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Effect of Constant Light on Fetal and Maternal Prolactin Rhythms in Sheep*

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ABSTRACT

A 24-h rhythm of plasma PRL is present in fetal sheep. This rhythm is synchronized to an environmental clue (*zeitgeber*). We determined whether the light-dark cycle (L:D) is a *zeitgeber* for the fetal PRL rhythm and, if so, whether the mother might convey this *zeitgeber* to the fetus. We kept nine ewes (twin pregnancies) in constant light (L:L) and five ewes (singleton) in 14:10 L:D from 110 days gestation. Fetuses and mothers were catheterized at 119 days gestation. Blood samples were taken hourly for 24 h after 16 days under L:L or L:D. A mean 24-h rhythm of PRL was found (by RIA) in fetuses under L:D, but not in those under L:L. However, fetuses under L:L showed individual 24-h PRL rhythms (cosinor analysis) whose acrophases were distributed around the clock. Nonsynchronized rhythms persisted after 23 and 30 days of L:L. Acrophases of PRL rhythms within a set of twins were closer than those between sets, suggesting that twins were responding to a common signal. These findings indicate that the L:D cycle is a *zeitgeber* for the PRL rhythm in fetal sheep and suggest that the mother might convey the *zeitgeber*. (*Endocrinology* **137:** 2355–2361, 1996)

CIRCADIAN RHYTHMS are endogenous biological rhythms with a cycle length close to 24 h. In mammals, these rhythms are generated by a pacemaker, a biological clock, located in the suprachiasmatic nucleus (SCN). This biological clock is entrained by environmental signals (*zeitgebers*), the most important of which is the light-dark (L:D) cycle. Information concerning the L:D cycle is conveyed to the SCN by the retinohypothalamic tract (1–3). The circadian system initiates its function during fetal life. In rodents, for example, the fetal SCN shows rhythms of metabolic activity (4). However, using rodents in this type of study imposes significant technical limitations due to their small body size. In contrast, using sheep, a large mammal, makes it possible to perform chronic fetal catheterization *in utero*, which has allowed exploration of the presence of different components of the circadian system during fetal life. In fetal sheep, the SCN shows circadian changes in the expression of c-*fos* consistent with a pacemaker function at 135 days gestation (gestation length, 147 days) (5). In this species, innervation by the retinohypothalamic tract is completed at 62 days gestation (6), providing a potential entrainment pathway. In addition, 24-h rhythms of vasopressin in the cerebrospinal fluid (7) and of PRL in the plasma (8, 9) have been demonstrated in fetal sheep. This 24-h PRL rhythm is abolished by lesions of the fetal hypothalamus that contain the SCN (10), suggesting that in sheep, the PRL rhythm is commanded by the fetal circadian system. Finally, synchronization to external signals (*zeitgebers*) is also present *in utero* in sheep, because in intact fetuses from different mothers kept under the same external conditions, the acrophases of the 24-h PRL rhythms (hours when the PRL concentration is highest) of individual fetuses are similar (9, 10).

The *zeitgeber* for the PRL rhythm in the fetal sheep has not been identified. A likely *zeitgeber* is the L:D cycle, as is the case for the PRL rhythms of the newborn and adult sheep (9, 11). If the L:D cycle is the *zeitgeber*, fetuses may receive the L:D signal either directly or through the mother. The direct route is unlikely. Although the retinohypothalamic tract is functional in the sheep fetus, as disruption of the tract decreased fetal PRL concentration, the acrophase of the fetal PRL rhythm was not altered (12). These results suggest that the fetal PRL rhythm does not use the L:D cycle as a *zeitgeber*, or that the mother conveys a signal that synchronizes the fetal PRL rhythm to the external L:D cycle.

The present study tested the hypothesis that the external L:D cycle is a *zeitgeber* for the fetal sheep PRL rhythm. We also determined whether the mother might convey this signal to the fetus. To explore the *zeitgeber*, we maintained pregnant sheep carrying twin fetuses in L:L. Under this condition, circadian rhythms persist, but the period deviates slightly from 24 h (13–15). In addition, because of the differences in period length, acrophases occur at different times of the day, and the 24-h rhythms are no longer synchronized among individuals (15). Thus, detection of nonsynchronized individual fetal 24-h PRL rhythms in the absence of a L:D cycle

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would suggest that the fetal PRL rhythm is missing this *zeitgeber*. We chose this experimental condition instead of continuous dark to avoid decreasing fetal PRL, a known effect of short days (16, 17), which would hinder the detection of 24-h PRL rhythms (17). Finally, the use of twin pregnancies indirectly adds insight into the ways that the *zeitgeber* is transmitted. If the L:D cycle is a *zeitgeber* and the mother conveys the information, we should find that the acrophases of the PRL rhythms are similar for twins (because they share the same maternal environment), but differ from the acrophases of the rhythms of fetuses of other mothers. The converse would be expected if each fetus directly detects the lack of a *zeitgeber*.

Materials and Methods

Animals

Fourteen time-dated pregnant ewes carrying a total of 23 fetuses were used in the experiments. The ewes, of mixed breed, were obtained from Nebeker Ranch (Lancaster, CA). At 110 days gestation, ewes were allocated to a control (L:D) or an experimental (L:L) group. The rooms housing the ewes were illuminated with white light, reaching 2300 lux of intensity at the head level of the ewes for 14 h (0700–2100; L:D group) or continuously (L:L group). Room temperature was maintained at 23 C, and food and water were available *ad libitum*. Experiments were performed between December and April at Loma Linda University. All procedures were approved by the Loma Linda University institutional animal care and use committee.

Experimental protocol

Beginning at 110 days gestation, five ewes carrying singleton fetuses were housed in a room under 14 h of light and 10 h of darkness (L:D). The other nine ewes, all carrying twin fetuses, were housed under continuous light (L:L). We felt that inclusion of twin pregnancies in the control group would be an unnecessary use of animals and effort, without adding new information; it has been amply demonstrated that a 24-h fetal PRL rhythm is present in the mean data under L:D conditions (8–12, 17), and no differences in 24-h rhythms of PRL (9) or vasopressin (7) have been observed between twin and singleton pregnancies housed under L:D. In addition, demonstration of the absence of a *zeitgeber* requires showing that a rhythm disappears in the mean data but persists in individual fetuses. Evidence of a maternal role in the transfer of a *zeitgeber* signal requires detecting an appropriate number of twin pregnancies in which both fetuses show individual rhythms. Therefore, to maximize the chances of answering these questions, we allocated all ewes carrying twins to the L:L group.

At 119 days gestation, arterial and venous catheters were surgically placed in ewes and fetuses, as described previously (9). Surgeries were performed between 0930–1300 h. After surgery, the ewes were brought back to their rooms. Catheters were pretreated with a heparin-binding resin (TDMC, Polysciences, Warrington, PA) and flushed daily with heparinized sterile 0.9 g/dl NaCl (1000 IU heparin/ml). Animal husbandry procedures, catheter maintenance, *etc.*, were performed randomly during the day and night, to eliminate additional external time cues.

Fetal and maternal blood samples were collected 7 days after surgery in the L:D and L:L groups (after 16 days of exposure to laboratory light conditions) for assay of PRL. In addition, samples were collected from one of the fetuses and its mother in four of the twin pregnancies after 23 and 30 days of exposure to L:L. Samples were drawn every hour for 24 h from maternal and fetal catheters using sterile procedures. Nocturnal samples were taken under a dim red light when sampling the L:D group and with the lights of the room on when sampling the L:L group. Blood was centrifuged at 1200 × *g* for 10 min at 4 C; plasma was harvested and stored at −20 C for later immunoassays. Red blood cells were resuspended in sterile 0.9 g/dl NaCl and reinfused into the fetuses every 4 h. In addition, fetal femoral artery pH, hematocrit, hemoglobin concentration, hemoglobin saturation, and pO_2 were measured every 6 h to assess fetal well-being.

Assays

PRL was measured by RIA, using NIDDK antiovine PRL-1 and ovine PRL for iodination and standard (provided by the National Hormone and Pituitary Program, Baltimore, MD). The method was previously described and validated (9). The sensitivity of the assay was 0.8 ng/ml (0.08 ng/tube). Intra- and interassay coefficients of variation were 6.8% and 12.7% for low (0.29 ng/tube), 5.0% and 10.6% for middle (0.67 ng/tube), and 6.4% and 16.0% for high (2.36 ng/tube) pools. Maternal and fetal samples from the same pregnancy were measured in the same assay. In experiments including twins, samples from both fetuses were measured in the same assay.

Statistical analysis

The PRL concentrations of fetuses and mothers were normalized by expressing each value as a percentage of the daily mean. For the mean data, the presence of 24-h rhythms of PRL concentration was determined by ANOVA and the least significant probability test (LSD) (18) as well as by cosinor analysis (19). Results are expressed as the mean ± SE. Results were considered significant when $P < 0.05$.

For data from individual fetuses, the presence of 24-h rhythms was tested using cosinor analysis. In this analysis, data are represented by the function: $Vt = M + A \cos 360/\tau\ (t - \phi)$, where Vt is the hormonal concentration at time t, M (mesor) is the mean hormonal concentration throughout the period, τ is the period of the rhythm (preestablished as of 24 h), ϕ (acrophase) is the time at which the function reaches a maximum, and A (amplitude) is the difference between the mesor and the value of the function at the time of the acrophase. With this method, a significant P value indicates that the data fit a cosine function with a period of 24 h. The randomness of the distribution of acrophases around the clock over 24 h was analyzed by Rayleigh's test (20). When individual rhythms were present but the acrophases showed a random distribution, the rhythms were aligned by considering all acrophases as 0100 h to calculate the mean rhythm of the group. The acrophases of the twins were compared by Wilcoxon signed ranks test, using as the null hypothesis the fact that values within twins were similar (21).

Differences in fetal and maternal PRL concentrations between the L:L and L:D groups and changes in PRL concentrations within the L:L group (16 *vs.* 30 days of exposure to L:L) were analyzed by Student's t test.

Results

Mean 24-h PRL rhythms

A 24-h rhythm was detected by ANOVA, LSD, and cosinor analysis in the mean PRL concentrations of the fetuses kept under L:D conditions (Fig. 1, *upper left panel*). Maximum values were obtained at 2200 and 2300 h ($P = 0.024$, by ANOVA and LSD). The rhythm fitted the function PRL (nanograms per ml) = 26.6 + 3.6 cos 15 (t − 23.3) ($P = 0.021$). In contrast, no rhythm was detected in the mean PRL concentrations of the fetuses kept under constant light for 16 days (Fig. 1, *upper right panel*) or for 23 or 30 days (data not shown).

No PRL rhythm was observed in the mothers under either L:D or L:L conditions (Fig. 1, *lower left and right panels*, respectively).

Individual 24-h PRL rhythms

Despite the absence of a 24-h rhythm in the mean PRL concentrations of fetuses kept under constant light, individual fetal 24-h PRL rhythms were not abolished. At all three times of exposure to L:L studied (16, 23, and 30 days), in-

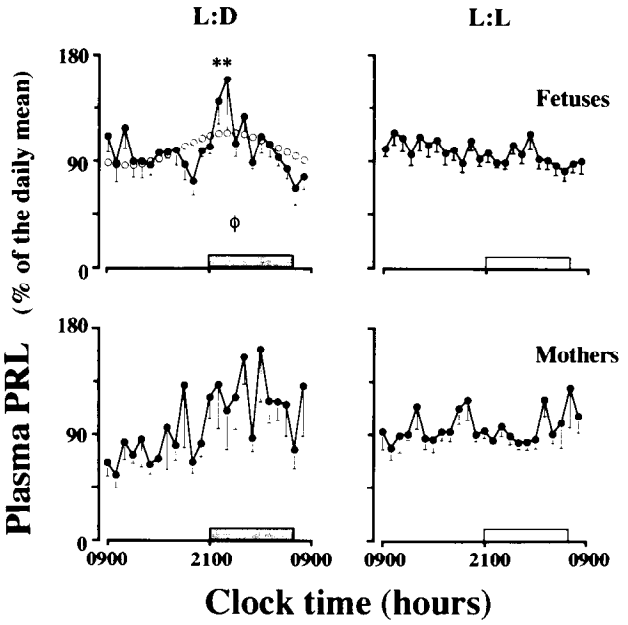

FIG. 1. Effect of exposure to constant light (L:L) on the variation in plasma PRL in fetal (*upper panels*) and maternal (*lower panels*) sheep during 24 h (*filled circles*). *Left*, Pregnancies kept under 14:10 L:D (n = 5). The *horizontal shaded bars* represent the time of day when the room lights were off. PRL was increased in the fetuses at 2200 and 2300 h (by ANOVA and LSD test). The data fitted a cosine function with a period of 24-h (cosinor analysis), represented by the *empty circles*. The ϕ indicates the acrophase of the function (hour of the day at which the function reached the maximum). *Right*, Pregnancies kept for 16 days under constant light (fetuses = 18; mothers = 9). The *empty horizontal bars* indicate constant light. Data are the mean ± SE.

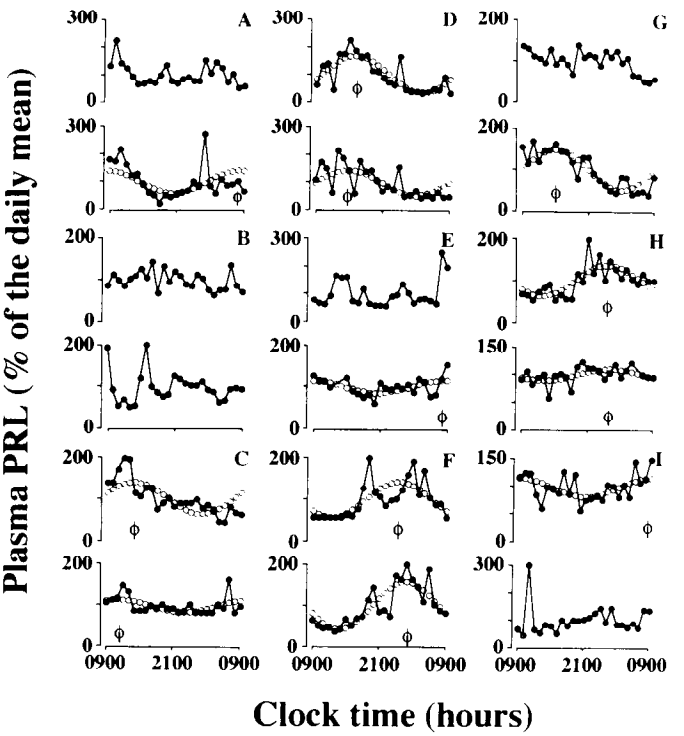

FIG. 2. Individual daily patterns of plasma PRL in the 18 fetuses whose mothers were maintained under constant light conditions. *Filled circles* represent the experimental values. *Open circles* represent the best-fitted cosinor function with a period of 24 h, depicted only in fetuses that showed a rhythm. The ϕ indicates the acrophase of the rhythms (hour of the day at which the function reached the maximum). A–I show sets of twins.

TABLE 1. Cosinor analysis of maternal and fetal PRL rhythms in pregnancies kept under L:L

Mother	Mesor (ng/ml)	Acrophase (clock hour)[a]	Fetus	Mesor (ng/ml)	Acrophase (clock hour)[a]
A	0.9 ± 0.1		A1	4.9 ± 0.5	8.4 ± 1.4
			A2	7.0 ± 0.6	
B	0.8 ± 0.1		B1	4.8 ± 0.4	
			B2	9.6 ± 0.4	
C	1.2 ± 0.4		C1	1.3 ± 0.1	10.8 ± 1.4
			C2	6.0 ± 0.4	13.7 ± 1.0
D	1.0 ± 1.0	10.1 ± 1.5	D1	12.9 ± 1.1	15.4 ± 1.0
			D2	6.7 ± 0.5	16.0 ± 0.6
E	1.8 ± 0.2	19.9 ± 1.3	E1	4.6 ± 0.2	8.6 ± 1.4
			E2	6.0 ± 0.6	
F	0.9 ± 0.1		F1	8.3 ± 0.5	1.8 ± 0.6
			F2	1.9 ± 0.1	0.4 ± 0.9
G	1.0 ± 0.1		G1	23.2 ± 1.2	15.0 ± 0.6
			G2	19.7 ± 1.0	
H	36.2 ± 5.9		H1	7.7 ± 0.2	0.9 ± 1.4
			H2	9.5 ± 0.5	0.6 ± 0.8
I	2.1 ± 0.1		I1	2.7 ± 0.3	
			I2	3.1 ± 0.1	8.9 ± 1.4

Data are expressed as the mean ± SE.

[a] Acrophase of rhythms detected by cosinor analysis. Acrophases do not appear in the table when rhythms were not detected.

dividual fetuses showed 24-h PRL rhythms by cosinor analysis.

After 16 days of exposure to L:L, 12 of the 18 fetuses demonstrated a 24-h PRL rhythm (Table 1 and Fig. 2, *open circles*). The acrophases of the rhythms of these 12 fetuses showed a random distribution around the clock (by Rayleigh's test). A 24-h rhythm was detected in the mean PRL when individual rhythms were aligned considering the acrophase of the cosine function as 0100 ($P < 0.05$, by ANOVA, LSD, and cosinor analysis). The rhythm fitted the function PRL (nanograms per ml) = 7.8 + 2.3 cos 15 (t − 1.2).

After 16 days of exposure to L:L, eight of the nine ewes had PRL concentrations below those of their fetuses and in the low range of the assay (<0.8 to 4 ng/ml). The remaining ewe had a high PRL level (>30 ng/ml). In contrast to the findings in the fetuses, only two ewes presented a rhythm by cosinor analysis (Table 1).

Analysis of the acrophases in sets of twins that both had a 24-h PRL rhythm (sets C, D, F, and H; Table 1 and Fig. 2) indicated that acrophases within twins were similar (by Wilcoxon ranks test), suggesting that the twins were synchronized to a common signal. In each of these sets, the acrophases of the PRL rhythms between twins were closer than those between sets. Fetal 24-h PRL rhythms persisted in the four fetuses that survived for 2 additional weeks under constant light (Fig. 3). Acrophases of the rhythms at 16, 23, and 30 days of L:L were: fetus A_1, 8.4, 1.3, and 1.8 h; fetus D_1, 15.4, 17.6, and 21.4 h; and fetus H_1, 0.9, 17.4, and 2.6 h. Fetus I_1 showed rhythms only at 23 and 30 days of L:L (acrophases were 20.2 and 20.9 h). The weekly drift in the acrophases of the PRL rhythms under constant light was calculated in each fetus as the difference in acrophases between 23 and 16 and between 30 and 23 days of exposure to L:L. Mean weekly

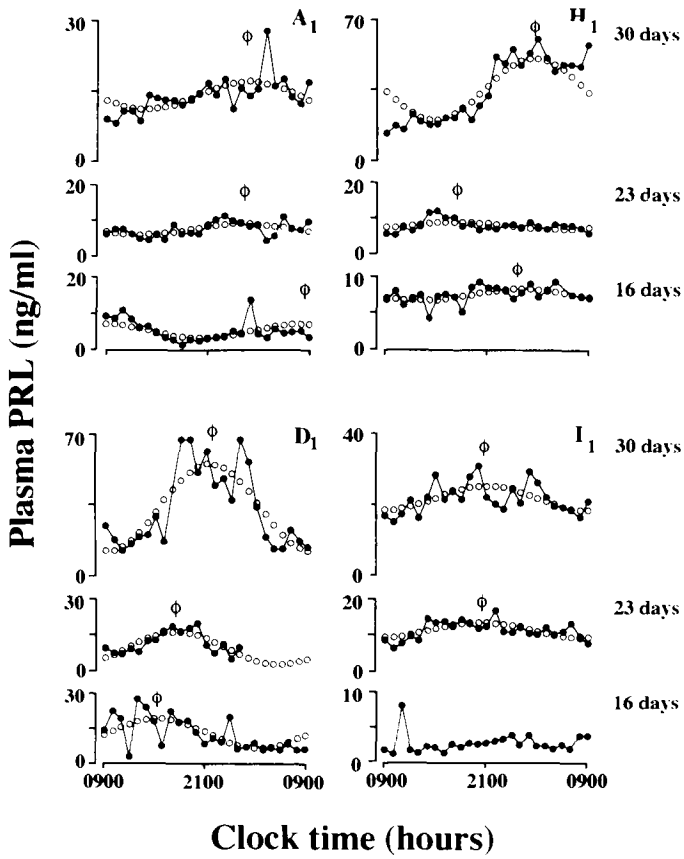

FIG. 3. Individual daily patterns of plasma PRL concentration (nanograms per ml) measured at weekly intervals in four fetuses. In each panel, the *lower graph* depicts measurements performed at 16 days; the *middle graph* shows those performed at 23 days, and the *upper graph* shows those performed at 30 days of exposure to constant light. *Filled circles* indicate experimental data. *Open circles* represent the best-fitted cosinor function with a period of 24 h, depicted only when the experimental data showed a rhythm. The ϕ indicates the acrophase of the rhythms (hour of the day at which the function reaches the maximum). See text for details.

drifts were 12.0 ± 4.8 h between 23 and 16 days (n = 3) and 3.5 ± 2.0 h between 30 and 23 days (n = 4; $P = 0.05–0.1$). The mean daily drifts in the acrophases (one seventh of the mean weekly drifts) were 1.7 and 0.5 h, giving estimated periods of the PRL rhythm of 25.7 and 24.5 h between 16–23 and between 23–30 days of exposure to L:L, respectively. These data suggested that the period of individual fetal PRL rhythms had not stabilized during the sampling period studied.

After 23 and 30 days of exposure to L:L, two and three ewes had 24-h PRL rhythms, respectively.

Fetuses kept under L:D conditions had individual rhythms. Two of the five fetuses had a 24-h rhythm of PRL, whereas in the remaining three fetuses, the rhythm fitted a cosine function, with P between 0.05–0.1.

PRL concentration

After 16 days of treatment, fetal and maternal concentrations of plasma PRL were higher in the L:D group (26.6 ± 6.7 and 39.3 ± 6.6 ng/ml, respectively) than in the L:L group (7.8 ± 1.4 and 5.1 ± 3.9 ng/ml; mean ± SE in fetuses and mothers; $P < 0.05$, by Student's t test). In the four pregnancies exposed to L:L for 30 days, fetal and maternal concentrations of plasma PRL increased to values similar to those found in the L:D group. In the fetuses, the plasma PRL concentration increased from 8.0 ± 2.6 at 16 days to 27.0 ± 5.4 ng/ml at 30 days; in the ewes, it increased from 10.2 ± 8.9 at 16 days to 190.2 ± 76.7 ng/ml at 30 days (mean ± SE; n = 4; $P < 0.05$, by Student's t test).

Cardiorespiratory variables and fetal outcome

Cardiorespiratory variables of fetuses whose mothers were kept in L:L were similar to those of fetuses kept in L:D and within the range previously reported from our laboratory (22). pH values were 7.34 ± 0.01 and 7.35 ± 0.01; hematocrits were 28.7 ± 0.9 and 29.8 ± 1.0%; hemoglobin values were 8.0 ± 0.3 and 9.8 ± 0.5 g/dl; hemoglobin saturation values were 56.9 ± 2.1 and 61.5 ± 2.7%; and pO_2 values were 22.0 ± 1.5 and 25.6 ± 0.9 torr (L:L and L:D fetuses, respectively). However, despite the normal cardiorespiratory variables, fetal outcome was poorer in the L:L group. Whereas all five ewes kept under L:D delivered live fetuses at term, three of the nine ewes kept under L:L delivered prematurely (126–130 days), and of the six ewes that delivered at term, only one delivered two live fetuses. The remaining fetuses died *in utero* after 126 days gestation. These effects of maternal exposure to L:L on fetal outcome are similar to those reported by Stark and Daniel (7).

Discussion

The present study supports the hypotheses that the L:D cycle is a *zeitgeber* for the circadian fetal PRL rhythm and that the ewe conveys this *zeitgeber* to the fetus. To test these hypotheses we studied the effect of the absence of a L:D signal on the fetal sheep PRL rhythm by transferring pregnant ewes from the natural winter photoperiod to constant light. Under this experimental condition, no mean 24-h rhythm of fetal PRL was detected, whereas a mean 24-h rhythm of PRL was present in fetuses of ewes transferred to L:D conditions. As expected from the known endogenous origin of the fetal sheep 24-h PRL rhythm (9, 10), fetuses of ewes kept under constant light showed changes in PRL over 24 h that can be described by a cosine function with a period preestablished as 24 h. The acrophases of the rhythms characterized by the cosine function were distributed at random. However, a mean 24-h fetal PRL rhythm became evident when we synchronized the acrophases of the individual rhythms. Our findings agree with those of previous studies. Differences like those we observed in the fetal sheep PRL rhythm under L:D and L:L have also been described for the rhythm of vasopressin in the cerebrospinal fluid of fetal sheep (7), for the corticosterone rhythm in adult rats (23), and for the rhythms of body temperature (24) and PRL (9) in newborn lambs. In all of these cases, as in our study, the rhythm in the group mean with respect to clock time disappeared under constant light. Also as in our study, individual rhythms of corticosterone in the rat (23) and of temperature (24) and PRL (9) in the newborn sheep were present,

and a rhythm became evident in the mean for the group of animals when calculated after aligning the acrophases of individual rhythms. Thus, our results indicate that under the conditions of the present experiment, the fetal sheep PRL rhythm, like the rhythms of temperature and PRL in the newborn lamb and the rhythm of corticosterone in the rat, uses the L:D cycle as a *zeitgeber*.

The lack of synchronization of the acrophases of PRL rhythms present in individual animals under constant light implies that the period of the rhythm deviates from 24 h; this deviation is known as free running (13–15). Under entrained conditions, *i.e.* animals kept under L:D, acrophases occur at the same clock time. Under constant light, as a consequence of free running, acrophases would not occur at the same clock time in daily measurements in the same individual. The time interval that separates two acrophases measured in hours is the free running period. After 16 days of exposure to L:L, fetuses had very different acrophases. It is unlikely that the presence of twins *vs.* singleton fetuses and the additional surgical stress involved in setting up chronic preparations in twin pregnancies are responsible for the desynchronization of the fetal PRL rhythms under L:L. Although data are scarce, catheterized twin fetuses kept under L:D showed synchronized PRL rhythms (9). In addition, constant light abolished the arginine vasopressin rhythm of singleton fetuses (7). It is reasonable to ask whether exposure to constant light abolishes the ability of the fetal pacemaker to detect the synchronizing signal. Although a PRL rhythm would still be present, it should not synchronize to any signal. The fact that the PRL rhythm is synchronized among twins refutes this interpretation. It is not known at which age an overt PRL rhythm is established in the fetal sheep. Nevertheless, at 90 days gestation, the fetal sheep SCN (the circadian pacemaker) is synchronized to the L:D cycle, showing day-night changes in c-*fos* expression (25). The rhythms of fetal PRL found after 16 days of treatment under constant light or L:D 14:10 may have emerged during the treatment period. Before our experiment began, ewes were exposed to the natural winter photoperiod, when it is known that the PRL concentration is low (16, 17) and no mean 24-h PRL rhythm is detected (17, 26). A likely interpretation of the lack of synchronization of the fetal PRL rhythm at 16 days of L:L is that this rhythm may have emerged during the experiment and could not synchronize because the *zeitgeber* is absent, *i.e.* the pacemaker controlling the PRL rhythm is free running. It is reasonable to assume that this effect of constant light will be detected during the fall and summer, when a fetal PRL rhythm is present. However, this possibility needs to be tested experimentally.

Once a rhythm is detected, successive measurements in the same animal allow calculation of the free running period. In this study we were not able to determine the free running period because of experimental limitations. One is the presence of a well developed response to hemorrhage in the fetal sheep (27), limiting the number of sampling intervals in our experiment. We, therefore, took blood samples at weekly intervals. Another limitation was that we could not continue the experiment long enough to establish a free running period, because births occur between 140–150 days gestation. Nevertheless, from our data we can estimate that the free running period of the fetal sheep PRL rhythm is slightly longer than 24 h. We base this estimate on inspection of the PRL rhythms observed after 16, 23, and 30 days of exposure to L:L, which showed a progressive decrease in the periods calculated from the differences in acrophases observed at each weekly interval. Between 16–23 days, the free running period was large and variable. Variability in the free running period has also been reported for rhythms such as locomotor activity, drinking, and temperature, which can be measured daily. Between 23–30 days, the estimated free running period was 24.50 h, which approaches the value of 24.57 h calculated from weekly measurements for the corticosterone rhythm of pups born to rats carrying a lesion of the suprachiasmatic nucleus (28). It is also within the range reported for the free running period of the rest-activity cycle in *Mesocricetus auratus*, *Rattus exulans*, *Saimiri sciureus*, and humans (13) calculated using continuous measurements over a long time period. Thus, although we were not able to obtain data beyond 30 days, because ewes reached term and delivered, our data suggest that under constant light, the fetal sheep PRL rhythm free runs with a period slightly longer than 24 h.

Our identification of the L:D cycle as the *zeitgeber* for the 24-h PRL rhythm in the fetal sheep allowed exploration of how this information is conveyed to the fetus. We observed that in twin fetuses of ewes kept under L:L, the acrophases of the PRL rhythms were closer than those between different sets of twins. The reason may be either a maternal signal or that one twin synchronizes the other. A maternal signal seems more likely. In rodents, a maternal signal synchronizes the rhythm of metabolic activity of the fetal SCN (29), and preliminary data from our group show that this is also the case for the Fos rhythm in the fetal sheep SCN (30). On the other hand, environmental light enters the uterus in pregnant sheep (31) and rodents (32). In adult lower vertebrates, there is evidence suggesting extraocular photoreception. Early in gestation (60 days), the fetal sheep has some structures analogous to those seen in the amphibian brain, although their function is not known nor whether they persist later in fetal life (33). However, the early development of the visual pathway *in utero* potentially allows direct detection of L:D by the fetus. This pathway is functional, as stimulation of the fetal eye elicits an evoked potential at 55 days gestation (34). In late gestation, the fetal sheep visual pathway is involved in PRL regulation, as blinding by optical enucleation decreased the fetal PRL concentration; however, these blinded fetal sheep (whose mothers were kept under L:D conditions) maintained synchronized 24-h PRL rhythms (12). Although extraocular photoreception cannot be ruled out, the persistence of a synchronized rhythm in the blind fetuses could suggest that a maternal signal conveyed the *zeitgeber* to the fetus. The present observation that the acrophases of the twins' PRL rhythms were closer than those between different sets of twins supports by independent means the former interpretation.

How the ewe conveys the *zeitgeber* to the fetus is unknown. The signals transducing L:D to the fetus may be biophysical, mechanical, metabolic, or hormonal. It is conceivable that the maternal temperature rhythm (35) could provide a signal to the fetus. In addition, the sheep fetus is subjected to mechanical stimulation by minimal, but regular, uterine con-

tractures. These events produce fetal biparietal electrocorticogram changes (36) and stimulate fetal movements (37). Metabolic signals derived from feeding may provide time signals to the fetus, as the fetal cortisol rhythm is detected in fetuses of ewes fed once a day, but not in those of ewes given multiple daily feedings (38). Lastly, hormones that are known to cross the placenta may also provide such signals. The effect of one such signal, melatonin, which conveys information about time and length of the day in adult ewes, has been explored extensively (39). In pregnant sheep, maternal melatonin crosses the placenta, generating a nocturnal rise in the fetal melatonin concentration that is proportional to the duration of the night (40–44). Furthermore, fetal PRL concentrations increase when melatonin decreases, for example after maternal pinealectomy (44). Similarly, in our experiments, the observed increases in fetal and maternal PRL after 30 days of exposure to constant light are probably mediated by a decreased melatonin (light is known to suppress melatonin synthesis) (45). Nevertheless, although melatonin may be involved in changes in PRL concentrations, it does not appear to convey the L:D cycle *zeitgeber* for the fetal sheep PRL rhythms. First, under L:D conditions, the fetal PRL rhythm remains synchronized after either maternal pinealectomy (44) or placement of a melatonin implant in the mother (46). Second, dissociation of the melatonin rhythm from the L:D cycle by infusion of melatonin in pinealectomized ewes showed that the fetal PRL rhythm remained synchronized with the external L:D cycle (47). Although in rats, isolated multiple maternal endocrine extirpations (pineal, pituitary, thyroid, or adrenal) did not affect the phase of circadian rhythms in the neonatal period (48), hormonal signals may be present in other species. In humans, the absence of a maternal cortisol rhythm (49) or treatment of the mother with exogenous glucocorticoids abolished the daily rhythms of fetal movements and fetal breathing (50). Another hormone, estradiol, also increases during pregnancy, and it could be a signal. In adult ovariectomized hamsters, estradiol treatment shortens the period of the activity rhythm (51). Although little is known about circadian placental function, changes in placental enzyme activities may provide signals to the fetus. An example may be 11-hydroxysteroid dehydrogenase, which regulates the conversion of cortisol to cortisone and, therefore, the passage of these steroids to the fetus. In the human placenta, this enzyme changes its activity throughout the day (52). Whatever the nature of the maternal entraining signal, the end pathway at the fetal SCN most likely involves dopamine, because in hamsters, injections during pregnancy of D1-dopamine receptor agonist to mothers with destroyed SCN, synchronize the circadian rhythm of running wheel activity of the pups at weaning. Dopamine may be acting on fetal circadian pacemakers, because the agonist induces c-*fos* expression in fetal SCN (53). Whether this mechanism is functional in the fetal sheep is unknown.

Another important finding of the present study is that placing pregnant ewes in constant light has a deleterious effect on fetal outcome. Despite not finding an effect on the cardiorespiratory variables studied, we found high fetal mortality in the fetuses of these ewes and a high incidence of preterm delivery, not seen in the control group. Although the fact that the fetuses in the L:L group were twins may make this group more labile, the deleterious effects of constant light were very similar to previous observations in singleton pregnancies (7). Our experiment and the previous one (7) cannot distinguish whether the former observations are due to constant light as such or to the absence of an environmental clue. Nevertheless, the possibility that, in an unknown manner, the availability of time clues may be important for fetal survival needs to be considered.

In summary, the present work provides evidence that the L:D cycle is a *zeitgeber* for the fetal sheep 24-h PRL rhythm. In addition, it provides evidence supporting a maternal role in conveying the L:D information to the fetus.

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