J. steroid Biochem. Vol. 22, No. 1, pp. 115–120, 1985
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0022-4731/85 $3.00 + 0.00
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OESTRADIOL AND PROGESTERONE RECEPTORS IN THE PIG OVIDUCT DURING THE OESTROUS CYCLE

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(*Received* 30 *December* 1983)

Summary—The concentrations of the tissue receptors for oestradiol (E) and progesterone (P) in the porcine oviduct at different stages on the oestrous cycle have been investigated by *in vitro* binding and exchange methods. Both hormones bound to specific cytoplasmic (Rc) and nuclear (Rn) receptor proteins with high affinity. The concentrations of ERc and ERn were two-fold higher in the ampulla as compared to the isthmus. The amount of ERc in the isthmic portion of the oviduct did not vary throughout the oestrous cycle. However, the ampullar ERc concentrations increased during prooestrus, showed a maximum at standing oestrus, thereafter decreasing. Significant variations in the amount of oviductal ERn were observed. Despite the differences in ERn amounts between segments, the concentration of ERn increased significantly during late prooestrus, attaining a three-fold elevation and remaining elevated during the period of standing oestrous and early luteal phase (days 3–4), thereafter returning to basal levels. No significant variations in the amount of isthmic PRc were found throughout the period studied. The ampulla, however, showed a significant increase in PRc concentrations during standing oestrus, thereafter decreasing. The concentrations of PRn in isthmus and ampulla were of about the same magnitude and varied significantly during the oestrous cycle, increasing in concentration from standing oestrous onwards. The temporal relationships between the variations in levels of oestradiol and progesterone receptors in oviductal tissues and those of the circulating plasma levels were established. The data obtained in this study suggest a relationship between the changes in the levels of oestradiol and progesterone oviductal binding during the first days of the oestrous cycle, and the gamete and embryo transport throughout the oviduct in the porcine species.

INTRODUCTION

Several crucial reproductive phenomena take place in the mammalian oviduct, e.g. sperm and ova transport to the fertilization site and posterior embryo transport to the uterus. The spontaneous motility of the pig myosalpinx appears to be decisive in regulating these transport mechanisms [1], but the mechanisms underlying this coordinated motor activity are not yet fully known. Hormonal factors might be involved, since changes in the circulating blood levels of the steroid hormones occur simultaneously with changes in the oviductal spontaneous motility. In the pig, the highest level of oestrogens occur just prior to standing oestrous. Significant blood levels of progesterone do not appear until ovulation, which occurs about 36 h after onset of heat [2]. The strongest contraction patterns both in the ampullar and the isthmic region of the tubes appear during oestrous [3, 4]. The porcine endosalpinx might also be under the influence of the ovarian steroids since it exhibits morphologic cyclic changes during the various phases of the oestrous cycle [5].

Steroid hormones exert their influence in target cells through interactions with specific protein receptors. The response of the target cell is dependent on the availability of cytosolic receptor and the retention of the hormone–receptor complex by intranuclear binding sites. In blood, the steroid hormones are largely bound to specific binding proteins [6]. From the blood, the steroids pass through the cell membrane [7] and bind to specific cytosolic receptors [8, 9]. If such cytosolic receptors are lacking, the tissue is refractory to the action of the hormone. The receptor–steroid complex is translocated to the cell nucleus, where it interacts with the chromatin, the net result being synthesis of new proteins [10].

A number of studies have been conducted to determine the presence and concentration of oestrogen and progesterone receptors during the sexual cycle in the oviduct of different species [11–17]. No corresponding studies have so far been made in the oviduct of the pig. Therefore, the present investigation was undertaken in order to determine the variations in the oestrogen and progesterone receptors in the pig oviduct during the different stages of the oestrous cycle.

EXPERIMENTAL

Experimental animals and sample collection

Twenty-seven 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. None of the animals had been used previously in any

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experimental work. Oestrus detection was performed twice daily in the presence of a boar. The gilts were slaughtered after they showed their first or later normal oestrous on days 16–17 ($n = 3$), 18–21 ($n = 3$), 1 (first day of standing oestrous, $n = 4$), 2 ($n = 3$), 3 ($n = 4$), 4 ($n = 5$), and 5–8 ($n = 5$) of the oestrous cycle. Blood samples were drawn by jugular venipuncture immediately prior to slaughter. The samples were centrifuged at 3000 rpm. Plasma was removed and stored at −20°C until analyzed for progesterone [18] and oestradiol-17β [19]. All hormone determinations were carried out in duplicate.

Immediately after slaughter, the genital tracts were removed, explored for confirmation of ovarian status and normality, and the oviducts dissected free from the mesosalpinx, separated into isthmic and ampullar segments and frozen in liquid nitrogen within 20 min after collection. The organs were coded and stored at −70°C until analysed.

Chemicals and buffers

The [2,4,6,7-^{3}H]oestradiol-17β (Sp. act. 104 Ci/mM), [^{3}H-17α-methyl]progesterone (R 5020) (Sp. act. 87 Ci/mM) and non-labeled R 5020 were obtained from New England Nuclear Corporation (Boston, MA). 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 and 1% bovine serum albumin (BSA). The subscript following the letter designation of a buffer (e.g. A_{30}) denotes the percentage 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 receptors of progesterone, PRc, and Buffer B for cytosolic receptors of oestradiol, ERc).

Receptor assay

The tissue samples from the ampullar and isthmic segments of the oviduct were divided into small portions and 400–800 mg were used for determination of progesterone receptors (PR) or oestrogen 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 noted. The tissue was homogenized by means of a motor-driven glass homogenizer with three 5–8 s bursts at 30 s intervals. A homogenate aliquot (0.2 ml) was taken and kept at −20°C for DNA measurement. The remainder was centrifuged at 800 g for 20 min. The supernatant was then centrifuged at 105,000 g for 60 min and used immediately for cytosolic receptor (Rc) determination. The crude nuclear pellet from the first centrifugation was washed twice by rehomogenization in 3 ml buffer (A_{30} for PR and B for ER) and centrifuged at 800 g for 15 min after each wash. The final pellet was resuspended in 6 ml of the same buffer.

The [^{3}H]oestradiol-17β and [^{3}H]17α-methyl (R 5020) were used as the labeled ligand. The saturation analyses were performed with six different concentrations of [^{3}H]oestradiol-17β (from 0.125 to 4 nM/l) for ER, or of [^{3}H] R 5020 (from 0.3 to 10 nM/l) for PR. Aliquots (0.25 ml) of the cytosolic or nuclear fraction were added to two parallel series of the tubes (in duplicate), one containing 0.1 ml of the labeled steroid and the other containing 0.1 ml of the same concentration of the labeled 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, ERn, PRc and PRn: ERc and ERn: 18 h at 4°C; PRc: 1 h at 20°C and PRn: 24 h at 4°C.

Bound and free steroid in the cytosol was separated by adding 0.5 ml DCC to each tube. After incubation for 10 min at 0°C they were centrifuged at 3,000 g for 10 min. The supernatant was added to 4 ml of liquid scintillation cocktail for radioactivity determination.

Incubations of the nuclear fractions were terminated by centrifugation at 3,000 g for 10 min at 4°C. The pellet was then washed three times with 1 ml buffer (C for PRn and B for ERn). The Rn-bound ligand was extracted with 1 ml absolute ethanol (overnight, room temperature) and counted for radioactivity.

Other analytical methods

DNA concentration was determined by the method of Burton [20] as modified by Richards [21]. The dissociation constant and binding data were determined by the method of Scatchard [22] corrected for non-specific binding according to Chamness and McGuire [23] as described by Snochowski *et al.* [24]. The receptor concentration was expressed as number of sites per cell. Conventional statistical methods were used for analysis of the results [25].

RESULTS

The dissociation constants (K_d) (Table 1) for cytosolic and nuclear pellet fractions for [^{3}H] oestradiol and [^{3}H] R 5020 in the ampullar and isthmic segments of the pig oviduct were estimated from Scatchard plot analysis. Representative plots are shown in Fig. 1. The Scatchard plots were linear and the K_d values differed for the two ligands. These binding data indicated a single class of high affinity, low capacity sites.

Plasma levels of progesterone and oestradiol-17β in the slaughtered gilts are shown in Fig. 2. The plasma level of oestradiol-17β showed values between 50 and 90 pmol/l during prooestrus, decreasing sharply thereafter during standing oestrous, and reaching basal levels (mean values below 30 pmol/l) from day 2 and onwards. The plasma level of progesterone remained low during prooestrus (days 18–21) and

Table 1. Dissociation constants (K_d) of cytosolic and nuclear oestradiol and progesterone receptors in the isthmus and ampulla of the pig oviduct ($n = 27$)

Tissue	Hormone	K_d (nM) Cytosolic	Nuclear
Isthmus	Oestradiol	0.37 ± 0.04	0.50 ± 0.06
Ampulla	Oestradiol	0.44 ± 0.04	0.44 ± 0.05
Isthmus	R5020	2.46 ± 0.20	2.39 ± 0.20
Ampulla	R5020	3.37 ± 0.30	2.52 ± 0.30

(Means ± SEM).

standing oestrus (days 1–2 of the cycle), increasing linearly thereafter.

The oestradiol binding to cytosol receptors in the isthmic and ampullar segments of the pig oviduct is shown in Fig. 3. There were substantial differences in the ERc amount between the oviductal segments, being 2-fold higher in the ampulla. The average concentration of isthmic ERc was $0.7–0.8 \times 10^4$ sites/cell at prooestrus. The isthmic ERc showed a tendency ($P > 0.005$) to increase up to the second day of standing oestrous, thereafter decreasing (n.s.). The ampullar ERc, however, increased ($P < 0.05$) from prooestrus (1.0×10^4 sites/cell), being present in maximum amounts of the first day of standing oestrous (average 2.5×10^4 sites/cell), thereafter decreasing.

The oestradiol binding to nuclear receptors in the pig oviduct is depicted in Fig. 4. The ERn levels were also 2-fold higher in ampulla than in isthmus, although the variations in ERn levels in both tubal segments followed a similar pattern when related to the stage of the oestrous cycle. In the isthmus the amount of ERn increased from days 16–17 (average 0.2×10^3 sites/cell) to late prooestrus ($0.6–0.7 \times 10^3$) ($P < 0.05$), maintaining those concentration levels through the period of standing oestrous and early luteal phase (days 3–4) (n.s.). A significant reduction of ERn concentrations occurred on days 5–8 ($P < 0.05$). The ampullar ERn concentrations followed a pattern similar to that in the isthmus (Fig. 4).

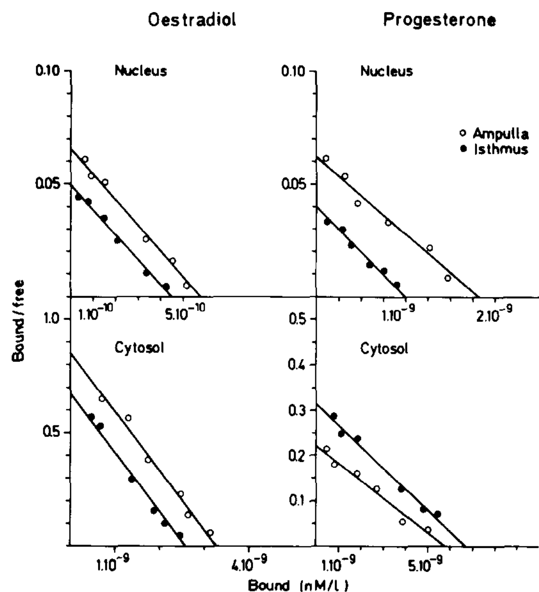

Fig. 1. Representative Scatchard plot analysis of [^{3}H] oestradiol and [^{3}H] R5020 binding in cytosolic and nuclear pellet fractions of pig oviductal isthmus and ampulla. Cytosolic and nuclear pellets (0.25 ml) were incubated with varying concentrations of [^{3}H] oestradiol and [^{3}H] R5020 as described in Experimental. Each point is the mean of the duplicate values.

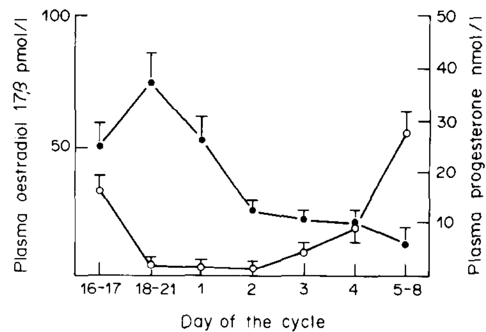

Fig. 2. Plasma levels of oestradiol (●——●) and progesterone (○——○) from the 27 slaughtered gilts at different stages of the oestrous cycle (means ± SEM).

The progesterone binding to cytosol receptors in the pig oviductal isthmus and ampulla is shown in Fig. 5. Progesterone binding was not detected in the porcine oviduct on days 16–17 of the oestrous cycle. The PRc levels in the isthmus did not reveal major changes during the rest of the period investigated (average $1–2 \times 10^4$ sites/cell). In the ampulla, the PRc concentration increased ($P < 0.05$) from late prooestrus (2×10^4 sites/cell) to the first day of standing oestrous (5.5×10^4), thereafter decreasing.

A similar pattern in the variations of PRn was seen in both tubal segments as depicted in Fig. 6. The relative concentration of tubal PRn were on average $0.1–0.3 \times 10^3$ sites/cell on the second day of oestrous

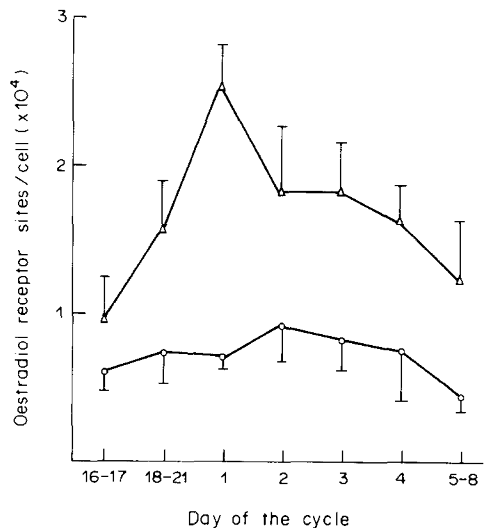

Fig. 3. Oestradiol in the cytosolic fraction of isthmic (○——○) and ampullar (△——△) segments of the porcine oviduct during the oestrous cycle (means ± SEM).

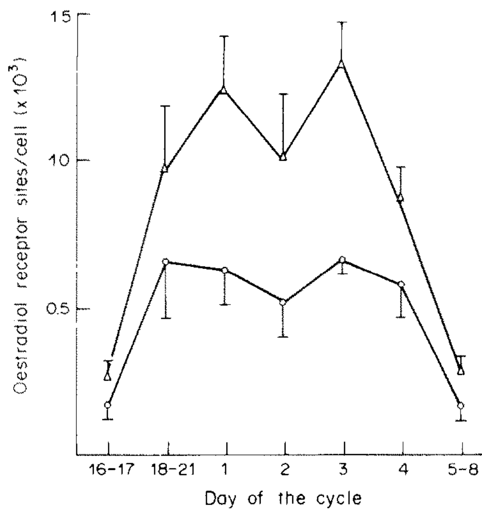

Fig. 4. Oestradiol binding in the nuclear fraction of the isthmus (O——O) and ampulla (△——△) of the pig oviduct during the oestrous cycle (means ± SEM).

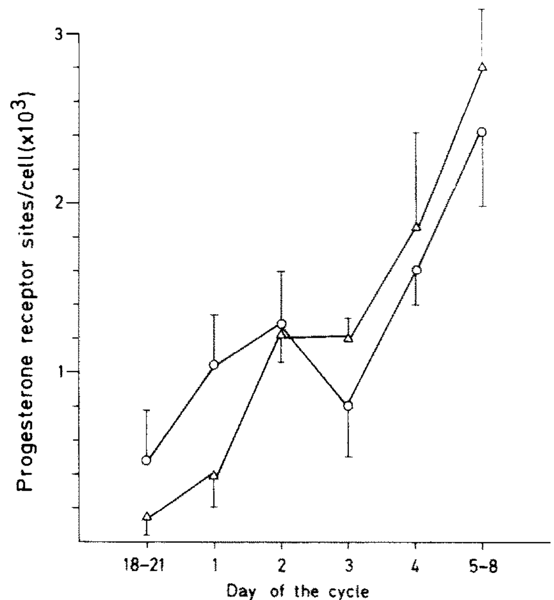

Fig. 6. Progesterone binding in the nuclear fraction of the isthmus (O——O) and ampulla (△——△) of the pig oviduct during the oestrous cycle (means ± SEM).

($P < 0.05$) and increasing linearly from days 3–4 onwards ($P < 0.05$).

DISCUSSION

The Scatchard analysis of ligand-receptors affinity for oestradiol-17β and R 5020 displayed linearity and indicated a single class of high affinity, low capacity binding sites. The K_d for oestradiol-17β lay in the range 3.7 to 5.0 × 10^{-10} for both ERc and ERn of the oviductal segments considered and is in agreement with the values obtained for the oestrogen receptor in rabbit [26] and rat [16] oviducts, but significantly higher than that reported for the human Fallopian tube [14]. The K_d for R 5020 lay in the range 2.4–3.4 × 10^{-9} for both oviductal ERc and ERn. The K_d for progesterone is similar to data reported for oviducts from humans [14].

The present study revealed not only the presence of specific oestrogen and progesterone receptors in the porcine oviductal isthmus and ampulla, but the presence of significant differences in their bindings in both nuclear and cytosolic compartments. Moreover, variations in the receptor concentrations along the oestrous cycle were also found.

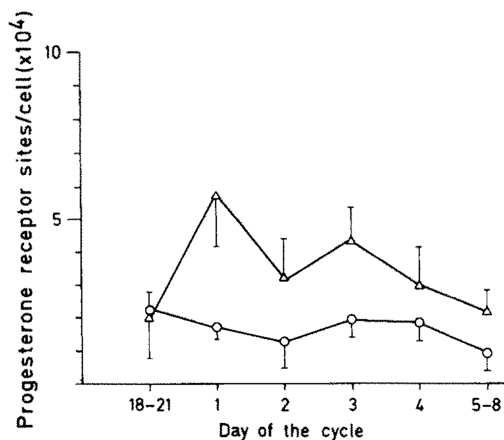

Fig. 5. Progesterone binding in the cytosolic fraction from isthmus (O——O) and ampulla (△——△) in the pig oviduct during the oestrous cycle (means ± SEM).

Peristaltic activity is present in the pig myosalpinx during standing oestrous in apparent relation to the sperm and ova transport to the fertilization site [4]. On the third day of the cycle, no peristalsis is recorded, but a sustained tonus in the isthmus and a low activity in the ampullar myosalpinx which seems to represent the locking mechanisms that retain the ova in the ampullary isthmic junction of the pig oviducts prior to their entrance into the uterus. During day 4, when ova/embryo normally pass down to the uterine cavity, peristalsis is present again, but merely in the isthmus, revealing an active participation of this segmental myosalpinx in ova descent.

In the pig the highest level of plasma oestrogens occurs during prooestrus, with a drastic drop on the first day of oestrous, thereafter maintaining low levels of the hormone until the next prooestrus [2, 24]. Circulating progesterone reaches significant levels on about day 4 of the oestrous cycle [28]. The plasma progesterone and oestradiol-17β levels found in the pig population used in this study agree with those in previous reports. Since the strongest oviductal contractions in the pig appeared during standing oestrous, after the sharp drop of plasma oestrogens, they might be provoked by oestrogen withdrawal as proposed to be the case in rabbits [29] or due to a phase difference between the concentrations of steroids in plasma and in target tissues.

Reports from studies on the rat uterus indicated that the ERc content increases during the follicular phase of the cycle in response to a rise in the circulating oestradiol levels [30, 31]. After translocation of the receptor–steroid complex to the cell nucleus (ERn) it initiates the specific metabolic changes that represent the target cell response to oestrogen [10]. A rapid and specific reduction of ERn in the hamster uterus is provoked by progesterone [32]. Although it is clear that progesterone and oestradiol have mutually counteractive effects on ERn, the mechanisms involved are less certain.

The concentrations of ERc in the ampulla followed the blood plasma concentrations of the hormone during the oestrous cycle studied. High levels of plasma oestradiol will cause a depletion of ERc, as seen in Fig. 3, since upon binding of the steroid to the receptor, the complex will be translocated to the cell nucleus where it appears as ERn complexes (Fig. 4) [33]. Such a depletion of ERc will be followed by replenishment through reactivation of ERn complexes and the *de novo* synthesis of ERc molecules. Nuclear receptor (ERn) concentrations for oestradiol, regardless of the variations in blood plasma concentrations of the hormone, showed a sustained level between late prooestrus and day 4 of the cycle, decreasing thereafter in both tubal segments. Progesterone counteracts the action of oestrogen by reducing the tissue level of oestrogen receptors [33].

The motility patterns present in corresponding periods of the oestrous cycle in the tubal isthmus and ampulla of the pig appear to be directly influenced rather than by the withdrawal of the hormone, which only occurs in blood. Evidence that oestrogen exerts a direct action on the rat oviduct during embryo transport and that the passage of the embryos to the uterus is preceded by a well defined increment of oestrogenic action on the oviduct has been reported [16].

The ampullar segment showed a higher extent of oestrogen cytosolic and nuclear binding than that of the isthmus. This finding agrees with those reported previously in humans [14, 34, 35]. Anatomical differences and variations of cellular components exist between the tubal segments concerned. The higher proliferative and secretory function of the ampulla that occurs during the perioestrous period might explain the differential distribution of the oestradiol receptors [36, 37].

Although the myosalpinx has been considered the major mechanical effector of the oviduct, the role of ciliary activity and flow of tubal secretions during the sperm and ova transport should not be overlooked [1]. Ciliated cells predominate in the pig endosalpinx during oestrous [5]. Secretory cells differentiate maximally during the follicular phase [38], when large concentrations of circulating oestrogens are present, and they are well developed in the ampulla and isthmus at days 3 and 9 of the cycle [5]. Our present results showed high concentrations of nuclear ER and PR occurring in porcine tubal tissues during corresponding periods of the cycle.

Oestrogen has been shown to increase the ciliary beat frequency *in vitro* in rabbit endosalpinx [39] and to decrease the viscosity of the tubal secretions [40]. The tubal epithelium of ovariectomized pigs showed absence of cilia and a depressed secretory activity. Ciliogenesis and maximal secretory cell differentiation occurred after 3 days of oestradiol treatment, increasing markedly on days 5 and 7 [41]. These data indicate that both ciliary and secretory activity might depend on oestrogen stimulation of the endosalpinx. The predominance of the secretory over the ciliated-cell population at days 3 and 9 of the cycle in swine tubes [5] may suggest a role for progesterone in maintaining the secretory activity induced by the oestrogens.

The concentration of cytosolic progesterone receptors in the ampullar segment was highest on day 1 of standing oestrous (Fig. 5), well in advance of the ovulation process and the presence of significant blood plasma levels of progesterone (Fig. 1). These low concentrations of circulating progesterone, with subsequent slow repletion of PRc, might explain these high PRc amounts during oestrous. Furthermore, it is known that oestrogens increase the tissue content of progesterone receptors [42, 43]. The high concentration of plasmatic oestradiol-17β present might explain this increment of PRc in the ampulla.

During the rest of the studied oestrous cycle the concentration of PRc in the porcine oviduct decreases slowly, showing a reverse trend when compared to the PRn concentrations. An increasing translocation of receptors from cytosolic to nuclear compartments during this period might explain the decrease in PRc receptors. Progesterone counteracts the action of oestrogen by reducing the tissue level of oestrogen receptors [33]. This effect of progesterone on the oestrogen receptor level is brought about by a mechanism by which progesterone blocks the second phase of the replenishment process, i.e. the *de novo* synthesis [33]. This might explain the decrease of ER after day 4 of the cycle seen in the present study.

Whether or not this increased effect of endogenous progesterone might also be related to the transport of ova/embryo through the oviduct to the uterus, as suggested in the rabbit [12], remains unclear and must be the subject of further investigation.

Acknowledgements—This work was supported by the Swedish Council for Forestry and Agricultural Research. Scholarships awarded to Dr Ph. Stanchev by IAEA, Vienna and to Dr H. Rodriguez-Martinez by the Swedish Institute, Stockholm, are acknowledged.

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