Theriogenology 97 (2017) 170–178

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Effects of environmental temperature and season on hair coat characteristics, physiologic and reproductive parameters in Shetland pony stallions

Kathrin Schmidt [a], Katharina Deichsel [b], Rodrigo Arruda de Oliveira [a], Jörg Aurich [b], Natascha Ille [a], Christine Aurich [a, *]

[a] Artificial Insemination and Embryo Transfer, Department for Small Animals and Horses, Vetmeduni Vienna, Austria
[b] Gynecology, Obstetrics and Andrology, Department for Small Animals and Horses, Vetmeduni Vienna, Austria

ARTICLE INFO

Article history:
Received 1 February 2017
Received in revised form
17 April 2017
Accepted 24 April 2017
Available online 27 April 2017

Keywords:
Stallion
Season
Environmental temperature
Body temperature
Semen

ABSTRACT

We hypothesized that housing of stallions in a thermoneutral temperature zone during autumn and winter does not only influence metabolism and hair shedding but also improves the characteristics of raw and processed semen. Fertile Shetland pony stallions were followed from October to June. This time coincided with the seasons autumn, winter and spring. Ponies were kept in outside paddocks (group CON, n = 8) or in indoor stables (group ST, n = 8) from October to March when ST stallions returned to outdoor paddocks, but ponies remained in the same groups. The rectal temperature was measured once weekly. Heart rate, heart rate variability, testosterone and cortisol concentration in blood as well as quality and length of the coat were determined. Semen was collected once weekly and raw semen characteristics were analyzed. The characteristics of cooled-stored and cryopreserved semen were determined once monthly. During the stabling period, environmental temperature for group ST averaged 13.6 ± 2.3 and for group CON 5.6 ± 4.2 °C. The mean rectal temperature was higher ($p < 0.05$) in ST than in CON stallions. All hair coat parameters underwent seasonal changes ($p < 0.001$) and differed between groups ($p < 0.05$) with shorter guard hair, slower hair regrowth and earlier hair change in ST stallions. Season influenced heart rate which was highest in autumn, lowest in winter and intermediate in spring but did not differ between groups. Testosterone and cortisol concentrations in blood as well as sexual behavior underwent seasonal changes but did not differ between CON and ST stallions. Gel-free semen volume and total sperm count were influenced by season ($p < 0.01$) and showed a more pronounced increase from winter to spring in CON than in ST stallions ($p < 0.05$) while no differences with regard to sperm concentration in raw semen were detected. Progressive motility of spermatozoa in raw semen was highest in spring ($p < 0.05$) but not affected by group. In cooled-stored and cryopreserved semen, neither season nor group affected total motility, progressive motility or membrane integrity. In conclusion, environmental temperature during autumn and winter had clear results on body temperature as well as hair coat characteristics in Shetland stallions. Simultaneously determined effects on semen characteristics were minimal indicating that reproductive function in the horse is more dependent on day length i.e. the geophysical year than on other environmental factors.

1. Introduction

Seasonal changes in reproduction in many species mainly depend on variation in day length. In the horse, long days stimulate gonadal function resulting in maximal reproductive activity in spring and summer [1,2]. Besides the reproductive system, also other organ systems of the horse are influenced by season. This results in pronounced changes in hair coat characteristics but also differences in energy metabolism when horses are kept outside throughout the year [3–5]. Not only in wild horses such as the Przewalski [3] but also in domesticated Shetland ponies kept outdoors, winter was associated with reduced locomotor activity and resting heart rate [4,6]. This hypometabolism has been interpreted

* Corresponding author. Artificial Insemination and Embryo Transfer, Department for Small Animals and Horses, Vetmeduni Vienna, Veterinärplatz 1, 1210 Vienna, Austria.
E-mail address: Christine.aurich@vetmeduni.ac.at (C. Aurich).

http://dx.doi.org/10.1016/j.theriogenology.2017.04.035
0093-691X/

as the capacity for seasonal adaptation to environmental conditions with the aim to reduce energy expenditure [4]. In addition to day length, factors such as environmental temperature [7] or energy intake [4,6,8–10] further modulate seasonal changes in different organ systems. However, domestication and breeding selection may have reduced seasonal changes not only in the adaptation of energy expenditure in livestock species [11], but also in reproductive functions in horses [12,13]. The Shetland pony is considered a robust horse breed at an early stage of domestication [14]. It is therefore an interesting model to study seasonal adaptation of horses because its capability to reduce metabolism during winter was not lost during domestication [4]. Season-related differences of reproductive function in Shetland ponies may thus be more distinct and therefore easier to assess than in more domesticated breeds.

In the stallion, seasonal changes in gonadal function do not only contribute to differences in the characteristics of raw semen, but may also affect the suitability of semen collected either in the breeding or non-breeding season for cooled-storage and cryopreservation. Semen cooled-stored or frozen in late winter and early spring has a reduced quality in comparison to other times of the year (reviewed by Ref. [13]). This may be of practical relevance because the breeding activity of stallions often already starts in early spring due to the demand of breeders aiming for foals to be born as early as possible in the year. Numerically, differences in semen characteristics among seasons are small and variation in the suitability of individual stallions for cryopreservation of their spermatozoa may be more important than seasonal variation [15]. However, taking into account seasonal differences in the characteristics of processed semen may be beneficial for fertility. We performed a series of experiments with the aim to improve the quality of processed stallion semen during early spring. A light program well established for advancing onset of the breeding season in seasonally acyclic mares [16,17] prevented a drop in the quality of cooled-stored but not of cryopreserved semen in Shetland pony stallions in late winter [18]. Similar effects were seen when stallions were fed linseed oil high in unsaturated fatty acids from November to February [19].

In the present investigation, we addressed the question of whether environmental temperature contributes to changes in semen characteristics of stallions kept under ambient photoperiod during winter. Such effects might be mediated via influences on metabolism that could affect fatty acid composition of spermatozoa. We hypothesized that housing of stallions in a thermoneutral temperature zone during autumn and winter does not only influence metabolism and hair shedding of the animals but also influences the characteristics of raw and processed semen.

2. Materials and methods

2.1. Animals

A total of 16 fertile Shetland pony stallions were included into the study. At the beginning of the study, age of the animals ranged from 5 to 24 years (11.3 ± 1.7; mean ± SD), range of weight was 116–207 kg (167.8 ± 7.3 kg; mean ± SD). Depending on the respective experimental group, the stallions were kept in outside paddocks or in indoor stables. They were fed hay *ad libitum* twice daily and they got water *ad libitum*. Similar amounts of hay were delivered to all animals and none had access to grass. During the entire study period the animals were not exercised.

2.2. Experimental design

The study was approved by the Austrian Federal Ministry for Science and Research (license number BMWFW-68.205/0150-WF/V/3b/2015) and was performed from 1 October to 10 June in Vienna, Austria (longitude 16.4°, northern latitude 48.3°). This period largely covered the seasons autumn (weeks 1–12, until winter solstice on 21 December), winter (weeks 13–24, until spring equinox on 22 March) and spring (weeks 25–36, i.e. until shortly before summer solstice on 21 June). Stallions were ranked according to age and semen quality and alternately assigned to two groups: The control group (CON, n = 8) was kept in outdoor paddocks with access to a barn closed on three sides providing shelter against wind and rain. The stallions were thus exposed to natural ambient temperature and humidity. The treatment group (ST, n = 8) was kept in spacious indoor stables (3 × 6 m for 4 stallions each) with large windows providing natural daylight and at a temperature of at least +5 °C from 1 October (week 1) to 25 March (week 25). All ponies lived in stable bachelor groups of 3–5 animals that were formed already well before the start of the study and maintained throughout. Both groups had similar visual, olfactory and auditory exposure to mares that were kept on the same premises but never in stables or paddocks directly adjacent to the stallions. Outdoor temperature was measured by a weather station on campus of the University of Veterinary Medicine Vienna operated by the Austrian Weather Service (Zentralanstalt für Meteorologie und Geodynamik, Wien, Austria) according to the standards of the World Meteorological Organisation (WMO Station no 11090). Indoor stable temperature was recorded by a mini datalogger (Testo 174H, Testo, Vienna, Austria). The indoor stable temperature was always maintained within the thermoneutral zone for horses and the stable was slightly heated whenever stable temperature started to decline below 10 °C. If outside temperature was at least +5 °C during daytime, ST group stallions were brought to outside paddocks for two hours a day, whereas at outdoor temperatures of less than +5 °C, they had the possibility for free movement in a closed riding arena for at least one hour per day. In all stallions, semen was collected once weekly. The rectal temperature was measured once weekly on a predefined day between 8 and 9 a.m. Blood samples for determination of testosterone and cortisol were collected by jugular venipuncture at 2 week intervals. Heart rate, heart rate variability as well as characteristics of the hair coat were determined at 4 week intervals.

2.3. Experimental procedures

2.3.1. Analysis of testosterone and cortisol in blood plasma

Blood samples (5 ml; Lithium Heparin Vacuette, Becton Dickinson, Schwechat, Austria) were collected by puncture of the right or left jugular vein at 2 week-intervals. Blood was centrifuged immediately after collection at 3000g for 10 min. The plasma was collected and stored at −20 °C until analysis. Testosterone concentration in plasma was determined by direct enzyme immunoassay (Testosterone ELISA, Demeditec Diagnostics, Kiel, Germany) without extraction. The assay has been validated for equine plasma in our own laboratory [5]. The intra-assay coefficient of variation was 5.3%, inter-assay variation was 5.1%, and the minimal detectable concentration was 0.03 ng/mL. Cortisol concentration in plasma was determined by direct enzyme immunoassay (Demeditec Diagnostics) validated for equine saliva in the authors' laboratory [20]. The intra-assay coefficient of variation was 6.6%, inter-assay variation was 7.0%, and the minimal detectable concentration was 1.13 ng/mL.

2.3.2. Analysis of heart rate and heart rate variability (HRV)

For determination of cardiac beat-to-beat (RR) intervals, a mobile recording system (V800, Polar, Kempele, Finland) was strapped to the thorax of the stallion with a flexible girth as described [21]. In brief, the heart rate sensor strap includes two electrodes. The