ABSTRACTS

ISCFR 2012

July 26-29, Whistler, Canada

7th International Symposium on Canine and Feline Reproduction

In a joint meeting with

EVSSAR 2012

15th Congress of the European Veterinary Society for Small Animal Reproduction

Editors: Gary England, Michelle Kutzler, Pierre Comizzoli, Wojciech Nizanski, Tom Rijsselaere and Patrick Concannon

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Effect of refractoriness to long photoperiod on sperm production and quality in tomcats

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INTRODUCTION: The queen is considered to be a seasonal animal and her reproductive season is under strong influence of the day light cycle. Additionally, some new studies also suggest seasonality of sperm production in tom cat (1, 2). In very recent studies, an artificial long photoperiod (12 h light-12 h dark) has been used as a model to assess the effects of melatonin implants to suppress estrus in the queen (3). In one previous study, a potential refractoriness to long photoperiod was observed in tomcats being subjected for 18 months to a long photoperiod (Nuñez Favre et al., unpublished observations). Therefore, the aim of this study was to follow-up on this previous study and assess if refractoriness to long photoperiod could be reversed by subjecting tomcats to short photoperiod and then when switched back to long photoperiods, sperm production and sperm quality could be set back to that before refractoriness. Our hypothesis was that subjecting tomcats to a short photoperiod followed by a long photoperiod would restore sperm quality and production to that before refractoriness.

MATERIALS AND METHODS: Six adult toms, mixed short hair breeds, aged between 2 and 4 year, and weighing between 3.5 and 4 kg, were used. All males were housed alone in stainless steel cages and were fed with commercial cat food (Fit 32° Royal Canin, Buenos Aires, Argentina) and water ad libitum. Animals were maintained in a controlled environment with artificial illumination (3). All males were fertile since they had fathered litters before the start of this experiment. At the beginning of the experiment the cats were housed in a conditioned room with long photoperiod (LP, 12 hours light-12 dark). After 45 d of acclimation (Period [P] 1, toms were maintained under this photoperiod for 19 months (P2) before they changed to a period of decreasing light (P3). The light decreased from 12 h to 8 h at a rate of 8 min/d during one month. Animals stayed one month with short photoperiod (SP, P4; 8 hours light-16 dark) and subsequently, the lighting increased at inverse rate as the decline (8 min/d, one month; P5). The experiment finished after the animals stayed in long photoperiod for 2 month (P6). Semen collection was done by electroejaculation. Toms were anesthetized with a combination of xilacine (0.5mg/kg im; Kensol®, Koning SA, Argentina) and ketamine (20 mg/kg im; Ketamina 50°, Holliday-Scott SA, Argentina). As previously described by Howard et al. (4), all cats received a total of 80 stimuli divided in three sets (30, 30 and 20) with 2–3 min of rest between sets. The first set consisted of 10 stimuli at 2 V, 10 at 3 V and 10 at 4 V. The second set consisted of 10 stimuli at 3 V, 10 at 4 V and 10 at 5 V. The third set consisted of 10 stimuli at 4 V and 10 at 5 V. The semen sample was collected into a 1.5 mL pre warmed plastic tube. After 136 d (45 d acclimation + 91 d [1.5 spermatogenesis cycles & maturation]), semen was-collected from all males every other week during 4 months (L12-1). Eight months after the end of L1, semen collection started again every other week for a period of 5 months (L12-2). In L12-1 and L12-2 animals were with LP. When males started with SP, semen samples were taken for two months every 4 weeks (L8-1). When males placed again under LP, semen samples were taken every other week until the end of the experiment (L12-3). All sperm samples were assessed for motility (MOT, %motile), velocity (VEL 0-5), volume (VOL), sperm concentration (CON, x10⁶), viability (V % alive, eosine-nigrosine stain), acrosome integrity (ACR, % intact; FITC-PSA), plasma membrane integrity (PMI, CFDA-PI) and sperm morphology (SM, % normal). Data were analyzed by the Mixed procedure of SAS⁶ (5). Sperm production and quality from a LP (Figure 1, L12-1&L12-3) were compared with a refractory LP (L12-2). Also sperm production and quality from a SP (Figure 1, L12-1&L12-3) were compared with a SP (L8-1). Finally, sperm production and quality from a refractory LP (Figure 1, L12-2) were compared with a SP (L8-1). Data are presented as LSM ± SEM. Significance was defined as P < 0.05.

RESULTS: Motility, velocity, volume, sperm concentration, total sperm count, viability, acrosome integrity, plasma membrane integrity, and sperm morphology were higher in a LP compared to a refractory LP (L12-1&L12-3 vs. L12-2; 86.9±2.1 vs. 72.2±2.7; 4.7±0.07 vs.3.8±0.09; 0.18±0.01 vs.0.12±0.01; 172.0±18.6 vs. 47.4±23.9; 25.2±2.4 vs. 5.6±3.1; 75.9±1.9 vs. 58.3±2.5; 77.7±2.0 vs. 53.8±2.7; 83.9±1.6 vs. 58.9±2.5; 63.7±1.4 vs. 48.3±1.8; P<0.01, respectively). Similarly, viability, velocity, acrosome integrity, plasma membrane integrity, and sperm morphology were higher in a LP compared to a SP (L12-1&L12-3 vs. L8-1; 4.7±0.07 vs.4.3±0.12; 75.9±1.9 vs. 61.2±3.6; 77.7±2.0 vs. 64.3±4.6; 83.9±1.6 vs. 71.6±3.6; 63.7±1.4 vs. 57.1±2.6; P<0.05, respectively). However, motility, volume, concentration, and total sperm count were similar between both groups (85.0±2.3; 0.18±0.01; 160.7±21.7; 27.7±4.2; P>0.20). Whereas motility, velocity, viability, acrosome integrity and plasma membrane integrity were similar in a refractory LP compared to a SP (L12-2 vs. L8-1; 77.6±4.5; 4.1±0.1; 59.7±3.7; 59.0±4.2; 65.3±5.1; P>0.05, respectively); volume, sperm concentration, total sperm count,
and sperm morphology were lower in a refractory LP compared to SP (L12-2 vs. L8-1; 0.12±0.01 vs.0.18±0.01; 47.4±15.6 vs. 149.3±24.8; 5.6±3.0 vs. 30.1±4.6; 48.3±1.8 vs. 57.1±2.76; P<0.05; respectively).

DISCUSSION: To our knowledge this is the first report to describe a decrease in sperm production and sperm quality caused by refractoriness due to long photoperiod in tomcats. This observation was first made in a control group of tomcats placed under a LP during 18 months period when the effect of melatonin implants was studied. Based upon this observation, tomcats were introduced first in a short photoperiod (8 h light-16 h dark) and then in a long photoperiod (12 h light-12 h dark) to reverse refractoriness and increase sperm production and sperm quality. In agreement with our hypothesis, sperm production and sperm quality was restored to levels similar to those at the beginning of the study. Therefore, we conclude that refractoriness and reduced sperm production and sperm quality induced by a prolonged long photoperiod of 18 month can be restored following subjecting tomcats to a short photoperiod.