Critical Roles of the Circadian Transcription Factor BMAL1 in Reproductive Endocrinology and Fertility

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Brain and muscle aryl-hydrocarbon receptor nuclear translocator like protein1 (BMAL1), a core component of circadian oscillation, is involved in many physiological activities. Increasing evidence has demonstrated the essential role of BMAL1 in reproductive physiology. For instance, BMAL1-knockout (KO) mice were infertile, with impaired reproductive organs and gametes. Additionally, in BMAL1-KO mice, hormone secretion and signaling of hypothalamus-pituitary-gonadal (H-P-G) hormones were also disrupted, indicating that H-P-G axis was impaired in BMAL1-KO mice. Moreover, both BMAL1-KO mice and BMAL1-knockdown by small interfering RNA (siRNA) in vitro cultured steroidogenic cells showed that BMAL1 was associated with gonadal steroidogenesis and expression of related genes. Importantly, BMAL1 also participates in pathogenesis of human reproductive diseases. In this review, we elaborate on the impaired reproduction of BMAL1-KO mice including the reproductive organs, reproductive endocrine hormones, and reproductive processes, highlighting the vital role of BMAL1 in fertility and reproductive endocrinology.

Keywords: circadian gene BMAL1, reproductive endocrine disorder, fertility, circadian rhythm, reproduction

INTRODUCTION

Circadian rhythms play essential roles in various physiological processes as well as the development of organisms, which are now known to be largely controlled by a group of transcription factor genes. In mammals, such molecular clock is expressed and operational in most cells of the body (1). The central clock in the SCN (suprachiasmatic nucleus) is the master circadian oscillator that synchronizes the peripheral clocks (2, 3). The circadian clock transcription factor, BMAL1 (brain and muscle aryl hydrocarbon receptor nuclear translocator like protein 1), also known as ARNTL1 or MOP3, which is expressed in most tissues especially in endocrine tissues, is a member of the bHLH-PAS (basic helix loop helix-period aryl hydrocarbon receptor nuclear translocator single-minded) family of transcription factors (4–7). BMAL1 and its partner, CLOCK (circadian
locomotor output cycles kaput), heterodimerize and bind to E-box elements in the promoter regions of clock-controlled genes to initiate their transcription, thereby driving the circadian rhythm (7).

A variety of physiological functions of BMAL1 have been identified. Studies have revealed that BMAL1-knockout (KO) mice have multiple defects including premature aging (8, 9), skeletal dysplasia (10–12), abnormal hair growth (13, 14), and especially poor reproduction (15–17). BMAL1-KO mice were infertile (15–17), with defective gonads and gametes (15, 18–20). Moreover, the hypothalamus–pituitary–gonad (H-P-G) axis was found dysfunctional in these animals, including the abnormal secretion and signaling of gonadal steroids (19, 21, 22), gonadotropins (15, 23), and gonadotropin-releasing hormones (GnRH) (24). Also, BMAL1-KO mice exhibited problematic mating behaviors (25), uterine decidualization (26, 27) and embryo implantation (22). Moreover, accumulating clinical evidence has suggested that BMAL1 also participates in human reproductive diseases (26–29), suggesting BMAL1 as a potential therapeutic target for related reproductive diseases.

**BMAL1 IN REPRODUCTIVE ORGAN DEVELOPMENT AND FERTILITY**

Various clues indicate that the development of the reproductive organs in BMAL1-KO mice is impaired (9, 16, 17, 22, 23, 30). Among such studies, these mice were reported to be arrhythmic under free-running conditions (constant darkness, constant temperature), with delayed puberty, irregular estrus cycles, and infertility (15, 16, 30). These fertility-impaired mice usually presented with structural and functional abnormalities in reproductive organs and gametes.

**Effect of BMAL1 Knockout on Female Reproduction in Mice**

Female BMAL1-KO mice displayed undersized and underdeveloped ovaries with a significant reduction in the number of corpora lutea and a higher proportion of atretic follicles (15). Despite these ovarian abnormalities, BMAL-KO female mice can ovulate; however, the quantity and quality of the oocytes are reduced (15, 16, 22, 31). A couple of studies reported that female BMAL1-KO mice had fewer ovulated oocytes and more abnormal oocytes after superovulation (15, 22, 32), which resembles the phenotypes of elder wild-type (WT) mice (15, 33), consistent with the premature aging feature of BMAL1-KO mice (9, 34). In addition, the BMAL1-KO mice exhibited abnormal follicle development, lower fertilization rate and retarded early embryo/blastocyst development (15, 32). Of note, although both in vivo fertilization in female BMAL1-KO mice and in vitro fertilization using BMAL1-KO oocytes were found to be impaired, the success rate of the in vitro fertilization was higher compared to that of in vivo fertilization in female BMAL1-KO mice (32), suggesting that the environment for fertilization and/or early embryo development might be affected by BMAL deletion. Further studies revealed high reactive oxygen species (ROS) levels in ovaries and oviducts of the female BMAL1-KO mice (32). Consistently, BMAL1 was found as a direct regulator for ROS homeostasis (9, 34). Although ROS are important regulators of various physiological processes (35), excessive ROS could be detrimental for normal reproductive activities (36–38). Therefore, the excessive ROS in ovaries and oviducts may account for the defective follicular development, fertilization and early embryo development in female BMAL1-KO mice.

In addition, female BMAL1-KO mice had undersized uteri (15). Recent studies have revealed that BMAL1 is associated with trophoblast invasion of the uterus decidualization (26, 27), which is a prerequisite for embryo implantation (39, 40). Furthermore, female BMAL1-KO mice were reported to experience an unsuccessful implantation (22, 32), since well-fertilized oocytes and well-developed preimplantation embryos could be observed in the reproductive tract on the implantation day (22). Therefore, the compromised implantation process of female BMAL1-KO mice can contribute to the impaired fertility of female BMAL1-KO mice (22). These structural abnormalities of the uterus and oviducts correlated with functional defects (Figure 1).

**Effect of BMAL1 Knockout on Male Reproduction in Mice**

Similar to the findings noted in females, male BMAL1-KO mice contained abnormal gonad and gametes. Male BMAL1-KO mice possessed slightly smaller testes, markedly smaller seminal vesicles, and generally reduced seminiferous tubule diameters (9, 23). The in vitro culture of TM3 Leydig cells have shown that silencing the BMAL1 gene might contribute to the apoptosis of Leydig cells which produce testosterone (41).

Male BMAL1-KO mice exhibited a nearly 70% reduction in sperm count (23). However, other characteristics, including sperm motility, sperm capacitation, and the outcome of in vitro fertilization were normal, although there was a trend of slightly decreased successful fertilization (23). Consequently, the aforementioned findings, such as the decreased sperm counts and quality, could not serve as solid evidence to explain the infertility of male BMAL1-KO mice. Apart from that, BMAL1 might also directly affect the structure of sperm. The chromatoid body (CB) is a special structure in pachytene spermatocytes and spermatids, which plays a pivotal role in gametogenesis during meiosis and spermatogenesis (42–44). Of interest, BMAL1 depletion converted the CB into fragments in round spermatids (18), and BMAL1 expression declined with abnormal CB structures and functions with age (45). As CB functions in mRNA silencing and translational regulation, which are important for spermatogenesis, BMAL1 might interfere with such procedures in spermatogenesis (18). The abnormalities of the sperm might also contribute to the reduced fertility of BMAL1-KO mice. Furthermore, male BMAL1-KO mice failed to mate with receptive WT female mice, showing no mating behaviours (23, 25). The underlying mechanism will be outlined in the following section.
In conclusion, male BMAL1-KO mice exhibit defective gonads and abnormal mating behavior, factors that might account for male infertility (Figure 1).

**BMAL1 IN HYPOTHALAMUS-PITUITARY-GONAD REGULATORY AXIS**

Poor gonadal development and dysfunction usually indicate abnormal gonadotropins and GnRH levels. The secretion of gonadotropins, including luteinizing hormone (LH) and follicle-stimulating hormone (FSH), is controlled by upstream signals derived from the hypothalamus. There, GnRH is consistently recognized as the master regulator for the secretion of downstream gonadotropin, including FSH and LH derived from the pituitary (46–51). Additionally, another stimulator of gonadotropins, kisspeptin derived from Kiss1 neurons, especially in the anteroventral periventricular nucleus of the hypothalamus, is significantly associated with a GnRH surge and induces gonadotropins secretion; thus it could directly affect pituitary gonadotrophs to release LH and FSH (47, 49, 52–56).

**Effect of BMAL1 Knockout on Hypothalamus/Pituitary Gonad Hormones in Mice**

BMAL1-KO mice exhibited abnormal pituitary gonadotropin levels (15, 31, 57). In female mammals, ovulation occurs after a pro-estrous LH surge triggered by estradiol peaks (58). However, female BMAL1-KO mice did not present with an LH surge (57). Meanwhile, male BMAL1-KO mice exhibited significantly altered FSH levels both in serum and in the whole pituitary, with reduced mRNA expressions of FSHb in the pituitary (23, 25). Thus, BMAL1 absence could affect the secretion of gonadotropin from the pituitary.

To determine the reasons for abnormal gonadotropin levels, more studies have been conducted by focusing on the signaling mechanisms in the hypothalamus. GnRH secretion in BMAL1-KO mice was in normal range (57). In the meantime, Kiss1 neurons show normal c-Fos expression in Female BMAL1-KO mice at the time of the LH surge, suggesting a normal responding state of these neurons (57). Furthermore, female BMAL1-KO mice showed an enhanced LH response to exogenous kisspeptin and a normally induced LH response to exogenous GnRH, suggesting unimpaired reactivity in the pituitary of these female mice and an even higher sensitivity to exogenous kisspeptin (57). Similarly,
male BMAL1-KO mice also exhibited higher sensitivity to kisspeptin and GnRH, with normal expression of GnRH and Kisspeptin in the hypothalamus (25). Notably, the GnRH receptor (GnRHR) gene was established to contain an E-box in the promoter region, and was under the regulation of BMAL1 (59). In Female BMAL1-KO mice, the expression of GnRHR in the pituitary remained unchanged, but that in Male BMAL1-KO mice was reduced (25). Therefore, BMAL1 participates in signaling from the hypothalamus to the pituitary. However, in terms of the lost LH surge and the abnormal reactivity of the pituitary to the hypothalamus in BMAL1-KO mice, there might be some additional mechanisms underlying the abnormal signaling from the hypothalamus to the pituitary.

Deleting BMAL1 specifically in important cells associated with the GnRH/LH pathways also resulted in alterations in gonadotropin release and response patterns (60). Conditional BMAL1 knockout in arginine vasopressin (AVP) neurons, which were demonstrated to play a specific role in the LH surge, resulted in a low and delayed LH surge; In KISS1 neuron conditional BMAL1-KO mice, the secretion pattern with double peaks and an altered surge time of induced LH were noted. Furthermore, in GnRH-neuron conditional BMAL1-KO mice, the LH surge was noted disappeared at a specific time, and the amplitude was found to be low (60). Such cells played important roles in hypothalamus endocrine signal transmission and were also proved to participate in mechanisms involving in GnRH/LH signaling (60). Therefore, the altered timing and pattern of the LH surge in these mice indicated that BMAL1 in these cells was involved in the transmission and feedback of hormones from the hypothalamus to the pituitary.

Altogether, BMAL1 may affect hormone secretion and signaling of hypothalamus and pituitary.

**Effect of BMAL1 Knockout on Hypothalamus-Pituitary Regulation of Reproduction in Mice**

LH and FSH are considered pivotal signals for gonadal development, follicle development, spermatogenesis, gonadal steroidogenesis, constitute the key steps for reproductive outcomes (61–67).

Female BMAL1-KO mice did not have an LH surge, an important stimulus for periodic ovulation. It is possible that the impaired quantity and quality of the superovulated oocytes of female BMAL1-KO mice were associated with the phenomenon (15). Apart from ovulation, LH also plays a significant role in the maintenance of corpora lutea (68, 69). This was consistent with the reduced number of corpora lutea and the disordered structure of the ovaries (15). Moreover, the delayed puberty and the disordered estrous cycle might also be attributed to the disrupted production of hormone signals (15). Such abnormal hypothalamus and pituitary hormone secretion and signaling in female BMAL1-KO mice might be associated with the impaired fertility.

Male BMAL1-KO mice had higher levels of LH, a hormone that primarily functions through Leydig cells to produce testosterone for male reproduction. Thus, the higher LH level in male BMAL1-KO mice was in line with the comparatively low testosterone levels. FSH facilitates male reproduction by promoting the proliferation of Sertoli cells and spermatogonia, thereby increasing sperm counts in synergy with testosterone (70–72). Therefore, it is possible that the lower levels of FSH and testosterone in male BMAL1-KO mice were responsible for the decreased sperm counts. Moreover, male BMAL1-KO mice showed reduced hypothalamic expressions of vasoactive intestinal peptide (Vip) and tyrosine hydroxylase (Th) (25), both of which are associated with the mating behaviors of male mice (73–75). The abnormal hypothalamus and pituitary hormone secretion and signaling of male BMAL1-KO mice were possibly associated with low sperm counts and impaired mating behaviors in these mice.

Altogether, BMAL1 may affect hormone secretion and signaling of hypothalamus and pituitary, thus affecting the fertility of BMAL1-KO mice.

**BMAL1 in Gonadal Hormone Production**

Reproductive activities are usually associated with gonadal hormones. Indeed, abnormal gonadal hormone levels might directly result in severe defects in the reproduction of BMAL1-KO mice.

**Effect of BMAL1 Deficiency on Gonadal Hormone Secretion and Actions**

In females, progesterone and estradiol are considered crucial hormones for embryo implantation and maintenance (39). However, BMAL1 depletion impaired female gonadal hormone secretion. Indeed, female BMAL1-KO mice showed decreased progesterone levels during the estrus cycle and early gestation (15, 22, 76). BMAL1 knockdown by small interfering RNA (siRNA) in *in vitro* cultured porcine granulosa cells and luteinizing granulosa cells of rat and human, also led to a decrease in progesterone and estradiol synthesis (21, 77, 78). Additionally, evidence suggests that leptin and leptin receptor (Lepr) exerted positive effects on estradiol synthesis in murine granulosa cells, whereas BMAL1 knockdown reduced estradiol synthesis and suppressed the stimulating effect of leptin and lepr on estradiol synthesis and associated gene expressions (20). Therefore, BMAL1 plays a role in the process by which leptin signaling induces estradiol synthesis (20). Another hormone, prostaglandin E2 (PGE2), was also found to be reduced in the reproductive tissues of female BMAL1-KO mice (21, 79).

Regulated by rate-limiting enzymes encoded by the gene prostaglandin-endoperoxide synthase 2/cyclooxygenase 2 (ptgs2 or cox2), PGE2 has been deemed vital for ovulatory cascade, sperm penetration, and fertilization, and decidual preparation for implantation (80–84). Moreover, *in vitro* cultured BMAL1 rat luteinizing granulosa cells and BMAL1-KO uterus cells exhibited considerably downregulated ptgs2 expression, and PGE2 secretion (21, 79). Therefore, BMAL1 is important for female gonadal hormone secretion, which could
directly account for the impaired fertility of female BMAL1-KO mice. Female BMAL1-KO mice were reported to undergo unsuccessful implantation. Exogenous supplementation with progesterone substantially rescued the implantation failure, although the recovered implantation sites were smaller and fewer compared to those noted in the WT group (22). Similarly, in female steroidogenic factor-1 (SF1) conditional BMAL1-KO mice, also characterized by poor steroidogenesis as well as failed implantation, exogenous progesterone supplement could greatly improve the implantation rate (76). Furthermore, progesterone was found to participate in the uterus decidualization pathways (39, 84–86), which are influenced by BMAL1 depletion (27). Thus, the impaired steroidogenesis due to BMAL1 loss might underlie the failed implantation of BMAL1-KO mice.

In males, testosterone also plays a crucial role in testis development and spermatogenesis (87–89). In line with the morphological abnormalities of the testis and seminal vesicles, male BMAL1-KO mice showed reduced testosterone secretion (23, 25, 90). In fact, BMAL1 was reported to be only expressed and oscillate in Leydig cells which account for testosterone production (19, 23), whereas BMAL1 oscillation in the whole testis was demonstrated to be arrhythmic (91, 92). In goat Leydig cells and in vitro cultured TM3 Leydig cells, BMAL1 knockdown led to a reduction in testosterone levels (41, 93). Thus, BMAL1 loss could be responsible for the poor gonadal hormone secretion in male BMAL1-KO mice.

Overall, the existing evidence for implantation related infertility in BMAL1-KO mice can be considered closely associated with the abnormal secretion of reproductive hormones.

**Effect of BMAL1 Deficiency on Gonadal Hormone Genes Expression**

In line with the impaired steroidogenesis, BMAL1-KO and interference altered the expression of most steroidogenic genes, including steroidogenic acute regulatory protein (StAR), cytochrome P450 family 11 subfamily A member 1 (Cyp11a1), 3β-hydroxysteroid dehydrogenase 2 (Hsd3b2), 17β-hydroxysteroid dehydrogenase 3 (Hsd17b3), cytochrome P450 family 17 subfamily A member 17 subfamily A member 1 (Cyp17a1), and cytochrome P450 aromatase (Cyp19a1). BMAL1-KO mice also altered the expression of related hormone receptor genes, including follicle-stimulating hormone receptor (FSHr), luteinizing hormone receptor (LHr, Lhgr), and estrogen receptor β (ERβ) (15, 20–23, 41, 77, 94, 95) (Table 1). For example, BMAL1 knockdown in goat Leydig cells reduced testosterone production and the expression of StAR and Hsd3b2, whereas BMAL1 overexpression enhanced testosterone production and the expression of StAR and Hsd17b2 (93). In rat luteinizing granulosa cells, BMAL1 knockdown suppressed the expression of a series of ovarian genes, such as StAR, Cyp19a1, Cyp11a1, Ptg2, Lhgr, and Hsd3b2, with reduced progesterone and PGE2.

Among steroidogenic genes, StAR is necessary for cholesterol transfer into the mitochondrial membrane (101). Both male and female BMAL1-KO mice reduced StAR protein and mRNA expression both in vivo and in vitro (15, 21–23, 41). Moreover, the STAR gene contains an E-box element near its promoter region, and BMAL1 can directly induce its transcription (23, 96, 97). Similarly, the expression of HSD17B3 was also demonstrated to be directly regulated by BMAL1 (93). Erβ, a major estradiol receptor type in the SCN, plays a role in reproduction, especially in follicle maturation (102–105). With an E-box in its promoter, BMAL1 was demonstrated to directly regulate Erβ expression (99). Lepr also contains an E-box in its promoter and is subjected to regulation by BMAL1 (20). In summary, BMAL1 can affect steroidogenesis and hormone signaling by directly affecting certain steroidogenesis related genes.

**Effect of BMAL1 Deficiency on Signaling Pathways Associated With Steroidogenesis**

Silencing information regulator 2 related enzyme 1 (sirtuin1, SIRT1) is an NAD⁺-dependent deacetylase; it collaborates with BMAL1 in the circadian regulation (106–109). It has already been established that SIRT1 participates in the steroidogenic function of estrogen-producing cells. Further evidence confirmed that SIRT1-BMAL1 signaling is involved in estradiol production in steroidogenic cells, by regulating the c-Jun N-terminal kinase (JNK) pathway (78). Moreover, BMAL1, SIRT1, and the JNK pathways seem to mutually affect each other, forming a BMAL1-SIRT1-JNK loop that functions in gonadal steroidogenesis (78).

Studies have revealed that the PI3K/AKT/mTOR pathway participates in the biological processes of apoptosis and

| Reference | Gene | E-box | Rhythmic |
|-----------|------|-------|----------|
| [19, 21, 77, 93, 95–97] | StAR (Steroidogenic acute regulatory protein) | Yes | Yes |
| [19, 21, 77, 93] | Cyp11a1 (Cytochrome P450 family 11 subfamily A member 1) | Yes | Yes |
| [19, 93, 95] | Cyp17a1 (Cytochrome P450 family 17 subfamily A member 1) | – | Yes |
| [21, 77, 95] | Cyp19a1 (Cytochrome P450 family 19 subfamily A member 1) | Yes | Yes |
| [21, 93, 95] | Hsd3b2 (3β-Hydroxy-Δ5-steroid dehydrogenase) | No | No |
| [19] | Hsd17b3 (3β-Hydroxysteroid dehydrogenase) | Yes | No |
| [21, 98] | Pgs2 (Prostaglandin-endoperoxide synthase 2/cyclooxygenase 2) | Yes | Yes |
| [77, 99, 100] | ERβ (Estradiol Receptor β) | Yes | Yes |
| [77] | FSHr (Follicle-stimulating hormone receptor) | No | Yes |
| [21, 77, 95] | LHr (Lhgr, Luteinizing hormone receptor) | No | Yes |
| [20] | Lepr (LeptinR, Leptin Receptor) | Yes | Yes |
| [59] | GnRHR (Gonadotropin-Releasing-Hormone Receptor) | Yes | Yes |
steroidogenesis in granulosa and Leydig cells (110–114). Coincidently, in the in vitro cell culture of porcine granulosa cells and the TM3 Leydig cell line, the PI3K/AKT/mTOR pathway was suppressed after intervention of BMAL1, with reduced steroidogenesis and increased apoptosis of steroidogenic cells (41, 77). Notably, this pathway was also reported to be involved in leptin-lepr signaling in the regulation of steroidogenesis (20). Therefore, BMAL1 might have functions in the PI3K/AKT/mTOR pathway and thus play a role in the apoptosis of gonadal steroid hormone-producing cells and steroidogenesis.

The PI3K/NF-κB pathway is also involved in steroidogenesis. NF-κB was demonstrated to participate in circadian rhythm generation and was also associated with the PI3K/AKT/mTORC1 pathway, which functions in steroid hormone production (110–114). Female BMAL1-KO mice exhibited impaired steroidogenesis and activated phosphorylation of the PI3K/NF-κB pathway. Moreover, data from the in vitro cultured mice theca and granulosa cells confirmed that the BMAL1 knockout increased phosphorylation of the PI3K/NF-κB pathway, along with severely impaired luteal steroidogenesis (95). Further evidence indicated that BMAL1 might directly establish negative interactions with NF-κB p (RelA), a subunit of NF-κB (95). Therefore, BMAL1 is also involved in the regulation of PI3K/NF-κB pathway, inducing steroidogenesis.

**BMAL1 IN THE ETIOLOGY OF HUMAN REPRODUCTIVE PATHOGENESIS**

Accumulating clinical evidence has demonstrated the role of BMAL1 in human reproductive pathologies. Single nucleotide polymorphisms (SNPs) of circadian genes revealed that BMAL1 is associated with all epithelial ovarian cancers (EOCs) and their histopathologic subtypes (28). Moreover, BMAL1 was obviously downregulated in an early stage transformed model of EOCs (28). One variant of BMAL1 was previously linked to a higher number of pregnancies and a higher number of miscarriages (29). In addition, BMAL1 expression was downregulated in recurrent miscarriage patients (26), and BMAL1 was revealed to affect trophoblast invasion during decidualization by regulating TIMP3, a protein that controls the invasiveness of trophoblast cells (26). Another mechanistic study revealed that BMAL1 facilitated the migration and invasion of the extra-villous trophoblast (EVT) via the SP1-DNMT1/DAB2IP pathway, which could be the underlying mechanism through which progesterone treatment prevents spontaneous abortion (27).

**CONCLUSIONS AND PROSPECTS**

As current review has indicated, BMAL1 knockout leads to impaired structures and functions of reproductive organs and gametes, signaling from the hypothalamus to pituitary impaired gonadal hormone secretion and impaired hormone secretion. Furthermore, dysfunctional BMAL1 was demonstrated to participate in human reproductive diseases. These findings revealed the critical role of BMAL1 in reproductive endocrinology and fertility. Still, the exact underlying mechanisms of how BMAL1 impacts on reproduction need to be further explored. The disparate phenotypes in different BMAL1-KO models (Table 2) have offered us deep insights into the role of BMAL1 in reproduction. Among these mice, global BMAL1-KO mice and steroidogenesis cells conditional BMAL1-KO (SF1-KO) mice were infertile, while other conditional BMAL1-KO mouse models were fertile such as the induced BMAL1-KO (iKO) mice (115). The iKO mice were treated with tamoxifen (TAM) at 8-weeks of age, thus losing BMAL1 in adulthood, and in these animals, reproductive tissues and reproductive endocrine systems are well-developed while devoid of BMAL1. However, BMAL1-KO mice had impaired reproductive tissues and gametes, as well as impaired reproductive endocrine systems. Thus, BMAL1 has a critical role in reproductive endocrinology and fertility.

As the core transcription factor of the circadian rhythm, BMAL1 also serves its circadian function in regulating the rhythm of reproductive endocrine hormones. Indeed, reproductive endocrine hormones like estradiol (20), and testosterone (118, 119), have been demonstrated to be rhythmically released. And the associated steroidogenic genes showed robust circadian rhythm in corresponding steroidogenic cells (20, 90, 93). Moreover, the disruption of BMAL1 by siRNA in mice steroidogenic cells led to the disrupted rhythm of associated genes including StAR, Cyp11a1, Hsd3b2, and Hsd17b3 (20, 90, 93). Additionally, another gene family crucial for cholesterol transportation to produce testosterone in the testis, namely apolipoprotein (Apo) genes

| Reference | Knockout Mice | The Reproductive Phenotypes |
|-----------|---------------|-----------------------------|
| (15, 22, 31, 115) | Global BMAL1-KO (BMAL1-KO) | Infertile in both female and male mice; delayed puberty in both sexes; prolonged progression through the estrous cycle in females |
| (116) | Myometrium-BMAL1-KO | Fertile; altered parturition time |
| (31) | Pituitary Gonadotrope-BMAL1-KO (GGMAL1-KO) | Fertile; successful and viable offspring; irregular estrous cycle |
| (76) | Steroidogenesis cells BMAL1-KO (SF1-BMAL1-KO) | Infertile; normal puberty; signs of early pregnancy loss and re-entry into estrus after mating |
| (95, 117) | Ovarian Theca Cell BMAL1-KO (TCKO) | Fertile; more mating failure with less viable litters; normal estrous cycle |
| (95, 117) | Ovarian Granular cell BMAL1-KO (GCKO) | Fertile; normal estrous cycle; normal ovary morphology |
| (115) | Inducible BMAL1 KO (KO) | Fertile; less fertile than normal mice, but comparable to the controls |
(including ApoA1, ApoA2 and ApoC3), was reported to be expressed in a robust circadian manner in WT mice (90). In BMAL1 KO mice, the rhythmicity of the expressions of Apo genes is lost, along with impaired metabolism of cholesterol, which might be associated with the reduced testosterone in Male BMAL1 KO mice (90). Accordingly, the circadian gene BMAL1 can also influence reproductive endocrinology by regulating the rhythmic expression of corresponding genes.

In conclusion, the established mouse model and the mechanistic studies have provided insights into the role of BMAL1 in reproduction. This could have clinical significance, as more reproductive pathologies can be attributed to defects at the gene level, such as the key role of BMAL1 outlined in this review. It is anticipated that a deeper understanding of the role of BMAL1 in reproduction could be applied to clinical practices in reproductive disease pathology and target therapies in the future.

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