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Effect of Refractoriness to Long Photoperiod on Sperm Production and Quality in Tomcats

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Contents
The aim of this study was to assess whether refractoriness to long photoperiod (LP) could be reversed by subjecting tomcats to a period of short days. Our hypothesis was that photoperiod changes can avoid refractoriness and restore sperm quality and production to that before refractoriness. Tomcats (n = 6) were housed in a conditioned room with LP (12L: 12D) for 45 days of acclimation and then maintained under LP for 18 month. Then, tomcats were changed to a period of decreasing light at a rate of 8 min/day for 1 month. Tomcats stayed for 1 month with short photoperiod (SP; 8L: 16D) and then were switched back to a period of increasing light at a rate of 8 min/day for 1 month. The experiment was completed after tomcats remained in LP for 2 months. Toms were anaesthetized and semen samples were collected by electroejaculation every 2 weeks. Sperm parameters were evaluated in all ejaculates, and data were analysed by ANOVA. Motility, velocity, volume, sperm concentration, total sperm count, viability, acrosome integrity, plasma membrane integrity and sperm morphology were higher during LP compared with a refractory LP ($p < 0.01$). Likewise, velocity, viability, acrosome integrity, plasma membrane integrity and sperm morphology were higher in a LP compared with a SP ($p < 0.05$). On the other hand, motility, volume, concentration and total sperm count were similar between LP and SP ($p > 0.20$).Whereas motility, velocity, viability, acrosome integrity and plasma membrane integrity were similar in a refractory LP compared with SP ($p > 0.05$), volume, sperm concentration, total sperm count and sperm morphology were lower in a refractory LP compared with SP ($p < 0.05$). In conclusion, refractoriness and reduced sperm production and quality induced by a prolonged LP of 18 month can be restored after placing tomcats to a SP.

Introduction

The queen is considered to be a seasonal animal and her reproductive season is under strong influence of the daylight cycle. Additionally, some new studies also suggest seasonality of sperm production in tomcat (Blottner and Jewgenow 2007; Stornelli et al. 2009). In very recent studies, an artificial long photoperiod (LP) (12 h light–12 h dark) has been used as a model to assess the effects of melatonin implants to suppress oestrus in the queen (Giménez et al. 2009). In one previous study, a potential refractoriness to LP was observed in tomcats being subjected for 18 months to a LP (R Nuñez Favre et al., unpublished observations). Therefore, the aim of this study was to follow up on this previous study and assess whether refractoriness to LP could be reversed by subjecting tomcats to short photoperiod (SP) and then when switched back to LP, sperm production and sperm quality could be set back to that before refractoriness. Our hypothesis was that subjecting tomcats to a SP followed by a LP 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 Cannin, Buenos Aires, Argentina) and water ad libitum. Animals were maintained in a controlled environment with artificial lighting (Giménez et al. 2009). All males were fertile as they had fathered litters before the start of this experiment. Animal care, housing and experimentation complied with the International Guiding Principles for Biomedical Research Involving Animals.

Light regimen

At the beginning of the experiment, the cats were housed in a conditioned room with LP [12 h light (L)–12 h dark (D)]. After 45 days of acclimation [Period (P) 1], toms were maintained under this photoperiod for 18 months (P2) before they changed to a period of decreasing light (P3). The light decreased from 12 to 8 h at a rate of 8 min/day over 1 month. Animals stayed 1 month with SP (P4; 8L: 16D) and subsequently, the lighting increased at the same but inverse rate as the decline (8 min/days, 1 month; P5). The experiment finished after the animals were maintained in LP for 2 month (P6, Fig. 1).

Semen collection and evaluation

Semen collection was performed by electroejaculation. Toms were anaesthetized with a combination of xylazine (0.5 mg/kg im; Kensol®, Köning SA, Argentina) and ketamine (20 mg/kg im; Ketamina 50®, Holliday-Scott SA, Argentina). As previously described by Howard et al. (1990), 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 days [45 days acclimation + 91 days (1.5 spermatogenesis cycles & maturation)], semen was collected from all males every other week (every 14th day) during 4 months [long photoperiod condition

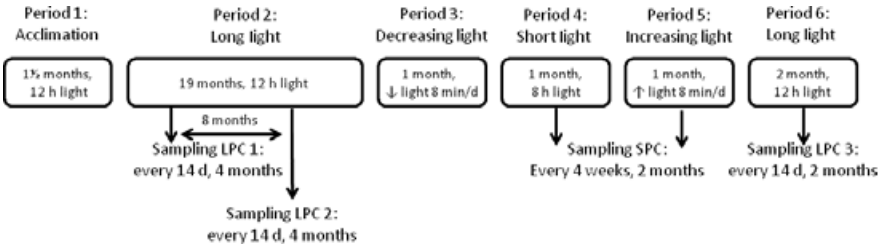

Fig. 1. Experimental details regarding design, light regimen and semen collection of study animals

(LPC) 1]. Eight months after the end of LPC 1, semen collection started again every other week for a period of 4 months (LPC 2). In LPC 1 and LPC 2, animals were with LP. When males started with SP, semen samples were taken for 2 months every 4 weeks [short photoperiod condition (SPC), Fig. 1]. When males were placed again under LP, semen samples were taken every 14 days until the end of the experiment (LPC 3). All sperm samples were assessed for motility (% motile), velocity (0–5), volume (μl), sperm concentration ($\times 10^6$), viability (% alive; eosin–nigrosin stain), acrosome integrity (% intact; FITC-PSA), plasma membrane integrity (% intact; CFDA-PI) and sperm morphology (SM,% normal; Tincion 15®, Biopur, Riccheri 195, Rosario, Santa Fe, Argentina). Data were analysed by the Mixed procedure of SAS® (SAS 1989). Sperm production and quality from a LP (Fig. 1, LPC 1 & LPC 3) were compared with a refractory LP (LPC 2). Also sperm production and quality from a LP (Fig. 1, LPC 1 & LPC 3) were compared with a SP (SPC). Finally, sperm production and quality from a refractory LP (Fig. 1, LPC 2) were compared with a SP (SPC). 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 with a refractory LP (LPC 1 & LPC 3 vs LPC 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, velocity, viability, acrosome integrity, plasma membrane integrity and sperm morphology were higher in a LP compared with a SP (LPC 1& LPC 3 vs SPC; 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 (LPC 1& LPC 3 vs SPC; 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 with SP (LPC 2 vs SPC; 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 with SP (LPC 2 vs SPC; 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 because of LP 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 SP (8 h light–16 h dark) and then in a LP (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 were restored to levels similar to those at the beginning of the study.

Our findings are in agreement with earlier reports in long-day breeder species like hamsters where the phenomenon of photorefractoriness has been reported (Stetson and Tate-Ostroff 1981). Likewise, photorefractoriness has been reported in short-day breeders too. In some short-day breeders like the male silver and blue fox, short days are necessary if gonadal stimulation is to occur (Smith et al. 1984, 1986; Forsberg et al. 1989). However, male silver fox show testicular regression after 1 year of SP (Forsberg et al. 1989). These results support our findings which show that tomcats maintained under LP for 18 months had good sperm quality at the beginning (LPC 1) but poor sperm production at the end (LPC 2). Similarly, other studies have shown that rams display reproductive photorefractoriness because the changes in testis activity proceed independently of photoperiod (Lincol 1977, Howles 1982). Almeida (1984) has shown that rams exposure to short days initially stimulated testis activity, but after 16 weeks, alternating periods of gonadal involution and redevelopment were observed. Therefore, we conclude that refractoriness and reduced sperm production and sperm quality induced by a prolonged LP of 18 month can be restored after placing tomcats to a SP.

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Conflicts of interest

None of the authors have any conflicts of interest to declare.

Author contributions

Nuñez-Favre R helped to design, conduct the experiment, process the samples, analysed data and drafted the manuscript. Bonaura MC, Tittarelli CM and Stornelli MC helped to conduct the experiment and process the samples. De la Sota RL and Stornelli MA helped to design and conduct the experiment and critically revised the manuscript.

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