Theriogenology 154 (2020) 212–222

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Changing the light schedule in late pregnancy alters birth timing in mice

Tomoko Amano [a, *], Jürgen A. Ripperger [b], Urs Albrecht [b]

[a] College of Agriculture, Food and Environment Sciences, Department of Sustainable Agriculture, Laboratory of Animal Genetics, Rakuno Gakuen University, 582 Midorimachi Bunkyodai, Ebetsu, Hokkaido, 069-8501, Japan
[b] Department of Biology/Unit of Biochemistry, Faculty of Sciences, University of Fribourg, Chemin du Musée 5, CH-1700, Fribourg, Switzerland

ARTICLE INFO

Article history:
Received 13 March 2020
Received in revised form
11 May 2020
Accepted 22 May 2020
Available online 24 May 2020

Keywords:
Light schedule
Birth timing
Mouse

ABSTRACT

In rats, birth timing is affected by changes in the light schedule until the middle of the pregnancy period. This phenomenon can be used to control birth timing in the animal industry and/or clinical fields. However, changes in the light schedule until the middle of the pregnancy period can damage the fetus by affecting the development of the major organs. Thus, we compared birth timing in mice kept under a 12-h light/12-h darkness schedule (L/D) throughout pregnancy with that of mice kept under a light schedule that changed from L/D to constant light (L/L) or constant darkness (D/D) from day 17.5 of pregnancy, the latter phase of the pregnancy period. On average, the pregnancy period was longer in D/D mice (19.9 days) than L/L or L/D mice (19.5 and 19.3 days, respectively, $P < 0.05$), confirming that light schedule affects birth timing. The average number of newborns was the same in L/L, L/D, and D/D mice (7.5, 7.8, and 7.9, respectively), but the average newborn weight of L/L mice (1.3 g) was lower than that of L/D and D/D mice (both 1.4 g, $P < 0.05$), indicating that constant light has detrimental effects on fetus growth. However, the percentage of dead newborns was the same between L/L, L/D, and D/D mice (11.1, 10.6, and 3.6%, respectively). The serum progesterone level on day 18.5 of pregnancy in L/D mice was 42.8 ng/ml, lower ($P < 0.05$) than that of D/D mice (65.3 ng/ml), suggesting that light schedule affects luteolysis. The average pregnancy period of mice lacking a circadian clock kept under D/D conditions from day 17.5 of pregnancy (KO D/D) (20.3 days) was delayed compared with wild-type (WT) D/D mice ($P < 0.05$). However, the average number of newborns, percentage of births with dead pups, and weight per newborn of KO D/D mice (7.6, 3.6%, and 1.4 g, respectively) were the same as WT mice kept under D/D conditions. A direct effect of the circadian clock on the mechanism(s) regulating birth timing was questionable, as the lighter average weight per KO fetus (0.6 g) versus WT fetus (0.7 g) on day 17.5 of pregnancy might have caused the delay in birth. The range of birth timing in KO D/D mice was the same as that of WT D/D mice, indicating that the circadian clock does not concentrate births at one time.

1. Introduction

A number of reports have indicated that there is a certain time period during the day at which birth frequently occurs at the end of the pregnancy period. In humans, birth frequently occurs at night (dark period) [1], whereas the birth of cows and rats frequently occurs during the day (light period) [2,3], indicating the presence of a specific mechanism that controls birth timing. Investigations of the factors affecting this mechanism could lead to improved techniques for controlling birth timing in farm animals, thus improving animal management. Such studies could also provide insights into methods to prevent premature birth, which is not only harmful to the health of newborn farm animals but also human neonates, affecting 10.6% of all live births in humans globally, according to a report by the World Health Organization in 2014 (https://www.who.int/reproductivehealth/global-estimates-preterm-birth/en/).

Environmental light reportedly has a strong effect on birth timing in rats. Between 60 and 90% of rats give birth during the day (light period) in the final 2–3 days of the pregnancy period (days 21–23) [3–7]. Moreover, a shift in the cycle of light and dark periods reportedly alters the timing of birth. Birth timing moves forward/backward with a shift of 4–18 h in the cycle of the light and dark periods, but after the shift, birth still tends to occur in the light

* Corresponding author.
E-mail address: amano@rakuno.ac.jp (T. Amano).

https://doi.org/10.1016/j.theriogenology.2020.05.032

period [3,4,7]. However, in previous reports, the shift in the cycle of the light and dark periods occurred during the early to middle phases of the pregnancy period, between days 2 and 12, corresponding to the phase in which important events take place that affect the survival of the embryo/fetus, such as implantation and the development of major organs [8,9]. As shifting the light schedule can have detrimental effects on the embryo/fetus, changes in light schedule for the purpose of controlling birth timing should be instituted in the later phase of the pregnancy period, when implantation and organ development are complete.

Other reports have indicated that changing the light schedule during the later phase of the pregnancy period (i.e., after day 19) does not affect birth timing in rats, as almost all of the animals in one study gave birth during the light period on day 21 or 22 of the pregnancy period [5]: Reppert et al. [5] suggested that this result occurred because birth time is controlled by the circadian clock, the molecular mechanism that functions in all organs and tissues to measure the time of day rhythmically based on the circadian clock in the brain suprachiasmatic nucleus (SCN), which is minimally affected by changes in light schedule over a couple of days because it is synchronized with long-term (more than 10 days) changes in light schedule [10,11]. In addition, a study examining the mechanism regulating birth timing in mice [12] reported that a 6-h shift in the cycle of light and dark periods from day 3 of the pregnancy period to birth (day 19–20) did not affect birth timing.

Based on the above observations, we conducted our study to clarify the effects of changes in light schedule on birth timing. We subjected mice to changes in the cycle of light and dark periods ranging from constant light to constant darkness during the late phase of the pregnancy period, after development of the major organs has finished [9], to examine the effect on birth timing and the health of newborn pups. Moreover, we examined the effect of the circadian clock on the regulation of birth timing in mice genetically lacking a circadian clock. For mice genetically lacking a circadian clock, we used a strain lacking the circadian genes *Per1* and *Per2* [10], the genomic absence of which is known to abolish circadian clock function throughout the body [13,14]. Moreover, we investigated the effect of changes in light schedule on the decrease in progesterone level immediately before birth. Progesterone is a hormone necessary to maintain pregnancy and prevent premature contraction of the uterus, but its levels are decreased at the start of parturition [15].

2. Material and methods

2.1. Animals

We have maintained a mouse strain derived from one pair of 129SV and C57BL/6 mice by random mating for 18 years in our laboratory. From this strain, females aged 8–12 weeks and males aged 10–14 weeks were randomly selected as wild-type (WT) mice for this study.

For mice lacking the circadian clock, we used a strain lacking both *Per1* and *Per2* derived from the same strain used as WT [13,14]. This strain has been maintained for 18 years via random mating of homozygous knockouts of both *Per1* and *Per2*. From this strain, females aged 8–12 weeks and males aged 10–14 weeks were randomly selected as circadian clock–deficient (KO) mice in this study. However, to maintain the genetic closeness of this *Per1*/*Per2* KO strain with the WT strain, we crossed the homozygous knockouts for both *Per1* and *Per2* with WT mice once per year. Specifically, the resulting heterozygous *Per1*/*Per2* knockouts from the aforementioned crossing were crossed with each other to obtain *Per1*/*Per2* homozygous knockouts. These mice were crossed to produce new generations. This procedure ensured that the genetic drift was similar in WT and *Per1*/*Per2* KO mice and that their genetic background was comparable.

All animal experiments were performed in accordance with the guidelines of the Schweizer Tierschutzgesetz (TSchG, SR455, Abschnitt 2: Art. 5 + 7, Abschnitt 5: Art. 11, and Abschnitt 6: Art. 12–19) and Declaration of Helsinki and approved by the State Veterinarian of Fribourg (permit FR 2015–33).

In this study, mice were given free access to food (Rat/Mouse-Maintenance gamma-irradiated No 3432, PROVIMI KLIBA AG, Vaud, Switzerland).

2.2. Preparation of pregnant mice and measurement of body weight

Pregnant female mice were prepared in an animal housing room in which a 12-h light and 12-h darkness cycle was maintained. One cycle of the light period and dark period was designated as 1 day, which began at 0000 h and ended at 2400 h (0:00); the starting times of the light and dark periods were designated as 0:00 and 12:00, respectively. The light intensity was 150 lux at the animals' eye level in the cage.

Pregnant mice were prepared according to Murray et al. [16]. Female WT or KO mice were co-housed with male WT or KO mice, respectively, at 12:00, the starting of the dark period. Female mice ovulate and accept male mice from midnight to dawn in the ovulation day in the estrus cycle, which spans 4–7 days. Thus, presence of the vaginal plug known to be generated immediately after copulation was checked every morning (at 2:00) from the day after cohabitation until the plug was observed in order to determine the copulation timing. The day the vaginal plug was observed was designated as day 1. Female mice with a vaginal plug were removed from the cage and housed individually in another cage until 12:00 on day 17 of the pregnancy period. During this period, body weight was recorded every day at 2:30. At 12:00 on day 17 of the pregnancy period, mice were left in the animal room to monitor the time of birth under different lighting conditions. The body weight of 5 non-copulated WT females and 5 KO females was also measured every day for 17 days at 2:30 as a control. Change in body weight was determined by subtracting the body weight measured on the first day from the body weight measured on each day of the pregnancy period.

In this study, the time points in the pregnancy period were defined as the combination of the day of the pregnancy period and the ratio of the time in the day. For example, 12:00 on day 17 of the pregnancy period would be described as day 17.5 (Fig. 1).

2.3. Lighting conditions from day 17.5 of the pregnancy period to birth

To control the lighting conditions, we prepared three boxes with 170-cm width, 65-cm depth, and 45-cm height in the animal housing room. Each box consisted of wooden boards that blocked light penetration and was able to contain the mouse cage, which was 21-cm wide, 20-cm deep, and 14-cm high. With these boxes, a door with insulation to block light was attached to one of the largest sides. For two of the boxes, two fluorescent lights emitting at a wavelength of 590 nm and color temperature of 6500K (L18W/865 LumiLux, OSRAM, Munich, Germany) were attached to the inside top panel. One of the two light-equipped boxes was set to repeat the 12-h lights on and 12-h lights off (L/D) cycle, and the other box was set to lights on constantly (L/L). These boxes were designated the L/D box and L/L box, respectively. The time schedule of lights on/lights off for the L/D box was set to be the same as that applied to the animal room used for the production of pregnant mice. The light intensity at mouse eye level in the mouse cage in the L/L box and the lighting period of the L/D box was 450 lux. No

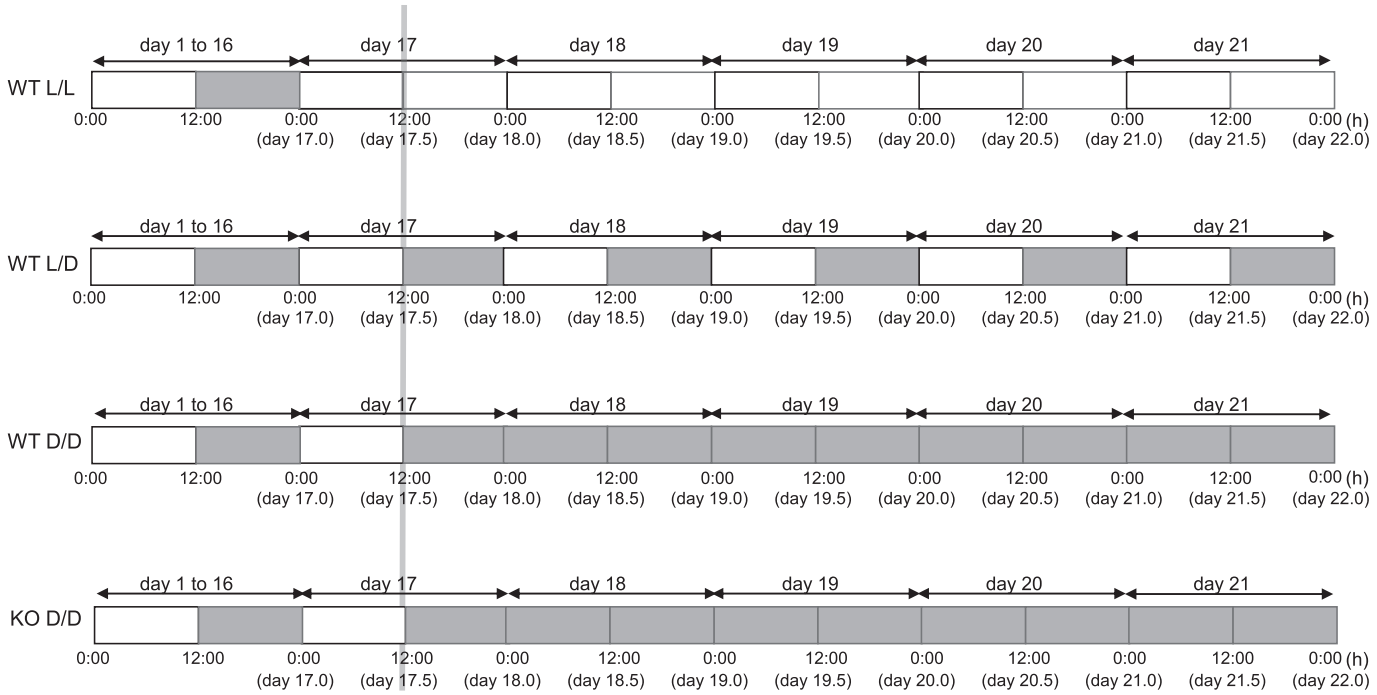

Fig. 1. Schematic illustration of the schedule of light cycling (WT L/L, WT L/D, WT D/D, and KO D/D) used in this study. Both the copulated wild-type females (WT L/D) and the circadian clock–deficient females (KO D/D) were placed under the repetition of 12 h of light and 12 h of darkness from days 1 through 16 of the pregnancy period; a day was defined as one repetition of the 12 h of light and 12 h of darkness cycle. The start of the day was designated as 0:00. Day 1 of the pregnancy period was set as the day that the vaginal plug (copulation) was observed. Hours in each day are shown under the bars. The time points during the pregnancy period were defined as the combination of the day of the pregnancy period and the ratio of the time of the day, as indicated in parentheses. On day 17.5 (at 12:00 on day 17) of pregnancy, the wild-type female mice were separated into three groups. One group was placed under continuous repetition of 12 h of light and 12 h of darkness until parturition, designated as WT L/D. The other groups were placed under constant light or constant darkness until birth, respectively, designated as WT L/L and WT D/D. The circadian clock–deficient females were placed under constant darkness from day 17.5 of the pregnancy period, designated as KO D/D. White bars denote periods of light; shaded bars denote periods of darkness. Black arrowheads denote the days in the pregnancy period. All of the pregnant female mice used in these experiments were kept individually from day 1 to the time of giving birth.

lighting equipment was attached to the other box, which was designated the D/D box. The light intensity inside the mouse cage in the D/D box and during the lights-off period in the L/D box was 0 lux. All of the boxes were equipped with a vent at a location that would not allow light penetration from outside the box. The temperature of the mouse room containing the L/L, L/D, and D/D boxes was maintained at 23–26 °C [17].

2.4. Determination of birth timing under L/L, L/D, and D/D lighting conditions

Birth timing was determined according to Murray et al. [16], as follows. The behavior of pregnant mice from day 17.5 of the pregnancy period to giving birth was monitored, and the time of birth was determined as the time that the first-born pup appeared in the mouse cage. In this study, a small (5-cm width, 5-cm depth, and 3-cm height) infrared camera (DCS-936L D-Link, Taipei, Taiwan) developed to monitor pets and/or young children in households was used to monitor the behavior of pregnant mice. One camera each was attached to the inside top of the L/L, L/D, and D/D boxes, and no more than two cages were set underneath. In this case, one cage contained one pregnant mouse. The distance from the camera to the mice was 35 cm, well within the distance that objects can be clearly observed using the camera (5 m) (Fig. S1). The camera was equipped with an SD card to store the resulting video data. The SD card could store an amount of data equivalent to 3 days.

On day 17.5 of the pregnancy period, the cage containing the mouse was placed under the camera in the L/L, L/D, and D/D boxes. After inserting a new SD card into the camera, the door of the box was closed. At 5 min before day 20.5 of pregnancy, the door of the box was opened, and the presence of pups in the cage was checked without touching the cage. If there were pups present, the cage was removed from the box, and the number, weight, and the sex of the pups and the body weight of the mother mouse were determined. Simultaneously, the SD card was removed from the monitoring camera attached above the cage. If there were no pups present, the cage was left undisturbed in the box, and the door of the box was closed. Videos made after day 20.5 of the pregnancy period were recorded on the same SD cards, after deletion of the old memory. If there were no pups in the other cage, a new SD card was inserted in place of the removed SD card.

The door of each box was re-opened on day 22.0 of the pregnancy period, and the mouse cage inside the box was checked once again for the presence of pups. If pups were present, the cage was removed from the box, and the number, weight, and sex of the pups and the body weight of the mother mouse were determined and the SD card collected. Pups were found in all of the cages examined in this study until day 22.0 of the pregnancy period. If any dead pups were found at the time of pup counting, the number was included with the number of the pups, and the females that had dead pups were counted as females with dead pups. The number of dead pups was less than two per female. The cages removed from the boxes after monitoring of birth on day 20.5 and day 22.0 of the pregnancy period were placed in different boxes under the same cycle of light and dark periods as the L/D box. The number of pups inside each cage was then determined 24 h after the time the cage was removed from the box. In all of the cages, the number of pups was the same as the number at the time the cage was removed from the box, confirming that no mother mice had been in the middle of giving birth when the door was opened on days 20.5 and 22.0 of the

pregnancy period. When observing the mouse cage in the D/D box, the room lights were turned off, and a lamp emitting dim red light (wavelength 680 nm) known not to affect mouse physiology [18,19] was used. The cage was illuminated using the lamp in a manner such that the mice were not directly exposed to the red light, and the cage was not illuminated with the dim red light for more than 3 s. The cages containing the pregnant mice were arranged so as not to interrupt monitoring by the camera. Specifically, the cages were covered with a large-meshed metal grating. Pellet feed was placed on the floor of the cages, and a water bottle was set vertical on each of the covers of the cages. All of the procedures in this experiment, such as opening/closing the box door, were done carefully so as not to stimulate the mice.

In this study, 27, 27, and 28 female WT mice were used for the L/L, L/D, and D/D groups, respectively. When the pregnant WT mice were produced, 3 female mice were co-habited with 3 male mice randomly selected from the 13 male WT mice, respectively, as one batch. The 3 pregnant mice in one batch were then randomly placed one each in the L/L, L/D, and D/D boxes. The next batch consisting of 3 females was then co-habited with the 3 males randomly selected from the same 13 male WT mice used for the former batch, respectively. This was carried out 2 days after confirmation of copulation (plug formation) by all of the females of the previous batch. Using this procedure, one WT male mouse produced 2 to 3 pregnant mice for each of the L/L, L/D, and D/D boxes. A total of 28 female KO mice were used in this study. In the same manner as the aforementioned method for WT mice, 1 female KO mouse was co-habited with 1 male KO mouse randomly selected from the 11 male KO mice, as one batch, when the pregnant KO mice were produced. The next batch consisted of 1 KO female co-habited with the 1 KO male randomly selected from the same 11 male KO mice used for the former batch, 2 days after confirmation of copulation (plug formation) by the female of the former batch. The pregnant mouse derived from each of these single batches was put into a D/D box. In this case, 1 KO male mouse was used to mate 2 to 3 KO females. All of the male and female pairs set in this study successfully mated, based on observation of vaginal plugs.

The WT females placed in the L/L, L/D, and D/D boxes were designated WT L/L, WT L/D, and WT D/D, respectively. As the KO females were placed in the D/D box, they were designated KO D/D. For each treatment (WT L/L, WT L/D, WT D/D, and KO D/D), the number of births occurring per hour during the observation period from days 17.5–22.0 of the pregnancy period was summed, and the results are shown as a percentage of the total number of female mice.

2.5. Comparison of fetal and uterine weight and morphology between WT and KO mice

A total of 7 pregnant WT and 7 pregnant KO mice produced according to the aforementioned methods were sacrificed by cervical dislocation on day 17.5 of the pregnancy period. The uterus was removed, and its weight and morphology were recorded. The morphology of each fetus and placenta and the number of fetuses and weight of each fetus and placenta were also recorded.

2.6. Measurement of serum progesterone level

A total of 6 WT L/D and 5 WT D/D mice were used for blood collection on day 18.5 of the pregnancy period. At the time of blood collection, the mice were anesthetized via isoflurane inhalation (3% in O_2, 1 L/min). After confirming that the mice had lost consciousness, the retro-orbital venous plexus was carefully cut using a hematocrit tube. The blood was collected from each mouse into a 1.5-ml disposable tube, and a total of 600–800 μl of blood was collected per mouse. The mice were sacrificed by cervical dislocation after blood collection, and the collected blood was stored at 4 °C for 12 h to clot. To separate the clot and the supernatant (serum), the tube was centrifuged at 3000 rpm for 20 min at 4 °C. The serum was transferred to a new 1.5-ml disposable tube immediately after centrifugation and stored at −80 °C until analysis. The volume of the resulting serum was >200 μl per mouse. The serum progesterone concentration was measured by the Nagahama Institute for Biochemical Sciences of Oriental Yeast Co., Ltd. (https://www.oyc.co.jp/en/business/bio.html) using LC-MS/MS, which can detect progesterone in the serum at concentrations above 10 pg/ml.

2.7. Statistical analysis

Table 1 shows the results of one-way ANOVA followed by Tukey's multiple comparison test for comparing the averaged values. Equal variance between the data was confirmed beforehand using Bartlett's test. As the variance of the average period from the start of cohabitation of male and female mice to the day of copulation was not equal between treatments, the Kruskal-Wallis test was used instead of one-way ANOVA. To compare data consisting of the ratio of a particular number of interest relative to the total population, the chi-square test was used. In this case, Bonferroni correction was used.

For the data shown in Table 2, the Student's t-test was used to compare differences in averaged data between treatments. An F-test was carried out beforehand to confirm equal variance between treatments. The chi-square test was used to compare data consisting of the ratio of a particular number of interest relative to the total population. For the data shown in Fig. 3, two-way ANOVA followed by Bonferroni's multiple comparisons test were used. For the data shown in Fig. 4, the Student's t-test was used to compare the data for different treatments, with an F-test performed beforehand to confirm equal variance. For the data shown in Fig. 6, Spearman's rank correlation coefficient was calculated to assess correlations between data. The null hypothesis that the overall slope was zero was evaluated using the F-test for each of the resulting regression lines.

The average data are presented with standard error of the mean (SEM) and standard deviation (SD) in the tables, but only SEM is shown in the text.

3. Results

3.1. Reproductive performance of WT and KO mice under different light schedules from day 17.5 of the pregnancy period

In general, female mice ovulate once in an estrus cycle (every 4–7 days) and copulate with male mice between midnight and dawn on the day of ovulation. In this study, we did not determine which stage in the estrus cycle the female mice were in and did not know when the females would ovulate at the start of cohabitation with the male mice. However, if the ovulation cycle of the females and the copulation ability of the co-habiting males were normal, the average period from the start of cohabitation to the day of copulation (the day that the vaginal plug was observed) should have been approximately 3 days. In our study, the average period from the cohabitation of males and females to the day of copulation was approximately 3 days for all of the treatments, WT, WT L/L, WT L/D, WT D/D, and KO D/D (3.3 ± 0.5, 2.7 ± 0.3, 3.2 ± 0.4, and 3.1 ± 0.5 days, respectively; Table 1), and the ovulation cycle and copulation ability of all of the female and male mice used in this study were thus determined to be normal.

Irrespective of the lighting conditions from day 17.5 of the

Table 1
Reproductive performance of wild-type female mice kept under different light conditions (constant light, repetition of 12 h of light and 12 h of darkness, and constant darkness) from 12:00 on day 17 of the pregnancy period (day 17.5), and circadian clock–deficient female mice (mice lacking both *Per1* and *Per2*) kept under constant darkness from 12:00 on day 17 of the pregnancy period.

	WT L/L	SEM	SD	WT L/D	SEM	SD	WT D/D	SEM	SD	KO D/D	SEM	SD
No. of examined females	27	–	–	27	–	–	28	–	–	28	–	–
Average period from the day of cohabitation of male and female to the day of copulation (days)	3.3	0.5	2.4	2.7	0.3	1.4	3.2	0.4	2.3	3.1	0.5	2.4
No. of delivered females (no. of delivered females/no. of copulated females [%])	27 (100.0)	–	–	27 (100.0)	–	–	28 (100.0)	–	–	28 (100.0)	–	–
Average period from copulation to delivery (days)	19.5^{ab}	0.1	0.5	19.3^{a}	0.1	0.5	19.9^{b}	0.1	0.6	20.3^{c}	0.1	0.7
No. of females with dead pups (no. of females with dead pups/no. of examined females [%])	3 (11.1)	–	–	1 (3.7)	–	–	1 (3.6)	–	–	1 (3.6)	–	–
Total no. of pups obtained from all examined pairs	202	–	–	211	–	–	220	–	–	213	–	–
Average no. of pups/delivery	7.5	0.5	2.5	7.8	0.3	1.5	7.9	0.4	1.9	7.6	0.4	2.0
Average rate of no. of male pups/total pups per delivery (%)	49.7	4.0	21.0	57.8	3.8	19.6	53.6	3.3	17.6	46.5	4.8	25.2
Average total weight of pups/delivery	9.7	0.5	2.8	10.6	0.4	1.9	11.0	0.5	2.4	10.5	0.4	2.3
Average weight/pup (g)	1.3^{a}	0.0	0.1	1.4^{b}	0.0	0.1	1.4^{b}	0.0	0.1	1.4^{b}	0.0	0.2

WT L/L: Wild-type female mice kept under constant light.
WT L/D: Wild-type female mice kept under repetition of 12 h of light and 12 h of darkness.
WT D/D: Wild-type female mice kept under constant darkness.
KO D/D: Circadian clock–deficient female mice kept under constant darkness.
Note: Females were placed under repetition of 12 h of light and 12 h of darkness immediately after copulation. Under these conditions, one cycle of the light and dark periods was designated as 1 day. For each day, the time points of the start of light and dark periods were designated as 0:00 and 12:00, respectively. The end of the day (starting time of the next day) was designated as 24:00 (0:00). The day that copulation (vaginal plug) was observed was set as day 1 of the pregnancy period. The females were kept under each lighting condition (L/L, L/D, or D/D) as shown in the table from the start (12:00) of day 17 of pregnancy. Values with different superscript lowercase letters in the same column differ significantly.
SEM (standard error of the mean) and SD (standard deviation) of each value are noted in the columns to the right labeled "SEM" and "SD".

pregnancy period, all of the females in the WT L/L, WT L/D, WT D/D, and KO D/D groups that copulated gave birth successfully (Table 1), and there were no signs of dystocia, such as heavy bleeding and/or death of a majority of the newborns, in any of the treatment groups. In contrast, the average day of birth in the WT D/D group (day 19.9 ± 0.1) was later compared with the WT L/L (day 19.5 ± 0.1) and WT L/D (day 19.3 ± 0.1) groups, and the difference between the WT L/D and WT D/D groups was significant ($P < 0.05$). The average birth time in the KO D/D group (day 20.3 ± 0.1) was the latest among all treatments ($P < 0.05$). The frequency of birth peaked in the afternoon (from 6:00 to 12:00) of day 19 of the pregnancy period in both the WT L/L and WT L/D groups, and the majority of the females gave birth by day 19.5 of the pregnancy period (59.3% and 77.8% in WT L/L and WT L/D, respectively) (Fig. 2). The frequency of birth in the WT D/D group also peaked around the afternoon of day 19 of the pregnancy period, but the majority (64.3%) of these females gave birth after day 19.5. Almost all of the KO D/D females gave birth after day 19.75 of the pregnancy period (96.4%), and there was no significant peak time in the frequency of birth.

There was no significant difference in the percentage of females with dead pups in the WT L/L, WT L/D, WT D/D, and KO D/D groups (11.1%, 3.7%, 3.6% and 3.6%, respectively, Table 1).

The average number of pups per birth in the WT L/L, WT L/D, WT D/D, and KO D/D groups was 7.5 ± 0.5, 7.8 ± 0.3, 7.9 ± 0.4, and 7.6 ± 0.4, respectively, and the differences were not statistically significant (Table 1). The average ratio of male pups among all pups in a single delivery did not differ between groups (49.7 ± 4.0%, 57.8 ± 3.8%, 53.6 ± 3.3%, and 46.5 ± 4.8% for the WT L/L, WT L/D, WT D/D, and KO D/D groups, respectively). The average total weight of pups per one birth in the WT L/L group was 9.7 ± 0.5 g, which was slightly lower than that of the WT L/D (10.6 ± 0.4 g), WT D/D (11.0 ± 0.5 g), and KO D/D (10.5 ± 0.4 g) groups, but the differences were not significant. The average weight per pup in the WT L/L group (1.3 ± 0.0) was lower ($P < 0.05$) than that in the WT L/D (1.4 ± 0.0 g), WT D/D (1.4 ± 0.0 g), and KO D/D (1.4 ± 0.0 g) groups (Table 1).

3.2. Increase in body weight during the pregnancy period

The increase in body weight of the female mice from day 1–17 of the pregnancy period was not significantly different between the WT L/L, WT L/D, and WT D/D groups (Fig. 3). However, the increase

Table 2
Average body weight, number of fetuses, fetal body weight, and weight of the placenta in wild-type female mice and circadian clock–deficient female mice (mice lacking both *Per1* and *Per2*) at 12:00 on day 17 of the pregnancy period (day 17.5).

	WT	SEM	SD	KO	SEM	SD
No. of examined females	7	–	–	7	–	–
No. of females that had both fetuses and placentas (no. of females that had both fetuses and placentas/no. of examined females [%])	7 (100.0)	–	–	7 (100.0)	–	–
Average body weight of the females (g ± SEM)	36.3	0.6	1.5	35.1	1.3	3.5
Average uterus weight (g ± SEM)	9.0	1.0	2.6	8.0	0.8	2.2
Average number of fetuses per female ± SEM	8.3	0.9	0.9	8.0	0.8	0.8
Average fetal body weight (g ± SEM)	0.7^{a}	0.0	0.0	0.6^{b}	0.0	0.0
Average placental weight (g ± SEM)	0.1	0.0	0.0	0.1	0.0	0.0

WT: Wild-type female mice.
KO: Circadian clock–deficient female mice (mice lacking both *Per1* and *Per2*).
SEM (standard error of the mean) and SD (standard deviation) of each value are noted in the columns to the right labeled "SEM" and "SD".
Note: All examined females had fetuses. In this experiment, the animals were kept under repetition of 12 h of light and 12 h of darkness. Under these conditions, one cycle of the light and dark periods was designated as 1 day. For each day, the time points of the start of the light and dark periods were designated as 0:00 and 12:00, respectively. The end of the day (starting time of the next day) was designated as 24:00 (0:00). The day that copulation (vaginal plug) was observed was set as day 1 of the pregnancy period. Values with different superscript lowercase letters in the same column differ significantly.

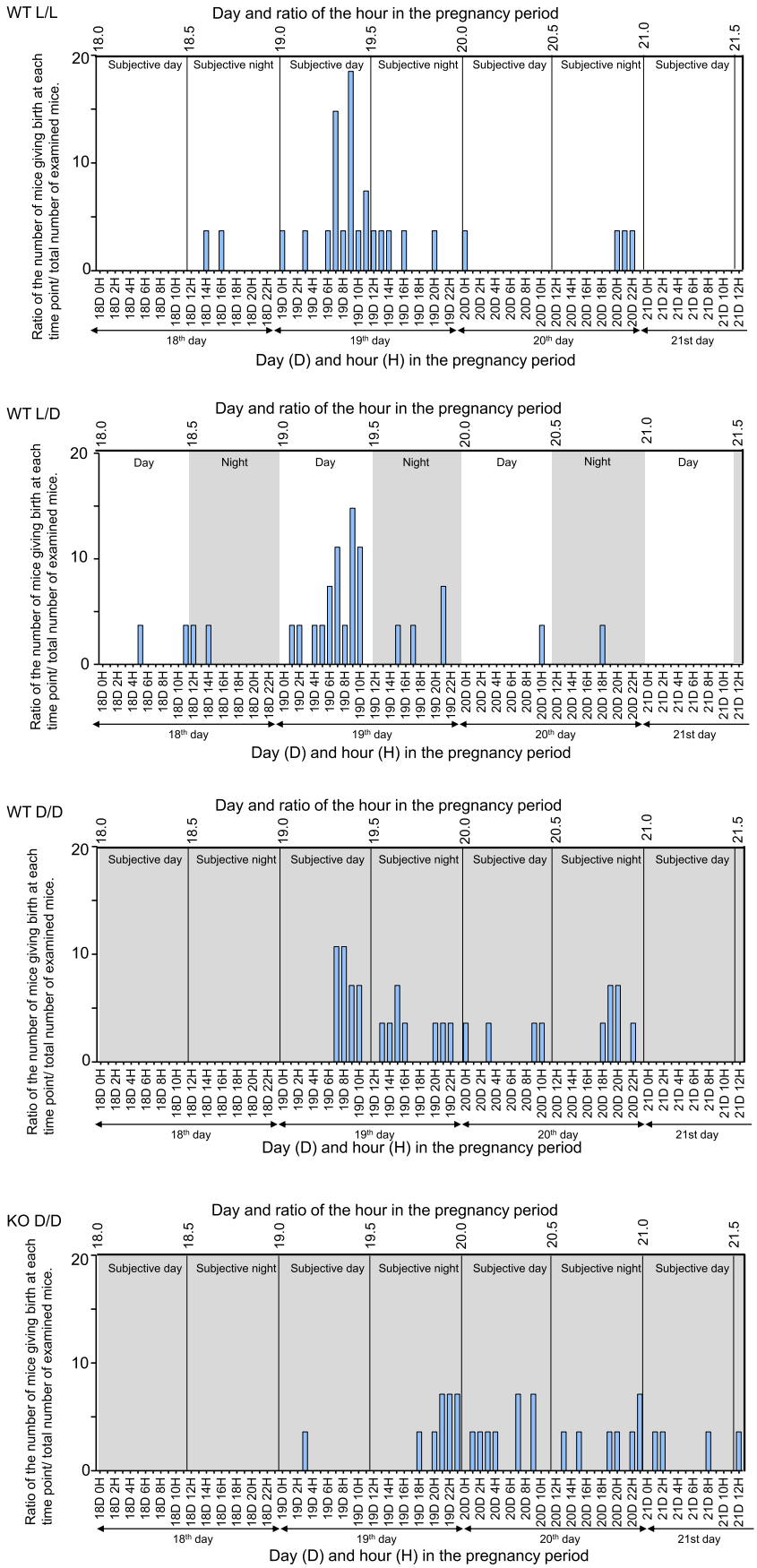

Fig. 2. Ratio of the number of mice giving birth to the total number of examined mice at each time point. WT L/L and WT D/D denote wild-type mice placed under constant light or constant darkness from the start of the night on day 17.5 (at 12:00 of day 17) of the pregnancy period, respectively. Subjective day and subjective night correspond to the period of

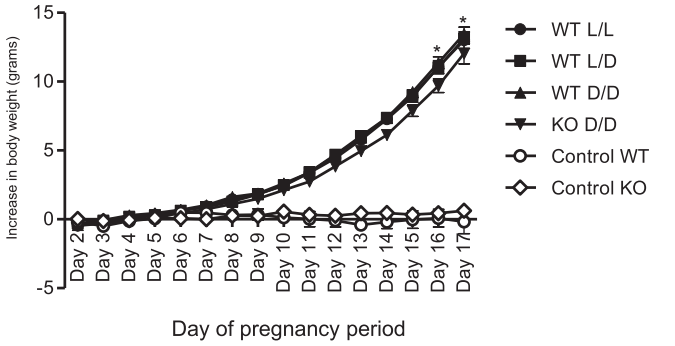

Fig. 3. Change in body weight of wild-type (WT) and circadian clock–deficient (KO) mice during the pregnancy period. All WT mice were held under repetition of 12 h of light and 12 h of darkness from day 1 to giving birth. WT L/L, L/D, and D/D denote mice placed under constant light, repetition of 12 h of light and 12 h of darkness, and constant darkness, respectively, from day 17.5 of the pregnancy period. KO D/D denotes circadian clock–deficient females that were placed under constant darkness from day 17.5 of the pregnancy period. The Y-axis indicates increase in body weight from day 1 of the pregnancy period; the value obtained by subtracting the body weight measured on the first day from the body weight measured on each day of the pregnancy period is plotted. Day 1 of the pregnancy period was set as the day that the vaginal plug (copulation) was observed. The X-axis indicates days 2 through 17 of the pregnancy period. Closed circles, squares, and upward-pointing triangles denote pregnant L/L, L/D, and D/D wild-type mice, respectively. Closed downward-pointing triangles denote pregnant circadian clock–deficient mice. Open circles and squares denote non-pregnant wild-type mice and circadian clock–deficient mice, respectively. The non-pregnant mice served as controls for the pregnant mice. Bars denote SEM. *Indicates a statistically significant difference between WT L/L, L/D, and D/D and pregnant KO mice ($P < 0.05$). The three lines indicating WT L/L, L/D, and D/D mice overlap along the top of the figure.

in body weight of KO D/D females was significantly less than that of the WT L/L, WT L/D, and WT D/D females on days 16 and 17 of the pregnancy period ($P < 0.05$). Neither the WT nor KO non-copulated females exhibited a significant increase in body weight during the 17 days of the observation period.

3.3. Comparison of uterine and fetal morphology and weight of WT and KO mice on day 17.5 of the pregnancy period

As shown in Fig. 3, the increase in body weight of KO females was significantly slower than that of WT females during the 17 days of the pregnancy period ($P < 0.05$), suggesting that slowed development of the fetuses caused the delay in delivery observed in the KO D/D group in this study (Table 1). To test this hypothesis, uterine and fetal morphology and weight were compared between the WT and KO mice on day 17.5 of the pregnancy period, immediately before exposure to the different light cycle conditions. The KO and WT mice exhibited similar uterine and fetal morphology (Fig. 4). Although there were no statistically significant differences, the average body weight of the females and average weight of the uterus on day 17.5 of the pregnancy period were slightly lower in KO females than WT females (36.3 ± 0.6 g and 35.1 ± 1.3 g; 9.0 ± 1.0 g and 8.0 ± 0.8 g, respectively). Regarding the difference with the results shown in Fig. 3, the significantly lower body weight of KO females on day 17 of the pregnancy period compared with WT females ($P < 0.05$) could have been due to the different number of mice used (27–28 vs. 7, respectively). However, the average

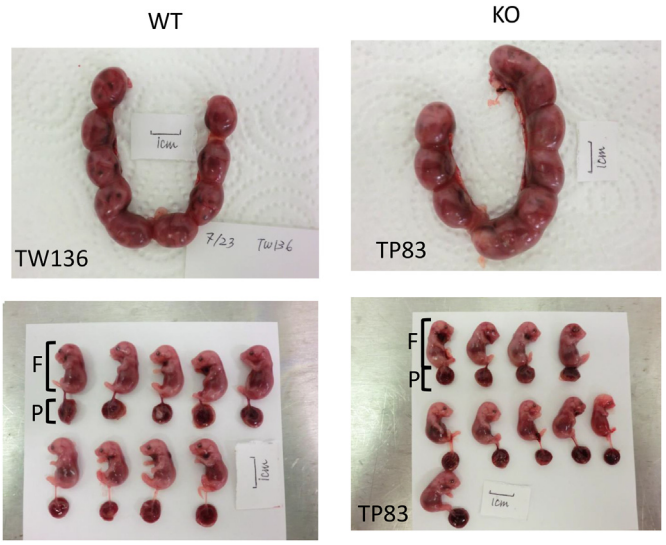

Fig. 4. Photographs of typical uteri and fetuses of wild-type (WT) and circadian clock–deficient (KO) mice on day 17.5 of the pregnancy period. The upper left photo shows a uterus of a WT mouse; the right upper photo shows a uterus of a KO mouse. The lower left photo shows the fetuses of a WT mouse; the lower right photo shows the fetuses of a KO mouse.

weight per fetus per KO female (0.6 ± 0.0 g) was significantly lower than that of the WT group (0.7 ± 0.0 g) ($P < 0.05$), although the average placenta weight of KO females (0.1 ± 0.0) was the same as that of WT females (0.1 ± 0.0 g). The average number of fetuses derived from one female was the same between the WT and KO groups (8.3 ± 0.9 g and 8.0 ± 0.8 g, respectively) (Table 2).

3.4. Serum progesterone level of WT L/D and WT D/D mice on day 18.5 of pregnancy

In this study, the average birth timing of WT L/D mice was day 19.5 of the pregnancy period, significantly earlier than that of WT D/D mice, day 19.9 of the pregnancy period ($P < 0.05$) (Table 1). This difference was thought to be the result of the different light cycle conditions between WT L/D and WT D/D mice, with L/D mice exposed to light between 0:00 and 12:00 on days 18 and 19 of the pregnancy period (Fig. 1). Birth is known to be triggered by a decrease in the secretion of progesterone from the corpus luteum, which is necessary for continuation of pregnancy via functional luteolysis [15]. In mice, the progesterone level in the blood decreases linearly from 60 to 10 ng/ml from the middle of the pregnancy period to the time of birth [20,21]. We therefore compared the level of progesterone in the serum on day 18.5 of the pregnancy period between WT L/D and WT D/D females to determine whether the light schedule on day 18 of the pregnancy period affected the functional regression of the corpus luteum. The average concentration of progesterone in serum collected from WT L/D mice at 12:00 on day 18 of pregnancy was 42.8 ± 7.2 ng/ml, significantly lower than that of WT D/D mice, 65.3 ± 4.2 ng/ml ($P < 0.05$) (Fig. 5).

light and darkness to which the mice were exposed before day 17.5 of the pregnancy period. WT L/D denotes wild-type mice held continuously under the same light condition (repetition of 12 h of light and 12 h of darkness) after day 17.5 of the pregnancy period. KO D/D denotes circadian clock–deficient mice placed under constant darkness from day 17.5 of the pregnancy period. Bottom X-axis indicates the day (D) and hour (H) in the pregnancy period. Upper X-axis indicates the combination of the day of the pregnancy period and the ratio of the time of day. The Y-axis indicates the ratio of the number of mice that gave birth at each time point to the number of mice examined. Day was defined as one repetition of 12 h of light and 12 h of darkness. The start of the day was designated as 0:00. Day 1 of the pregnancy period was set as the day that the vaginal plug (copulation) was observed.

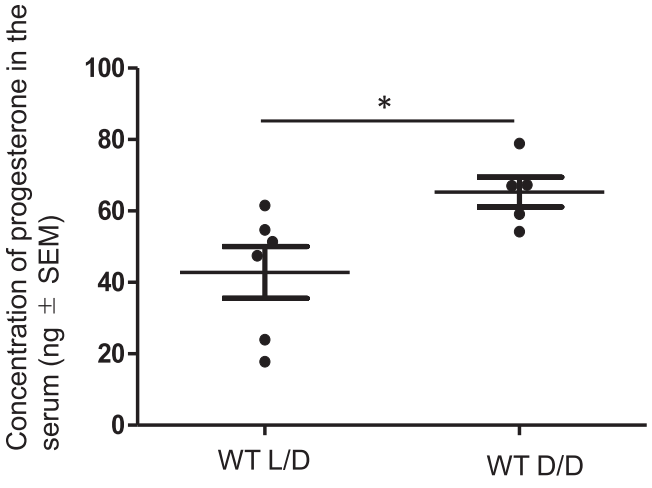

Fig. 5. Concentration of progesterone in the serum of WT L/D and WT D/D mice collected on day 18.5 of the pregnancy period. Each closed circle denotes the progesterone concentration of an individual female. The statistical comparison was done between WT L/D and WT D/D mice on day 18.5 of the pregnancy period. *Indicates a statistically significant difference ($P < 0.05$). Horizontal bars in each treatment denote the average concentration of progesterone. Vertical bar in each treatment denotes SEM.

3.5. Correlation between the total number and weight of pups in one birth and the timing of birth

Spearman correlation coefficients for the association between the weight of pups and the timing of birth were −0.3, 0.3, 0.1, and −0.5 in the WT L/L, WT L/D, WT D/D, and KO D/D groups, respectively. A statistically significant correlation was observed only in the KO D/D group ($P < 0.05$). Spearman correlation coefficients for the association between the number of pups and birth timing were −0.4, 0.2, 0.0, and −0.5 in the WT L/L, WT L/D, WT D/D, and KO D/D groups, respectively. Statistically significant correlations were observed only in the WT L/L and KO D/D groups ($P < 0.05$) (Fig. 6).

3.6. Comparison of the increase in body weight of female mice from the day of copulation (plug confirmation) to immediately after parturition in the WT L/L, L/D, D/D, and KO D/D groups on days 19 and 20

The body weight of female mice in the WT L/L, L/D, D/D, and KO D/D groups on the day of copulation was subtracted from that measured immediately after parturition and averaged for days 18, 19, and 20. Comparison of the data for day 18 was not possible because there were no data for the WT D/D and KO D/D groups. The comparison of data for day 20 was done without the data for the WT L/D group, because there were data for only two mice in the WT L/D group. There were no statistically significant differences between groups on days 19 and 20 (Fig. S2).

4. Discussion

In this study, we demonstrated that a change in the lighting schedule from L/D to D/D in the later phase of the pregnancy period, from day 17.5, affected the timing of birth in mice. Moreover, our data provided no clear indication of involvement of the

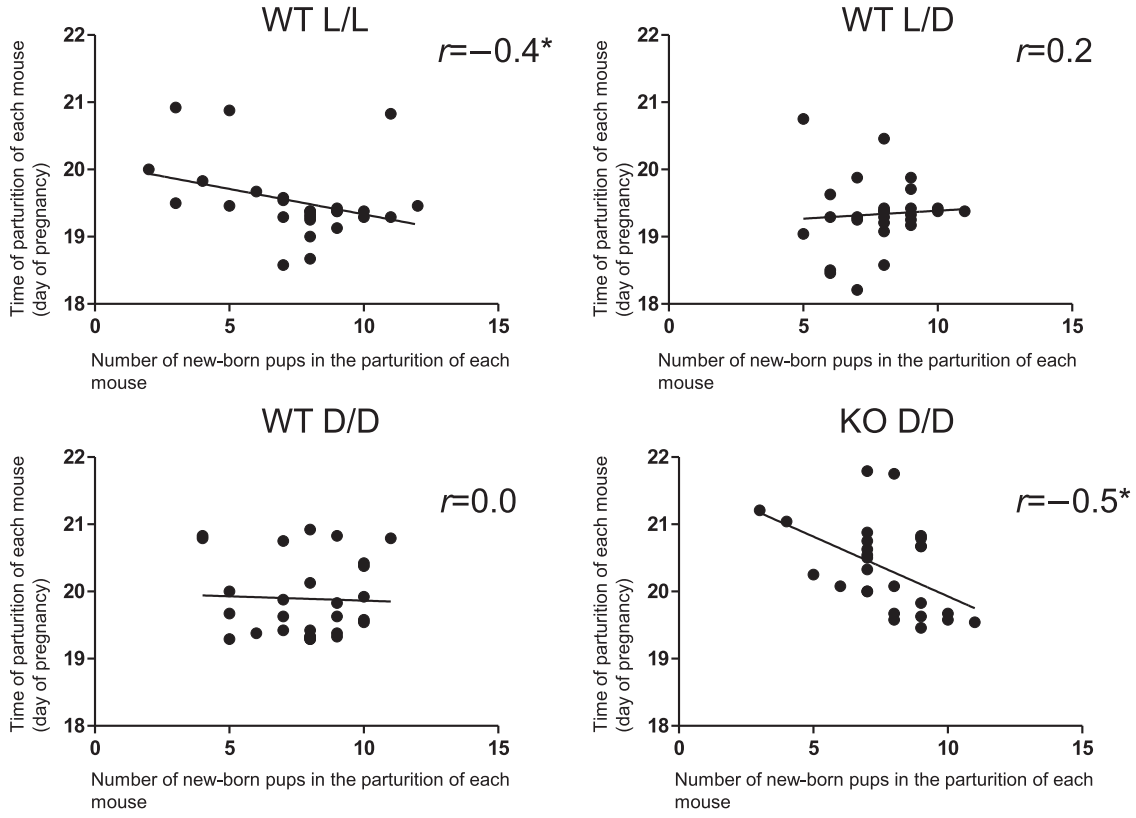

Fig. 6. Correlation between the total number of newborns and time of birth. Each closed circle represents an individual female. WT L/L, WT L/D, and WT D/D denote wild-type mice placed under constant light, repetition of 12 h of light and 12 h of darkness, and constant darkness, respectively, from day 18.0 of the pregnancy period. KO D/D denotes circadian clock–deficient mice placed under constant darkness from the start of day 18.0 of the pregnancy period. *Indicates a statistically significant difference ($P < 0.05$). 'r' indicates the Spearman correlation coefficient.

circadian clock in birth timing.

Evidence suggesting the involvement of the circadian clock in birth timing is derived from observations that there is often a certain period during the day when birth frequently occurs in several species. For example, humans and rats frequently give birth during the dark and light periods of the day, respectively [1,22]. Moreover, the time of day during which birth frequently occurs is spread over several specific days at the end of the pregnancy period. For example, birth frequently occurs during the light period between days 21 and 23 of the pregnancy period in rats, suggesting control of birth timing by the circadian clock [3,5,6]. Similar to previous reports in rats, in the present study, the majority of female mice in the WT L/D group (17/27, 63.0%) gave birth during the light period (0:00 to 12:00) of day 19 of the pregnancy period (Fig. 2). However, this frequent occurrence of birth during the light period was observed transiently on day 19 of the pregnancy period and did not occur again in our data. Therefore, this high frequency of birth during the light period could be related to the physiologically normal pregnancy period of mice.

In mammals, the length of one cycle of the circadian clock depends on feedback loop regulation of the transcription of the circadian genes *Clock*, *Bmal1*, *Pers* (*Per1* and *Per2*), and *Crys* (*Cry1* and *Cry2*) in the middle of the circadian rhythm within the SCN. The length of the period is from the start of transcription of *Pers* and *Crys* by CLOCK and BMAL1 to when the derived proteins, PERs and CRYs, terminate the transcription-inducing activity of CLOCK and BMAL1. Transcription of *Pers* and *Crys* then re-starts, and a new transcription feedback loop commences when suppression of the transcription activity of CLOCK and BMAL1 by PERs and CRYs is released. Moreover, as CLOCK and BMAL1 down-regulate the transcription of many genes (10% of transcribed genes) other than *Pers* and *Crys*, a daily physiologic rhythm in patterns such as sleep is generated [10]. The length of one cycle of the feedback loop in animals, including mice, is approximately (but not exactly) 24 h [10,11]. However, the length of the period of one cycle of the circadian clock is adjusted to 24 h when animals are exposed to repetition of 12 h of light and 12 h of darkness, because the transcription of *Per1* is controlled not only by the transcription feedback loop of the circadian genes but also by the light cycle to which the animals are exposed [23]. Thus, if repetition of environmental exposure to light and darkness is lost, that is, the animals are exposed to constant darkness, the animals revert to the circadian clock with the original cycle that repeats almost every 24 h. The original length of one cycle of the circadian clock in mice is reportedly slightly less than 24 h (between 23.5 and 24 h) [11,24]. Hence, if birth timing is controlled by the circadian clock in mice, the birth timing of the WT D/D mice should have been forwarded approximately 0.5 h compared with that of WT L/D mice. On the contrary, the average timing of birth in WT D/D mice was day 19.9 of the pregnancy period, later than that of WT L/D mice (day 19.5 of the pregnancy period) by 0.4 day, or 9.6 h. Thus, it is difficult to conclude that birth timing was strongly affected by the circadian clock in our study.

As mentioned above, our data suggest that birth timing is independent of the circadian clock in mice. However, it is still possible that the circadian clock concentrates the occurrence of the births at a certain time point. With respect to this point, to obtain more insights about the involvement of the circadian clock with birth timing in mice, we compared the average birth timing and dispersion of birth timing between KO D/D and WT D/D mice. The average birth timing in the KO D/D group was significantly delayed compared with that of the WT D/D group ($P < 0.05$) (Table 1 and Fig. 2). As suggested in a previous report [5], this result was thought to be due to the slower growth of the fetuses in the KO D/D group. Although the weight of the newborns in the KO D/D group was the same as that of WT D/D newborns, the weight of the fetuses of the KO D/D group on day 17.5 of the pregnancy period was significantly lower than that of WT D/D newborns (Table 1 and Fig. 3). In this case, the loss of the circadian clock in KO D/D females was thought to first affect the growth of the fetuses and thus result in a delay in birth timing. It is therefore difficult to conclude that the delay in birth timing observed in the KO D/D group was due to any direct effect associated with the loss of the circadian clock on the mechanism(s) controlling birth timing. The range of the dispersion in birth timing in the KO D/D group was the same as that observed in the WT D/D group, as determined by ANOVA (Table 1 and Fig. 3). This indicates that the circadian clock does not set the birth timing of individuals to a particular time period. It is possible that the abnormal development of the fetuses in the KO D/D group affected the birth timing. However, the morphology of the fetuses on day 17.5 of the pregnancy period was the same as that of the fetuses of WT females (Fig. 4).

In our study, the average birth timing in the WT L/D and WT D/D groups was day 19.5 and 19.9 of the pregnancy period, respectively, which was significantly different (Table 1). This was speculated as being primarily caused by the different light schedule of the WT L/D and WT D/D groups: lights on versus off, respectively, from day 18.0–18.5 of the pregnancy period (Fig. 1). To clarify whether the delay in birth timing observed in the WT D/D group was caused by delayed or slowed decrease in the blood progesterone level in the WT D/D group in this study, we compared the progesterone level in the blood between WT L/D and WT D/D females on day 18.5 of the pregnancy period, corresponding to the end of the light or subjective light period of the WT L/D and WT D/D groups, respectively. As a result, the level of progesterone in the blood was higher in WT D/D than WT L/D females, indicating that constant darkness during the first half of day 18 of the pregnancy period slowed or delayed functional luteolysis.

Involvement of the circadian clock with the timing of functional luteolysis has been reported in rats. A significant decrease in the progesterone level in the blood occurs at a certain time in rats, from the late darkness period to the early light period, in the final few days of the pregnancy period. Moreover, a gene regulating the circadian clock, *Bmal1*, reportedly attenuates progesterone biosynthesis in rat luteinizing granulosa cells [25]. In contrast, in our study using mice, a strong involvement of the circadian clock in regulating the mechanism of functional luteolysis was questionable for the aforementioned reasons. The birth timing of WT D/D females was significantly delayed compared with that of WT L/D females, and the expected dispersion of birth timing of each individual was not observed in the KO D/D group. Souman JL [26] suggested that changes in environmental light affect the physiology of an organism by modifying the circadian clock and/or directly giving rise to acute effects apart from modification of the circadian clock. From our results, the birth timing of mice appears to be controlled more strongly by the acute effects of changes in the environmental light cycle than by effects associated with modification of the circadian clock due to changes in the environmental light cycle. Some reports have suggested that animals have been genetically selected (evolved) to give birth during resting times in the day, possibly to avoid predation [1,2,27]. However, Edwards [2] speculated that birth timing is more likely affected by environmental factors in domesticated animals, such as the prevailing management routine, than by genetic factors. Genetic selection of the trait of giving birth at a certain time period of the day has been absent for many generations in domesticated animals, and therefore, they tend to harbor genetic defects that disturb the occurrence of birth at a certain time of day. This may be one of the reasons why changes to the light cycle schedule (an environmental factor) affected the birth timing of mice in this study.

Secretion of melatonin from the pineal gland is promoted in the dark period of the day because it is regulated by environmental light through the SCN in many animal species [28–30]. An effect of melatonin on birth timing has been reported in several species; melatonin suppresses contraction of the uterus in rats and promotes birth during the light period, but although melatonin induces contraction of the uterus in humans, birth frequently occurs during the dark period [1]. However, Kasahara et al. [31] reported that almost all laboratory mouse strains, including the ancestral strains of the mice used in this study, cannot produce melatonin because they only possess mutated alleles for the gene encoding the indispensable enzyme for melatonin synthesis, hydroxyindole *O*-methyltransferase. For this reason, it is unlikely that the difference in birth timing between the WT D/D and WT L/D groups observed in this study was due to perturbation of melatonin secretion.

The fact that almost all mouse strains lack the ability to produce melatonin suggests that mice are not good model animals to study the mechanism(s) controlling birth timing in other species [30]. However, the involvement of melatonin with birth timing is not clear in some domesticated animal species; the injection of pregnant animals with melatonin at different time points during the day immediately before birth was shown to affect birth timing in rats [22] but not hamsters [32]. In cows, there are several conflicting reports regarding restricted birth timing [2]. These reports may indicate that the genetic ability to give birth at a certain time period in the day due to the daily rhythm of melatonin secretion is now genetically degenerating in humans and domesticated animals because of the absence of genetic selection for many generations [2,30,31,33]: a group of humans or domesticated animals would include individuals that are genetically prone to give birth at a certain time of day and individuals that are not genetically prone to give birth at a certain time of day. Indeed, even in reports demonstrating a restricted time during which birth frequently occurs in humans and cows, the percentage giving birth during the restricted time, night, was only approximately 60%, with the remaining 40% giving birth at another time [1,2]. This 40% of animals or humans may harbor some genetic mutations that suppress melatonin production. This possibility may be supported by the suggestion that the aforementioned mutation in the gene encoding hydroxyindole *O*-methyltransferase that abolishes the ability to produce melatonin in mice arose as a result of domestication, that is, the loss of genetic selection of individuals giving birth at certain time [31]. Thus, mice can be regarded as a good model for some humans and domesticated animals that give birth at times outside the restricted time.

In our study, the irregular light schedule affected the metabolic status of mice and altered the timing of birth [34–36]. It is thus possible to hypothesize that increased/decreased eating time (the time to ingest calories) or time of active movement (the time to expend calories) in the pregnant mice caused by the irregular light schedule caused a change in birth timing. To examine this hypothesis, we subtracted the body weight of pregnant mice at copulation from the body weight immediately after parturition and compared the average values between WT L/L, L/D, D/D, and KO D/D mice on days 19 and 20. No significant difference was observed between groups (Fig. S2), indicating that the total number of calories taken in and expended by the pregnant mice after the change in light schedule was not significantly affected by the light schedule. However, although the total number of calories taken in and expended by the mice was the same, the eating time (the time to ingest calories) or the time of active movement (the time to expend calories) might have differed between WT L/L, L/D, D/D, and KO D/D mice, and this difference could have affected the birth timing of each group. Thus, the possibility that changes in the metabolic profile caused by the change in the light schedule altered the time of parturition remains to be elucidated.

In our study, the body weight of newborns in the WT L/L group was significantly lower than that of newborns in the WT L/D and WT D/D groups (Table 1). In our experiments, we measured the body weight of the newborns only twice (on days 20.5 and 22.0 of the pregnancy period) during the observation period to avoid disturbing the mice. Thus, measurement of the body weight of the newborns was not always done immediately after birth, and the period from birth to the measurement of body weight was approximately 2 days at the longest (Fig. 2). For this reason, the body weight of the newborns could have been affected by the mother's ability to care for them under the different lighting schedules used in the study. However, our data may indicate a detrimental effect of constant light exposure on the growth of the fetuses immediately before birth. Sherwood et al. [37] reported that functional luteolysis is delayed in rats in births involving small litter sizes, resulting in a delay in birth timing. This phenomenon is also known in mice; the birth timing of strains that give birth to a small number of pups is later than that of strains that give birth to a large number of pups [16]. In our study, we observed a tendency for births of a small number of pups to occur significantly later in the WT L/L and KO D/D groups (Fig. 6).

The observed negative correlation coefficient (r) between pup number and birth timing observed in the WT L/L group could have been due to the chance presence of mice that had an extremely larger/smaller number of pups than mice of the WT L/D and D/D groups. However, if the data were reassessed after omitting that obtained for 7 mice that gave birth to fewer than 5 pups (2–4 pups) and more than 10 pups (11–12 pups), the correlation for the L/L group was still negative ($r = -0.4$), although the difference was no longer statistically significant. The reason why births involving a small number of pups were delayed only in the WT L/L and KO D/D groups remains to be elucidated. However, our data at least suggest that the light cycle schedule and circadian clock are involved with the mechanisms controlling birth timing along with the number of pups.

5. Conclusions

In summary, we demonstrated that changes in the light cycle schedule during the later phase of the pregnancy period affect birth timing in mice. Moreover, our data do not provide any clear indication that the circadian clock has a direct effect on the regulation of birth timing similar to that previously reported in rats.

CRediT authorship contribution statement

Tomoko Amano: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing - original draft. **Jürgen A. Ripperger:** Resources, Methodology. **Urs Albrecht:** Writing - review & editing.

Declaration of competing interest

None.

Acknowledgement

The authors would like to thank Ms. Antoinette Hayoz, Ms. Stéphanie Aebischer and Mr. Ashot Sargsyan for technical assistance with the experiments. This work was supported by a Grant-in-Aid for Scientific Research (18K05951) from the Japanese Ministry of Education, Culture, Sports, and Technology, a grant from the

Swiss National Science Foundation (31003A_166682) and the program of long-term residency research of Rakuno Gakuen University. The funding sources had no involvement in study design, data collection, data analysis, data interpretation, or writing of the article.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.theriogenology.2020.05.032.

References

[1] Olcese J. Circadian aspects of mammalian parturition: a review. Mol Cell Endocrinol 2012;349:62–7.
[2] Edwards SA. The timing of parturition in dairy cattle. J Agric Sci 1979;93:359–63.
[3] Lincoln DW, Porter DG. Timing of the photoperiod and the hour of birth in rats. Nature 1976;260:780–1.
[4] Sherwood O, Downing S, Golos T, Gordon W, Tarbell M. Influence of light-dark cycle on antepartum serum relaxin and progesterone immunoactivity levels and on birth in the rat. Endocrinology 1983;113:997–1003.
[5] Reppert SM, Henshaw D, Schwartz WJ, Weaver DR. The circadian-gated timing of birth in rats: disruption by maternal SCN lesions or by removal of the fetal brain. Brain Res 1987;403:398–402.
[6] Rowland D, Wagonblast A, Dykstra T. Timing of parturition in the rat: an analysis of successive births. Chronobiologia 1991;18:31–8.
[7] Plaut S, Grota L, Ader R, Graham III G. Effects of handling and the light-dark cycle on time of parturition in the rat. Lab Anim Care 1970;20:447–53.
[8] Carney EW, Scialli AR, Watson RE, DeSesso JM. Mechanisms regulating toxicant disposition to the embryo during early pregnancy: an interspecies comparison. Birth Defects Res Part C Embryo Today - Rev 2004;72:345–60.
[9] Nagy A, Gertsenstein M, Vintersten K, Behringer R. Manipulating the mouse embryo: a laboratory manual; chaptor 2; summary of mouse development. NY: Cold Spring Harbor Laboratory Press Cold Spring Harbor; 2003.
[10] Ko CH, Takahashi JS. Molecular components of the mammalian circadian clock. Hum Mol Genet 2006;15:R271–7.
[11] Ripperger JA, Jud C, Albrecht U. The daily rhythm of mice. FEBS Lett 2011;585:1384–92.
[12] Roizen JD, Asada M, Tong M, Tai H-H, Muglia LJ. Preterm birth without progesterone withdrawal in 15-hydroxyprostaglandin dehydrogenase hypomorphic mice. Mol Endocrinol 2008;22:105–12.
[13] Zheng B, Albrecht U, Kaasik K, Sage M, Lu W, Vaishnav S, et al. Nonredundant roles of the mPer1 and mPer2 genes in the mammalian circadian clock. Cell 2001;105:683–94.
[14] Zheng B, Larkin DW, Albrecht U, Sun ZS, Sage M, Eichele G, et al. The mPer2 gene encodes a functional component of the mammalian circadian clock. Nature 1999;400:169.
[15] Grazzini E, Guillon G, Mouillac B, Zingg HH. Inhibition of oxytocin receptor function by direct binding of progesterone. Nature 1998;392:509.
[16] Murray SA, Morgan JL, Kane C, Sharma Y, Heffner CS, Lake J, et al. Mouse gestation length is genetically determined. PloS One 2010;5. e12418.
[17] Jud C, Schmutz I, Hampp G, Oster H, Albrecht U. A guideline for analyzing circadian wheel-running behavior in rodents under different lighting conditions. Biol Proced Online 2005;7:101–16.
[18] Fall MW. The use of red light for handling wild rats. Lab Anim Sci 1974;24:686–7.
[19] Dauchy RT, Wren MA, Dauchy EM, Hoffman AE, Hanifin JP, Warfield B, et al. The influence of red light exposure at night on circadian metabolism and physiology in Sprague–Dawley rats. J Am Assoc Lab Anim Sci 2015;54:40–50.
[20] Miyado M, Miyado K, Katsumi M, Saito K, Nakamura A, Shihara D, et al. Parturition failure in mice lacking Mamld1. Sci Rep 2015;5:14705.
[21] Piekorz RP, Gingras Sb, Hoffmeyer A, Ihle JN, Weinstein Y. Regulation of progesterone levels during pregnancy and parturition by signal transducer and activator of transcription 5 and 20α-hydroxysteroid dehydrogenase. Mol Endocrinol 2005;19:431–40.
[22] Takayama H, Nakamura Y, Tamura H, Yamagata Y, Harada A, Nakata M, et al. Pineal gland (melatonin) affects the parturition time, but not luteal function and fetal growth, in pregnant rats. Endocr J 2003;50:37–43.
[23] Shigeyoshi Y, Taguchi K, Yamamoto S, Takekida S, Yan L, Tei H, et al. Light-induced resetting of a mammalian circadian clock is associated with rapid induction of the mPer1 transcript. Cell 1997;91:1043–53.
[24] Ebihara S, Tsuji K, Kondo K. Strain differences of the mouse's free-running circadian rhythm in continuous darkness. Physiol Behav 1978;20:795–9.
[25] Chen H, Zhao L, Kumazawa M, Yamauchi N, Shigeyoshi Y, Hashimoto S, et al. Downregulation of core clock gene Bmal1 attenuates expression of progesterone and prostaglandin biosynthesis-related genes in rat luteinizing granulosa cells. Am J Physiol Cell Physiol 2013;304:C1131–40.
[26] Souman JLTA, Susan F, Pas te, Raymond van Ee, Bjorn NS. Vlaskamp. Acute alerting effects of light: a systematic literature review. Behav Brain Res 2018;337:228–39.
[27] Longo L, Yellon S. Biological timekeeping during pregnancy and the role of circadian rhythms in parturition. In: The endocrine control of the fetus. Springer; 1988. p. 173–92.
[28] Ho AK, Chik CL. Modulation of Aanat gene transcription in the rat pineal gland. J Neurochem 2010;112:321–31.
[29] Schomerus C, Korf HW. Mechanisms regulating melatonin synthesis in the mammalian pineal organ. Ann N Y Acad Sci 2005;1057:372–83.
[30] McCarthy RT, Jungheim ES, Fay JC, Bates K, Herzog ED, England SK. Riding the rhythm of melatonin through pregnancy to deliver on time. Front Endocrinol 2019;10:616.
[31] Kasahara T, Abe K, Mekada K, Yoshiki A, Kato T. Genetic variation of melatonin productivity in laboratory mice under domestication. Proc Natl Acad Sci U S A 2010;107:6412–7.
[32] Viswanathan N, Davis FC. Timing of birth in Syrian hamsters. Biol Reprod 1992;47:6–10.
[33] Kistka ZA-F, DeFranco EA, Ligthart L, Willemsen G, Plunkett J, Muglia LJ, et al. Heritability of parturition timing: an extended twin design analysis. Am J Obstet Gynecol 2008;199:43. e1-. e5.
[34] Fonken LK, Workman JL, Walton JC, Weil ZM, Morris JS, Haim A, et al. Light at night increases body mass by shifting the time of food intake. Proc Natl Acad Sci U S A 2010;107:18664–9.
[35] Coomans CP, van den Berg SA, Houben T, van Klinken J-B, van den Berg R, Pronk AC, et al. Detrimental effects of constant light exposure and high-fat diet on circadian energy metabolism and insulin sensitivity. Faseb J 2013;27:1721–32.
[36] Gonzalez M. Dim light at night and constant darkness: two frequently used lighting conditions that jeopardize the health and well-being of laboratory rodents. Front Neurol 2018;9:609.
[37] Sherwood O, Downing S, Rieber A, Fraley S, Bohrer R, Richardson B, et al. Influence of litter size on antepartum serum relaxin and progesterone immunoactivity levels and on birth in the rat. Endocrinology 1985;116:2554–62.