J. Steroid Biochem. Molec. Biol. Vol. 37, No. 4, pp. 509–511, 1990
Printed in Great Britain.

0960-0760/90 $3.00 + 0.00

ENZYME IMMUNOASSAY OF OESTROGEN AND PROGESTERONE RECEPTORS IN UTERINE AND INTRAUTERINE TISSUE DURING HUMAN PREGNANCY AND LABOUR

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(Received 27 March 1990)

Summary—Oestrogen and progesterone receptors were studied in the non-pregnant state, in early pregnancy and at term using monoclonal antibody enzyme immunoassays. Receptors for both steroids were found in tissues from non-pregnant patients and patients in early pregnancy. At term oestrogen receptors were undetectable in all tissues studied. Progesterone receptors were undetectable in chorion, amnion and placenta at term, while present in extremely low concentrations in decidua and myometrium.

INTRODUCTION

It has been reported previously that the levels of oestrogen receptors (ER) and progesterone receptors (PR) in human uterine and intra-uterine tissues decrease with the advance of gestation and are undetectable at term [1, 2]. In those reports results were obtained either by multipoint titration analysis of both cytosolic and nuclear fractions or by sucrose density gradient analysis. However, both methods involve binding of a radiolabelled ligand to the steroid binding site on the receptor and detection of the receptor may be influenced by the increased levels of endogenous steroids during pregnancy.

The development of monoclonal antibodies to steroid receptor proteins presents an opportunity to study these receptors regardless of the state of occupancy [3]. The aim of this study, therefore, was to re-examine uterine and intrauterine tissues obtained during human pregnancy for the presence of ER and PR using monoclonal antibodies in an enzyme immunoassay (EIA) to establish whether the previously observed decrease and disappearance of receptors during pregnancy is a real phenomenon or merely reflects a methodological inadequacy.

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MATERIALS AND METHODS

Materials

Informed consent for the removal of tissue was obtained from all patients in the study. Tissue was obtained from 3 groups of patients:

(i) Endometrial and myometrial samples were collected during hysterectomy from non-pregnant patients during the luteal phase of the menstrual cycle ($n = 4$);
(ii) Decidual tissue was collected by curettage from patients with tubal pregnancies ($n = 4$) with amenorrhoea ranging between 6 and 8 weeks;
(iii) Amnion, chorion, placenta, decidua and myometrium were collected at elective Caesarian section ($n = 7$) and following emergency Caesarian section ($n = 6$) in term pregnant patients.

All samples were transported on ice in 0.15 mol/l NaCl to the laboratory, gently washed with cold saline, dried and stored in liquid nitrogen. Portions of decidual tissue were examined histologically to confirm the nature of the samples.

Assays

ER and PR were measured by Abbott ER-EIA Monoclonal and Abbott PgR-EIA Monoclonal kits, respectively. The assays were performed according to the manufacturer's

Instructions; the control values were within the specified range and the intra- and interassay variations were below 10%.

Statistics

Significance of differences between the concentrations of receptors measured during the various stages of gestation was assessed by the Mann–Whitney U-test; differences were considered significant when $P < 0.05$.

RESULTS

Changes in ER concentrations with the advance of pregnancy are presented in Table 1. In non-pregnant patients, high concentrations of ER were found in both the endometrium and myometrium; receptor levels decreased significantly in the decidua during the first trimester of pregnancy and were undetectable in all tissues studied at term.

High levels of PR were also demonstrated in the myometrium and endometrium from non-pregnant patients and in decidua obtained during the first trimester of pregnancy (Table 2). At term, PR were either undetectable or found in extremely low concentrations in the samples assayed. Pre-labour PR levels at term in the myometrium and decidua (11.9 ± 2.0 and 13.7 ± 2.4 fmol/mg protein, respectively), were not significantly different to those found after the onset of labour (11.9 ± 1.5 and 10.1 ± 2.6 fmol/mg protein, respectively).

Table 1. Oestrogen receptor concentrations (fmol/mg protein) in tissues from non-pregnant and pregnant patients

Non-pregnant		Ectopic	Term
E	M	D	ACPDM
>322	329	70	
309	133	27	
137	122	30	Undetectable
120	97	22	

E = endometrium; M = myometrium; D = decidua; A = amnion; C = chorion; P = placenta.
Non-pregnant vs ecotopic and term, $P = 0.014$.
Ectopic vs term, $P = 0.014$.

Table 2. Progesterone receptor concentrations (fmol/mg protein) in tissues from non-pregnant and pregnant patients

Non-pregnant		Ectopic	Term
E	M	D	ACP
218	220	139	
146	170	127	
88	52	18	Undetectable
84	48	14	

E = endometrium; M = myometrium; D = decidua; A = amnion; C = chorion; P = placenta.
Non-pregnant vs ectopic, $P = 0.171$.
Non-pregnant and ectopic vs term, $P = 0.014$.

DISCUSSION

The current study is the only report on the use of monoclonal antibodies in an EIA for the measurement of ER and PR during pregnancy. The technique has been well validated in tissue from non-gravid patients with good correlations reported between EIAs and titration analyses [4, 5], although EIAs tend to yield higher results [6]. The monoclonal antibodies to ER and PR used in these assays are directed towards epitopes away from the DNA and steroid binding regions of the receptor protein and therefore detect both proteins that bind ligand (biologically active) and those that are unable to bind ligand (biologically inactive). The advantage of the use of monoclonal antibodies is that they do not compete for binding with endogenous steroids [3] and thus allow reliable detection of receptor proteins in a high steroid milieu such as pregnancy. The controls and standards used in the kits do not yield any binding on titration analysis, confirming that the two methods identify different components of the receptor molecule.

With respect to ER, the present study supports the previous reports of decreases in ER with the advance of human gestation. Similarly the enzyme immunoassay of PR has also confirmed the presence of receptors in non-pregnant and early pregnancy tissue, while at term these receptors were absent in the amnion, chorion and placenta, and either absent or present in very low concentrations in the decidua and myometrium. The significance, if any, of these extremely low levels of PR as measured by EIA in normal tissue at term pregnancy is unknown but most likely represents biologically inactive remnants of the receptor molecule.

The current study using a method independent of steroid occupancy confirms earlier observations, based on ligand binding techniques, of the gradual disappearance of steroid receptors during pregnancy. Precautions taken in the previous studies [1, 2] to deal with the elevated hormone levels, included removal by dextran-coated charcoal of free steroids in the cytosol and nuclear receptor assay incubation conditions which allowed exchange between unlabelled and labelled steroid in intact nuclei [7]. The favourable comparison of the results obtained by the two techniques is, therefore, not unexpected. Clearly these results reflect a real loss of steroid receptors, biologically active and inactive with the advance of pregnancy and

argue against a receptor mediated role for either oestrogen or progesterone in the initiation of human parturition.

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