REVIEW

The role of circadian rhythm in male reproduction

Ana Peterlin[a], *Tanja Kunej*[b], *and Borut Peterlin*[c]

Purpose of review
To integrate evidence on the role of circadian rhythm in male reproduction. Several studies report on various aspects of the association between the circadian system and male reproductive function in animals and humans both in physiological condition as well as in the case of subfertility.

Recent findings
Epidemiological data demonstrate diurnal and seasonal changes as well as the effect of sleep/wake cycles on the quality of semen. Rare and common genetic variation in circadian clock genes in humans and animal models support the role of circadian rhythms in male fertility in humans.

Summary
Current data support the modest effect of the circadian clock on male reproductive potential; however, the evidence available is still fragmented and inconclusive. Additional well designed and sufficiently powered studies are needed to delineate the role of the circadian clock both in cause and potential interventional and preventive approaches in male subfertility.

Keywords
animal models, circadian clock, epidemiology, genetics, male infertility

INTRODUCTION

Cause of male subfertility is still poorly understood. Moreover, reasons for the seemingly increased burden of subfertility in modern society are still mostly unknown. The circadian clock, which is a temporal program that developed as an adaptation on earth's rotation, is one of the most basic physiological mechanisms exerting its effect through the control of metabolism, endocrine and immune function as well as behavior. Due to its numerous systemic effects it would be reasonable to expect that circadian clock is also involved in mechanisms involved in human male reproduction. Disrupted eigen and external zeitgebers, which are increasingly prevalent in modern societies could well contribute through circadian disruption, social jet lag and circadian misalignment to impairment of male reproduction. Namely, when perturbed, impairment of the system may through the (dis)regulation of physiological processes such as cell cycle progression, hormone secretion, immune regulation contribute to several human diseases including cancer, diabetes, obesity, cardiovascular and neurodegenerative diseases and sleep disorder [1,2].

The circadian clock is both autonomous, based on cellular clocks which build rhythmic activity of tissues, organs and entire organism as well as controlled by synchronization and entrainment processes through environmental signals of the central pacemaker rooted in the suprachiasmatic nucleus. Molecular components of the circadian clock network represent positive and negative transcriptional–translational feedback loops of many genes. Testis a complex organ in which several cell types directly produce gametes, but are also the major source of circulating androgens with ultradian (multihour), infradian (multiday) and seasonal periodicity of the seminiferous epithelium, Sertoli and Leydig cells [3].

The current review article brings together evidence to stimulate further research on the role of the circadian system in male reproduction function in humans.

EPIDEMIOLOGY

Epidemiological evidence supports the role of the circadian clock on the normal male reproduction in

[a]Faculty of Medicine, Institute of Histology and Embryology, [b]Department of Animal Science, Biotechnical Faculty, University of Ljubljana and [c]Clinical Institute of Medical Genetics, University Medical Centre Ljubljana, Ljubljana, Slovenia

Correspondence to Borut Peterlin, MD, PhD, Clinical Institute of Medical Genetics, University Medical Centre Ljubljana, Ljubljana, Slovenia.
Tel: +386 1 5401137; fax: +386 1 5401137;
e-mail: borut.peterlin@guest.arnes.si

Curr Opin Endocrinol Diabetes Obes 2019, 26:313–316
DOI:10.1097/MED.0000000000000512

KEY POINTS

- Epidemiological studies support diurnal and seasonal changes as well as the effect of sleep/wake cycles on the quality of semen.
- Rare and common genetic variation in circadian clock genes in humans and animal models were associated with subfertility in males.
- Existing evidence available is still fragmented and inconclusive and additional studies are needed to delineate the role of the circadian clock both in cause and potential interventional and preventive approaches in male subfertility.

humans. Several studies suggested that annual rhythms of human conception rates were correlated with the season [4,5] as well as photoperiod, monthly averages of daily hours of sunshine, minimum and maximum temperature and humidity [6]. Moreover, the quality of semen changed both diurnally and seasonally [7]: semen samples collected in the early morning showed the highest levels in sperm concentration, total sperm count and normal morphology. Evidence for daily diurnal variance in sperm DNA fragmentation index was demonstrated both in man and mice [8]. In addition to diurnal variation in sperm parameters, seasonal variation has been reported in several studies. Increase in sperm concentration and total sperm count was reported in spring, while summer was associated with the highest percentage of normal morphology and significant decreases in sperm concentration and total sperm count [7]. Similarly, other studies reported better sperm parameters in normozoospermic and oligozoospermic men in spring in winter [9,10], while Giorgi *et al.* [11] provide evidence for a higher prevalence of samples with normal sperm pH during spring and higher volume of sperm in winter.

An additional level of evidence for the role of the circadian clock in male reproduction comes from circadian rhythm disruptors including sleep. Sleep/wake cycles are intertwined with the circadian system and several lines of evidence link sleep with male reproduction [12]. Sleep duration was associated with testis volume [13], male fecundability [14▪], total sperm number and semen volume [15,16], sperm chromatin integrity [17], while the effect of sleep duration on testosterone concentrations is not conclusive [18,19]. Significantly, another circadian clock disruptor, shift work, was so far not convincingly associated neither with semen parameters nor testosterone levels [20–23].

To convincingly detect relatively small effects of the circadian clock on male fertility, future epidemiological studies should involve large groups of men and control for relevant cofounders.

ANIMAL STUDIES

Strong evidence for the involvement of circadian clock in male reproduction stems from the animal models. Mutations in some key regulators of the circadian clock are associated with reduced male fertility in animal models although the mechanisms are not totally elucidated yet. Mice with homozygous mutations in the *Clock* gene have reduced male fertility [24,25] and it was proposed that the serine protease inhibitor *Serpina3k* is involved in the pathogenic mechanism by regulating acrosin activity [26]. Furthermore, it was suggested that CLOCK can interact with RANBP9 and bind several key transcripts in mouse spermatogenesis [27].

Mutations in another central circadian clock genes, *Bmal1* are associated with male infertility [28]. Male knockout mice had low-testosterone and high-luteinizing hormone concentrations suggesting steroidogenesis impairment in testes and other steroidogenic tissues. Peruquetti *et al.* [29] provided evidence that both BMAL1 and CLOCK proteins contribute to assembly and physiology of the chromatoid body, the cytoplasmic organelle playing an important role during the late steps of germ-cell differentiation. It was also reported that BMAL1 protein contributes to the maintenance of neural circuits that drive pheromone-mediated mating behaviors [30].

The abnormal testicular function was also found in knockout mice for cryptochrome 1 (*Cry1*) gene [31]. Namely, *Cry1* deficiency increased testicular germ cell apoptosis and decreased sperm count and was associated with differential expression of 375 testicular genes. In Drosophila melanogaster loss of circadian clock function related to the mutated period (per0), timeless (tim0) genes lead to decreased reproductive function in males [32].

GENETIC STUDIES IN HUMANS

There is limited evidence for the involvement of rare, functional genetic variation in male infertility. Whole exome sequencing revealed a homozygous mutation in *NPAS2* gene in a single Turkish family with nonobstructive azoospermia [33]. Rare functional genetic variation in human circadian genes have been reported in human Cryptochrome 1 (*CRY1*) [34], *CRY2* [35] genes, *TIM* (*TIMELESS*) [36] and *CKIδ* [37] genes, that resulted in delayed sleep phase disorder and familial advanced sleep phase syndrome. In none of them, male subfertility was reported; however, the number of families was too

small to formally evaluate male reproductive potential.

Further evidence for the impact of human genetic variation on male fertility comes from genetic association studies. Genetic variability in the *CLOCK* gene was associated with semen quality in idiopathic infertile men [38] and with increased risk for male infertility [39,40].

CONCLUSION

Presented epidemiological data, as well as genetic studies in humans and animals support the contribution of circadian clock in male fertility. Based on the current data, we could hypothesize that both circadian rhythm disruptors as well as genetic variation in genes coding for key regulators of circadian clock could modestly modify male reproductive potential. Nevertheless, the evidence available is still fragmented and inconclusive, studies are frequently underpowered. Therefore, additional well designed studies are needed to delineate the role of the circadian clock both in cause and potential interventional and preventive approaches in male subfertility.

Acknowledgements

None.

Financial support and sponsorship

The current study was supported by Slovenian Research Agency.

Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- ■ of special interest
- ■■ of outstanding interest

1. Xie Y, Tang Q, Chen G, *et al.* New insights into the circadian rhythm and its related diseases. Front Physiol 2019; 10:682.
2. Roenneberg T, Merrow M. The circadian clock and human health. Curr Biol 2016; 26:R432–R443.
3. Bittman EL. Timing in the testis. J Biol Rhythm 2016; 31:12–36.
4. Cowgill UM. Season of birth in man. Contemporary situation with special reference to Europe and the Southern Hemisphere. Ecology 1966; 47:614–623.
5. Cypryjański J. Changes in seasonality of births in Poland in the years 1900–2009. Demogr Res 2019; 40:1441–1454.
6. Roenneberg T, Aschoff J. Annual rhythm of human reproduction: II. Environmental correlations. J Biol Rhythm 1990; 5:217–239.
7. Xie M, Utzinger K, Blickenstorfer K, Leeners B. Diurnal and seasonal changes in semen quality of men in subfertile partnerships. Chronobiol Int 2018; 35:1–10.
8. Ni W, Liu K, Hou G, *et al.* Diurnal variation in sperm DNA fragmentation: analysis of 11,382 semen samples from two populations and in vivo animal experiments. Chronobiol Int 2019; 36:1455–1463.
9. Ozelci R, Yılmaz S, Dilbaz B, *et al.* Seasonal variation of human sperm cells among 4,422 semen samples: a retrospective study in Turkey. Syst Biol Reprod Mec 2016; 62:1–8.
10. Pol P, Beuscart R, Leroy-Martin B, *et al.* Circannual rhythms of sperm parameters of fertile men. Fertil Steril 1989; 51:1030–1033.
11. Giorgi DA, Volpi R, Tiseo R, *et al.* Seasonal variation of human semen parameters: a retrospective study in Italy. Chronobiol Int 2015; 32:711–716.
12. Liu PY. A clinical perspective of sleep and andrological health: assessment, treatment considerations and future research. J Clin Endocrinol Metab 2019; 104:4398–4417.
13. Zhang W, Piotrowska K, Chavoshan B, *et al.* Sleep duration is associated with testis size in healthy young men. J Clin Sleep Med 2018; 14: 1757–1764.
14. ■ Wise L, Rothman K, Wesselink A, *et al.* Male sleep duration and fecundability in a North American preconception cohort study. Fertil Steril 2018; 109:453–459.

Supporting the evidence of the influence of sleep-wake cycle on male fertility. Short sleep duration in men was associated with reduced ability to conceive in the North American cohort of couples.

15. Chen Q, Yang H, Zhou N, *et al.* Inverse U-shaped association between sleep duration and semen quality: longitudinal observational study (MARHCS) in Chongqing, China. Sleep 2016; 39:79–86.
16. Jensen T, Andersson A-M, Skakkebæk N, *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: 1027–1037.
17. Wang X, Chen Q, Zou P, *et al.* Sleep duration is associated with sperm chromatin integrity among young men in Chongqing, China. J Sleep Res 2018; 27:e12615.
18. Leproult R, Cauter E. Effect of 1 week of sleep restriction on testosterone levels in young healthy men. JAMA 2011; 305:2173–2174.
19. Smith I, Salazar I, RoyChoudhury A, St-Onge M-P. Sleep restriction and testosterone concentrations in young healthy males: randomized controlled studies of acute and chronic short sleep. Sleep Health 2019. [Epub ahead of print]
20. Irgens Å, Krüger K, Ulstein M. The effect of male occupational exposure in infertile couples in Norway. J Occup Environ Med 1999; 41:1116–1120.
21. El-Helaly M, Awadalla N, Mansour M, El-Biomy Y. Workplace exposures and male infertility – a case–control study. Int J Occup Med Env 2011; 23:331–338.
22. Axelsson J, Ingre M, Åkerstedt T, Holmbäck U. Effects of acutely displaced sleep on testosterone. J Clin Endocrinol Metabolism 2005; 90:4530–4535.
23. Deng N, Kohn TP, Lipshultz LI, Pastuszak AW. The relationship between shift work and men's health. Sex Med Rev 2018; 6:446–456.
24. Liang X, Cheng S, Jiang X, *et al.* The noncircadian function of the circadian clock gene in the regulation of male fertility. J Biol Rhythm 2013; 28: 208–217.
25. Dolatshad H, Campbell EA, O'Hara L, *et al.* Developmental and reproductive performance in circadian mutant mice. Hum Reprod 2006; 21:68–79.
26. Cheng S, Liang X, Wang Y, *et al.* The circadian Clock gene regulates acrosin activity of sperm through serine protease inhibitor A3K. Exp Biol Med 2015; 241:205–215.
27. Yang J, Zhang Z, Zhang Y, *et al.* CLOCK interacts with RANBP9 and is involved in alternative splicing in spermatogenesis. Gene 2018; 642 (J. Physiol. Paris 100 2006):199–204.
28. Alvarez JD, Hansen A, Ord T, *et al.* The circadian clock protein BMAL1 is necessary for fertility and proper testosterone production in mice. J Biol Rhythm 2008; 23:26–36.
29. 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:e42695.
30. Schoeller EL, Clark DD, Dey S, *et al.* Bmal1 is required for normal reproductive behaviors in male mice. Endocrinology 2016; 157:4914–4929.
31. Li C, Xiao S, Hao J, *et al.* 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; 85:325–335.
32. Beaver L, Gvakharia B, Vollintine T, *et al.* Loss of circadian clock function decreases reproductive fitness in males of *Drosophila melanogaster*. Proc National Acad Sci U S A 2002; 99:2134–2139.
33. Ramasamy R, Bakırcıoğlu EM, Cengiz C, *et al.* Whole-exome sequencing identifies novel homozygous mutation in NPAS2 in family with nonobstructive azoospermia. Fertil Steril 2015; 104:286–291.
34. Patke A, Murphy PJ, Onat O, *et al.* Mutation of the human circadian clock gene CRY1 in familial delayed sleep phase disorder. Cell 2017; 169: 203–215.e13.
35. Hirano A, Shi G, Jones CR, *et al.* A cryptochrome 2 mutation yields advanced sleep phase in humans. Elife 2016; 5:e16695.
36. Kurien P, Hsu P-K, Leon J, *et al.* TIMELESS mutation alters phase responsiveness and causes advanced sleep phase. Proc Nat Acad Sci U S A 2019; 116:12045–12053.

37. Xu Y, Padiath QS, Shapiro RE, *et al.* Functional consequences of a CKIdelta mutation causing familial advanced sleep phase syndrome. Nature 2005; 434:640.

38. Zhang J, Ding X, Li Y, *et al.* Association of CLOCK gene variants with semen quality in idiopathic infertile Han-Chinese males. Reprod Biomed Online 2012; 25:536–542.

39. Hodžić A, Ristanović M, Zorn B, *et al.* Genetic variation in circadian rhythm genes CLOCK and ARNTL as risk factor for male infertility. PLoS One 2013; 8:e59220.

40. Shen O, Ding X, Nie J, *et al.* Variants of the CLOCK gene affect the risk of idiopathic male infertility in the Han-Chinese population. Chronobiol Int 2015; 32:959–965.