Animal Reproduction Science 167 (2016) 68–73

Contents lists available at ScienceDirect

Animal Reproduction Science

journal homepage: www.elsevier.com/locate/anireprosci

Effects of a long-day light programme on the motility and membrane integrity of cooled-stored and cyropreserved semen in Shetland pony stallions

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ARTICLE INFO

Article history:
Received 25 November 2015
Received in revised form 3 February 2016
Accepted 5 February 2016
Available online 8 February 2016

Keywords:
Stallion
Photoperiod
Semen
Cryopreservation
Cooled storage

ABSTRACT

Increasing day length in spring stimulates reproductive functions in horses. In this study, we have analysed the effect of artificial long days on the quality of cooled-stored and cryopreserved semen in Shetland stallions. Stallions of the treatment group (AL, n = 8) were exposed to 16 h light and 8 h darkness from 15th December to 20th March while control stallions (CON, n = 7) were kept under natural photoperiod. Semen was collected once weekly and processed for cooled-storage and cryopreservation once per month. Total and progressive motility and percentage of membrane intact spermatozoa were analysed at 24, 48 and 72 h of cooled-storage and after freezing-thawing, respectively. Total and progressive motility and membrane integrity decreased during cooled-storage for 72 h in each month and both groups ($p < 0.001$). All these parameters were lower in CON versus AL stallions ($p < 0.05$) and the decrease was more pronounced in group CON (storage time x group $p < 0.05$). Differences between groups decreased throughout the observation period from January ($p < 0.05$ between groups) to July (e.g. total motility after 72 h of cooled-storage in January for group AL 80 ± 3 and group CON 49 ± 12%, respective values in July, 83 ± 2 and 72 ± 6%). Neither total and progressive motility nor percentage of membrane-intact and morphologically defect spermatozoa in frozen–thawed semen differed between groups and months. In conclusion, motility of cooled-stored semen was reduced in January and increased in stallions kept under a long day light programme for at least 30 days.

1. Introduction

Horses are seasonal breeders and respond to decreasing daylength in autumn and winter with a reduction in reproductive functions while increasing daylength in spring stimulates reproductive activity (Gerlach and Aurich, 2000; Aurich, 2011). Reproductive seasonality is most pronounced in less domesticated breeds such as ponies (Ginther, 1993). While in Shetland pony mares a strict anovulatory season lasts from October to March (unpublished own observations) 20–30% of Warmblood mares

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http://dx.doi.org/10.1016/j.anireprosci.2016.02.008
0378-4320/

continue to cycle throughout the year (Aurich et al., 1994). Nevertheless, despite a reduction in gonadotropin release during winter (Irvine and Alexander, 1982), reproductive seasonality in stallions is less pronounced than in mares as they remain fertile throughout the year.

Artificial light programmes are well established to stimulate ovulatory cycles in seasonally acyclic mares and thus advance the onset of the breeding season (Sharp et al., 1975; Oxender et al., 1977). Exposure of stallions to a complex experimental light programme with short daylength from July to December followed by 16 h of light per day until March resulted in a higher total sperm count in February compared to controls kept under natural photoperiod (Clay et al., 1987). In contrast, exposure of stallions to a prolonged daylength starting in December, a protocol effective in advancing the breeding season in mares (Sharp et al., 1975; Oxender et al., 1977) had neither an effect on raw semen parameters nor on plasma testosterone concentration when compared to control stallions kept under natural photoperiod (Schrammel et al., 2015). Because photoperiod may affect composition of the sperm cell membrane and/or constituents of seminal plasma which affect semen quality during storage (Aurich, 2005, 2008), the absence of a light programme effect on raw semen does not exclude effects on cooled or cryopreserved semen. Thus, the percentage of motile spermatozoa was higher in semen cryopreserved in autumn compared to spring and summer and sperm membrane integrity was lowest in summer, indicating seasonal differences in the quality of frozen–thawed semen (Janett et al., 2003a,b). In another study, addition of linseed oil and antioxidants to the diet of stallions attenuated the decline in motility and membrane integrity during cooled-storage of stallion semen from November to February (Schmid-Lausigk and Aurich, 2014).

With the increasing use of artificial insemination in horses (Aurich and Aurich, 2006; Aurich, 2012), reducing the loss in motility and membrane integrity during cryopreservation or cooled-storage of semen economically might be as important as improvements in raw semen quality. If seasonal changes in quality of stored semen could be overcome by light programmes, this might increase the number of cryopreserved semen doses which can be obtained throughout the year. In this study, we have analysed the effects of artificial long days on seminal parameters in cryopreserved and cooled-stored semen from Shetland stallions. We hypothesized that seasonal changes in semen parameters after storage depend on photoperiod-regulated differences in the composition of sperm membranes and/or seminal plasma and therefore will be influenced by a long day light programme.

2. Material and methods

2.1. Animals

A total of 15 Shetland pony stallions were included into this study. All stallions had produced pregnancies in previous projects or during the time of the present study with ejaculates collected in the weeks without cooled-storage and cryopreservation of semen. At the beginning of the experiment, stallions were between 3 and 22 years old (mean 9.2 ± 1.7 years) and weighed between 116 and 183.5 kg (mean 156 ± 6.7 kg). For evaluation of frozen–thawed semen only 12 stallions were taken into account because in three of them raw semen quality was far from fulfilling the minimum quality requirements for the production of frozen–thawed semen (sperm concentration >200 × 106/ml, total motility >50%, morphologically normal spermatozoa >70%; Sieme, 2011). The stallions were kept in one outdoor paddock between 8 am and 4 pm and were separated by group in two spacious stables for the remainder of the day. Hay was fed twice daily and water was freely available. The stallions were neither trained nor exercised during the study period and were used for regular semen collections.

2.2. Experimental design

The experiment took place in Vienna, Austria (longitude 16.4°, northern latitude 48.3°) between 1 December and 31 July. For the study, stallions were ranked by age and assigned in alternating order to two groups. The control group (CON, n = 7) was kept under natural light conditions whereas the treatment group (AL, n = 8) received additional light from 15 December to 20 March (spring equinox). Semen was collected once weekly for evaluation of raw semen (Schrammel et al., 2015). Processing of cooled-stored and frozen–thawed semen was performed once a month with the ejaculate collected in week 1 processed for cooled-storage and the ejaculate collected in week 2 of the respective months for cryopreservation. The study was approved by the competent authority for animal experimentation (Austrian Federal Ministry for Science and Research, license number BMWF-68.205/0194-II/3b/2013).

2.3. Light programme

All stallions were exposed to natural light during daylight hours. Stallions of group AL were exposed to artificial light each day from 15 December to 20 March. The daily period of artificial light begun at 4 p.m. and was continued until a total length of 16 h of light after sunrise was reached. The daily distribution of hours of light to hours of darkness was therefore 16:8. Light was provided by two standard lamps (Ritos R7s, 500W, Ritter Leuchten, Mömbris, Germany) and size of the stable was 12 × 7 m. Light was dispersed by reflecting screens to provide a near-homogenous light distribution in the stable. Group CON stallions were kept in a separate, identical barn compartment and any artificial light was prevented by covering all windows facing the hallway of the stable with light-proof material. After termination of artificial illumination in group AL on 20 March, all stallions were kept under natural light only until the end of the experiment on 31 July.

2.4. Semen collection and preservation

Semen was collected with a Hannover model artificial vagina (Minitube, Tiefenbach, Germany) on a dummy as