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Sperm morphology in the southern white rhinoceros (Ceratotherium simum)

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The white rhinoceros is classified as near threatened in the International Union for Conservation of Nature red list, mainly due to poaching and habitat destruction. Recent success in breeding this iconic animal using assisted reproductive intervention has focussed attention on the lack of information on sperm morphology. Semen samples (native and frozen) from five animals were routinely prepared for light microscopy (LM) and transmission electron microscopy (TEM) and sperm structure was digitally recorded. The cells were approximately 48μm in length with a relatively long midpiece and displayed a degree of head pleomorphism. Typical mammalian ultrastructural features were apparent, most notable of which was a gently swollen apical acrosomal ridge, a large number (approximately 90) of small round to elongated mitochondria (depending on the plane of section) with one or two cristae forming the midpiece, and a prominent annulus. The implantation fossa was shallow and the various components of the neck region (basal plate, proximal centriole, segmented columns of the connecting piece, terminating dense fibres and *pars ascendens* mitochondria) clearly visible. The capitulum was not always obvious and the short distal centriole contained no organised cellular components. The axoneme running the length of the flagellum presented the typical microtubular arrangement and was supported along much of its length by prominent outer dense fibres. A high incidence (50 – 70%) of abnormal sperm were observed in the material studied, reflecting the full range of defective mammalian sperm. The most prominent anomalies were the knobbed acrosome defect and the "Dag" defect, both of which are linked to impaired fertility. Knobbed sperm showed an eccentric knob-like thickening of the apical acrosome, often accompanied by cystic inclusions filled with cellular material. In some cells the acrosome was absent but with its contents abnormally concentrated to form the knob. Most sperm appeared to display this defect when examined by TEM although subtle forms of the defect could not be detected by LM with obvious implications for the abnormality count. The "Dag" defect demonstrated the tail coiling and displacement of axonemal elements characteristic for this anomaly. Based on these observations, an accurate assessment of sperm morphology should be an integral part of breeding programmes for these endangered animals.

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Frozen-thawed semen quality: its relationship with the conception rate in tropical beef cattle herds using fix time artificial insemination

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Conception rate (CR) in AI programs is affected by many factors including female, nutrition, sanitary, environment, management and synchronization protocols among others. The role of the frozen semen sample quality is of outmost importance. Even though testing of the semen donor before freezing should be a routinely step, finding samples with questionable quality is not unusual in tropical areas. This report aims to quantify the CR obtained at first insemination in beef cattle after using frozen semen straws of different quality. 2496 cows distributed in 18 herds in tropical Costa Rica were inseminated through fix time AI protocols by the same skilled operator. Frozen semen straws from 57 (local or imported) different bulls were used in the study. At least one straw from each different stud was thawed and tested conventionally according to the Andrology Lab (UNA) for motility, sperm number/straw, morphology under phase contrast microscopy and carbol fuchsin staining (1000x) and total viable spermatozoa (VS) (motile and morphologically normal). Thereafter, samples were categorized as G1: ≤15% uncompensable sperm defects (USD) and ≥10 million VS, G2: ≤15% USD but <10 million VS, and G3: >15% USD and <10 million VS. CR was determined by rectal palpation 40 days after fix time AI. Percentage of studs categorized as G1, G2 and G3 was respectively 49.1 (n=28/57), 31.6 (n=18/57) and 19.3 (n=11/57). Motility (%), NTS ($x10^6$), USD (%) and VS ($x10^6$) were respectively 74.3±15.2 (25-90), 30.5±14.8 (15-107.5), 4.4±3.4 (0-15) and 16±3.9 (10.2-26.1) for G1, 63.9±15.6 (35-85), 18.8±6.8 (8.8-36.5), 6.1±4.6 (0-15) and 6.7±2 (2.7-9.6) for G2, and 66.6±5.5 (55-80), 22.6±6.3 (8.1-42.5), 27.5±15 (16-71) and 6.1±2.3 (0-14) for G3. Conception rate in fix time AI protocols was significantly higher ($P<0.0001$) in cows inseminated with G1 samples compared to those bred with straws ranked as G2 and G3 (58.8±12.9 versus 45.0±16.2 and 37.8±11.0 respectively). No differences in CR ($P>0.05$) were found between G2 and G3 groups. Regardless we performed semen evaluation using conventional methods, it was shown that seminal parameters such as sperm morphology and number of viable sperms per straw are essential issues that influence in a big extent the conception rate of fix time AI programs in tropical beef cattle farms.