

121 Anti-Müllerian hormone cutoff values for the selection of oocyte donors in the Gir (*Bos indicus*) breed

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Abstract

Anti-Müllerian hormone (AMH) has a well-known association with antral follicle count (AFC) and the number of oocytes recovered by OPU in cattle. However, to be used as a criterion to select candidate donors for ART, AMH reference values must be established for each breed. The aim of this study was to estimate AMH cutoff values for selecting oocyte donors in Gir (*Bos indicus*) cattle. A blood sample was collected at a random day of oestrous cycle from Gir heifers ($n = 120$) at 23.3 ± 0.5 months of age, and the plasma was stored for further AMH analysis by ELISA, using a commercial kit (AL114, AnshLabs) in a private laboratory (LEAC, Sao Paulo, Brazil). Data from 506 ovum pickup-*in vitro* embryo production (OPU-IVEP) sessions (4.2 ± 0.2 per donor) performed in these heifers from 2017 to 2019, starting 120 days after blood sampling, were then analysed. Donors were ranked in quartiles according to the number of total oocytes recovered, viable oocytes recovered, and embryos produced. Those classified in the first quartile for each endpoint were considered poor responders. The AMH values were then analysed using the receiver operating characteristic (ROC) curve, and cutoff values for each endpoint were estimated based on best values for sensitivity and specificity. A second analysis was performed to check which cutoff value would result in a significant increase in average for each endpoint. The OPU-IVEP data were ranked according to corresponding AMH values (smaller to greater), and the average of the selected group of donors was compared with the original group by ANOVA, using the PROC GLM of SAS (SAS Institute Inc.), after successive exclusion of donors with lower AMH concentrations. Results are shown as mean \pm s.e.m. On average, heifers presented AMH concentrations of 932.9 ± 48.4 pg mL⁻¹ (ranging from 112.7 to 2044.8 pg mL⁻¹) and produced 32.3 ± 1.5 total oocytes, 25.9 ± 1.4 viable oocytes, and 5.3 ± 0.4 embryos per OPU-IVEP session. The heifers ranked in the third and last quartiles yielded more total oocytes, viable oocytes, and blastocysts than those ranked in the first quartile ($P < 0.05$). Based on the total number of oocytes recovered (59.2 ± 3.1), heifers of the last quartile had an estimated AFC >60 . The area under the ROC curve (AUC) for total oocytes, viable oocytes, and embryos produced were 0.77 ($P < 0.0001$), 0.75 ($P < 0.0001$), and 0.72 ($P = 0.0003$), and the estimated cutoffs for AMH concentration were 761.4, 622.5, and 681.5 pg mL⁻¹, respectively. After successive exclusions of donors with lower AMH values, averages were greater ($P < 0.05$) at a cutoff value of 632.4 pg mL⁻¹ for total oocytes (36.9 ± 1.6 vs. 32.3 ± 1.5 ; +15.3%), 672.3 pg mL⁻¹ for viable oocytes (30.3 ± 1.4 vs. 25.9 ± 1.4 ; +19.4%), and 776.8 pg mL⁻¹ for embryos produced (6.6 ± 0.3 vs. 5.3 ± 0.4 ; +23.4%). However, the use of these cutoff values to increase the averages would result in the exclusion of 32.8, 37.9, and 50.0% of the potential donors for each endpoint, respectively. In summary, these results demonstrated that an AMH concentration of approximately 700 pg mL⁻¹ can be used to select donors with higher oocyte yield and thus to increase IVEP outcomes in Gir heifers.

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