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EFFECTS OF DIFFERENT LIGHT INTENSITIES ON QUALITY OF SPERMATOZOA IN RABBITS

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Abstract: The present study evaluated the effects of light intensity on semen production in rabbits. Bucks were allocated to two groups which were exposed to a lighting program (16L:8D) with different light intensities (Group 1: <100 Lux; Group 2: >200 Lux). Prior to the thirteen weeks duration of the examination period, bucks were adapted to the light system for two months and conditioned for semen collection for one month. There was no effect of light intensity on libido, concentration and motility of spermatozoa. However, there were more bucks in group 1 delivering ejaculate with the gel fractions (P = 0.0065) and more foreign particles were observed in the ejaculates (P = 0.0007) compared to male rabbits from group 2. The first week of semen collection also revealed a light intensity effect on spermatozoa obtained by swim-up procedure. The concentrations of spermatozoa from group 2 were higher than those from group 1 (P < 0.0001). This effect was only slightly visible for motility after swim-up (P = 0.067).

Key words: light intensity, semen collection, rabbit.

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INTRODUCTION

One of the major tools in male rabbit reproduction is to perform semen collection under defined or standardized conditions. However, there is a plethora of prerequisites and factors which finally make each collection session unique. Among several elementary topics the photoperiod focuses on environmental stimuli (Theau-Clément *et al*., 1998) which daily, seasonally and annually exert positive as well as negative influences on reproduction. Even the onset of puberty is influenced by the season and light exposure (Kamwanja and Hauser, 1983). In male rabbits testicular activity showed photosensitive changes within the season (Ben Saad, 1997). There is also a time dependent relation between the duration of artificial daylight and testicular regression (Boyd, 1986a). Changes in the photoperiod are thought to be reflected by changes in synaptic processes in the pineal gland (Martinez-Soriano *et al.*, 1999). The neuronal regulation following pineal stimulation is carried out via the superior cervical sympathetic ganglia (Ben Saad, 1997).

The present study was carried out in order to evaluate elementary photosensitive factors influencing male rabbit reproduction. Preliminary work has been done based upon the effect of light intensity on libido and the posterior semen collection, including in vitro assessment of spermatozoa quality.

MATERIAL AND METHODS

Animals

Twenty bucks from the INRA-line 1077 of the same age were transported from Toulouse to the Institute of Agrobiotechnology, Tulln, Austria, at weaning. The rabbits were housed in groups until the age of three months. In the following, male rabbits were separated into single cages and exposed to the final light program. Animals were adapted to routine semen collection at the age of 5 months. The experiment was started one month later.

Experimental design

The light program was fixed for the total duration of animal experimentation at 16 hours of light and 8 hours of darkness. The wooden cages used were closed on all sides except from the front. The light source consisted of ten neon lamps positioned in two rows in the middle of the room so that lighting shone into the front of the cages. The distances from the rows of the neon lamps to the front of the cages were 0.80 m and 1.30 m, respectively. Light was measured at the center of the front door by a photometer (Light Meter, RS 180-7133). Animals were allocated to two groups (Group 1: n = 10; Group 2: n = 10) according to the measured light intensity values (Factor of light colour: 33): Group 1: < 100 Lux (46 – 97 Lux); Group 2: > 200 Lux (210 – 590 Lux). Before starting to collect semen, a female rabbit was presented to all bucks until the bucks accepted the doe and attempted to mount. Semen collection was performed once a week over a period of 13 weeks. After each ejaculation samples were immediately transferred (2-3 minutes) to the lab followed by examination of the traits.

Traits subjected to examination

The following data were collected weekly: Libido until mounting (s, libido 1), libido until ejaculation (s, libido 2), colour of the ejaculate (transparent, white, yellow, red), amount of ejaculate (ml, with/without gel fraction), concentration, motility, particles and concentration and motility after swim-up procedure. Concentration was measured in a Neubauer chamber by using 1:100 diluted semen in 0.9 % NaCL. Motility was estimated, when semen was mixed 1:50 with TRIS-buffer and examined under an invert microscope (magnification 100 fold). Swim-up technique was performed under standard conditions described by Parrish *et al*., (1995). Briefly, aliquots of 100 µl of semen were layered under 1 ml of TALP (Tyrode-albumin-lactate-pyruvate) medium in Falcon tubes and incubated at 37°C. After 45 minutes 800 µl of semen from each tube were removed and used for evaluation of concentration and motility. To quantify the number of particles (polymorphic fragments of the dimension of spermatozoa) a subjective scale was developed regarding the ratio of the number of particles per 9 spermatozoa.

Statistical analysis

All traits shown in the table are expressed by means, standard error means and percentage. Equality of variance was checked using a Levene´s test. Light exposition (< 100 Lux and > 200 Lux) and week (wk 1-13) were treated as fixed effects in the analysis of variance. Differences among groups were evaluated by use of a t-test. Libido (1 and 2); Volume; Concentration native, Motility native, Concentration swim-up; Motility swim-up, by use of Kruskal-Wallis 1-Way (particles) and by Chi-square (Pearson, gel fractions) tests. Additionally, multivariate analysis of variance was used to test the interaction of Group x Gel Fractions x Volume.

RESULTS

The present study summarizes data from male reproduction within a period of three months (13 weeks). Data from 260 sessions of semen collection were collected and 10 traits were examined. The mean time for mounting and ejaculation was 4.4 ± 0.24 s and 8.25 ± 0.36 s and 0.9 ± 0.02 ml of ejaculate were delivered. No effect of light intensity on these traits could be observed. However, there were significant differences among animals which produced supplementary gel fractions in their ejaculates in the two groups. In group 1 there were nearly twice as many bucks with gel fractions in the ejaculates than in group 2 (P = 0.0065, see Table 1). This difference was characterized throughout the experimentation period by a continuous weekly increase of bucks producing this additional gel fraction (Figure 1).

Table 1: Effect of light intensity on male reproduction traits.

	Libido 1(s) Mean ± SEM	Libido 2 (s) Mean ± SEM	Volume (ml) Mean ± SEM	Gel fraction (%)
Group 1< 100 Lux	4.67 ± 0.40	8.65 ± 0.53	0.9 ± 0.03	32[a]
Group 2> 200 Lux	4.15 ± 0.25	7.85 ± 0.49	0.9 ± 0.03	18[b]

SEM: Standard error od the mean.
Means with different superscript differ. (P<0.01).

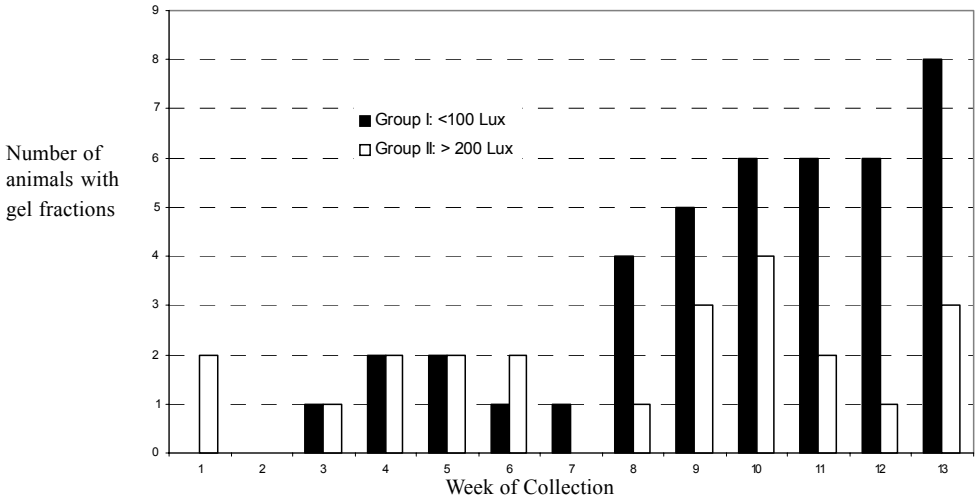

Figure 1: Effect of light intensity on gel fractions in the ejaculates.

There was no effect of light intensity on concentration of spermatozoa in the ejaculates or on the sperm motility. The number of particles related to spermatozoa was significantly different in the two groups. Animals from group 1 had 2.85 ± 0.15 particles related to 9 spermatozoa in the ejaculates, which was about 25 % higher than the number of particles in the ejaculates in group 2 ($P = 0.0007$). The appearance of particles was influenced in tendency by the volume of the ejaculates ($P = 0.076$) but not by the gel fractions ($P = 0.36$).

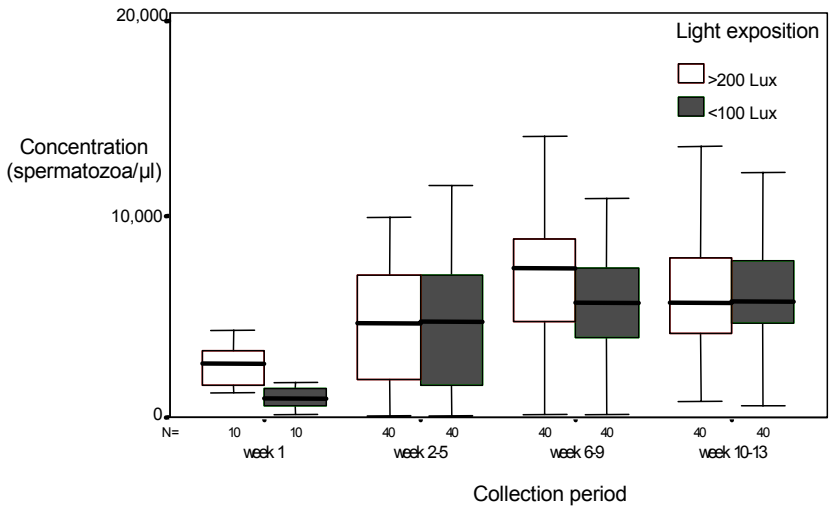

Figure 2: Effect of light intensity on concentration of spermatozoa following swim-up.

Table 2: Effect of light intensity on concentration of spermatozoa and motility before and after swim-up and on the number of particles.

	Concentration, native, Spz/µl Mean ± SEM	Motility %	Particles Mean±SEM	Concentration, swim-up Spz/µl Mean ± SEM	Motility after swim-up, %
Group 1<100 Lux	427,275 ± 13,364	50.4	2.85 ± 0.15[a]	5,145 ± 256	74.5
Group 2> 200 Lux	466,261 ± 12,481	52.2	2.19 ± 0.12[b]	5,562 ± 292	74.9

SEM: Standard error od the mean.
Means with different superscript differ. (P<0.001).

After swim-up the concentration of spermatozoa and the sperm motility only showed differences between the two groups during semen collection in the first week. The concentration of spermatozoa derived by swim-up was significantly higher in animals exposed to higher light intensity ($P < 0.0001$, Figure 2). The same effect was visible regarding the motility rate after swim-up ($P = 0.067$, Figure 3). These effects disappeared throughout the whole period of the study (Table 2). The colour of the ejaculate remained constant over the 13 weeks and in both groups.

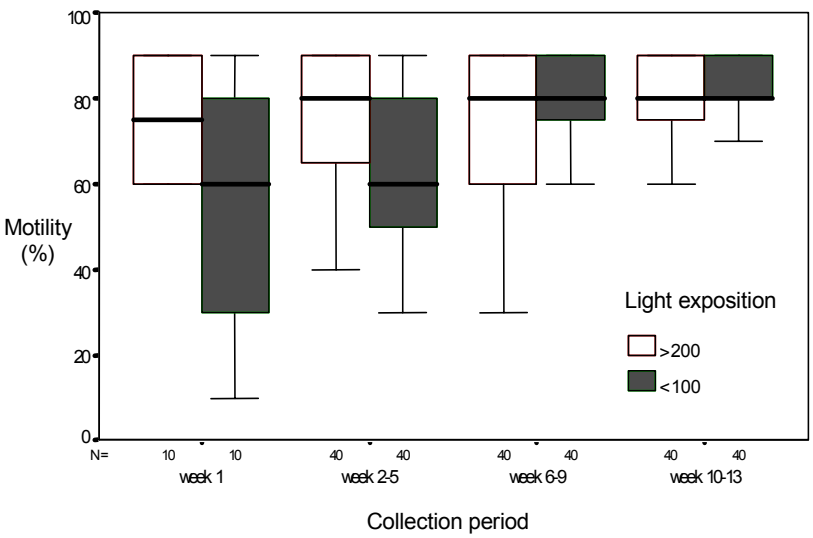

Figure 3: Effect of light intensity on motility of spermatozoa following swim-up.

DISCUSSION

The present study was performed in order to get preliminary information about the influence of light intensity on male reproduction. Within the framework of this experimental design there was no obvious effect of the different light exposures on animals in group 1 and 2 on main traits such as concentration and motility of spermatozoa. However, an important effect was observed regarding ejaculates having gel fractions and the number of particles, which appeared to be dependent on the volume of the ejaculate. These figures do not give a clear answer as to the origin of this effect. One possibility is a relationship between time dependent sexual maturity and light intensity. Kamwanja and Hauser (1983) studied the effect of artificial lighting on the onset of puberty in female rabbits. Rabbits exposed to 18 hours of light from weaning reached puberty earlier than rabbits housed under a short lighting program (6 hours light). Hence, increasing daily light exposure from 6 to 18 hours/ day may improve litter size in pubertal does. Boyd (1986b) described a defined seasonal rhythm in wild rabbits (*Oryctolagus cuniculus*). Maximum fertility occurs with increasing light, i.e. increasing day length and light intensity. Comparable conclusions may be drawn from the results of the present study, where the first sampling of ejaculates including separation by swim-up showed light intensity dependent differences in concentration and motility. In the following weeks the effect disappeared. The higher number of particles, which have been observed in group 1 (light intensity < 100 Lux) may fit in with this hypothesis inasmuch as sexual maturity including spermiogenesis probably did not reach the complete developmental capacity. Vesicles, droplets and residues of somatic cells could be an indicator of early semen production. Further studies have to be performed in order to evaluate the influence of light intensity on the composition of the ejaculates with special regard to the gel fraction. Although semen collection was performed over a period of 13 weeks, it must be emphasized that the duration of light exposure is the most important factor. Boyd (1986a) kept male rabbits in 3-day treatments (20L:4D; 8L:16D; 13L:11D). When the light system was changed after 8 weeks there was no regression of testes. A delayed change (after 16 weeks), however, resulted in testicular regression.

CONCLUSION

The present study demonstrated the effect of light intensity on some male reproduction traits. However, this preliminary experiment does not explain the reproduction traits which are directly affected by light intensity. Further studies should investigate the seasonal rhythm by increasing the duration of the experimental period and the change of light intensity within a long-term physiological interval.

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