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Daily exposure to summer circadian cycles affects spermatogenesis, but not fertility in an *in vivo* rabbit model

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ABSTRACT

Heat stress (HS) in mammals is a determining factor in the deterioration of spermatogenesis and can cause infertility. The aim of this study was to evaluate the effect of continuous summer circadian cycles on semen production, sperm cell features, fertility, prolificacy, and fecal cortisol metabolites from rabbits kept under an *in vivo* HS model. We split randomly 60 New Zealand White rabbits into two temperature-controlled rooms: The control group was maintained at comfort temperature (18 °C–22 °C) and an HS group, where the environmental temperature was programmed to increase from 22 °C to 31 °C and be maintained for 3 hours to this temperature at the central part of the day. Fecal cortisol metabolites were assessed to evaluate the stress conditions. Seminal parameters were analyzed. Although animals exposed to HS showed higher values of fecal cortisol metabolites ($P = 0.0003$), no differences were detected in fertility or prolificacy. Semen samples from HS males showed a significant decrease ($P < 0.05$) with respect to the controls in the percentage of viable spermatozoa (80.71% vs. 74.21%), and a significant ($P \leq 0.01$) increase in the percentage of acrosomic abnormalities (22.57% vs. 36.96%) and tailless spermatozoa (7.91% vs. 12.83). Among motility parameters, no differences were found. This study describes a model of HS simulating a continuous summer daily cycle that allows periods of time to recover as it occurs under natural conditions. Although negative effects have been detected in several sperm parameters, fertility and prolificacy were not affected, suggesting a recovery of the reproductive function when normal conditions are reestablished.

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1. Introduction

Global warming is having many ramifications on the planet's weather conditions. The effects of this phenomenon are an increase in air temperature, shortening of winters, loss of the temperate seasons, an increase in periods of drought and flooding, and as a result, a decrease in periods of bioclimatic comfort. Forecasts indicate that there will be a rise in the maximum and mean temperatures

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around the year 2020 of 1.5 °C to 2.1 °C, which will significantly increase the heat stress (HS) on the world [1].

One of the biggest problems in most domestic animals during summer is the period of infertility or subfertility caused by HS [2,3]. Heat stress has negative effects on a large number of physiologic functions, because all tissues are susceptible to its effects. The reproductive function is the first physiologic feature impaired in adverse situations. Thus, the study of reproductive function could be a direct indicator of conditions affecting animal welfare, such as maintenance under HS conditions [4]. Testes are particularly sensitive to HS because they can be influenced by the physiologic abdominal temperature [2,5]. The scrotal temperature of male mammals should be about 2 °C to 8 °C below the body temperature to achieve a normal spermatogenesis [6,7]. An high testicular temperature, either acute or chronic, impairs spermatogenesis and results in a reduction of the spermatozoa number, associated with a transitional period of partial or complete infertility in several species [2,3,6,8,9]. Some studies have detected that the most important effects of HS are changes in ejaculate volume, sperm concentration, and motility. Heat stress also produces an increase in morphologic abnormalities and dead spermatozoa [2,3,6,10–12]. Heat stress applied on the day of artificial insemination (AI) cause a 6% of decrease in male fertility rate respect thermoneutrality [13]. However, many of these experiments consisted in exposing the animals to very high temperatures, in some instances above physiologic range, that is, temperatures higher than 40 °C, usually for long periods of time. To better represent real weather conditions, we aimed to analyze the effects of a continuous exposure to a summer circadian cycle on sperm cell characteristics and fertility. No previous studies in the rabbit have analyzed the effects of a continuous summer circadian cycle with exposure to high temperatures in the central part of the day, followed by a recovery period to comfort temperatures. In addition, to the best of our knowledge, no studies have performed crossed AI between rabbits submitted to HS and control conditions. These cross-inseminations could assess if the effect of HS in fertility and prolificacy is caused by one of the two genders or by the interaction of both.

Rabbits are very sensitive to HS owing to their difficulty in eliminating excess body heat; rabbits have few functional sweat glands. Exposure to high levels of humidity and temperature causes a negative effect on their growth and reproduction, and reduces their resistance against diseases [8]. However, rabbits can adapt to adverse situations after exposure to HS conditions, as we have reported recently [14]. In addition, the impact of HS on prolificacy could be alleviated if the temperature decreases during the day [15].

Stress has been defined as a state that occurs when an animal is required to make abnormal or extreme adjustments to cope with the adverse aspects of its environment and management [16]. At high temperatures, rabbits react showing physiologic and behavioral changes [17]. Physiologically, stress agents promote the activation of the hypothalamic–pituitary–adrenal axis, which significantly increases corticosteroid levels [18]. A plasmatic cortisol secretion peak provokes an increase in cortisol metabolites, which are detectable in feces some hours later [19]. Fecal cortisol assessment has the advantage of providing an integrated measure of cortisol secretion over one or two previous days [20,21] and the time of day does not affect its measurement.

The aim of the present study was to evaluate the effect of continuous summer circadian cycles on semen production and sperm cell features, such as viability, morphology, and motility. Additionally, fecal cortisol metabolites, fertility, and prolificacy of rabbits kept under an *in vivo* HS model were also assessed.

2. Materials and methods

All experiments were approved by the Institutional Animal Care and Use Committee of the Institut de Recerca i Tecnologia Agroalimentàries (IRTA).

2.1. Animals and experimental conditions

The study was performed at the experimental farm of the Institut de Recerca i Tecnologia Agroalimentàries (IRTA, Torre Marimon) from February 2010 through July 2011 using White New Zealand breed (71 males and 161 females). The animals belonged to the Caldes line selected for growth rate during the fattening period [22].

At 2 months of age, bucks and does were randomly split and housed in two identical, closed rooms (46.5 m^2) with ventilation and cooling/heating systems. Rooms only differed in the environmental conditions as described. Daily climate variables of the rooms (temperature and relative humidity) were automatically recorded every 4 minutes using a data logger (Tinytag, Gemini Data Loggers, Chichester, UK) located inside the chambers during the experiment.

Animals located in the HS room were continuously exposed to a summer temperature daily cycle, with a period of high temperature at the central part of the day for 3 hours. The resultant environmental conditions were as follows: The temperature in the HS room at 9 am was programmed to start to increase from 22 °C to reach a maximum temperature of 30 °C at 12 pm. This temperature was maintained for 3 hours and then started to decrease to a temperature of 22 °C until 9 am of the following day. Animals located in the control room were exposed to a daily constant temperature of the thermo neutral zone (from 18 °C to 22 °C).

The temperature humidity index (THI) was estimated from the following equation described by Marai et al. [8] for rabbits:

THI = db °C – [(0.31–0.31 (RH)) (db °C–14.4)]

Where db °C is dry bulb temperature and RH is relative humidity percentage/100. Figure 1 shows the THI values in the HS and control rooms. The THI of the HS room from 16 to 9 hours ranged from 18.3 to 20.2. Thereafter, the THI was gradually increased from 9 to 16 hours from 23.6 to 28.2. Animals located in the control room were exposed to a daily constant THI between 16.5 and 19.3. The values for the temperatures established in the present study were chosen based on the average minimum and maximum temperature values registered during the three previous summers on the farm where the current experiment was performed.

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