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Research Article
Effect of Photoperiod with Sunlight at Thermal Stress and Sperm Parameters in Guinea Pigs

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Abstract

Background and Objective: Photoperiod can regulate reproductive physiological processes in mammals, in which improvements in testosterone concentration, testicular volume and seminal quality have been reported. The aim was to evaluate the influence of photoperiod treatments on guinea pigs' spermatic parameters. **Materials and Methods:** Thirty guinea pigs, between males and females, were distributed in two rooms with the photoperiodic treatment of 10 hrs light and 14 hrs dark (PT_1 with artificial photoperiod and PT_2 photoperiod with sunlight by opening windows from 08:00-18:00) and one without any direct light stimulus (PT_0) for 78 days. The temperature and humidity were recorded and the TH index was calculated for each room. The sperms were recovered in Tris base medium from the epididymis of 16 males to determine sperm concentration, motility, kinetic parameters, vitality, HOST, acrosomal integrity and DNA fragmentation. **Results:** Sperm values in PT_1 and PT_0 were similar but PT_2 obtained values lower in sperm concentration, non-progressive motility, total motility, VCL, ALH, vitality, HOST+, acrosomal integrity, sperm with non-fragmented DNA and no pregnancies were reported (0/5). A 100% pregnancy was observed in PT_0 (4/4) and 50% in PT_1 (2/4). However, precocity was evidenced in PT_1 compared to PT_0. PT_2 recorded higher peaks in temperature (33.8°C, THI 81, considered as thermal stress) compared to PT_0 (32.65°C, THI 81.8) and PT_1 (32.75°C, THI 81.6). **Conclusion:** An artificial photoperiod can improve sperm characteristics and reproductive precociousness of guinea pigs, unlike the photoperiod with sunlight, which generated low spermiogram values and absence of pregnancy due to thermal stress.

Key words: *Cavia porcellus* L., photoperiod, sunlight, thermal stress, TH index, spermiogram, kinetic parameters, HOST, acrosome, DNA fragmentation

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Breeding guinea pigs in family rearing, in Latin American countries, are usually confined to small dark spaces devoid of sunlight but field observations showed a predilection of these animals to rest in spaces with sunlight. The photoperiod is a significant environmental stimulus that regulates essential physiological processes in mammals, such as reproduction[1]. In mammals, eyes detect the light and that information is transmitted to the pineal gland through the suprachiasmatic nucleus, which regulates circadian rhythms. The pineal gland secretes melatonin, encoding the night length and regulates the secretion of Thyroid-Stimulating Hormone (TSH) in the pars tuberalis of the pituitary gland[2]. Melatonin has hormone receptors in a wide range of organs, including those of the male reproductive tract and spermatozoa[3]. Some studies about photoperiod have reported higher testosterone concentration and precociousness in male guinea pigs[4,5] as well as testicular size, sperm concentration and morphologically normal sperms in goats[6], rams[7,8] and equines[9,10].

Sunlight can create a photoperiodic effect in nature, however, this is accompanied by infrared radiation, which is capable of generating heat when absorbed. In animal housing, environmental conditions of high temperature and humidity could lead to heat stress, negatively affecting sperm parameters and fertility. Heat stress can be estimated as a function of ambient temperature and relative humidity (temperature-humidity index), using the Thorn equation (1959) and is generally applied to grazing cattle. In guinea pigs subjected to heat stress, reduced motility, greater abnormalities and DNA alteration have been reported, due to apoptosis in germ cells due to an increase in scrotal temperature and alteration of pulsatile gonadotropin secretion, which in turn will reduce FSH and LH secretion, causing spermatogenesis dysfunction[11,12].

For this reason, this study aimed to evaluate the effect of a photoperiod with sunlight on sperm parameters of guinea pigs.

MATERIALS AND METHODS

Experimental location: The study was carried out from February-December, 2020 at a guinea pig breeding module and the Laboratory of the Semen Collection Center and the Laboratory of Animal Biotechnology, Reproduction and Genetic Improvement of the Institute of Livestock and Biotechnology, of the Universidad Nacional Toribio Rodríguez de Mendoza de Amazonas, Chachapoyas District, Amazonas Region, Peru.

The area is located at an altitude of 2442 m above sea level, between 6°12'29.88" south latitude and 77°52'1.62" west longitude. It is characterized by a temperate-cold climate, with an average annual temperature of 15°C and average annual rainfall of 1578 mm (UNTRM Meteorological Station, 2016).

Animals and experimental design: Twenty guinea pigs of Peru phenotype (5 males and 15 females), all from a commercial farm located in Pedro Ruiz District, Bongará Province, Amazonas Region, were breeding from February-June, 2020 (mid-summer and late fall). Five-month-old male guinea pigs were located with three females of the same age, in a cage of 1.0×1.0×0.45 m, each. The pregnancies were carried from July-September, 2020 (winter). In each cage, six to eleven pups were born (mean of 2.9 pups per mother) and were weaned at twenty-one days of age.

At age of 25±4 days and weighing 262±60 g, thirty guinea pigs (17 males and 13 females) were randomly assigned to the three experimental photoperiod treatments in fifteen cages with dimensions 1.0×0.5×0.45 m (2 subjects per cage). Before the assignment to experimental photoperiods, parents and pups were kept in a common room with a photoperiod of 12:02±00.09 hrs light (without direct exposure to sunlight) to establish a common photoperiod history. Five cages were assigned to each treatment, corresponding to the litters of each male (block) to reduce possible variability of parental effect. The experimental treatments were carried out from September-December, 2020 (spring season).

Housing and feeding: A guinea pig breeding module, 6.0×4.5 and 2.5 m high were installed with fibro-cement walls and polypropylene calamine roof (Fibraforte®). Three independent rooms of 2.8×1.5×2.3 m high, with a soil flattened floor, were installed inside the breeding module. Each room had five wells of 1.0×0.5×0.45 m, separated by a steel mesh net covered with polyethylene. A passive ventilation system was installed using curtains on the upper sides of the shed. An automatic drinkers system was installed and fresh drink water was supplied twice a day, in 10-litre tanks. The cleaning routine was daily for the front of the cages to avoid stress but a total cleaning was done once a week with an application of lime on the floor for disinfection and humidity reduction.

Before the allocation of the exposure regimens, Daimeton® T (Sulfamonomethoxine+Trimethoprim) were given in the food (2 g kg^{-1} food) for three days for the prevention of diarrhoea and complex B (1 g per litre of drinking water). During growth up to 78±5 days of age, they were provided with a commercial concentrate (10% of live

weight daily), alfalfa forage (*Medicago sativa*), Guatemala pasture (*Tripsacum laxum*) and water in an automatic waterer drinker. Weekly weights were performed to recalculate the food supply.

Photoperiod treatments and environmental monitoring: Each room was assigned to a different type of Photoperiod Treatment (PT). PT_0 was a room without any direct light stimulus. PT_1 was a room with an artificial photoperiod of 10 hrs of light and 14 hrs of darkness (10L/14D), generated with a 62-lamp LED light bulb of 3.1 W power (CN-L862Y, CAFINI, China) on at 08:00 am and off at 06:00 pm. Finally, PT_2 was a room with a photoperiod with sunlight, from direct incidence of sunlight through the opening of side windows located at 1.2 m from the ground and covered with a transparent polyethylene sheet to prevent currents of air and rain. Windows, located on the east and northeast side of the shed, was opened at 08:00 am and closed at 06:00 pm.

Moreover, environment temperature and relative humidity were recorded at 50 cm above the soil every 10 min with thermo-hygrometers containing a datalogger (HT71N, PCE Instruments, Germany). The Temperature-Humidity Index (THI) was determined to estimate the level of thermal stress using the following Thom Eq.[13]:

$$THI = (0.8)T_{\alpha} + \frac{HR}{100} \times (T_{\alpha} - 14.3) + 46.4$$

where, THI is the temperature-humidity index, T_{α} is the air temperature in °C and HR is the percentage value of relative air humidity. Where, 72-79 THI is mild stress, 80-89 THI is moderate stress and 90 THI or more is severe stress in cattle.

Sacrifice and epididymis recovery: The animal sacrifice protocols in this study were carried out according to the ARRIVE 2.0 guidelines (Animal Research: Reporting of *in vivo* Experiments, https://arriveguidelines.org)[14] to produce the least discomfort[15]. At 103±5 days of age, male guinea pigs were slaughtered by cutting the jugular vein and bled for 30 sec. The testes of the guinea pigs were recovered immediately after the slaughter as follows: A cut was made with a scalpel blade on the left side of the scrotal pouch. Then, the inguinal area was pressed and the testicles were removed. Organs were transported in polyethylene labelled bags at 37°C to the Laboratory of the Semen Collection Center. The epididymis' caudal portion was sectioned with a scalpel blade in a petri dish on a thermal plate at 37°C. The Petri dish contained 0.4 mL of tempered Tris medium (3.028 g molecular grade Tris (hydroxymethyl) aminomethane, 1.7 g citric acid, 1.25 g D-fructose and 100 mL distilled water)[16]. Slight cuts were made to promote the exit of the sperm into the medium[17]. Another 0.6 mL of the same medium was added and the liquid portion was recovered in 1.5 mL microtubes.

Sperm analysis: Motility and sperm concentration was determined in a computerized semen analysis system Sperm Class Analyzer (SCA®, Spain) using Makler chamber and 5 µL of the sample at 37°C. Besides, the following kinetic parameters were obtained:

- Curvilinear velocity (VCL, µm s^{-1})
- Straight-line velocity (VSL, µm s^{-1})
- Average path velocity (VAP, µm s^{-1})
- $$\text{Linearity (LIN \%)} = \frac{VSL}{VCL} \times 100$$
- $$\text{Straightness (STR \%)} = \frac{VSL}{VAP} \times 100$$
- $$\text{Wobble (WOB \%)} = \frac{VAP}{VCL} \times 100$$
- Amplitude of lateral head displacement (ALH, µm)
- Beat-cross frequency (BCF, Hz)

Vitality was evaluated by eosin-nigrosin staining by doing a phrotis with 5 µL of sample and adding 5 µL of 5% eosin Y solution and 5 µL of 5% nigrosin solution (Sigma-Aldrich, USA). Both solutions were used preheated to 37°C. The phrotis were observed after drying with a blue filter in a phase-contrast microscope with a 40× objective. Not less than 200 cells were counted. We expressed the percentage of dead sperm to those with cytoplasm not stained by eosin.

To evaluate membrane functionality, we use a hypoosmotic swelling test (HOST), mixing 25 µL of sperms recovered from the epididymis with 100 µL of 50 mOsm solutions (2.45 mg of D-fructose, 4.5 mg of sodium citrate and 1 mL of bidistilled water) tempered at 37°C. After incubation at 37°C for 5 min, 31 µL of the formaldehyde solution was added to stop the reaction. A 5 µL aliquot was observed under a phase-contrast microscope with a 40× objective, counting no less than 200 spermatozoa with some degree of tail coiling (HOST+) expressed in percentage.

A Coomassie Blue 0.22% staining determined acrosomal integrity[18], prepared with 0.11 g Brilliant Blue for Coomassie G250 (Merck), 25 mL methanol, 5 mL glacial acetic acid and 20 mL distilled water. Phrotices from each sample were horizontally fixed for 15 min in 4% formaldehyde in PBS (10 mL of 40% formaldehyde and 90 mL of PBS) and finally

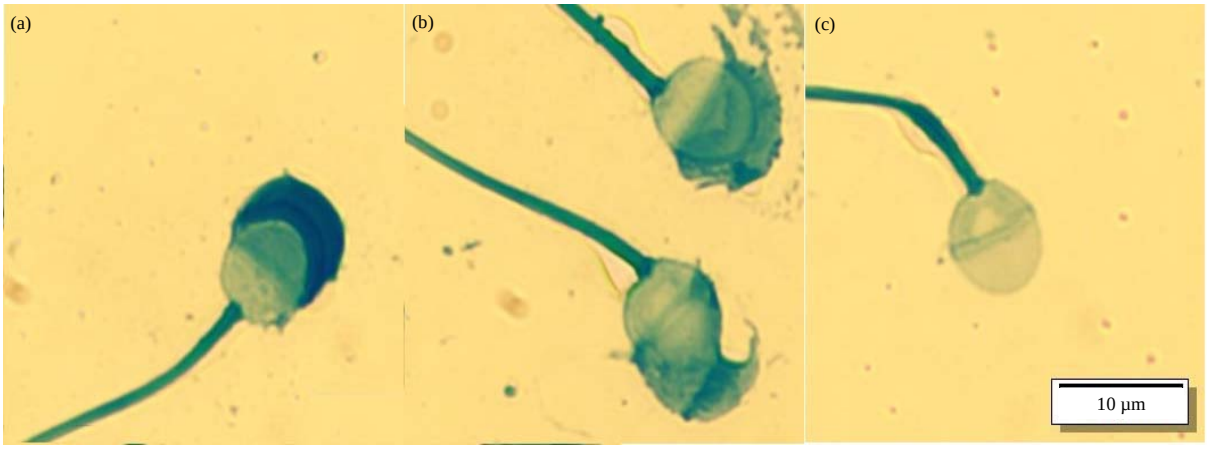

Fig. 1(a-c): Guinea pig epididymal spermatozoa stained with coomassie blue 0.22%, (a) Intense blue staining of the acrosomal cap (CB++), (b) Weak blue staining of the acrosomal cap (CB+) and (c) Absence of the acrosomal cap (CB-) 100× objective

washed in PBS (5 immersions of 1 sec each). Then, they were placed horizontally in a tray and Coomassie Blue 0.22% stain was applied slowly with a syringe. After 5 min, the stain was allowed to drain and they were washed in distilled water (5 immersions of 1 sec each). Once dried, it was observed under phase-contrast microscopy with 40× objective and no less than 250 cells were counted to express the percentage of spermatozoa with blue staining. For classification, Fig. 1a shows spermatozoa with intense blue staining of the acrosomal cap (CB++), Fig. 1b shows sperm with weak blue staining of the acrosomal cap (CB+) and Fig. 1c shows spermatozoa with the absence of the acrosomal cap (CB-).

The sperm DNA fragmentation was evaluated by the Sperm Chromatin Dispersion test (SCD) with Halomax® kit (MM-40HT, Halotech DNA, Spain). Briefly, samples were diluted with Tris medium to 20×10^6 spermatozoa mL^{-1}. In a 25 µL of the new concentration, 50 µL of agarose was added, previously heated at 86°C until completely liquefied and then tempered at 37°C. It was slightly homogenized and 2 µL was placed in the wells of the pretreated agarose slides, covered with a coverslip and lightly pressed to spread the sample on the slide well and it was refrigerated horizontally at 4°C for 5 min. The coverslip was carefully removed by lateral sliding and the slide well was immersed in a horizontal cuvette with a Lysis Solution (10 mL Base Lysis Solution+70 µL Reducing Agent) for 5 min. Subsequently, it was allowed to drain and then it was immersed in distilled water for 5 min. Finally, the slide well was dehydrated in 70% ethanol for 2 min, 96% ethanol for 2 min and dyed with Diff-Quick stain (6 min Diff-Quick I and then 6 min Diff-Quick II) and Wright stain (15 min) and washed in tap water by immersion. All this process was carried out by horizontal immersion inside Petri dishes. Sperm without fragmented DNA nucleoids showed a large and spotty halo of chromatin dispersion, but sperm with fragmented DNA did not exhibit a halo around the nucleoid, only a small core, whose percentage was the Sperm DNA Fragmentation Index (SDFI).

Statistical analysis: The experiment was conducted under a DBCA with three photoperiod treatments (PT_0, PT_1 and PT_2) and five replicates (N = 5) or litters from the different parent (blocks), randomly assigned to each PT. Normal distribution and homogeneity of variances were verified using the Shapiro-Wilk and Levene tests ($p<0.05$), respectively. To determine the independent effect of PT, an ANOVA ($p<0.05$) and Bonferroni correction and Kruskal-Wallis test for SDFI were run in the SPSS v.15.0 program.

RESULTS

Sperm analysis of sixteen adult male guinea pigs 107±9.8 days of age, with an average liveweight of 1163.18±66.17 g (PT_0), 1264.38±48.73 g (PT_1) and 1036.82±83.05 g (PT_2) and testicular weight of 12.24±1.16 g (PT_0), 14.72±1.29 g (PT_1) and 13.70±2.17 g (PT_2), are shown in Table 1. Guinea pigs subjected to the three treatments showed non-significant differences in live or testicular weight ($p>0.05$) but significant differences in sperm concentration, with PT_0 and PT_1 higher than PT_2 ($p<0.05$). Similarly, PT_0 and PT_1 had a higher number of

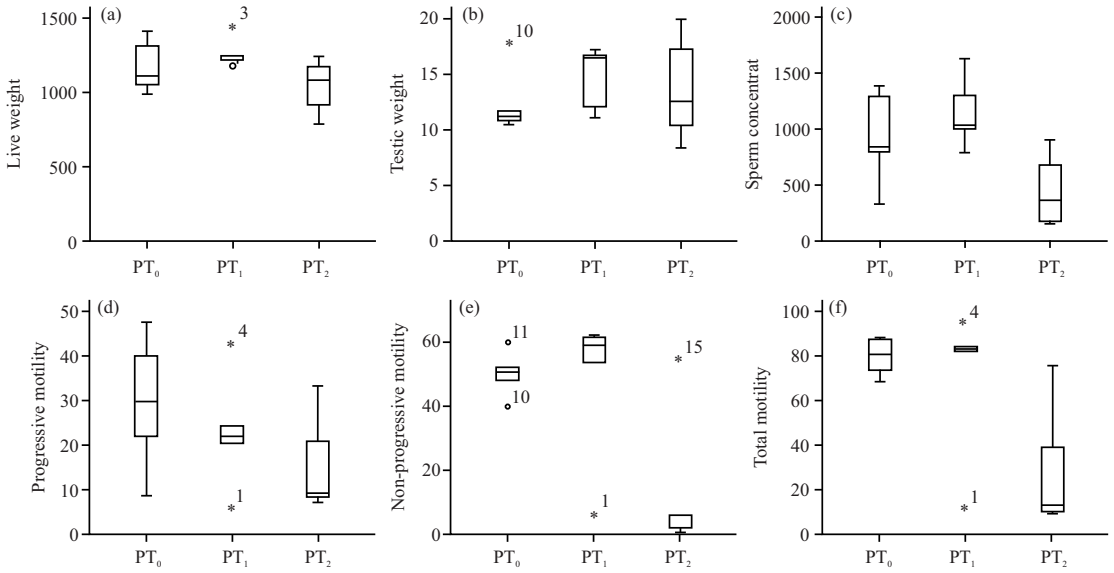

Fig. 2(a-f): Boxplot of weights, concentration, and motility of guinea pigs epididymal spermatozoa, subjected to different light stimuli, (a) Live weight (g), (b) Testicular weight (g), (c) Sperm concentration (M mL^{-1}), (d) Progressive motility (%), (e) Non-progressive motility (%) and (f) Total motility (%)

PT_0: Without direct light stimulation, PT_1: Artificial photoperiod and PT_2: Photoperiod with sunlight

Table 1: Guinea pigs sperm parameters (107±9.8 days) were subjected to different light stimuli (Mean±SE)

Parameters	Without direct light (PT_0)	Artificial photoperiod 10L/14D (PT_1)	Photoperiod with sunlight 10L/14D (PT_2)	p-value	Sig.
Samples	6	5	5		
Live weight (g)	1163.18±66.17	1264.38±48.73	1036.82±83.05	0.141	NS
Testicular weight (g)	12.24±1.16	14.72±1.29	13.70±2.17	0.601	NS
Sperm concentration (M mL^{-1})	915.23±154.97^{ab}	1151.10±144.26^{a}	456.19±146.57^{b}	0.022	*
Progressive motile (%)	29.44±5.59	22.88±5.86	15.61±5.03	0.265	NS
Non-progressive motile (%)	50.19±2.67^{a}	48.49±10.63^{a}	13.89±10.25^{b}	0.015	*
Total mótiles (%)	79.63±3.12^{a}	71.37±15.03^{a}	29.50±12.70^{b}	0.011	*
VCL (μm s^{-1})	86.44±6.26^{a}	96.62±6.79^{a}	57.66±8.45^{b}	0.028	*
VSL (μm s^{-1})	23.52±2.57	26.87±1.90	17.32±4.01	0.145	NS
VAP (μm s^{-1})	50.04±3.41	50.72±4.25	33.94±5.17	0.083	NS
LIN (%)	26.64±1.04	26.60±1.38	27.85±3.22	0.985	NS
STR (%)	44.94±2.78	48.86±1.59	47.55±3.82	0.437	NS
WOB (%)	58.47±1.40	52.69±2.44	56.63±2.04	0.074	NS
ALH (μm)	2.98±0.17^{a}	3.41±0.23^{a}	2.10±0.21^{b}	0.011	*
BCF (Hz)	6.87±0.52	7.23±0.33	5.98±0.90	0.4	NS
Vitality (%)	34.86±3.43^{a}	32.73±2.31^{a}	20.70±2.05^{b}	0.001	**
HOST+ (%)	60.49±3.81^{a}	59.76±3.91^{a}	36.63±4.94^{b}	0.003	**
CB++ (%)	66.43±2.85^{a}	28.67±7.52^{b}	36.51±11.04^{ab}	0.023	*
CB+ (%)	21.38±2.53^{b}	59.12±7.82^{a}	36.46±6.19^{ab}	0.007	**
CB total (%)	87.81±2.06^{a}	87.79±2.89^{a}	72.97±6.19^{b}	0.013	*
SDFI (%)	10.26±1.27^{a}	12.49±3.29^{a}	53.04±3.04^{b}	0.014	**

HOST+: Positive reaction to hypoosmotic swelling test, CB++: Coomassie blue strong staining, CB+: Coomassie blue weak staining, CB total: Coomassie blue total staining, VCL: Curvilinear velocity, VSL: Straight-line velocity, VAP: Average path velocity, LIN: Linearity, STR: Straightness, WOB: Wobble, ALH: Amplitude of lateral head displacement, BCF: Beat-cross frequency, SDFI: Sperm DNA fragmentation index. Different letters superscript in rows$^{(a,b)}$ indicate significant differences. *Significant differences at the level of p<0.05, **Significant differences at the level of p<0.01. NS: Non-significant differences (p>0.05)

spermatozoa with non-progressive motility and total motility compared to PT_2 (p<0.05) (Table 1). The boxplot of these variables is shown, live weight (Fig. 2a) and testicular weight (Fig. 2b) showed high variability but without significant differences among groups (p>0.05), sperm concentration (Fig. 2c) showed less variability and significant

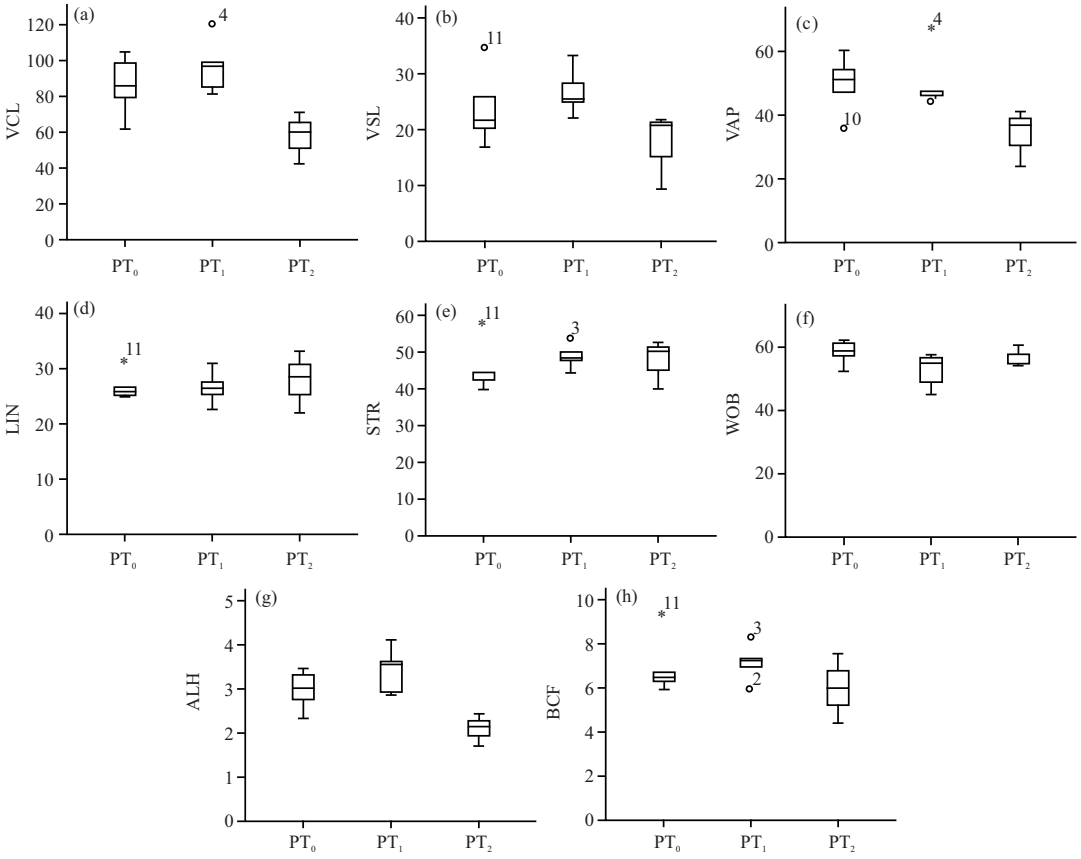

Fig. 3(a-h): Boxplot of sperm kinetic parameters of guinea pigs epididymal spermatozoa, subjected to different light stimuli, (a) Curvilinear velocity (VCL, μm s^{-1}), (b) Straight-line velocity (VSL, μm s^{-1}), (c) Average path velocity (VAP, μm s^{-1}), (d) Linearity (LIN (%) = VSL/VCL×100), (e) Straightness (STR (%) = VSL/VAP×100), (f) Wobble (WOB (%) = VAP/VCL×100), (g) Amplitude of lateral head displacement (ALH, μm) and (h) Beat-cross frequency (BCF, Hz)

PT_0: Without direct light stimulation, PT_1: Artificial photoperiod and PT_2: Photoperiod with sunlight

differences among groups ($p<0.05$). Progressive motility (Fig. 2d) also showed high variability and non-significant differences among groups ($p>0.05$) and non-progressive motility (Fig. 2e) and total motility (Fig. 2f) also showed less variability and significant differences among groups ($p<0.05$).

Sperm concentration was improved in PT_0 and PT_1 (without direct light stimulus and artificial photoperiod), compared to PT_2 (photoperiod with sunlight). Moreover, Table 2 shows the Pearson coefficients that measure the correlation between these sperm parameters, where we observed that sperm concentration was significantly correlated with progressive ($p<0.05$), non-progressive ($p<0.05$) and total motility ($p<0.01$) parameters. Also, a similar correlation was found between live weight with testicular weight, sperm concentration ($p<0.01$), non-progressive and total motility ($p<0.05$).

Among sperm kinetic parameters analyzed, only VCL and ALH of PT_0 and PT_1, were significantly higher compared to PT_2 ($p<0.05$) (Table 1). In Fig. 3a, the VCL boxplot with less variability than previous variables is shown, in Fig. 3(b-c) the boxplot of VSL and VAP, respectively is shown and there is heterogeneity but without significant differences among groups. There is greater homogeneity in LIN (Fig. 3d), STR (Fig. 3e), WOB (Fig. 3f), ALH (Fig. 3g) and BCF (Fig. 3h) and the absence of differences among groups except in ALH. A high correlation was found between VCL, VSL and VAP with ALH ($p<0.01$) and VCL, VSL, LIN, STR and ALH with BCF ($p<0.01$ and $p<0.05$). Besides, VAP and WOB correlated with the percentage of non-progressive motile and total motility, respectively (Table 2).

In Table 1, we also see other sperm characteristics: Vitality and positive reaction to hypoosmotic swelling tests

Table 2: Pearson's coefficients between spermatic parameters of guinea pigs epididymal spermatozoa

Parameters	WTesti	Conc.	MPro	MNPro	MTot	Vital	HOST+	CB++	CB+	CBTot	VCL	VSL	VAP	LIN	STR	WOB	ALH	BCF
LWeight	0.68**	0.68**	0.42	0.51*	0.56*	0.22	0.44	0.21	0.07	0.57*	-0.02	0	-0.02	0.11	0.03	0.07	0.03	0.17
WTesti		0.36	0.3	0.29	0.35	-0.13	0.14	0.1	0.06	0.32	-0.21	0	-0.14	0.5	0.28	0.24	-0.19	0.2
Conc.			0.50*	0.59*	0.65**	0.46	0.47	0.17	0.11	0.57*	-0.03	-0.12	0.03	-0.13	-0.27	0.21	0.01	-0.08
MPro				0.42	0.73**	0.24	0.39	0.3	-0.08	0.49	-0.14	-0.31	0.03	-0.26	-0.53	0.49	-0.1	-0.41
MNPro					0.93**	0.39	0.81**	0.58*	-0.22	0.84**	0.41	0.48	0.54*	0.5	0.24	0.4	0.37	0.53
MTot						0.39	0.77**	0.56*	-0.2	0.84**	0.26	0.23	0.43	0.26	-0.07	0.55*	0.25	0.21
Vital							0.56*	0.1	0.07	0.34	0.53	0.38	0.59*	-0.15	-0.19	0.14	0.49	0.24
HOST+								0.4	-0.04	0.79**	0.67**	0.66**	0.69**	0.27	0.25	0.11	0.65*	0.63*
CB++									-0.88**	0.52*	-0.16	-0.09	-0.01	0.28	-0.05	0.53	-0.2	0.16
CB+										-0.06	0.31	0.23	0.19	-0.23	0.05	-0.43	0.34	-0.05
CBTot											0.39	0.36	0.51	0.23	-0.01	0.41	0.36	0.36
VCL												0.90**	0.92**	0.1	0.31	-0.24	0.99**	0.61*
VSL													0.83**	0.5	0.66*	-0.22	0.84**	0.86**
VAP														0.19	0.15	0.15	0.86**	0.51
LIN															0.78**	0.23	0.01	0.71**
STR																-0.41	0.27	0.83**
WOB																	-0.33	-0.21
ALH																		0.55*

LWeight: Live weight, WTesti: Testicular weight, Conc: Concentration, MPro: Progressive motility, MNPro: Non-progressive motility, MTot: Total motility, Vital: Vitality, HOST+: Positive reaction to hypoosmotic swelling test, CB++: Coomassie blue strong staining, CB+: Coomassie blue weak staining, CBTot: Coomassie blue total staining, VCL: Curvilinear velocity, VSL: Straight-line velocity, VAP: Average path velocity, LIN: Linearity, STR: Straightness, WOB: Wobble, ALH: Amplitude of lateral head displacement, BCF: Beat-cross frequency, **Significant correlation at the level p<0.01 (bilateral), *Significant correlation at the level p<0.05 (bilateral)

(HOST+) revealed that PT_0 and PT_1 vitality were significantly higher than PT_2 ($p<0.01$) as seen in Fig. 4a-b. Furthermore, Coomassie Blue 0.22% cationic staining (CB) showed the acrosomal integrity variation correlated to bluish staining intensity. We found a higher percentage of spermatozoa with intense blue acrosome staining (CB++, $p<0.05$) in PT_0 compared to PT_1 but similar to PT_2 (Fig. 4c). Conversely, PT_0 spermatozoa exhibited a lower percentage of weak acrosome staining (CB+, $p<0.01$) compared to PT_1 and similar to PT_2 (Fig. 4d). The result of Fig. 4e shows the percentages of total reaction to CB staining (total CB) for acrosomal integrity, where these varied among groups ($p<0.05$), with no difference between PT_0 and PT_1 but significantly higher to PT_2. We also found that the percentage of HOST+ correlated directly with vitality ($p<0.05$) and with total CB ($p<0.01$) but the relationship between CB++ with CB+ was significantly inverse ($p<0.01$) (Table 2). On the other hand, the percentage of non-progressive motility and total motility correlated significantly with HOST+ ($p<0.01$), CB++ ($p<0.05$) and total CB ($p<0.01$). In the same way, HOST+ correlated with VCL, VSL and VAP ($p<0.01$) and with ALH and BCF ($p<0.05$), therefore it can be considered as an adequate predictor of sperm functionality. Also, a correlation of guinea pig live weight and sperm concentration with total CB was observed ($p<0.05$) (Table 2).

Sperm DNA Fragmentation Index (SDFI) was higher in PT_2 but there were no differences between PT_0 and PT_1 (Table 1 and Fig. 4f). During the exposure period, each male guinea pig was housed with females of the same age to standardize sexual stimulation. At the end of the analysis, no PT_2 females became pregnant (0/5), while all PT_0 females became pregnant (4/4) and only half of the PT_1 females became pregnant (2/4). However, births in PT_1 occurred earlier than in PT_0 females, showing greater sexual precocity for the onset of reproductive activity.

From the record of environment temperature and relative humidity, in Fig. 5a-c, the temperature and Temperature-Humidity Index (THI) plots in three compartments with different light stimuli (October-December, 2020), are shown. In PT_0 (Fig. 5a) lower temperature peaks and higher THI were reached (32.65°C and 81.8), in PT_1 (Fig. 5b) the temperature and THI peaks were intermediate (32.75°C and 81.6) but in PT_2 (Fig. 5c) reached high-temperature peaks and lower THI (33.8°C and 81.0).

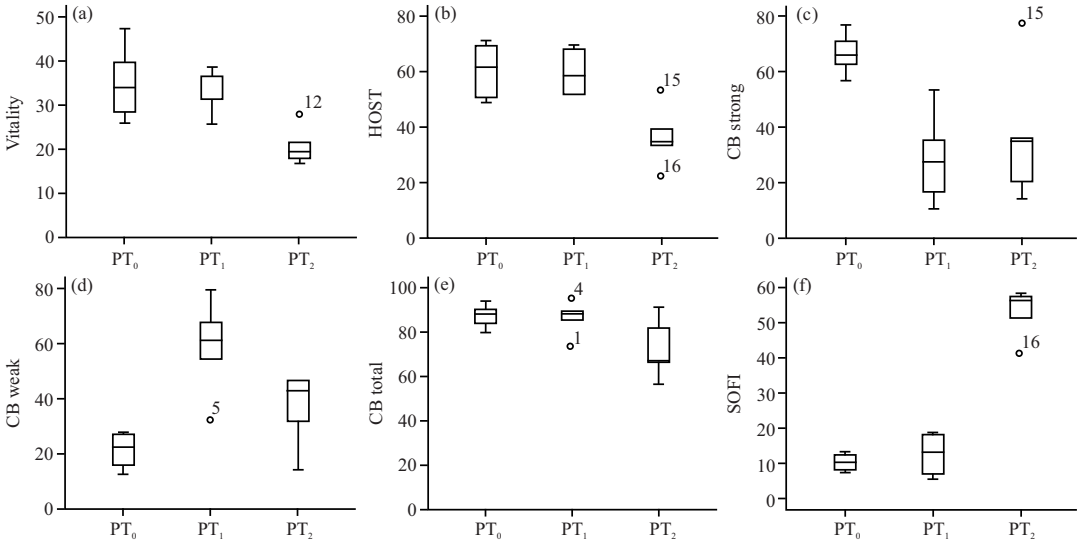

Fig. 4(a-f): Boxplot of characteristics of guinea pigs epididymal sperm subjected to different light stimuli, (a) Vitality (%), (b) Hypoosmotic swelling test (HOST, %), (c) CB strong staining (CB++, %), (d) CB weak staining (CB+, %), (e) CB total staining (%) and (f) Sperm DNA fragmentation index (SDFI, %)

PT_0: Without direct light stimulation, PT_1: Artificial photoperiod and PT_2: Photoperiod with sunlight

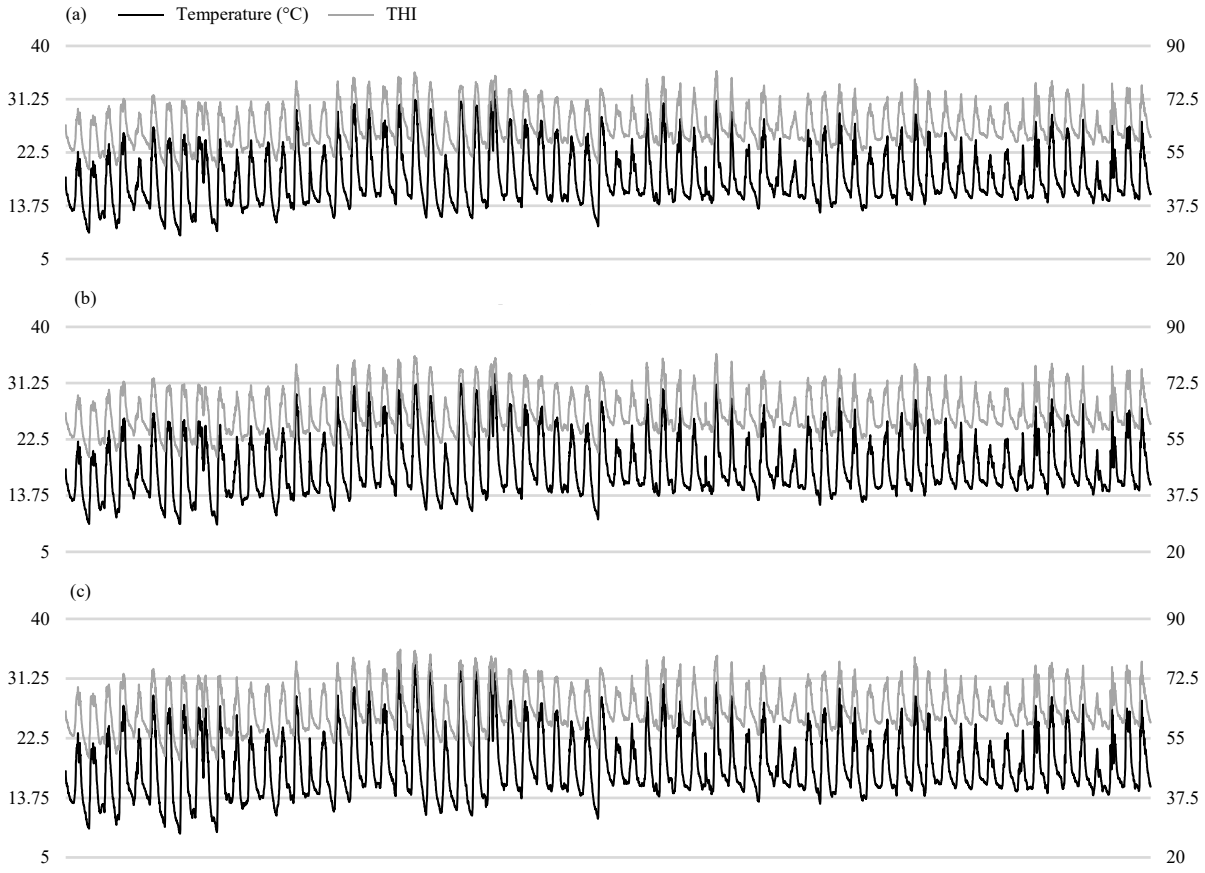

Fig. 5(a-c): Environment temperature and temperature-humidity index (THI) from October-December, 2020, in three compartments with different light stimuli, (a) PT_0 or room without direct light stimulus, (b) PT_1 or artificial photoperiod of 10L/14D, generated with LED lamp and (c) PT_2 or photoperiod with the sunlight of 10L/14D

DISCUSSION

In this study, the effect of photoperiod treatments (without direct light stimulus or PT_0, artificial photoperiod or PT_1 and photoperiod with sunlight PT_2) on guinea pigs sperm parameters was evaluated, where most of these were less in PT_2.The values of PT_0 concentration and total motility are higher than those reported by Ayala Guanga *et al.*[19] ($418.0\pm57.0\times10^6$ mL^{-1} and $58\pm5.39\%$), less total motility than Rodriguez *et al.*[20] (95%) and higher concentration than Ferdinand *et al.*[21] ($149.85\pm5.07\times10^6$) for epididymal spermatozoa. Our findings are higher than reported by Cabeza *et al.*[22] (47.33×10^6 mL^{-1} and 69.40%) and Benavides *et al.*[23] ($36.7\pm28.4\times10^6$ mL^{-1}) for spermatozoa collected by electroejaculation, although the latter reported higher motility ($90.86\pm6.64\%$) than ours. In PT_1, live weight, testicular weight and sperm concentration were higher than in PT_0, although not significantly. In seasonal breeding species (sheep or goats), the photoperiodic effect on testosterone levels, testicular volume and sperm concentration has been demonstrated[6,8], attributed to the increase in melatonin secretion due to the reduction of day length and the intensity of light on the eye retina[3,24]. Melatonin has a role as a hormonal messenger in the environment-animal relationship in mammals since its receptors were found in reproductive organs and spermatozoa[25]. Guinea pigs could be sensitive to photoperiod as documented in males and females by Bauer *et al.*[4] and Trillmich *et al.*[26]. In their study, the effect of light stimulus with long photoperiods of up to 14 and 16 hrs achieved earlier puberties. In the same way, Bauer *et al.*[4] reported earlier puberty and earlier serum testosterone peaks in a 16L/8D regime compared to an 8L/16D regime. In this study, sexual activity and successful pregnancies occurred earlier in PT_1 (artificial photoperiod with 10L/14D) than PT_0 (no direct light stimulation). However, in a photoperiod with sunlight (PT_2), sperm concentration and motility parameters were lower. In PT_2, with a photoperiodic regimen 10L/14D, similar to PT_1 but using sunlight was established.

The direct solar radiation incidence was only from 8:00 to 10:00 am in 20% of the cage's surface and the rest of the day, the windows stayed open (until 18:00), reaching higher temperature peaks in PT_2 (33.8°C) compared to PT_1 (32.75°C) and PT_0 (32.65°C). The Temperature-Humidity Index (THI) was determined to estimate thermal stress based on the environmental temperature and relative humidity records. Peaks of 81.0, 81.6 and 81.8 were obtained in PT_2, PT_1 and PT_0, respectively. THI values of 80-89 are categorized as severe stress experiences in cattle, however, these indices were higher in PT_0 and PT_1, so perhaps the equation should be adjusted to guinea pigs' thermoregulatory strategy. It was probably PT_2 guinea pigs who experienced frequent thermal stress episodes caused by sunlight, explaining the reduction in sperm concentration, motility and kinetic parameters. Daily high environmental temperatures in guinea pigs can alter thermoregulation at a scrotal level, generating thermal stress and consequently negative impacts on spermatogenesis and seminal quality, such as lower sperm concentration, lower individual motility, higher abnormal spermatozoa rate and even DNA alteration[11,27]. In rabbit spermatozoa, *in vitro* temperatures of 42°C reduced total motility, VCL and VAP values[28]. In our study, the temperature peaks reached midday (33.8°C to an optimal scrotal temperature of 32.5°C) were probably able to compromise sperm functionality.

About spermatic kinetic parameters, in PT_1, VCL, VSL, VAP, STR, ALH and BCF were higher than PT_0, although not significantly, these parameters reflect the vigorousness of progressive sperm movement[29]. The fertility rate can be positively correlated with VCL, VSL and VAP[30]. Furthermore, an efficient velocity, a LIN greater than 50% and an ALH of 4.8 μm are related to increased migration and penetration of cervical mucus in goat spermatozoa[31]. In our study, fertility was higher in PT_0 but in PT_1, pregnancies were earlier. Furthermore, because of the photoperiod synchronizing effect of endogenous melatonin secretion, melatonin was *in vitro* supplemented in bovine spermatozoa and a positive impact on sperm motility and velocity was found. That is due to the stimulation of cellular Ca2+ influx regulated by calmodulin present in the head and flagellar zones of the spermatozoa, influencing the cytoskeleton and intracellular ATP concentration[29,32,33].

Sperm vitality tests may reveal if a non-motile sperm has or does not have a functional membrane. In our study, vitality rates by eosin-nigrosin staining of epididymal spermatozoa are lower than reported by Ayala Guanga *et al.*[19] ($60.2\pm4.0\%$) and Cabeza *et al.*[22] ($72.65\pm8.19\%$, obtained by electroejaculation). Similarly, Mutwedu *et al.*[34] reported high HOST+ reaction rates in epididymal spermatozoa ($74.66\pm9.68\%$). Between PT_0 and PT_1, there were no significant differences but in PT_2 lower vitality and HOST+ rates were found. We believe that PT_2 experienced thermal stress and that sperm viability and fertility were negatively impacted as low motility was also strongly correlated with

HOST+ but further research is still required. Supravital staining measures the plasma membrane's physical integrity, while HOST assesses its functional integrity[35]. Optimal sperm plasma membrane functional integrity plays a key role during capacitation, acrosomal reaction and fertilization[36]. Due to photoperiod variation could promote alterations in testosterone secretion and consequently changes in the spermatogenesis[37] and maturation process, we presume this could be happening in our study since in PT_1, the pregnancies were early compared to PT_0, despite the absence of differences in the spermiogram.

Also in PT_2, higher SDFI than PT_0 and PT_1 was found and we could attribute it to the adverse effect of thermal stress on scrotal temperature. Thermal stress can induce apoptosis in germ cells and spermatogenesis dysfunction due to an increase in scrotal temperature in guinea pigs and mice. Furthermore, oxidative stress could lead to DNA breakdown and therefore sperm DNA degradation[11,12].

Previous andrological studies have already used Coomassie blue staining to evaluate the integrity of the acrosomal cap of camelid spermatozoa[18,38]. Compared to us, Benavides[24] found 6.51±6.3% acrosomal absence. A higher percentage of CB++ acrosomal staining pattern was observed in PT_0 than in PT_1. Conversely, the CB+ pattern was higher in PT_1 than in PT_0. Kim *et al.*[39] classified guinea pig sperm according to acrosomal domains, which vary according to the state of acrosomal protein release or exocytosis. Coomassie blue is a cationic dye with an affinity for protein elements such as acrosomal enzymes so that it could be an indicator of the state of exocytosis in the acrosome matrix of guinea pig spermatozoa. CB++ could be indicative of the acrosomal matrix integrity and the soluble compartment in three morphological domains of the acrosome (M_1, M_2 and M_3) classified by Hardy *et al.*[40] and Class 1 according to the acrosomal protein release model in guinea pigs[39,41]. On the other hand, CB+ seems to indicate the beginning of acrosomal contents release since we observed less staining intensity, expansion of the acrosome and even remnants of membrane and acrosomal contents around the nucleus, coinciding with Class 2 and Class 3 of the transition states model. The exocytotic process was probably earlier in PT_1 than in PT_0, which could be explained by a possible photoperiod variation effect on testosterone secretion and spermatogenesis[38] as well as the earlier precocity and pregnancy rates found in this study. However, ChaithraShree *et al.*[29] too reported adverse effects of excessive melatonin concentration on the plasma membrane and acrosomal i ntegrity in cattle. Therefore, further studies of the photoperiodic effect on the structure and acrosomal function of guinea pig sperm are necessary.

It is important to remember that, the acrosomal reaction allows penetration of the spermatozoa into the zona pellucida during oocyte fertilization. This process involves fenestration and vesiculation of the plasma and external acrosomal membrane, triggering the acrosomal contents' release[42]. In PT_2, a low acrosomal integrity rate (total CB reaction) was found, probably due to the thermal stress experienced, correlated with sperm concentration, motility and membrane functionality, which finally resulted in the absence of pregnancies.

CONCLUSION

Photoperiods with sunlight (PT_2) could generate thermal stress and, consequently, low spermiogram values and absence of pregnancies. An artificial 10L/14D photoperiod (PT_1) improved some microscopic characteristics of guinea pig spermatozoa, although not significantly compared to a room without any direct light stimulus (PT_0). However, although the fertility rate was not improved, pregnancies were earlier in guinea pigs subjected to artificial photoperiod. Further studies need to be considered.

SIGNIFICANCE STATEMENT

This study discovers the possible effect on artificial photoperiod and photoperiod with sunlight on sperm quality and fertility in guinea pigs, which can be beneficial for improving the reproductive activity of captive-bred guinea pigs. This study will help the researcher to uncover the critical area of photobiology in guinea pig reproduction that many researchers were not able to explore. Thus, a new theory on these environmental factors on breeding males may be arrived at.

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REFERENCES

1. Goldman, B.D., 1999. The circadian timing system and reproduction in mammals. Steroids, 64: 679-685.
2. Nakane, Y. and T. Yoshimura, 2019. Photoperiodic regulation of reproduction in vertebrates. Annual Rev. Anim. Biosci., 7: 173-194.
3. Lincoln, G.A., 1992. Photoperiod-pineal-hypothalamic relay in sheep. Anim. Reprod. Sci., 28: 203-217.
4. Bauer, B., I. Womastek, J. Dittami and S. Huber, 2008. The effects of early environmental conditions on the reproductive and somatic development of juvenile guinea pigs (*Cavia aperea f. porcellus*). Gen. Comp. Endocrinol., 155: 680-685.
5. Guenther, A., R. Palme, M. Dersen, S. Kaiser and F. Trillmich, 2014. Photoperiodic effects on reproductive development in male cavies (*Cavia aperea*). Physiol. Behav., 123: 142-147.
6. Zarazaga, L.A., M.C. Gatica, I. Celi, J.L. Guzmán and B. Malpaux, 2010. Effect of artificial long days and/or melatonin treatment on the sexual activity of mediterranean bucks. Small Ruminant Res., 93: 110-118.
7. Dacheux, J.L., C. Pisselet, M.R. Blanc, M.T. Hochereau-de-Reviers and M. Courot, 1981. Seasonal variations in rete testis fluid secretion and sperm production in different breeds of ram. J. Reprod. Fertil., 61: 363-371.
8. Santiago-Moreno, J., A. Gomez-Brunet, A. Gonzalez-Bulnes, A. Toledano-Diaz, B. Malpaux and A. Lopez-Sebastian, 2005. Differences in reproductive pattern between wild and domestic rams are not associated with inter-specific annual variations in plasma prolactin and melatonin concentrations. Domestic Anim. Endocrinol., 28: 416-429.
9. Gamboa, S., A.S. Rodrigues, L. Henriques, C. Batista and J. Ramalho-Santos, 2010. Seasonal functional relevance of sperm characteristics in equine spermatozoa. Theriogenology, 73: 950-958.
10. Leme, D.P., F.O. Papa and J.F. Roser, 2012. Reproductive characteristics of stallions during the breeding and non-breeding season in a tropical region. Trop. Anim. Health Prod., 44: 1703-1707.
11. Ferdinand, N., G.T. Maryvonne, K. Augustave, T.T.C. D'Alex and N. Sandrine *et al*., 2017. Effects of heat stress on some reproductive parameters of male cavie (*Cavia porcellus*) and mitigation strategies using guava (*Psidium guajava*) leaves essential oil. J. Ther. Biol., 64: 67-72.
12. Dantzer, R. and K.W. Kelley, 1989. Stress and immunity: An integrated view of relationships between the brain and the immune system. Life Sci., 44: 1995-2008.
13. Thom, E.C., 1959. The discomfort index. Weatherwise, 12: 57-61.
14. Kilkenny, C., W. Browne, I.C. Cuthill, M. Emerson and D.G. Altman, 2010. Animal research: Reporting *in vivo* experiments: The arrive guidelines. Br. J. Pharmacol., 160: 1577-1579.
15. Everitt, J.I. and E.A. Gross, 2007. Euthanasia and Necropsy. In: The Laboratory Rat, Suckow, M.A., S.H. Weisbroth and C.L. Franklin (Eds.)., Elsevier, London, Pages: 14.
16. Quispe, H.A., R. Ciprián, C. Ordoñez, E. Ampuero and H. Cucho, 2015. Hypoosmotic swelling test in alpaca (*Vicugna pacos*) spermatozoa recovered the vas deferens. Spermova, 5: 10-14.
17. Ugarelli, A., S. Evangelista-Vargas and A. Santiani, 2017. Evaluation of acrosome integrity in epidydimal alpaca sperm by flow cytometry. Rev. Investigaciones Vet. Perú, 28: 130-140.
18. Fumuso, F.G., M.L. Giménez, D.M. Neild, S.M. Giuliano, M.G. Chaves and M.I. Carretero, 2014. Comparison of washing methods and smear conservation periods for llama sperm acrosome assessment using the coomassie blue stain. Spermova, 4: 50-53.
19. Guanga, L.E.A., R.R. Carpio, A. Almeida, C.S.T. Inga and P.E.N. Escandón, 2017. Influence of penis spicule of cavy (*Cavia porcellus*) on their sexual behavior, fertility and sperm quality. Rev. Prod. Anim., 29: 36-42.
20. Rodríguez, G.J., M. Barrios-Arpi, L.W. Huanca, G.A. Rodríguez and L. Revuelta, 2016. Levels of glucose and triglycerides in seminal plasma and spermatic motility in guinea pig fed with 10% more in digestible energy. Rev. Complutense Cienc. Vet., Vol. 10. 10.5209/rev_rccv.2016.v10.n1.52501.
21. Ngoula, F., S.C. Nouboudem, A. Kenfack, C.D. Tadondjou and J.R. Kana *et al*., 2014. Effect of guava (*Psidium guajava*) leaves essential oil on some reproductive parameters in male guinea pig (*Cavia porcellus*). Biol. Syst., Vol. 3. 10.4172/2329-6577.1000125.
22. Cabeza, U., C. Ordóñez, A. Meza and H. Cucho, 2020. Morphological and morphometric characterization of the guinea pig sperm (*Cavia porcellus*). Spermova, 10: 94-101.
23. Benavides, F., P. Sutovsky, V. López, C. Kennedy and L. Echevarría, 2020. Semen parameters of fertile guinea pigs (*Cavia porcellus*) collected by transrectal electroejaculation. Animals, Vol. 10. 10.3390/ani10050767.
24. Kozioł, K., D. Broda, M. Romerowicz-Misielak, S. Nowak and M. Koziorowski, 2020. Melatonin concentration in peripheral blood and melatonin receptors (MT1 and MT2) in the testis and epididymis of male roe deer during active spermatogenesis. Theriogenology, 149: 25-37.
25. Correa, L.M. and J.L. Fernández, 2017. Influence of melatonin on the physiology and behavior of ungulates. J. High Andean Res., 19: 351-358.
26. Trillmich, F., B. Mueller, S. Kaiser and J. Krause, 2009. Puberty in female cavies (*Cavia aperea*) is affected by photoperiod and social conditions. Physiol. Behav., 96: 476-480.
27. Durairajanayagam, D., R.K. Sharma, S.S. du Plessis and A. Agarwal, 2014. Testicular Heat Stress and Sperm Quality. In: Male Infertility, Stefan, S., D.P. Ashok A. Edmund and S. Sabanegh (Eds.)., Springer, Cham, New York, ISBN-13: 978-1-4939-1040-3, pp: 105-125.

28. Sabés-Alsina, M., O. Tallo-Parra, M.T. Mogas, J.M. Morrell and M. Lopez-Bejar, 2016. Heat stress has an effect on motility and metabolic activity of rabbit spermatozoa. Anim. Reprod. Sci., 173: 18-23.
29. ChaithraShree, A.R., S.D. Ingole, V.D. Dighe, A.S. Nagvekar and S.V. Bharucha *et al*., 2020. Effect of melatonin on bovine sperm characteristics and ultrastructure changes following cryopreservation. Vet. Med. Sci., 6: 177-186.
30. Verstegen, J., M. Iguer-Ouada and K. Onclin, 2002. Computer assisted semen analyzers in andrology research and veterinary practice. Theriogenology, 57: 149-179.
31. Cox, J.F., V. Alfaro, V. Montenegro and H. Rodriguez-Martinez, 2006. Computer-assisted analysis of sperm motion in goats and its relationship with sperm migration in cervical mucus. Theriogenology, 66: 860-867.
32. Tash, J.S. and A.R. Means, 1983. Cyclic adenosine 3`,5` monophosphate, calcium and protein phosphorylation in flagellar motility. Biol. Reprod., 28: 75-104.
33. Perumal, P., S. Chang, C.T.R. Sangma, N. Savino and K. Khate, 2016. Effect of melatonin on mobility and velocity parameters of mithun (*Bos frontalis*) semen preserved in liquid state (5°C). J. Exp. Biol. Agric. Sci., 4: S95-S102.
34. Mutwedu, V.B., A.W. Nyongesa, P.C. Azine, D.K. Chiregereza, V.H. Ngoumtsop, Y. Mugumaarhahama and R.B.B. Ayagirwe, 2021. Growth performance and reproductive function impairment of glyphosate based herbicide in male guinea pig (*Cavia porcellus*). Vet. Med. Sci., 7: 1047-1055.
35. Sánchez, R.A. and D.P. Zamora, 2016. Effect of hypoosmotic environment on canine semen sperm viability. Rev. Investigaciones Vet. Perú, 27: 288-293.
36. Rota, A., V. Bastianacci, C. Magelli, D. Panzani and F. Camillo, 2010. Evaluation of plasma membrane integrity of donkey spermatozoa. Reprod. Domest. Anim., 45: 228-232.
37. Andersson, H., M. Wallgren, L. Rydhmer, K. Lundström, K. Andersson and M. Forsberg, 1998. Photoperiodic effects on pubertal maturation of spermatogenesis, pituitary responsiveness to exogenous GnRH and expression of boar taint in crossbred boars. Anim. Reprod. Sci., 54: 121-137.
38. Carretero, M.L., C. Pigretti, M. Bertuzzi and F. Fumuso, 2018. Hypoosmotic test combined with coomassie blue staining in llama sperm. Spermova, 8: 129-132.
39. Kim, K.S., J.A. Foster and G.L. Gerton, 2001. Differential release of guinea pig sperm acrosomal components during exocytosis. Biol. Reprod., 64: 148-156.
40. Hardy, D.M., M.N. Oda, D.S. Friend and T.T.F. Huang, 1991. A mechanism for differential release of acrosomal enzymes during the acrosome reaction. Biochem. J., 275: 759-766.
41. Kim, K.S., J.A. Foster, K.W. Kvasnicka and G.L. Gerton, 2011. Transitional states of acrosomal exocytosis and proteolytic processing of the acrosomal matrix in guinea pig sperm. Mol. Reprod. Dev., 78: 930-941.
42. Larson, J.L. and D.J. Miller, 1999. Simple histochemical stain for acrosomes on sperm from several species. Mol. Reprod. Dev., 52: 445-449.