

1 follicle measured  $\geq 15$  mm in diameter, 50  $\mu$ g deslorelin in aqueous solution (Precision Pharmacy, Bakersfield, CA) was given intramuscularly every 12 hours until at least 1 follicle reached 32 mm in diameter with uterine edema. Subsequently, the LDD protocol was discontinued, allowing for 24 hours without hormone treatment. After collection, oocytes deemed mature (expanded cumulus) or immature (compact cumulus) were handled accordingly, prior to ICSI with frozen semen from a single ejaculate. Presumptive zygotes were evaluated at 24 hours for evidence of cleavage and again at 4, 6, and 8 days. Comparisons were made utilizing a Student's and Welch's *t*-tests with significance established at  $p \leq 0.05$  and reported as the mean  $\pm$  standard deviation. In total, 41 estrous cycles were included (24 control and 17 treated). On average,  $7.41 \pm 2.3$  injections of LDD were given during treated estrous cycles. Average number of follicles observed or number of follicles  $> 25$  mm in diameter at OPU did not differ between groups ( $p = 0.13$  and  $p = 0.46$ , respectively). Average number of follicles aspirated in the treated group ( $2.1 \pm 1.9$ ) tended to be higher ( $p = 0.09$ ) than control ( $1.9 \pm 1.4$ ); however, the number of oocytes collected in treated cycles ( $1.2 \pm 1.4$ ) compared to control ( $0.75 \pm 1.0$ ) did not differ ( $p = 0.28$ ). A total of 36 oocytes were subjected to ICSI from the treated and 38 from control estrous cycles. The cleavage rate was similar ( $p = 0.89$ ) for treated (56%; 20/36) compared to control (61%; 23/38). Finally, the blastocyst rate for treated was similar ( $p = 0.66$ ) (19%; 7/36) compared to control (24%; 9/38). In conclusion, the utilization of a low dose deslorelin protocol did not affect the number of follicles or oocytes collected within estrous cycles for OPU/ICSI. Additionally, cleavage and blastocyst rates were similar between the 2 groups indicating that this protocol did not affect the quality of oocytes. More research is necessary to identify an ovarian superstimulation protocol effective for OPU/ICSI.

**Keywords:** Mare, oocytes, follicles, deslorelin

### **Pregnancy rates and ovarian activity after uterotubal infusion of n-butyl cyanoacrylate via a hysteroscopic approach in mares: a pilot study**

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Sterilization of mares requires surgical removal of ovaries or ligation of uterine tubes and is performed for behavioral reasons, treatment of ovarian pathology, and preparation of tease or mount mares. However, it is invasive, costly, and may be associated with adverse outcomes. Objective was to determine the effect of bilateral uterine tube infusion with n-butyl

cyanoacrylate on fertility and estrous cycle activity of mares. We hypothesized that bilateral infusion of n-butyl cyanoacrylate in the uterine tubes causes infertility but does not affect mare's estrous cycle activity. Light horse mares ( $n = 8$ ) ranging in age from 5 - 23 years, were selected for potential fertility using transrectal ultrasonography examination, uterine culture and cytology. A proven fertile Thoroughbred stallion that successfully bred mares in 2015 was used. Mares were sedated, perineum cleansed, and the uterine tubes of treated mares ( $n = 6$ ) were infused with 0.5 ml n-butyl cyanoacrylate, and control mares ( $n = 2$ ) were infused with 10 ml saline, using the endoscopic hydrotubation method.<sup>1</sup> Treated mares were hand mated through 2 or 3 breeding seasons ( $n = 78$  estrous cycles), and control mares were hand mated through 1 breeding season ( $n = 4$  estrous cycles). Biweekly blood samples were obtained from mares during the breeding season for progesterone ( $P_4$ ) concentrations by RIA. Per cycle pregnancy rate, established by transrectal ultrasonography of the uterus and interestrus intervals, were recorded. Stallion's seasonal pregnancy rate (SPR) in 2015 ( $n = 24$  mares) was compared to the SPR of treated ( $n = 6$ ) and control mares ( $n = 2$ ) for years 2016 - 2018. Data were evaluated using Fisher's Exact test at  $p < 0.05$ . Per cycle pregnancy rates of treated (0/78) and control mares (1/4) were different ( $p = 0.048$ ). SPR for the stallion in 2015 prestudy breeding season (20/24 mares pregnant) was different ( $p < 0.001$ ) than the SPR of treated mares, wherein 0/6 became pregnant in each year. Interestrus intervals of 6 treated mares averaged 20 days (range 18 - 22 days), and serum  $P_4$  profiles were consistent with estrous cycle activity. The n-butyl cyanoacrylate may function to physically obstruct sperm, oocyte and embryo movement in the uterotubal junction or uterine tube, disturb the milieu required for sperm capacitation, oocyte fertilization, and embryonic development, or a combination of these. Uterotubal infusion of n-butyl cyanoacrylate via a hysteroscopic approach may serve as an effective management strategy for induced infertility in mares of at least 3 years duration and may cause permanent sterilization.

**Keywords:** Mare, infertility, n-butyl cyanoacrylate, uterine tube, progesterone, estrous cycle

### **Reference**

1. Inoue Y, Sekiguchi M: Clinical application of hysteroscopic hydrotubation for unexplained infertility in the mare. *Equine Vet J* 2018;50:470-473.

### **Daily sperm output, spermatogenic efficiency, and sexual behavior of donkey jacks mounting jennies in estrus**

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Assessment of daily sperm output (DSO) and spermatogenic efficiency are critical components of breeding soundness evaluation

to diagnose reproductive diseases and estimate the number of females that may be bred in a season. However, there is no consensus on how to determine these parameters in stallions or donkeys. Part of the discrepancies is due to estimation of testicular volume (TV) by several ways. Additionally, donkeys are thought to be less consistent breeders when required to collect daily. We hypothesized that a donkey's DSO varies with the equation utilized for calculation of TV and his behavior parameters are influenced by increased numbers of collection days. Aim was to assess the sexual behavior of jacks mounting jennies in estrus and determine the DSO and spermatogenic efficiency. Eight sexually rested jacks had semen collected once a day for 10 consecutive days using jennies in good standing estrus for mounting. Sexual behavior and semen parameters were assessed during each collection. Testicular measurements of height, width, and length were taken immediately before the first semen collection, and these measurements were used to calculate the TV using a nonellipsoid equation (TV1 [cm<sup>3</sup>]:  $33.57 \times \text{height [in cm]} - 56.57$ ) and an ellipsoid equation (TV2 [cm<sup>3</sup>]:  $4/3\pi \text{ length}/2 \times \text{height}/2 \times \text{width}/2$ ) used for stallions. After that, the TVs were used to predict the DSO. The average total sperm number (TSN) obtained on days 8 -10 was deemed the actual DSO. Differences in the predicted versus the actual DSO were used to calculate the spermatogenic efficiency. In addition, the actual DSO was also used to calculate the number of inseminating doses a jack could produce for both on- and off-site breeding. Data were analyzed with the Shapiro-Wilk

test and then with ANOVA followed by Tukey's test or Kruskal-Wallis. Sexual rest did not affect ( $p < 0.05$ ) sperm motility. Jack's sexual behavior did not vary across collection days ( $p < 0.05$ ). Sperm concentration and TSN reduced ( $p < 0.05$ ) over time. The actual DSO was  $9.1 \pm 4.1 \times 10^9$ , and the predicted DSO varied from  $4.7$  to  $18 \times 10^9$ . Spermatogenic efficiency ranged from 50 to 150% based on the jack and the equation used to calculate TV. The predicted-DSO obtained with TV1 demonstrated a strong and positive correlation with the actual-DSO ( $r = 0.76$ ,  $p < 0.05$ ); however, there was no significant correlation between predicted-DSO and TV2 ( $p > 0.05$ ). The number of inseminating doses ranged from 15 to 47 at  $300 - 500 \times 10^6$  progressively motile sperm (PMS) /dose for on-site breeding. In contrast, the number of breeding doses with cooled-shipped semen ( $1 \times 10^9$  PMS/dose) varied from 4 to 14 doses across donkeys. In conclusion, sexual behavior was not affected by daily semen collections. Sexual rest did not affect sperm motility. The predicted DSO varied with the equation used to determine TV. Clinically normal donkeys have high spermatogenic efficiency that confirms previous histology reports. A distinct approach to calculate DSO in donkeys is described. Further studies are warranted to apply the findings of the present study in reproductively abnormal donkeys.

**Keywords:** Sexual behavior, daily sperm output, spermatogenic efficiency