

easy to administer, reversible to allow the owner to change his or her mind or to better organize and control reproduction of his or her pet. Particularly important was that it should be devoid of significant side effects. Our goal was to develop a device responding to these requirements and by using available technologies to reduce development costs.

The development of these implants was realized in four different phases:

(1) *in vivo* determination of the minimum effective progestin dose (MED) to control estrus cycles in dogs and cats; (2) definition of a prototype formulation—*in vitro* and *in vivo* releases; (3) clinical validation trials; (4) *in vitro* optimization of the implant and *in vivo* efficacy and tolerance studies.

After 4 years of development, we produced a 2 years contraceptive implant, effective both in dogs and cats and devoid of significant side effects during a 4 years follow-up study. Its contraceptive effects were reversible. The cat implant is ± 2 cm long and 0.24 cm of diameter; the implants for dogs are ± 4 cm long and 0.32 cm diameter. The implant is not associated with any local or general reactions, and, it is easily detected by radiography, allowing its removal when reversibility is needed.

Based on these results, we conclude that progestins administered consistently at low dose still are a viable option for contraception in dogs and cats. This approach also helps to better understand the mode of action of progestins and certainly lead to a new perspective for the use of these agents in pets. This allows us to reconsider the use of older drugs with new perspectives.

Keywords: Dog; Cat; Contraceptive implant; Clinical trial

EVALUATION OF THE “MINITUBE SPERMVISION COMPUTER-BASED AUTOMATED SYSTEM” FOR DOG SEMEN ANALYSIS

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An objective evaluation of semen is required to assess the canine fertility and to select appropriate techniques for semen preservation. With conventional microscopic evaluation, the subjectivity of the analysis results in significant variability. In the present study, we validated the Minitube SpermVision Computer Based-Automated System for objective assessment of canine semen. A description of the fertile canine motility parameters using this analyzer is reported. The repeatability

between fields is automatically controlled by the system, which does not allow a variation of more than 20% in the evaluation of any single sample to allow for reliability of the results. The SpermVision is effective over a large range of concentration without major significant differences; however, based on the concentration studies realized, it clearly appears that the ideal range for measurement is probably between 50 and 100 million sperm cells/ml. Highly concentrated semen should then be diluted to fit in this optimum range, if the concentration is lower, variability per field can be too important, if the concentration is too high the effects of sperm cells superposition and sperm collision decrease the reliability of the analyses. The setting up of the system is easy and can rapidly be adapted to any species of interest. The major factor impacting the sperm cell recognition is the light intensity which should ideally check and set up before every analysis. The validity of the measurement over time has been determined with semen preserved at two temperature 20 and 38 °C before analyses at 38 °C. Semen analysis at 20 °C appears to allow for better and longer preservation of non-diluted semen during viability studies reducing significantly semen agglutination observed over time. The makler chamber gave similar results as the leja cell which both were adapted to dog semen evaluation whereas the cell-vu which induced a decrease of all sperm motility parameter was considered as inadapted. Generated from the analysis of six mature fertile Anatolian Shepherd Dogs and pools of semen, the SpermVision analyzer represents a useful tool for andrological studies in dogs.

Keywords: Canine semen; CASA; motility parameters

Equine

UTERINE INFLAMMATORY RESPONSE TO FROZEN SEMEN, *STREPTOCOCCUS EQUI* SUBSPECIES *ZOOEPIDEMICUS*, OR BOTH AT 72 h POST-TREATMENT IN YOUNG, REPRODUCTIVELY SOUND MARES

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In vivo, bacteria and spermatozoa are simultaneously present or introduced into the mare's uterus at breeding, however there is little information on the combined

effects of frozen semen and bacteria on uterine inflammation in resistant or susceptible mares. The objective of this study was to compare the uterine inflammatory response (total and percent neutrophils) to frozen semen, bacteria, or both at 72 h post-treatment in young, reproductively sound mares. Thirteen mares (mean age 3 years, range 2–6 years), were randomly assigned to three unbalanced treatment groups ($n = 20$ cycles). A rest cycle was given between each treatment. The treatments were: 1×10^9 frozen-thawed spermatozoa (FS) in lactose EDTA extender ($n = 8$), 5×10^6 frozen-thawed *Streptococcus equi* subspecies *zooepidemicus* (FB) ($n = 6$), and the aforementioned doses of frozen thawed semen and bacteria together (FSB) ($n = 8$). Treatment samples were thawed, and extended to make 10 mL with Kenney extender (EZ Mixin—Basic[®], ARS, Chino, CA). Estrus mares were monitored daily with rectal palpation/ultrasound, administered 2000 IU hCG IM when a 35 mm follicle was detected, and treated on the day of ovulation (ov). A single 72 h post-ov low volume uterine lavage sample was collected for culture and cytology by inserting a catheter into the uterus, infusing 60 mL PBS, massaging the uterus per rectum, and then collecting the fluid back into sterile tubes. The fluid volume recovered was recorded and centrifuged at $350 \times g$ for 15 min, the supernatant removed, the pellet resuspended in 1 mL, and the cell concentration determined using a hemocytometer. The cell concentration was used to determine the total cells in the original 60 mL of PBS. The pellet suspension was used to make cytology slides which were stained with a rapid Wright Giemsa stain (Protocol, Fischer Diagnostics, Middletown, VA). The percent neutrophils was determined from differential counts of 300 cells, at 1000 times by two investigators blinded to sample identity. Total numbers of neutrophils were determined by multiplying the percent neutrophils by the total cell count of a sample. Kruskal–Wallis one-way ANOVA analysis was performed using Statistix version 8.0 software (Analytical Software, Tallahassee, FL). Data below are listed as median (first, third quartile). There were no differences in neutrophil percent: FS 4% (3, 10%); FB 11% (3, 24%); FSB 5% (2, 12%) or total neutrophil number (10^6) FS 1.78 (0.02, 7.8); FB 1.56 (0.22, 5.27); or FSB 0.36 (0.15, 2.73) between groups. Based on previously published reports this population of young mares would be considered “resistant,” with <5% neutrophils at 72 h post-treatment with *Streptococcus equi* subspecies *zooepidemicus*. In conclusion, there were no significant differences induced by the three treatments at 72 h, which indicated that there were no additive or synergistic effects of intrauterine chal-

lenge with bacteria and frozen semen together. Future studies of “susceptible” mares that are unable to clear bacteria by 72 h post-infusion, should be performed to determine if the interactions between frozen semen and bacteria are different in these mares compared to resistant mares.

Keywords: Mare; Uterus; Semen; Inflammation; Bacteria

EVALUATION OF THREE FIXATIVES FOR REPRODUCTIVE BIOPSIES IN THE MARE AND STALLION

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Bouin's and 10% buffered neutral formalin have typically been used as fixatives of choice when evaluating endometrial and testicular biopsies in the veterinary specialty of theriogenology. However, formalin has been associated with creating artifacts and Bouin's contains picric acid which poses both a health and disposal hazard. Additionally, Bouin's requires several additional washes and steps in biopsied tissue preparation. This study was done to assess histomorphology with three different fixatives. A board-certified pathologist (TS) examined endometrial biopsies from ten mares and testicular biopsies from four stallions. All tissue samples were obtained from abattoir specimens immediately after death, therefore eliminating potential autolysis while facilitating precise duplication of biopsy site and adequate size for complete histomorphologic examination. The biopsies were divided equally and fixed in each of three fixatives—10% buffered neutral formalin (F), Bouin's (B) and modified Davidson's solution (MD). In a blinded study, the pathologist graded the endometrial biopsies for overall clarity of morphological detail, creation of artifacts, and preservation of nuclear chromatin in luminal epithelium and glands, stratum compactum and spongiosum, vessels and overall staining. The testicular biopsies were graded for overall clarity of morphological detail, capsule integrity, creation of artifacts and preservation of nuclear chromatin in Leydig cells, seminiferous tubules, epididymis and overall staining. The results of this study for fixation of endometrial biopsies revealed no significant ($p < 0.05$) difference (the GLM procedure for least squares means) between the three fixatives in overall