EFFECTS OF PHOTOPERIOD ON TESTICULAR FUNCTIONS IN MALE SPRAGUE-DAWLEY RATS

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Summary: Variation in reproductive status in response to photoperiods has been observed in laboratory rats. We investigated the effects of photoperiod on testicular activity in Sprague-Dawley rats (Rattus norvigicus) maintained in experimental photoperiodic condition. Twenty four adult male rats weighing 170±10g were conditioned to different lighting conditions of Light/Dark (LD) Cycle for 6 weeks. Group 1, Control group (LD12:12, light on from 07:00hr to 19:00hr). Group 2, Short Photoperiod group (LD 8:16hr, light on from 09:00hr to 17:00hr). Group 3, Long Photoperiod group (LD 16:8hr, light on from 05:00hr to 21:00hr). A significant influence of different lighting conditions on the testicular parameters was observed. Short photoperiod showed a suppressing effect (P<0.001) on testicular weight, sperm motility sperm viability and sperm counts, while long photoperiod had an inducing, though insignificant, effect on the measured parameters. The results confirmed that Sprague-Dawley rats are photoresponsive and changes in the photoperiod could influence their reproductive functions.

Key Words: Photoperiod, Sperm motility, Sperm viability, Sperm counts, Testicular weight.

Introduction

In some mammals, reproduction follows a seasonal pattern that is often under photoperiodic control. Such patterns have evolved so that animals give birth during period when environmental conditions are favourable, maximizing the chances that the young will survive. One of the most reliable seasonal predictors appears to be photoperiod (Bronson, 1989; Boissin and Canguilhem, 1998). Depending on the species, photoperiod may either trigger onset of the reproductive period (a stimulating effect), or initiate gonadal regression (an inhibitory effect). In long-day breeding species, the seasonal increase in sexual activity occurs when the amount of daylight increases, and in short-day breeding species, the reproductive season is triggered by the shortening of day length, (Ben Saad and Maurel, 2002). Melatonin, a 5-methoxyindole synthesized by the pineal gland, plays a major role in photoperiod-mediated control of reproduction in mammals with seasonal breeding patterns determined by day length in their natural environment, and the circadian pattern of melatonin secretion constitute an endocrine message that provides information regarding the photoperiod (Reiter, 1986; Reiter, 1991; Arendt, 1995; Goldman, 1999).

Variation in reproductive status and body mass in response to short photoperiod has been observed in laboratory rats (Leadem, 1988; Heideman and Sylvester, 1997). Studies have shown that the Fischer 344 (F344) and Brown Norway (BN) inbred rat strains exhibit robust obligate photosensitivity, repressing reproduction, food intake, and somatic growth in the absence of light (Leadem, 1988). However, short photoperiods (Heideman and Sylvester, 1997; Lorincz et al., 2001; Shoemaker and Heideman, 2002). In contrast, other strains of laboratory rats have not been considered functionally photosensitive because unmanipulated rats of these strains show little or no marked differences in body mass, gonad size, or food intake in response to short photoperiod (Nelson et al, 1994). However, photoreponsiveness in rats does not fall neatly into two phenotypes, for example in some of the rat strains considered nonphotoperiodic, including Wistar and Sprague-Dawley outbred strains, photoperiodic response can be unmasked by treatments such as administration of androgen (Wallen and Turek, 1981; Wallen et al., 1987). In view of the variation in the response to changes in the photoperiod among rat strains, further investigation into this phenomenon becomes worthwhile.

The present study was therefore designed to investigate the effects of photoperiod on testicular functions in Sprague-Dawley rats. In this study, we investigated young males of Sprague-Dawley rat. This strain was chosen because it is the most commonly used type of rats in our laboratory. The objectives of the study were to test whether photoperiodic responses might be
widespread in this strain of rats and to assess
the magnitude of any photoperiodic responses
on reproductive functions.

Materials and Methods
Twenty four Sprague-Dawley rats were
obtained from Animal Breeding Unit of the
Department of Biochemistry, University of
Ilorin, Nigeria. The rats weighed 170 ± 10g
and were conditioned to different lighting
conditions for 6 weeks. All animals were
housed in plastic cages with stainless steel
mesh cover under standard laboratory
conditions in photoperiod-control chambers.
Lighting in photoperiod chambers was
provided by 6-watt fluorescent tubes at
illuminance of 100-250 lux, 5cm above each
cage. The experiment was conducted during
the raining season. Rats pellet and tap water
were provided *ad libitum*. All animals received
human care. The animals were divided into 3
groups of 6 animals per group, with groups I,
II and III subjected to photoperiodic conditions
of light/dark cycle of 12:12h, 8:16h, and 16:8h
respectively, as shown in Table 1. At the end
of the experiment, (6 weeks), the rats were
anaesthesized with urethane (5mg/kg), body
weight was measured, both testes were
excised, and wet weight was recorded.

Sperm Motility, Viability and Counts
The caudal epididymis was immediately
dissected. An incision (about 1mm) was then
made in the caudal epididymis. A drop of
sperm fluid was squeezed onto the microscope
slide and 2 drops of normal saline were added
to mobilize the sperm cells. Epididymal sperm
motility was then assessed by calculating
motile spermatozoa per unit area and was
expressed in percentage. Epididymal sperm
counts were done by first homogenizing the
epididymis in 5ml of normal saline. The
counting was then done using the counting
chamber in the haemocytometer (Adeeko and
Dada, 1998). The sperm viability was also
determined using Eoisin/Nigrosin stain as
earlier described (Raji *et al*, 2003).

Statistical Analysis Data were expressed as
mean ± SEM. Statistical significance was
determined using the student’s t-test. P<0.05
was considered significant.

Table 1: Animal Groups (Control and
Experimental), Light/Dark Cycle, and
Photoperiod

<table>
<thead>
<tr>
<th>Groups</th>
<th>Study</th>
<th>Control</th>
<th>Experiment</th>
<th>Experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light/Dark Cycle (hrs)</td>
<td>Light/Dark Cycle</td>
<td>12:12h</td>
<td>8:16h</td>
<td>16:8h</td>
</tr>
<tr>
<td>Time</td>
<td>7:00-1900h</td>
<td>9:00-17:00h</td>
<td>5:00-21:00h</td>
<td></td>
</tr>
</tbody>
</table>

Results
The results (Table 2), showed that there
was a significant decrease (P<0.005) in
testicular-body-weight ratio from 0.01 ±
0.001g to 0.004 ± 0.001g in short photoperiod
(SP) group compared to control, about 60%
reduction. Long photoperiod (LP) did not
affect the testicular-body weight ratio.

SP significantly reduced sperm motility
(P<0.005) from 72.60 ± 8.44% in the control
group to 29.00 ± 5.42% in the SP group. LP
increased sperm motility from 72.60 ± 8.44%
in the control group to 74.00 ± 6.52% in LP
group, but this was not statistically significant
(P=0.72). SP showed a significant effect on
sperm viability, which was reduced from 57.00
± 11.51% in the control group to 23.00 ±
3.42% in the SP group (P<0.005), while it was
insignificantly (P<0.42) increased to 64.00 ±
14.36% in LP group.

Moreover, SP significantly reduced sperm
counts from 41.60 ± 7.89 x 10⁶/ml in the
control group, to 17.70 ± 3.56 x 10⁶/ml in the
SP group, (P<0.001) while LP slightly
increased the sperm count to 44.60 ± 9.86 x
10⁶/ml, but this was not statistically significant
(P=0.24).

Table 2: Effect of Photoperiod on testicular Weight, Sperm Motility, Viability, and Count in Control, SP, and LP

<table>
<thead>
<tr>
<th>Groups</th>
<th>Left &amp; Right testes/Body Weight (g)</th>
<th>Sperm Motility (%)</th>
<th>Sperm Viability (%)</th>
<th>Sperm Count (10⁶/mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (12D:12L)</td>
<td>0.01±0.001</td>
<td>72.60±8.44</td>
<td>57.00±11.51</td>
<td>41.60±7.89</td>
</tr>
<tr>
<td>SP (16D:8L)</td>
<td>0.004±0.001</td>
<td>29.00±5.42</td>
<td>23.00±3.42</td>
<td>17.70±3.56</td>
</tr>
<tr>
<td>LP (8D:16L)</td>
<td>0.01±0.001</td>
<td>74.00±6.52</td>
<td>64.00±14.36</td>
<td>44.60±9.86</td>
</tr>
</tbody>
</table>
Discussion

The results of this study show that male Sprague-Dawley rats are photoresponsive. The rats showed significantly lower reproductive organ masses, sperm motility, viability, and counts following exposure to short photoperiod (SP). There was also insignificant increase in sperm motility, viability, and counts, but not testicular-body weight ratio on exposure to long photoperiod (LP). Previous work on young male F344 and BN rats indicated that reproductive and body masses were reduced by SP (Heideman and Sylvester, 1997; Lorincz et al., 2001). SP has also been observed to have an inducing effect on male reproductive parameters in Zembla Island wild rabbits (Oryctolagus cuniculus) (Ben Saad and Maurel, 2002).

Earlier studies on wister and Sprague-Dawley rats showed that they were nonphotoperiodic and responded to photoperiod manipulation only after administration of androgen (Sorrentino et al., 1971; Wallen and Turek, 1981; Wallen et al., 1987) but the present study has shown that in the absence of any hormonal manipulation, photoperiod has significant effects on the measured reproductive parameters in the Sprague-Dawley rats. Exposure of hamsters to short photoperiods inhibits their reproductive system until there is testicular involution in males and anoestrous in females (Hoffman, 1973; Lerchl and Nieschlag, 1992). Pinealectomy, however, prevents gonadal regression in hamsters exposed to a short photoperiod (Hoffman, 1979), implicating melatonin as the hormone responsible for the effects of photoperiod on reproductive parameters. Melatonin administration in hamsters mimics all the effects of short photoperiod on reproduction (Duncan et al., 1990; Buchanan and Yellon, 1991; Badra and Goldman, 1992; Pevet, 1993). The observed suppression of male reproductive parameters in SP group in our study could be due to actions of melatonin, which is known to be secreted at a very high rate during darkness due to 30-to 70-fold increase in activity of N-acetyltransferase, the enzyme that catalyses the penultimate step in the biosynthesis of melatonin (Ebadi, 1984).

Available evidence indicates that melatonin regulates the reproductive function in seasonal mammals by its inhibitory action at various levels of the hypothalamic-pituitary-gonadal axis. By acting on melatonin receptors (MT1 and MT2) in the hypothalamus, anterior pituitary and reproductive organs, melatonin inhibits the reproductive system (Vanecek and Klein, 1992; Zemkova and Vanecek, 1997; Balik et al., 2004; Soares et al., 2003; Frungier et al., 2005). Melatonin is also known to reduce body weight by suppressing intraabdominal fat, plasma leptin, and plasma insulin in rats (Wolden-Hanson et al., 2000). Our study showed testicular-body weight ratio reduction in the SP group, suggesting that the effect of melatonin and possibly, photoperiod, is more pronounced on the gonadal weight than on the general body weight. Our observation of an insignificant increase in sperm parameters is consistent with earlier observation that light exposure and pinealectomy are associated with an enhancement in gonadal function (Kinson and Peat, 1971). We also observed an increase in sperm motility, viability and sperm count. But these increments were not statistical significant.

The present study confirmed that Sprague-Dawley rats are functionally photoresponsive and that in the absence of any hormonal manipulation, changes in the photoperiod could influence their reproductive functions.

References


