Seasonal changes in oestrogen receptor affinity in the domestic rabbit, *Oryctolagus cuniculus**

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Summary. Over the course of 1 year the K_a value of uterine oestrogen receptor for oestradiol varied 10-fold from a low of $0.259 \pm 0.065 \times 10^9 \text{ M}^{-1}$ in early spring to $2.21 \pm 0.21 \times 10^9 \text{ M}^{-1}$ in fall. There were no significant changes in receptor number. In addition, administration of oestradiol or progesterone to ovariectomized rabbits resulted in significant reductions in measureable oestrogen receptor affinity. Although these variations in oestrogen receptor K_a values parallel reproductive success in the domestic rabbit, no causal relationship has been established.

Introduction

Seasonal changes in reproduction, pronounced in wild rabbits (Sadleir, 1969; Conaway *et al.*, 1974), have been reduced, but not eliminated, in domestic stocks which are productive throughout the year. During the spring months, females accept males readily and copulations, in most cases, produce sizable litters. In fall and winter months even domestic animals are less productive (Reed, 1979). Rabbits, induced ovulators with a short gestation period (about 31 days), have been used extensively in investigations of implantations, pregnancy, and parturition, but little attention has been given to the phenomenon of seasonality. This is largely because a biochemical basis and confirmation of the observed seasonal variation has not been available. In the course of studying oestrogen receptor properties of rabbit endometrium and myometrium (Stern & Vaughan, 1978, 1979) we have observed a seasonal shift in oestrogen receptor affinity (K_a) which is accompanied by a difference in fecundity between the spring and fall months.

Materials and Methods

New Zealand White rabbits, weighing about 2.7 and 4.1 kg and at least 6 months of age, were obtained from Graves Rabbitry of Knoxville, Tennessee, where they had been exposed to prevailing seasonal conditions. These animals were subsequently housed in an authorized animal facility and used within 2 weeks of purchase. Mature, virgin females were mated with males of proven fertility and killed at 12:00 h during the first week of pregnancy. Uterine tissue was removed, freed of connecting fat, placed on ice, and flushed with cold TEM buffer (40 mM-Tris, 1.5 mM-EDTA, 15 mM-monoethioglycerol, 50 mM-KCl and 10% (v/v) glycerol). since K_a values for oestrogen receptor were reduced by 40 to 80% in the few frozen samples measured, only fresh tissue was used in this study. Endometrium was separated from myometrium by gentle scraping. Receptor properties in the two tissues were measured separately. A high speed centrifugation of tissue homogenates ($100\,000 \text{ g} \times 10 \text{ min}$) produced a particle-free cytosol in TEM, samples of which were incubated (0°C , 16 h) with various concentrations of [^3H]oestradiol-17 β (serial concentrations covering the range (10^{-10} to 10^{-8} M), or with [^3H]oestradiol-17 β and diethylstilboestrol in 100-fold excess as competitor. Receptor-bound and free hormone were separated by an hydroxylapatite (HAP) batch separation procedure (Pavlik & Coulson, 1976) where the hormone-receptor complex binds to HAP. [^3H]Oestradiol was routinely purified on an LH₂₀ mini-column eluted with benzene:methanol (90:10, v/v) (Wu & Lundy, 1971). Data were analysed by the method of Scatchard (1949) to derive values for receptor titre and oestradiol affinity.

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To evaluate the effect for oestradiol and progesterone on uterine oestrogen receptors animals were ovariectomized and treated with hormone. For surgery, the rabbits were injected with fentanyl and droperidol (Innovar-Vet: Pittman-Moore), at a concentration of 0.3 ml per kg body weight 10 min before operation. Immediately before surgery, 0.2 ml (10 mg) pentobarbitone sodium was administered. A midventral incision was made and a double thickness of 00 silk was pulled through the fatty tissue on either side of the mesentery and tied securely to restrict the blood vessels to the ovary. The ovary was then excised. Each animal was given 300 units of antibiotic containing benzathine penicillin and procaine penicillin G, and allowed to recover from anaesthesia under a heat lamp. A cardboard neck collar was attached to each animal to prevent biting of the stitches and removal of the suture.

Oestradiol-17 β (100 μ g/ml) and progesterone (3 mg/ml) were dissolved in unrefined sesame oil previously filtered through Fisher filter paper. Gentle heating and stirring were required to fully dissolve the steroid in the oil. Oil for control groups was treated in the same manner. Subcutaneous injections were given twice daily in the loose skin behind the neck using a 20-gauge needle. The dose was calculated on the basis of body weight (1 ml/kg) giving a concentration of 100 μ g/kg/day for oestradiol and 3 mg/kg/day for progesterone. Half of this dose was given at 10:00 h and half at 17:00 h on each day of injection. Animals were injected on Days 2–5 after ovariectomy and killed at 12:00 h on Day 6. Tissues were treated as described above.

Results

Seasonal changes in oestrogen receptor K_a value are shown in Fig. 1. Receptor K_a value was lowest in February, increased through early summer and remained at constant high levels through late summer and fall. Both endometrial and myometrial values are included in the figure as no significant difference in the seasonal variation was seen between the two tissues.

Figure 2 shows representative Scatchard plots for animals in the spring and fall months. Data are from myometrial samples of 1-day pregnant rabbits assayed in March or November. There was a significant difference between the affinity of binding in the two seasons ($0.73 \times 10^8 \text{ M}^{-1}$ and $2.36 \times 10^9 \text{ M}^{-1}$ respectively). No difference was observed, however, in total binding (0.297 vs 0.222 fmol/ μ g protein). Both lines show good correlation coefficients (0.941 and 0.987) and the change in affinity did not appear to be related to an increase in non-specific binding.

Fig. 1. Seasonal changes in oestrogen receptor affinity constant (K_a). Receptor K_a values were derived from the slope of the line of the bound versus bound over free plot of data from samples assayed over a 16-month period. The values December through April represent a composite of data from two separate years. Numbers of individuals are shown for each point. Vertical bars are s.e.m. (except for the February point in which the vertical bars represent the range of the values).

Fig. 2. Scatchard plot analysis of binding parameters of oestrogen receptors in the myometrium of Day-1 pregnant rabbits in the spring and fall. Values represent duplicate points from which competition from diethylstilboestrol has been subtracted.

Table 1. Comparison of spring and fall titre and K_a value from 1-day pregnant rabbits*

Tissue	Season	$K_a (\times 10^{-9}) \text{ M}^{-1}$	Titre	
			fmol/ μg DNA	fmol/ μg protein
Endometrium	Fall	2.23 ± 0.33	1.27 ± 0.30	0.362 ± 0.030
	Spring	$0.77 \pm 0.51^*$	1.70 ± 0.62	0.352 ± 0.040
Myometrium	Fall	2.43 ± 0.42	2.74 ± 0.67	0.265 ± 0.044
	Spring	$0.40 \pm 0.17^{**}$	3.66 ± 0.37	0.317 ± 0.010

Each value represents the mean $\pm$ s.e.m. from 3 animals.

* $P < 0.05$; ** $P < 0.01$ compared with K_a values for fall.

Table 2. Oestrogen receptor concentration after in-vivo hormone treatment

Treatment	fmol/ μg protein		fmol/ μg DNA		Total receptor (pmol)	
	Endometrium	Myometrium	Endometrium	Myometrium	Endometrium	Myometrium
Oil	0.624 ± 0.091	0.390 ± 0.027	1.21 ± 0.53	2.53 ± 0.13	2.4 ± 0.8	25.8 ± 2.1
Oestradiol	0.125 ± 0.035	$0.130 \pm 0.084^*$	0.77 ± 0.34	2.63 ± 1.73	$6.2 \pm 0.2^{**}$	37.7 ± 5.4
Progesterone	0.450 ± 0.095	$0.222 \pm 0.056^*$	2.78 ± 0.74	5.31 ± 1.64	$34.8 \pm 10.6^*$	25.8 ± 8.1

Values are the mean $\pm$ s.e.m. for 3 animals.

* $P < 0.05$; ** $P < 0.01$ compared to value for oil-treated animals.

Table 1 compares the titres and affinity constants of oestrogen receptors for one group of fall and one group of spring animals. Although the K_a value of the receptor was depressed during the spring in both tissues, the titres of receptors were not significantly different from spring to winter. Annual changes in affinity were not accompanied by changes in receptor titre.

Figure 3 shows the influence of in-vivo administration of oestradiol and progesterone on endometrial and myometrial oestrogen receptor affinity. Both hormones significantly reduced the K_a value in myometrium ($P < 0.05$ for progesterone and $P < 0.01$ for oestradiol) while only oestradiol

Fig. 3. Influence of in-vivo oestrogen or progesterone on oestrogen receptor affinity constant (K_a). Oestrogen (100 μ g/kg/day) or progesterone (3 mg/kg/day) was administered to ovariectomized animals for 4 days. There were 3 samples per treatment group.

significantly depressed endometrial receptor affinity ($P < 0.05$). Both hormones decreased receptor titre when normalized to μ g protein, but an apparent increase in receptor titre occurred in response to progesterone when reported as a function of DNA (Table 2).

Discussion

Domestic rabbits of the species *Oryctolagus cuniculus* are commonly assumed to display no seasonal breeding behaviour. Seasonal changes in litter size, percentage pregnancies, and properties of the reproductive tract, have, however, been reported for wild rabbits. Wild rabbits produce more offspring during mild spring months than during fall or late winter months (Elder & Finerty, 1943; Marsden & Conaway, 1963; Sadleir, 1969).

K_a values, in this study, were lowest in February, increased through June, and remained high throughout the late summer and fall. Tennessee has four distinct seasons, with the summer months of July and August being particularly hot (26–32°C), the winter mild, and spring weather beginning late February and mid-March. The period of low receptor K_a value, in our animals, matched the productive spring season. The increase in K_a value between February and June correlated with increasing daylength and increasing temperatures.

Batra & Kallstrand (1979) have demonstrated that mean oestrogen concentrations of oestrous female rabbits are lower in September than in January. Although we were working with pregnant animals, these data correlate well with Fig. 1 and with our observation (Fig. 3) that oestrogen administered to ovariectomized animals reduced cytoplasmic oestrogen receptor K_a value. Critical levels of oestrogen may be needed for normal development of corpora lutea during early pregnancy in the rabbit (Miller & Keyes, 1975). High levels of oestrogen are, however, inhibitory to uterine development in this progesterone-dependent animal (Hilliard & Eaton, 1971; Kwun & Emmens, 1974). Perhaps, when oestrogen levels are higher in spring, corpus luteum development in early pregnancy is facilitated while lower uterine receptor K_a values protect the uterus, enabling implantation to proceed.

Several studies have reported progesterone-sensitive variations in uterine oestrogen receptor titre during pregnancy in the rabbit (Lee *et al.*, 1971; Jacobson *et al.*, 1972; Quirk & Currie, 1984).

Evans *et al.* (1980) and Okulicz *et al.* (1981) describe a progesterone-controlled regulatory factor for oestrogen receptor in the hamster. Boyd & Spelsberg (1980) report seasonal rhythms in oestrogen and progesterone receptor translation and nuclear binding in the chick oviduct. They suggest that the variation in progesterone receptor is a function of a rhythm of oestrogen action. None of these studies reported a significant variation in oestrogen receptor K_a values. This may, when oestrogen receptor K_a value was examined, be a reflection of the fact that tissues were usually frozen before assay.

Changes in oestrogen receptor K_a values need not be causally related to seasonal changes in reproductive success. It is possible that oestrogen receptor K_a value and reproductive behaviour were both responding to an additional seasonally-modified agent. Photoperiod influences attainment of puberty, length of oestrous cycles, and duration of pregnancies in many mammalian species. These functions reflect LH and FSH concentration and involve the pineal gland (Hoffman, 1973). It is possible that fluctuations in pituitary or pineal output could be influential in producing the observed seasonal variation.

Functions for shifts in receptor K_a values are not known. Nevertheless, changes in K_a value of the progesterone receptor have been previously reported for rabbits (Rao *et al.*, 1973; Davies *et al.*, 1974). Our demonstration that oestrogen and progesterone administered *in vivo* can produce such changes in rabbit oestrogen receptors suggest that modifications in receptor affinity may have a specific function in this species. Seasonal changes in receptor may result from seasonal variations in the levels of reproductive hormone and should not be ignored in studies of reproductive physiology.

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