Uterine Uptake of Progesterone and Estradiol in Young and Aged Rabbits (36799)

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(Introduced by W. Hansel)
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Progesterone is required for the maintenance of pregnancy in nearly all species. The amount of progesterone reaching the target tissue, the binding ability of the target tissue and the ability of the target tissue to respond to the hormone influence the total response in that tissue (1). Target tissues can selectively bind both progesterone (2–4) and estrogen (5, 6). Pregnancy maintenance in the rat and rabbit was found to be correlated more closely with levels of uterine progesterone than plasma progesterone (7, 8).

Our previous studies showed that the decline in reproductive performance in a group of aged does could not be attributed to changes in ovulation rate or ovum potential (9, 10). Luteal function was normal (11) and pregnancy maintenance was not improved by giving supplemental progesterone and/or estradiol (10). The amount of progesterone reaching the uterus was not known but uterine blood flow rate in these aged does was only half of the rate found in young does (12). The ability of the uteri of these rabbits to utilize steroids was not known. Therefore, experiments were designed to test the theory that low reproductive performance in aged does might be related to the ability of the uterus to bind estradiol and progesterone.

Materials and Methods. Each of 18 young does (6–13 mo old) and 28 aged does (49–72 mo old) were randomly assigned to three treatment groups to provide uteri under different conditions. Does in group 1 were ovariectomized and sacrificed 14 days later. Does in treatment groups 2 and 3 were bred naturally and sacrificed 12 and 24 days postcoitum (pc), respectively. Does were randomly started on the experiment so that does from each age and treatment group were sacrificed simultaneously. Treatment details and data at sacrifice have been published elsewhere (10–12).

Does were sacrificed by cervical dislocation. The reproductive tract was quickly exposed and its condition recorded. The uterine horns were trimmed of excess tissue, slit longitudinally and conceptuses when present, were removed. Six strips of uterine wall of similar size were obtained. Six strips of leg skeletal muscle also were taken from each doe as controls.

In vitro steroid uptake was determined by a modification of procedures previously described (13–15). Progesterone uptake was determined on three uterine as well as three skeletal muscle samples from each doe. The six tissue samples from each doe were blotted and placed in one incubation flask containing 20 ml of Krebs–Ringer phosphate buffer (pH 7.4) to which 1×10^{-8} *M* progesterone-7-^{3}H had been added. The specific activity of the labeled progesterone was 20.0 Ci/mmole.

Incubation was for 0, 2 and 4 hr in a Dubnoff metabolic shaking incubator at 37° with 100 shakes/min. After each incubation period one uterine and one muscle sample from each doe were removed from the medium with forceps, rinsed twice with tracer-free buffer, and once with 70% ethanol, blotted dry, weighed, placed in separate counting

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vials and stored frozen until digested. Estrogen uptake was determined similarly excepting that 1×10^{-8} *M* 17β-estradiol-4-^{14}C (sp act, 52 mCi/mmole) was added to the buffer.

Tissue samples were minced with small scissors and Nuclear-Chicago solubilizer (NCS) was added at a rate of 6 ml/g tissue. Samples were digested initially at room temperature with occasional shaking. Samples not digested in 48 hr were heated to 50° and shaken frequently until digestion was complete. Glacial acetic acid equal to 1/30 of the volume of NCS was added following digestion.

Duplicate 0.5 ml aliquots of each tissue digest were transferred to separate counting vials and 10 ml of counting fluid was added. The counting fluid consisted of toluene containing 4 g/liter of 2,5-diphenyloxazole (POP) plus 100 mg/liter of 1,4-bis-2-(5-phenyloxazolyl)-benzene (POPOP). All samples were counted three times for 1 min in a liquid scintillation counter.

Unlabeled control uterine and muscle digests containing known quantities of each radioisotope in combination with an external standard were used to determine the quench correction curve for each radioisotope for both uterine and muscle tissue. Unknown samples were corrected to 100% counting efficiency by use of the external standard. Net activity was determined by subtracting the skeletal muscle uptake from the corresponding uterine uptake. Results were expressed as cpm per mg wet weight of tissue.

Results. All young does in both mated groups had viable embryos. Eight of 10 and 7 of 9 aged does in the 12 and 24 day pc treatment groups, respectively had implantation sites but in each case only four does had viable embryos at the time of sacrifice (10). Uterine steroid uptake appeared to be relatively high in the pseudopregnant aged does compared to pregnant does but variation was great and the difference was not significant. Therefore, the data from all aged does within each treatment group were combined and compared to the young does. Ovariectomized does, particularly in the young age group, had a greater net uterine *in vitro* uptake of progesterone-7-^{3}H and 17β-estradiol-4-^{14}C than did other does at either 12 or 24 days pc (Table I). Uterine uptakes of progesterone and 17β-estradiol in young does were markedly and significantly ($p < .05$) greater than in aged does in the ovariectomized group after both 2 and 4 hr of incubation (Fig. 1). The difference in steroid uptake between age groups was reduced by 12 days pc and disappeared by 24 days pc (Table I). By 24 days pc uterine tissue from both young and old age groups showed minimal uptake of progesterone and estradiol.

Steroid uptake by skeletal muscle tissue was greater in young does than in aged does. Therefore, the uncorrected differences in steroid uptake between age groups was larger than the corrected values reported.

An attempt was made to determine total progesterone content of the uterus in order to determine if *in vitro* progesterone uptake was influenced by the initial content. However, interfering substances were high relative to the progesterone concentration and the various extraction procedures resulted in recoveries too low to provide meaningful data.

Discussion. These results clearly indicate that the uterine capacity for taking up ovari-

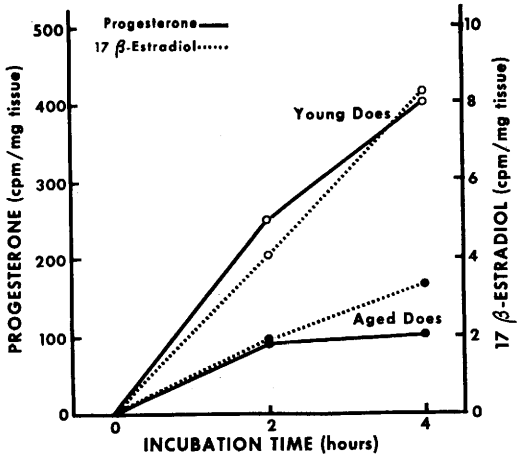

FIG. 1. Age effect on *in vitro* net uterine uptake of progesterone-7-^{3}H and 17β-estradiol-4-^{14}C in ovariectomized rabbits. Differences in cpm between the two hormones reflect, in part, differences in their specific activity.

TABLE I. Net *In Vitro* Uterine Uptakes of Progesterone and Estrogen by Age and Reproductive Status.[a]

Treatment group and incubation time	Progesterone-7-^{3}H		17β-estradiol-4-^{14}C	
	Young does[b]	Aged does[c]	Young does[b]	Aged does[c]
Ovariectomized				
2 hr	249 ± 24[e]	87 ± 26[e]	4.1 ± 0.4[e]	1.9 ± 0.4[e]
4 hr	401 ± 90[e]	101 ± 40[e]	8.3 ± 1.1[e]	3.3 ± 0.6[e]
12 day pc				
2 hr	131 ± 34	72 ± 39	1.8 ± 0.7	1.5 ± 0.5
4 hr	164 ± 31[e]	26 ± 15[d,e]	4.1 ± 0.8[e]	1.9 ± 0.4[e]
24 day pc				
2 hr	44 ± 25	62 ± 18	1.2 ± 0.3	1.4 ± 0.3
4 hr	144 ± 57	95 ± 31	1.3 ± 0.3	1.6 ± 0.4

[a] Group mean ± SE expressed as corrected cpm/mg uterine tissue.
[b] Six young does/treatment.
[c] Nine aged does/treatment except 10 does in the 12 day pc group.
[d] Unusually low value apparently due to sampling error.
[e] $p < .05$.

an steroids was altered by age and reproductive status in female rabbits. The pattern of progesterone and estradiol uptakes were remarkedly similar, although there is evidence for a general progestin receptor (4) and a specific estrogen receptor (16). The binding proteins for progesterone and estradiol are reported to be different in the rat (17). The concentration of specific progesterone binding sites was very low in prepuberal rat uteri compared to adults (3). Incorporation of ovarian steroids by bovine endometrium *in vitro* varied according to the reproductive state of the female at the time of tissue removal, but incorporation apparently was not influenced by the concentration of circulating hormones (15). There was no difference in peripheral plasma progestin levels between the young and aged does used in the present study when measured at 6, 12, 18 and 24 days pc (11). Therefore, the differences between age groups in uterine uptake of steroid hormones *in vitro* does not appear to have resulted from any difference in endogenous hormone levels and possible previous degree of saturation of receptor sites. The reduced steroid uptakes at 24 days paralleled the reduced circulating progestin levels (11).

In the present study estrogen presumably was greater in the young does, particularly at 24 days pc, because they had more follicles than the aged does (10). Several investigators have shown that estradiol binding by the rat uterus was inversely proportional to the expected estrogen titer during the estrous cycle, maximum uptake occurring in late diestrus and least uptake occurring at estrus (18–20). Since endogenous hormone concentration was insignificant compared with the concentration of receptor sites, it was believed that the changes in binding capacity was not due to saturation of the receptors (20, 21). Estradiol receptor concentration was also reported to be considerably higher in immature than in adult rat uteri (20).

Net uptake of progesterone and estrogen was greatest in the ovariectomized does. In the rat ovariectomy resulted in degeneration of both progesterone (3) and estrogen (18, 20) receptors in the uterus. Estrogen treatment restored the receptor concentration. The ovariectomized does in the present study were not estrogen primed but the receptor concentration apparently remained relatively high for 14 days after ovariectomy, at least in the young does.

Rabbit uterine uptake of progesterone and estradiol was higher in early pregnancy and decreased with increasing time pc. This agrees with information in the rat of a high estradiol receptor concentration in early preg-

nancy during placental development and a reduction at later stages (20). These authors found that the receptor concentration varied with stage of pregnancy but was relatively high in the placenta and consistently low in the uterine wall of the rat.

Since total uterine progesterone could not be measured in the present studies it is not known for certain whether the differences in hormone uptake with doe age were due to differences in saturation of receptor sites or due to the total number of receptors present. Because the uteri of young and aged does were exposed to similar levels of circulating progestins (11) *in vivo* it is assumed that the receptor sites were similarly saturated at the time of removal. If so, the differential uptake *in vitro* would represent a difference in the total capacity of uteri from young and old rabbits to take up progesterone. This presumed difference in capacity to bind progesterone and estrogen could account for the difference in reproductive rates in these groups of rabbits (10) and is consistent with other reports that pregnancy maintenance in the rat and rabbit is more closely correlated with uterine than plasma progesterone levels (7, 8). From these and other data it is concluded that an important factor in limiting the capability of aging rabbit uteri to maintain pregnancy is a reduction in uterine capacity to take up progesterone and 17β-estradiol.

Summary. Eighteen young female rabbits 6–13 mo old, and 28 aged rabbits 49–72 mo old, were randomized into three treatment groups. Group 1 does were ovariectomized and sacrificed 14 days later. Groups 2 and 3 were sacrificed 12 and 24 days post-coitum (pc), respectively. Net progesterone-7-^{3}H and 17β-estradiol-4-^{14}C uptakes were determined for uterine tissue strips incubated at 37° for 0, 2 and 4 hr by correcting for skeletal muscle incubated similarly.

Net uterine uptakes of progesterone-7-^{3}H and 17β-estradiol-4-^{14}C were greatest in the ovariectomized does and decreased particularly in the young does with increased time pc. Of most significance was the two- to four-fold greater uptake of both steroid hormones by uteri from young does than old does after 2 and 4 hr of incubation in the ovariectomized group and after 4 hr of incubation in the 12 day pc group. By 24 days pc steroid hormone uptake was low in both groups and not significantly different. The reduced capacity of uterine tissue in aged rabbits to take up steroid hormones is interpreted to be one of the uterine factors limiting reproductive performance in aged does.

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