ELSEVIER

Steroids 65 (2000) 409–414

Steroids

Estrogen and progesterone modulation of eosinophilic infiltration of the rat uterine cervix☆

Jorge G. Ramos[a], Jorgelina Varayoud[a], Laura Kass[a], Horacio Rodriguez[a], Mónica Muñoz de Toro[a], Gregorio S. Montes[b], Enrique H. Luque[a,*]

[a]*Department of Human Physiology, Faculty of Biochemistry and Biological Sciences, Universidad Nacional del Litoral, C. C. 530, Paraje El Pozo, Ciudad Universitaria, 3000 Santa Fe, Argentina*

[b]*Laboratory for Cell Biology, The University of São Paulo School of Medicine, São Paulo, Brazil*

Received 22 December 1999; received in revised form 15 February 2000; accepted 22 February 2000

Abstract

Ripening of the rat cervix involves widespread collagenolysis that follows an eosinophilic leukocyte infiltration. The hormonal control of these events is not well understood. The aims of this study were to investigate the mechanism through which progesterone (P) and 17β-estradiol (E_2) modulate eosinophilic invasion and to determine if this event is protein synthesis mediated. Cervical eosinophilic invasion was measured in intact rats during the second half of pregnancy and compared with values from ovariectomized (O) pseudopregnant (PSP) rats treated with P and E_2 in doses that mimicked the levels of pregnancy. Other O-PSP rats were treated with an E_2 antagonist (tamoxifen) and the antiprogestin RU-486. To study the role of protein synthesis in eosinophilic invasion of the cervix, rats were treated with actinomycin-D (an inhibitor of mRNA synthesis), and animals were sacrificed on D21 or D22 to evaluate eosinophilic invasion. Rats treated with E_2 showed high levels of infiltration and tamoxifen blocked this E_2 effect. On the other hand, P antagonized the stimulatory effects of E_2 on eosinophilic invasion, however when the P and E_2 treated rats were injected with RU-486 the inhibitory effect of P was reversed. In intact pregnant rats a sharp rise in eosinophilic infiltration was detected on D23, 20 h after the fall of serum P. Finally, E_2 treated rats injected with actinomycin-D had no invasion of eosinophils. In conclusion, the estrogen-triggered eosinophil invasion is affected by the classic estrogen receptor antagonist tamoxifen and by the mRNA synthesis blocker actinomycin-D suggesting a genomic action of E_2. Furthermore, the estrogen effect is blocked by P and this inhibition is reversed by RU-486.

Keywords: Parturition; Estrogen; Progesterone; Eosinophils; Cervix; Tamoxifen; RU-486

1. Introduction

During ripening, marked biochemical changes take place in the uterine cervix, causing it to became soft and dilatable at the time of parturition [1]. The physiological mechanism involved in the ripening process at term is still uncertain [2]. This process involves a disruption of the ordered collagen bundles that occurs after polymorphonuclear leukocyte invasion [3–5]. Neither the process of polymorphonuclear leukocyte infiltration, nor the physiological role of this invasion, is clearly defined. Though some authors suggest that polymorphonuclear leukocyte infiltration of the cervix at term may be incidental [6], it is generally accepted that this infiltration may be an important aspect of the parturition process.

In previous studies we found that eosinophilic infiltration and collagen remodeling of the rat cervix at term are under differential hormonal control [5,7]. When studying the eosinophilic invasion, it was found that 17β-estradiol (E_2) stimulated (whereas P blocked) infiltration of the cervix [7,8]. Thus, if E_2 and P modulate eosinophilic infiltration of the cervix by acting through their classic receptors, pretreatment with antiprogestin (RU-486), or antiestrogen (tamoxifen) should reverse the above-mentioned events. On the other hand, previous studies using an in vitro chemotactic assay on immature rat uterus demonstrated that the promotion effect of E_2 on eosinophilic infiltration depends on the synthesis of a specific chemotactic protein factor [9].

☆ This study was supported by grants from CONICET (PIP 528/98), CONICET/CNPq (N° 1786), SECYT-CAPES (BR04/99/OG), and Universidad Nacional del Litoral (CAI+D 027-195).

* Corresponding author. Fax: +54-342-4550944.

E-mail address: eluque@fbcb.unl.edu.ar (E.H. Luque).

0039-128X/00/$ – see front matter
PII: S0039-128X(00)00098-2

Table 1
Experimental protocol designed to study the kinetics of eosinophilic invasion of the uterine cervix at the end of pregnancy and in pseudopregnancy (PSP)-ovariectomized, 17β-estradiol (E_2)-treated rats

Group	D9	D20	D21	D22	D23
PSP[a]-D20[b]	E_2 (0.05 μg)[c]	E_2 (0.05 μg)	—	—	—
PSP-D21	E_2 (0.05 μg)	E_2 (0.05 μg)	E_2 (0.5 μg)	—	—
PSP-D22	E_2 (0.05 μg)	E_2 (0.05 μg)	E_2 (0.5 μg)	E_2 (1 μg)	—
PSP-D23	E_2 (0.05 μg)	E_2 (0.05 μg)	E_2 (0.5 μg)	E_2 (1 μg)	E_2 (1 μg)
Pregnant[d]	—	Sacrifice	—	—	—
Pregnant	—	—	Sacrifice	—	—
Pregnant	—	—	—	Sacrifice	—
Pregnant	—	—	—	—	Sacrifice

[a] All PSP rats were ovariectomized on day 9; D1 of pseudopregnancy is the day after cervicovaginal stimulation.
[b] Indicates the day when experimental animals were sacrificed.
[c] From D9 to D20, the same dose of E_2 was administered daily.
[d] D1 of pregnancy is the day when sperm was present in vaginal smears.

The fact that, at term, the caudal portion of the uterus (the cervix) softens while its cranial regions (uterine horns) strongly contract, suggests that the same hormones at identical concentrations, may have different effects on these distinct segments of the uterus [10,11]. In fact, regionally specific changes might be expected, because the different parts of the uterus in term pregnancy should serve the dual but conflicting functions of acting as a barrier to retain the conceptus and opening the cervix at the time of delivery [4]. Thus, though the effect of E_2 [12] and P [13,14] on eosinophilic infiltration of the uterine *horn* have already been reported, it appeared expedient to investigate whether the same results would be demonstrated in the uterine *cervix*.

It should also be remarked that, whereas the studies by King et al. [12] and Howe et al. [13] used immature rat uterus to evaluate the steroid regulation of eosinophilic migration, the present paper reports our observations on this process using the cervix of adult rats, that were ovariectomized (O) during pregnancy or pseudopregnancy (PSP). The widely used experimental model of the O-PSP rat [7] is particularly useful for these determinations. To investigate whether our observations in the present studies were a result of pharmacological effects or due to physiological levels of the hormones administered, O pregnant rats treated with the same protocol were allowed to complete pregnancy, indicating that exogenous hormonal levels were physiological.

To study the effect of P on the O-PSP rat model, an anti-progesterone (RU-486) was administered to O rats treated with E_2 and P. To evaluate E_2 action on the eosinophilic infiltration of the uterine cervix, an anti-estrogen (tamoxifen) was administered to O rats treated with E_2. To study the estrogenic effect on protein synthesis, O rats treated with E_2 received an inhibitor of mRNA synthesis, actinomycin D (ACT-D).

The aim of this study was to achieve better insight into the mechanism through which E_2 and P modulate eosinophilic invasion of the uterine cervix and to determine whether the eosinophilic invasion of the cervix is dependent, as is the case in the uterine horn [9], on protein synthesis.

2. Experimental

2.1. Animals

Female adult rats (over 200 g of body weight) of a Wistar-derived strain bred at the Department of Human Physiology (Santa Fe, Argentina) were used. Animals were maintained in a controlled environment (22 ± 2°C; lights on from 6:00 a.m. to 8:00 p.m.). Animals had free access to pellet laboratory chow (Nutric, Argentina) and tap water.

Vaginal smears were used to confirm normal cycling [15]. To obtain pregnancy, proestrous females were caged overnight with males of proven fertility. The presence of sperm in the vaginal smear taken on the following morning was the criterion for designating day 1 (D1) of pregnancy.

To obtained pseudopregnancy, virgin cycling rats received artificial cervicovaginal stimulation on the evening of proestrous [16], and the following day was considered as D1 of PSP. On D9 of PSP, bilateral O was performed under ether anesthesia. In PSP rats, a functional *corpus luteum* is formed [17] and serum P levels are equivalent to those of pregnant rats until the moment that O is performed (D9) [18]. After O, PSP animals were assigned to different experimental groups to receive various hormone treatment regimens (see Table 1).

2.2. Tissue preparation and light microscopy

Cervical tissue was obtained on D23, unless otherwise specified. Tissue samples were fixed by immersion in 10% buffered formalin for 6 h, dehydrated in graded concentrations of alcohol, embedded in paraffin, and sectioned at 5 μm. Serial sections were stained for 60 min in 0.5% Sirius Red (Direct Red 80, Aldrich, Milwaukee, WI, USA) dissolved in alkaline solution (NaOH, pH 10.5) followed by

counterstaining with Harris' hematoxylin (Biopur, Argentina) for 10 min. This method permitted distinct characterization of rat eosinophils in tissue sections: their specific granules were deeply stained red, which strongly contrasted against a pale background [4]. Taking into account that, in the stroma of the cervix, eosinophilic infiltration assumes a heterogeneous pattern, quantification in each section, was performed on the mucosal lamina propria and on the fibrous submucosal layer of the whole cervical wall.

2.3. Kinetics of eosinophilic invasion of the uterine cervix at the end of pregnancy and in O-PSP estrogen-treated rats (Table 1)

The pattern of eosinophilic infiltration in the uterine cervix of intact pregnant rats at the end of pregnancy was compared with that of O-PSP steroid-treated rats. Pregnant rats were sacrificed at 5:00 p.m. on D20, D21, and D22 and at 1:00 p.m. on D23. O-PSP animals were treated with E_2 (Sigma Chemical Co., St. Louis, MO, USA) following a protocol previously described [7,19]. Daily subcutaneous (s.c.) injections of 0.05 μg of either E_2 or its vehicle (0.1 ml sesame oil) were administered at 10:00 a.m. from the time of O (D9) until D20. To mimic the increase of E_2 levels before the onset of rat parturition, the doses were increased to 0.5 μg on D21, and to 1.0 μg on D22 and D23. O-PSP animals were sacrificed following the same schedule used for the intact, pregnant rats. Uterine cervices were collected at sacrifice and processed as mentioned above.

2.4. Evaluation of the effect of estrogen antagonist (tamoxifen) on eosinophilic invasion

To examine the effect of tamoxifen (Tx) on E_2-stimulated eosinophilic infiltration of the rat cervix, a group of O-PSP rats treated with E_2, as mentioned above, received 1 mg/0.2 ml s.c. of Tx, 90 min before each E_2 dose on D21, 22, and 23. The corresponding control groups were conducted: one group was injected with E_2 and vehicle of Tx, another with Tx and vehicle of E_2, and the third with both vehicles.

2.5. Evaluation of the effect of progestin antagonist (RU-486) on eosinophilic invasion

To study the effect of P on E_2-induced eosinophilic infiltration, we used the synthetic anticorticoid/antiprogestin RU-486 [20]. E_2 was administered as described above, and P was given in the form of implants made from Silastic tubing (internal diameter, 0.15 × 5 cm; Dow–Corning, Midland, MI, USA), each containing 60 mg crystalline P (4-pregnene-3,20-dione; Sigma Chemical Co.) [7]. Two Silastic implants were inserted s.c. in the back of the neck at the time of O. Both implants were removed at the time of sacrifice (1:00 p.m. of D23). RU-486 was dissolved in sesame oil + 5% ethanol, and 0.04 mg RU-486/0.2 ml was given s.c. at 10:00 a.m. on D20, 21, 22, and 23. Control groups received empty implants and/or RU-486 vehicle.

2.6. Evaluation of the role of protein synthesis in eosinophilic invasion

To determine if the cervical eosinophilic infiltration induced by E_2 requires mRNA synthesis, we evaluated the effect of actinomycin-D (ACT-D, Cosmegen®, Sidus, Argentina) administration on E_2-treated O-PSP rats. A single injection of 100 μg/100 g body weight of ACT-D was administered intraperitoneally (i.p.) 30 min before E_2 administration on D21. Animals were sacrificed either on D21 (7 h after inhibitor injection) or on D22 (31 h after injection). Another group was treated with ACT-D and E_2 vehicle. Animals were sacrificed at 5:00 p.m. on D20, D21, and D22.

2.7. Stereology

The point counting procedure [21] was used to obtain data concerning the morphometric analysis of the number of eosinophils invading the cervix. Eosinophils were counted using a glass disc with a squared grid inserted in a focusing eyepiece and a 100× immersion objective [22]. The fraction of points occurring within the structure of eosinophils (stained in red) was determined and then compared to the total number of points lying within the cervical stroma. The volume fraction was calculated by applying the formula given by Weibel [23]:

$$Vv = \frac{\mathrm{Pi}}{\mathrm{P}}$$

where *Vv* is the estimated volume fraction of the object (eosinophils); Pi is the number of incident points over the eosinophils; and P is the total number of incident points over the volume unit (stroma of the uterine cervix).

To investigate the differences in eosinophilic infiltration of the cervical stroma among experimental groups, values were subjected to the Kruskal–Wallis one-way ANOVA. Probabilities were assigned using the Mann–Whitney *U*-test [24].

3. Results

3.1. Kinetics of eosinophilic invasion of the uterine cervix at the end of pregnancy and in O-PSP estrogen-treated rats

Consistent with previous studies [7] measurement of estrogen-induced eosinophilic invasion in O-PSP E_2-treated rats showed very high values of volume density compared

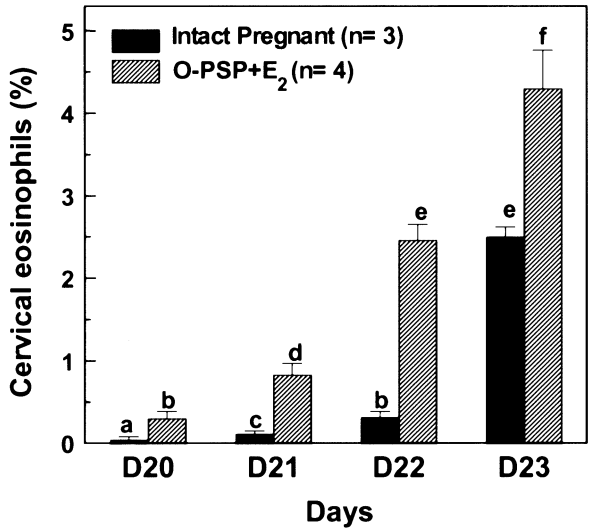

Fig. 1. Kinetics of eosinophilic invasion of the uterine cervix in intact rats at the end of pregnancy and in ovariectomized pseudopregnant 17β-estradiol-treated (O-PSP+E_2) animals treated with increasing doses of E_2 (as detailed in Section 2). Means with different letters differ significantly ($P < 0.01$).

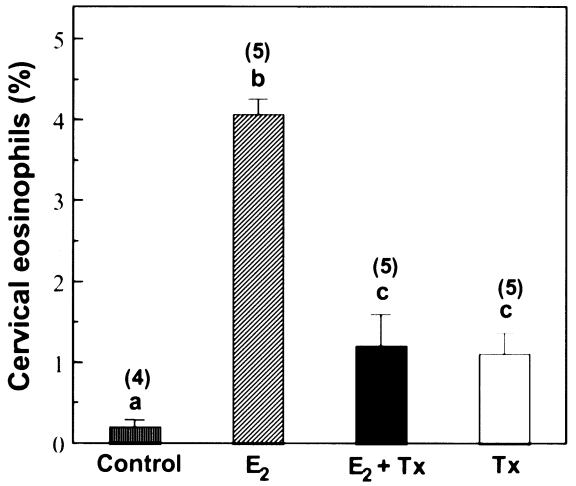

Fig. 2. Effect of tamoxifen (Tx) on eosinophilic infiltration of the rat uterine cervix. Ovariectomized pseudopregnant rats treated with E_2 showed a dramatic eosinophilic invasion that was blocked by the antiestrogen Tx. Tx alone induced a slight, but significant, eosinophilic invasion. Control: vehicle-injected. Animals were sacrificed on D23. Number of animals per group is indicated in brackets. Means with different letters differ significantly ($P < 0.01$).

with O-PSP controls on D23 (4.06 ± 0.19 versus 0.2 ± 0.09; $P < 0.05$).

As shown in Fig. 1 O-PSP animals injected with E_2 (7 h before sacrifice) demonstrated an increased effect on eosinophilic infiltration from D21 onwards. The time course of eosinophilic infiltration in the intact pregnant control group gradually increased from D20 to D22 and showing an acute eosinophilic invasion on D23.

3.2. Evaluation of the effect of estrogen antagonist (tamoxifen) on eosinophilic invasion

Because most E_2-induced events are regulated by an initial interaction with classic estrogen receptors, we examined the effect of an antiestrogen on eosinophilic infiltration of the uterine cervix. As shown in Fig. 2, in animals treated with E_2 + Tx a drastic fall of cervical eosinophil levels was observed as compared with rats treated only with E_2. On the other hand, animals injected only with Tx showed a small but significant eosinophilic invasion compared with vehicle-injected control rats.

3.3. Evaluation of the effect of progestin antagonist (RU-486) on eosinophilic invasion

When O-PSP rats were treated with E_2 plus P they showed a very poor eosinophilic infiltration similar to that found in control O group (Fig. 3). To further understand the hormonal regulation of this event, the P antagonist RU-486 was administered simultaneously with E_2 and P. This coadministration reversed the P inhibition of eosinophilic infiltration.

3.4. Evaluation of the role of protein synthesis in eosinophilic invasion

As shown in Fig. 1, O-PSP animals treated on D20 to D23 with increasing doses of E_2 demonstrated an increase in eosinophilic infiltration with increasing days of treatment. Thus, the number of eosinophils observed on D23 was significantly higher than those found on D20, D21, and

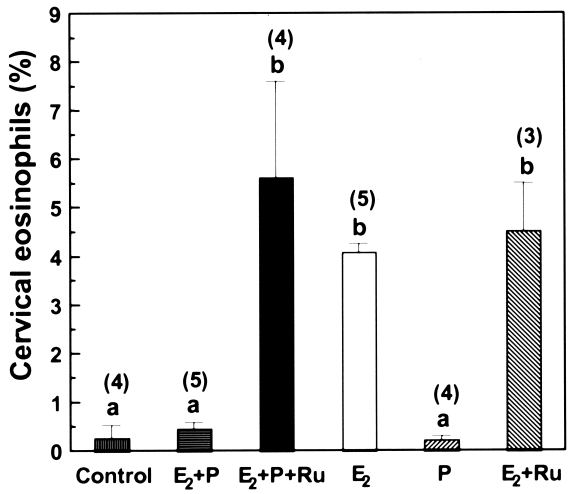

Fig. 3. Effect of the progesterone antagonist RU-486 on eosinophilic invasion of the rat uterine cervix. In ovariectomized (O) pseudopregnant rats, progesterone (P) blocked estradiol (E_2)-induced eosinophilic infiltration and this effect was completely reversed with RU-486 (Ru). The control group contained empty implants and was injected with vehicles of E_2 and RU-486. Animals were sacrificed on D23. Number of animals per group is indicated in brackets. Means with different letters differ significantly ($P < 0.01$).

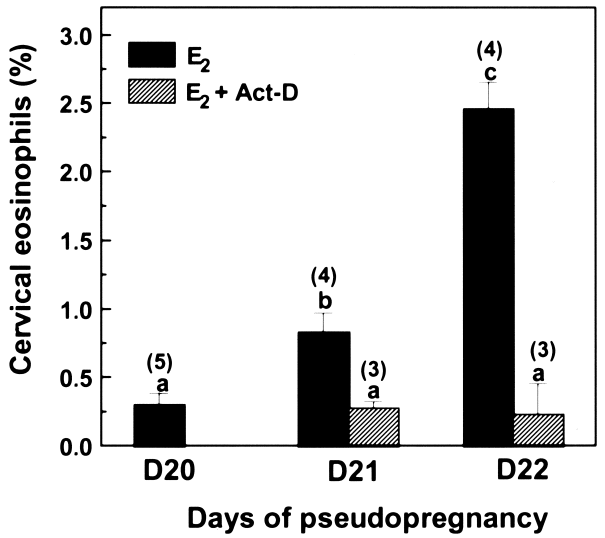

Fig. 4. Effect of actinomycin-D on eosinophilic invasion of the rat uterine cervix. Ovariectomized pseudopregnant rats received increasing doses of E_2 (that mimicked plasma levels at term) that induced a dose-response effect on the eosinophilic invasion of the cervix. The stimulatory effect of E_2 is clearly inhibited by actinomycin-D (ACT-D). Number of animals per group is indicated in brackets. Means with different letters differ significantly ($P < 0.01$).

D22. To understand if this response pattern was protein-synthesis mediated, the E_2 doses were coadministered with ACT-D on D21. Animals treated with ACT-D showed a dramatic inhibition of the estrogen-stimulated leukocyte invasion of cervical tissues on both D21 and 22 (Fig. 4).

4. Discussion

A variety of early responses to E_2 administration were reported in the rat uterus and cervix, such as changes in vascular permeability [25], water imbibition's [26] and eosinophilic infiltration [7,9]. The rat cervical stroma at parturition is an excellent model to study P and E_2 interaction. However, because the majority of the above-mentioned results were obtained using the immature rat uterus and/or pharmacological doses of steroid hormones [4,9,13], little is known regarding the physiological conditions of this interaction in the pregnant intact rat. Previous works have shown that rat cervix at term is invaded by eosinophilic leukocytes and a similar pattern of infiltration is obtained in O-PSP E_2-treated rats [5,7]. The E_2 therapy used in these studies maintained physiological plasma levels similar to those of pregnant intact control rats [19]. In the present work we used the same model of O-PSP steroid treated rats; therefore, we can postulate that the results obtained here are in close correlation with those found in pregnant intact animals at the time of parturition. In this work we show that the capacity of E_2 to promote eosinophilic infiltration of the cervix is blocked by the anti-E_2 Tx. The small but significant Tx-induced effect on eosinophil invasion was interpreted as an estrogenic activity of Tx at the given doses [20]. Taken together, these results show that eosinophilic infiltration is an estrogen-regulated event that seems to require an initial interaction with estrogen receptors.

Eosinophilic invasion found on D21, D22, and D23 in the cervical stroma of O-PSP E_2-treated rats, showed a direct correlation with increasing doses of E_2 (Fig. 1). This mechanism did not demonstrate saturation within the range of the doses utilized and the infiltration pattern differed from that observed in pregnant animals. In the latter, an acute eosinophilic invasion of the cervix was observed on D23, coincident with P declination [27]. The data presented here show that P antagonizes the effect of E_2 on eosinophilic infiltration. The animals treated with P + E_2 did not show significant infiltration, however, when these rats were injected with RU-486 the eosinophilic invasion was evident. The finding that RU-486 blocks the inhibitory action of P on eosinophilic infiltration of the cervix suggests that the P effect is mediated through the progestin receptor. These experiments confirm the negative modulation that P exerts on E_2-stimulated eosinphilic infiltration of rat cervical stroma [7,8]. The antagonistic actions of both steroids explains the time course of the leukocyte invasion in the intact pregnant rat during the last days of pregnancy (Fig. 1). When the P decrease takes place during the last 36 h of pregnancy [27], increased E_2 levels act through the E_2 receptor promoting the massive infiltration observed on D23 of pregnancy (Fig. 1).

The effect of estradiol on cervical eosinophilic invasion was blocked by ACT-D, an inhibitor of mRNA synthesis. These results strongly suggest that the E_2-stimulated eosinophilic infiltration could be protein-synthesis mediated. This finding is in agreement with the results found in the immature rat uterine horn [9]. These authors demonstrated the existence of an eosinophil chemotactic protein that mediates the E_2 stimulation of eosinophilic infiltration.

In summary, the physiological conditions used in our work permit an interesting approach to the real events that occur in the cervix of pregnant rats at term. Our results demonstrate that the eosinophilic infiltration of the uterine cervix at the time of parturition is under complex hormonal control. This physiological control has similarities with the results found in the uterine horns of immature rats, showing that in both regions (cervix and uterine horns) the estrogen-triggered eosinophilic infiltration is P inhibited and receptor-ligand and protein-synthesis mediated.

Acknowledgments

G.S.M. is Career Investigator of the Brazilian National Council for Scientific and Technological Development (CNPq) and E.H.L. is Career Investigator of the Argentine National Council for Science and Technology (CONICET). We are very grateful to SIDUS (Argentina) for providing actinomycin-D used in this study.

References

[1] El Maradny E, Kanayama N, Kobayashi H, et al. The role of hyaluronic acid as a mediator and regulator of cervical ripening. Hum Reprod 1997;12:1080–8.

[2] Kelly RW. Pregnancy maintenance and parturition: the role of prostaglandin in manipulating the immune and inflammatory response. Endocr Rev 1994;15:684–706.

[3] Junqueira LCU, Zugaib M, Montes GS, Toledo OMS, Kriszian RM, Shigihara KM. Morphologic and histochemical evidence for the occurrence of collagenolysis and for the role of neutrophilic polymorphonuclear leukocytes during cervical dilation. Am J Obstet Gynecol 1980;138:273–81.

[4] Luque EH, Montes GS. Progesterone promotes a massive infiltration of the rat uterine cervix by the eosinophilic polymorphonuclear leukocytes. Anat Rec 1989;223:257–65.

[5] Luque EH, Muñoz de Toro M, Ramos JG, Rodriguez HA, Sherwood OD. Role of relaxin and estrogen in the control of eosinophilic invasion and collagen remodeling in rat cervical tissue at term. Biol Reprod 1998;59:795–800.

[6] Leppert PC. Cervical softening, effacement, and dilatation: a complex biochemical cascade. J Matern Fetal Med 1992;1:213–23.

[7] Luque EH, Ramos J, Rodriguez H, Muñoz de Toro M. Dissociation in the control of cervical eosinophilic infiltration and collagenolysis at the end of pregnancy or after pseudopregnancy in ovariectomized steroid-treated rats. Biol Reprod 1996;55:1206–12.

[8] Duchesne MJ, Badia E. Immunohistochemical localization of the eosinophil major basic protein in the uterus horn and cervix of the rat at term and after parturition. Cell Tiss Res 1992;270:79–86.

[9] Lee YH, Howe RS, Sha SJ, Teuscher C, Sheehan DM, Lyttle R. Estrogen regulation of an eosinophil chemotactic factor in the immature rat uterus. Endocrinology 1989;125:3022–8.

[10] Huszar GB, Walsh MP. Relationship between myometrial and cervical functions in pregnancy and labor. Semin Perinatol 1991;15:97–181.

[11] Buhimschi I, Ali M, Jain V, Chwalisz K, Garfield RE. Differential regulation of nitric oxide in the rat uterus and cervix during pregnancy and labor. Hum Reprod 1996;11:1755–66.

[12] King WJ, Allen TC, DeSombre ER. Localization of uterine peroxidase activity in estrogen-treated rats. Biol Reprod 1981;25:859–70.

[13] Howe R, Lee Y, Fischkoff S, Teuscher C, Lyttle C. Glucocorticoid and progestin regulation of eosinophil chemotactic factor and complement C3 in the estrogen treated rat uterus. Endocrinology 1990; 126:3193–9.

[14] Hasty LA, Lyttle CR. Progesterone and RU486 regulation of uterine complement C3 after prior induction with estradiol. Biol Reprod 1992;47:285–90.

[15] Montes GS, Luque EH. Effects of ovarian steroids on vaginal smears in the rat. Acta Anat 1988;133:192–9.

[16] Luque EH, Castro–Vazquez AC. Sensory mechanisms involved in the induction of pseudopregnancy by progesterone: increased sensitivity to stimulation of the pudendal sensory field. Endocrinology 1983; 113:385–90.

[17] Smith MS, Freeman ME, Neill JD. The control of progesterone secretion during the estrous cycle and early pseudopregnancy in the rat: prolactin, gonadotropin, and steroid levels associated with rescue of the corpus luteum of pseudopregnancy. Endocrinology 1975;96: 219–26.

[18] Rothchild I. Role of progesterone in initiating and maintaining pregnancy. In: Bardin CW, Milgrom E, Mauvais–Jarvis P, editors. Progesterone and progestins. New York: Raven Press, 1983. pp. 219–29.

[19] Downing SJ, Sherwood OD. The physiological role of relaxin in the pregnant rat. IV. The influence of relaxin on cervical collagen and glycosaminoglycans. Endocrinology 1986;118:471–9.

[20] Fuhrmann U, Parczyk K, Klotzbücher M, Klocker H, Cato ACB. Recent developments in molecular action of antihormones. J Mol Med 1998;76:512–24.

[21] Gundersen HJG, Bendtsen TF, Korbo L, et al. Some new, simple and efficient stereological methods and their use in pathological research and diagnosis. APMIS 1988;96:379–94.

[22] Anderson JM. Histometry. In: Bancroft JD, Stevens A, editors. Theory and practice of histological techniques, 2nd ed. London: Churchill Livingston, 1982. pp. 428–57.

[23] Weibel ER. Stereological principles for morphometry in electron microscopic cytology. Int Rev Cytol 1969;26:235–302.

[24] Siegel S. Nonparametric statistics for the behavioral sciences. New York: McGraw–Hill, 1956.

[25] McRae A, Kennedy TG. Estrogen, progesterone, and the blood-uterine lumen permeability barrier in rats. Biol Reprod 1981;25: 314–8.

[26] Tchernitchin A. The role of eosinophil receptors in the non-genomic response to estrogen in the uterus. J Steroid Biochem 1979;11:417–24.

[27] Morishige WK, Pepe GJ, Rothchild I. Serum luteinizing hormone, prolactin, and progesterone levels during pregnancy in the rat. Endocrinology 1973;92:1527–30.