COMPARATIVE EVALUATION OF LLAMA SPERMATOZOA MORPHOLOGICAL ABNORMALITIES USING LIGHT MICROSCOPY AND TRANSMISSION ELECTRON MICROSCOPY (TEM)

E. James[1], B. Stang[2], M. Kutzler[2]

[1]Department of Animal Science, Oregon State University, Corvallis, OR 97331, USA, [2]Department of Clinical Sciences, Oregon State University, Corvallis, OR 97331, USA

Several morphologic abnormalities have been identified in llama sperm when evaluated using light microscopy, ranging from 0 to 79% morphologically normal [MN; Lichtenwalner AB, et al. Theriogenology 1996;46:293–305]. However, ultrastructural examination of camelid spermatozoal defects has not been reported. Our objective is to evaluate llama spermatozoal morphology using light microscopy and TEM. The hypothesis is that TEM will identify additional morphologic abnormalities and better characterize abnormalities previously described in llama sperm using light microscopy. In September 2004, semen was collected using an artificial vagina from five male llamas. A total of 23 collections were made over a 3-week period. Volume, progressive forward motility (PFM), pH and concentration were determined for each collection. Semen samples were stained with eosin and nigrosin and air-dried on a slide at 37 °C for light microscopic evaluation (1000×). One representative sample from each male was also evaluated by TEM. The mean volume, PFM, pH and concentration for the semen samples were 3 ml, 21%, 7.8 and 32×10^6 ml^{-1}, respectively. The number of MN spermatozoa from these collections ranged from 35 to 69% with a mean of 53%. In addition to highly variable sperm head size (length and width) within and between samples, several head and midpiece abnormalities were identified on both light microscopy and TEM. Nuclear vacuoles were evident in sperm from all five llamas. Size and position of the nuclear vacuoles varied within each sample from a single large apical lesion to several small apical and/or post acrosomal vacuoles. Acrosomal membrane bound vacuoles were also present and acrosomal plasma membranes were often irregular. Thickened midpieces with Dag-like defects commonly occurred with nuclear vacuoles. Midpiece abnormalities included mitochondrial sheath lesions, irregular midpiece globules and abnormal microtubules, including some midpieces with a total lack of microtubules. Vacuolation, acrosomal membrane abnormalities, and variable head sizes have been linked to poor thermoregulation, stress, injury, illness and abnormal climate conditions in bovine sperm [Barth AD, et al. Abnormal morphology of bovine spermatozoa. Ames, Iowa: Iowa State University Press; 1989]. Poor thermoregulation would likely be a factor for the abnormalities observed in these samples due to the perineal, non-pendulous scrotal position in llamas and the elevated ambient temperature in the summer months. Also, scrotal trauma is common to camelids from fighting and evidence of scars was present on the scrotal skin. In cattle, the Dag defect is a recessive inherited condition. If this is true for camelids, selection against the Dag defect would reduce the occurrence of this abnormality. Males with exceptional fertility should be compared to males with questionable fertility using both light microscopy and TEM.

Keywords: Llama; Spermatozoa; Morphologic abnormality; Transmission electron microscopy