THE ANATOMICAL RECORD 245:519–524 (1996)

Effect of Exogenous Melatonin on the Onset of Puberty in Female Albino Rats

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ABSTRACT ***Background:*** **The influence of melatonin (MT) on the onset of puberty has attracted many researchers leading to many contradictory claims. Although some researchers believe that exogenous MT delays the onset of puberty in female juvenile rats, others dispute this finding. We explored the effects of exogenous MT, administered for different periods of time, on very young pups.**

Methods: **Groups of 5- and 10-day-old rat pups were given daily 100 μg of MT subcutaneously for 10, 15, and 20 days at 1500 hours. On the vaginal opening day (VOD), rats were sacrificed for collection of ovary and thymus. Besides measuring ovarian and thymus weights, Graafian follicular ratio and thymic corticomedullary ratio were computed.**

Results: **MT caused an advancement of VOD in 5-day-old pups but delayed it in 10-day-old ones. Other parameters studied revealed an exciting correlation with this reversed trend in VOD. In the 5-day-old groups, the ovaries and thymuses showed an increase in weights, but the 10-day-old groups recorded a fall. Follicular and thymic corticomedullary ratios also displayed corresponding increases and decreases.**

Conclusions: **MT administered to younger pups (5 day old) causes an earlier VOD probably due to an ovarian stromal inhibition. In slightly older pups (10 days old), where most secretory mechanisms are progressing from an immature to an adult type, perhaps exogenous MT depresses the developing and sensitive thymus-hypothalamus-pituitary-gonadal axis (THPGA) and causes a delay in VOD.**

Key words: Melatonin, Puberty, Rats, Thymus, Ovary

Topics in medical research, such as production and physiology of melatonin (MT), have brought out an enormous number of reports, which are largely contradictory to each other. Although it is broadly agreed that MT exerts an inhibitory effect on the reproductive axis in mammals (Minneman and Wurtman, 1975), its reproductive role in nonseasonal breeders, such as the laboratory rat, continues to be elusive. Exogenous MT seems to be ineffective in adult male rats (Lang, 1983), whereas in adult female rats, its effects vary from disrupting the estrous cycle (Chu et al., 1964) to exerting a blockade of ovulation (Collu et al., 1971; Sorrentino, 1975). The time of injection, with respect to photoperiod, appears to be crucial since an obvious diurnal pattern in the sensitivity of the reproductive axis to MT has been noticed. Rivest et al. (1986) have demonstrated that MT acts most efficiently when administered either shortly before the onset of darkness or late in the light period.

Badawi and Wilkinson (1988), despite adhering to different accepted time schedules, have denied such actions of MT on the reproductive system in many strains of female laboratory rats, including Sprague-Dawley and Wistar. MT was not found to be effective when administered through drinking water. An interesting correlation between the removal of the olfactory bulbs and increased sensitivity of the reproductive system in female rats has been observed by Reiter (1980). Yamada et al. (1992) demonstrated that, in pinealectomised rats, MT inhibits the reproductive behaviour of male rats. In all, what appear crucial are the age of the rats and the time of administration of MT (Karsch, 1986; Reiter, 1987).

Recently, strong evidence has emerged indicating a physiological link between the pineal gland and the immune system. Such a link might reflect the evolutionary connection between self-recognition and reproduction (Maestroni, 1993). With so many conflicting reports available, it would be significant to probe, at the time of onset of puberty, the histological and histometric changes in ovary and thymus that could be brought about by daily administration of MT.

Received September 12, 1994; accepted October 4, 1995.
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MATERIALS AND METHODS

Animals

Inbred strains of pregnant female Wister rats were used for the entire study. The pregnant rats and the pups that were subsequently delivered were housed, as a matter of acclimatization, under controlled lighting (fluorescent 40 W cool white) of 12 h light: 12 h dark (12L : 12D), lights on at 0600 h. They received rat pellets and water ad libitum. Litters obtained from the pregnant rats provided sufficient numbers of pups for experimental and control groups. Pups remained with their mothers until 22 days of age when they were separated and housed five per cage.

Animal Groups

Five and 10-day-old pups were used for the study. In each age group, there were three subgroups, each of which received MT for 10, 15, or 20 days. Similar control groups received the vehicle alone. Each subgroup consisted of 10 rat pups.

PROTOCOLS FOR MELATONIN ADMINISTRATION

The dose of MT injected was 100 μg by subcutaneous route.

Starting on day 5 or on 10, depending on the age group, (day of birth = day 0), MT (100 μg) was administered subcutaneously to pups for varying periods of time (vide supra), 9 hr after the onset of light (1500 h). Fresh solutions of MT (Sigma Chemical Co., St. Louis, MO) dissolved in saline-ethanol (9:1 V/V; 0.9% NaCL and 100% ethanol), were prepared every day immediately before injections.

Control pups received subcutaneous injections of the vehicle only.

Starting from day 29, rats were checked daily for vaginal opening. On the day of vaginal opening, rats were sacrificed, ovaries and thymus glands, cleaned of the surrounding fat, removed and weighed in an electronic balance. The tissues were subsequently fixed in Bouin's fluid and processed for routine histological examination. Sections (5 μm thick) were taken, stained with haematoxylin and eosin, and examined under Leitz research microscope.

HISTOMETRIC ANALYSIS OF THE HAEMATOXYLIN AND EOSIN STAINED SECTIONS

Ovary

Number of follicles per unit volume (follicular ratio)

The number of follicles per unit volume of the ovary was calculated using the point count method and applying the mathematical formula (Smith, 1991). Point counting of five random histological sections was done by direct microscopy using an eye piece graticule. The mathematical formula used for counting the number of follicles/unit volume of ovary is:

$$F_v = \frac{T_F 3/2}{1.38/P_F/P_O}$$

Where 'F_V' is the total number of follicles per unit volume of the ovary

T_F = Total number of follicles in the section of the ovary.

P_F = Total number of points falling over the follicles.

P_O = Total number of points falling over the ovary.

1.38 = Constant for the section.

Thymus

The sections of thymus were histometrically analyzed for the area occupied by cortex and medulla, in 10 regularly spaced thymic sections, by planimetry using the point count method. This was achieved by superimposing a grid of lines over the sections and counting the grid intersections that hit the medulla and the cortex. The cortico-medullary volume ratio was thus obtained.

Statistical Analysis

Statistical significance was computed using Student's t-test.

RESULTS

Organ Weights and Vaginal Opening Time

Five-day-old pups

Daily MT injection for 10 days had significantly advanced the age at which the vaginal opening occurred. MT treatment of these pups also caused a rise in the weight of the thymus glands and ovaries. Such an increase in weight was proportionately larger in thymus than that in ovary (Table 1).

In rats treated for 15 days as well, there was a significant advancement in VOD and an increase in the thymus and ovarian weights.

VOD was advanced significantly in rats that received MT for 20 days too. Whereas the weight of thymus was observed to be raised, that of the ovary was reduced.

Ten-day-old pups

There was no significant effect on VOD in pups receiving MT for 10 days. The ovarian weight registered a slight fall, whereas thymus weight showed a negligible upward trend in experimental pups compared to the control ones. However, these differences were found to be statistically insignificant.

As for VOD, unlike in other groups, there was an interesting delay in experimental pups treated with MT for 15 days. The mean weights of ovary and thymus were also observed to be significantly lower than the control values.

In pups that received MT for 20 days as well, there was a highly significant delay in VOD. Regarding the thymus and ovarian weights, there was a significant fall, the reduction in the weight of the thymus being more significant.

Histometric Observations

Ovary

Follicular Ratio. The number of ovarian follicles per unit volume of the ovary was observed. It was found that in 5-day-old experimental groups, these values were higher than those in the respective control groups (Table 1, Fig. 1a,b). Ten-day-old experimental groups showed a reverse trend with their values being lower than those in the corresponding control groups (Fig. 1c), the only point of difference being at the level of significance. The reduction in the follicular ratio was highly significant in 10-day-old pups treated for 15 and

TABLE 1. Showing the age of the animals at the beginning of the experiment, the number of days of MT injections administered along with the comparison of control and experimental values, obtained by student's t-test, of various parameters employed[1]

Initial age (days)	Duration of treatment (days)	Vaginal opening time (days)		Ovary weight (mgms.)		Follicular ratio		Thymus weight (mgms.)		Thymus thickness Cortex		Thymus thickness Medulla		Cortico medullary ratio	
		Control	Exp.	Control	Exp.	Control	Exp.	Control	Exp.	Control	Exp.	Control	Exp.	Control	Exp.
5	10	44.00	37.00*	23.33	35.00****	58.26	127.60*	130.00	155.00*	38.00	49.80*	23.80	16.20*	1.63	3.4*
		0.00	0.00	1.67	2.89	14.45	20.55	5.77	2.89	2.3	2.4	2.15	2.94	0.14	0.48
	15	41.67	36.00*	38.33	50.00****	38.93	55.78	165.00	270.00*	46.40	58.80*	23.00	13.4*	2.13	4.67*
		0.03	0.00	1.87	2.09	5.25	6.75	2.89	5.79	4.34	3.37	2.98	2.06	0.3	0.51
	20	42.67	40.00*	43.33***	26.67	56.88	65.29	186.67	235.00*	52.8	72.4*	27.00	10.4*	2.12	7.24*
		0.03	0.00	3.33	1.67	7.58	14.32	6.67	2.89	4.8	4.87	5.12	1.21	0.28	0.66
10	10	44.33	44.00	41.00	36.67	77.89	47.78	190.00	206.67	47.20	51.60	27.20	16.2	2.77	4.32
		0.33	0.00	2.08	4.41	14.68	7.08	5.77	6.67	2.24	0.45	2.63	3.18	0.10	0.60
	15	42.33	45.00***	39.00	20.00**	97.86	44.92*	253.00	183.30*	56.80	37.20***	10.40	16.60***	3.65	2.38*
		0.33	0.58	3.21	0.00	7.09	5.28	10.93	1.67	2.94	2.06	1.75	2.32	0.42	0.25
	20	44.67	50.00*	43.67	40.00*****	54.69	25.31*	270.00	215.00*	61.60	49.01*	10.60	18.80*	5.92	2.71*
		0.33	0.00	0.67	1.15	8.40	4.76	5.77	2.89	2.93	1.00	0.87	1.71	0.40	0.28

[1]Ten animals in each group. Endpoints were measured on the day of vaginal opening. All values are mean ± SEM.
*P < 0.001 **P < 0.004 ***P < 0.01 ****P < 0.025 *****P < 0.05.

20 days and insignificant in pups receiving MT for 10 days only, just as it was so for elevated values in 5-day-old pups getting MT for 10 and 15 days.

Thymus gland

The cortex and medulla were well developed in the thymus glands (Fig. 2a). The corticomedullary ratio was higher and statistically significant in all 5-day-old experimental pups, higher but insignificant in 10-day-olds receiving MT for 10 days only and lower in the other two groups (Table 1, Fig. 2b,c).

There was no significant difference in the appearance and number of Hassall's corpuscles.

DISCUSSION

Thus the pineal gland, once sidelined as a mere vestige, is now a centre of active investigation. Most research on the functions of pineal gland and its active secretion, MT, has dealt with its major role in the regulation of sexual development and function. However, the basic controversy whether the pineal gland has any reproductive effect or not was eliminated with the discovery of a window of maximum sensitivity to administration of MT 9–11 hours after the onset of light. The next issue that had to be sorted out was whether MT had any influence in the reproductive functions of non-seasonal breeders, such as laboratory rats.

There are those who argue that chronic MT administration delays sexual maturation in female rats, probably by retarding maturation of hypothalamic GnRH-producing cells and thus modify basal GnRH secretion or its pulsatile release. A characteristic of the advent of final stages of sexual maturation in the rat, the equivalent of puberty in the human, is the development of a specific pattern of pulsatile GnRH secretion, reflected by an equivalent pattern of LH secretion. Many hypotheses have been put forward to explain these changes, including one at the hypothalamic level and a modification of the ovary. These models are developed to include MT, which appears to play a role in the establishment of the final pattern of LH secretion, thus affecting both vaginal opening and subsequent oestrous cycles. Prior to first ovulation, pulsatile release of LH acquires at least two types of patterns, one with low amplitude, high frequency pulses, announcing a pattern observed in the follicular phase of primates, and the other with high amplitude, low frequency pulses, mimicking what is seen in the luteal phase of primates. Rivest (1987) thus argued that chronic MT administration increases the number of patterns typical of luteal phases and reduces those of the follicular type, resulting in a decreased frequency of LH pulses and longer intervals between estrous cycles.

In vitro studies, where granulosa cells harvested from immature Sprague-Dawley rats, when incubated for a short period (90 minutes) with MT and hCG, caused a significant rise in estrogen. When incubated with ovine LH, MT led to a substantially greater increase in the production of progesterone. Thus according to Fiske et al. (1984), MT is progonadal in that it augments gonadotropic stimulation of granulosa cells and leads to increases synthesis of estrogen or progesterone.

Culture of neonatal anterior pituitary glands in the laboratory with luteinising hormone release factor (LRF) showed a 10-fold increase in median luteinizing hormone (LH) concentration over control values. But addition of MT significantly reduced the stimulatory effect of LRF on release of LH. Thus, Martin and Klein (1976) observed that MT can act directly on the neonatal hypothalamus to inhibit the LH response to LRF.

In contrast, some researchers (Badawi and Wilkinson, 1988) have declared that daily injections of MT, at the appropriate time, in appropriate dose under controlled temperature and lighting arrangements failed to delay puberty, irrespective of the colony of rats used. As a matter of fact, Badawi and Wilkinson (1988) found that the vaginal opening was significantly advanced by 1.5 days. In our experiment, 5-day-old rats treated with MT for 10, 15, and 20 days all had a VOD earlier than their corresponding control rats with values ranging from 2.67–7 days, which are all statistically significant. In the same group, it was observed that rats that received MT for the shortest duration (10 days) had VOD 7 days in advance, whereas those that received

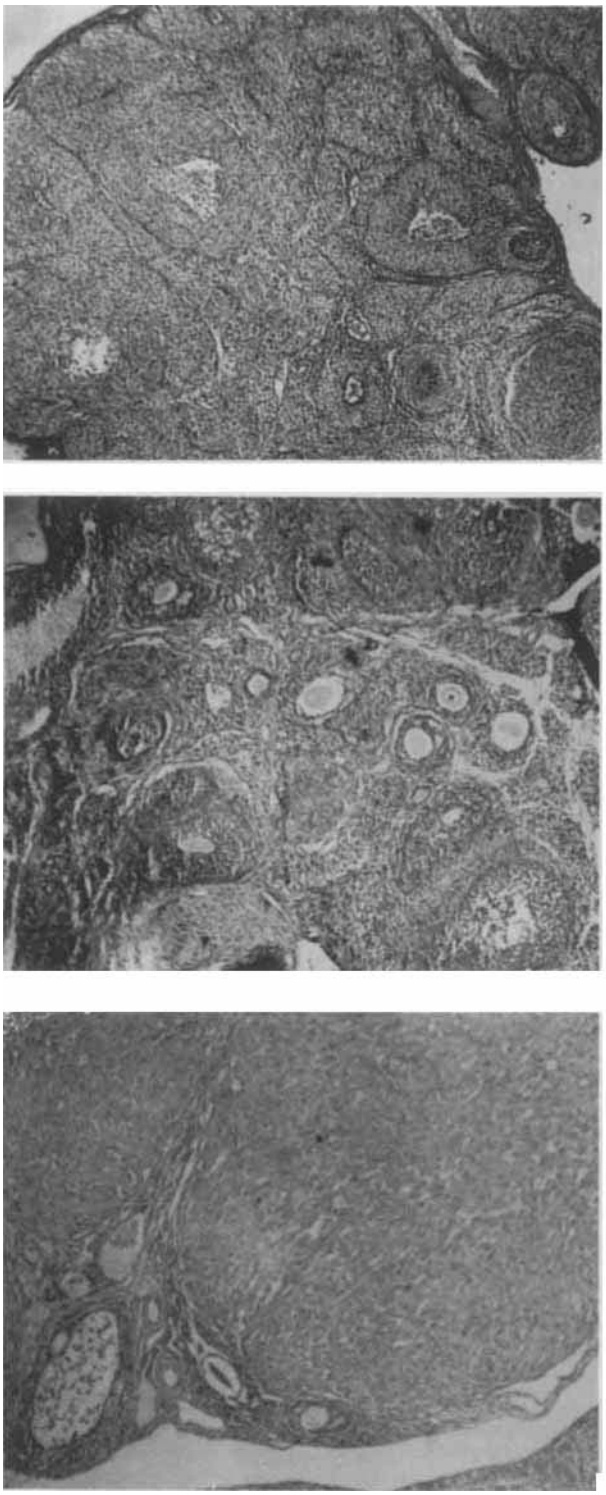

Fig. 1. (a) Section of a control ovary with Graafian Follicles at various stages of maturation. (b) Appearance of a section of ovary in 5-day-old experimental rats. The number of follicles per unit volume is more than that in control. (c) Section of ovary in 10-day-old rats. Very few follicles can be seen. H & E stain (a–c, ×40).

Fig. 2. (a) Section of a thymus from a control rat. Note the well-developed and delineated cortex and medulla. (b) Appearance of thymus in all 5-day-old and 10-day-old experimental rats that received MT for 10 days only. Note the increase in thickness of cortex. The subcapsular cortex is densely packed with lymphocytes. (c) Appearance of thymus in 10-day-old experimental rats that received MT for 15 and 20 days. The cortex is considerably thin and the medulla is very extensive. The density of lymphocytes in the cortex is also strikingly less. H & E stain (a–c, ×40).

for 15 and 20 days showed an earlier onset by 5.67 and 2.67 days, respectively. Thus there appears to be a graded response depending upon the period of exposure to exogenous MT (Table I). This observation could indicate that the MT effect is being gradually neutralized or antagonised by other factors probably belonging to the continuously maturing thymus-hypothalamus-pituitary-gonadal axis (THPGA). This finding correlates with the views of Sizoneko (1985), who observed that MT does not permanently inhibit sexual maturation, since normal but delayed sexual maturation occurs after 45 days of life whether MT administration is discontinued or maintained indefinitely. This means in younger rats that a shorter exposure is good enough for MT to act. Probably with a not-so-fully mature THPGA (Laceta et al, 1988), it is possible that exogenous MT could cause an ovarian stromal inhibition leading to depleted estrogen secretion and alteration of negative feedback effect on this axis. Once the negative feedback effect is altered, an augmented secretion of FSH may occur, leading to ovulation and vaginal opening. As a matter of fact, the results of the follicular ratio carried out in this study point quite well in this direction (vide infra). The "progonadal" effect of exogenous MT, as suggested by Fiske et al. (1984) based on their in vitro studies, could be another mechanism behind the occurrence of early VOD.

Until puberty, ovulation is prevented by means of negative feedback effect of estrogen on hypothalamo-pituitary-gonadal axis. Estrogen produced from ovary exerts its inhibitory effect on both hypothalamus and pituitary. Negative feedback is evidensed by increase in LH and FSH that occur after the decrease in ovarian estrogen secretion after castration or menopause.

In case of 10-day-old rats, the effect of MT seems to range from no effect in 10-day-old rats (treated for 10 days) to a delay in VOD in the same group of rats exposed to MT for 15 and 20 days. Many researchers (e.g., Sizoneko, 1985; Rivest et al., 1986) have reported that the critical period for effect of MT, on THPGA is between 20–30 days. This reversed effect of MT, seen in our experiment, on midprepubertal rats seems very interesting and seems to indicate that this specific age (20 days) is a very critical period where probably the site of action of MT switches from ovary to the fast-developing THPGA. Since it is known that around this period most secretory mechanisms develop from an immature to an adult type of control when markedly increased FSH secretion and elevated pituitary GnRH receptor numbers were observed (Dalkin et al., 1981). By directly acting on THPGA, from this time onward, MT may cause a reduction in the secretion of GnRH, followed by diminished LH surge and hence delayed ovulation resulting in postponement of VOD. Incidentally, Martin and Klein (1976) had observed that MT inhibited the pituitary response to GnRH in rats within 15 minutes of its administration.

This delay in VOD is in agreement with the results of Rivest et al. (1985), who conducted the experiment in 15-day-old rats. Although little is known about the triggering system for vaginal opening, pulsatile administration of GnRH release is thought to be a part of it. Pulsatile administration of GnRH advances the first ovulation in immature female monkeys (Wildt et al., 1980) and is being used as therapy for hypogonadotropic hypogonadism in man (Hoffman and Crowley, 1982). Besides, it also has been shown that GnRH agonist, given to women, can decrease the ovulatory peak of FSH and lead to insufficient follicular maturation (Sheenan et al., 1982).

Vaginal smears taken at VOD showed diestrous cell patterns since the normal association between vaginal opening and the first proestrous is broken with MT administration.

A statistically significant increase in the follicular ratio also fits in place. Quite surprisingly, the level of significance/insignificance (indirectly the follicular ratio) also shows a graded variation, going parallel to the values of VOD. In 5-day-old pups receiving MT for 20 days and 10-day-olds receiving for 10 days, there is no significant increase in the follicular ratio, whereas in other 10-day-old groups, there is statistically significant fall, which explains the delay in VOD in these pups. This observation reinforces our view that the site of action of MT is perhaps different at different age (vide supra), stimulating or inhibiting the growth and maturation of follicles through FSH from two different sites of action.

The data in the follicular ratio are also reflected on the gross ovarian weight, where 5-day-old pups on MT injection for 10 and 15 days recorded an increase in the weight of the ovary, whereas 10-day-olds on MT for 15 and 20 days registered a significant fall.

In all 5-day-old experimental pups, the weight of the thymus gland recorded an increase in comparison with the control pups. The cortices in these rats were also wider and more densely packed with lymphocytes. Very few Hassall's corpuscles were noticed. This increased activity of the thymus gland offers further evidence in support of an early VOD in these pups.

Experimental evidence abounds revealing that MT is of major importance to thymus development (Heradon et al, 1991). It has been shown that low levels of testosterone, demonstrated in decapitated embryos, "shoot" a permanent production of thymosin beta 4, which would in turn explain the high activity of the thymic cortex observed in these embryos (Grossman, 1985).

Thus the presence of a bi-directional communication between the thymus and the hypothalamo-hypophysial-gonadal axis has been established underlying the ability of the thymus to secrete hormones capable of regulating the reproductive axis. Conversely, products of reproductive organs, i.e., gonadal steroids, are important modulators of immune function, and LHRH is an important factor involved in this communication. Moreover, in these interconnections between the immune and the reproductive systems, recent studies have clearly revealed the ability of the thymus to secrete hormones capable of regulating the reproductive axis. Thymosin beta 4 is a potent inducer of LHRH release from hypothalamus, thus stimulating pituitary LH release (Hall et al., 1992).

It, therefore, follows that the earlier VOD in 5-day-old pups could be brought about by an LH surge through a direct inhibitory action on the ovarian stroma and through stimulatory action on thymic cortex. The cortex, incidentally, is more sensitive to thymic microenvironmental changes (Morale et al., 1991).

It was interesting to observe that in 10-day-old pups

on MT for 15 and 20 days, in which animals VOD was delayed, along with a fall in ovarian weight and follicular ratio, the thymus glands also showed a reduction in their own weight and cortical thickness. This finding also correlates well with others, since the basic phenomenon is one of direct inhibition of the reproductive axis, at the hypothalamic level reducing GnRH output. LHRH is known to exert a direct effect on the thymus gland, increasing the weight and thymocyte proliferative capacity as well as functional activation of cortical thymocytes. The low level of circulating LHRH could well abolish this stimulatory effect on thymus (Marchetti et al., 1989).

LITERATURE CITED

Badawi, H., and M. Wilkinson 1988 Lack of effect of melatonin on sexual maturation in female rats. J. Reprod. Fert., *83(1)*:273–278.

Chu E.W., R.J. Wurtman, and J. Axelrod 1964 An inhibitory effect of melatonin on the oestrous phase of the oestrous cycle of the rodents. Endocrinology, *75*:238–242.

Collu, R., F. Fraschini, and L. Martini 1971 Blockade of ovulation by melatonin. Experientia, *27*:844.

Dalkin, A.C., G.A. Bowne, D.R. Pieper, S. Regiani, and J.C. Marshall 1981 Pituitary and gonadal gonadotropin releasing hormone receptors during sexual maturation in the rat. Endocrinology, *108*: 1658–1664.

Fiske, V.M., K.L. Parker, R.A. Ulmer, O.W. Cheng Hoon, and N. Aziz 1984 Effect of melatonin alone or in combination with human chorionic gonadotropin or ovine luteinizing hormone on the in-vitro secretion of oestrogen or progesterone by granulosa cells of rats. Endocrinology, *114*:407–410.

Grossman, C.J. 1985 Interaction between gonadal steroids and the immune system. Science, *227*:435–455.

Hall NR, O'Grady MP. and Menzies R.A. 1992 Thymic regulation of the hypothalamic-pituitary-gonadal axis Int. J. Immunopharmacol *14(3)*:353–359.

Heradon, P.G., B. Razqun, and A.G. Zapata 1991 Effects of early partial decapitation on the ontogenic development of chicken lymphoid organs: 1. Thymus. Am. J. Anat., *191*:57–66.

Hoffman, A.R., and J.R. Crowley 1982 Induction of puberty by long term pulsatile administration of low-dose gonadotropin-releasing hormone. N. Engl. J. Med., *307*:1237–1241.

Karsch, F.J. 1986 A role for melatonin as a time keeping hormone in the ewe. J. Neuro. Trans., *21*:109–124.

Laceta, J., C. Fernandez, C. Sanchez, R. Navrro, R.P. Gomariz, A. Zapata, and A. Tejero 1988 Effects of neonatal treatment with estrogens on the development of the thymus in rats. Dev. Com. Immunol., *12*:375–383.

Lang, U., 1983 Influence of exogenous melatonin on melatonin secretion and on the neuroendocrine reproductive axis of intact male rats during sexual maturation. Endocrinology, *112*:1578–1584.

Maestroni, G.J.M. 1993 The immunoendocrine role of melatonin. J. Pineal. Res., *14*:1–10.

Marchetti, B., U. Guarcello, M.C. Morale, G. Bartolini, Z. Farinella, S. Cordaro, and T. Scapagmini 1989 Luteinizing hormone releasing hormone binding sites in the rat thymus: Characterization and biological function. Endocrinology, *125*:1025–1036.

Martin, J.E., and D.C. Klein 1976 Melatonin inhibition of the neonatal pituitary response to luteinizing hormone releasing factor. Science, *191*:301–302.

Minneman, K.P., and R. Wurtman 1975 Effects of pineal compounds on mammals. Life Sci., *17*:1189–1200.

Morale, M.C., N. Batticane, G. Bartolini, U. Guarcello, Z. Farinella, M.G. Galasso, and B. Marchetti 1991 Blockade of central and peripheral LHRH receptors in neonatal rats with a potent LHRH antagonist inhibits the morphofunctional development of the thymus and maturation of the cell. Endocrinology, *128*:1073–1085.

Reiter, R.J. 1980 The pineal and its hormones in the control of reproduction in mammals. Endocr. Rev., *1*:109–131.

Reiter, R.J. 1987 The melatonin message: Duration versus coincidence hypothesis. Life Sci., *40*:2119–2131.

Rivest, R.W. (1987). The female rat as a model describing patterns of pulsatile LH secretion during puberty and their control by melatonin. Gynecol. Endocrinol., *1(3)*:279–293.

Rivest, R.W., M.L. Aubert, U. Lang, and P.C. Sizonenko 1986 Puberty in rat: Modulation by melatonin and light. J. Neurol. Trans.-Supplementum, *21*:81–108.

Rivest, R.W., U. Lang, M.L. Aubert, and P.C. Sizonenko 1985 Daily administration of melatonin delays rat vaginal opening and disrupts the first estrous cycles evidence that these effects are synchronized by the onset of light. Endocrinology, *116(2)*:779–787.

Sheenan, K.L., R.F. Casper, and S.S. Yew 1982 Luteal phase defects induced by an agonist of luteinizing hormone-releasing factor: A model of fertility control. Science, *215*:170–172.

Sizoneko, P.C. 1985 The pineal and pubertal development. Ciba Foundation Symposium, *177*:208–230.

Smith, B.J. 1991 Comparison of random and serial sections in assessment of ovarian toxicity. Reproductive Toxicol., *5(4)*:379–383.

Sorrentino, S. Jr., 1975 Ovulation in PMS-treated rats with gonadotropin releasing hormone after pentobarbital and melatonin block. Neuroendocrinology, *19*:170–176.

Wildt, L., G. Maishall, and E. Knobil 1980 Experimental induction of puberty in the infantile female rhesus monkey. Science, *207*: 1373–1375.

Yamada, K., K. Maroyama, S. Mogam, N. Miyagawa, and M. Tsubn 1992 influence of melatonin on reproductive behavior in male rats. Chem. Pharmaceut. Bull., *40(8)*:2222–2223.