*Pakistan J. Zool., vol. 57(3), pp 1117-1121, 2025* **DOI**: https://dx.doi.org/10.17582/journal.pjz/20231014095339

# Effect of Permanent Darkness on Rabbit Male Reproductive Function

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## ABSTRACT

Biological rhythms control many physiological and behavioral functions in mammals, including reproduction. During the development of the reproductive system, testicular growth is primarily influenced by photoperiod, which is influenced by the circadian release of melatonin. The purpose of this study was to determine the effect of darkness on the gonadotropic axis in mature male rabbits. This study involved ten male rabbits five of which were placed in complete darkness for 15 days and another five were kept as a control group. After the sacrifice, using the IRMA method, blood samples were collected to estimate the serum LH and FSH hormone levels. Sperm smears were also obtained in order to investigate the morphological structure of the sperm collected. The results revealed morphological alterations in treated rabbits as well as a significant decrease in blood FSH and LH levels when compared to controls, suggesting a relationship between the gonadotropic axis and the pineal gland. The results of this study reveal that factors that activate the gonadotropic axis, such as darkness, can cause alterations in reproductive function, and that this is also dependent on photoperiod duration.

**Article Information**
**Received 14 October 2023**
**Revised 15 December 2024**
**Accepted 27 December 2024**
**Available online 22 March 2025**
**(early access)**
**Published 29 April 2025**

**Authors' Contribution**
**AK and LL contributed to the analysis and interpretation of results of the manuscript. ZH-S contributed to the data search and selection. All authors reviewed the results and approved the final version of the manuscript.**

**Key words**
**Spermatozoa, Biological rhythms, FSH, LH, Darkness, Rabbit**

## INTRODUCTION

Several environmental factors, such as as lighting, temperature, food, and soil type, might have an impact on the animals well-being. Many mammalian species with photoperiodic reproductive cycles live in temperate climate zones (Chemineau *et al*., 1992). Most mammals reproductive functions have a seasonal rhythm, which is often under photoperiodic regulation. Animal rhythmicity, particularly circadian rhythms, is based on the interaction of physiological systems known as the internal clock or biological clock. These rhythms control an extensive variety of physiological and behavioral processes in mammals, including reproduction, so that calving occurs when environmental conditions are favorable, increasing the chance of survival for the young animals.

In comparison to hormonal treatments, light programs are less expensive and easier to implement. To reduce the negative consequences of decreasing day duration, commercial rabbit breeders in Europe adopt a 16 h light and 8 h dark (16HL:8HD) continuous lighting cycle (Alvarino and Ubilla, 1993). The photoperiod therefore has an essential influence in animal reproduction. The cycle of reproduction of the domestic rabbit *Oryctolagus cuniculus* begins with the lengthening of the light period in spring and concludes with an increase in the number of h of darkness during the day (Lebas *et al*., 1997). During the development of the reproductive system, testicular growth is primarily determined by the photoperiod, which has a strong connection to the circadian release of melatonin (Czeisler and Klerman, 1999). According to Lebas *et al*. (1996) changing from 8 to 16 h of light causes an increase in testicular weight and the proportion of viable spermatozoa, while changing from 8 to 16 h of light causes a drop in these same parameters. The testis, which is composed of seminiferous tubules, is the tubular compartment responsible for the production of spermatozoa: spermatogenesis, which is primarily sustained by testosterone, which was previously synthesized by the Leydig cells (Curtis and Amann, 1981; Eurell and Frappier, 2006). The rabbit is a hardy species that is regarded an important model in scientific research because of its various advantages in the field

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0030-9923/2025/0003-1117 $ 9.00/00

of reproductivity, which allows particular reproductive processes to be highlighted (Ewuola and Equnike, 2010).

The biological clock that determines circadian rhythmicity has been the most studied; at present, there is no complete comprehension of these distinct levels in any experimental model. A few studies have examined the influence of circadian rhythm disruption on sperm abnormalities, testicular capacity and reproductive hormone levels in rabbits to assess the impact of darkness on the development of the reproductive system (Moustafa, 2020). The aim of our study is to determine the effect of darkness on sperm morphology and serum gonadotropin levels in mature domestic rabbits (*Oryctolagus cuniculus*) after a 15 days period of permanent darkness.

## MATERIALS AND METHODS

This study was conducted in Tizi Ouzou (a rural town) in northern Algeria. Eight-month-old New Zealand White male rabbits (n= 20) were included in the study, which has a mature weight of about 3.5 kg. The animals were divided into two groups. The animals in the first group I (control) were exposed to 8 h light and 16 h darkness (8HL:16HD), the animals in the second group II were exposed to permanent darkness for 15 days.

All experimental male rabbits were raised in the same rabbit house by the same breeder and management mode of large-scale rabbit farms to maintain the same environmental conditions. Each male rabbit was raised in a single cage measuring and a nipple drinker was provided for clean water. The rabbits were fed twice a day commercial pellets (crude protein 16,1%, crude fiber 18%, crude ash 12%, calcium 1,3%; dry matter 89.6 %; digestible protein 13,5% ; mineral matter 7%). The male rabbits were acclimated to the environment for 8 days prior to the start of the experiment. Changes in body weight of rabbits were assessed during the experimentation.

Hematoxylin and eosin 1% using for smear sperm were provided from Sigma Aldrich. LH and FSH levels were measured using Immuno Radio Metric Assay (IRMA; Immunotech Inc. Beckman Coulter, France).

*Smear sperme analysis*

Sperm quality is usually assessed in spermatozoa collected from the cauda epididymidis of freshly sacrificed males rabbit and were performed using the hematoxylin and eosin staining. Smears were prepared for morphological evaluation using slides precleaned with 70% ethanol. 5µL aliquot of semen was placed on each slide, which was air-dried at 37°C in a warm tray. The slides were stained with Hematoxylin and Eosin.

The slides were fixed in 95% etathol/methanol and then stained with hematoxylin eosin following routine established protocol.

All the slides were viewed with a X100 oil-immersion objective under the Optica microscope, using immersion objective lenses. The images of sperm were examined on the computer screen.

*Hormone assays*

The blood samples were collected into a set of sterile plastic bottles and allowed to coagulate to produce sera for hormonal analyses. Plasma follicle stimulating hormone (FSH) and luteinizing hormone (LH) levels were measured in duplicate samples by specific IRMA methods (Immunotech Inc. Beckman Coulter, France. The volume of plasma used was 75 µl (FSH assay) and 100 µl (LH assay). All samples were measured in the same assay run to avoid inter-assay variations.

*Histological studies*

The staining of a seminal smear (hematoxylin and eosin) allows the qualitative evaluation of normal and abnormal sperm morphological forms in smear sperme. Smears can be scored for morphology using the World Health Organization (WHO) classification, or by Kruger's strict criteria classification (WHO, 1992; Kruger *et al*., 1995). WHO method classifies abnormally shaped sperm into specific categories based on specific head, tail, and midpiece abnormalities.

In contrast, Kruger's strict criteria classifies sperm as normal only if the sperm shape falls within strictly defined parameters of shape and all borderline forms are considered abnormal (>14% normal forms).

*Statistical analysis*

Data are presented as means ± standard deviation. Statistical comparisons between group means were performed using a Origin LAB, 2007 using Student's t-test. The level of significance was $< 0.05$.

## RESULTS

The change in body weight during the experiment (15 days) showed an increase in body weight in group II exposed to permanent darkness compared with group I (control). It was evaluated at 3.70 ± 0.28 Kg in group I at the start of the experiment and 4.35 ± 0.28 Kg in group II exposed to darkness for 15 days.

The smear sperm analysis shows several abnormalities in the head, midpiece and flagellum, compared to the controls where most spermatozoa were normal (Fig. 1).

Figure 2 shows the effect of the obscurity for 15 days on serum hormones profile of male rabbits. The LH levels were found ranged within 0.09 to 0.120 IU/L. The

obscurity had a negative impact on LH levels ($p<0.05$) (0.10 ± 0.009 IU/L V*s* 0.125 ± 0.003 IU/L). During the period of obscurity, FSH levels in the Rabbit male ranged from 0.10 to 0.25 IU/L (0.17 ± 0.03 IU/L V*s* 0.23 ± 0.010 IU/L). The progressive decrease in FSH and LH values suggests that exposing rabbits to darkness for 15 days reduces serum circulating FSH and LH levels.

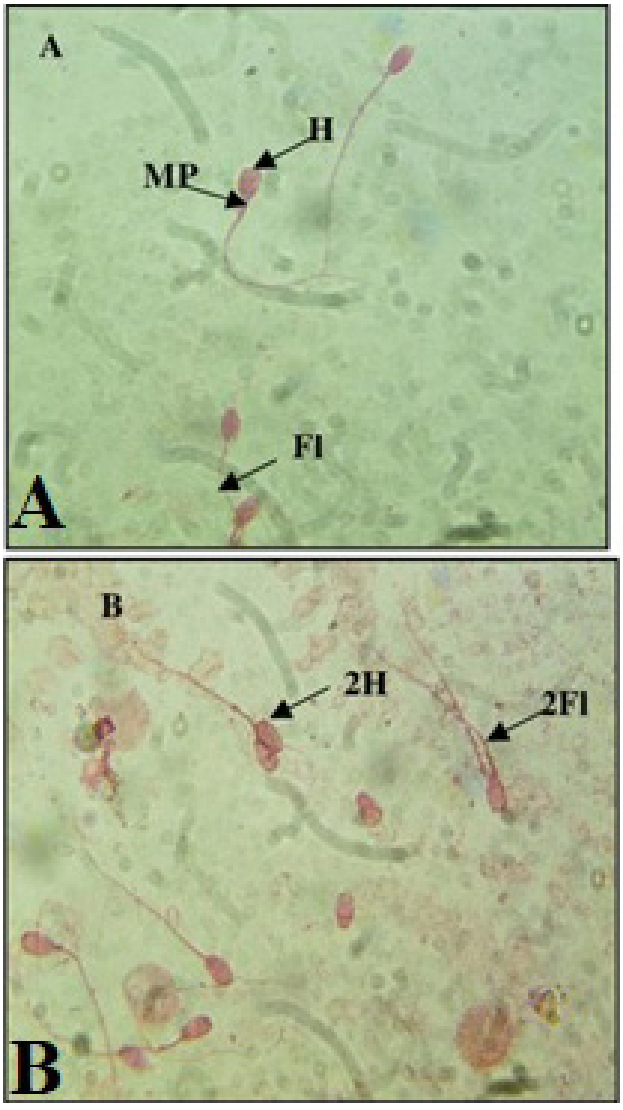


Fig. 1. Hematoxylin and Eosin sperm stained smear Rabbit (1000× magnification) of the described Group I control (A) and Group II (B) under darkness. H, head; MP, midpiece; Fl, flagellum.

## DISCUSSION

The primary mechanism governing annual coat change is photoperiodism. Growth is controlled by the pineal gland, which generates melatonin, and the pituitary gland,

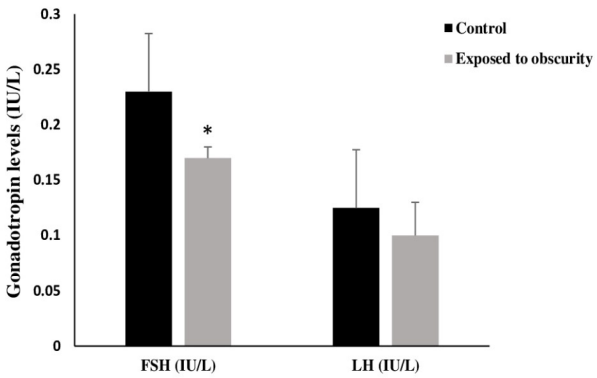


Fig. 2. Changes in the levels of gonadotropin hormone (FSH and LH) in male Rabbit after prolonged dark exposure for 15 days. Values are expressed as means ± SEM. *P <0.05 vs the control group by student's t-test.

which produces prolactin (Allain *et al*., 2002). Melatonin (also known as the hormone of darkness) is produced throughout the night and has an impact in the synchronization of the circadian cycles of physiological functions such as growing. The effect of melatonin treatment is similar to what happens when animals spend extended periods of time in the dark. Testis and epididymis weights, as well as daily sperm production and ovary weights, revealed significant seasonal change in the Mediterranean climate, with peak values in March and April (Gonçalves *et al*., 2002). Melatonin plays an essential function in reproduction in addition to its role in protecting against cellular damage (Reiter *et al*., 2000) by enhancing the activity of antioxidant enzymes and removing free radicals, particularly in the female and male gonads (Lampiao and Plessis, 2013). It's production by the pineal gland follows a circadian cycle, with low levels during the day and high levels at night (Brzezinski, 1997). Melatonin has been shown in animal studies to impact testicular function and there can be evidence that the pattern of melatonin secretion, which is controlled by photoperiod, has a direct impact on reproductive function. Seasonally reproducing mammals have provided much of the evidence (Malpaux *et al*., 1999; Yu *et al*., 2018).

On the other hand, melatonin, has been shown to inhibit reproductive activity particularly, Leydig cells in mice and rats (Ng and Lo, 1988; Persengiev *et al*., 1991). There are contradictory research findings regarding melatonin's effect on spermatozoa function. In seasonally breeding Syrian hamsters, day length altered copulatory behavior, as males stopped ejaculating after many weeks of being exposed to short day lengths (Powers *et al*., 1989; Miernicki *et al*., 1990).

In our study, exposure to permanent darkness for 15

days appeared to affect most reproductive characteristics in male rabbits. Sperm morphological modifications are defined by morphological anomalies in the spermatozoa's several sections, including the head, midpiece, and flagellum. Our results were confirmed by Luboshitzky *et al*. (2002) who showed that melatonin's effect on spermatozoa function has been reported to be variable. The administration of Melatonin to healthy males over time has been associated to lower sperm quality and significant impact on sperm concentration, motility and testosterone levels in healthy men. An in vitro study, on the other hand, discovered the administration of melatonin to human spermatozoa, increased progressive motility and decreased the number of static cells (Ortiz *et al*., 2011). This suggests that exposure to excessive levels of melatonin (permanent darkness) may be the cause of reproductive system damage.

Serum FSH and LH levels in exposed rabbits to permanent darkness, were significantly lower than in controls. Melatonin treatment inhibited the production of FSH and LH in male rats, affecting sexual maturation via decreasing FSH activation of Sertoli cells (Li and Zhou, 2015). Melatonin has been shown *in vitro* to suppress the stimulation of LH release by luteinising hormone-releasing hormone (LHRH) in pituitary cells from rat foetuses (Hattori *et al*., 1995; Li and Zhou, 2015). Changes in Ca2+ concentrations and cAMP accumulation may be associated with melatonin-induced declines in LH production by pituitary cells (Vanecek, 1998). $Ca^{2+}$ inflow or concentrations in pituitary cells increased the release of GnRH-induced LH, while melatonin administration partially inhibited this effect. Melatonin treatment partially inhibited this response, implying that melatonin's suppression of LH release is mediated by melatonin. Melatonin's effects may be mediated by decreases in intracellular concentrations of these second messengers. This might explain the decline in LH and FSH levels in rabbits exposed to complete darkness for 15 days.

Finally, natural light and artificial illumination have a variety of effects on reproductive parameters in farmed rabbits. Permanent darkness for 15 days results in high melatonin levels, which affects reproductive function (Sperm quality). This exploratory experiment, however, does not explain the reproduction characteristics that are directly influenced by permanent darkness.

## DECLARATIONS

*Acknowledgements*

We thank the team of Laboratory BPO/Endocrinology of the Faculty of Biological Sciences, USTHB. We also thank departement of Biology, Faculty of Biological Sciences and Agricultural Sciences, UMMTO and the team of the Laboratory Research on Dry Zones, Faculty of Biology, University Algers 1 and all authors contributed to the study.

*Ethical statement*

All experimental procedures were conducted in strict accordance with protocols approved by the institution and the guidelines for experimental animals. No death or disease in the rabbits were observed during the experimental period.

*Statement of conflicts of interest*

The author have declared no conflict of interest.

## REFERENCES

Allain, D., Malpaux, B., Puechal, F., Thébault, R.G., de Rochambeau, H. and Chemineu, P., 2002. Genetic variability of the pattern of night melatonin blood levels in relation to coat changes development in rabbits. *Genet. Sel. Evol.,* **36**: 207–216. https://doi.org/10.1051/gse:2003059

Alvarino, M.R. and Ubilla, E., 1993. Female reproduction physiology. In: *The control of the reproduction in the rabbit* (ed. M.R. Alvarino). Ediciones Mundi-Prensa, Madrid, Spain. pp. 33–50.

Brzezinski, A., 1997. Melatonin in humans. *N. Engl. J. Med*., **336**: 186-195. https://doi.org/10.1056/NEJM199701163360306

Chemineau, P., Malpaux, B., Delgadillo, J.A., Guerin, Y., Ravault, J.P., Thimonier, J. and Pelletier, J., 1992. Control of sheep and goat reproduction: Use of light and melatonin. *Anim. Reprod. Sci*., **30**: 157–184. https://doi.org/10.1016/0378-4320(92)90010-B

Curtis, S.K. and Amann, R.P., 1981. Testicular development and estabilishment of spermatogenesis in Holstein bulls. *J. Anim. Sci*., **53**: 1645-1657. https://doi.org/10.2527/jas1982.5361645x

Czeisler, C.A. and Klerman, E.B., 1999. Circadian and sleep-dependent regulation of hormone release in humans. *Recent Prog. Horm. Res*., **54**: 97-130.

Eurell, J.A. and Brian, L.F., 2006. *Dellmann's textbook of veterinary histology*. John Wiley & Sons.

Ewuola, E.O. and Equnike, G.N., 2010. Effects of dietary fumonisin B1 on the onset of puberty, semen quality, fertility rates and testicular morphology in male rabbits. *Reprodocution*, **139:** 439–445. https://doi.org/10.1530/REP-09-0077

Gonçalves, H., Alves, P.C. and Rocha, A., 2002. Seasonal variation in the reproductive activity of the wild rabbit (*Oryctolagus cuniculus algirus*) in a Mediterranean ecosystem. *Wildl. Res.,* **29**: 165–

173. https://doi.org/10.1071/WR00048

Hattori, A., Herbert, D.C., Vaughan, M.K., Yaga, K. and Reiter, R.J., 1995. Melatonin inhibits luteinizinghormone releasing hormone (LHRH) induction of LH release from fetal rat pituitary cells. *Neurosci. Lett*., **184**: 109–112. https://doi.org/10.1016/0304-3940(94)11181-H

Kruger, T.F., du Toit, T.C., Franken, D.R., Menkveld, R. and Lombard, C.J., 1995. Sperm morphology: Assessing the agreement between the manual method (strict criteria) and the sperm morphology analyzer IVOS. *Fertil. Steril*., **63**: 134-141. https://doi.org/10.1016/S0015-0282(16)57308-7

Lampiao, F. and Du Plessis, S.S., 2013. New developments of the effect of melatonin on reproduction. *World J. Obstet. Gynecol*., **2**: 8-15. https://doi.org/10.5317/wjog.v2.i2.15

Lebas, F., Coudert, P., De Rochambeau, H. and Thebault, R.G., 1996. *Le lapin: Elevage et pathologie*. Collection F.A.O: Production et santé animal. France. pp. 53-54.

Lebas, F., Coudert, P., Rouvier, R. and deRochambeu, H., 1997. *The rabbit husbandry, health and production*. Food and Agriculture organization of the United Nations. Rome. pp. 21-48.

Li, C. and Zhou, X., 2015. Melatonin and male reproduction. *Clin. Chim. Acta*, **446**: 175–180. https://doi.org/10.1016/j.cca.2015.04.029

Luboshitzky, R., Shen-orr, Z.I.L.L.A., Nave, R., Lavi, S. and Lavie, P., 2002. Melatonin administration alters semen quality in healthy men. *J. Androl*., **23**: 572-578. https://doi.org/10.1002/j.1939-4640.2002.tb02281.x

Malpaux, B., Thiéry, J.C. and Chemineau, P., 1999. Melatonin and the seasonal control of reproduction. *Reprod. Nutr. Dev.,* **39**: 355-366. https://doi.org/10.1051/rnd:19990308

Miernicki, M., Pospichal, M.W. and Powers, J.B., 1990. Short photoperiods affect male hamster socio-sexual behaviors in the presence or absence of testosterone. *Physiol. Behav*., **47**: 95–106. https://doi.org/10.1016/0031-9384(90)90046-7

Moustafa, A., 2020. Effect of light-dark cycle misalignment on the hypothalamic-pituitary-gonadal axis, testicular oxidative stress, and expression of clock genes in adult male rats. *Int. J. Endocrinol*., **2020**: 1-17. https://doi.org/10.1155/2020/1426846

Ng, T.B. and Lo, L.L., 1988. Inhibitory actions of pineal indoles on steroidogenesis in isolated rat Leydig cells. *J. Pineal Res*., **5**: 229-243. https://doi.org/10.1111/j.1600-079X.1988.tb00649.x

Ortiz, A., Espino, J., Bejarano, I., Lozano, G.M., Monllor, F., García, J.F. and Rodríguez, A.B., 2011. High endogenous melatonin concentrations enhance sperm quality and short-term *in vitro* exposure to melatonin improves aspects of sperm motility. *J. Pineal Res*., **50**: 132-139. https://doi.org/10.1111/j.1600-079X.2010.00822.x

Persengiev, S. and Kehajova, J., 1991. Inhibitory action of melatonin and structurally related compounds on testosterone production by mouse Leydig cells *in vitro*. *Cell Biochem. Funct*., **9**: 281-286. https://doi.org/10.1002/cbf.290090410

Powers, J.B., Steel, E.A., Hutchison, J.B., Hastings, M.H., Herbert, J. and Walker, A.P., 1989. Photoperiodic influences on sexual behavior in male Syrian hamsters. *J. Biol. Rhythms*., **4**: 61–78. https://doi.org/10.1177/074873048900400105

Reiter, R.J., Tan, D.X., Osuna, C. and Gitto, E., 2000. Actions of melatonin in the reduction of oxidative stress: A review. *J. Biomed. Sci*., **7**: 444-458. https://doi.org/10.1007/BF02253360

Vanecek, J., 1998. Melatonin inhibits release of luteinizing hormone (LH) via decrease of [Ca2+] and cyclic AMP. *Physiol. Res*., **47**: 329–335.

WHO, 1992. *World Health Organization. Laboratory manual for the examination of human semen and semen-cervical mucus interaction*. 3rd ed., Cambridge University Press. Cambridge.

Yu, K., Deng, S.L., Sun, T., Li Y.Y. and Liu, Y.X., 2018. Melatonin regulates the synthesis of steroid hormones on male reproduction: A review. *Molecules*, **23**: 447. https://doi.org/10.3390/molecules23020447