ABSTRACTS

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Evaluation of vaginal cytology with BS200Pro software in digital format for determination of cycle stage in bitches

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OBJECTIVES AND METHODS: Vaginal cytology is a widely used diagnostic tool in clinical canine reproduction. The aim of present study was thus to investigate the usefulness of BS200Pro Software for objective determination of the sexual cycle stage in the bitch by means of computer associated digital measurements. Eighty clinically healthy bitches from different breeds aged 19-32 months were used in this study. The bitches were randomly assigned to 8 groups: early proestrus (n=10), late proestrus (n=10), early estrus (n=10), estrus (n=10), late estrus (n=10), early metestrus (n=10), late metestrus (n=10), and anestrus (n=10). In all groups, cycle stage was determined by anamnesis, vaginoscopic and vaginal cytological examinations. Blood samples and vaginal swabs were taken daily, from early proestrus to anestrus and serum progesterone (P4) concentrations measured by radioimmunoassay (Immunotech® RIA progesterone kits; Canada). A total of 11423 images from 80 bitches were analysed. Bitmap (bmp) image format was used to avoid compression algorithms. Data obtained from cells and their nuclei were selected manually and automatically by BS200Pro® software and transferred to MS Excel® software. Values were processed with following algorithms: Cell Area (µm²) = Pixel number in selected cells x Calibration coefficient. Nucleus Area (µm²) = Pixel number in selected nucleus x Calibration coefficient. Cell average R (Red), G (Green) and B (Blue) Channel Values were automatically calculated by BS200Pro software®. R, G and B values of all pixels in selected cell were calculated and the average R, G and B channel value was used as R, G and B value of that cell.

RESULTS: The serum P4 concentration progressively increased, averaged 0.78 ng/ml in early proestrus, 7.79 ng/ml in estrus, and reached highest levels in early metestrus (31.14 ng/ml). During anestrus, P4 values decreased to basal levels. Cell area measurements of small and large intermediate cells in late metestrus and anestrus, as well as ceratinised and nucleated superficial cells in late metestrus were significantly lower in comparison to other cycle stages (P<0.001). Large intermediate cells reached highest values in early proestrus with an area measurement of on average 1970.96 µm². Besides, nucleated and ceratinised superficial cells reached highest values in estrus (3438.82 and 3961.11 µm², respectively). A linear increase in cell area values of nucleated and ceratinised superficial cells were determined from the beginning of early proestrus to estrus. Similarly, nucleus area values of nucleated superficial cells increased from early proestrus to highest levels in early estrus. During mentioned stages, positive correlations were calculated between serum P4 concentration and cell area values of nucleated superficial cells (P<0.01) towards estrus. In late metestrus, G channel values of nucleated and ceratinised cells were significantly higher then in other stages, especially in comparison to estrus (P<0.001). In early estrus and estrus, R channel values of ceratinised superficial cells were significantly lower then they were in other phases.

CONCLUSION: In conclusion, by performing cell and nucleus area measurements we achieved reference values for different cycle stages. When proestrus and estrus were compared, a progressive increase of cell area together with low RGB values of superficial cells was characteristic for the estrus stage. However, more results comparing the classical microscopy method with digital image processing are necessary to proof the accuracy of this protocol.