Journal of Infertility and Reproductive Biology
Volume 13, Issue 1, DOI 10.18502/jirb.v13i1.18779
Production and Hosting by KnE Publishing

KnE Publishing

Check for updates

Minireview

Sleep and Circadian Rhythm Association with Sperm Head Defects in Infertile Males

Anmol Garg and Mona Sharma*

Department of Reproductive Biology, AIIMS, New Delhi, India

Abstract

Male factor infertility can be due to defects in the semen quality and sperm parameters. The sperm head contains the acrosomal enzymes and the DNA required for fertilization. Defects in the sperm head morphology can be a major factor associated with male infertility. Lifestyle changes alter sleep and circadian rhythm which has shown to affect the quality of semen. Circadian rhythm is the normal cycle of physical, emotional, and behavioural changes the body undergoes every 24 hours. Circadian rhythm has an impact on sleep quality and spermatogenesis, thus disrupted circadian rhythm can alter sperm production. Sperm acrosome contain many proteins responsible for fertilization, acrosin being commonest of the proteases. Acrosin activity is essential for fertilization thus changes in the acrosin activity can affect fertilization outcome. This review will highlight the association of sleep and circadian rhythm with sperm acrosin activity and head defects.

Keywords: circadian rhythm, sleep, acrosin, clock

Corresponding Author: Mona Sharma; email: dr.mona18sharma@gmail.com

Received: 5 January 2025
Accepted: 7 March 2025
Published: 20 March 2025

Production and Hosting by KnE Publishing

OPEN ACCESS

1. INTRODUCTION

Infertility is a disease of the male or female reproductive system defined by the failure to achieve a pregnancy after 12 months or more of regular unprotected sexual intercourse [1]. Infertility is experienced by approximately 1 in 6 couples globally. Infertility is caused by male factors in 30%-50% percent of cases [2]. Male factor infertility can be due to defects in sperm structure and function. Sperm-related factors are among the most common reproductive challenges. These factors include abnormalities in sperm count, motility, morphology, and DNA integrity. A low sperm count (oligozoospermia), poor sperm motility (asthenozoospermia), and abnormal sperm shape (teratozoospermia) can all hinder the ability of sperm to successfully fertilize the egg. A man is considered to have teratozoospermia if the number of sperm with normal morphology in his ejaculate is less than 4% [3]. Structural components of mature spermatozoa include head and tail. Sperm head consists of nucleus and acrosome whereas tail has got 4 parts: connecting piece, midpiece, principal piece and end piece.

A normal sperm has an oval head (2.5-3.5μm wide, 5-6μm long), a tail (50μm), and an acrosome covering 40-70% of head area [4]. Morphology of the sperm plays a crucial role in fertilization as defect in morphology would affect sperm function. Defects in the tail (flagellum) can impair motility, reducing the sperm's ability to move towards the egg whereas defects in the head reduces sperm's ability to bind and penetrate the protective layers of egg and deliver its genetic material into the ooplasm. It has also been observed that sperm structure and function may get altered during infections or surgical procedures [5,6]. Sperm is also prone to undergo oxidative induced damage during cryostorage where sperm is stored under subzero temperatures. Depending on the methods of cryostorage, the impact may differ but functional compromise is sure to occur [7,8]. Studies have shown that oxidative stress alters sperm chromatin including telomeres and shelterin components [9,10]. Lifestyle changes can also lead to the defects in sperm production. Among the other lifestyle changes sleep has been found to be affecting the sperm physiology. Sleep, including bedtime, length, and quality greatly influence seminal quality influencing sperm parameters [11].

1.1. Effect of sleep on sperm parameters

Studies demonstrate that sleep plays a crucial role in regulating sperm function, as it influences hormonal balance, cellular repair, and overall reproductive health. Higher levels of testosterone are produced during sleep [12]. Disturbed testosterone levels will affect the sperm formation. In sleep deprived male rats lower levels of testosterone and higher levels of progesterone and glucocorticoids were seen [13]. Low subjective sleep quality was seen in the OAT (Oligo/astheno/teratozoospermia) patients [14]. The study showed that patients with high sleep disturbances assessed by the Karolinska sleep questionnaire had a 29% lower adjusted sperm concentration with scores>50 and 1.6% less morphologically normal sperm than those with scores of 11-20 [15]. Low numbers of sperm cells, their survival rates, and decreased number of progressively motile sperms were observed in those men sleeping for a short duration (<7h) and also in those sleeping for a long duration (>9h) [16]. A study found an inverted U-shaped association between

sleep duration and male fertility. Men who slept less than 6 hours or more than 9 hours per night had lower fertility ratios than those who slept for average 8 hours. Additionally, men who experienced sleep difficulties had lower fertility ratios compared to those who had no sleeping issues [17]. Sleep disturbances also affect the internal biological clock/circadian rhythm of the body.

1.2. Circadian Rhythm and Spermatogenesis

Circadian Rhythm is a biological clock maintained within the body of an organism. The biological clock helps organisms to recognize and act upon the changes in the environment. Internal chronological order is maintained and internal changes occur simultaneously due to the biological clock [18]. The transcriptional-translational feedback loop mechanism is exhibited by clock genes where CLOCK and BMAL1 are the positive regulators and period (Per 1, Per2, and Per 3) and Cryptochrome (Cry1 and Cry2) are the negative regulators. CLOCK/BMAL1 heterodimer binds to the E-Box of the DNA and regulates the transcription of various genes. The expression of other clock-related proteins, like Rev-erbα is also promoted by CLOCK/BMAL1, which further inhibits the formation of BMAL1.Thus many biological processes related to these factors are affected by the change in any of these factors [19] (Figure **1**). Spermatogenesis is among these biological factors that is affected by clock genes. Studies in different clock gene knock-out models have shown various defects in spermatogenesis [20].

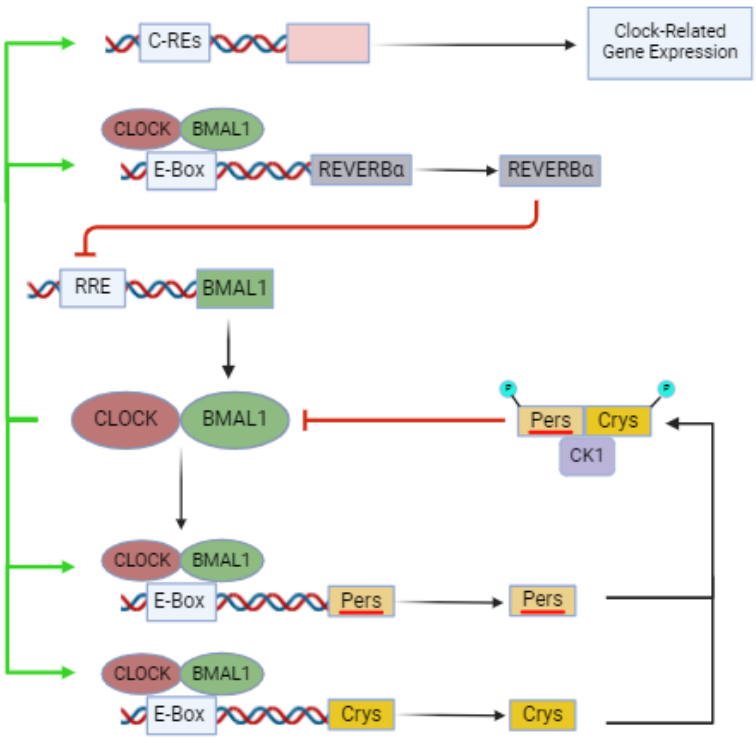

Figure 1: *Molecular signalling pathway of circadian rhythm: CLOCK/BMAL1heterodimer binds to E-box sites present in tshe promoter region, stimulating the production of essential clock transcription factors Pers and Crys which further inhibits binding of CLOCK/BMAL1 heterodimer to E-box. CLOCK/BMAL1 heterodimer also regulates the formation of REVERBα which further inhibits the formation of BMAL1. Clock transcription factors also control clock-controlled gene expression.*

Spermatogenesis is a biological process through which male germline cells develop into mature spermatozoa. Sperm mixed with secretions from accessory glands such as prostate and seminal vesicles released during ejaculation [21–24]. The process of spermatogenesis consists of mitotic division where spermatogonium undergoes mitosis to form primary spermatocyte and then meiotic divisions where primary spermatocyte undergoes meiosis I to form secondary spermatocyte and meiosis II to form spermatid and then differentiation process known as spermiogenesis to form mature sperm (Figure **2**). During spermiogenesis nuclear modification, acrosome formation and assembly of tail structures takes place. In nuclear modifications spermatid nucleus undergoes condensation and elongation. Histone proteins are replaced by protamines which allow the DNA to become highly compacted and transcriptionally inactive. Nuclear modification is followed by acrosome formation. The acrosome originates from the Golgi apparatus in early spermatids. Proacrosomal granules accumulate within the Golgi complex and fuse to form a large acrosomal vesicle. This vesicle attaches to the anterior end of the nucleus. Further acrosomal vesicle flattens and spreads over the nuclear surface to form the acrosomal cap. During development of acrosome the centrosome, pair of centrioles proximal and distal to the nucleus along with the accessory proteins, move to the side of nucleus which is opposite to the acrosomal development. Proximal centriole anchors the tail to the sperm head and participates in the formation of the connecting piece and the distal centriole initiates the formation of the axoneme, the basic structural unit of the sperm tail. Elongation of axoneme takes place away from the nucleus. Sperm mitochondria divide and elongate to form a mitochondrial sheath in the midpiece of tail. During tail formation excess cytoplasm is also removed as residual body [25].

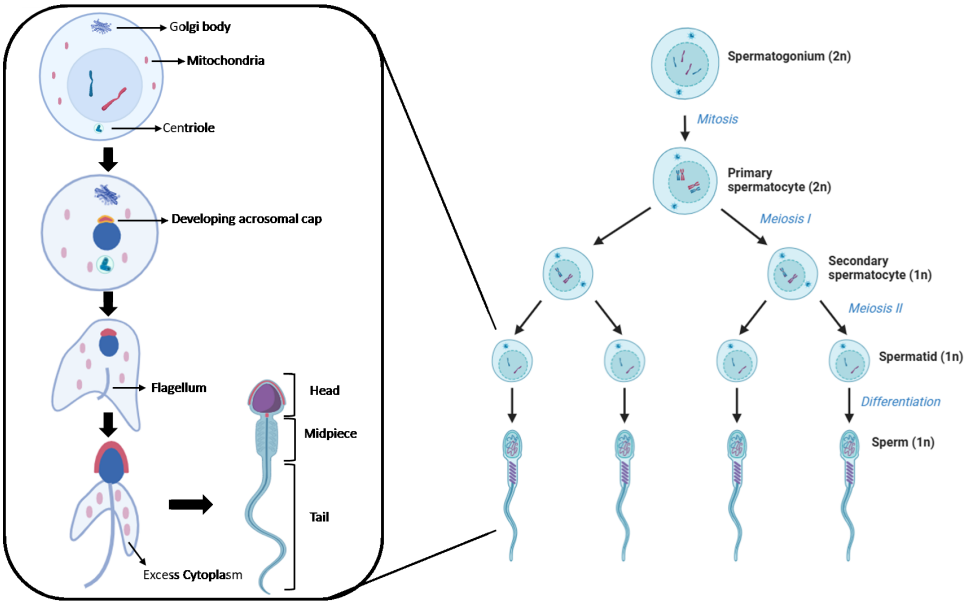

Figure 2: *Schematic diagram of spermatogenesis including changes during spermiogenesis.*

Spermatogenesis can be affected due to impaired circadian rhythm by various signalling pathways. The suprachiasmatic nucleus (SCN), the central circadian pacemaker in mammals, coordinates the timing of peripheral clocks by releasing signalling molecules, including hormones, to various organs. In male

reproductive system, the SCN receives environmental cues and transmits this temporal information to the testes to regulate spermatogenesis via the hypothalamic-pituitary-gonadal (HPG) axis. Changes in the light-dark cycle prompt the SCN to synchronize gonadotropin releasing hormone (GnRH) neurons in the brain, either through neural pathways or Kisspeptin neurons. GnRH then modulates testosterone production in Leydig cells by influencing luteinizing hormone (LH) signalling, which in turn impacts spermatogenesis (Figure **3**a) [26–28]. During spermatogenesis, homologous recombination takes place in the first meiotic division, enabling the exchange of genetic material between non-sister chromatids on homologous chromosomes. If homologous recombination is inhibited, it prevents the repair of DNA double-strand breaks in meiosis I, leading to spermatocyte death and a reduction in sperm count [29,30]. In prostate cancer cell lines Cry1 operate as a transcription factor to regulate homologous recombination (Figure **3**b) so it might be possible that presence of Cry1 gene also affect the homologues recombination occurring in spermatogenesis [31]. A germ granule, that is, chromatid body is located in the cytoplasm of mammalian germ cells, with a structure that changes throughout spermatogenesis. It first appears in late pachytene spermatocytes and persists until the late stages of elongating spermatids [32,33]. Researchers studying the chromatid body found the presence of BMAL1 and CLOCK proteins. In knockdown mice models for Bmal1 or Clock genes it was found that the chromatid body became fragmented, suggesting that these proteins are essential for its proper assembly (Figure **3**c) [34]. In mice spermatogenesis retinoic acid signalling is thought to play a crucial role by forming differentiated A1-type spermatogonia from undifferentiated A-type spermatogonia, also initiating meiosis through the regulation of genes such as Stra8, and influencing spermatid elongation (Figure **3**d) [35].

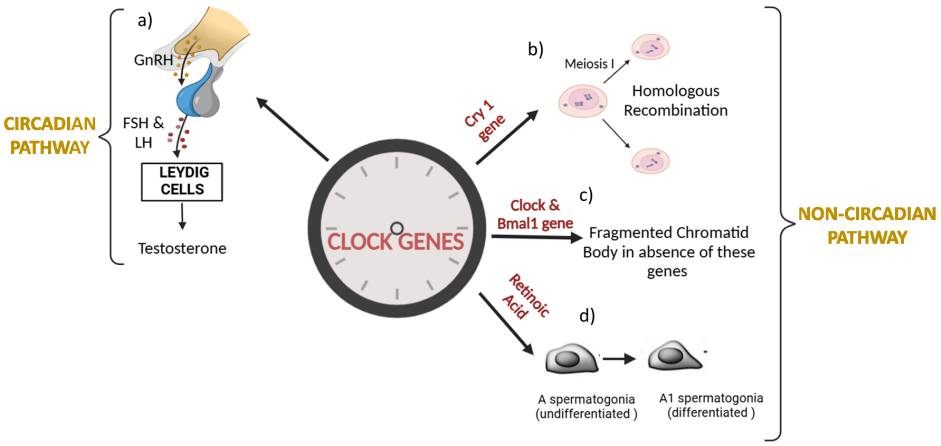

Figure 3: *Schematic Diagram of circadian and non-circadian pathway regulating spermatogenesis. a) Regulation of GnRH neurons by clock genes. b) Effect of Cry1 on homologous recombination as seen in prostate cancer cell lines. c) Effect of CLOCK and Bmal1 gene on chromatid body as seen in KO mice models. d) Effect of Retinoic Acid signalling on A type spermatogonia in mice.*

BMAL1, CLOCK, CRY1, PER1, and PER2 have been shown to be expressed in Sertoli cells, spermatogonia, spermatocytes, and the interstitial tissue of mouse testes, suggesting that these clock genes may play a crucial role in the regulation of spermatogenesis [36]. Various experimental studies conducted on clock gene knockout models have demonstrated disruptions in spermatogenesis, leading to altered sperm development and function. Double knockout model for Global Per1/Per2 in elderly male mice (15 months)

lead to decreased weight of testis and sperm motility, impaired testosterone synthesis and disordered spermatogenic cells [37]. Study on CRY1 knockout mice model revealed that the absence of CRY1 gene led to an increase in degenerated and apoptotic germ cells in the testis, as well as a reduced sperm count. Also, the mRNA expression of PER2 was elevated in the testis of CRY1 knockout mice compared to wild-type controls [38]. Decreased sperm count and quality can be related to disturbed circadian desynchrony. Circadian desynchrony means adapting sleep/wake cycles inconsistent with the inner biological clock. It can occur from many reasons such as job-related in the case of shift workers and non-job-related due to the use of mobile phones or other sleep disorders [39,40]. Circadian desynchrony affects the biological clock genes which can be linked to male infertility. A study demonstrated that infertile men with asthenozoospermia (low sperm motility) had lower expression levels of five clock genes, that is, BMAL1, Clock, CRY1, PER1, and PER2 as compared to the expression of these genes in healthy fertile men [41]. These findings suggest that clock genes play a critical role in regulating the processes involved in male fertility, and their absence can result in significant reproductive impairments.

1.3. Sperm Head Defects, Altered Circadain Rhythm and Acrosin activity

Abnormalities in sperm head can impair ability of sperm to bind and penetrate the egg barriers, essential for successful fertilization. The head of the sperm contains enzymes that help it pass through these layers. Sperm head is an important region bearing oocyte activation factor PLC zeta in the post-acrosomal area. Studies have correlated PLC zeta with sperm head morphology. Sperm extra-mitochondrial citrate synthase has also found to activate oocyte and its levels were found to be decreased in patients with ICSI failure [42–44]. Various sperm head defects include: sperm with abnormally large head (*macrocephaly*), sperm smaller head size (*microcephaly*), sperm head is too small and looks like a pin, *tapered* head where sperm head narrows toward the tip, *amorphous* head where sperm head has an irregular appearance without a defined structure, *pyriform* head where sperm head is pear shaped, *globozoospermia* where sperm head is round instead of oval with very less or no acrosome, vacuoles constituting more than $1/5^{th}$ of the head area or presence of any vacuoles in post acrosomal area, sperm head with acrosomal area less than 40% or more than 70% of head area or absence of acrosomal area (Figure **4**). These morphological defects can alter the acrosomal content thereby compromising the sperm fertilization potential.

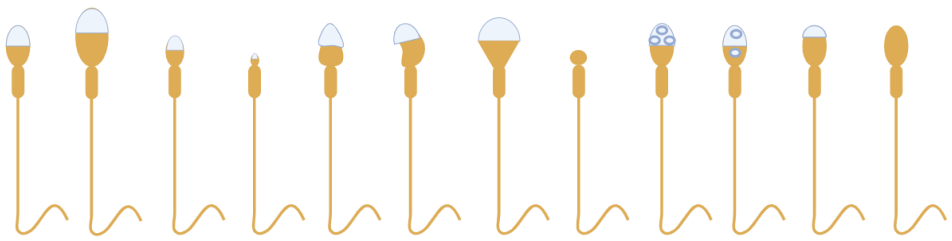

Figure 4: *Schematic Diagram of various sperm head defects: a) Normal Head shape; b) Macrocephaly; c) Microcephaly; d) Pin head; e) Tapered head; f) Amorphous head; g) Pyriform head; h) Globozoospermia (Round head); i) >2 Vacuoles; j) Vacuole in Post acrosomal area; k) Less acrosomal area; l) No acrosomal area.*

Acrosin is a serine protease present in the acrosomal area of the sperm head in proacrosin form (Figure **5**). It is required for the penetration of zona pellucida during fertilization. Studies showed that subjects having low acrosin-proacrosin activity were infertile irrespective of normal sperm motility. In vitro fertilization (IVF) can also fail due to aberrant proacrosin activation and lower acrosin activity [45–48]. Acr knockout hamsters showed the failure of sperm to penetrate the zona pellucida but no effect was seen on sperm and oolemma fusion [49]. Measuring acrosin activity can be useful in determining the best approach for couples undergoing ART (IVF or ICSI) [50]. As acrosin activity is important for fertilization many factors leading to male infertility can be by affecting this acrosin activity. Recent research studies have pointed out that sleep patterns and circadian rhythm may significantly impact male fertility. Irregular sleep cycles can potentially disrupt the quality and quantity of sperm produced this disruption can be by affecting the acrosin activity.

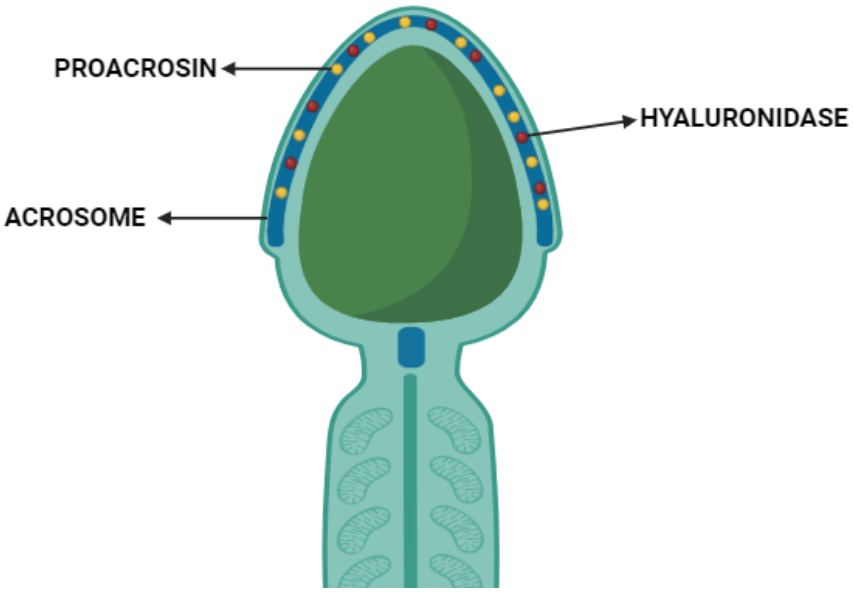

Figure 5: *Schematic Diagram of sperm head showing acrosome containing proteolytic enzymes hyaluronidase and proacrosin (inactive form of acrosin).*

Sperm head defects, such as abnormal morphology can be associated with reduced acrosin activity. Defects such as globozoospermia lack acrosome thus acrosin enzyme is absent and no acrosin activity has been observed [51]. Defects in the formation of acrosome in the sperm also decreases acrosin activity as low or no acrosin will be present.

During spermatogenesis spherical spermatids and growing acrosome are the only stages linked to CLOCK genes [52]. Studies performed in mice model with knockout CLOCK expression in round spermatids and testes revealed no significant changes in testes' weight and testosterone but CLOCK knockdown sperms had decreased acrosin activity and affected blastula development [53]. CLOCK knockdown animals were also checked for changes in the protein expression by proteomics technique. Serine protease inhibitors were found to be the two affected clusters focusing on Serpina3k, a serine/cysteine peptidase inhibitor from clade A. Increased Serpina3k was found in knockdown CLOCK

sperm. Serpina3k decreases the acrosin activity thus low acrosin activity is found in CLOCK knockdown sperm [54].

2. CONCLUSION

Lifestyle changes including sleep affects sperm parameters. Circadian Rhythm regulates spermatogenesis and therefore any disturbance in sleep/circadian rhythm can affect sperm parameters that would lead to male infertility. Studies in the animal models has shown, knockdown CLOCK genes affect the acrosin activity. Studies have shown that low acrosin activity is responsible for male infertility. Studies done in animal models have revealed that acrosin activity is also affected by the changes in the clock genes causing fertilization failure but no information is available for humans. As head defects are more common in infertility cases and as the disturbances in sleep/circadian rhythm are also causing male infertility it can be studied whether the defect in clock genes is one of the factors affecting acrosin activity in human infertility cases having sperm head defects.

References

[1] Infertility [Internet]. [cited 2024 Nov 27]. Available from: https://www.who.int/health-topics/infertility

[2] Eisenberg ML, Esteves SC, Lamb DJ, Hotaling JM, Giwercman A, Hwang K, et al. Male infertility. Nat Rev Dis Primer. 2023;9(1):1–22.

[3] WHO Laboratory Manual for the Examination and Processing of Human Semen. 6th ed. Geneva: World Health Organization; 2010. 1 p.

[4] Ammar O, Mehdi M, Muratori M. Teratozoospermia: Its association with sperm DNA defects, apoptotic alterations, and oxidative stress. Andrology. 2020;8(5):1095–106.

[5] Asuri K, Mohammad A, Prajapati OP, Sagar R, Kumar A, Sharma M, et al. A prospective randomized comparison of sexual function and semen analysis following laparoscopic totally extraperitoneal (TEP) and transabdominal pre-peritoneal (TAPP) inguinal hernia repair. Surg Endosc. 2021;35(6):2936–41.

[6] Dipankar SP, Kumar T, Itagi ABH, Naik BN, Kumar Y, Sharma M, et al. Semen Quality in Males Suffering From COVID-19: A Pilot Study. Cureus. 2022;14(11):e31776.

[7] Sharma Y, Sharma M. Biophysics of Cryopreservation. Int J Thermodyn. 2022;25(1):17–27.

[8] Sharma Y, Sharma M. Sperm Cryopreservation: Principles and Biology. J Infertil Reprod Biol. 2020;8(3):43–8.

[9] John A, Sharma M, Dada R, Mahey R, Halder A, Pandey D. Effect of Oxidative Stress on Sperm Shelterin TRF1 Expression and Chromatin Function in Infertile Males: Preliminary Findings. Glob J Reprod Med. 2024;10(5):GJORM.MS.ID.555800.

[10] Ankur, Sharma M, Halder A, Dada R, Mahey R, Kumar N. Effect of Oxidative Stress on Sperm TERRA Expression and Chromatin Function in Infertile Males: A Preliminary Finding. J Sci Res Rep. 2024;30(10):947–59.

[11] Wang Y, Chen Q, Liu K, Wang X, Yang H, Zhou N, et al. Sleep behavior is associated with over two-fold decrease of sperm count in a chronotype-specific pattern: path analysis of 667 young men in the MARHCS study. Chronobiol Int. 2021;38(6):871–82.

[12] Axelsson J, Ingre M, Akerstedt T, Holmbäck U. Effects of acutely displaced sleep on testosterone. J Clin Endocrinol Metab. 2005;90(8):4530–5.

[13] Andersen ML, Martins PJF, D'Almeida V, Bignotto M, Tufik S. Endocrinological and catecholaminergic alterations during sleep deprivation and recovery in male rats. J Sleep Res. 2005;14(1):83–90.

[14] Cai XF, Zhao JM, Yang YS, Wang CY, Nian MX, Huang JF, et al. Self-reported sleep quality and oligo/astheno/teratozoospermia among men attending an infertility clinic: a longitudinal study. Sleep Breath. 2023;27(4):1465–71.

[15] Jensen TK, Andersson AM, Skakkebæk NE, Joensen UN, Blomberg Jensen M, Lassen TH, et al. Association of sleep disturbances with reduced semen quality: a cross-sectional study among 953 healthy young Danish men. Am J Epidemiol. 2013;177(10):1027–37.

[16] Liu MM, Liu L, Chen L, Yin XJ, Liu H, Zhang YH, et al. Sleep Deprivation and Late Bedtime Impair Sperm Health Through Increasing Antisperm Antibody Production: A Prospective Study of 981 Healthy Men. Med Sci Monit. 2017;23:1842–8.

[17] Wise LA, Rothman KJ, Wesselink AK, Mikkelsen EM, Sorensen HT, McKinnon CJ, et al. Male sleep duration and fecundability in a North American preconception cohort study. Fertil Steril. 2018;109(3):453–9.

[18] Vitaterna MH, Takahashi JS, Turek FW. Overview of Circadian Rhythms. Alcohol Res Health. 2001;25(2):85–93.

[19] Nader N, Chrousos GP, Kino T. Interactions of the Circadian CLOCK System and the HPA Axis. Trends Endocrinol Metab. 2010;21(5):277–86.

[20] He M, Liu K, Cao J, Chen Q. An update on the role and potential mechanisms of clock genes regulating spermatogenesis: A systematic review of human and animal experimental studies. Rev Endocr Metab Disord. 2023;24(4):585–610.

[21] Kumar A, Sharma M, editors. *Basics of Human Andrology* [Internet]. Singapore: Springer; 2017 [cited 2024 Dec 1]. Available from: http://link.springer.com/10.1007/978-981-10-3695-8

[22] Sharma M, Gupta S, Dhole B, Kumar A. The Prostate Gland. In: Kumar A, Sharma M, editors. *Basics of Human Andrology: A Textbook* [Internet]. Singapore: Springer; 2017 [cited 2024 Dec 1]. p. 17–35. Available from: https://doi.org/10.1007/978-981-10-3695-8_2

[23] Gupta S, Sharma M, Kumar A. Seminal Vesicles. In: Kumar A, Sharma M, editors. *Basics of Human Andrology: A Textbook* [Internet]. Singapore: Springer; 2017 [cited 2024 Dec 1]. p. 37–46. Available from: https://doi.org/10.1007/978-981-10-3695-8_3

[24] Sharma M, Kumar A. The Sperm. In: Kumar A, Sharma M, editors. *Basics of Human Andrology: A Textbook* [Internet]. Singapore: Springer; 2017 [cited 2024 Dec 1]. p. 171–204. Available from: https://doi.org/10.1007/978-981-10-3695-8_12

[25] Dhole B, Kumar A. Spermatogenesis. In: Kumar A, Sharma M, editors. *Basics of Human Andrology* [Internet]. Singapore: Springer; 2017 [cited 2024 Nov 26]. p. 135–60. Available from: http://link.springer.com/10.1007/978-981-10-3695-8_10

[26] Baburski AZ, Andric SA, Kostic TS. Luteinizing hormone signaling is involved in synchronization of Leydig cell's clock and is crucial for rhythm robustness of testosterone production. *Biol Reprod*. 2019;100(5):1406–15.

[27] Walker WH. Testosterone signaling and the regulation of spermatogenesis. *Spermatogenesis*. 2011;1(2):116–20.

[28] Yao Y, Silver R. Mutual Shaping of Circadian Body-Wide Synchronization by the Suprachiasmatic Nucleus and Circulating Steroids. *Front Behav Neurosci*. 2022;16:877256.

[29] Xu X, Aprelikova O, Moens P, Deng CX, Furth PA. Impaired meiotic DNA-damage repair and lack of crossing-over during spermatogenesis in BRCA1 full-length isoform deficient mice. *Development*. 2003;130(9):2001–12.

[30] Zickler D, Kleckner N. Recombination, Pairing, and Synapsis of Homologs during Meiosis. *Cold Spring Harb Perspect Biol*. 2015;7(6):a016626.

[31] Shafi AA, McNair CM, McCann JJ, Alshalalfa M, Shostak A, Severson TM, et al. The circadian cryptochrome, CRY1, is a pro-tumorigenic factor that rhythmically modulates DNA repair. *Nat Commun*. 2021;12(1):401.

[32] Meikar O, Da Ros M, Korhonen H, Kotaja N. Chromatoid body and small RNAs in male germ cells. *Reproduction*. 2011;142(2):195–209.

[33] Parvinen M. The chromatoid body in spermatogenesis. *Int J Androl*. 2005;28(4):189–201.

[34] Peruquetti RL, de Mateo S, Sassone-Corsi P. Circadian proteins CLOCK and BMAL1 in the chromatoid body, a RNA processing granule of male germ cells. *PLoS One*. 2012;7(8):e42695.

[35] Endo T, Freinkman E, de Rooij DG, Page DC. Periodic production of retinoic acid by meiotic and somatic cells coordinates four transitions in mouse spermatogenesis. *Proc Natl Acad Sci U S A*. 2017;114(47):E10132–41.

[36] Morse D, Cermakian N, Brancorsini S, Parvinen M, Sassone-Corsi P. No circadian rhythms in testis: Period1 expression is clock independent and developmentally regulated in the mouse. *Mol Endocrinol*. 2003;17(1):141–51.

[37] Liu Q, Wang H, Wang H, Li N, He R, Liu Z. Per1/Per2 Disruption Reduces Testosterone Synthesis and Impairs Fertility in Elderly Male Mice. *Int J Mol Sci*. 2022;23(13):7399.

[38] Li C, Xiao S, Hao J, Liao X, Li G. Cry1 deficiency leads to testicular dysfunction and altered expression of genes involved in cell communication, chromatin reorganization, spermatogenesis, and immune response in mouse testis. *Mol Reprod Dev*. 2018;85(4):325–35.

[39] Liu S, Wing YK, Hao Y, Li W, Zhang J, Zhang B. The associations of long-time mobile phone use with sleep disturbances and mental distress in technical college students: a prospective cohort study. *Sleep*. 2019;42(2):zsy213.

[40] Roenneberg T, Wirz-Justice A, Merrow M. Life between clocks: daily temporal patterns of human chronotypes. *J Biol Rhythms*. 2003;18(1):80–90.

[41] Zhang P, Li C, Gao Y, Leng Y. Altered circadian clock gene expression in the sperm of infertile men with asthenozoospermia. *J Assist Reprod Genet*. 2022;39(1):165–72.

[42] Sharma Y, Sharma M, Halder A, Mahey R, Kumar N, Dipankar S. Temporal analysis of cryopreservation effects on human sperm phospholipase C zeta expression profile: Laboratory preliminary findings. *J Lab Physicians*. 2024;16(3):328–37.

[43] Kang W, Harada Y, Yamatoya K, Kawano N, Kanai S, Miyamoto Y, et al. Correction: Extra-mitochondrial citrate synthase initiates calcium oscillation and suppresses age-dependent sperm dysfunction. *Lab Invest*. 2020;100(4):665.

[44] Joginder AH, Sharma JB, Mahey R, Gupta S, Sharma M. A preliminary study of human sperm citrate synthase expression in patients with failed ICSI cycles. *J Res Biol*. 2020;10:2888–97.

[45] Chaudhury K, Das T, Chakravarty B, Bhattacharyya AK. Acrosin activity as a potential marker for sperm membrane characteristics in unexplained male infertility. *Fertil Steril*. 2005;83(1):104–9.

[46] Cui YH, Zhao RL, Wang Q, Zhang ZY. Determination of sperm acrosin activity for evaluation of male fertility. *Asian J Androl*. 2000;2(3):229–32.

[47] De Jonge CJ, Tarchala SM, Rawlins RG, Binor Z, Radwanska E. Acrosin activity in human spermatozoa in relation to semen quality and in-vitro fertilization. *Hum Reprod*. 1993;8(2):253–7.

[48] Menkveld R, Rhemrev JP, Franken DR, Vermeiden JP, Kruger TF. Acrosomal morphology as a novel criterion for male fertility diagnosis: relation with acrosin activity, morphology (strict criteria), and fertilization in vitro. *Fertil Steril*. 1996;65(3):637–44.

[49] Hirose M, Honda A, Fulka H, Tamura-Nakano M, Matoba S, Tomishima T, et al. Acrosin is essential for sperm penetration through the zona pellucida in hamsters. *Proc Natl Acad Sci U S A*. 2020;117(5):2513–8.

[50] Langlois MR, Oorlynck L, Vandekerckhove F, Criel A, Bernard D, Blaton V. Discrepancy between sperm acrosin activity and sperm morphology: significance for fertilization in vitro. *Clin Chim Acta*. 2005;351(1–2):121–9.

[51] Kruger TF, Haque D, Acosta AA, Pleban P, Swanson RJ, Simmons KF, et al. Correlation Between Sperm Morphology, Acrosin, and Fertilization in an IVF Program. *Arch Androl*. 1988;21(1):1–9.

[52] Alvarez JD, Chen D, Storer E, Sehgal A. Non-cyclic and Developmental Stage-Specific Expression of Circadian Clock Proteins During Murine Spermatogenesis. *Biol Reprod*. 2003;69(1):81–91.

[53] Liang X, Cheng S, Jiang X, He X, Wang Y, Jiang Z, et al. The Noncircadian Function of the Circadian Clock Gene in the Regulation of Male Fertility. *J Biol Rhythms*. 2013;28(3):208–17.

[54] Cheng S, Liang X, Wang Y, Jiang Z, Liu Y, Hou W, et al. The circadian Clock gene regulates acrosin activity of sperm through serine protease inhibitor A3K. *Exp Biol Med*. 2016;241(2):205–15.