



A164 Physiology of Reproduction in Male and Semen Technology

Identification of seminal parameters predictive of conception rates in *Bos indicus* cows submitted to timed-artificial insemination- partial results

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Ability to predict male fertility is highly desirable for bulls used in timed-artificial insemination (TAI) to achieve better conception rates, consequently reducing reproductive program costs. Our goal was to correlate different methods of post-thaw semen evaluation with the pregnancy (P/AI) of Nelore (zebu) cows subjected to TAI to identify candidate predictors of sire conception rate. The P/AI data from 43231 Nelore cows, inseminated in TAI with basic protocols with application of Estradiol Benzoate on D0 (2 mg im) and use of the intravaginal device with 1 g of P4 for 8 days, followed by application of 1 mg of estradiol cypionate, 150µg of d-cloprostenol and 300 IU eCG on D8. The TAI was performed 40-56 h after, with frozen-thawed semen from 21 Nelore and 50 Angus bulls (P/AI from experimental data and fertility index (IFERT®-Lagoa da Serra). Three samples were evaluated from each bull, with semen batches analyzed for physical, functional and morphological aspects, including subjective means [gross motility, thermal resistance test (TRT), morphology, sperm concentration per ml (total and viable)], sperm tail mitochondrial sheath (MS) length stained with aggresome probe; Computer Assisted Semen Analysis [CASA- total motility, progressive motility, VAP, VSL, linearity, STR, ALH and VCL], hyposmotic swelling test (HOST), and image-based flow cytometry: mitochondrial membrane potential (JC-1), and over 1,300 image-based calculations from nuclear stain DAPI, acrosome status/integrity-detecting lectin PNA (*Arachis hypogaea*/peanut agglutinin) aggresome-detecting probe (AGG), bright field, and side scatter. Data was analyzed using ANOVA (GLIMMIX), Partial Least Squares (PLS) regression with use of Wolds criterion to explore the relative importance of individual sperm variables related to fertility (P/AI). The differences in P/AI were found between bulls ($P < 0.001$), and between breeds – Nelore: 54.44%, and Angus: 49.23% ($P < 0.001$). The following *in vitro* sperm variables were determined to be important predictors of P/AI with negative coefficient: total and tail defects, AGG minor axis intensity standard deviation (SD); AGG width SD; AGG minor axis intensity median absolute deviation (MAD); AGG width mean; PNA H entropy mean; PNA H energy mean; PNA H variance SD. Predictors with positive coefficient included: MS length, polarized (JC-1), gross motility, vigor-TRT, CASA variables (progressive motility, VAP, VSL and VCL), viable sperm concentration, DAPI Elongatedness Head MAD; side scatter H contrast mean; PNA H Variance Mean; PNA H Entropy MAD; PNA H Entropy median; AGG Width MAD; AGG H Entropy SD; PNA +++ Gated; AGG Grad MAX Mean; AGG H Contrast Median; AGG H Contrast Mean. In conclusion, Angus and Nelore bulls differ in P/AI when mated to *Bos indicus* cows. Such multiplex studies correlating sperm parameters and differences in fertility rates observed in TAI are under way and provide an advancement in better understanding sperm fertility potential.