0013-7227/04/$15.00/0
Printed in U.S.A.

Endocrinology 145(4):1546–1549
Copyright © 2004 by The Endocrine Society
doi: 10.1210/en.2003-1593

Photoperiodic Regulation of Type 2 Deiodinase Gene in Djungarian Hamster: Possible Homologies between Avian and Mammalian Photoperiodic Regulation of Reproduction

MIWA WATANABE, SHINOBU YASUO, TSUYOSHI WATANABE, TAKASHI YAMAMURA, NOBUHIRO NAKAO, SHIZUFUMI EBIHARA, AND TAKASHI YOSHIMURA

Division of Biomodeling, Graduate School of Bioagricultural Sciences, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-8601, Japan

The molecular mechanisms responsible for seasonal time measurement have yet to be fully described. Recently, we used differential analysis to identify that the type 2 iodothyronine deiodinase (*Dio2*) gene is responsible for the photoperiodic response of gonads in Japanese quail. It was found that expression of *Dio2* in the mediobasal hypothalamus is induced by light and that T_3 content in the mediobasal hypothalamus increased under long day conditions. In addition, we showed that intracerebroventricular infusion of T_3 mimics photoperiodically induced testicular growth. Because it is well known that thyroid hormone is also essential for the maintenance of the seasonal reproductive changes in a number of mammals, we examined expression of *Dio2* in Djungarian hamsters and found expression in the ependymal cell layer lining the infralateral walls of the third ventricle and the cell-clear zone overlying the tuberoinfundibular sulcus. Signal intensity was high under long days and weak under short days. Although light pulse did not affect *Dio2* expression, melatonin injections decreased *Dio2* expression under long days. These results indicate that Dio2 may be involved in the regulation of seasonal reproduction in mammals in the same way as observed in birds. (*Endocrinology* 145: 1546–1549, 2004)

APPROPRIATE TIMING OF various seasonal processes is crucial to the survival and reproductive success of animals living in temperate regions. Accordingly, seasonal reproduction is conserved among various organisms. Although being the focus of a considerable amount of research, the mechanism of photoperiodism is not well described. Japanese quail are an excellent model for studying photoperiodism because of their rapid and dramatic response to the photoperiod. In birds, the mediobasal hypothalamus (MBH) is considered to be the center for photoperiodic time measurement because: 1) lesion blocks photoperiodic response of gonads even though the GnRH system of the lesioned animal has been left intact (1–3); 2) electrical stimulation increases LH secretion and induces testicular growth (4); 3) c-Fos is expressed by long day stimulus (5, 6); 4) deep brain photoreceptors are thought to be localized (7); and 5) the circadian clock is localized (8). These observations indicate that all of the essential machinery for photoperiodic time measurement is localized in the MBH. If the center for photoperiodic time measurement is localized in the MBH, then it is expected that some molecular events may occur in the MBH when birds receive long day stimulus. In our recent study on Japanese quail, we used differential subtractive hybridization analysis to identify a gene that is responsible for photoperiodic response of gonads (9). We found that the expression of this gene, type 2 iodothyronine deiodinase (*Dio2*), is induced by long day stimulus in the MBH. Dio2 is an enzyme which converts prohormone T_4 to bioactive T_3 within a narrow range of concentration (10). T_3 content in the MBH was increased under long days compared with short days, and such differences were not observed in other regions of the brain or in blood. In addition, intracerebroventricular infusion of T_3 mimics photoperiodically induced testicular growth (9). Although thyroid hormones were known to be somehow involved in photoperiodism, our recent study determined both the hormone target site and the molecular events that take place in the brain. Despite the conservation of seasonal reproduction among various species, regulatory mechanism of seasonal reproduction between mammals and birds seems to be quite different. That is, melatonin is critical for mammals, whereas it has no effect in birds (11). Because many studies have indicated that thyroid hormones are also essential for the maintenance of seasonal reproductive changes in a number of mammals (12, 13), we hypothesized that mammals use a similar mechanism for the photoperiodic response of gonads. In this study, we have tested this hypothesis by examining expression of *Dio2* in the long day breeder Djungarian hamster.

Abbreviations: ARC, Arcuate nucleus; *Dio2*, 2 iodothyronine deiodinase; MBH, mediobasal hypothalamus; RXR, retinoid X receptor; THR, thyroid hormone receptor.

***Endocrinology* is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.**

Materials and Methods

Animals and housing

Djungarian hamsters (*Phodopus sungorus*) were kept in our colony under 14-h light, 10-h dark conditions. Food and water were provided *ad libitum*, and sunflower seeds were given once per week. After weaning at 3 wk of age, males were group housed and moved to 8-h light, 16-h dark conditions in light-tight boxes (55 × 210 × 62 cm) to induce testicular regression, according to Milette and Turek (14) with modification. The boxes were placed in a room temperature of 24 ± 1 C. Light was supplied by fluorescent lamps with a light intensity of 200 lux. Short-day animals were kept under short days (8-h light, 16-h dark), and long day animals were transferred to long days (16-h light, 8 h dark) from short days (8-h light, 16-h dark) at 7 wk of age. Brains were collected at 9 wk of age. Animals were treated in accordance with the guidelines of Nagoya University.

Light pulse

Animals were kept under 8-h light, 16-h dark conditions. A single 15-min light pulse was given at zeitgeber time 16, which is within the photoinducible phase. No light was given to control animals. Brains were collected 1 h after the beginning of the light pulse.

Melatonin injection

Eight-week-old hamsters kept under 16-h light, 8-h dark conditions were given daily ip injections of melatonin (Sigma, St. Louis, MO) (10 μg in 0.05 ml 5% ethanol-0.9% NaCl) or vehicle (0.05 ml 5% ethanol-0.9% NaCl) 2 h before lights-out. Injections were given for 8 wk, and animals were killed 8 h after lights-on.

In situ hybridization

In situ hybridization was carried out according to Yoshimura *et al.* (15). Antisense 45-oligomer oligonucleotide probes of Djungarian hamster *Dio2* (5′-tgcttgagtagaatgaccgagtcatagagcgccaggaagaggcag-3′) were labeled with [^{33}P]deoxy-ATP (NEN Life Science Products, Boston, MA) using terminal deoxyribonucleotidyl transferase (Life Technologies, Inc., Frederick, MD). Coronal sections (20 μm thick) were prepared using a Cryostat (Leica CM3050S, Nussloch, Germany). Hybridization was carried out overnight at 42 C. Two high-stringency posthybridization washes were performed at 55 C. The sections were air-dried and apposed to Biomax-MR film (Kodak, Rochester, NY) for 2 wk. ^{14}C Standards (American Radiolabeled Chemicals, St. Louis, MO) were included in each cassette, and the relative OD was measured using a computed image-analyzing system (MCID, Imaging Research, St. Catherine's, Ontario, Canada) and converted into the radioactive value (nanocuries) using the ^{14}C standard measurements. Data were normalized by subtracting the value at the lateral hypothalamic area, which is located in the same section and does not exhibit a hybridization signal. After the exposure to x-ray film, each slide was dipped in type NTB2 autoradiography emulsion (Kodak) and the sections observed with a photomicroscope.

Results

Testicular weight was increased when animals were transferred to long days (Mann-Whitney *U* test, $P < 0.05$, n = 3) (Fig. 1). Expression of *Dio2* was observed in the ependymal cell layer lining the infralateral walls of the third ventricle and the cell-clear zone overlying the tuberoinfundibular sulcus (Fig. 2A). Signals were also associated with the blood vessels in the arcuate nucleus (ARC). No signal was observed in the sense control (data not shown). Signals were strong under long days and weak under short days in the ependymal cell layer and the cell-clear zone overlying the tuberoinfundibular sulcus (Fig. 2, C–F). A single light pulse at photoinducible phase did not induce *Dio2* expression (Mann-Whitney *U* test, $P > 0.05$) (see supplemental Fig. 1 published as supplemental data on The Endocrine Society's Journals Online web site at http://endo.endojournals.org). However, daily melatonin injections significantly reduced the *Dio2* expression in the cell-clear zone overlying the tuberoinfundibular sulcus (Mann-Whitney *U* test, $P < 0.05$, n = 6) (Fig. 3, A–C). Although similar tendency was observed, no significant difference between melatonin and vehicle injections was observed in the ependymal cell layer (Fig. 3D).

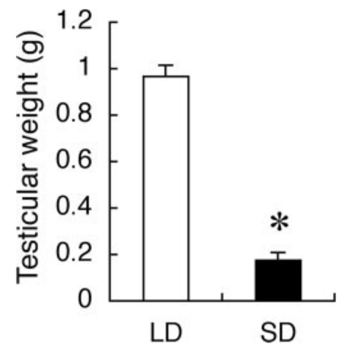

FIG. 1. Effect of day length on testicular weight. Mean ± SEM of paired testes weight for 9-wk-old Djungarian hamsters exposed for 2 wk to either 16-h light, 8-h dark conditions [long day (LD)] or 8-h light, 16-h dark conditions [short day (SD)]. *Asterisk*, $P < 0.05$ (Mann-Whitney *U* test, n = 3).

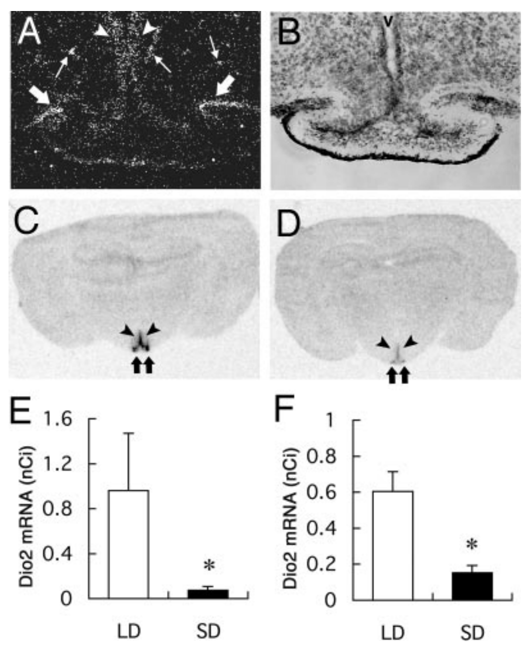

FIG. 2. Expression of *Dio2* in the hamster hypothalamus. A, Representative dark-field photomicrograph showing the distribution of *Dio2* mRNA of long-day animal. Expression of *Dio2* was observed in the ependymal cell layer lining the infralateral walls of the third ventricle (*arrowhead*) and the cell-clear zone overlying the tuberoinfundibular sulcus (*bold arrow*). Signals were also associated with the blood vessels in the ARC (*thin arrow*). B, Light-field photomicrograph of serial section stained by Nissl staining. V, Third ventricle. C and D, Representative autoradiograms for *Dio2* expression under long days (C) and short days (D). *Arrowhead*, Ependymal cell layer; *arrow*, cell-clear zone overlying the tuberoinfundibular sulcus. E and F, Expression of *Dio2* is high under long days and low under short days in cell-clear zone overlying the tuberoinfundibular sulcus (E) and the ependymal cell layer (F). Relative OD was measured using computed image-analyzing system and converted into the radioactive value (nanocuries) using the ^{14}C standard measurements. *Asterisks*, $P < 0.05$ (Mann-Whitney *U* test, n = 3).

Discussion

In the present study, we found expression of *Dio2* gene in the ependymal cell layer lining the infralateral walls of the third ventricle, the cell-clear zone overlying the tuberoinfundibular sulcus and surrounding the blood vessels in the

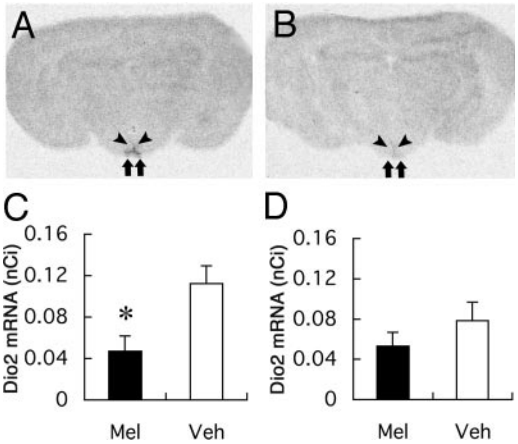

FIG. 3. Effect of melatonin injections on *Dio2* expression. Representative autoradiograms from animals given vehicle (A) or melatonin (B) injections under 16-h light, 8-h dark conditions. Vehicle or melatonin injections were given 2 h before lights-out for 8 wk. *Arrowhead*, Ependymal cell layer; *arrow*, cell-clear zone overlying the tuberoinfundibular sulcus. *Dio2* expression in cell-clear zone overlying the tuberoinfundibular sulcus (C) was decreased in animals that received melatonin injections, whereas no statistical difference was observed in the ependymal cell layer (D). *Asterisk*, $P < 0.05$ (Mann-Whitney *U* test, n = 6).

ARC. These results were consistent with the previous reports on rat kept under 12-h light, 12-h dark, suggesting the possibility that *Dio2* is expressed in the tanycyte in the Djungarian hamster (16). Expression levels were high under long-day conditions and low under short-day conditions. It is noteworthy that in the infundibular nucleus and the median eminence of Japanese quail, *Dio2* is strongly expressed under long-day conditions and weakly expressed in short-day conditions (9). These results suggest the possibility that the molecular mechanism for photoperiodic response of gonads may be conserved between Japanese quail and hamsters.

Recently, a number of groups have tried to identify photoperiodically regulated genes in the Djungarian hamster hypothalamus using cDNA microarrays (17, 18). Prendergast *et al.* (17) found a class of genes encoding T_4-binding proteins (transthyretin, T_4-binding globulin, and albumin) whose expression is associated with reproductive refractoriness to short-day length. In addition, they found reduced hypothalamic T_4 uptake in reproductive refractory animals. It is noteworthy that we found increased T_4 uptake under long photoperiod in Japanese quail (9). In addition, Ross *et al.* (18) found that genes of the retinoid-signaling pathway, encoding nuclear receptors [retinoid X receptor (RXR)/retinoic acid receptor] and retinoid binding proteins (CRBP1 and CRABP2), are photoperiodically regulated in the hypothalamus and suggested that changes in RXR expression may be associated with seasonal changes in body weight and energy metabolism. RXRs are known to form heterodimers with thyroid hormone receptors (THRs). Distribution of THR (α) is reported in the hypothalamus of hamster and the sheep (19). Although we did not find photoperiodic regulation of THR and RXR expression, these genes are indeed expressed in the infundibular nucleus and median eminence of Japanese quail (9). It is particularly interesting that results from different species and with different experimental designs all indicate the involvement of thyroid hormone.

It has been suggested that photoperiodic GnRH release could be controlled at the GnRH terminals by glia (6), and thyroid hormones are known to have a critical involvement in the development, plasticity of the central nervous system (10). Recently, we found that GnRH nerve terminals are in close proximity to the basal lamina in birds subjected to long days using immunoelectron microscopy (Yamamura, T., K. Hirunagi, S. Ebihara, and T. Yoshimura, unpublished observations). We also found that the nerve terminal was enclosed by the end feet of glia in short day animals. It is particularly noteworthy that plasticity of GnRH afferents are reported in sheep and hamster, and possible involvement of thyroid hormones are indicated (20).

Although there is little evidence that melatonin controls the reproductive cycles in birds (11), melatonin is a critical factor controlling the photoperiodic response of gonads in mammals (21). Although *Dio2* expression was induced by light pulse in the dorsal hypothalamus in the Japanese quail, we found no light induction in the hamster hypothalamus. Therefore, we next examined the effect of melatonin on *Dio2* expression. Because chronic melatonin release from beeswax or SILASTIC brand implants (Dow Corning, Midland, MI) can prevent short-day-induced testicular regression (22, 23), we performed daily injection of melatonin according to methods published in previous reports (24, 25). After 8 wk, *Dio2* expression in the cell-clear zone overlying the tuberoinfundibular sulcus of melatonin injected animals was decreased compared with expression in the vehicle-injected animals. Although acute effects of melatonin remains to be clarified in future studies, the present study clearly demonstrates that melatonin injections reduce *Dio2* expression in the cell-clear zone overlying the tuberoinfundibular sulcus. Recently, it is suggested that different expression profiles of clock genes in the pars tuberalis under different photoperiods decode photoperiodic information (26, 27). Activation of *Per* genes occur in the early day, whereas activation of *Cry* genes occur in the early night. This temporal association is conserved even in short and long photoperiods, which result in differences in *Per-Cry* intervals depending on photoperiod. In mammals, expression of *Cry* seems to be regulated by melatonin (26), whereas it is driven by light in birds (27). When taken together with the results from the present study, it seems that mammals and birds use similar mechanism for the photoperiodic regulation of reproduction with different mediator (*i.e.* melatonin and light).

The mechanism that determines long-day breeder or short-day breeder remains unknown. In the short-day breeder ewe, thyroid hormones are required for the seasonal suppression of GnRH and LH secretion, thereby maintaining an annual rhythm in reproductive activity (13). Malpaux *et al.* (28) have demonstrated that melatonin acts in the premammillary hypothalamic area to control reproduction. Recently, Anderson *et al.* (29) have demonstrated that chronic T_4 microimplants to premammillary region (including posterior arcuate and ventral premammillary nuclei) in thyroidectomized ewe allow the termination of the breeding season. It seems possible to speculate that expression of Dio2 is photoperiodically regulated around this region in the ewe, and melatonin and T_4 act via Dio2 expressed in this region. This hypothesis remains to be tested in the future study.

Possible homologies between mammalian and avian seasonal reproduction have been discussed for several decades (12, 13). Although many questions remain to be answered, our present study now provides a way in which to analyze homologies between mammalian and avian photoperiodism at the molecular level.

Acknowledgments

We thank Nagoya University Radioisotope Center for use of facilities and Drs. N. Ibuka, K. I. Maeda, and K. Hirunagi for helpful advice.

Received November 24, 2003. Accepted January 6, 2004.

Address all correspondence and requests for reprints to: Takashi Yoshimura, Ph.D, Division of Biomodeling, Graduate School of Bioagricultural Sciences, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-8601, Japan. E-mail: takashiy@agr.nagoya-u.ac.jp.

This work was supported by a supporting grant from the Program for Promotion of Basic Research Activities for Innovative Biosciences (PROBRAIN) (to T.Yo.).

References

1. **Sharp PJ, Follett BK** 1969 The effect of hypothalamic lesions on gonadotrophin release in Japanese quail (*Coturnix coturnix japonica*). Neuroendocrinology 5:205–218
2. **Davies DT, Follett BK** 1975 The neuroendocrine control of gonadotrophin release in Japanese quail. I. The role of the tuberal hypothalamus. Proc R Soc Lond B 191:303–315
3. **Juss TS** 1993 Neuroendocrine and neural changes associated with the photoperiodic control of reproduction. In: Sharp PJ, ed. Avian endocrinology. Bristol, UK: Society for Endocrinology; 47–60
4. **Ohta M, Wada M, Homma K** 1984 Induction of rapid testicular growth in quail by phasic electrical stimulation of the hypothalamic photosensitive area. J Comp Physiol A 154:583–589
5. **Meddle SL, Follett BK** 1995 Photoperiodic activation of Fos-like immunoreactive protein in neurons within the tuberal hypothalamus of Japanese quail. J Comp Physiol A 176:79–89
6. **Meddle SL, Follett BK** 1997 Photoperiodically driven changes in Fos expression within the basal tuberal hypothalamus and median eminence of Japanese quail. J Neurosci 17:8909–8918
7. **Silver R, Witkovsky P, Horavath P, Alones V, Barnstable CJ, Lehman MN** 1988 Coexpression of opsin- and VIP-like-immunoreactivity in CSF-contacting neurons of the avian brain. Cell Tissue Res 253:189–198
8. **Yasuo S, Watanabe M, Okabayashi N, Ebihara S, Yoshimura T** 2003 Circadian clock genes and photoperiodism: comprehensive analysis of clock genes expression in the mediobasal hypothalamus, the suprachiasmatic nucleus and the pineal gland of Japanese quail under various light schedules. Endocrinology 144:3742–3748
9. **Yoshimura T, Yasuo S, Watanabe M, Iigo M, Yamamura T, Hirunagi K, Ebihara S** 2003 Light-induced hormone conversion of T_4 to T_3 regulates photoperiodic response of gonads in birds. Nature 426:178–181
10. **Bernal J** 2002 Action of thyroid hormone in brain. J Endocrinol Invest 25: 268–288
11. **Gwinner E, Heigel M, Hau S** 1997 Melatonin: generation and modification of avian circadian rhythms. Brain Res Bull 44:439–444
12. **Nicholls TJ, Follett BK, Goldsmith AR, Pearson H** 1988 Possible homologies between photorefractoriness in sheep and birds: the effect of thyroidectomy on the length of the ewe's breeding season. Reprod Nutr Dev 28:375–385
13. **Karsch FJ, Dahl GE, Hachigian TM, Thrun LA** 1995 Involvement of thyroid hormones in seasonal reproduction. J Reprod Fertil Suppl 49:409–422
14. **Milette JJ, Turek FW** 1986 Circadian and photoperiodic effects of brief light pulses in male Djungarian hamsters. Biol Reprod 35:327–335
15. **Yoshimura T, Suzuki Y, Makino E, Suzuki T, Kuroiwa A, Matsuda Y, Namikawa T, Ebihara S** 2000 Molecular analysis of avian circadian clock genes. Mol Brain Res 78:207–215
16. **Tu HM, Kim S-W, Salvatore D, Bartha T, Legradi G, Larsen PR, Lechan RM** 1997 Regional distribution of type 2 thyroxine deiodinase messenger ribonucleic acid in rat hypothalamus and pituitary and its regulation by thyroid hormone. Endocrinology 138:3359–3368
17. **Prendergast BJ, Mosinger Jr B, Kolattukudy PE, Nelson RJ** 2002 Hypothalamic gene expression in reproductively photoresponsive and photorefractory Siberian hamsters. Proc Natl Acad Sci USA 99:16291–16296
18. **Ross AW, Webster CA, Mercer JG, Moar KM, Ebling FJ, Schuhler S, Barrett P, Morgan PJ** 2004 Photoperiodic regulation of hypothalamic retinoid signaling: association of RXRγ with body weight. Endocrinology 145:13–20
19. **Jansen HT, Lubbers LS, Macchia E, DeGroot LJ, Lehman MN** 1997 Thyroid hormone receptor (α) distribution in hamster and sheep brain: colocalization in gonadotropin releasing hormone and other identified neurons. Endocrinology 138:5039–5047
20. **Lehman MN, Goodman RL, Karsch FJ, Jackson GL, Berriman SJ, Jansen HT** 1997 The GnRH system of seasonal breeders: anatomy and plasticity. Brain Res Bull 44:445–457
21. **Arendt J** 1995 Melatonin and the mammalian pineal gland. London: Chapman, Hall
22. **Reiter RJ, Vaughn MK, Blask DE, Johnson LY** 1974 Melatonin: its inhibition of pineal antigonadotrophic activity in male hamsters. Science 185:1169–1171
23. **Turek FW, Desjardins C, Menaker M** 1975 Melatonin antigonadal and progonadal effects in male golden hamsters. Science 190:280–282
24. **Wade GN, Bartness TJ** 1984 Effects of photoperiod and gonadectomy on food intake, body weight, and body composition in Siberian hamsters. Am J Physiol 246:R26–R30
25. **Yellon SM, Goldman BD** 1987 Influence of short days on diurnal patterns of serum gonadotrophins and prolactin concentrations in the male Djungarian hamster, *Phodopus sungorus*. J Reprod Fertil 80:167–174
26. **Lincoln G, Messager S, Andersson H, Hazlerigg D** 2002 Temporal expression of seven clock genes in the suprachiasmatic nucleus and the pars tuberalis of the sheep: evidence for an internal coincidence timer. Proc Natl Acad Sci USA 99:13890–13895
27. **Yasuo S, Watanabe M, Tsukada A, Takagi T, Iigo M, Shimada K, Ebihara S, Yoshimura T** 18 December 2003 Photoinducible phase specific light induction of *Cry1* gene in the pars tuberalis of Japanese quail. Endocrinology 10.1210/en.2003-1285
28. **Malpaux B, Daveau A, Maurice-Mandon F, Duarte G, Chemineau P** 1998 Evidence that melatonin acts in the premammillary hypothalamic area to control reproduction in the ewe: presence of binding sites and stimulation of luteinizing hormone secretion by *in situ* microimplant deliverly. Endocrinology 139:1508–1516
29. **Anderson GM, Hardy SL, Valent M, Billings HJ, Connors JM, Goodman RL** 2003 Evidence that thyroid hormones act in the ventromedial preoptic area and premammillary region of the brain to allow the termination of the breeding season in the ewe. Endocrinology 144:2892–2901

***Endocrinology* is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.**