

Species abstracts

Small animal

ENDOMETRIAL CYTOLOGY IN THE BITCH NUCLEI MEASUREMENT IN DIFFERENT PHASES OF THE ESTROUS CYCLE AND IN UTERINE DISORDERS

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The cytology of the canine uterus is a relatively new research field and the nuclear appearance of the endometrial cellular population has not yet been examined. The objective of this study was to investigate aspects of the endometrial cell nuclei throughout the reproductive cycle and in cases of uterine disorders. A total of 46 dogs that had undergone ovariohysterectomy were used, including 35 healthy bitches (5 prepubertal, 6 in proestrus, 6 in estrus, 9 in diestrus and 9 in anestrus) and 11 bitches with uterine pathologies (2 cystic endometrial hyperplasia, 1 hydrometra, 5 pyometra, 1 uterine stump and 2 subinvolution of placental sites). Stage of the estrous cycle was determined by progesterone concentrations (ELFA system, miniVidas, Biomerieux, France). The cytologic uterine samples were collected immediately after surgery by apposition of the endometrial surface on a slide, stained with hematoxylin and eosin, and examined under an Olympus BX51 light microscope. Each slide was analysed for the presence of: single endometrial epithelial cells, groups of normal and degenerate endometrial epithelial cells, naked nuclei, erythrocytes, leucocytes, macrophages, plasma cells and bacteria. The cells present in 20 fields of each slide, at 400 $\times$ magnification, were counted and the mean $\pm$ S.D. number of cells for field calculated. Computer morphometry evaluation, expressed as mean $\pm$ S.D. of the nuclear area, perimeter, diameter, density, aspect and roundness of the endometrial epithelial nuclei, was performed at 400 $\times$ using software for image analysis (Image-Pro Plus, Media Cybernetics, Inc). For each sample, a total of 100 nuclei were evaluated. Histological examinations confirmed the diagnosis of the uterine state. Data were analyzed with a Student's *t*-test. Proestrus and

estrus had similar distribution of endometrial cell population and analogous nuclear features ($P > 0.1$). The nuclear morphometry was significantly different among the remaining stages of the reproductive cycle. Cytological specimens from middle diestrus to early anestrus had epithelial signs of involution, with foamy cells and pyknotic nuclei and predominant macrophages. The uterine cytological appearance of late anestrus abruptly changed; a great number of intact cell groups with round-shaped nuclei and uniform cytoplasm were present. Uterine pathologies were always characterised by degenerate endometrial cell groups with cytoplasmic large vacuoles and nuclear alterations. In conclusion, nuclei morphometric data of endometrial epithelial cells differed significantly among phases of the reproductive cycle in healthy dogs (no difference between proestrus and estrus) and allowed to discriminate among uterine disorders. The potential role of the nuclear morphometry of the canine endometrium should be further investigated in view of a useful reproductive soundness examination of the bitch.

Keywords: Endometrial cytology; Nuclear morphometry; Bitch; Canine

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INDUCTION OF ABORTION IN QUEENS BY ADMINISTRATION OF AGLEPRISTONE (ALIZIN[®]): PRELIMINARY RESULTS

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Aglepristone is a well-known drug used for pregnancy termination in bitches with satisfactory efficacy and excellent general safety. Nevertheless, pregnancy termination in the queen is less documented, especially with antiprogesterone. The aim of this study was to assess the efficacy of aglepristone to induce abortion in queens. The hypothesis was that aglepristone would induce termination of mid-pregnancy in queens without side effects. Six adult queens (age, 24–36 months; weight 3–4 kg) were maintained under artificial illumination (14-h light:10-h dark) in cages and monitored daily for estrous behavior and vaginal cytology. At evidence of estrus, the queens were placed with a tom cat for 48 h. First mating was documented. Twenty-five days after mating, ultrasonography was

performed to confirm pregnancy (linear array transducer, Tringa[®], Pie Medical; Maastricht, Holland), and on Days 35–38, pregnant queens ($n = 6$) were injected subcutaneously with 10 mg/kg aglepristone (Alizin[®], Virbac, Germany) on two consecutive days. Blood samples were taken before the administration of aglepristone (Day 0), during treatment (Days 1 and 2), the day after treatment (Day 3), the day of abortion, and the two following days; to measure progesterone (P_4) by RIA (Coat-A-Count, Diagnostic Product Corporation, Los Angeles, CA, USA). The general condition, vaginal cytology and presence and character of vaginal discharge were recorded daily. The condition of the uterus was assessed by ultrasonography the day before and the days after the administration of aglepristone until confirmation of complete fetal expulsion. Progesterone concentrations were analyzed by least squares means analysis of variance using the GLM procedure of SAS[®] (Statistical Analysis System, Cary, NC, USA). Termination of pregnancy was achieved in all queens; the mean interval between administration of aglepristone and fetal expulsion was 5 ± 2 days. Progesterone plasma concentration increased after aglepristone injection (15.0 ng/mL versus 28.0 ± 2.5 ng/mL; $P > 0.004$). Mean progesterone concentration started to increase 28 h after aglepristone injection and peaked 77 h after drug administration. On the day of abortion, mean serum P_4 concentration was 19.5 ± 3.9 ng/mL. There were no significant differences in P_4 plasma concentration between Day 0 and the two consecutive days after the abortion (15.0 ± 4.0 ng/mL versus 18.4 ± 4.9 ng/mL). Hemorrhagic vaginal discharge was observed in all queens during the abortion and during the following week. The mean interval between the abortion day and the beginning of the next estrus was 24.6 ± 6.6 days. Few negligible side effects were observed; two queens had a slight decrease in feed intake and three had increased vocalization. In conclusion, the use of aglepristone in queens at Days 35–38 of pregnancy induced abortion 3–7 days later without side effects.

Keywords: Queen; Abortion; Aglepristone; Pregnancy termination; Progesterone receptor antagonist

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DETECTION OF REACTIVE OXYGEN SPECIES IN CANINE SEMEN

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The presence of reactive oxygen species (ROS) have been documented in canine semen. In humans, centrifugation of semen has resulted in increased iatrogenic ROS concentrations during laboratorial processing. The purpose of this study was to determine the concentrations of ROS, in raw and diluted canine ejaculates prior to and post-centrifugation. The study was designed to test the hypothesis that centrifugation would increase the concentration of ROS in semen. Ejaculates ($n = 21$) were collected via digital manipulation from sexually mature dogs ($n = 6$). Raw semen was immediately evaluated for motility, viability, morphology, membrane integrity (hypo-osmotic swelling test, HOST) and presence of leukocytes with Hema 3[®] (Fisher Diagnostics, Middletown, VA, USA). Sperm concentration and motility were determined by computer-assisted sperm analyzer (CASA, IVOS; Hamilton-Thorne). Viability and morphology were determined under light microscopy at $1000\times$ magnification using eosin-nigrosin stain. Membrane integrity was evaluated with a 100 mM sucrose solution (HOST) and visualized under phase contrast microscopy at $400\times$. Concentrations of ROS were determined using a luminometer (automatic measuring system for chemiluminescence; Lumat LB 9507, Berthold Technology Oak Ridge, TN, USA). Each ejaculate was divided into two fractions, raw and diluted 1:1 with a skim milk-based diluent (Har-vet, Spring Valley, WI, USA). Following initial analysis, each fraction was then centrifuged at $700 \times g$ for 10 min (Eppendorf Centrifuge 5702, Eppendorf North America; Westbury, NY, USA) and reassessed for ROS. For each chemiluminescence analysis, 20 μ L of luminol (Sigma Aldrich, St. Louis, MO, USA) were added to 500 μ L of semen and placed in the luminometer. Eleven, relative light unit (RLU) measurements, taken over the course of 5 min every 30 s, were converted into $RLU/10^6$ spermatozoa for each sample and statistical analyses. Data were analyzed using a two-way ANOVA with repeated measures to evaluate the effects of diluent and centrifugation on seminal concentrations of ROS, and expressed as means $\pm$ S.E.M. Concentrations of RLU in raw and post-centrifugation raw ejaculates were 975.0 ± 292.9 and 1804.2 ± 579.7 , respectively