Acta vet. scand. 2001, *42,* 161-169.

Regulation by Gonadal Steroids of Estrogen and Progesterone Receptors Along the Reproductive Tract in Female Lambs

By *A. Meikle[1,2,3], E.G. Garófalo[1], M. Rodríguez-Piñón[1], C. Tasende[1]* and *L. Sahlin[3]*

[1]Biochemistry, Molecular and Cellular Biology, Veterinary Faculty, Montevideo, Uruguay, [2]Clinical Chemistry, Swedish University of Agricultural Sciences, Uppsala, and [3]Division for Reproductive Endocrinology, Department of Woman and Child Health, Karolinska Institutet, Stockholm, Sweden.

Meikle A, Garófalo EG, Rodríguez-Piñón M, Tasende C, Sahlin L: Regulation by gonadal steroids of estrogen and progesterone receptors along the reproductive tract in female lambs. Acta vet. scand. 2001, 42, 161-169. – The regulation of estrogen and progesterone receptor (ER, PR) expression by estradiol (E2) and progesterone (P4) in the oviduct, uterus and cervix of female lambs was studied. The animals received three intramuscular injections of E2, P4 or vehicle with an interval of 24 h and they were slaugthered 24 h after the third injection. Determinations of ER and PR were performed by binding assays and mRNAs of ERα and PR by solution hybridization. High levels of ER and PR in both cervix and oviduct were found in the female lamb, differing from other mammalian species. No significant effects by either E2 or P4 treatment on ER and PR levels in the cervix and oviduct could be observed. E2 treatment increased the mRNA levels of ERa and PR more than 3-fold in the cervix, while P4 treatment increased the mRNA levels of ERa and PR in the uterus. The results show differential effects of gonadal steroids on sex steroid receptor expression along the reproductive tract in female lambs, suggesting that steroid target tissues can modulate responses to the same circulating levels of steroid hormones.

sex; steroid; receptors; uterus; lambs.

Introduction

Estrogens and progesterone are the main hormones modulating the function of the female reproductive tract by operating through specific intracellular receptors (ER and PR, respectively). The importance of estrogens for normal growth and differentiation of the reproductive tract has been reported, thus the presence of ER for this action is also needed (*Greco et al.* 1993, *Gorski & Hou* 1995). Of the two isoforms of ER described - ERα and the recently discovered ERß (*Kuiper et al.* 1996) - ERα is predominant in the oviduct, uterus and cervix of the rat (*Wang et al.* 1999, 2000). It has been demonstrated in ERα knock-out mice that although they have a normal appearance, abnormalities exist in the reproductive tract (*Lubahn et al.* 1993). *Yamashita et al.* (1989) demonstrated in 1-day-old mice the presence of epithelial ER in the oviduct and cervix, but the receptors could not be detected in uterine epithelia until day 4. Regarding sheep, previous studies showed high concentrations of ER and PR in the uterus of prepubertal ewes (*Garófalo & Tasende* 1996) differing from some species in which PR expression is low or undetectable at this stage of development (guinea pig: *Pasqualini et al.* 1980, dog: *Lessey et al.* 1981). A recent study has described the uterine changes in ER and PR

expression during the early postnatal period in the lamb and the results support the hypothesis that ERα is also necessary for normal uterine growth and development in ovines (*Taylor et al.* 2000). *Zhao et al* (1999) detected few ER positive nuclei in cervical cells of prepubertal ewes, but no quantitative studies were performed. Although sex steroid hormones are known to act in unity with their respective receptor molecule to induce cellular effects, little or no information exists concerning the presence of ER and PR in the cervix and oviduct of immature ewes. In the ovariectomized adult ewe, sex steroid receptor levels in the isthmic oviduct are equal to or slightly higher than in the uterus (*Stone & Miller* 1978). Similar findings were reported for receptor levels in the cervix vs uterus of the postpartum ewe (*Rodriguez-Piñon et al.* 2000). The regulation of sex steroid receptors in the oviduct and cervix is similar to that reported for the uterus, i.e., E2 stimulates the expression while P4 downregulates it; although in primates the changes in cervical ER and PR throughout the menstrual cycle are more subtle than in the endometrium and myometrium (*Gorodeski* 1996, *Brenner & Slayden* 1996). *Miller et al.* (1977) have found high rates of protein synthesis and increased RNA:DNA ratios in the ovine endometrium and oviduct at or shortly after estrus, associated with an increased E2 level in plasma. Progesterone action on RNA and protein synthesis differs in the ovine endometrium and isthmic oviduct, with highly significant increases in the former but no effect in the latter (*Miller* 1976, *Miller et al.* 1977). Similar findings were described in the immature ewe (*Meikle et al.* 1997), since the administration of P4 increased uterine weight but had no effect on the oviductal and cervical weights, differing from E2 treatment after which all parts of the reproductive tract increased in weight. Antagonistic effects of progesterone on estrogen action, like inhibition of E2-induced uterine growth, have been demonstrated in rodents (*Clark et al.* 1977), but P4 does not decrease the weight of the uterus in E2-treated ovariectomized ewes (*Miller et al.* 1979, *Stone et al.* 1982). Furthermore, it was shown that P4 alone could increase uterine weight in 2 months old lambs without the need of previous estrogen priming (*Meikle et al.* 1997). The different actions of the steroid hormones along the reproductive tract are dependent on the presence of the hormone and its specific receptor concentration in the target tissue, which is also regulated by E2 and P4 (*Clark et al.* 1992). In this study, we focused on the regulation of ER and PR expression by gonadal steroids in the cervix, uterus and oviduct of female lambs.

Materials and methods

Animals and treatments

Eleven 2 months old Corriedale female lambs (body weight, mean ± SEM: 11.4 ± 0.3 kg) born in September were used. The experiment was carried out in November which corresponds to the nonbreeding season for the Corriedale breed in the experimental field of Veterinary Faculty, Montevideo, Uruguay (35° SL, spring). The lambs were maintained under natural environmental conditions and they were allowed to nurse freely during the experiment. The animals received daily injections of 17 ß-estradiol (E2) (1 μg/kg, group E, n=4), progesterone (P) (0.3 mg/kg, group P, n=4) or corn oil vehicle (0.1 ml/kg group C, n=3) i.m. on Days 0, 1 and 2. Blood samples for P4 and E2 determinations were collected daily by jugular venipuncture before, during and after the treatment, and on Day 3 all lambs were slaughtered. The endocrine data as well as the uterine receptor determinations by binding assays have been published previously (*Meikle et al.* 1997). Serum levels of E2 and P4 were significantly different in accordance with the treatments used in the different groups. Uteri, cervices and oviducts

were dissected at 0-4 °C and weighed. Treatment with E2 significantly increased uterine, cervical and oviductal weights while P4 only affected uterine weight (*Meikle et al.* 1997). The tissues were frozen in liquid nitrogen and stored at -80 °C until assayed. ER and PR determinations by binding assays were performed in cervices and oviducts, as well as mRNA determinations of ERa and PR by solution hybridization. Uterine samples from the lower uterine zone, defined as the third portion next to the cervix, were taken to perform mRNA determinations for ERα and PR.

Assays of steroid receptors

Ligand-binding assays were performed on the cytosolic fractions from the cervix and oviduct of each animal as described previously (*Garófalo & Tasende* 1996, *Meikle et al.* 1997). In the ligand-binding assay both ERa and ERb are determined (i.e., the sum of their binding activity is measured). The term cytosolic receptors is used in this study to indicate receptors found in the supernatant fraction of a tissue homogenate after a high-speed centrifugation. Briefly, the cytosolic fraction was incubated with 5 to 6 increasing concentrations of [2,4,6,7-^{3}H]-estradiol-17ß 86 Ci/mmol (0.3-15 nM), or ^{3}H-ORG-2058, (16α-ethyl-21-hydroxy-19-nor[6,7-^{3}H] pregn-4en-3,20-dione 40 Ci/mmol (0.5-30 nM) for 18 h with or without 200-fold molar excess of either unlabeled diethylstilbestrol or unlabeled ORG-2058, respectively. The separation of free hormone was by dextran-coated charcoal and radioactivity was measured by liquid scintillation counting. Protein concentrations were determined by the method of *Lowry et al.* (1951), using BSA as the standard. A linear regression test of the inverse Scatchard model (*Braunsberg* 1984) analysis of the data was performed to obtain the dissociation constant (Kd, nM), and the concentration of receptor sites at the intercept, B max, expressed in fmol/mg protein. Dissociation constants (Kd) for ER were 0.57 ± 0.07 nM for the oviduct and 0.30 ± 0.22 nM for the cervix (n=11), while PR Kd for the oviduct was 1.15 ± 0.16 nM and for the cervix 1.31 ± 0.22 nM (n=11). All Kds were well within the range reported for uterine receptors (*Garófalo & Tasende* 1996, *Meikle et al*. 1997).

Hybridization analysis of mRNA

A solution hybridization assay of specific mRNAs for ovine ERa and PR was performed on samples of cervices, uteri and oviducts. The method was essentially performed as in *Persson et al.* (1997). In short, total nucleic acids were prepared and the concentration of DNA in the TNA samples was measured fluorometrically. The probes used for ERα mRNA and PR mRNA determinations were derived from plasmids containing 360 and 314 bp cDNAs from the ovine ERα and PR, respectively, supplied by Dr. N. Ing, Texas A & M University, TX, USA (*Ing et al.* 1996). Probes were synthesized in vitro and radiolabeled with ^{35}S-UTP as described by *Melton et al.* (1984). Overnight incubation was performed at two different concentrations, and samples were then treated with RNase to digest unhybridized RNA. Labeled hybrids were precipitated with trichloroacetic acid and collected on filters, whereafter radioactivity was determined in a liquid scintillation counter. All the samples from the experiment were determined in the same assay. Receptor mRNA levels were expressed as cpm in relation to DNA content (cpm/μg DNA). In Figure 2, the mRNA levels of ERa and PR are expressed as percentages of the control group average to show changes induced by the treatment.

Statistical analysis

Statistical analyses were carried out using the procedures of the Statistical Analysis Systems Institute Inc. (1994). Differences between groups concerning receptor and mRNA deter-

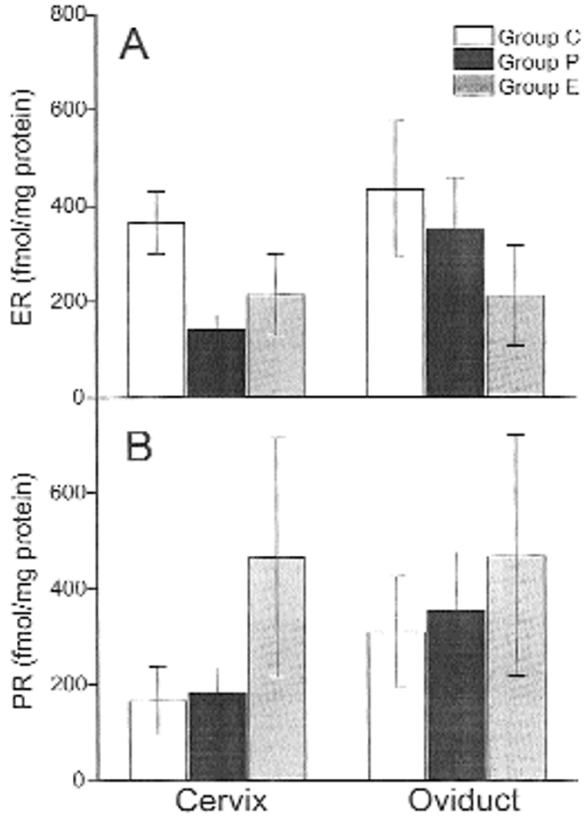

Figure 1. Concentrations (fmol/mg protein) by ligand-binding assay of A) estrogen (ER) and B) progesterone (PR) receptor in the cervix and oviduct of 2 months old lambs after i.m. injections of oil (Group C), estradiol (-Group E) or progesterone (Group P). The bars represent least square mean ± SEM. There were no significant differences between the treatments.

minations were analyzed by the General Linear Model procedure for analysis of variance according to a model including the treatment (groups C, E and P), region of the reproductive tract (cervix, oviduct and uterus), and the interaction between treatment and region. Data are presented as least-square means ± standard errors for each treatment group and $P<0.05$ is considered as significant. Normality of distribution of the residuals were analyzed using the Univariate Procedure.

Results

ER and PR

Concentrations of ER and PR (fmol/mg protein) in the oviduct and cervix of control and treated lambs are shown in Figure 1A and 1B respectively. There was a significant effect of region of the reproductive tract on both ER and PR levels ($P=0.03$ and $P<0.005$, respectively). In control lambs, ER concentrations in the cervix and oviduct were similar (365 ± 65 and 433 ± 142), but significantly lower than in the mid-

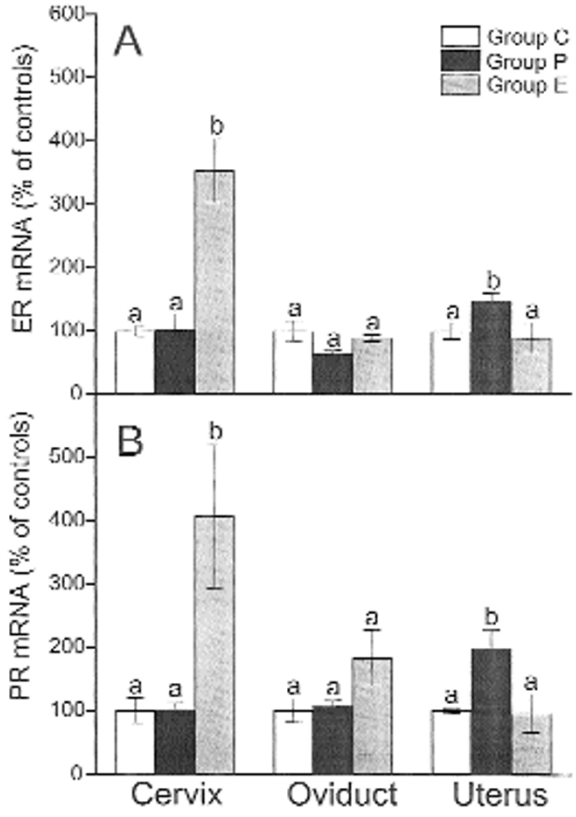

Figure 2. Levels of mRNA of A) estrogen receptor α (ERα mRNA) and B) progesterone receptor (PR mRNA) in the cervix, oviduct and uterus of 2 months old lambs after i.m. injections of oil (Group C), estradiol (Group E) or progesterone (Group P). The results are presented as percentages of Group C. Bars (least square mean ± SEM) with different superscripts are significantly different (P< 0.05).

dle zone of the uterus of the same lambs, (682 ± 53) (*Meikle et al.* 1997). The same occurred with PR of which the cervix and oviduct had lower concentrations (167 ± 71 and 308 ± 117) than the uterus (1479 ± 153) (Meikle et al. 1997). No significant effects by either E2 or P4 treatment on ER and PR levels in the cervix and oviduct could be observed. No significant interaction between treatment and region was found.

mRNA of ER_α and PR

Concentrations of mRNA of ERα and PR in the cervix, oviduct and uterus of the lambs are presented as percentage of controls in Figure 2A and 2B respectively. There was a significant effect of region of the reproductive tract on both mRNA levels (P<0.0005 and P<0.005, respectively). The cervix of control lambs had a lower ERα mRNA level (cpm/mg DNA) than the oviduct and uterus (34.7 ± 3.7, 79.7 ± 11.5 and 80.7±3.2 respectively). PR mRNA concentra-

tions (cpm/μg DNA) were lower in the cervix and oviduct than in the uterus (63.0 ± 8.6, 64.0 ± 11.7 and 161 ± 6.7 respectively). Significant interaction between treatment and region was found for ERα mRNA (P=0.0001) and PR mRNA (P=0.0016).

The ERα mRNA level increased more than 3-fold in the cervix after E2 treatment as compared to both the control group and P4 treatment (Figure 2A). No differences were found in the ERα mRNA levels in the oviduct, although the levels in the P4 treated group tended to be lower than in the controls. The ERα mRNA level in the uterus was higher after P4 treatment as compared to the control group.

The cervical PR mRNA level increased 4-fold after E2 treatment as compared to the control group (Figure 2B). There were no significant changes seen in the oviduct. In the uterus, P4 treatment increased the PR mRNA level 2-fold, whereas E2 showed no effect.

Discussion

This is the first report of expression of sex steroid receptors in the oviduct of the immature ewe. *Zhao et al.* (1999), reported few ER immunopositive cells in the cervix of two prepubertal ewes. In contrast, in this study, high levels of ER and PR were found in both cervix and oviduct, although less than in the uterus of the same animals (*Meikle et al.* 1997). The receptor proteins are functional since treatment induced changes in receptor and mRNA expression. The presence of ER was expected since it is known that ER are needed for normal development of the reproductive tract (*Lubahn et al.* 1993), but it is not clear why PR levels were so high at this stage of development. Similarly, no clear biological role could be attributed to the presence of high affinity PR in the oviduct in concentrations equal to or sligthly higher than those found in endometrium or whole uterus in the adult ewe (*Stone & Miller* 1978). In the immature dog, both the uterus and oviduct contain relatively low levels of ER while PR is absent (*Lessey et al.* 1981). In contrast, the present study and other findings in sheep (*Garófalo & Tasende* 1996) show that ruminants differ from other mammals in sex steroid receptor expression along the reproductive tract.

No effect of the hormone treatment on receptor concentrations in the cervix and oviduct could be demonstrated and this contrasts with the downregulation of ER and PR expression after E2 and P4 treatment seen in uteri of the same animals (*Meikle et al.* 1997). *Scharl et al.* (1988) found that the endocervix - unlike endometrium and myometrium - undergoes minimal changes with regard to nuclear receptor content during the human menstrual cycle. In the oviduct, a clear steroid hormone dependence of ER and PR expression has been found in primates and rodents (*Brenner & Slayden* 1996). On the other hand, no differences in ER content were found after E2 treatment in the oviduct of immature dogs (*Lessey et al.* 1981) and PR downregulation in the rabbit oviduct was delayed and not so pronounced as in the uterus (*Muechler et al.* 1976). In the present study, the tissues were sampled 3 days after the first E2 injection and the dynamics of the receptor contents could not be observed during the period of treatment.

This study shows that E2 and P4 have different effects on the regulation of mRNA expression of sex steroid receptors along the reproductive tract in prepubertal ewes. E2 treatment induced an increase in mRNA levels of ERα and PR in the cervix, but no such effect was observed in the oviduct and uterus. In uteri of ovariectomized ewes, E2 increased mRNA levels of ERα and PR with maximal concentrations 24 h after the E2 injection (*Ing et al.* 1996). We have recently showed that mRNA levels of ERα and PR increase 2-fold 12 h after E2 injection and levels remain high during daily injections of E2 (*Meikle et al.* 2000a). As for the receptor deter-

minations, mRNAs were measured at only one point of the treatment period, and an earlier increase in mRNA concentrations could have been missed. Nevertheless, the 3 to 4-fold increase in mRNA levels of ERα and PR after E2 treatment found only in the cervix, suggests that E2 in this organ maintains the stimulus on mRNA expression for a longer period or that the cervix is able to respond to repeated hormone treatments.

An interesting finding was the P4-induced increase in mRNA levels in the uterus, different from the action on the cervix and oviduct. These results agree with previous reports in sheep, which showed that P4 increases RNA and protein synthesis in the endometrium but have no effect in the isthmic oviduct (*Miller* 1976, *Miller et al.* 1977). The inability to influence nucleic acid synthesis by P4 in the oviduct was also reported for the mouse (*Bronson & Hamilton* 1972). In the oviduct of macaques, E2 stimulated differentiation of a fully ciliated-secretory epithelium primed for gamete transport, whereas P4 induced cell atrophy (*Brenner et al.* 1990). In the primate endometrium, E2 stimulates epithelial and stromal proliferation while P4 suppresses proliferation and induces differentiation by converting the tissue to a hypertrophied and secretory state able to support implantation (*Slayden et al.* 1993). This difference in the action by P4, i.e., suppressive effects in the oviduct and inductive in the endometrium, agrees with the tendency to lower levels of ERα mRNA found in the oviduct and the increased ERα mRNA and PR mRNA expression in the uterus observed after P4 treatment as seen in the present study. This is consistent with the effect of P4 on the weight of the oviduct and uterus found in these animals, with no change in the former and a significant increase in the latter (*Meikle et al.* 1997).

The overall response of the uterus to steroid stimulation will be the product of the combined responses through sex steroid receptor levels in various cell types. Immunohistochemical studies on ER and PR expression during the ovine estrous cycle (*Cherny et al.* 1991; *Spencer and Bazer* 1995), showed that in the majority - but not all - of the uterine compartments, receptor levels were high shortly after estrus in the different compartments and then declined to negligible levels at mid luteal phase. For example, ER levels in the innermost region of the basalis zone are not suppressed by the end of the luteal phase (*Cherny et al.* 1991). Likewise, it was shown that E2 regulation of ERα immunostaining showed a similar pattern in epithelial and stromal cells in the endometrium of prepubertal lambs, but there were cell type specific differences in timing and strength of E2 action (*Meikle et al.* submitted 2000b).

In summary, this study showed that gonadal steroids regulate ER and PR expression differently along the reproductive tract in female lambs, suggesting that peripheral steroid target tissues can modulate responses to the same circulating levels of steroid hormones.

Acknowledgments

The authors want to thank Prof. M. Forsberg for constructive criticism of this manuscript. We also want to thank P. Tummaruk for help in statistical analyses, P. Rubianes and I. Sartore for their technical assistance and M. Lindberg for performing the mRNA determinations. The ovine ER and PR cDNAs were a generous gift from Dr. N. Ing Texas A &M University, TX, USA. The present study received financial support from the CSIC, the Veterinary Faculty, University of Uruguay, the Swedish Medical Research Council (grant 03972) and the Swedish Society for Medical Research.

References

Braunsberg H: Mathematical analysis of data from receptor assay. In: Leclerq G, Toma S, Paridaens R, Heuson JC (Eds): Clinical Interest of Steroid Hormone Receptors in Breast Cancer. Recent Results in Cancer Research, Springer-Verlag, Berlin, 1984, p. 91.¡Error!Marcador no definido.

Brenner RM, West NB, McClellan MC: Estrogen and progestin receptors in the reproductive tract of male and female primates. Biol. Reprod. 1990, *42,* 11-19.

Brenner RM, Slayden OD: The fallopian tube cycle. In: Adashi EY, Rock JA, Rosenwaks Z (Eds), Reproductive Endocrinology, Surgery and Technology, Lippincott-Raven Publishers, Philadelphia, 1996, 325-340.

Bronson FH, Hamilton TH: A comparison of nucleic acid synthesis in the mouse oviduct and uterus: interactions between estradiol and progesterone. Biol. Reprod. 1972, *6,* 160-167.

Cherny RA, Salamonsen LA, Findlay JK: Immunocytochemical localization of oestrogen receptors in the endometrium of the ewe. Reprod. Fert. Dev. 1991, *3,* 321-331.

Clark JH, Hsueh AJW, Peck EJ: Regulation of estrogen replenishment by progesterone. Ann. NY Acad. Sci. 1977, *286,* 161-179.

Clark JH, Schrader WT, O'Malley BW: Mechanisms of steroid hormones action. In: Wilson JD and Foster DW (Eds), Textbook of Endocrinology, WB Saunders, Philadelphia 1992, 35-90.

Garófalo EG, Tasende C: Uterine estrogen and progesterone receptors in prepubertal ewes: distribution in myometrium, endometrium and caruncles. Vet. Res. 1996, *27,* 177-183.

Gorodeski GI: The cervical cycle. In: Adashi EY, Rock JA, Rosenwaks Z (Eds), Reproductive Endocrinology, Surgery and Technology, Lippincott-Raven Publishers, Philadelphia 1996, 301-324.

Gorski J, Hou Q: Embryonic estrogen receptors: Do they have a physiological function?. Environ. Health. Perspect. 1995, *103,* 69-72.

Greco TL, Duello TM, Gorski J: Estrogen receptors, estradiol, and diethylstilbestrol in early development: The mouse as a model for the study of estrogen receptors and estrogen sensitivity in embryonic development of male and female reproductive tracts. Endocr. Rev. 1993, 59-71.

Ing NH, Spencer TE, Bazer FW: Estrogen enhances endometrial estrogen receptor gene expression by a posttranscriptional mechanism in the ovariectomized ewe. Biol. Reprod. 1996, *54,* 591-599.

Kuiper GGJM, Enmark E, Pelto-Huikko M, Nilsson S, Gustafsson JA: Cloning of a novel estrogen receptor expressed in rat prostate and ovary. Proc. Natl. Acad. Sci. USA 1996, *93,* 6925-6930.

Lessey BA, Wahawisan R, Gorell TA: Hormonal regulation of cytoplasmic estrogen receptor and progesterone receptors in the beagle uterus and oviduct. Mol. and Cell. Endocr. 1981, *21,* 171-180.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ: Protein measurement with the folin phenol reagent. J. Biol. Chem. 1951, *193,* 265-275.

Lubahn DB, Mayer JS, Golding TS, Couse JF, Korach KS, Smithies O: Alteration of reproductive function but not prenatal sexual development after insertional disruption of the mouse estrogen receptor gene. Proc. Natl. Acad. Sci. USA 1993, *90,* 11162-11166.

Meikle A, Tasende C, Rodriguez M, Garófalo EG: Effects of estradiol and progesterone on the reproductive tract and on uterine sex steroid receptors in female lambs. Theriogenology 1997, *48,* 1105-1113.

Meikle A, Forsberg M, Sahlin L, Masironi B, Tasende C, Rodríguez-Piñón M, Garófalo EG: A biphasic action of estradiol on estrogen and progesterone receptor expression in the lamb uterus, Reprod. Nutr. Dev. 2000a, *40,* 283-293.

Meikle A, Bielli A, Masironi B, Pedrana G, Wang H, Forsberg M, Sahlin L: An immunohistochemical study on the regulation of estrogen receptor α by estradiol in the endometrium of the immature ewe. Submitted to Reprod. Nutr. Dev 2000b.

Melton DA, Krieg PA, Rebagliati MR, Maniatis R, ZinnK, Green MR: Efficient in vitro synthesis of biologically active RNA and RNA hybridization probes from plasmids containing bacteriophage SP6 promotor. Nucl. Acids. Res. 1984, *12,* 7035-7056.

Miller BG: RNA and protein metabolism in the oviduct and endometrium of the ewe at pro-estrus: regulation by estradiol and progesterone. J. Endocrinol. 1976, *69,* 57-66.

Miller BG, Murphy L, Stone GM: Hormone receptor levels and hormone, RNA and protein metabolism in the genital tract during the oestrous cycle of the ewe. J. Endocrinol. 1977, *73,* 91-98.

Miller BG, Wild J, Stone GM: Effects of progesterone on the oestrogen-stimulated uterus: a comparative study of the mouse, guinea pig, rabbit and sheep. Aust. J. Biol. Sci. 1979, *32,* 649-560.

Muechler EK, Flickinger GL, Mastroianni L Jr, Mikhail G: Progesterone binding in rabbit oviduct and uterus. Proc. Soc. Exp. Biol. Med. 1976, *151,* 275-279.

Pasqualini JR, Sumida C, Nguyen BL, Gulino A, Gelly G: Estrogens, estrogen receptors and progesterone receptors in fetal and newborn guinea pigs. A model for the study of the biological ef-

fect of estrogens. In: Bresciani F (Ed), Perspectives in Steroid Receptor Research, Raven Press, New York 1980, 183-192.

Persson E, Sahlin L, Masironi B, Dantzer V, Eriksson H, Rodriguez-Martinez H: Insuline-like growth factor-I in the porcine endometrium and placenta. Localization and concentration in relation to steroid influence. Anim. Rep. Sci. 1997, *46,* 261-281.

Rodríguez-Piñón M, Tasende C, Meikle A, Garófalo EG: Sex steroid receptors in ovine cervix during the postpartum period. Theriogenology 2000, *53,* 743-750.

Scharl A, Vierbuchen M, Graupner J, Fischer R, Bolte A: Immunohistochemical study of distribution of estrogen receptors in corpus and cervix uteri. Arch. Gynecol. Obstet. 1988, *241,* 221-233.

Slayden OD, Hirst JJ, Brenner RM: Estrogen action in the reproductive tract of rhesus monkeys during antiprogestin treatment. Endocrinology 1993, *132,* 1845-1856.

Spencer TE, Bazer FW: Temporal and spatial alterations in uterine estrogen receptor and progesterone receptor gene expression during the estrous cycle and early pregnancy in the ewe. Biol. Reprod. 1995, *53,* 1527-1543.

Stone GM, Miller BG: The isthmic oviduct of the ewe: What is the biological significance of high affinity cytosol receptors for estradiol and progesterone? Biol. Reprod. 1978, *19,* 653-656.

Stone GM, McCaffery C, Miller BG: Effects of progesterone on nuclear and cytosol steroid receptor levels in the oestrogen-stimulated uterus: comparison of the sheep and mouse. Aust. J. Biol. Sci. 1982, *35,* 403-415.

Taylor KM, Gray A, Joyce MM, Stewart MD, Bazer FW, Spencer TE: Neonatal ovine uterine development involves alterations in expression of receptors for estrogen, progesterone and prolactin. Biol. Reprod. 2000, *63,* 1192-1204.

Wang H, Masironi B, Eriksson H, Sahlin L: A comparative study of estrogen receptors a and b in the rat uterus. Biol. Reprod. 1999, *61,* 955-964.

Wang H, Eriksson H, Sahlin L: Estrogen receptors α and b in the female reproductive tract of the rat during the estrous cycle. Biol. Reprod. 2000, in press.

Yamashita S, Newbold RR, McLachlan JA, Korach KS: Developmental pattern of estrogen receptor expression in female mouse genital tracts. Endocrinology 1989, *125,* 2888-2896.

Zhao Y, Williams LM, Hannah LT, Ross AW, McKelvey WAC, Robinson JJ: Oestrogen and progesterone receptor ummunoreactivity and c-fos expression in the ovine cervix. J. Reprod. Fert. 1999, *115,* 287-292.

Sammanfattning

Östradiol och progesteron regleringen av östrogen och progesteronreceptorer.

Könskörtelsteroidernas reglering av östrogen- och progesteronreceptorer i reproduktionsorganen hos tacklamm Östradiol- (E2) och progesteron-regleringen (P4) av östrogen- och progesteronreceptor (ER och PR) expressionen har studerats i äggledare, livmoder och livmodermun hos prepubertala tacklamm. Djuren behandlades i 24 timmars intervall med tre i.m. injektioner av E2, P4 eller vehikel och avlivades 24 timmar efter sista behandlingstillfället. Mätningar av ER- och PR-nivåerna gjordes med bindningsstudier, och deras respektive mRNA-nivå bestämdes med hjälp av lösningshybridisering. De nivåer av ER och PR som uppmättes i livmodermun och äggledare hos lammen var högre än vad som tidigare visats hos andra prepubertala däggdjur. Inga signifikanta effekter på ER- och PR-nivåerna i livmodermun och äggledare erhölls med E2 eller P4 behandling. E2 behandling ökade mRNA-nivåerna av ERa och PR mer än 3 gånger i livmodermunnen. P4 behandling ökade mRNA-nivåerna för ERα och PR i livmodern. Resultaten visar att man får olika effekter av de två könshormonerna på ER- och PR-expressionen i fortplantningsorganen, vilket tyder på att målorganen kan modulera svaret på de cirkulerande nivåerna av steroidhormoner.

(Received July 3, 2000; accepted October 30, 2000).

Reprints may be obtained from: Ana Meikle, Dept of Clinical Chemistry, Faculty of Veterinary Medicine, Swedish University of Agricultural Sciences, P.O. Box 7038, S- 750 07 Uppsala, Sweden. E-mail: anamei@adinet.com.uy, tel: +46-18-671613, fax: +46-18-309565.