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Bovine Uterine, Cervical and Ovarian Androgen Receptor Concentrations

Correlation with Estrogen and Progesterone Receptor Concentrations

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Vesanen, M., V. Isomaa, M. Alanko and R. Vihko: Bovine uterine, cervical and ovarian androgen receptor concentrations. Correlation with estrogen and progesterone receptor concentrations. Acta vet. scand. 1992, 33, 379-386. – Bovine cytosol androgen receptor (ARC) concentrations were examined simultaneously in various regions of the uterus and in ovarian tissues of cows, and were related to cytosol estrogen (ERC) and progesterone receptor (PRC) concentrations and circulating steroid levels. ERC concentrations were 3-7-fold and PRC concentrations 13-29-fold those of ARC in bovine endometrial and myometrial tissues. When serum progesterone levels were low, both endometrial and myometrial ARC, endometrial ERC, and endometrial and myometrial PRC concentrations were higher ($p<0.05$) than those observed during higher progesterone concentrations. Because serum 5α-dihydrotestosterone (5α-DHT) concentrations were higher during the luteal phase, it is possible that ARC was down-regulated by this natural ligand at this phase of the cycle. There were no differences between uterine horns in endometrial or myometrial ARC concentrations. Bovine cervical and ovarian stromal tissue also contained ARC, and the concentrations were about the same as in the endometrium and the myometrium. The relative binding affinities (RBAs) of some steroid hormones towards ARC in vitro were: the synthetic compound R1881 (146%), 5α-dihydrotestosterone (100%), testosterone (75%) while estradiol-17ß, progesterone and dexamethasone had lower RBAs (2, <1, <1% respectively). Cytosol androgen receptor concentrations correlated significantly with cytosol progesterone (PRC) and estrogen receptor (ERC) concentrations, both in the endometrium and myometrium. These data show that androgens, such as 5α-DHT, may participate the endocrine regulation of bovine reproductive tissues.

estrogen receptor; progesterone receptor.

Introduction

Uterine steroid hormone receptors, especially those for progesterone and estrogen, have been objects of intensive study in the investigation of sex steroid hormone actions as reviewed by *Katzenellenbogen* 1980, *Leavitt et al.* 1983, *Clark et al.* 1985. Characterization of androgen receptor properties in vitro has been performed using human and animal uterine tissues (*Tamaya et al.* 1979, *Chang & Tindall* 1983, *de Boer et al.* 1986, *Muechler* 1987), but variations of androgen receptor concentrations in normal bovine reproductive organs have not been well established. *Büchi & Weber* (1983) studied alterations of rat uterine cytoplasmic androgen receptor concentrations during the estrous cycle and found higher contents at proestrus than at

metestrus. On the other hand, *Tamaya et al.* (1986) reported that androgen receptor sites did not change noticeably in human endometrium throughout the normal menstrual cycle.

In our previous studies (*Vesanen et al.* 1988, *Vesanen et al.* 1991), bovine endometrial cytoplasmic estrogen and progesterone receptor concentrations were shown to vary in relation to the phase of the normal estrous cycle. To clarify the sensitivity of bovine genitals to androgenic stimulation, cytosol androgen receptor (ARC) concentrations were measured in this study simultaneously with cytosol estrogen (ERC) and progesterone receptor (PRC) contents from various parts of uteri and ovaries. Such information is believed to be needed for a better understanding of endocrine associations in bovine reproductive endocrinology.

Material and methods

Animals and collection of samples

The animals were Finnish dairy cows delivered to slaughter-houses. Cows were examined by rectal palpation of the genitalia to exclude those which showed abnormalities. In the slaughter-houses, blood samples were collected from jugular veins without anticoagulant. Uteri and ovaries were removed after stunning and examined macroscopically, recording uterine and ovarian findings. Tissue specimens (wall of uterine horn, *portio vaginalis cervicis* and ovaries) were packed in aluminium foil and stored in liquid nitrogen within 15 min of stunning, for receptor analyses. Serum was separated by centrifugation from blood samples and stored at -20°C for steroid hormone assays.

Measurement of cytosol androgen, estrogen and progesterone receptor concentrations

In this paper, cytosol steroid hormone receptors (androgen receptor ARC, estrogen receptor ERC, progesterone receptor PRC) refer to those found in the cytosol fraction following homogenization of the tissue in buffer and ultracentrifugation of the homogenate.

Endometrium and myometrium were separated from frozen tissue samples using a scalpel. Endometrial samples were scraped from the inner surface of the uteri with a scalpel. Myometrial samples were cut from the middle stratum of the uterine wall. Macroscopically selected stromal tissue from ovari was taken, and corpora lutea, follicles with their walls and the ovarian capsule removed from these specimens.

Preparation of cytosol was carried out as described previously (*Vesanen et al.* 1988). Briefly, tissue pieces were weighed, minced and homogenized in TETMO 10 G buffer at 4°C. The homogenates were centrifuged and the supernatant saved for receptor assays and the measurement of protein concentration.

Cytosols for ARC assay were treated with dextran-charcoal pellets (*Isomaa et al.* 1987), which were prepared by centrifuging 2 ml dextran-charcoal suspension (2.5 g Norit A, 150 mg Dextran T-70, and 1 g gelatin per 1 liter of a buffer composed of 0.1 mol/l phosphate, 15 mmol/l NaN3, and 0.15 mol/l NaCl, pH 7.4) at 1500 × g for 10 min and decanting the supernatants. The samples were incubated at 4°C for 10 min with the charcoal, which was then removed by centrifugation (1500 × g for 10 min). Aliquots of cytosol were incubated with [^{3}H]methyltrienolone (0.3-10 nmol/l) with and without a 500-fold molar excess of testosterone at 4°C for 18 h. The synthetic progestin, ORG 2058 (500 nmol/l), a highly specific ligand to PRC (*Jänne et al.* 1976), was used to prevent the binding of the tracer to PRC. Bound and free steroids were

separated using hydroxyl apatite, as described previously (*Isomaa et al.* 1982). Cytosol ERC and PRC concentrations were measured using methods described previously (*Vesanen et al.* 1988). The intra-assay coefficients of variation were 5%, 2% and 4% for ARC, ERC and PRC determinations, respectively. The corresponding values for inter-assay coefficients of variation were 10%, 10% and 9%.

The concentrations of cytosol protein were measured according to *Bradford* (1976) using BIO-RAD protein assay. Bovine serum albumin was used as the standard. The method of *Scatchard* (1949) was used to calculate the binding data, corrected for non-specific binding, for all 3 receptors. The receptor concentrations are expressed as fmol per mg cytosol protein.

Measurement of relative binding affinities (RBAs)

Measurement of RBAs of various ligands towards ARC was performed using a constant molar concentration of the labeled tracer ([^{3}H]methyltrienolone (5 nmol/l)) with ORG 2058 (200 nmol/l) and various quantities of non-labeled competitors in receptor assay incubations. RBAs of the competitors were estimated from the amounts of ligands required to decrease the binding of the tracer by 50 %.

Measurement of serum steroid hormone concentrations

The serum concentrations of progesterone and 5α-dihydrotestosterone (5α-DHT) were measured by individual radioimmunoassays after solvent extraction and chromatographic

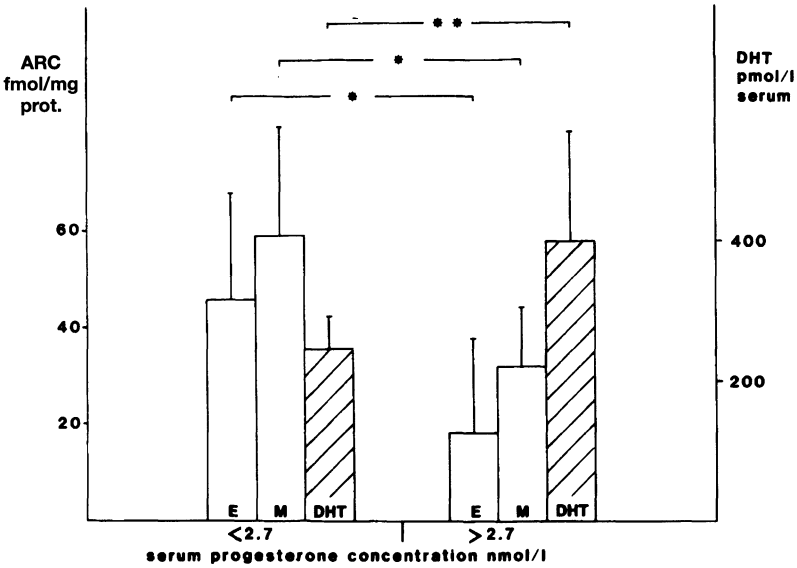

Figure 1. Bovine uterine cytosol androgen receptor (ARC) concentrations (mean±S.D.) in endometrium (E) and myometrium (M) of cows with low serum progesterone concentrations (<2.7 nmol/l) or during the production (>2.7 nmol/l) of endogenous progesterone as indicating the luteal phase (open columns). Serum 5α-dihydrotestosterone (DHT) concentrations are expressed (oblique-lined columns) respectively. Significances: *p<0.05, **p<0.01.

purification of samples as described previously (*Apter et al.* 1976, *Hammond et al.* 1977). Briefly, unconjugated steroids were extracted from serum and then fractionated on Lipidex-5000™ microcolumns (Packard-Becker B.V., Chemical Operations, Netherlands) followed by radioimmunoassay of each steroid from the appropriate fraction using antisera of defined specificity (*Jänne et al.* 1974, *Apter et al.* 1976). The intra-assay coefficient of variation and the inter-assay coefficient of variation were less than 10% and less than 15%, respectively (*Apter et al.* 1976).

Other methods

Statistical comparisons of the results were performed by one-way analysis of variance and t-statistics by the Bonferroni method (*Wallenstein et al.* 1980). Linear regression analysis was used to evaluate the correlations.

Results

The animals were divided into 2 groups, based on serum progesterone concentrations. Group 1 (follicular phase) consisted of animals having serum progesterone concentrations below 2.7 nmol/l, which corresponds to a progesterone value of 10 nmol/l in residual milk, the limit used in our previous study (*Vesanen et al.* 1988). The second group was composed of cows with serum progesterone concentrations above 2.7 nmol/l indicating progesterone production (luteal phase) by the corpus luteum. Serum 5α-dihydrotestosterone concentrations were higher in the luteal phase than in the follicular phase ($p<0.01$) (Fig. 1). This may also indicate 5α-dihydrotestosterone production by the cor-

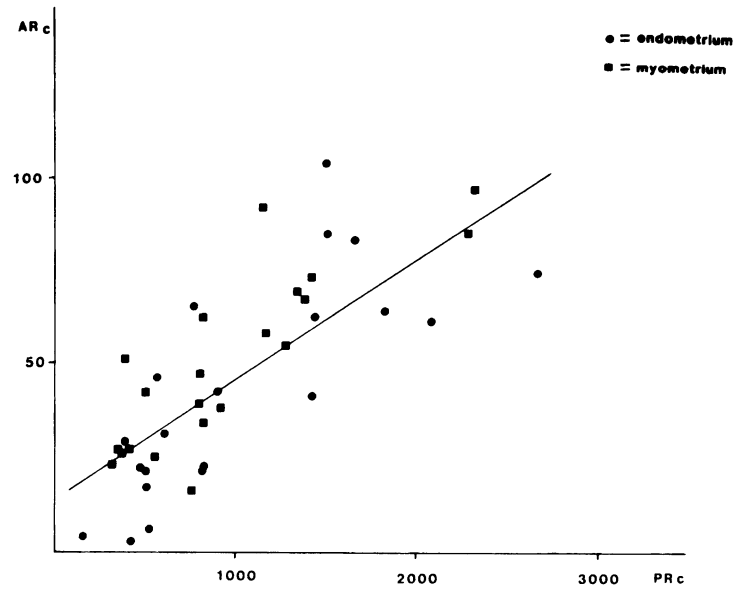

Figure 2. Comparison of bovine uterine cytosol androgen receptor (ARC) and progesterone receptor (PRC) concentrations (fmol/mg protein) in the endometrium (circles) and myometrium (squares) of individual animals.

Table 1. Simultaneous ARC, PRC and ERC concentrations (fmol/mg cytosol protein; mean±S.D.) in bovine endometrium and myometrium.

		Group 1	Group 2
Serum progesterone concentration (nmol/l)		<2.7	>2.7
Number of cows		n=12	n=4
ARC	Endometrium	46±22[a]	18±19[a]
	Myometrium	59±22[b]	32±13b
PRC	Endometrium	1319±682[c]	443±181[c]
	Myometrium	1237±574[d]	413±93[d]
ERC	Endometrium	316±136[e]	116±109[e]
	Myometrium	208±105	104±27

Significances: [a, b, c, d, e] $p<0.05$
ARC = Cytosol androgen receptor
PRC = Cytosol progesterone receptor
ERC = Cytosol estrogen receptor

pus luteum as already noted in our previous study (*Vesanen et al.* 1990). During follicular phase, when serum 5α-DHT concentrations were low, ARC contents in both endometrium (46±22 fmol/mg cytosol protein) and in myometrium (59±22, n=12) were higher ($p<0.05$) than those observed during the luteal phase (in endometrium 18±19, and in myometrium 32±13, n=4) (Fig. 1).

Table 2. Bovine ARC concentrations (fmol/mg cytosol protein; mean±S.D.) in the endometrium and myometrium of the uterine horns (ipsi- and contralateral) of individual animals.

Uterine horns		Ipsilateral n=5	Contralateral n=5
ARC	Endometrium	45±40	35±22
	Myometrium	43±28	47±14

The ipsilateral horn is defined as that on the ovarian side with a dominant follicle in cows with low (<2.7 nmol/l) and as that on the ovarian side with a corpus luteum in cows with elevated (>2.7 nmol/l) serum progesterone concentrations and thus the contralateral horn was that on the reverse side.

Table 3. Relative binding affinities (RBAs) of various ligands to bovine myometrial cytosol androgen receptor.

Ligand	RBA(%)
5α-Dihydrotestosterone	100
Methyltrienolone (R1881)	146
Testosterone	75
Estradiol-17ß	2
Progesterone	<1
Dexamethasone	<1

Simultaneous measurements of PRC and ERC with ARC from the same bovine endometrial and myometrial tissue specimens showed that both PRC and ERC concentrations were much higher than those of ARC and they were related to circulating progesterone contents (Table 1). In addition, a clear correlation was observed between ARC and PRC ($p<0.001$) (Fig. 2) as well as between ARC and ERC concentrations ($p<0.001$) in bovine uterus. No differences were observed in ARC concentrations in endometrium or in myometrium of the 2 uterine horns. The ipsilateral horn was defined as that on the side with a dominant ovarian follicle in the low progesterone group and as that on the side with a corpus luteum in the cows producing progesterone (>2.7 nmol/l) (Table 2).

Bovine cervical and ovarian ARC concentrations were similar to those observed in endometrium and myometrium. ARC concentrations in the cervix were 59±15 fmol/mg cytosol protein (n=4) and in ovaries 32±19 (n=13).

The relative binding affinities (RBAs) of various ligands to bovine myometrial ARC were measured to characterize the binding proper-

ties of the receptor. The synthetic compound, methyltrienolone (R1881), showed the highest RBA, followed by 5α-DHT and testosterone. Binding of estradiol-17ß, progesterone and dexamethasone to androgen receptor was minimal (Table 3).

Discussion

Together with elevated serum progesterone concentrations, 5α-DHT concentrations increase in bovine (*Vesanen et al.* 1990) and in human (*Apter et al.* 1976) sera during the luteal phase of the cycle. In this study, variations of bovine serum progesterone and 5α-DHT concentrations between luteal and follicular phases are distinct, indicating the great ability of the ovarian corpus luteum to produce steroid hormones.

Increased circulating 5α-DHT concentrations were associated with decreased ARC concentrations in bovine uterine endometrium and myometrium. Previously, similar receptor down-regulation has been observed in the transmission of intra-cellular progesterone effects in other mammalian species (*Walters & Clark* 1987, *Chen & Leavitt* 1979, *Isomaa et al.* 1979). Thus, target tissue (bovine uterus) can control the transmission of androgen action by regulating receptor concentrations in target cells. Except for the ligand, 5α-DHT, progesterone would be responsible for the down-regulation of ARC in analogy with down-regulation of the ERC (for review, see *Leavitt et al.* 1983).

Tamaya et al. (1979) compared estrogen, progesterone and androgen receptor concentrations in human myoma and myometrium and found no differences in receptor contents between these tissues. According to *Muechler* (1987), human myometrial tissue samples were not different in androgen binding properties from endometrial specimens. In our study, ARC concentrations were very close to each other in bovine endometrium and myometrium and no significant differences were observed in ARC contents between the uterine horns. This observation indicates that androgens transported in the systemic blood circulation are bound by the androgen receptor and the presence of a corpus luteum or dominant follicle had no local effect on ARC concentrations and thereby on the androgenic sensitivity of target tissues.

Prodi et al. (1978) observed significant relationships in the occurrence of estrogen versus progesterone receptor, estrogen versus 5α-DHT receptor, and 5α-DHT versus progesterone receptor in normal and pathological human uterine tissues. Our data are in harmony with these observations, indicating similarity in bovine and human endocrine functions in relation to steroid hormone receptor concentrations in target tissues.

The relative binding affinities (RBAs) of various steroidal ligands to mammalian ARC differ to some degree according to the tissue source of receptor. Methyltrienolone (R1881) has a higher affinity to ARC than the endogenous androgens 5α-DHT and testosterone, in human (*Ekman et al.* 1982) and rat prostatic cytosol (*Bonne & Raynaud* 1976, *Gaubert et al.* 1980), in porcine *Leydig* cells (*Isomaa et al.* 1987) and in calf uterus (*de Boer et al.* 1986). 5α-DHT bound with higher affinity to ARC than did testosterone in bovine and human uterine tissue (*de Boer et al.* 1986, *Muechler* 1987), in rat (*Gaubert et al.* 1980) and human prostatic cytosol (*Ekman et al.* 1982) but not in porcine *Leydig* cells (*Isomaa et al.* 1987). The binding properties of estradiol, progesterone and dexamethasone to ARC have been reported to be distinctly weaker than those of 5α-DHT and testosterone in various mammalian tissues (*Bonne & Raynaud* 1976, *Tamaya et al.* 1979, *Ekman et al.* 1982, *Isomaa et al.*

1987, *Muechler* 1987). In our study, RBAs to bovine uterine ARC are similar to those reported above in various mammalian tissues.

In conclusion, ARC concentrations in various parts of the bovine uterus changed according to the phase of the estrous cycle, indicating the role of circulating steroid hormones in the regulation of ARC concentrations. The changes in the concentrations of this receptor at the follicular and luteal phases of the cycle were identical to those of estrogen and progesterone receptors.

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References

Apter D, Jänne O, Karvonen P, Vihko R: Simultaneous determination of five sex hormones in human serum by radioimmunoassay after chromatography on Lipidex-5000. Clin. Chem. 1976, *22,* 32-38.

de Boer W, Lindh M, Bolt J, Brinkmann A, Mulder E: Characterization of the calf uterine androgen receptor and its activation to the deoxyribonucleic acid-binding state. Endocrinology 1986, *118,* 851-861.

Bonne C, Raynaud J-P: Assay of androgen binding sites by exchange with methyltrienolone (R1881). Steroids 1976, *27,* 497-507.

Bradford MM: A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 1976, *72,* 248-254.

Büchi KA, Weber E: Variation of cytoplasmic rat uterine androgen receptors. Moll. Cell. Endocrinol. 1983, *29,* 295-307.

Chen TJ, Leavitt WW: Nuclear progesterone receptor in hamster uterus. Measurement by [^{3}H]progesterone exchange during the estrous cycle. Endocrinology 1979, *104,* 1588-1597.

Chang CH, Tindall DJ: Physicochemical characterization of the androgen receptor in rat uterine cytosol. Endocrinology 1983, *113,* 1486-1493.

Clark J, Schrader W, O`Malley B: Mechanisms of steroid hormone action. In: Wilson J, Fortec D (eds): Textbook of Endocrinology. WB Saunders Company, Philadelphia 1985, pp. 33-75.

Ekman P, Barrack E, Walsh P: Simultaneous measurement of progesterone and androgen receptors in human prostate: A microassay. J. clin. Endocr Metab. 1982, *55,* 1089-1099.

Gaubert CM, Tremblay RR, Dube` JY: Effect of sodium molybdate on cytosolic androgen receptors in rat prostate. J. Steroid Biochem. 1980, *13,* 931-937.

Hammond G, Ruokonen A, Kontturi M, Koskela E, Vihko R: The simultaneous radioimmunoassay of seven steroids in human spermatic and peripheral venous blood. J. clin. Endocr. Metab. 1977, *45,* 16-24.

Isomaa V, Isotalo H, Orava M, Jänne O: Regulation of cytosol and nuclear progesterone receptors in rabbit uterus by estrogen, anti-estrogen and progesterone administration. Biochim. Biophys. Acta 1979, *585,* 24-33.

Isomaa V, Pajunen A, Bardin C, Jänne O: Nuclear androgen receptors in the mouse kidney: Validation of a new method. Endocrinology 1982, *111,* 833-843.

Isomaa V, Orava M, Vihko R: Evidence for an androgen receptor in porcine Leydig cells. Acta Endocrinol. 1987, *115,* 119-124.

Jänne O, Apter D, Vihko R: Assay of testosterone, progesterone and 17α-hydroxyprogesterone in human plasma by radioimmunoassay after separation on hydroxyalkoxypropyl Sephadex. J. Steroid Biochem. 1974, *5,* 155-162.

Jänne O, Kontula K, Vihko R: Progestin receptors in human tissues: concentrations and binding kinetics. J. Steroid Biochem. 1976, *7,* 1061-1068.

Katzenellenbogen B: Dynamics of steroid hormone action. Ann. Rev. Physiol. 1980, *42,* 17-35.

Leavitt W, MacDonald R, Okulicz W: Hormonal regulation of estrogen and progesterone receptor systems. In: Litwack G (ed): Biochemical Action of Hormones. Academic Press, New York 1983, pp. 32-356.

Muechler E: The androgen receptor of human endometrium. Endocr. Res. 1987, *13,* 69-84.

Prodi G, de Giovanni C, Galli M, Gola G, Grilli S, Rocchetta R, Orlandi C: 17ß-estradiol, 5α-dihydrotestosterone, progesterone and cortisol

receptors in normal and neoplastic human endometrium. Tumori 1979, *65*, 241-253.

Scatchard G: The attractions of proteins for small molecules and ions. Ann. N.Y. Acad. Sci. 1949, *51*, 660-672.

Tamaya T, Motoyama T, Ohono Y, Ide N, Tsurusaki T, Okada H: Estradiol-17ß, progesterone and 5α-dihydrotestosterone receptors of uterine myometrium and myoma in the human subject. J. Steroid Biochem. 1979, *10*, 615-622.

Tamaya T, Murakami T, Okada H: Concentrations of steroidreceptors in normal human endometrium in relation to the dayof the menstrual cycle. Acta Obstet. Gynecol. Scand. 1986, *65*, 195-198.

Walters M, Clark J: Cytosol and nuclear compartmentalization of progesterone receptor of the rat uterus. Endocrinology 1978, *103*, 601-609.

Wallenstein S, Zucker CL, Fleiss JL: Some statistical methods useful in circulation research. Circ. Res. 1980, *47*, 1-9.

Vesanen M, Isomaa V, Alanko M, Vihko R: Cytosol estrogen and progesterone receptors in bovine endometrium after uterine involution postpartum and in the estrous cycle. Anim. Reprod. Sci. 1988, *17*, 9-20.

Vesanen M, Isomaa V, Bolton N, Alanko M, Vihko R: Bovine steroid hormone and SHBG concentrations postpartum and during the oestrous cycle. Acta vet. scand. 1990, *31*, 459-569.

Vesanen M, Isomaa V, Alanko M, Vihko R: Bovine uterine, cervical and ovarian estrogen and progesterone receptor concentrations. Anim. Reprod. Sci. 1991, *26*, 61-71.

Sammanfattning

Koncentrationen av androgen receptorer i livmodern, livmoderhalsen och ovarierna hos kor

Koncentrationerna av cytoplasmiska androgen receptorer (ARC) i kornas livmoder och ovarier studerades samtidigt med estrogen (ERC) och progesteronreceptorer (PRC) och jämfördes med steroid hormoner i blodserum. Koncentrationerna av ERC var 3-7 och PRC 13-29 gånger högre än motsvarande ARC koncentrationer både i endometrium och myometrium. Koncentrationerna av cytoplasmiska ARC i de undersökta kornas livmödrer varierade enligt brunstcykeln. Då progesteronhalten i blodserum var låg, var även 5α-dihydrotestosteronhalten (5α-DHT) låg - livmoderns ARC-koncentrationer i endometrium och myometrium var däremot på en högre nivå. Också ERC koncentrationerna i endometrium och PRC både i endometrium och myometrium var högre under denna period. Vid höjda serum progesteron och 5α-DHT koncentrationer minskade emellertid livmoderns ARC-halt samtidigt med ERC och PRC koncentrationerna. Antagligen berodde minskningen av ARC på att höjda 5α-DHT-koncentrationer verkade hämmande på ARC-nivån. ARC koncentrationerna i olika delar av livmodern befanns vara på samma nivå. En positiv korrelation mellan mängderna av androgen-, progesteron- och estrogenreceptorer i livmodern kunde påvisas. Syntetisk metyltrienolon (R1881) hade en högre och testosteron en mindre affinitet till ARC än 5α-DHT in vitro. Estradiol-17ß, progesteron och dexametason hade endast en minimal affinitet till ARC.

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