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CHARACTERIZATION OF UTERINE SEX STEROID RECEPTORS IN THE PIG AND THEIR VARIATION DURING THE OESTROUS CYCLE

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Summary—The present study establishes and validates an *in vitro* binding and exchange assay for tissue receptors for oestradiol (E) and progesterone (P) in pig uterus. Both hormones bound to specific cytoplasmic (Rc) and nuclear (Rn) receptor proteins with high affinity. The relative concentrations of the receptors were measured in dissected samples from endometrium and myometrium obtained at late prooestrus, oestrus, and luteal phases of the oestrous cycle. The Scatchard analysis of the oestradiol and R 5020–receptor complex displayed linearity and indicated a single class of high affinity, low capacity binding sites. Significant variations were seen in the binding of E and P to their cytosolic and nuclear receptors, following the changes in the circulating levels of the hormones in blood plasma during the oestrous cycle. Both tissue components, i.e. endometrium and myometrium followed a similar pattern when related to the stage of the oestrous cycle considered. The ERc increased from prooestrus, reaching a maximum at standing oestrus, thereafter decreasing. The concentrations of ERn increased from prooestrus towards the early luteal phase, with a significant reduction by day 8 of the cycle. The amounts of PRc were maximal at standing oestrus, remaining high during the early luteal phase, while the PRn showed a linear increase from oestrus onwards throughout the luteal phase.

INTRODUCTION

The structure and function of the uterus is controlled by the ovarian secretion of oestrogen and progesterone which act through specific tissue receptors [1, 2]. The steroids, which are lipophilic diffuse through the plasmalemmae, into the cytoplasm and the nuclear envelope and bind to the specific receptor protein. As a result of this interaction rapid changes occur in the conformation of the receptor protein [3–5].

Steroid receptors exist in the soluble fraction obtained after homogenization of target tissues/cells in hypotonic buffers. However, after *in vivo* injection of [^{3}H]oestradiol, radioactivity was predominantly found in the nuclear fraction [6, 7]. According to a recently proposed model for the action of steroid hormones [4, 8, 9] there are at least three stages of solubilized receptors: unoccupied, non-transformed (occupied) and transformed. All of them are assumed to be present in the nuclear compartment of the cell. In most reported studies, only the non-transformed (cytosolic) and the transformed (nuclear) receptors have been investigated. The transformed receptor shows marked differences in sedimentation velocity, DNA binding, and other characteristics as compared to the non-transformed receptor [4].

The estimation of these receptor forms is necessary to evaluate functional aspects of their role(s) in the mechanism of hormone action in different species, sex, organs and tissues. Recent information available from experiments with pigs carried out by us [10–14] and others [15–18] showed that the concentrations of oestradiol and progesterone receptors in several target tissues changed specifically during the oestrous cycle.

The aim of the present study was to characterize and quantify the receptor forms (transformed and non-transformed) for oestradiol and progesterone in the endometrium and myometrium of the pig during prooestrus, oestrus and the early luteal phases.

MATERIAL AND METHODS

Experimental animals and sample collection

Eighteen sexually mature, crossbred gilts, 6–8 months of age, with body weights ranging from 80 to 100 kg, were used. The animals were housed indoors, 3–4 gilts per pen and were fed a commercial pig feed. Oestrus detection was performed twice daily in the

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presence of a boar. The gilts were slaughtered after showing their first or later normal oestrus on days 19–20 ($n = 5$), 1 (first day of standing oestrus, $n = 4$), 4 ($n = 4$) and 8 ($n = 5$) of the oestrous cycle. Blood samples were drawn by jugular venipuncture immediately prior to slaughter. The samples were centrifuged at 3000 rpm. The plasma was removed and stored at −20°C for hormone analysis. Immediately after slaughter, the genital tracts were removed, explored for confirmation of ovarian status and normality. A 5 × 5 cm sample from the middle part of each uterine horn was dissected out, coded and stored at −70°C until analysed for the contents of oestradiol and progesterone receptors.

Chemicals and buffers

The [2,4,6,7-^{3}H]oestradiol-17β (SA 104 Ci/mM), [^{3}H-17α-methyl]progesterone (R 5020) (SA 87 Ci/mM) and non-labeled R 5020 were obtained from New England Nuclear Corporation (Boston, Mass., U.S.A.). Diethylstilbestrol (DES) and liquid scintillation cocktail were obtained from Sigma Chemical Corporation (St Louis, Mo., U.S.A.) and Koch-Light Lab Ltd (Colnbrook, Berks, England) respectively. All other chemicals were of reagent grade or better.

Buffer A was 10 nM Tris–HCl (pH 7.4), *Buffer B* was 10 nm Tris–HCl and 1.5 nM EDTA (pH 7.4) and *Buffer C* was buffer A with 1% bovine serum albumin (BSA). The subscript following the letter designation of a buffer (e.g. A_{30}) denotes the percentage of glycerol (v/v) in the buffer. Dextran-coated charcoal (DCC) consisted of 1 g Norit-A and 50 mg Dextran T70 in 100 ml buffer (Buffer A_{10} for cytosolic (non-transformed) receptors of progesterone, PRc, and Buffer B for cytosolic receptors of oestradiol, ERc).

Tissue fractionation

Each uterine sample was taken from the freezer and kept in the refrigerator (approx. 4°C) until ice started to melt. It was then easy to separate, by dissection,the endometrium from the myometrium, which were divided into small pieces (1–2 mm). A portion (400–800 mg) from each tissue area was used for determination of progesterone (PR) or oestradiol receptors (ER). The samples were weighed and placed in 6 ml buffer (A_{30} for PR and B for ER). All subsequent steps were carried out at 0–4°C except where otherwise quoted. The tissue was homogenized in a glass homogenizer (Ultra-Turax®, Labassco, Stockholm) by three 5–8 s bursts at 30 s intervals. An homogenate aliquote (0.2 ml) was taken and kept at −70°C for DNA measurement. The remainder was centrifuged at 800 *g* for 20 min. The supernatant was then centrifuged at 104,000 *g* for 60 min to obtain the cytosol supernatant, and used immediately for cytosol receptor (Rc) determination. A fraction (0.5 ml) of the cytosol was kept for determination of total protein. The crude nuclear pellet from the first centrifugation was washed twice by rehomogenization in 3 ml buffer (A30 for PR and B for ER) and centrifuged at 800 *g* for 20 min after each wash. The final pellet was resuspended in 7 ml buffer.

Receptor assay

Experiments were conducted in order to determine optimum conditions for assay of the Rc (non-transformed) and Rn (transformed) from both endo- and myometrium.

To choose the optimum incubation time and temperature aliquots of cytosol or nuclear pellets (250 μl) were incubated (in duplicate) with either 4 nM [2,4,6,7-^{3}H]oestradiol-17β in a total volume of 350 μl buffer B at 4, 20 and 37°C or [^{3}H]R 5020 in a total volume of 350 μl buffer A10 at 4, 20 and 30°C. To asses nonspecific binding (NSB), a series of control tubes were included in each assay. In these, the cytosol or the pellet was incubated with a 100-fold excess of either DES for ER or non-labeled R 5020 for PR. At various times ranging from 5 min to 48 h, the bound ligand was separated from the free ligand either by adding 500 μl DCC to the cytosol incubates or for the nuclear fraction by centrifugation at 3000 *g* followed by washing three times with 1 ml buffer (buffer C30 for PRn and buffer B for ERn).

To discriminate between high and low affinity binding of [^{3}H]R 5020 in the cytosol, a study of the influence of DCC incubation time and DCC glycerol content was performed at ice-bath temperature.

Finally, to determine a range of ligand concentrations useful for saturation analysis, pools of cytosol and pellet preparations were incubated with [^{3}H]oestradiol-17β ranging from 0.1 nM to 30 nM and [^{3}H]R 5020 ranging from 0.1 nM to 40 nM. Specific receptor-binding activity was obtained by subtraction of the radioactivity in the NSB tubes from that in the total binding tubes. From the Scatchard plot it was possible to determine the concentrations of the hormones that approached saturation of the high affinity receptors with minimal contribution of binding occurring at the low affinity, non-specific binding. The reliability of the assays was assessed by assaying serial dilutions of cytosol preparations. The specificity of the ER and PR was investigated by competitive inhibition of both the [^{3}H]oestradiol-17β and [^{3}H]R 5020 by various non-radioactive steroids.

Assay procedure

The assay procedure finally adopted was as follows: each cytosol or nuclear preparation, in aliquots of 250 μl, were added to two parallel series of the tubes (in duplicate), one containing 100 μl of the labeled steroid (4, 2, 1, 0.5, 0.25 and 0.125 nM [^{3}H]oestradiol-17β or 10, 5, 2.5, 1.25, 0.6 and 0.3 nM [^{3}H]R 5020) and the other containing 100 μl of the same concentration of the label steroid plus a 100-fold molar excess of unlabeled steroid (DES for ER and R 5020 for PR).

The following incubation times were used for ERc and PRn 24 h at 4°C; PRc in the myometrium 18 h at 4°C; PRc in the endometrium 2 h at 20°C.

Bound from free steroid in the cytosol was separated by adding 500 μl DCC to each tube. After incubation for 10 min at 4°C they were centrifuged at 3000 *g* for 10 min. The supernatant was added to 4 ml of liquid scintillation cocktail for radioactivity determination.

The incubation of the nuclear fraction was terminated by centrifugation at 3000 *g* for 10 min at 4°C. The pellet was washed three times with 1 ml buffer (buffer B for ERn and buffer C_3O for PRn). The Rn-bound ligand was extracted with 1 ml absolute ethanol (overnight, 23°C), the tubes were centrifuged and the supernatant added to 4 ml of liquid scintillation cocktail for determination of radioactivity.

Other analytical methods

The concentrations of oestradiol-17β and progesterone in peripheral blood plasma were determined by radioimmunoassay [19, 20]. The concentration of DNA was determined by the method of Burton [21] as modified by Richards[22] using calf thymus DNA as standard. The protein content of the cytosol preparations was determined by the Lowry method using BSA as standard. The dissociation constant and binding data were determined by the method of Scatchard[23] corrected for non-specific binding according to Chamness and McGuire as described by Snochowski *et al.*[24]. The receptor concentration was expressed as pmol per mg protein for Rc and pmol per mg DNA for Rn. Conventional statistical methods were used for analysis of the results [25].

RESULTS

Cytosol receptors (oestradiol receptor, ERc; progesterone receptor, PRc) refer to these non-transformed forms found in the cytosol fraction following homogenization of the tissue in buffer and ultracentrifugation of the homogenate. Nuclear receptors (oestrogen receptor, ERn; progesterone receptor, PRn) refer to these transformed forms found in the nuclear pellet fraction following homogenization of the tissue in the buffer.

Conditions for assay of Rc

The degree of hormone association with cytosolic receptors in endometrium and myometrium using 4 nM [^{3}H]oestradiol-17β was temperature-dependent (Fig. 1). Maximum specific binding in the endometrium and myometrium cytosol required 24 h at 4°C and 18 h at 4°C respectively, but occurred more rapidly at 20 or 37°C. There was a rapid loss of specific binding activity at the higher temperatures. Therefore, 4°C for 24 h was chosen as the incubation conditions for quantifying the ER concentration in all cytosol samples.

Maximum specific binding using [^{3}H]R 5020 was obtained in the endometrium cytosol at 20°C for 2 h and in the myometrium cytosol at 4°C for 18 h (Fig. 2). Therefore, these incubation conditions were chosen for measurements of progesterone receptor concentrations in the cytosol fractions.

The time-course of dissociation of [^{3}H]R 5020 from binding components in the cytosol was determined by incubation of cytosol with DCC suspension on an ice bath. The dissociation of specific binding was higher during a period between 10 and 20 min and a 12 min incubation with DCC suspension was chosen for separation of bound from free forms of the hormone in the cytosol samples.

A pooled sample of both endometrial and myometrial cytosols was incubated with a range of increasing quantities of both ligands [^{3}H]estradiol-17β (0.125–4 nM) or [^{3}H]R 5020 (0.625–10 nM). The non-specific binding value was substracted and the Scatchard plot was determined (Fig. 3). Saturation of high affinity binding is evident at the concentration range of [^{3}H]oestradiol-17β (0.125–4 nM/350 μl) or [^{3}H]R 5020 (0.625–10 nM/350 μl) and they were chosen for saturation analysis of the cytosol samples. The Scatchard plot analysis of a diluted cytosol showed the same slope as the undiluted one but the binding capacity was lower (for example K_d for ERc endometrium was 6.2^{-10} and 6.3^{-10} but B_{max} were 4.2×10^{-10} and 7.95×10^{-10} respectively). The interassay coefficients of variation were less than 10% regardless of tissues and ligands.

Conditions for assay of Rn

Time studies of hormone association with endometrial and myometrial nuclear receptor using the same ligand concentration as with the cytosol were temperature-dependent (Fig. 4). The oestradiol receptors required 18 h at 4°C to reach maximum specific binding and maintained it for the next 6 h. Maximum [^{3}H]R 5020 binding required incubation for 24 h at 4°C. Therefore, 21 and 24 h at 4°C were chosen as the incubation conditions for estimating the concentration of nuclear oestradiol and progesterone receptors respectively.

The Scatchard plot analyses (Fig. 5) showed high affinity binding with K_d between 6.02^{-10} and 1.07^{-9} for oestradiol receptors and 2.9^{-9} for progesterone receptors.

Specificity of ER and PR

The specificity of oestradiol or progesterone binding in the endometrium and myometrium were determined by incubating a cytosol pool with 4 nM [^{3}H]oestradiol-17β or 10 nM [^{3}H]R 5020 and increasing levels of unlabeled steroids. Of the unlabeled steroids tested, only oestrogens were able to compete with the labeled [^{3}H]oestradiol-17β for the oestrogen receptors (Fig. 6, upper). In both tissues, DES and R 5020 were the strongest competitors for oestrogen or progesterone receptors, respectively (Fig. 6, lower).

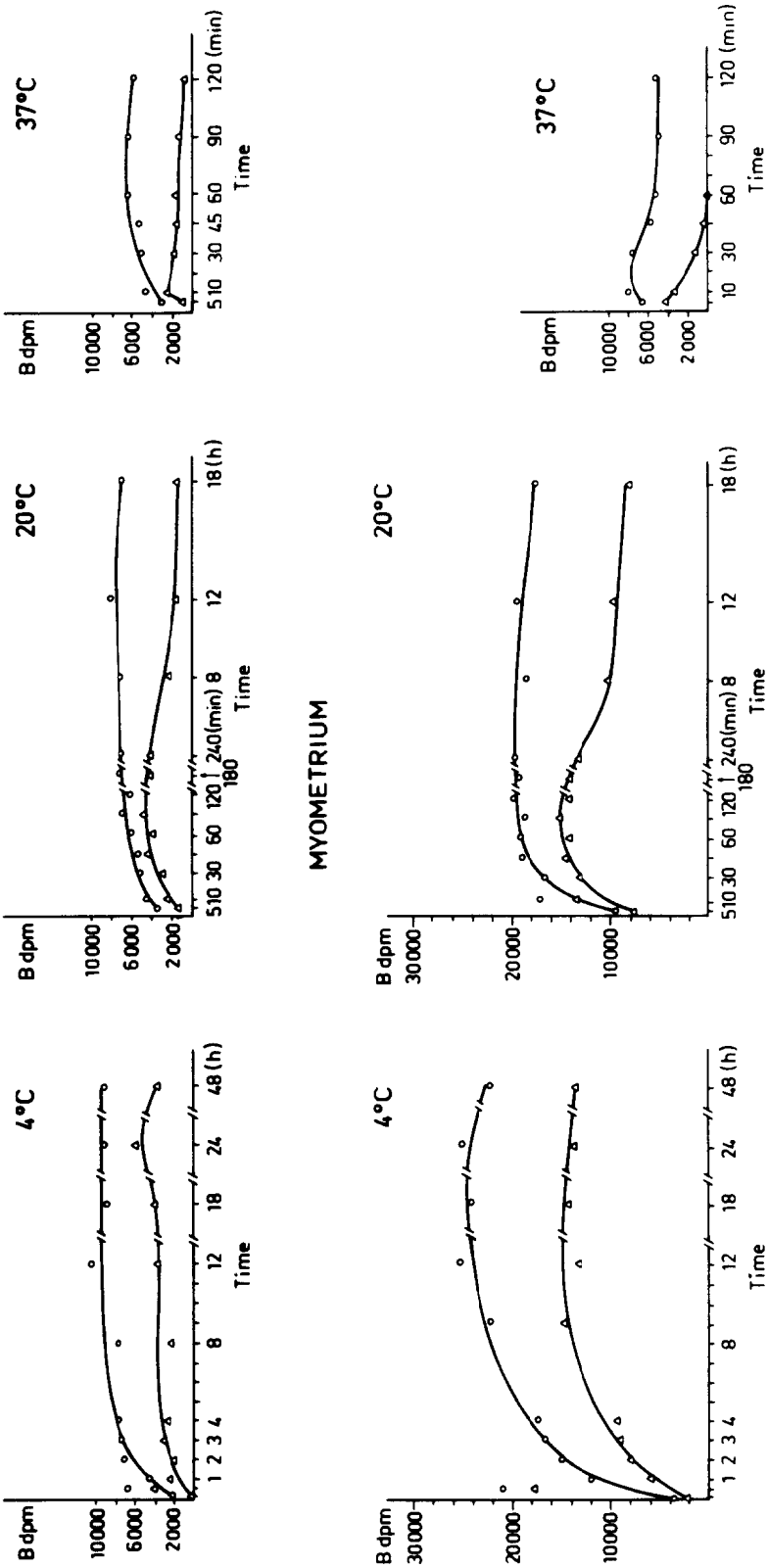

Fig. 1. Degree of [^{3}H]oestradiol-17β (4 nM) binding with cytosolic receptors (ERc) in endo- and myometrium when incubated *in vitro* at different temperatures and times (○: total binding, △: non-specific binding).

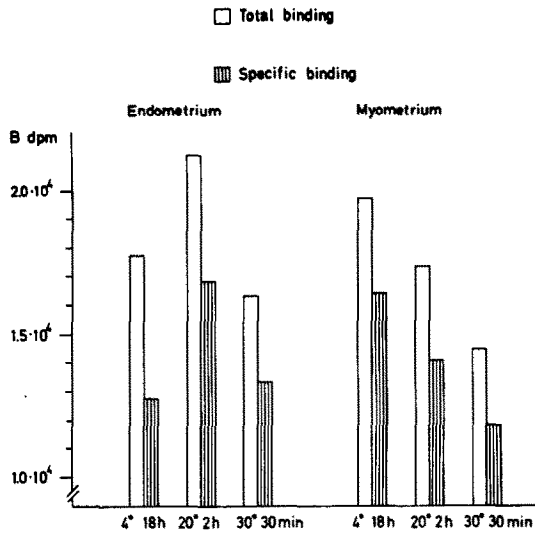

Fig. 2. Degree of [^{3}H]R 5020 (10 nM) binding with cytosolic receptors in endo- and myometrium, when incubated *in vitro* at different temperatures and times (open bars: total binding; lined bars: specific binding).

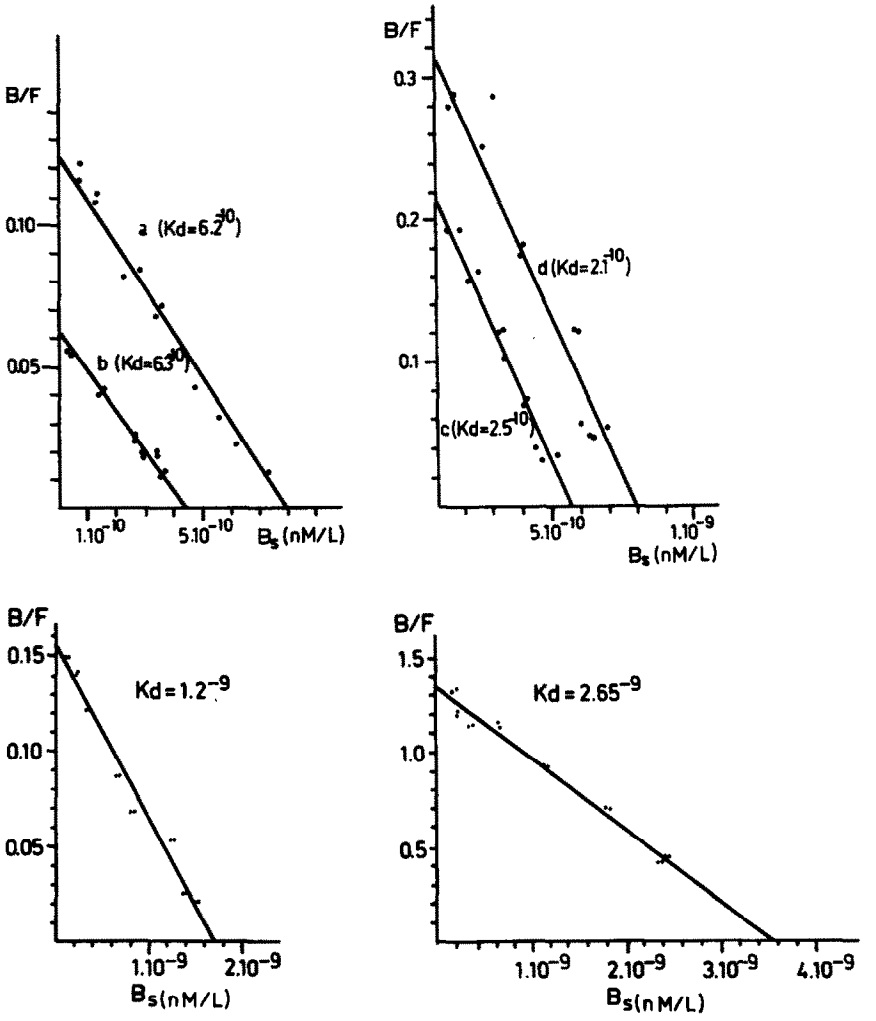

Fig. 3. Representative Scatchard plot analysis of [^{3}H]oestradiol-17β (upper graphs) and [^{3}H]R 5020 (lower graphs) binding in cytosol fractions from the endometrium (left graphs) and the myometrium (right graphs) in cycling gilts. Cytosols (0.25 ml) were incubated with varying concentrations of [^{3}H]oestradiol-17β and [^{3}H]R 5020 as described in Material and Methods. Each point is the mean of the duplicate values (B/F: bound/free, B_s: bound steroid).

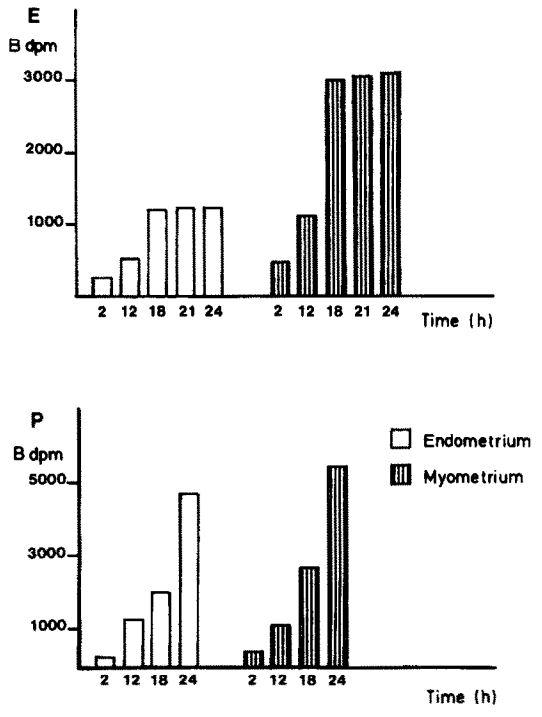

Fig. 4. Degree of specific binding with nuclear receptors in endo- and myometrium, when incubated *in vitro* at 4°C for different time periods with [^{3}H]oestradiol-17β (4 nM, upper histogram) and [^{3}H]R 5020 (10 nM, lower histogram).

Plasma hormone levels

Plasma levels of oestradiol-17β and progesterone in the slaughtered gilts are shown in Fig. 7. The plasma levels of oestradiol-17β were between 50 and 90 pmol/l during prooestrus, decreased sharply thereafter during standing oestrous and reached basal levels (mean values below 30 pmol/l) at days 4 and 8. The plasma level of progesterone remained low during prooestrus (days 19–20) and standing oestrus (day 1 of the cycle) and increased almost linearly thereafter.

Receptor concentration

The oestradiol binding to cytosol receptors in the endometrium and myometrium of the pig uterus showed marked variations ($P < 0.05$) during the different stages of the oestrous cycle investigated, as shown in Fig. 8 (upper). Differences existed also in the relative amount of receptors between the two tissue components. At prooestrus, the average concentrations of receptors were 0.8 pmol/mg for endometrium and 1.9 pmol/mg for myometrium. The ERc increased significantly ($P < 0.05$) at standing oestrus, for both tissues (5.2 and 9.0 pmol/mg for the endo- and myometrium, respectively), and decreased thereafter.

The oestradiol binding to nuclear receptors in the pig uterus is depicted in Fig. 8 (lower). The ERn also

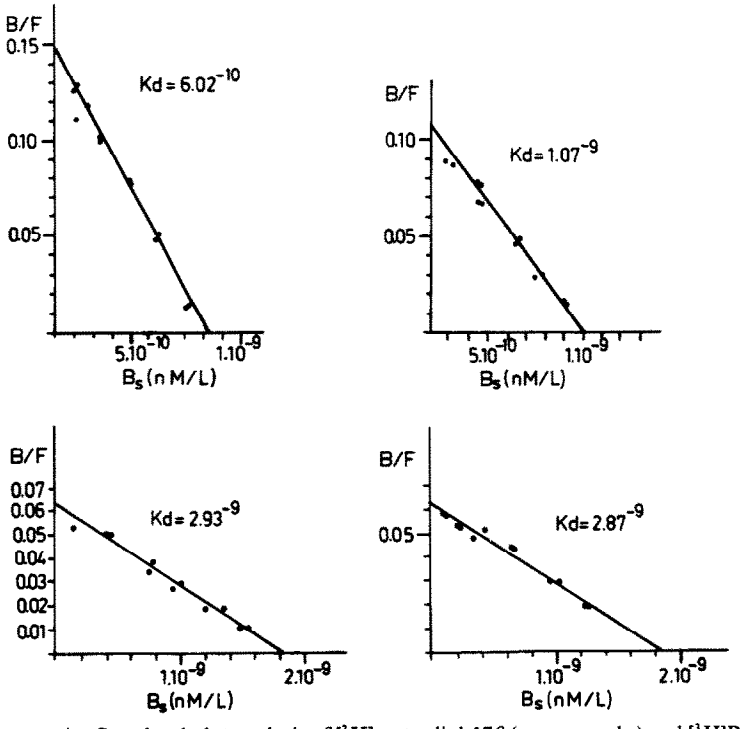

Fig. 5. Representative Scatchard plot analysis of [^{3}H]oestradiol-17β (upper graphs) and [^{3}H]R 5020 (lower graphs) binding in nuclear fractions from endometrium (left graphs) and myometrium (right graphs) in cycling gilts. Nuclear pellets (0.25 ml) were incubated with varying concentrations of [^{3}H]oestradiol-17β and [^{3}H]R 5020 as described in Material and Methods. Each point is the mean of the duplicate values (B/F: bound/free, B_s: bound steroid).

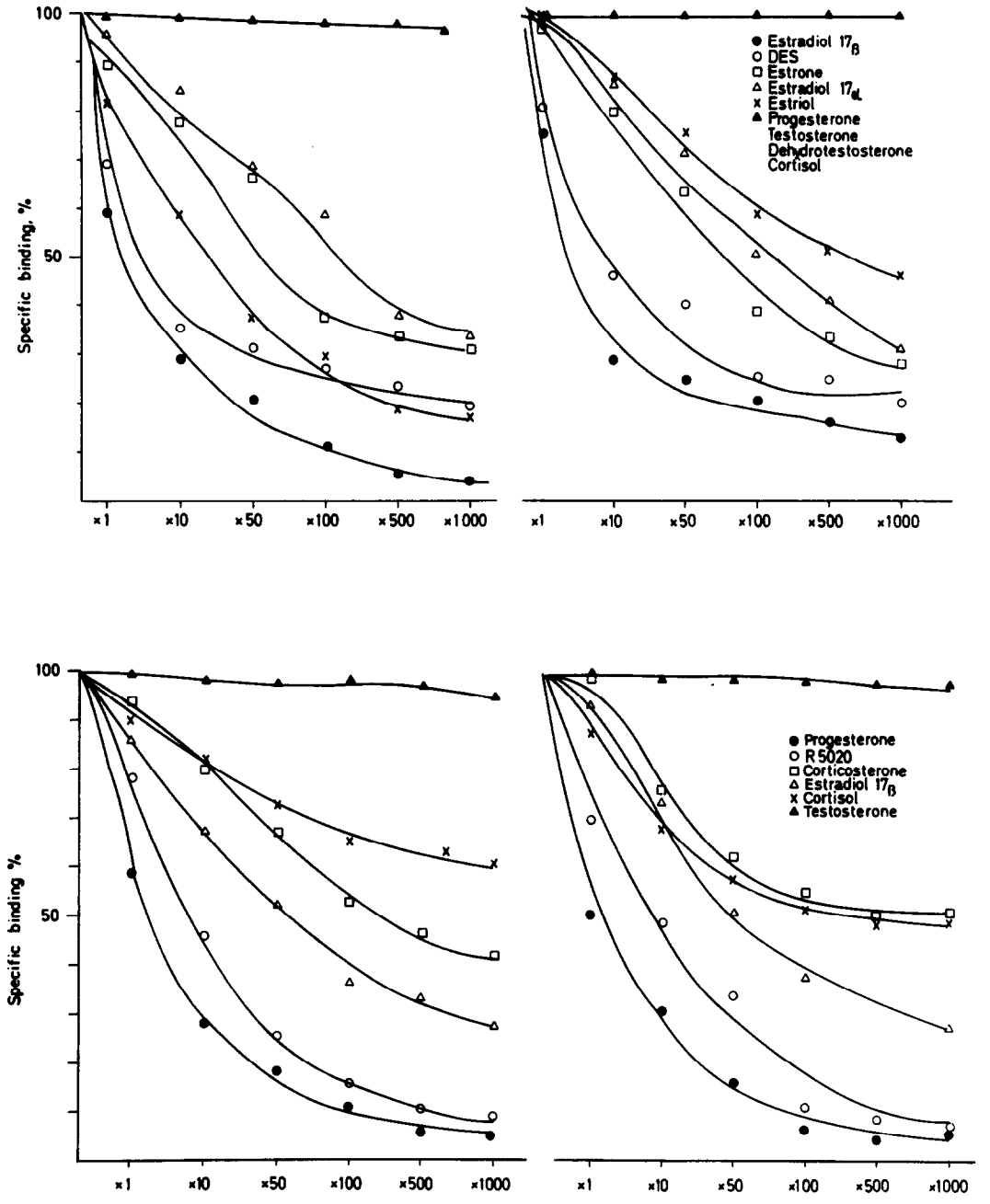

Fig. 6. Steroid specificity of ER (upper curves) and PR (lower curves) in the endometrial (left) and myometrial (right) nuclear fractions.

showed significant changes ($P < 0.05$) between the tissues during the oestrous cycle. Both tissue components followed a similar pattern when related to the stage of the oestrous cycle. The ERn levels increased from days 19–20 (0.2–0.3 pmol/mg) to standing oestrus (1.1–0.7 pmol/mg), the concentrations decreased thereafter for the endometrium or remained high up to day 4 for the myometrium. A significant reduction of ERn concentrations had occurred by day 8 of the cycle ($P < 0.01$).

The profile of the cytosol progesterone receptor in the porcine uterus is depicted in Fig. 9. Receptor levels were very low (0.2–0.3 pmol/mg) at days 19–20 of the cycle (Fig. 9, upper) and increased thereafter (oestrus onwards). However, no significant changes were recorded until day 4 of the cycle. In the myometrium, the PRc concentration increased significantly ($P < 0.01$) from late prooestrus to oestrus, decreased thereafter ($P < 0.05$), and remained at a similar level during the early luteal phase.

A similar pattern in the variations of PRn was seen in both tissue components of the pig uterus throughout the oestrous cycle explored, as seen in Fig. 9 (lower). The relative concentration of uterine PRn

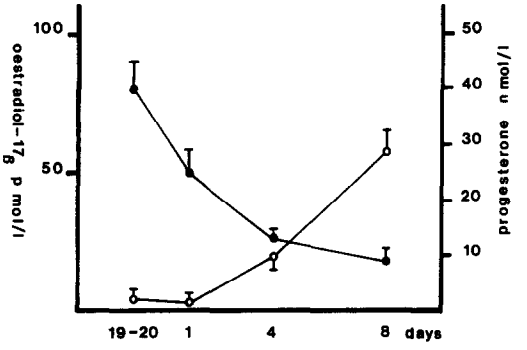

Fig. 7. Plasma levels of oestradiol-17β (●——●) and progesterone (○——○) from the slaughtered gilts at different stages of the oestrous cycle (means ± SEM).

was low during days 19–20 (0.5–0.8 pmol/mg) and increased significantly thereafter ($P < 0.05$).

DISCUSSION

In the present study, proper assay conditions were established to distinguish between high and low affinity binding for oestradiol and progesterone in the pig uterus. Scatchard analysis of ligand–receptor complex for oestradiol and R 5020 displayed linearity and indicated a single class of high affinity, low capacity binding sites. The K_d values for both ligands were in the ranges previously reported for the pig oviduct [12], the pig cervix [11] and other similar tissues [26–28].

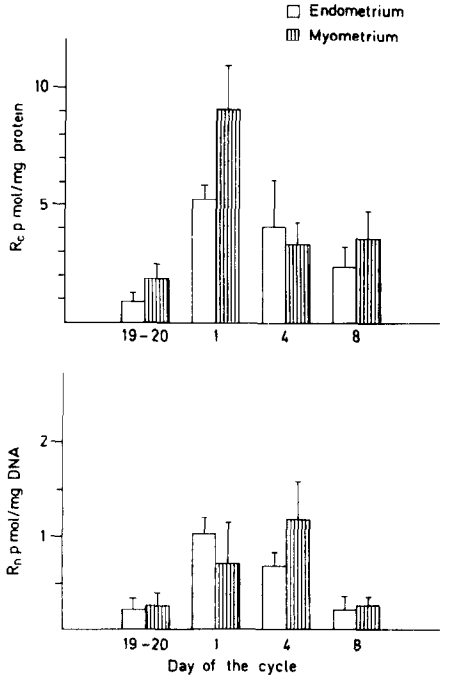

Fig. 8. Oestradiol binding (means ± SEM) in the cytosolic (pmol/mg protein, upper histogram) and the nuclear (pmol/mg DNA, lower histogram) fractions of the endometrium (open bars) and the myometrium (lined bars) of gilts during different stages of the oestrous cycle.

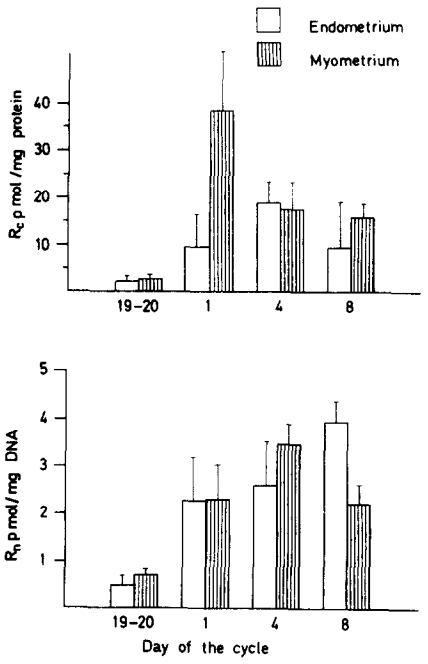

Fig. 9. Progesterone binding (means ± SEM) in the cytosolic (pmol/mg protein, upper histogram) and the nuclear (pmol/mg DNA, lower histogram) fractions of the endometrium (open bars) and the myometrium (lined bars) of gilts during the different stages of the oestrous cycle.

Not only were there specific tissue receptors for oestradiol and progesterone present in the porcine endometrium and myometrium, but there were also significant differences in their binding in nuclear and cytosolic compartments. Moreover, variations in the concentrations of receptors during the oestrous cycle were found. In the pig, the highest level of plasma oestradiol occurs during prooestrus, with a drastic drop on the first day of standing oestrus, thereafter maintaining low levels of the hormone until the next prooestrus [29]. The levels of circulating progesterone attain significant values on about day 4 of the oestrous cycle [30]. The plasma concentrations for oestradiol-17β and progesterone found in the group of pigs used in the present study were in the same range as those previously reported.

Regular, highly coordinated, spontaneous muscle activity have been reported in the porcine uterus during the oestrous cycle. Sustained peristaltic activity is present in the pig myometrium during oestrus. At this stage, the waves of contraction are mainly of cervico-tubal direction, presumably facilitating the rapid transport of semen towards the oviducts [31]. This coordinated muscle activity becomes more irregular during metaoestrus, to regain regularity during the period of reallocation of preimplantational embryos, i.e. between days 10 and 12 post-mating.

Since the strongest uterine contractions in the pig appeared during oestrus, following the sharp drop in plasma oestrogens, they might be provoked by either oestrogen withdrawal [31], or due to a phase

difference between the concentrations of steroids in plasma and in target tissues, as in the pig oviduct [12].

Changes in the secretory activity of the endometrium have been reported in the cycling pig [32]. Secretory cells differentiate maximally during the follicular phase while the maximal secretory activity, even of specific proteins [33], is present during the early luteal phase, a stage dominated by progesterone levels in plasma [34]. Similar findings have been defined by our group in the porcine oviduct [14]. Nevertheless, it appears clear that both ciliary and secretory activity depend upon an oestrogen stimulation of these target organs, followed by progesterone, which role, through its general metabolic effect, is to maintain the secretory activity induced by the oestrogens [14, 35, 36]. The ERc content in rat [37, 38] and pig [10] uterus increases during the follicular phase of the cycle in response to a rise in the circulating oestradiol levels. After binding of the receptor–steroid complex to the cell nucleus (ERn) it initiates the specific metabolic changes that represent the target cell response to oestrogen [39, 40]. Oestrogens increase progesterone receptor synthesis in mammals [41–43] and birds [44]. A rapid and specific reduction of ERn in the uterus of hamster [45] and rabbit [36] is provoked by progesterone.

The concentrations of ERc in the endometrium and myometrium of the pig correlated well with the blood plasma concentrations of oestradiol-17β during the stages of the oestrous cycle. High levels of plasma oestradiol causes a depletion of ERc, which is followed by replenishment through reactivation of ERn complexes and the *de novo* synthesis of ERc molecules [12, 46]. The concentrations of ERn, regardless of the changes in the plasma levels of the corresponding hormone, showed a sustained level between late prooestrus and day 4 of the cycle, and decreased thereafter in both layers of the uterine wall. This decrease is due to the counteraction of progesterone upon the action of oestradiol by the reduction of the amount of ER in the involved tissues [46]. Upon oestradiol administration in the ovariectomized pig, oestradiol receptors shift to a tight nuclear association (Rodriguez-Martinez, unpublished results). Following hormone dissociation, nuclear receptors are degraded and there is a long lag before "cytosolic" receptors return to their initial level [36].

The progesterone binding to PRc was very low in the endometrium until day 4 of the oestrous cycle, i.e. after ovulation occurred and the circulating levels of progesterone increased. In the myometrium however, the PRc concentrations increased significantly to reach the highest level during oestrus, well in advance of the ovulation process. An explanation of these results can either reside in the slow repletion of PRc that follow the presence of low levels of circulating progesterone [12] or by the fact that oestradiol increases the tissue content of progesterone receptors [47], bearing in mind the high concentrations of oestradiol-17β in blood plasma during these stages of the cycle. The PRc concentrations decreased during the rest of the cycle, when the levels of PRn increased linearly in both the endometrium and the myometrium. An increasing *de novo* synthesis of receptors during these periods might explain the changes mentioned above. Progesterone receptors are controlled by their own ligand, that is, progesterone decreases progesterone receptor concentration in animals previously stimulated by oestradiol [48], thus autolimiting its own action by decreasing its binding sites [36].

It has been shown that the levels of ER and PR depend not only upon the concentrations of the respective hormones in blood. Some other factors might be involved in the mechanisms regulating the steroid action in the target tissues. As an example, Sumida and Pasqualini[49] found that the epidermal growth factor (EGF) could be an autocrine or paracrine factor in estrogen-sensitive cells due to its capacity to increase the binding sites of progesterone receptor, which is an oestrogen-inducible protein. Preliminary results in pigs (Rodriguez-Martinez, unpublished) indicate that the insulin-like growth factor (IGF-I) does represent a similar factor, modulating the action of oestradiol-17β upon the oestrogen-sensitive uterine cells.

There are differences in sensitivity between various target tissues which may provide the animal with a mechanism by which it can differentially regulate the steroid responses [50]. If all target tissues were identical in sensitivity, each would respond equally to the same level of circulating hormones. However, if some target tissues are less sensitive to the steroids than others, it is possible to activate only a subset of the steroid target tissues and in this manner an additional level of regulation is achieved. Thus, the differential activation of steroid hormone target tissues may be a general and vitally important principle of endocrine regulation.

The present study shows that the assay system is suitable for studying oestradiol and progesterone receptors in endometrium and myometrium of the pig at various stages of the oestrous cycle. Furthermore, the data obtained suggest a relationship between the changes in the levels of oestradiol and progesterone uterine binding during the oestrous cycle, the spontaneous motility and the secretory activity of the uterus in the pig.

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