# Impairment of male reproductive function after sleep deprivation

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**Objective:** To evaluate the influence of sleep loss on sexual behavior, hormone levels, sperm parameters, and testis-specific gene expression in male rats.
**Design:** Experimental research.
**Setting:** Animal laboratory.
**Animal(s):** Male adult Wistar-Hannover rats.
**Intervention(s):** Sexually experienced rats were subjected to paradoxic sleep deprivation (PSD) for 96 hours or sleep restriction (SR) for 21 days or kept in their home cage as control (CTRL).
**Main Outcome Measure(s):** Sexual behavior, hormone levels, sperm parameters and expression of stress and nitric oxide–related genes were evaluated.
**Result(s):** PSD significantly decreased sexual behavior compared with the CTRL group, whereas SR had no effect. The PSD group had significantly lower testosterone levels than the CTRL group. Both PSD and SR groups had lower sperm viabilities than the CTRL group. The decrease in the number of live sperm compared with the CTRL group was larger in the PSD group than in the SR group. Regarding testicular gene expression, both PSD and SR led to an increase of iNOS and hydroxysteroid 11$\beta$-dehydrogenase 1 expressions compared with the CTRL group. These changes were more pronounced in the PSD group. A significant increase in endothelial nitric oxide synthase expression was observed in the PSD groups compared with the CTRL group. No changes were observed in dimethylarginine dimethylaminohydrolase 1 and casein kinase 2$\beta$-polypeptide expressions.
**Conclusion(s):** Sleep loss can promote marked changes in the male reproductive system of rats, particularly affecting spermatic function in part by interfering in the testicular nitric oxide pathway. (Fertil Steril® 2015;103: 1355–62. ©2015 by American Society for Reproductive Medicine.)
**Key Words:** Sleep restriction, sexual behavior, testosterone, progesterone, sperm, reproduction, nitric oxide, male rat, iNOS, eNOS

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Reproductive function in humans has been of particular concern in recent years. Diseases, psychologic factors, stress, and hormonal changes are some of the factors that contribute to the appearance of dysfunction in the male reproductive system (1). Studies have shown that sperm concentration has been decreasing over the years (2–5) and a high prevalence of erectile dysfunction complaints has been observed in men 18–40 year old in association with psychosocial but not organic problems (6). The stress resulting from socioeconomic pressure combined with increased workload lead to a decrease of total sleep time. Because it is difficult to study the isolated role of sleep loss in human reproduction, it becomes valuable to use nonhuman models.

In rats, adverse effects of sleep deprivation (SD) have been well documented and include changes in functional parameters of male sexual behavior, leading to increased frequency of spontaneous erections and ejaculations (7, 8). To comprehensively assess sexual behavior, one must scrutinize other motivational behaviors, expressed as performance, such as the numbers of mounts, intromissions, and ejaculations. Alvarenga et al. (9) demonstrated that rats exposed to 96 hours of paradoxic SD (PSD) displayed reduced sexual performance, as evidenced by an increased latency for intromission initiation and a reduction in the

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number of intromissions compared with a control group. In addition to altering sexual behavior, PSD has been found to influence sex hormones. Male rats exhibit marked hormonal alterations when subjected to PSD, with decreases in testosterone (T) and $E_2$ concentrations, as well as increases in progesterone (P) and glucocorticoid levels (10–12).

Progesterone, LH, FSH, and T are all intimately involved in the process of cellular division and spermatogenesis, which ultimately leads to spermatozoid production (13–17). Some specific genes are also responsible for spermatogenesis and infertility (18, 19). In addition to the important role of nitric oxide (NO) in male fertility, mainly on the effectiveness of erection for sexual intercourse, NO can be toxic to cells at high levels, owing to the inhibition of DNA replication and lipid peroxidation (20). Inducible nitric oxide synthase (iNOS), a calcium-independent NOS present in the testis, is implicated in spermatogenesis and apoptosis of Sertoli and germ cells (21). Therefore, we hypothesized that both acute and chronic SD may lead to endocrine alterations, adversely affecting sexual behavior and sperm quality and quantity in association with molecular modulation of the NO pathway in the reproductive system of male rats.

## MATERIALS AND METHODS

### Animals

Adult male Wistar-Hannover rats were bred and raised in the animal facility of the Centro de Desenvolvimento de Modelos Experimentais para Medicina e Biologia of the Universidade Federal de São Paulo. The animals were housed in a colony with a constant temperature of 22 ± 1°C and a 12-h/12-h light/dark cycle (lights on at 07:00) and had free access to water and food. All animals were treated in accordance with the National Institutes of Health guidelines, and all procedures were approved by the university's Ethics Committee (CEP no. 09/071).

### Training and Sexual Behavior Evaluation

Before sexual behavior was evaluated, the rats acquired sexual experience through training. Because sexually inexperienced male rats can display low performance, we followed an established protocol that standardizes the degree of copulatory activity and avoids possible bias (22). Twenty-four hours after the last training session, the rats with excellent sexual performance (i.e., animals that showed >70% ejaculation frequency during the training) were selected and subjected to PSD for 96 hours or sleep restriction (SR) for 21 days. After these periods, sexual behavior was reevaluated immediately.

Training and testing of sexual behavior was performed with the use of a Plexiglas cylinder arena with a 45-cm diameter. Dim red lights shone during the dark phase of the light/dark cycle. A male rat was introduced into the arena 5 minutes before a female rat. Sexual receptivity in the female rats was established by subcutaneous administration of $E_2$ benzoate (10 μg/0.1 mL sesame oil; Sigma Chemical Co.) 48 hours and 24 hours before testing, followed by subcutaneous administration of P (500 μg/0.1 mL sesame oil; Sigma Chemical Co.) 4 hours before testing sexual behavior. Each test of sexual behavior lasted for 30 minutes after the introduction of the female rat, during which the following variables were recorded: time to first mount; intromission and ejaculation latencies; total numbers of mounts (i.e., mounts with pelvic thrusting); intromissions (mounts with pelvic thrusting and penile insertion); and ejaculations. The copulation rate (number of intromissions/[number of mounts + number of intromissions]), inter-intromission interval (ejaculation latency/number of intromissions) and intercopulatory interval (ejaculation latency/[number of intromissions + number of mounts]) were also calculated (23).

### Protocol Designs

The animals that displayed excellent performance after sexual training were randomly assigned to one of the following three groups (n = 10 per group): CTRL, control rats maintained in their home cage; s PSD, rats submitted to 96 hours of PSD; and SR, rats submitted to 21 days of SR.

### Paradoxic Sleep Deprivation

Rats were subjected to 96 hours of PSD by means of the modified multiple-platform method. The 96-hour length of the PSD was chosen based on previous studies showing that the most dramatic alterations in behavior (24) and hormone concentrations (11) occur for this period of PSD. The ten rats were individually placed inside a tiled water tank (143 × 41 × 30 cm) containing 14 circular platforms (each 6.5 cm in diameter) with the water level within 1 cm of the upper surface. The rats could move within the tank by jumping from one platform to another. When they reached the paradoxic phase of sleep, muscle atonia caused them to fall into the water and awaken. Throughout the study, the experimental room was maintained at a controlled temperature (22 ± 1°C) with a 12-h/12-h light/dark cycle (lights on from 07:00 to 19:00). The rats had free access to food and water located on a grid at the top of the tank. The water in the tank was changed daily during the PSD period. All animals began their PSD period at the same time in the dark phase of the light/dark cycle (19:00). Because we elected not to invert the light/dark cycle, the rats were trained and tested during a dark phase.

### Sleep Restriction

The SR protocol was based on the technique used for the PSD conditions. The difference in the SR protocol was that the rats were kept on the platforms for 18 hours (beginning at 16:00) and allowed to sleep for 6 hours (10:00–16:00) every day for 21 days, providing partial compensation for sleep loss (24). The time interval of 10:00–16:00 was chosen because it represented the time when paradoxic sleep is at its highest (25).

### Sperm Evaluation

Immediately after male ejaculation, the female rat was killed and the seminal fluid directly removed from the uterine horns. Seminal fluid was stored in Eppendorf tubes at 37°C and subjected to microscopic and macroscopic analyses (26).