanisms that underlie the differences among individuals with distinct AFC remains to be elucidated.

The original articles regarding AFC pointed to several advantages for females with a larger number of antral follicles. However, a relationship with low AFC and better fertility, such as observed in recent studies with TAI in *Bos indicus* seems to be more easily explained. The first results about follicular dynamics and AFC have shown larger ovulatory follicles in low-AFC cows compared with those with a high AFC (Morotti *el al*., 2017; unpublished data). When considering fewer number of follicles to receive gonadotropin stimulation, it is possible to expect that each follicle would obtain a larger amount of FSH at the emergence of the follicular wave. Conversely, those females with a high AFC would have more follicles to share the same amount of gonadotropins. After comparing the diameter of the ovulatory follicle, many articles have described better pregnancy rates for cows with moderate to large pre-ovulatory follicle diameter compared with females with small follicle diameter at ovulation (Sá Filho *et al*., 2010; Pfeifer *et al*., 2012). In this way, the better pregnancy rates in cows with a low AFC compared with cows with a high AFC might be explained by those with low AFC having larger diameter of the ovulatory follicle.

Regarding the IVP, the majority of the studies in the literature did not present a clear advantage for any AFC category, or the studies' results are controversial. This might be explained by the artificial steps the oocytes undergo with *in vitro* embryo production. The nuclear and cytoplasmic maturation of oocytes are finished *in vitro* aside from the follicular environment. In this way, it is possible to believe that the influence of the follicle on the oocyte will be lower than in *in vivo* conditions, such as artificial insemination. We believe this possibility could at least partly explain the differences of AFC between artificial insemination and IVP programs.

Challenges related to AFC

The largest problem in AFC studies has been the significant variation in the evaluation criteria used to establish the categories of high, intermediate, or low counts, making it difficult to compare data (Morotti *et al*., 2015). For example, an intermediate category in one published paper may be considered a low or even a high category for another paper. This question becomes more critical when comparing AFC between *taurus* and *indicus* cattle. Zebu cattle usually show two, three, or even four times more follicles than female cattle of European breeds. Therefore, it is not surprising that differences in follicullogenesis between species or subspecies of domestic cattle might also affect how AFC is associated with reproduction, and extrapolation of data from *Bos taurus taurus* to *Bos taurus indicus* breeds might be incorrect and further work is warranted to identify the underlying mechanisms that explain such differences in responses.

Another critical question is the interval among AFC categories. Several articles have considered specific cut-points with only one follicle to distinguish one AFC category from another. For example, a low AFC as being fewer than 15 follicles and an intermediate AFC as being equal to or greater than 15 follicles. We believe that it is highly critical to keep the interval close, but at the same time it is easy to imagine that one follicle could be missed during an ultrasound evaluation. Therefore, we suggest a more conservative

approach with a safety interval of AFC categories, eliminating a portion of the animals with AFC in between categories to maintain a more accurate classification of individuals. In other words, low AFC as fewer than 15 follicles and the intermediate category of AFC could start at greater than 20 or even greater than 25 follicles. The total number of animals in the experiment would be less because many cows would be discarded. Conversely, it would be possible to introduce more consistency in the AFC comparisons, because individuals with borderline values for AFC would be excluded.

Another critical challenge regarding AFC is related to the technique used. For example, females with high AFC are advantageous when submitted to OPU and IVP. For *taurus* donors, there are benefits regarding total numbers of embryos, as well as better rates of embryonic development. For *indicus* cows, it is well-accepted that there is a better performance only on the number of embryos. However, for pregnancy rates after AI, the results between *indicus* and *taurus* cattle are notably conflicting. For *taurus* herds, it is well-established that high AFC will be associated with best pregnancy rates in AI programs. On the other hand, for Zebu females, the relationship between AFC and pregnancy per AI remains equivocal. In fact, several studies have shown improved proportion of pregnancy in cows with low or intermediate AFC (Jimenez-Krassel et al., 2017; Santos *et al*., 2013, 2016).

Conversely, there are many factors that warrant further study, such as the relation of AFC with follicular growth, quality of oocytes, nutritional and health status of the female. Additionally, the maternal environment during gestation seems to determine the size of the ovarian reserve in their offspring (Ireland *et al*., 2011; Evans *et al*., 2012), and this aspect of maternal imprinting merits better understanding.

Final remarks

The relationship between AFC and reproductive performance represents a great challenge to the current reproductive scenario in cattle. Studies with *Bos taurus* herds have pointed to better reproductive performance in females with high AFC, regardless of biotechnology. However, studies conducted with *Bos indicus* animals have shown that AFC may be associated with performance differently according to the technique used (i.e., AI *vs*. IVP). Additionally, a weak correlation between genetic merit and number of antral follicle has been demonstrated. This finding may be used when considering AFC and the biotechnology to be used, as no interferences on genetic merit would be expected. We predict that AFC may be another tool for improving reproductive efficiency in cattle, perhaps something less determinant, as initially proposed years ago. However, AFC clearly remains a useful strategy for improving programs of reproductive performance in cattle.

References

Batista EOS, Macedo GG, Sala RV, Ortolan MDDV, Sá Filho MF, Del Valle TA, Jesus EF, Lopes RNVR, Renn FP, Baruselli PS. 2014. Plasma antimullerian hormone as a predictor of ovarian antral follicular population in *Bos indicus* (Nelore) and *Bos taurus* (Holstein) heifers. *Reprod Domest Anim*, 49:448-452.

Baruselli PS, Batista EOS, Ferreira RM. 2016. Plasma anti-müllerian hormone allows reproductive selection of donors with greater potential of embryo production. *Spermova*, 6:1-13.

Bazer FW, Wu G, Spencer TE, Johnson GA, Burghardt RC, Bayless K. 2010. Novel pathways for implantation and establishment and maintenance of pregnancy in mammals. *Mol Hum Reprod*, 16:135-152.

Bó GA, de la Mata JJ, Baruselli PS, Menchaca,A. 2016. Alternative programs for synchronizing and resynchronizing ovulation in beef cattle. *Theriogenology*, 86:388-396.

Burns DS, Jimenez-Krassel F, Ireland JLH, Knight PG, Ireland JJ. 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*, 73:53-62.

Cate RL, Mattaliano RJ, Hession C, Tizard R, Farber NM, Cheung A, Ninfa EG, Frey AZ, Gash DJ, Chow EP, Fisher RA, Bertonis JM, Torres G, Wallner BP, Ramachandran KL, Ragin RC, Manganaro TF, MacLaughlin DT, Donahoe PK. 1986. Isolation of the bovine and human genes for mullerian inhibiting substance and expression of the human gene in animal cells. *Cell*, 45:685-698.

Diskin MG, Morris DG. 2008. Embryonic and early foetal losses in cattle and other ruminants. *Reprod Domest Anim*, 43:260-267.

Erickson BH. 1966. Development and senescence of the postnatal bovine ovary. *J Anim Sci*, 25:800-805.

Evans ACO, Mossa F, Walsh SW, Scheetz D, Jimenez-Krassel F, Ireland JLH, Smith GW, Ireland JJ. 2012. Effects of maternal environment during gestation on ovarian folliculogenesis and consequences for fertility in bovine offspring. *Reprod Domest Anim*, 47:31-37.

Guerreiro BM, Batista EOS, Vieira LM, Sá Filho MF, Rodrigues CA, Castro Netto A, Silveira CRA, Bayeux BM, Dias EAR, Monteiro FM, Accorsi,M, Lopes RNVR, Baruselli PS. 2014. Plasma anti-mullerian hormone: an endocrine marker for in vitro embryo production from *Bos taurus* and *Bos indicus* donors. *Domest Anim Endocrinol*, 49:96-104.

Hansen P. 2014. Current and future assisted reproductive technologies for mammalian farm animals. *Adv Exp Med Biol*, 752:1-22.

Hunter J. 1787. An experiment to determine the effect of extirpating one ovarium upon the number of young produced. *Philos Trans R Soc Lond*, 77:233-239.

Inskeep EK. 2004. Preovulatory, postovulatory, and postmaternal recognition effects of concentrations of progesterone on embryonic survival in the cow. *J Anim. Sci,* 82:24-39.

Ireland JJ, Ward F, Jimenez-Krassel F, Ireland JLH, Smith GW, Lonergan P, Evans ACO. 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 JJ, Smith GW, Scheetz D, Jimenez-Krassel F, Folger JK, Ireland JLH, Mossa F, Lonergan P, Evans ACO. 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.

Ireland JLH, Scheetz D, Jimenez-Krasse F, Themmen APN, Ward F, Lonergan P, Smith GW, Perez GI, Evans ACO, Ireland JJ. 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 JK, Ireland JLH, Smith GW, Hou X, Davis JS, Lonergan P, Evans ACO, Ireland JJ. 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.

Jimenez-Krassel F, Scheetz DM, Neuder LM, Ireland JLH, Pursley JR, Smith GW, Tempelman RJ, Ferris T, Roudebush WE, Mossa F, Lonergan P, Evans, ACO, Ireland JJ. 2015. Concentration of anti-Müllerian hormone in dairy heifers is positively associated with productive herd life. *J Dairy Sci*, 98:3036-3045.

Jimenez-Krassel F, Scheetz DM, Neuder LM, Pursley JR, Ireland JJ. 2017. A single ultrasound determination of≥25 follicles ≥3 mm in diameter in dairy heifers is predictive of a reduced productive herd life. *J Dairy Sci*, 100:5019-5027.

Mapletoft RJ, Hasler JF. 2005. Assisted reproductive technologies in cattle: a review. *Rev Sci Tech*, 24:393-403.

Martinez MF, Sanderson N, Quirke LD, Lawrence SB, Juengel JL. 2016. Association between antral follicle count and reproductive measures in New Zealand lactating dairy cows maintained in a pasture-based production system. *Theriogenology*, 85:466-475.

McNeel AK, Cushman RA. 2015. Influence of puberty and antral follicle count on calving day in crossbred beef heifers. *Theriogenology*, 84:1061-1066.

Mendonça HGR, Santos GMG, Silva-Santos KC, Barreiros TRR, Blaschi W, Morotti F, Moraes FLZ, Seneda MM. 2013. Pregnancy rates following FTAI of Nelore heifers (*Bos indicus*) with high, intermediate and low numbers of antral follicles. *Anim Reprod*, 10:452. Abstract.

Monteiro FM, Batista EOS, Vieira LM, Bayeux BM, Accorsi M, Campanholi SP, Dias EAR, Souza AH, Baruselli PS. 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.

Morotti F, Santos GMG, Silva-Santos KC, Moritz Y, Marcantonio TN, Seneda MM. 2014. Follicular dynamics in Nelore females (*Bos indicus*) with high or low numbers of antral follicles. *Anim Reprod*, 11:352. Abstract.

Morotti F, Barreiros TRR, Machado FZ, González SM, Marinho LSR, Seneda MM. 2015. Is the number of antral follicles an interesting selection criterium for fertility in cattle? *Anim Reprod*, 12:479-486.

Morotti F, Santos GMG, Koetz Júnior C, Silva-Santos KC, Roso VM, Seneda MM. 2017. Correlation between phenotype, genotype and antral follicle population in beef heifers. *Theriogenology*, 91:21-26.

Mossa F, Duffy P, Naitana S, Lonergan P, Evans AC. 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, Jimenez-Krassel F, Folger JK, Ireland JL, Smith GW, Lonergan P, Evans AC, Ireland JJ. 2010. Evidence that high variation in antral follicle count during follicular waves is linked to alterations in ovarian androgen production in cattle. *Reproduction*, 140:713-720.

Mossa F, Walsh SW, Butler ST, Berry DP, Carter F, Lonergan P, Smith GW, Ireland JJ, Evans ACO. 2012. Low numbers of ovarian follicles ≥3 mm in diameter are associated with low fertility in dairy cows. *J Dairy Sci,* 95:2355-2361.

Mossa F, Carter F, Walsh SW, Kenny DA, Smith GW, Ireland JLH, Hildebrandt TB, Lonergan P, Ireland JJ, Evans ACO. 2013. Maternal undernutrition in cows impairs ovarian and cardiovascular systems in their offspring. *Biol Reprod*, 88:92. doi: 10.1095/biolreprod.112.107235.

Pfeifer LFM, Leal SdCBdS, Schneider A, Schmitt E, Corrêa MN. 2012. Effect of the ovulatory follicle diameter and progesterone concentration on the pregnancy rate of fixed-time inseminated lactating beef cows. *Rev Bras Zootec*, 41:1004-1008.

Pohler KG, Geary TW, Atkins JA, Perry GA, Jinks EM, Smith MF. 2012. Follicular determinants of pregnancy establishment and maintenance. *Cell Tissue Res*, 349:649-664.

Pontes JHF, Melo-Sterza FA, Basso AC, Ferreira CR, Sanches BV, Rubin KCP, Seneda MM. 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.

Rico C, Fabre S, Médigue C, Di Clemente N, Clément F, Bontoux M, Touzé JL, Dupont M, Briant E, Rémy B, Beckers JF, Monniaux D. 2009. Antimullerian hormone is an endocrine marker of ovarian gonadotropin responsive follicles and can help to predict superovulatory responses in the cow. *Biol Reprod*, 80:50-59.

Rico C, Drouilhet L, Salvetti P, Dalbiès-Tran R, Jarrier P, Touzé J-L, Pillet E, Ponsart C, Fabre S, Monniaux D. 2012. Determination of anti-müllerian hormone concentrations in blood as a tool to select Holstein donor cows for embryo production: from the laboratory to the farm. *Reprod Fertil Dev*, 24:932-944.

Rosa CO, Marinho LSR, Lunardelli PA, Machado

FZ, Gonzalez SM, Rosa PRA, Cesaro MP, Bueno JVR, Bordignon V, Seneda MM. 2015. *In vitro* embryo development and gene expression in granulosa and cumulus cells from Bos indicus cows with different numbers of antral follicles. *Anim Reprod*, 12:762. Abstract.

Sá Filho MF, Crespilho AM, Santos JE, Perry GA, Baruselli PS. 2010. Ovarian follicle diameter at timed insemination and estrous response influence likelihood of ovulation and pregnancy after estrous synchronization with progesterone or progestin-based protocols in suckled *Bos indicus* cows. *Anim Reprod Sci*, 120:23-30.

Santos GMG, Silva-Santos KC, Siloto LS, Morotti F, Marcantonio TN, Marinho LSR, Thasmo RLO, Koetz JRC, Cintra DML, Seneda MM. 2012. Dinâmica folicular em fêmeas bovinas de alta, média e baixa contagem de folículos antrais: resultados preliminares. *Acta Sci Vet*, 40:422. Abstract.

Santos GMG, Silva-Santos KC, Barreiros TRR, Blaschi W, Morotti F, Silva CB, Moraes FLZ, Giudicissi DPL, Seneda MM. 2013. Conception rates following FTAI of Nelore cows (*Bos indicus*) with high, intermediate and low numbers of antral follicles. *Anim Reprod*, 10:451. Abstract.

Santos GMG, Silva-Santos KC, Barreiros TRR, Morotti F, Sanches, BV, Moraes FLZ, Blaschi W, Seneda MM. 2014. High numbers of antral follicles influence the in vitro embryo production, but not the conception rate of fixed-time artificial insemination in nelore cattle. *Reprod Fertil Dev*, 27:206.

Santos GMG, Silva-Santos KC, Barreiros TRR, Morotti F, Sanches BV, de Moraes FLZ, Blaschi W, Seneda MM. 2016. High numbers of antral follicles are positively associated with *in vitro* embryo production but not the conception rate for FTAI in Nelore cattle. *Anim Reprod Sci*, 165:17-21.

Silva-Santos KC, Santos GMG, Siloto LS, Hertel MF, Andrade ER, Rubinb MIB, Sturionc L, Melo-Sterza FA, Seneda MM. 2011. Estimate of the population of preantral follicles in the ovaries of *Bos taurus indicus* and *Bos taurus taurus* cattle. *Theriogenology*, 76:1051-1057.

Silva-Santos KC, Santos GMG, Koetz Júnior C, Morotti F, Siloto LS, Marcantonio TN, Urbano MR, Oliveira RL, Lima DCM, Seneda MM. 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 KC, Siloto LS, Santos GMC, Morotti F, Marcantonio TN, Seneda MM. 2014b. Comparison of antral and pre-antral ovarian follicle populations between *Bos indicus* and *Bos indicus-taurus* cows with high or low antral follicles counts. *Reprod Domest Anim*, 49:48-51.

Singh J, Dominguez M, Jaiswal R, Adams GP. 2004. A simple ultrasound test to predict the superstimulatory response in cattle. *Theriogenology*, 62:227-243.

Taneja M, Bols PE, Van De Velde A, Ju JC, Schreiber D, Tripp MW, Levine H, Echelard Y, Riesen J, Yang X. 2000. Developmental competence of juvenile calf oocytes in vitro and in vivo: influence of donor animal variation and repeated gonadotrophin stimulation. *Biol Reprod*, 62:206-213.

Walsh SW, Mossa F, Butler ST, Berry DP, Scheetz D, Jimenez-Krassel F, Tempelman RJ, Carter F, Lonergan P, Evans ACO, Ireland JJ. 2014. Heritability and impact of environmental effects during pregnancy on antral follicle count in cattle. *J Dairy Sci*, 97:4503-4511.