Netherlands Journal of Agricultural Science 42-3 (1994) 217-223

Oestrogen receptors in endometrial cytosol of gilts on days 10–13 of oestrous cycle and pregnancy

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Received 18 August 1993; accepted 12 March 1994

Abstract

Cytosolic oestrogen receptors (ER_c) were determined with a Dextran-coated charcoal assay and Scatchard plot analysis in endometrial tissue of non-pregnant and pregnant gilts on Days 10–13 after standing oestrus. The Kd of the ER_c was 0.40 ± 0.04 nM (mean ± SEM) and was not affected by day or reproductive state. The endometrial ER_c concentration was affected by day and reproductive state ($P<0.001$). During pregnancy, the endometrial ER_c concentration declined from Day 10 (48.1 ± 14.1 fmol/mg cytosol protein) to 13 (9.7 ± 2.0 fmol mg^{-1} cytosol protein), but did not change in non-pregnant gilts. On Day 12 of pregnancy, the endometrial ER_c concentration was significantly greater ($P<0.01$) in gilts with spherical blastocysts (30.2 ± 5.4 fmol mg^{-1} cytosol protein) than in those gilts with filamentous blastocysts (9.8 ± 2.6 fmol mg^{-1} cytosol protein).

Keywords: oestrogen receptor, endometrium, gilt, oestrus cycle, pregnancy

Introduction

In the pig oestrogens from blastocyst origin are supposed to be responsible for the maternal recognition of pregnancy. The oestrogens alter the direction of $PGF_{2\alpha}$ secretion by the uterine endometrium, and in this way $PGF_{2\alpha}$ is prevented to enter the uterine vasculature and to exert its luteolytic effect on the corpora lutea (Bazer & Thatcher, 1977). In vitro oestrogen synthesis is started by spherical blastocysts on Day 11 (Perry et al., 1976; Fischer et al., 1985; Mondschein et al., 1985), and in vivo prolongation of corpus luteum function is achieved from Day 12 by filamentous and spherical blastocysts ≥8 mm (Van der Meulen et al., 1988).

Oestradiol and progesterone are important in regulating oestrogen receptor concentration in uterine endometrium (Deaver & Guthrie, 1980). During the period of the maternal recognition of pregnancy, oestrogen released by blastocysts within the uterine lumen may interact with the endometrial oestrogen receptor and thereby mediate the anti-luteolytic effect (Deaver & Guthrie, 1980).

So far, no study has focused on endometrial oestrogen receptor concentrations around the time of maternal recognition of pregnancy, and the data available are partly contradictory (Pack et al., 1978; Deaver & Guthrie, 1980; Rexroad & Guthrie, 1984). In this study oestrogen receptor concentrations in endometrial cytosol of non-pregnant and pregnant gilts were measured on Days 10–13 when a first signal for corpus luteum (CL) maintenance is generated.

Materials and methods

Animals and sample collection

Sixty-nine crossbred gilts (Great Yorkshire x Dutch Landrace) which had shown 2 or more consecutive normal oestrous cycles (18–22 days) were used. The gilts were checked for oestrus with a vasectomized boar twice daily (09:00 and 15:00 h). The day of observing first standing oestrus was designated Day 0. Forty-one gilts were artificially inseminated on Day 1, 24 h after the onset of standing oestrus. The gilts were slaughtered on Day 10 (N = 8), 11 (N = 6), 12 (N = 7) and 13 (N = 7) of cycle and Day 10 (N = 9), 11 (N = 11), 12 (N = 9) and 13 (N = 12) of pregnancy. The uterus was removed immediately after stunning and exsanguination. Uterine horns of the pregnant gilts were flushed twice with 30 ml Dulbecco's phosphate-buffered saline (Gibco, Paisley, UK) and size of the recovered blastocysts was measured. Endometrium was dissected from the myometrium at the mesometrial side in the middle of the left uterine horn. The endometrial tissue was frozen in dry ice and stored until processing.

Receptor assay

All procedures were performed at 0–4 °C. Endometrial tissue (500 mg) was minced in small pieces and pulverized with a microdismembrator (Braun, Melsungen, FRG). The tissue powder was extracted with 0.01 M phosphate buffer, (pH 7.5), containing ethylendiaminetetraacetate (EDTA, 1 mM: Merck, Darmstadt, FRG). Cytosol was produced by centrifugation of the suspension for 1 h at 85000 g. Aliquots of the (250 µl) cytosol were incubated overnight with 100 µl of one of 6 dilutions of [2,4,6,7-^{3}H]oestradiol (specific activity 3.44 TBq/mmol: Amersham International, Amersham, UK), resulting in final [^{3}H]oestradiol concentration of 0.3–2.0 nM. Competition was studied with 100-fold molar excess of non-radioactive diethylstilbestrol (DES: Sigma, St. Louis, MO, USA) in parallel series of the two highest [3H]oestradiol concentrations. Receptor-bound and unbound steroids were separated by centrifugation (10 min, 2000 g) after incubation for 10 min with a suspension of Dextran-coated charcoal (5% Norit A and 0.5% Dextran T-70: Pharmacia, Uppsala, Sweden) in buffer. An aliquot of the supernatant (750 µl) was added to 3 ml scintillation cocktail (Maxifluor: Baker, Deventer, The Netherlands) and radioactivity was determined by liquid scintillation counting (1900 CA Tri-Carb Liquid Scintillation Analyzer: Packard, Downers Grove, IL, USA). A control sample was used in every

measurement to expose possible measurement errors; the inter-assay coefficient of variation was 11.3%. The concentration of cytosolic protein was measured according to the method described by Bradford (1976), using bovine serum albumin as a standard. The dissociation constant (Kd) and binding data were calculated by Scatchard plot analysis (Scatchard, 1949) after correction for non-specific binding according to Chamness & McGuire (1975). The cytosolic oestrogen receptor (ER_c) concentration was expressed as fmol/mg cytosol protein.

Statistical analysis

After ln transformation of the data, differences in endometrial ER_c concentrations and Kd were tested for significance by analysis of variance and differences in endometrial ER_c concentration between gilts with spherical and gilts with filamentous blastocysts were tested for significance by Student's *t* test (SPPS Inc, 1988). Data were expressed as mean ± SEM.

Results

Scatchard plot analysis indicated the presence of high-affinity cytosolic oestrogen receptors (ER_c) in endometrial tissue of both non-pregnant and pregnant gilts on Days 10–13. Some Scatchard plots, representative for those obtained during Days 10–13 of cycle and pregnancy are shown in Figure 1. The Kd of the endometrial ER_c was 0.40 ± 0.04 nM (n = 69) and was not significantly affected by day or reproductive state.

Endometrial ER_c concentration, however, was significantly affected by day and reproductive state ($P<0.001$). In non-pregnant gilts endometrial ER_c concentration was not significantly different between Days 10–13 (Table 1). During pregnancy endometrial ER_c concentration declined from Day 10 (48.1 fmol mg^{-1} cytosol protein) to Day 13 (9.7 fmol mg^{-1} cytosol protein; Table 1). On Day 12 of pregnancy spherical blastocysts were recovered in 5 gilts and filamentous blastocysts were recovered in 4 gilts. On this day endometrial ER_c concentration was significantly greater ($P<0.01$) in gilts with spherical blastocysts (30.2 ± 5.4 fmol mg^{-1} cytosol protein; n = 5) compared to gilts with filamentous blastocysts (9.8 ± 2.6 fmol mg^{-1} cytosol

Table 1. Endometrial ER_c concentration (fmol mg^{-1} cytosol protein) in pregnant and non-pregnant gilts on Days 10 to 13 after standing oestrus.

Day	Non-pregnant	Pregnant
10	54.1 ± 12.1	48.1 ± 14.1[a]
11	35.5 ± 8.3	41.3 ± 8.9[ab]
12	40.6 ± 9.8	21.1 ± 4.7[b]
13	40.9 ± 6.0	9.7 ± 2.0[c]

Column means without a common superscript differ significantly ($P<0.05$).

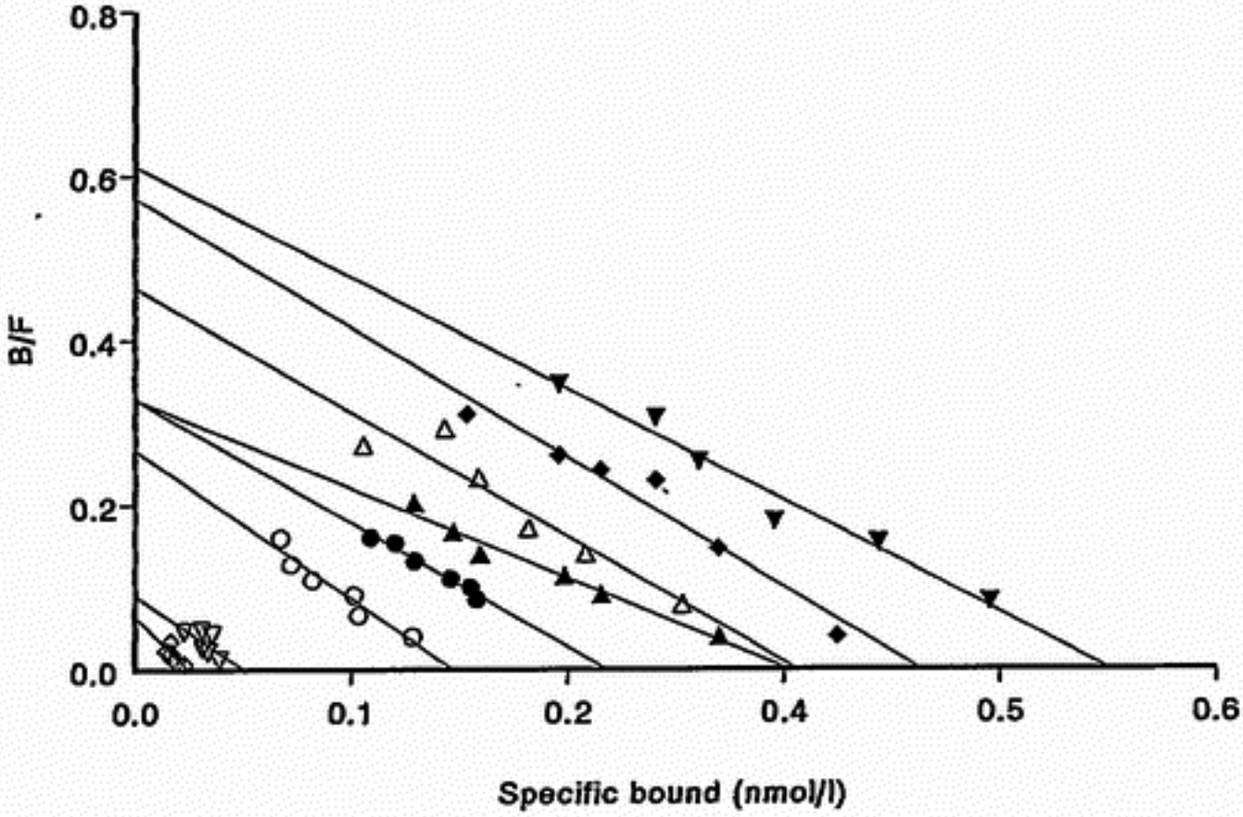

Fig. 1. Representative scatchard plots of the binding of [3H]oestradiol to endometrial cytosol of non-pregnant gilts on Days 10 (▲), 11 (●), 12 (▼) and 13 (◆) and pregnant gilts 10 (△), 11 (○), 12 (▽) and 13 (◇) days after standing oestrus.

protein; n = 4; Fig. 2). On Day 13 of pregnancy filamentous blastocysts were recovered in all gilts and endometrial ER_c concentration was 9.7 ± 2.0 fmol mg^{-1} cytosol protein.

Discussion

The high-affinity cytosolic non-steroid-bound oestrogen receptors (ER_c) with a Kd of on average 0.40 ± 0.04 nM measurted in endometrial tissue of both non-pregnant and pregnant gilts on Days 10–13, are comparable to those earlier reported for pig endometrium (Pack et al., 1978; Deaver & Guthrie, 1980; Rexroad & Guthrie, 1984; Koziorowski et al., 1984). In addition to these high-affinity oestrogen receptors also low-affinity high-capacity oestradiol binding sites may be present in endometrial cytosols of pigs (Tolton et al., 1985).

In most studies endometrial ER_c concentrations in non-pregnant gilts were higher in the luteal phase than in the early follicular phase (Deaver & Guthrie, 1980; Rexroad & Guthrie, 1984; Koziorowski et al., 1984), although in 2 studies no differences were found (Pack et al., 1978; Diekman & Anderson, 1979). In non-pregnant gilts endometrial ER_c concentrations have been reported on Day 12 which had not changed (Pack et al., 1978), which were not significantly lower (Deaver & Guthrie,

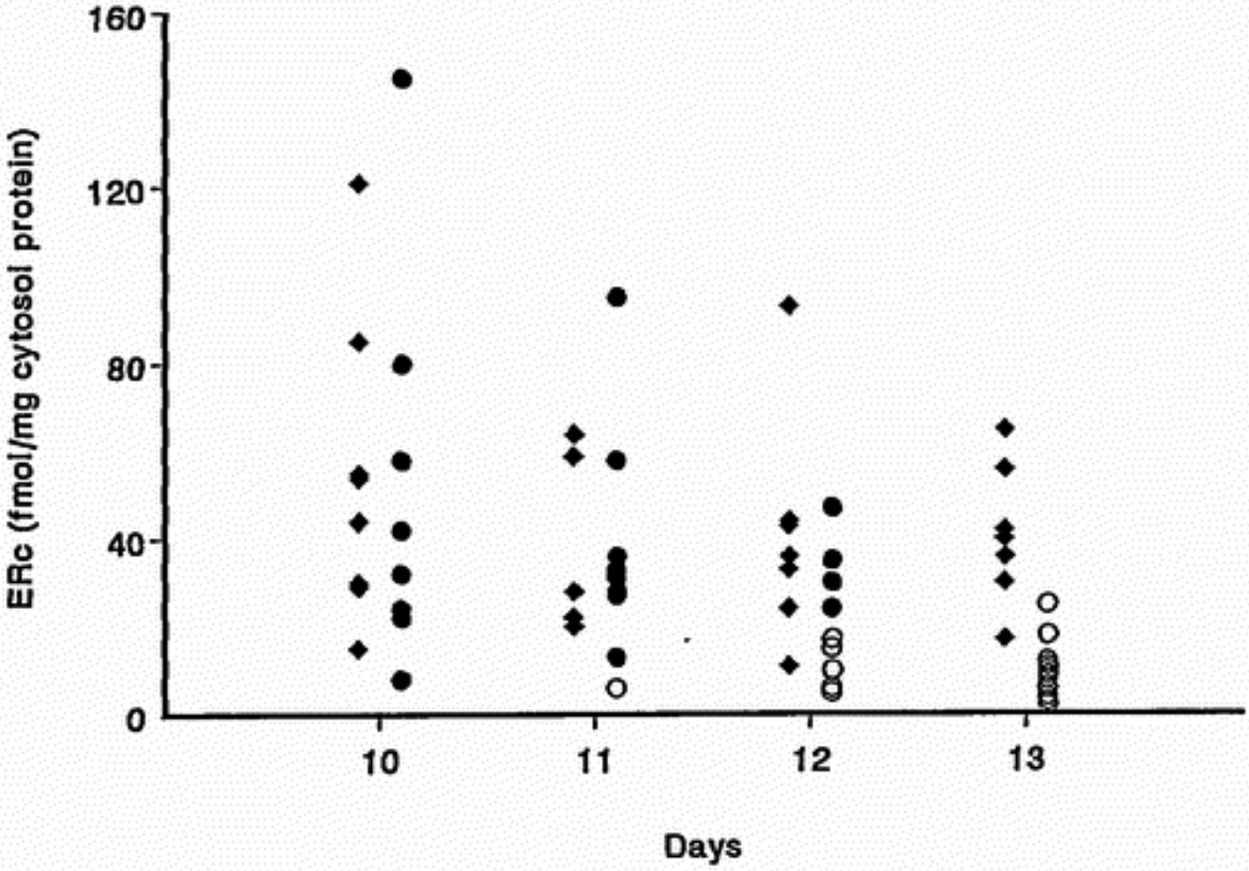

Fig. 2. Endometrial ER_c concentration on Days 10–13 in non-pregnant (◆) and pregnant gilts with spherical (●) and filamentous (○) blastocysts.

1980) or which were significantly lower than on Day 10 (Rexroad & Guthrie, 1984). Part of these differences may be explained by the fact that in the studies of Deaver & Guthrie (1980) and Rexroad & Guthrie (1984) gilts of different breeds had been used. Since differences may exist in endometrial ER_c concentrations of non-pregnant gilts and non-pregnant sows of the same breed (Koziorowski et al., 1984), differences between gilts of different breeds may also exist. In our study endometrial ER_c concentrations in non-pregnant gilts of the same breed measured on subsequent days, did not change from Days 10 to 13, which is in agreement with the data of Pack et al. (1978) for Days 10 and 12.

During pregnancy on Day 12 lower (Rexroad & Guthrie, 1984) or similar (Deaver & Guthrie, 1980) endometrial ER_c concentrations compared with Day 10 have been reported. In both studies endometrial ER_c concentrations on Days 16 and 19 were lower than on Day 10 or 12 (Deaver & Guthrie, 1980; Rexroad & Guthrie; 1984). In our study endometrial ER_c concentrations declined significantly from Days 10 to 13 of pregnancy and were significantly affected by the development stage of the blastocysts.

At the time oestrogen synthesis by spherical blastocysts starts (Day 11; Fischer et al., 1985; Mondschein et al., 1985), endometrial ER_c concentrations is high. While blastocysts develop into filamentous forms and oestrogen synthesis increases, endometrial ER_c concentrations declines. Occupied nuclear ER concentration also seems

to decrease during this period, since it is lower on Day 12 than on Day 10 (Rexmond & Guthrie, 1984) or even absent on Day 10 and Day 13 (Pack et al., 1978; 1979).

Oestrogen interaction with genetic material in the nucleus has been shown to stimulate cytosolic oestrogen receptor resynthesis and synthesis of progesterone receptor (Döhler, 1987). Declining endometrial ER_c concentrations from Days 10 to 13 suggest a down-regulation of ER concentration. Progesterone-induced endometrial oestrogen sulfotransferase seems partially responsible for the downregulation of ER in secretory porcine endometrium, by interfering with oestradiol-dependent replenishment of ER (Saunders et al., 1989). As a consequence of down-regulation the action of oestrogens during Days 10–13 of pregnancy is probably a temporary effect of short duration. One of those effects is the oestrogen induction of calcium cycling across the endometrial epithelum (which in turn redirects the secretion of $PGF_{2\alpha}$ toward the uterine lumen, Gross et al., 1990).

The results of our study demonstrate the existence of differences in ER_c concentrations between non-pregnant and pregnant gilts at the time blastocysts generate a first signal for the maternal recognition of pregnancy. The physiological significance of these differences, however, remains to be investigated.

Acknowledgements

We wish to acknowledge the technical assistance of Mrs. D. Sijpkens and Mrs. C. Frijters. This work was carried out as a project of the Research Group on Early Pregnancy at the Wageningen Agricultural University.

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