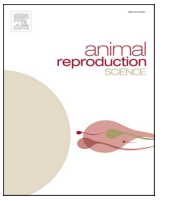
Animal Reproduction Science 266 (2024) 107491

Contents lists available at ScienceDirect

Animal Reproduction Science

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



Selecting oocyte donors based on anti-Müllerian hormone (AMH) concentrations: A critical analysis of using cutoff values as exclusion criterion for an *in vitro* embryo production program in Gir cattle

Luiz Fernando Rodrigues Feres [a], Luiz Gustavo Bruno Siqueira [b], Miller Pereira Palhao [c], Livia Loiola dos Santos [d], Luiz Francisco Machado Pfeifer [e], Carlos Antônio de Carvalho Fernandes [a], Joao Henrique Moreira Viana [a,f,*]

[a] *Universidade José do Rosário Vellano, Alfenas, MG 37130-000, Brazil*
[b] *Embrapa Gado de Leite, Juiz de Fora, MG 36038-330, Brazil*
[c] *Universidade Federal de Lavras, Lavras, MG 37200-900, Brazil*
[d] *Universidade Federal de Minas Gerais, Belo Horizonte, MG 31270-901, Brazil*
[e] *Embrapa Rondonia, Porto Velho, RO 76815-800, Brazil*
[f] *Embrapa Recursos Genéticos e Biotecnologia, Brasília, DF 70770-901, Brazil*

ARTICLE INFO

Keywords:
Antral follicle count
Bos taurus indicus
Endocrine markers
Oocytes

ABSTRACT

The aims of this study were to determine anti-müllerian hormone (AMH) cutoff values for selecting Gir (*Bos taurus indicus*) oocyte donors and estimate the impact of using AMH concentrations as a selection criterion. In Exp. 1, Gir heifers (n=120) were sampled for AMH analysis and submitted to ovum pick-up and *in vitro* embryo production (OPU-IVEP). AMH cutoff values were calculated using ROC analysis or, alternatively, by the successive exclusion of heifers with the lowest AMH values. The correlations between AMH and OPU-IVEP outcomes were significant (P<0.001), though low or moderate (r= 0.34–0.52). We estimated an improvement (P<0.05) after the use of AMH cutoff values to select donors of +15.3% for total oocyes, +19.4% for viable COC, and +23.4% for blastocysts. This selection pressure, however, led to the exclusion of 32.8%, 37.9%, and 50.0% of the initial potential donors, respectively. In Exp. 2, we analyzed data from OPU-IVEP sessions of 658 Gir donors with known genomic values for predicted transmitting ability for milk (GPTAm) and age at first calving (GPTAafc). The selection based on the number of oocytes recovered had no effect (P>0.05) on the average GPTAm nor GPTAafc values of the remaining donors. In summary, plasma AMH ≥700 pg/mL is a cutoff value that can be used to select Gir heifers with a greater potential as oocyte donors. Nevertheless, this selection leads to the exclusion of up to 50% of potential donors. Finally, exclusion of poor responders had no effect on mean genomic estimates for milk production or age at first calving in the selected subset of donors.

* Correspondence to: PqEB, Av, W5 Norte, Brasília, DF 70770-917, Brazil.
E-mail address: henrique.viana@embrapa.br (J.H.M. Viana).

https://doi.org/10.1016/j.anireprosci.2024.107491
Received 30 September 2023; Received in revised form 13 March 2024; Accepted 28 April 2024
Available online 7 May 2024
0378-4320/© 2024 Elsevier B.V. All rights reserved.

1. Introduction

The anti-müllerian hormone (AMH) is a dimeric glycoprotein produced by the granulosa cells from healthy growing follicles in the ovaries and, thus, reflects the size of the ovarian reserve (Visser et al., 2006). Preantral follicles cannot be directly assessed by current imaging technologies *in vivo*; the total number of follicles on a given ovary, however, is highly associated with the number of antral follicles detected by ultrasonography, referred to as antral follicle count (AFC) (Ireland et al., 2008). Plasma AMH concentration is also positively associated with AFC (Batista et al., 2014). Because AFC has a direct impact on the outcomes of assisted reproductive technologies (ART), AMH concentrations have been proposed by many authors as a potential marker of oocyte and embryo yield in both humans (Gleicher et al., 2010; Irez et al., 2011; La Marca et al., 2016) and farm animals, such as cattle (Monniaux et al., 2010; Guerreiro et al., 2014; Krause et al., 2022), sheep (Lahoz et al., 2014; McGrice et al., 2020), and goats (Monniaux et al., 2011).

In human medicine, circulating AMH is used to predict ART outcomes as well as to adjust ovarian stimulation protocols, with cycle cancellation occurring only if AMH values are very low (La Marca et al., 2010). In farm animals, on the other hand, AMH has been proposed as a criterion for the selection of superior embryo or oocyte donors, aiming to increase the efficiency of embryo production. In previous studies, however, this possibility has only been inferred based on the positive associations between AMH and superovulation or *in vitro* embryo production outcomes (Karl et al., 2022; Krause et al., 2022) or indirectly demonstrated by differences in average outcomes between groups categorized according to AMH concentrations (Monniaux et al., 2010; Baldrighi et al., 2014; Guerreiro et al., 2014; Ghanem et al., 2016) rather than objectively evaluated. From a clinical perspective, however, the use of AMH concentrations as a selection criterion requires the establishment of threshold reference values. In humans, for example, different cutoff values have been proposed for plasma AMH, depending on the definition of a 'poor response' and the type of AMH assay (La Marca et al., 2010). In cattle, there is a lack of research studies establishing reference threshold values and this also remains to be determined for different breeds. Moreover, AMH cutoff values were only evaluated for *Bos taurus taurus* breeds (Rico et al., 2012).

The exclusion of potential donors based on such biological marker may have other implications for ovum pick-up (OPU) and *in vitro* embryo production (IVEP) programs. The impact of donor selection on a given characteristic (e.g., number of oocytes retrieved, embryos produced, etc.) will depend both on the type of distribution and range of values of each endpoint within a given population, as well as on the cutoff value used. Thus, the latter needs to be determined for each breed and animal category. Additionally, the goals of each embryo transfer program may vary, particularly regarding the relative importance of the efficiency of embryo production and of the genetic merit of the donors. In its early years, IVEP was adopted mainly by animal breeding programs to increase the number of offspring from high genetic merit dams, regardless of embryo production efficiency. Over the past decades, however, we have witnessed an increase in the use of IVEP in large-scale programs focused on, for example, the production of replacement heifers for dairy herds (Pontes et al., 2010). One example is the extensive use of Gir donors, a *Bos taurus indicus* dairy breed, to produce crossbred cattle for the dairy industry in South American tropical countries (Viana et al., 2017). In the latter situation, embryo production costs are highly important and so is the embryo production potential of the aspirated donors.

The aims of the present study were to 1) propose a cutoff value for the use of AMH as a biological marker to anticipate oocyte and embryo yield in Gir heifers; 2) estimate the potential impact of the use of AMH as a selection criterion in an IVEP program on embryo outcomes and on the average genetic value for other production traits within selected donors. We hypothesized that selection of donors based on AMH concentrations increases average embryo production, but results in the exclusion of a significant proportion of potential oocyte donors, with a possible impact on overall absolute embryo production. A second hypothesis was that the exclusion of potential donors due to low AMH values would impact the average genomic PTAs for milk production or age at first calving.

2. Material and methods

Unless otherwise indicated, all reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.1. Animals and location

The study was conducted at Fazendas do Basa, a private dairy farm that raises a Gir (*B. taurus indicus*) herd. The farm is located in Leopoldina, MG, Brazil (21°31'55" S; 42°38'35" W). The climate classification of the region is Aw, tropical with dry winter (Alvares et al., 2013). Cows and heifers used as cumulus-oocyte complexes (COC) donors in the IVEP program were raised in *Cynodon nlemfuensis* pastures with *ad libitum* access to water and minerals and were supplemented during the dry season with corn silage, Tifton 85 (*Cynodon dactylon*) hay and a 22% crude protein concentrate. The sanitary calendar includes vaccinations against IBR, BVD, and leptospirosis, along with treatments for endo- and ectoparasites. All procedures using research animals were conducted in accordance with the Brazilian Ethics, Bioethics, and Animal Care Committee (CEBEA) guidelines and were approved by the Ethics in the Use of Animals Committee from the Universidade José do Rosário Vellano (Protocol CEUA-16A/2017).

2.2. Experimental design

This study was subdivided into two parts. In Experiment 1, we analyzed the association between AMH concentration, AFC, and IVEP outcome results and estimated potential cutoff values for the use of AMH as a selection criterion for oocyte donors. For that, 120 heifers, 23.3±0.5 months old, were sampled for AMH analysis and evaluated by ultrasonography to estimate individual AFC. Then, they were enrolled as oocyte donors in an IVEP program. Numbers of total oocytes and viable COC collected by transvaginal ultrasound-guided follicle aspiration, also known as OPU, and of blastocysts produced *in vitro*, were recorded for each donor.

In Experiment 2, we analyzed whether oocyte and embryo yields were associated with the genomic predicted transmitting ability values for milk production (GPTAm) and age at first calving (GPTAafc), and thus the potential impact of the selection for donors based on IVEP outcomes on the genetic progress for other production traits. In this experiment, we used data from 658 Gir cows with known genomic values that were enrolled in the farḿs OPU-IVEP program between 2008 and 2020. Because some donors were submitted to multiple OPU, we used only data from the first OPU session for each cow.

2.3. Blood sampling and AMH assay

Blood samples were collected by puncture of the coccygeal vein, using double-ended 21 G needles and 5 mL vacuum tubes with EDTA (BD Vacutainer EDTA K2; Becton Dickinson, Sao Paulo, SP, Brazil). Samples were centrifuged at 900 χ g for 15 min at room temperature and the harvested plasma kept frozen at −20°C until further analysis. Plasma AMH concentrations were determined by enzyme linked immunosorbent assay (ELISA) using a commercial kit for bovine AMH (AL114, AnshLabs, Webster, TX, EUA). This kit has been previously used for AMH evaluation in the Gir breed (Baldrighi et al., 2014). The nominal concentration working range was 13.5–2240 pg/mL, the limit of detection was 11 pg/mL, and the intra-assay and inter-assay CVs were 2.8% and 4.9%, respectively. The CV form the standards (0.0, 16.5, 68.0, 203.0, 642.0, an 2050.0 pg/mL) ranged from 0.0% to 2.4%. Absorbance was read in a micro-plaque reader Stat Fax model 2100 (Awareness Technology, Fisher Bioblock Scientific, FL, EUA). Concentrations were estimated using the HIDA software. All analysis were conducted by a private laboratory specialized in scientific analysis (LEAC, São Paulo, SP, Brazil).

2.4. Ovarian ultrasonography

Heifers from Experiment 1 had their ovaries scanned by transrectal ultrasonography at a random day of the estrous cycle using a portable ultrasound device equipped with a 7.5 MHz linear probe (Mindray DP2200 Vet, Shenzhen, China). During each exam, the number of follicles $\geq$ 3 mm in diameter was recorded for each ovary. The variable AFC corresponded to the total number of follicles recorded on both ovaries of each donor.

2.5. Oocyte recovery and in vitro embryo production

Oocyte retrieval by OPU and IVEP were carried out by a commercial laboratory, following their standard operational procedures and protocols. Briefly, OPU was performed by a single technician, at a random day of the estrous cycle, without any hormonal treatment. All visible follicles were aspirated using a 20 G needle and a vacuum pressure equivalent to an aspiration rate of 12 mL/min. The recovered immature oocytes underwent morphological evaluation according to the number of cumulus cells layers surrounding the oocyte and the aspect of the cytoplasm, as described elsewhere (Viana et al., 2004). The COC classified as viable were transported to the laboratory in tubes containing maturation medium (Hepes-buffered TCM 199, Gibco Life Technologies, Grand Island, NY, EUA) supplemented with 10% fetal calf serum (Vitrocell, Campinas, SP, Brazil), 50 UI/mL hCG (Sigma C-1063), 0.5 μg/mL FSH (Folltropin, Vetoquinol Saúde Animal, Aparecida de Goiânia, GO, Brazil), 1 μg/mL estradiol (Sigma E-8875), 2.2 μg/mL pyruvate (Sigma P-4562), and 70 μg/mL amikacin (Sigma A-2324) in a portable incubator (Minitube, Tiefenbach, Germany) at 38 °C.

In vitro maturation (IVM) was carried out for 22 h, including the transportation period. Semen from Gir sires with known records on IVEP were prepared by centrifugation in percoll (Sigma-P1644) gradient (45% and 90%) and adjusted for a final concentration of 1.2×10^6 sperm/mL in the fertilization droplet. The matured COC were denuded by repeated gentle pipetting and then co-incubated with sperm in TALP medium 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. Presumptive zygotes were cultured in 50 μL of modified synthetic oviduct fluid (SOFaa) medium, under mineral oil at 38.5°C and 5% CO_2, 5% O_2 and 90% N_2 humidified atmosphere for 6 days. Medium feeding occurred at 72 hpi and formation of blastocysts was assessed at day 7 (168 hpi). Blastocyst rate was calculated based on the number of COC submitted to IVM.

2.6. Genotyping

Genomic estimates for predicted transmitting ability for milk production (GPTAm) and age at first calving (GPTAafc) of animals enrolled in experiment 2 were determined for each donor using the GeneSeek-Genomic-Profiler (GGP) Bovine 50 K microchip (Neogen Corporation, Lincoln, NE, USA), as previously described elsewhere (Boison et al., 2017). Briefly, blood samples were collected following the same procedures described in experiment 1. The genomic DNA was extracted from blood leucocytes using the modified phenol/chloroform method described by Sambrook and Russell (2001). After centrifugation, the fraction containing leucocytes (buffy coat) was transferred to a 2 mL tube, washed with lysis buffer, centrifuged, and the resulting pellet treated with a saline buffer containing proteinase K. DNA quality and quantity were assessed using a NanoDrop spectrophotometer. Individual genotyping was then carried out using the 50 K SNP microchip with 47.843 SNPs. These SNPs were uniformly distributed along the genome, with an average space of 59 kb among them. For quality control, data were analyzed using the PLINK v1.07 software (Purcell et al., 2007). All individuals either lacking more than 10% of the SNPs, or with a call rate lower than 95%, or minor allele frequency lower than 5%, or with markers deviating from Hardy-Weinberg equilibrium (Fisher's exact test; *P*-value $<10-7$) were excluded from the study.

2.7. Data analysis

Experiment 1: the commercial kit used for AMH analysis indicated values above the detection range in samples from four heifers and, therefore, data from these donors were excluded from further analysis. Thus, data from the remaining 116 heifers enrolled in 506 OPU-IVEP sessions were analyzed. Data of AFC from 38 heifers were also excluded, due to an inconsistency in ultrasound device settings that might have caused a bias in these results. All endpoints were examined for normality using the Kolmogorov-Smirnov test, and homogeneity of variances were checked using the Levene's test. Data was first stratified into quartiles (smaller to greater) according to the average total number of oocytes retrieved by OPU for each donor. The outcome variables donor age, number of OPU sessions, AFC, total oocytes, viable COC, number of blastocysts, and AMH concentration were compared among quartiles by ANOVA, using the GLM procedure of SAS (SAS Studio 3.8. University Edition; SAS Institute Inc., Cary, NC, USA) and differences among means contrasted using the post hoc Tukey's test. The associations between AMH concentrations and the variables AFC, number of total oocyte and viable COC and number of blastocysts were calculated using the Pearson's correlation test.

Two approaches were used to establish optimum AMH concentrations cutoff values for donor selection: 1) donors within the first quartile were arbitrarily classified as of 'low potential as donors'. The AMH values were then used to establish a ROC (Receiver Operating Characteristics) curve for each of the following outcome variables: total oocytes, viable COC, and number of blastocysts using the Prism 8 software (GraphPad Software, CA, EUA). We calculated sensitivity and specificity of AMH cutoff concentrations using AUC (area under curve) values. Cutoff values were defined as those corresponding to the highest sensitivity and specificity. 2) The second approach defined cutoff values as those that would result in statistically significant increases in mean values of each endpoint. OPU-IVEP results were ranked based on the corresponding AMH value (lower to greater) and data from donors with the lowest AMH concentrations were successively excluded. After each exclusion, the resulting 'new' mean was compared with the 'original' mean using the GLM procedure of SAS. Exclusions were discontinued when statistical significance ($P<0.05$) was observed between 'new' and 'original' means. Cutoff values calculated by the two distinct approaches were then used to simulate OPU-IVEP

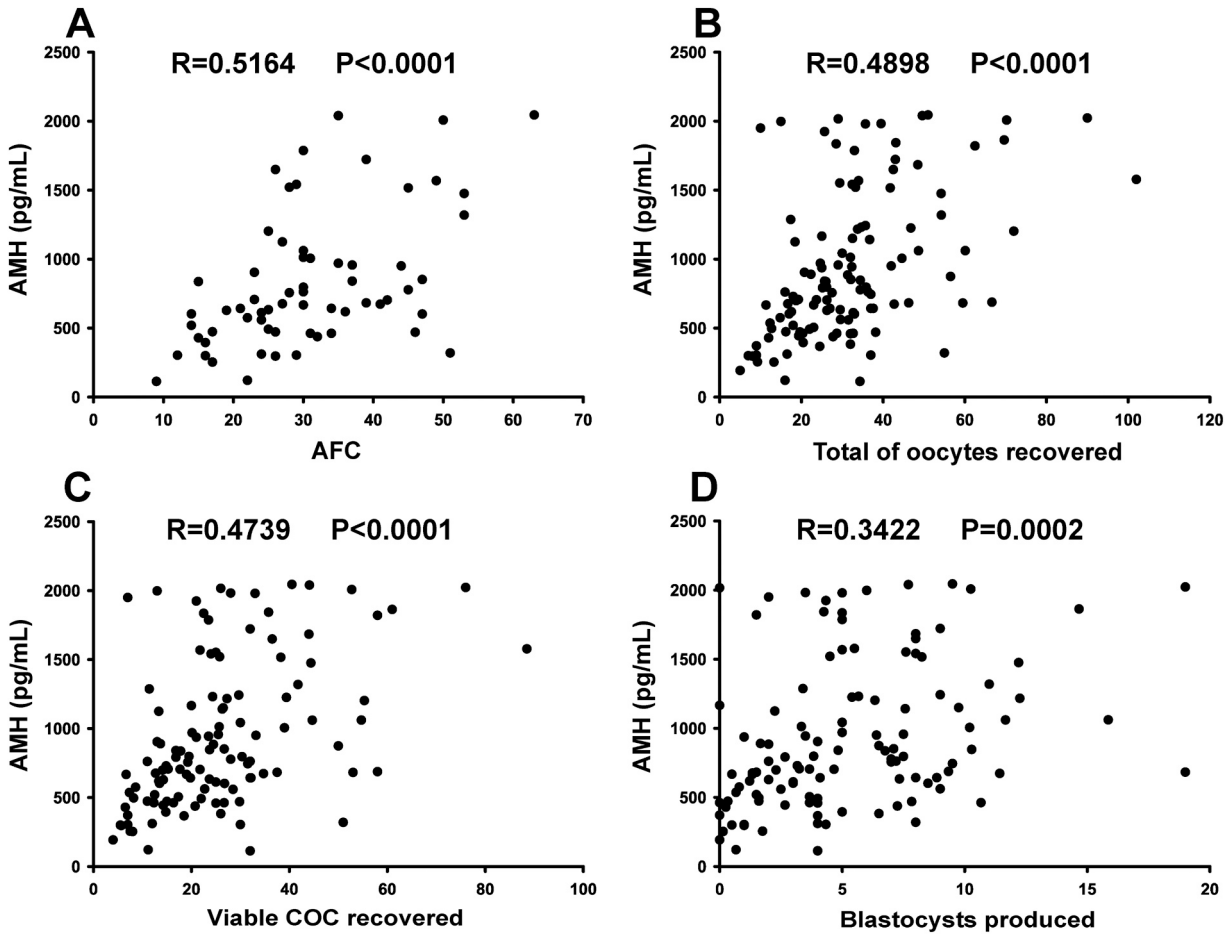


Fig. 1. (A-D). Associations between AMH concentrations and ovarian antral follicle count (AFC) or OPU-IVEP outcomes in Gir heifers. A) AMH vs. AFC; B) AMH vs. total oocytes retrieved by OPU; C) AMH vs. number of viable COC; and D) AMH vs. number of blastocysts produced *in vitro*. Pearson's correlation coefficients (R) and respective *P*-values are shown for each association.

outcomes if they were to be used as donor selection criteria. Means ('original' and 'projected') were also compared using the GLM procedure of SAS.

Experiment 2: data from the first OPU-IVEP session from 658 Gir donors previously genotyped and with known GPTAm and GPTAafc values were retrospectively analyzed. Donors were ranked into quartiles, as described in Exp. 1, and OPU-IVEP outcome variables and GPTAm and GPTAafc values were compared among quartiles by ANOVA using the GLM procedure of SAS. Associations between numbers of total oocytes, viable COC, blastocysts and GPTAm or GPTAafc were calculated using the Pearson's correlation test, as previously described for experiment 1. In addition, we simulated the effect of excluding donors, based on a cutoff value calculated for the total of oocytes retrieved, on OPU-IVEP outcomes. Means ('original' and 'projected') were compared using the GLM procedure. Results are shown as mean±SEM. A *P*-value <0.05 was considered to determine statistical significance.

3. Results

3.1. Experiment 1

We did not observe any differences in donor age nor number of OPU sessions/donor among quartiles ($P>0.05$). We observed differences in all other examined endpoints, i.e., the greater the total number of oocytes retrieved, the greater the AFC, the number of viable COC and blastocysts, and the AMH concentrations ($P<0.0001$; Table S1, supplemental file). Fig. 1 depicts the associations between AMH and AFC or OPU-IVEP outcome variables. All correlations were significant ($P<0.001$); coefficients of correlation (r values) were, however, low to moderate (0.34–0.52).

The results of the ROC analysis are shown in Figure S1 (supplemental file) and Table 1. Based on each ROC curve we estimated AMH cutoff values ranging from 622.5 (for viable COC) to 761.4 pg/mL (for total oocytes). The effect of the successive exclusion of donors with lowest AMH values on the changes of average outcomes (% increase) is shown in Fig. 2. Based on this approach, we estimated cutoff values ranging from 632.4 (for total oocytes) to 776.8 pg/mL (for number of blastocysts produced).

Results of the simulated effect of donor exclusion based on the estimated cutoff values are shown in Table 2. By definition, the selection of donors based on cutoff values estimated by the criterion of successive exclusions resulted in increments ($P<0.05$) in the number of oocytes and viable COC retrieved and blastocysts produced. In contrast, donor selection based on values estimated by the ROC curve resulted in a significant increase in the number of total oocytes retrieved, but not for viable COC and blastocysts produced. It is noteworthy that to increase the original mean in approximately one embryo per OPU-IVEP session, 50% of the potential donors needed to be excluded, resulting in a estimated 39.1% reduction in absolute blastocyst production.

The stratification of donors into quadrants based on their total number of oocytes and AMH concentrations (above or below average; 32.0 oocytes and 932.9 pg/mL AMH, respectively) and the comparison of blastocyst production among quadrants (low-low, low-high, high-low, high-high) are shown in Fig. 3 and Table 3, respectively. We did not observe a difference ($P>0.05$) in blastocyst production nor blastocyst rate among heifers with similar number of oocytes retrieved, but distinct AMH concentrations.

3.2. Experiment 2

The mean values for GPTAm and GPTAafc for genotyped Gir cows were 235.2±8.1 kg of milk (range:-385 to +815) and −5.7±0.7 days at first calving (range: −47 to +68), respectively. Ranking cows based on their total number of oocytes per OPU did not result in any difference ($P>0.05$) in GPTAm nor in GPTAafc among quartiles (Table 4).

We also investigated the associations between GPTAm, GPTAafc and OPU-IVEP outcomes (Figure S2, supplemental file). Overall, correlations were weak ($r<0.15$) and only significant ($P<0.05$) for number of oocytes (total and viable) and GPTAm. Results of the simulated selection of oocyte donors based on the method of successive exclusions of donors with the lowest numbers of oocytes per OPU are shown in Table 5. No differences ($P>0.05$) were observed between mean GPTA values before or after selection.

4. Discussion

Circulating plasma AMH has been proposed as a potential endocrine marker for the selection of oocyte and embryo donors due to its known association with ovarian reserve. In the current study, we proposed cutoff values for the use of plasma AMH concentrations as a selection criterion for Gir (*B. taurus indicus*) females and addressed the potential impact of using plasma AMH to select donors from the perspective of animal breeding programs. Our results supported our initial hypothesis that selection of donors based on AMH would

Table 1
Outcomes of the ROC analysis for total oocytes, viable COC, and number of blastocysts produced *in vitro* in Gir heifers (n=116).

Endpoint	Total oocytes	Viable COC	Blastocysts
Area under curve (AUC)	0.7748	0.7547	0.7225
P-value	<0.0001	<0.0001	0.0003
Confidence interval (95%)	0.6720–0.8776	0.6464–0.8629	0.6118–0.8331
Sensitivity	63.9%	79.3%	70.9%
Specificity	86.6%	65.5%	73.3%
AMH cutoff value (pg/mL)	761.4	622.5	681.5

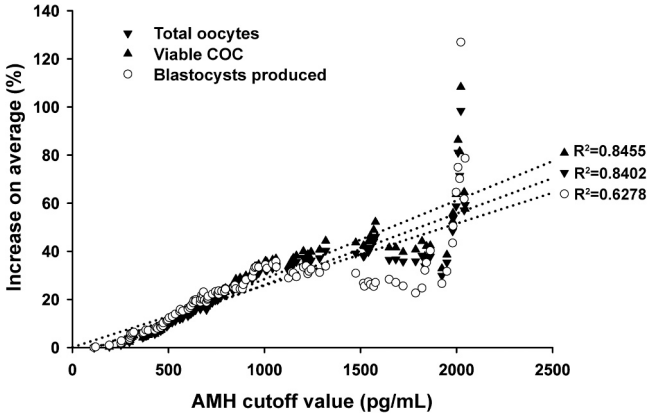


Fig. 2. Effect of the successive exclusion of donors ranked based on AMH concentration (from the lowest to the highest) on the mean values for total oocytes retrieved (▼), viable COC (▲), and blastocysts produced *in vitro* (○)in Gir heifers. Note that the mean moves up linearly until AMH ~1500, thereafter the sinificant reduction in the number of donors causes averages to fluctuate.

Table 2
Simulation of OPU-IVEP outcomes in Gir heifers (n=116) if donors were selected based on AMH cutoff values obtained by the ROC curve or by the method of successive exclusions.

Endpoint	Total oocytes	Viable COC	Blastocysts
Cutoff value[1] (pg/mL)	761.4	622.5	681.5
Original mean	32.0±1.5[a]	25.7±1.4[a]	5.3±0.4[a]
Projected mean after selection	39.1±2.1[b]	29.8±1.7[a]	6.5±0.5[a]
Projected increase on mean	22.2%	16.1%	23.1%
Potential donors excluded	49.1%	31.9%	40.5%
Donors excluded from Q3 or Q4[2]	14.7%	8.6%	12.1%
Reduction in overall blastocyst production	37.8%	21.0%	30.2%
Cutoff value[3] (pg/mL)	632.4	672.3	776.8
Original mean	32.0±1.5[a]	25.7±1.4[a]	5.3±0.4[a]
Projected mean after selection	36.9±1.6[b]	30.3±1.4[b]	6.6±0.3[b]
Projected increase on mean	15.3%	19.4%	23.4%
Potential donors excluded	32.8%	37.9%	50.0%
Donors excluded from Q3 or Q4[2]	8.6%	10.3%	15.5%
Reduction in overall blastocyst production	22.4%	26.9%	39.1%

[a,b]Within a column and under the same criterion (1 or 2), means without a common superscript differ (P<0.05).
[1] Determined using the ROC curve.
[2] Donors excluded due to low AMH, regardless of being classified within Q3 or Q4 according to total oocyte production.
[3] Determined by the successive exclusions of donors with lowest AMH values.

improve OPU-IVEP outcomes, but could lead to an intended exclusion of a significant proportion of potential donors. In this regard, we indicate that the decision about whether AMH should be considered as a selection criterion for oocyte donors will depend on the main goals of the IVEP program, i.e., if the goal is to prioritize overall embryo production efficiency or the multiplication of offspring from specific individuals.

Both the average results of OPU-IVEP and the individual variation among donors observed in the current experiment were consistent with those previously reported for Gir cattle (Pontes et al., 2010; Baldrighi et al., 2014; Feres et al., 2018). The number of growing follicles in the ovaries and, thus, the AFC, is affected by numerous factors such as age, nutritional and health status, and even by epigenetic effects during early gonad development (Ireland et al., 2011). The genetic background, however, is the major cause of differences in AFC, which varies significantly among breeds of cattle. Zebu breeds (*B. taurus indicus*) are known for their greater mean AFC compared with European breeds (*B. taurus taurus*), which results in a greater number of oocytes retrieved per OPU in Zebu and, consequently, a greater number of embryos produced *in vitro* per OPU session in *B. taurus indicus* cattle (Pontes et al., 2010). Not surprisingly, the mean AMH values observed in the current study was greater than those previously reported for European cattle (Monniaux et al., 2010; Vernunft et al., 2015; Ghanem et al., 2016; Alward et al., 2021).

Nevertheless, in both *B. taurus taurus* and *B. taurus indicus* breeds, a substantial individual variation is observed in AFC and, thus, oocyte and embryo yield among donors within the same breed (Burns et al., 2005; Watanabe et al., 2017). For instance, a previous study from our group demonstrated that the mean number of oocytes retrieved per OPU session was 24.8±0.6, but ranged from 0 to 112 in Gir cattle (Feres et al., 2018). It is possible that this variation can be even greater in other breeds, considering that there are reports of donors yielding nearly 500 oocytes in a single OPU session (Resende et al., 2021). The use of biomarkers such as AMH to predict embryo production could, thus, allow the identification of animals with the greatest potential as donors and eventually lead to

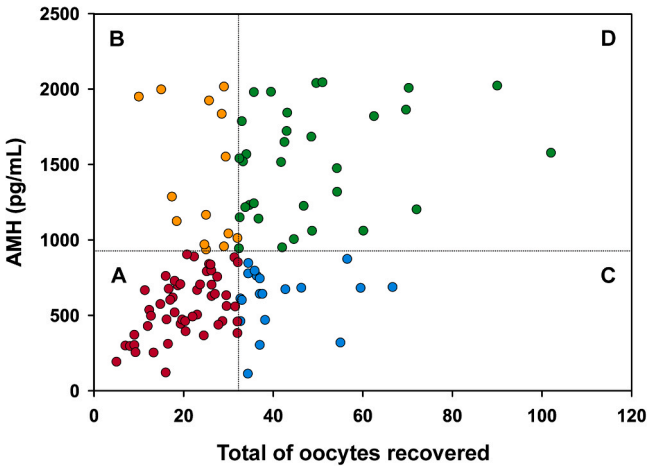


Fig. 3. Distribution of Gir donors in quadrants based on their respective values of total number of oocytes and AMH concentrations (above or below means, 32.0 oocytes and 932.9 pg/mL AMH, respectively).

Table 3
Number of blastocysts produced *in vitro* and blastocyst rate from Gir heifer donors (n=116) allocated into quadrants based on the outcome results of number of oocytes and AMH concentrations (data as shown graphically in Fig. 3).

Quadrant*	Oocytes	AMH	n (%)	Blastocysts (n)	Blastocyst rate (%)
A	Low	Low	51 (44.0)	3.1±0.3[a]	19.4±2.0[a]
B	Low	High	14 (12.1)	3.7±0.7[a]	20.2±3.5[a]
C	High	Low	19 (16.4)	7.5±0.9[b]	22.4±2.9[a]
D	High	High	32 (27.6)	8.2±0.7[b]	21.9±1.5[a]

[a,b]Within a column, means with different superscriptsdiffer (P<0.05)
* According to means (932.9 pg/mL for AMH concentration and 32.0 for the total number of oocytes retrieved)

Table 4
OPU-IVEP outcomes and genomic values for milk production (GPTAm) and age at first calving (GPTAafc) in Gir (*B. taurus indicus*) cows (n=658) classified into quartiles according to the total number of oocytes per OPU session. Data are mean±SEM.

	Quartile				
Endpoint	Q1	Q2	Q3	Q4	*P*-value
Total oocytes (n)	11.4±0.3[a]	22.4±0.2[b]	33.2±0.3[c]	54.8±1.2[d]	<0.0001
Viable COC (n)	8.2±0.3[a]	17.3±0.3[b]	26.0±0.4[c]	44.1±1.2[d]	<0.0001
Viable COC (%)	70.6±1.6[a]	77.0±1.2[b]	78.5±1.0[b]	80.3±1.0[b]	<0.0001
Grade 1 COC (n)	0.5±0.1[a]	0.8±0.1[a]	1.3±0.1[b]	2.0±0.2[c]	<0.0001
Blastocysts (n)	1.6±0.1[a]	2.0±0.2[a]	3.3±0.3[b]	4.7±0.5[c]	<0.0001
Blastocyst rate (%)	25.2±2.2[a]	12.4±1.1[b]	13.0±1.2[b]	11.1±1.2[b]	<0.0001
GPTAm (kg of milk)	216.1±14.9	245.1±15.8	233.0±16.3	257.9±18.3	0.2721
GPTAafc (days)	-5.5±1.3	-8.1±1.3	-5.1±1.4	-4.1±1.4	0.2178

[a,b,c,d]Within a row, means without a common superscriptdiffer (P<0.05)

Table 5
Simulation of projected OPU-IVEP outcomes and genomic estimates if Gir donors (n=658) were selected based on a cutoff value (19) for the number of total oocytes recovered by OPU, calculated by the method of successive exclusions.

Endpoint	Original mean	Mean after selection	*P*-value
Total oocytes (n)	29.4±0.4	37.6±0.6	<0.0001
Viable COC (n)	23.1±0.6	29.9±0.5	<0.0001
Blastocysts (n)	2.8±0.2	3.4±0.2	0.0026
GPTAm (kg of milk)	237.0±8.2	248.8±8.3	0.4593
GPTAafc (days)	-5.8±0.7	-5.5±0.7	0.5744

a potential increase in embryo production efficiency.

In the current study, we used two methodological approaches to estimate AMH cutoff values and both ended up indicating similar values, approximately 600–700 pg/mL (depending on the reference endpoint, e.g., total oocytes, viable COC or blastocysts produced). These values are greater than those reported by Rico et al., (2012), but consistent with the differences in AMH mean and range reported for *B. taurus taurus* and *B. taurus indicus* (Guerreiro et al., 2014), as well as among animal categories and age (Krause et al., 2022). These particular differences highlight the importance of determining the mean AMH values for each cattle breed and category. Moreover, the use of reference values shall also take into account the kind of assay used to evaluate circulating AMH (La Marca et al., 2010).

A second aim of the present study was to retrospectively investigate the potential impact of using the obtained AMH cutoff values to select Gir donors. The hypothetical exclusion of donors with AMH concentrations below the calculated cutoff values resulted in a significant increase in the mean number of oocytes retrieved and blastocysts produced. The use of AMH as a selection criterion, however, would also result in the exclusion of approximately 30–50% of the potential donors initially enrolled in the OPU-IVEP program, including 8–15% of heifers actually ranked in quartiles Q3 and Q4 for total oocyte recovery based on real data, i.e., those with greater oocyte and embryo yield. In fact, when we plot the data into quadrants considering only AMH concentrations and total number of oocytes retrieved, 16.4% of the heifers fall below the average AMH but above the mean COC number, whereas 12.1% of the heifers fall into the exact opposite situation. An increase of ~23% in embryo production efficiency would, therefore, be compensated by an overall reduction of 30–40% in total embryo production. To the best of our knowledge, this is the first study to estimate the impacts of donor selection using AMH cutoff values on an OPU-IVEP program overall performance.

The considerable percentage of donors that would have to be excluded from the OPU-IVEP program to increase the mean embryo production reflects the low to moderate association between AMH and AFC or OPU-IVEP outcomes (r values ranging from 0.30 to 0.50) observed both in the current and previous studies in cattle (Rico et al., 2012; Guerreiro et al., 2014; Vernunft et al., 2015). The presence of heifers with AMH above average but low number of oocyte recovered can be easily explained, as the OPU recovery rate can be affected by technical aspects such as vacuum pressure (Bols et al., 1996) or the size of the aspirated follicles (Seneda et al., 2001). A similar inconsistency is observed when AHM is used as a marker for superstimulation, because individuals with good AFC can fail to respond to FSH treatment, as described for the poor response syndrome in both human (Tarlatzis et al., 2003) and cattle (Wohlres-Viana et al., 2019). The explanation for the heifers with low AMH but great number of oocyte recovered, on the other hand, is more speculative. Perhaps the explanation lies on the role of bone morphogenetic proteins (BMPs) on the control of AMH production. For instance, in ewes carrying mutations in the gene encoding the FecB/BMP receptor, the granulosa cells secrete low AMH amounts, despite high number of follicles in the ovaries (Estienne et al., 2015).

Interestingly, *B. taurus indicus* cattle are known by their greater number of antral follicles growing on the ovaries compared with *B. taurus taurus*, in spite of both having a similar number of primary and secondary follicles per ovary (Silva-Santos et al., 2011). This paradox is not yet fully understood and may involve sensitivity to FSH or particularities of the ovarian IGF system (Satrapa et al., 2013). Lower rates of follicle atresia throughout follicular development, however, could also result in a proportionally greater AFC relative to AMH concentration. In humans, inconsistencies between AFC and AMH have also been observed and linked to a range of factors such as body mass index, menstrual cycle length, FSH and testosterone serum concentrations, all associated with AMH values lower than those expected if AFC alone was to be considered (Alebic et al., 2018). Clinical assessment of AMH has also been suggested as a marker of oocyte quality (Borges et al., 2017). In our study, however, we were not able to find differences in embryo production between heifers with similar number of oocytes retrieved, but contrasting AMH values. Moreover, there are evidences of a low but significant negative correlation between the number of oocytes retrieved and subsequent blastocyst rates (Watanabe et al., 2017; Feres et al., 2018). Such findings highlight the limitations of AMH as a sole marker for the selection of potential donors.

In the present study we also evaluated the association between OPU-IVEP outcomes and the genomic predicted transmitting ability value for milk production (GPTAm) and for age at first calving (GPTAafc). Coefficient of correlations between both genetic estimates and IVEP perfomance were at the very low end ($r<0.13$) and the only significant associations detected were between GPTAm and number of total oocytes or viable COC. This low, yet significant, association between GPTAm and oocyte yield suggests a possible common physiological mechanism affecting milk production and ovarian activity, e.g., IGF concentrations (Lucy et al., 1993). Coherently, we did not observe any differences in GPTAm nor GPTAafc among quartiles when cows were ranked by number of total oocytes. Whether selection for donor potential would affect puberty, however, remains inconclusive, as previously studies found positive (Snelling et al., 2012) or negative (Faria et al., 2021) associations between AFC and age at puberty. Similarly, there is no consensus regarding the potential effects on fertility, and both positive (Mossa et al., 2012; Martinez et al., 2016) and negative (Moraes et al., 2019; Bonato et al., 2022) associations between AFC on fertility traits have been reported. The results of the current study suggests that the adoption of a selection pressure for embryo production similar to the one simulated in this study for heifers using AMH cutoff values would result in a significant increase in oocyte and embryo yield, without significantly affecting the average GPTAm or GPTAafc in a given oocyte donor population. Caution, however, is required to generalize our findings to other breeds, particularly taking into account the conflicting reports on literature on the association between AFC and other genetic traits.

5. Conclusion

In summary, AMH concentration can be used as an endocrine marker for donor selection if the goal is to improve OPU-IVEP outcomes. One shall consider that this is an indirect clinical marker and, thus, may lead to selection or exclusion of animals misidentified as potentially good or poor donors, which reduces its accuracy if used as a single donor selection criterion. The evaluation of individual AFC by ultrasonography seems to be a cheaper and more straightforward method for donor selection with relatively high accuracy. Nevertheless, if the latter is not available or feasible, AMH concentration offers a clinical reference to identify donors with a

predicted superior performance. Whether or not animals should be selected solely based on their potential as oocyte donors will depend on the main goals of the OPU-IVEP program. In a large-scale IVEP program, with many potential available donors, embryo production efficiency (and cost-benefit) may be more important than the actual individual genetic merit of a given donor and genetic gain may be guaranteed based mostly on the sire. In this case, AMH would offer an additional tool to pre-select donors and possibly improve outcomes. Conversely, for animal breeding programs the opposite may be true, i.e., the focus on genetic merit and progress is the goal and individual embryo production efficiency might be nearly disregarded.

Funding

This project received financial support from FAPEMIG Project CVZ APQ 03430–17.

Declaration of Competing Interest

None

Acknowledgements

The authors thank Evandro do Carmo Guimarães and the Fazendas do Basa for the animals and infrastructure used.

Declaration of competing interest

The authors declare that there are no conflicts of interest.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.anireprosci.2024.107491.

References

Alebic, M.Š., Stojanovic, N., Dewailly, D., 2018. Discordance between serum anti-Müllerian hormone concentrations and antral follicle counts: not only technical issues. Hum. Reprod. 33 (6), 1141–1148. https://doi.org/10.1093/humrep/dey098.

Alvares, C.A., Stape, J.L., Sentelhas, P.C., Gonçalves, J.L.M., Sparovek, G., 2013. Köppen's climate classification map for Brazil, 2013 Meteorol. Z. 22, 711–728. https://doi.org/10.1127/0941-2948/2013/0507.

Alward, K.J., Graves, W.M., Palomares, R.A., Ely, L.O., Bohlen, J.F., 2021. Characterizing Anti-Müllerian Hormone (AMH) concentration and change over time in Holstein dairy cattle. Theriogenology 168, 83–89. https://doi.org/10.1016/j.theriogenology.2021.03.007.

Baldrighi, J.M., Sá Filho, M.F., Batista, E.O., Lopes, R.N., Visintin, J.A., Baruselli, P.S., Assumpção, M.E., 2014. Anti-Mullerian hormone concentration and antral ovarian follicle population in Murrah heifers compared to Holstein and Gyr kept under the same management. Reprod. Dom. Anim. 49 (6), 1015–1020. https://doi.org/10.1111/rda.12430.

Batista, E.O., Macedo, G.G., Sala, R.V., Ortolan, M.D., Sá Filho, M.F., Del Valle, T.A., Jesus, E.F., Lopes, R.N., Rennó, F.P., Baruselli, P.S., 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 (3), 448–452. https://doi.org/10.1111/rda.12304.

Boison, S.A., Utsunomiya, A.T.H., Santos, D.J.A., Neves, H.H.R., Carvalheiro, R., Mészáros, G., Utsunomiya, Y.T., do Carmo, A.S., Verneque, R.S., Machado, M.A., Panetto, J.C.C., Garcia, J.F., Sölkner, J., da Silva, M.V.G.B., 2017. Accuracy of genomic predictions in Gyr (Bos indicus) dairy cattle. J. Dairy Sci. 100 (7), 5479–5490. https://doi.org/10.3168/jds.2016-11811.

Bols, P.E., Van Soom, A., Ysebaert, M.T., Vandenheede, J.M., de Kruif, A., 1996. Effects of aspiration vacuum and needle diameter on cumulus oocyte complex morphology and developmental capacity of bovine oocytes. Theriogenology 45 (5), 1001–1014. https://doi.org/10.1016/0093-691x(96)00028-3.

Bonato, D.V., Ferreira, E.B., Gomes, D.N., Bonato, F.G.C., Droher, R.G., Morotti, F., Seneda, M.M., 2022. Follicular dynamics, luteal characteristics, and progesterone concentrations in synchronized lactating Holstein cows with high and low antral follicle counts. Theriogenology 179, 223–229. https://doi.org/10.1016/j.theriogenology.2021.12.006.

Borges, E., Braga, D.P.A.F., Setti, A., Figueira, R.C., Iaconelli Jr., A., 2017. The predictive value of serum concentrations of anti-Müllerian hormone for oocyte quality, fertilization, and implantation. JBRA Assist. Reprod. 21 (3), 176–182. https://doi.org/10.5935/1518-0557.20170035.

Burns, D.S., Jimenez-Krassel, F., Ireland, J.L., 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 (1), 54–62. https://doi.org/10.1095/biolreprod.104.036277.

Estienne, A., Pierre, A., di Clemente, N., Picard, J.Y., Jarrier, P., Mansanet, C., Monniaux, D., Fabre, S., 2015. Anti-Müllerian hormone regulation by the bone morphogenetic proteins in the sheep ovary: deciphering a direct regulatory pathway. Endocrinology 156 (1), 301–313. https://doi.org/10.1210/en.2014-1551.

Faria, A.C.F., Moraes, G.F., Pereira, L.S., Cunha, R.R., Santos, R.M., 2021. Influence of antral follicle count on *in vitro* embryo production, sexual precocity and conception rate of Senepol cattle. Anim. Prod. Sci. 61, 1418–1424. https://doi.org/10.1071/AN20355.

Feres, L.F., Siqueira, L.G.B., Palhao, M.P., Dos Santos, L.L., Brandao, F.Z., Viana, J.H.M., 2018. Likelihood of pregnancy after the transfer of embryos derived from follicle aspiration and in vitro embryo production sessions with different relative efficiencies. Anim. Reprod. Sci. 193, 165–170. https://doi.org/10.1016/j.anireprosci.2018.04.065.

Ghanem, N., Jin, J.I., Kim, S.S., Choi, B.H., Lee, K.L., Ha, A.N., Song, S.H., Kong, I.K., 2016. The anti-müllerian hormone profile is linked with the in vitro embryo production capacity and embryo viability after transfer but cannot predict pregnancy outcome. Reprod. Domest. Anim. 51 (2), 301–310. https://doi.org/10.1111/rda.12681.

Gleicher, N., Weghofer, A., Barad, D.H., 2010. Anti-Müllerian hormone (AMH) defines, independent of age, low versus good live-birth chances in women with severely diminished ovarian reserve. Fertil. Steril. 94 (7), 2824–2827. https://doi.org/10.1016/j.fertnstert.2010.04.067.

Guerreiro, B.M., Batista, E.O., Vieira, L.M., Sá Filho, M.F., Rodrigues, C.A., Castro Netto, A., Silveira, C.R., Bayeux, B.M., Dias, E.A., Monteiro, F.M., Accorsi, M., Lopes, R.N., Baruselli, P.S., 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. https://doi.org/10.1016/j.domaniend.2014.07.002.

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), 1–14. https://doi.org/10.1071/RD10226.

Ireland, J.L., Scheetz, D., Jimenez-Krassel, F., Themmen, A.P., Ward, F., Lonergan, P., Smith, G.W., Perez, G.I., Evans, A.C., 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 (6), 1219–1225. https://doi.org/10.1095/biolreprod.108.071670.

Irez, T., Ocal, P., Guralp, O., Cetin, M., Aydogan, B., Sahmay, S., 2011. Different serum anti-Müllerian hormone concentrations are associated with oocyte quality, embryo development parameters and IVF-ICSI outcomes. Arch. Gynecol. Obstet. 284 (5), 1295–1301. https://doi.org/10.1007/s00404-011-1979-6.

Karl, K.R., Ireland, J.L.H., Clark, Z.L., Tempelman, R.J., Latham, K.E., Ireland, J.J., 2022. Limitations in use of ovarian reserve biomarkers to predict the superovulation response in small ovarian reserve heifers. Theriogenology 182, 53–62. https://doi.org/10.1016/j.theriogenology.2022.01.033.

Krause, A.R.T., Dias, F.C.F., Caunce, S.L., Adams, G.P., Mapletoft, R.J., Singh, J., 2022. Predictors of the ovarian superstimulatory response and oocyte collection in prepubertal heifers. Domest. Anim. Endocrinol. 81, 106729 https://doi.org/10.1016/j.domaniend.2022.106729.

La Marca, A., Sighinolfi, G., Radi, D., Argento, C., Baraldi, E., Artenisio, A.C., Stabile, G., Volpe, A., 2010. Anti-Mullerian hormone (AMH) as a predictive marker in assisted reproductive technology (ART). Hum. Reprod. Update 16 (2), 113–130. https://doi.org/10.1093/humupd/dmp036.

La Marca, A., Ferraretti, A.P., Palermo, R., Ubaldi, F.M., 2016. The use of ovarian reserve markers in IVF clinical practice: a national consensus. Gynecol. Endocrinol. 32 (1), 1–5. https://doi.org/10.3109/09513590.2015.1102879.

Lahoz, B., Alabart, J.L., Cocero, M.J., Monniaux, D., Echegoyen, E., Sánchez, P., Folch, J., 2014. Anti-Müllerian hormone concentration in sheep and its dependence of age and independence of BMP15 genotype: an endocrine predictor to select the best donors for embryo biotechnologies. Theriogenology 81 (2), 347–357. https://doi.org/10.1016/j.theriogenology.2013.09.033.

Lucy, M.C., De La Sota, R.L., Staples, C.R., Thatcher, W.W., 1993. Ovarian follicular populations in lactating dairy cows treated with recombinant bovine somatotropin (sometribove) or saline and fed diets differing in fat content and energy. J. Dairy Sci. 76 (4), 1014–1027. https://doi.org/10.3168/jds.S0022-0302(93)77429-9.

Martinez, M.F., Sanderson, N., Quirke, L.D., Lawrence, S.B., Juengel, J.L., 2016. Association between antral follicle count and reproductive measures in New Zealand lactating dairy cows maintained in a pasture-based production system. Theriogenology 85 (3), 466–475. https://doi.org/10.1016/j.theriogenology.2015.09.026.

McGrice, H., Kelly, J.M., Kleemann, D.O., Kind, K.L., Hampton, A.J., Hannemann, P., Walker, S.K., van Wettere, W.H.E.J., 2020. Plasma anti-Müllerian hormone concentration as a predictive endocrine marker for selection of donor lambs to improve success in juvenile in vitro embryo transfer programs. Reprod. Fertil. Dev. 32 (4), 383–391. https://doi.org/10.1071/RD18498.

Monniaux, D., Barbey, S., Rico, C., Fabre, S., Gallard, Y., Larroque, H., 2010. Anti-Müllerian hormone: a predictive marker of embryo production in cattle? Reprod. Fertil. Dev. 22 (7), 1083–1091. https://doi.org/10.1071/RD09279.

Monniaux, D., Baril, G., Laine, A.L., Jarrier, P., Poulin, N., Cognié, J., Fabre, S., 2011. Anti-Mullerian hormone as a predictive endocrine marker for embryo production in the goat. Reproduction 142 (6), 845–854. https://doi.org/10.1530/REP-11-0211.

Moraes, F.L.Z., Morotti, F., Costa, C.B., Lunardelli, P.A., Seneda, M.M., 2019. Relationships between antral follicle count, body condition, and pregnancy rates after timed-AI in Bos indicus cattle. Theriogenology 136, 10–14. https://doi.org/10.1016/j.theriogenology.2019.06.024.

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 (5), 2355–2361. https://doi.org/10.3168/jds.2011-4325.

Pontes, J.H., Silva, K.C., Basso, A.C., Rigo, A.G., Ferreira, C.R., Santos, G.M., Sanches, B.V., Porcionato, J.P., Vieira, P.H., Faifer, F.S., Sterza, F.A., 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 (8), 1349–1355. https://doi.org/10.1016/j.theriogenology.2010.06.004.

Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M.A., Bender, D., Maller, J., Sklar, P., de Bakker, P.I., Daly, M.J., Sham, P.C., 2007. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am. J. Hum. Genet. 81 (3), 559–575. https://doi.org/10.1086/519795.

Resende, A.O., Bohrer, R.C., Viana, J.H.M., 2021. A record of 485 viable cumulus–oocyte complexes recovered and 165 viable embryos produced in a single ovum pickup session from a Senepol breed donor. Reprod. Fertil. Dev. 33 (2), 111. https://doi.org/10.1071/RDv33n2Ab7.

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 (7), 932–944. https://doi.org/10.1071/RD11290.

Sambrook, J., Russell, D., 2001. Molecular Cloning: A Laboratory Manual, 3rd edn. Cold Spring Harbour Laboratory Press, Cold Spring Harbour, NY.

Satrapa, R.A., Castilho, A.S., Razza, E.M., Pegorer, M.F., Puelker, R., Barros, C.M., 2013. Differential expression of members of the IGF system in OPU-derived oocytes from Nelore (Bos indicus) and Holstein (Bos taurus) cows. Anim. Reprod. Sci. 138, 155–158. https://doi.org/10.1016/j.anireprosci.2013.02.023.

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 (1-2), 37–43. https://doi.org/10.1016/s0378-4320(01)00113-0.

Silva-Santos, K.C., Santos, G.M., Siloto, L.S., Hertel, M.F., Andrade, E.R., Rubin, M.I., 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 (6), 1051–1057. https://doi.org/10.1016/j.theriogenology.2011.05.008.

Snelling, W.M., Cushman, R.A., Fortes, M.R., Reverter, A., Bennett, G.L., Keele, J.W., Kuehn, L.A., McDaneld, T.G., Thallman, R.M., Thomas, M.G., 2012. Physiology and endocrinology symposium: how single nucleotide polymorphism chips will advance our knowledge of factors controlling puberty and aid in selecting replacement beef females. J. Anim. Sci. 90 (4), 1152–1165. https://doi.org/10.2527/jas.2011-4581.

Tarlatzis, B.C., Zepiridis, L., Grimbizis, G., Bontis, J., 2003. Clinical management of low ovarian response to stimulation for IVF: a systematic review. Hum. Reprod. Update 9 (1), 61–76. https://doi.org/10.1093/humupd/dmg007.

Vernunft, A., Schwerhoff, M., Viergutz, T., Diederich, M., Kuwer, A., 2015. Anti-Muellerian hormone levels in plasma of Holstein-Friesian heifers as a predictive parameter for ovum pick-up and embryo production outcomes. J. Reprod. Dev. 61 (1), 74–79. https://doi.org/10.1262/jrd.2014-091.

Viana, J.H., De Almeida Camargo, L.S., De Moraes Ferreira, A., De Sa, W.F., De Carvalho Fernandes, C.A., De Pinho Marques Junior, A., 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 (1-2), 1–12. https://doi.org/10.1016/j.anireprosci.2003.12.002.

Viana, J.H.M., Figueiredo, A.C.S., Siqueira, L.G.B., 2017. Brazilian embryo industry in context: pitfalls, lessons, and expectations for the future. Anim. Reprod. 14, 476–481. https://doi.org/10.21451/1984-3143-AR989.

Visser, J.A., de Jong, F.H., Laven, J.S., Themmen, A.P., 2006. Anti-Müllerian hormone: a new marker for ovarian function. Reproduction 131 (1), 1–9. https://doi.org/10.1530/rep.1.00529.

Watanabe, Y.F., Souza, H.A., Mingoti, R.D., Ferreira, R.M., Batista, E.O.S., Dayan, A., Watanabe, O.Y., 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. https://doi.org/10.21451/1984-3143-AR1008.

Wohlres-Viana, S., Arashiro, E.K.N., Minare, T.P., Fernandes, C.A.C., Grazia, J.G.V., Siqueira, L.G.B., Machado, M.A., Viana, J.H.M., 2019. Differential expression of LHCGR and its isoforms is associated to the variability in superovulation responses of Gir cattle. Theriogenology 126, 68–74. https://doi.org/10.1016/j.theriogenology.2018.