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**STANDARDISATION AND APPLICATION OF COMPUTER AUTOMATED BULL SPERM
HEAD MORPHOMETRIC ANALYSIS**

Curtis G. Gravance

School of Medicine

Department of Obstetrics and Gynaecology

A thesis submitted in partial fulfillment of the requirements for the degree of
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ABSTRACT

The goal of the contemporary cattle breeder is to maximize the efficiency of production, whether it is meat or milk. Reproductive efficiency is a major component of this objective. With the use of cryopreserved semen and artificial insemination, bull fertility is a large factor in herd reproduction. General semen characteristics such as volume, sperm concentration, sperm motility and sperm morphology characteristics are classic methods of assessing fertility. For over 50 years, scientists have attempted to correlate normal sperm morphology with male fertility. Confounding reports as to this correlation may be due to the variability associated with the methods used to determine normal morphology.

Due to subjectivity and variability, development of objective and consistent analysis methods receive considerable attention. One subjective method is computer assisted sperm head morphometry analysis (ASMA). Precise and objective sperm head size and shape can be quantified using ASMA. The objectives of this dissertation were to 1) develop processing and analysis methods for the accurate quantification of bull sperm heads, 2) assess the inherent instrument, sample and observer variability associated with the analysis of bull sperm heads and, ultimately, 3) quantify the effects of cryopreservation on bull sperm head size and shape and 4) determine the relationship of sperm-head morphometry and post-thaw fertility of cryopreserved bull semen.

A standard semen sample dilution, sperm staining method and microscope magnification level improved the instrument performance in assessing bull sperm head morphometry. There were no significant replication or observer effects detected on sperm head measurements. The heads of bull spermatozoa did show a significant decrease in dimensions after cryopreservation, indicating an effect among all bulls. Furthermore, in a

subsequent study, no significant differences between pre-freeze and post-thaw sperm head measurements of two groups of bulls with high and low non-return to oestrus rates were detected. However, the change in sperm head width/length pre-freeze and post-thaw was weakly correlated with the post-thaw fertility among bulls.

These results indicate that when standard methods of sample preparation and analysis are used, ASMA is an objective method of analysing bull sperm head dimensions. Measurements provided by the ASMA system show that uniform results are acquired among different observers. A single analysis of one slide, analysing 100 sperm heads, provides precise information regarding the sample. Because sperm head dimensions are affected by cryopreservation, samples of fresh semen will have to be utilised in future studies to determine whether differences in sperm head morphometry are detectable between fertile and subfertile bulls and to quantify the dimensions of spermatozoa of fertile bulls. Furthermore, pre-freeze measurements of morphometry do not appear to be correlated with post-thaw fertility of cryopreserved bull semen, based on non-return to oestrus rates.