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# The Tissue Distribution of Porcine 17β-Estradiol Dehydrogenase and its Induction by Progesterone*

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**Porcine 17β-estradiol dehydrogenase (EDH) was recently purified and cloned. It catalyzes the $NAD^+$-dependent oxidation of estradiol to estrone 360-fold more efficiently than the back reaction with NADPH. The 32 kDa EDH is cut from an 80 kDa primary translation product with a multidomain structure unknown for other hydroxysteroid dehydrogenases. The highest EDH activities and strongest immunoreactions are found in liver (hepatocytes) and kidney (proximal tubuli) followed by uterus (luminal and glandular epithelium), lung (bronchial epithelium). Progesterone treatment of ovariectomized gilts stimulates oxidative EDH activity in uterus, anterior pituitary, skeletal muscle (diaphragm) and kidney. Constitutive levels of EDH activity were seen in the adrenals, the lung and the liver.**

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## INTRODUCTION

Porcine tissues express 17β-estradiol dehydrogenase (EDH) activity which is structure-associated and catalyzes the $NAD^+$-dependent oxidation of 17β-estradiol ($E_2$) [1]. The reduction of estrone ($E_1$), measured in homogenates or in subcellular fractions of porcine uterus epithelium, is about 30-fold less efficient and resides largely in a different enzyme. Recent purification and cloning of the EDH revealed that it is coded by an 2.9 kb mRNA translated to an 80 kDa protein that is cleaved to a N-terminal 32 kDa fragment corresponding to EDH [2, 3]. The 80 kDa protein consists of three domains revealing similarities to: N-terminal (EDH), short chain alcohol dehydrogenase family (27% similarity); central, enzymes of peroxisomal β-oxidation of fatty acids from *Candida tropicalis* or *Saccharomyces cerevisiae* (40%); and C-terminal, sterol carrier protein 2 (36%) [4–8]. The purified porcine EDH has a 360-fold preference for oxidation ($K_m = 0.5\ \mu M$) and is compounded in peroxisomes [9]. It is kinetically related to an EDH activity found in cytoplasmic membranes of human endometrium [10]. This enzyme and several other hydroxysteroid dehydrogenases were reported to respond to stimulation by progesterone [11–13]. We present a study on the tissue distribution of the enzyme and the effect of progesterone on EDH-activity in porcine target and non-target tissues.

## MATERIALS AND METHODS

### *Animals*

Prepubertal German landrace gilts (*Sus scrofa*) aged 5–6 months were kept at the Federal Institute for Animal Husbandry and Animal Behaviour at Mariensee, Germany. The pigs were ovariectomized as described previously [14]. A silastic tubing containing a crystalline suspension of estradiol in propylene glycol was implanted subcutaneously behind the right ear 10 days before the ovariectomy. Progesterone (10 mg in 1 ml sesame oil) was injected i.m. on 3 or 7 consecutive days before slaughter. The tissues were quickly collected and either frozen in liquid nitrogen or fixed in Bouin's solution for paraffin embedding.

### *Immunocytochemistry*

Rehydrated 3 μm sections of tissues were either incubated with mouse monoclonal antibody F1 conjugated with peroxidase (4 μg/ml) and the color devel-

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*Abbreviations*: EDH, porcine 32 kDa 17β-estradiol dehydrogenase; $E_2$, 17β-estradiol; $E_1$, estrone.

oped with diaminobenzidine/$H_2O_2$ or incubated with F1 (15 $\mu$g/ml) followed by goat anti-mouse-F(ab')$_2$-Cy3 (200-fold diluted) fluorescent secondary antibody (Dianova, Hamburg) as described [15, 16]. Controls were performed by preadsorption of primary F1 antibody with antigen, by the omission of primary antibody and by the observation of autofluorescence.

*Assay of EDH*

Frozen tissues were pulverized in a dismembrator (Braun, Melsungen) and the powder resuspended in 5 vol. of 50 mM $KH_2PO_4$, 5 mM $NaN_3$, pH 7.8. Oxidation of $E_2$ was measured in 100 mM phosphate buffer, pH 7.8 with $NAD^+$ as a cofactor, the reduction of estrone at pH 6.6 with NADPH using reversed phase HPLC for the separation of tritiated substrates and products [2]. Protein content was measured according to a modified Lowry procedure [17].

*Western blotting*

For SDS–PAGE and Western blotting samples (20 $\mu$g protein) were denatured in 1.5% (w/v) SDS, 0.02 M $Na_2$ EDTA, 0.1 M thioglycolic acid, 10% (w/v) glycerol for 5 min at 95°C before application to the gels. The 0.75 mm 8% slab gels were cast according to Laemmli [18]. The gels were run at 100 V for 2 h in 0.05 M Tris, 0.384 M tricine, 0.2% (w/v) SDS, pH 8.8 in Mighty Small Apparatus (Serva, Heidelberg). Western blotting from SDS–gels was on 0.2 $\mu$m nitrocellulose membranes (Schleicher und Schüll, Dassel) and immunoreactions were performed with primary antibody KV (rabbit anti-EDH IgG, 5 $\mu$g/ml) followed by porcine secondary anti-rabbit antibody SAK # 221 (0.1 $\mu$g/ml) peroxidase conjugates [16].

## RESULTS

Immunocytochemical studies on porcine uterus restricted the enzyme to luminal and glandular epithelium [16]. Treatment of ovariectomized gilts with progesterone increased the oxidative EDH activity in the uterus to the levels observed in intact gilts during the luteal phase [16]. Immunoperoxidase reactions in uterine sections are most prominent at day 7, both in the luminal (Fig. 1) and the glandular epithelium (not shown). Corresponding studies were performed with other target and non-target tissues.

The comparison of the influence of progesterone on oxidative and reductive activities in homogenates of different porcine tissues is summarized in the Table 1. In all tissues studied, the oxidation of $E_2$ was induced to a much higher extent than the reduction of $E_1$. Note that the $E_1$-reductase activity in many tissues is close to the detection limit (0.01 $\mu$U/mg prot.) and in all cases the oxidation prevails. Tissues which respond to progesterone include uterus, anterior pituitary, skeletal muscle (diaphragm) and kidney. Not responding were adrenals, lung and liver. The highest oxidative

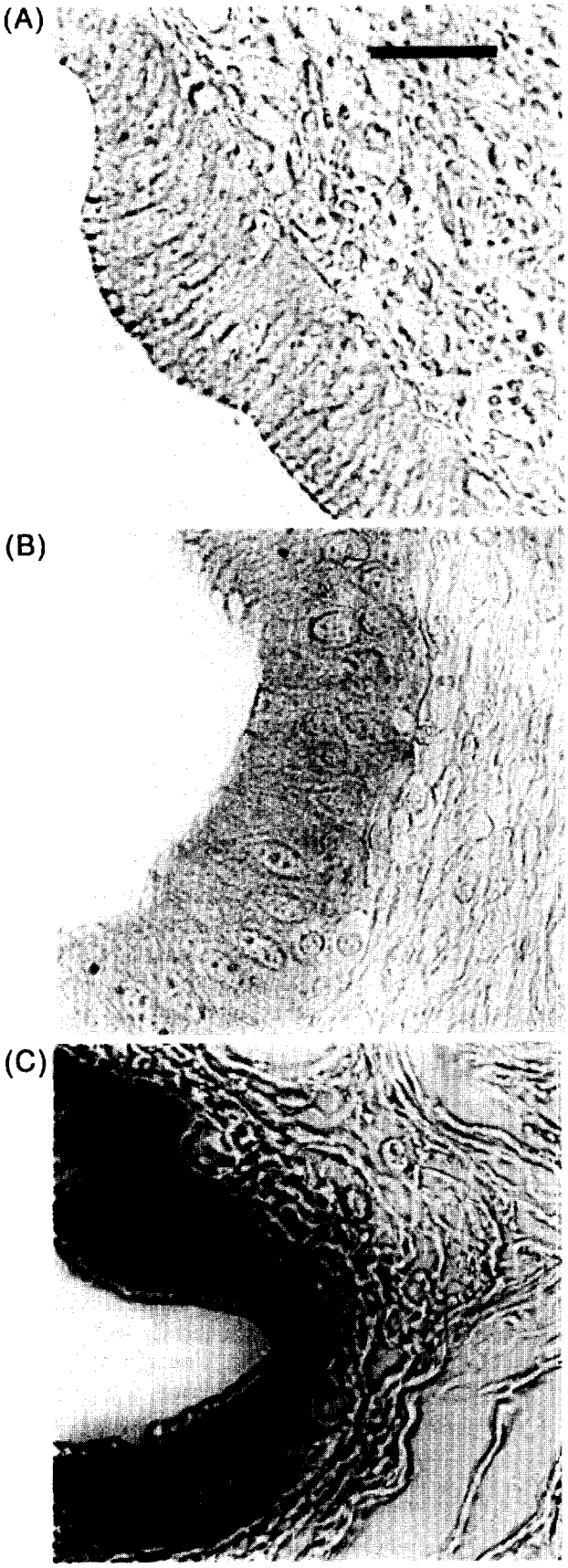


**Fig. 1. Stimulation of 17$\beta$-estradiol dehydrogenase in uterine epithelium. Rehydrated paraffin sections were incubated with anti-EDH antibody F1 (4 $\mu$g/ml) conjugated with peroxidase and the color reaction developed with diaminobenzidine/$H_2O_2$. (A) Uterus of untreated animal; (B) uterus collected after 3 days of progesterone treatment; (C) after 7 days. Bar 20 $\mu$m.**

*Table 1. Influence of progesterone on $E_2$ oxidation and $E_1$ reduction in porcine tissues*

| | $E_2 \rightarrow E_1$ | | | $E_2 \leftarrow E_1$ | | |
|---|---|---|---|---|---|---|
| | Fold induction | | | Fold induction | | |
| Tissue | 3 days | 7 days | Max. value (μU/mg prot) | 3 days | 7 days | Max. value (μU/mg prot) |
| Anterior pituitary | 2.4 | 3.8 | 0.2 | 1.0 | 1.0 | 0.01 |
| Adrenals | 1.0 | 1.0 | 0.3 | 1.0 | 0.9 | 0.02 |
| Skeletal muscle | 4.7 | 11.9 | 6.8 | 2.0 | 5.0 | 0.05 |
| Uterus | 3.8 | 12.9 | 9.0 | 2.5 | 7.0 | 0.10 |
| Mammary gland | 20.6 | 10.1 | 10.3 | 9.0 | 7.0 | 0.13 |
| Lung | 0.8 | 1.0 | 18.4 | 1.0 | 1.0 | 0.15 |
| Kidney | 2.5 | 2.8 | 70.3 | 1.3 | 1.6 | 0.13 |
| Liver | 0.8 | 1.1 | 573.1 | 0.9 | 0.9 | 0.95 |

Steroid conversions were assayed in homogenates as described in Materials and Methods. The fold of induction is given in comparison to untreated animals.

activities were observed after 7 days of prolonged progesterone treatment in all tissues except for the mammary glands which showed a maximum at day 3. This might be due to the heterogeneity of the samples with respect to adipose tissue admixtures.

Tissues of intact gilts collected during the luteal phase contained both the 80 kDa primary translation product and the 32 kDa EDH as demonstrated by Western blots (Fig. 2). In target tissues (uterus or mammary glands) the 32 kDa EDH was more prominent, whereas in non-target tissues (liver, kidney and lung) the 80 kDa protein prevailed.

Immunofluorescence and immunoperoxidase reactions recognized the same cell types in all non-target tissues collected during the luteal phase of intact gilts. EDH was found in hepatocytes, in the bronchial epithelium and in the proximal tubuli in the kidney (glomeruli were not stained, Fig. 3).

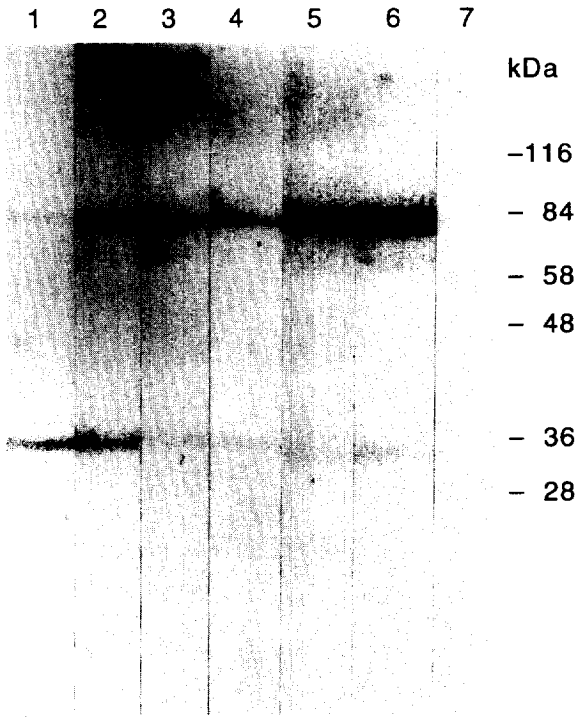


**Fig. 2. The presence of 80 and 32 kDa forms of $E_2$ dehydrogenase in tissues of intact gilts. Homogenates (20 μg per lane) were subjected to SDS-PAGE, blotted on nitrocellulose membranes and incubated with rabbit anti-EDH antibody KV (5 μg/ml) followed by a porcine secondary antibody SAK # 221 (0.1 μg/ml) conjugated with peroxidase. Lane 1, uterus; lane 2, mammary glands; lane 3, skeletal muscle; lane 4, lung; lane 5, kidney cortex; lane 6, liver. Control in lane 7, kidney with secondary antibody only. Molecular mass standards are marked.**

## DISCUSSION

The kinetic parameters of porcine 32 kDa 17β-estradiol dehydrogenase suggests that this enzyme catalyzes the inactivation of $E_2$ and $\Delta^5$-androstene-3β,17β-diol [2]. The release of the N-terminal EDH from the 80 kDa protein seems to proceed with different efficiencies in the various tissues. This and the further processing of the 80 kDa protein could control the multifunctionality, suggested by the presence of three domains [4]. Protection against onflowing $E_2$ could be exerted in target tissues such as uterus and breast and would involve the 32 kDa EDH and/or the 80 kDa protein. Western blots of homogenates of target tissues such as uterus and breast show prominent EDH bands and weak 80 kDa bands, whereas in non-target tissues (liver, muscle) the 80 kDa band prevails. A related murine enzyme could be responsible for similar distribution of $E_2$ oxidation in rat tissues [19]. The occurrence of the 80 kDa protein and its recently cloned human homologue [20] must at least in part reflect the ability to participate in the β-oxidation of fatty acids and in sterol transfer. Its localization in peroxisomes [9] supports the presumption of a direct involvement in all these activities. Human, rat and porcine oxidative 17β-hydroxysteroid dehydrogenase activities share a common distribution pattern: they are most prominent in liver and kidney followed by ovary, lung, testes and prostata [19, 20 and manuscript in preparation]. The activities other than steroid inactivation, which are present in the 80 kDa protein, could explain the high

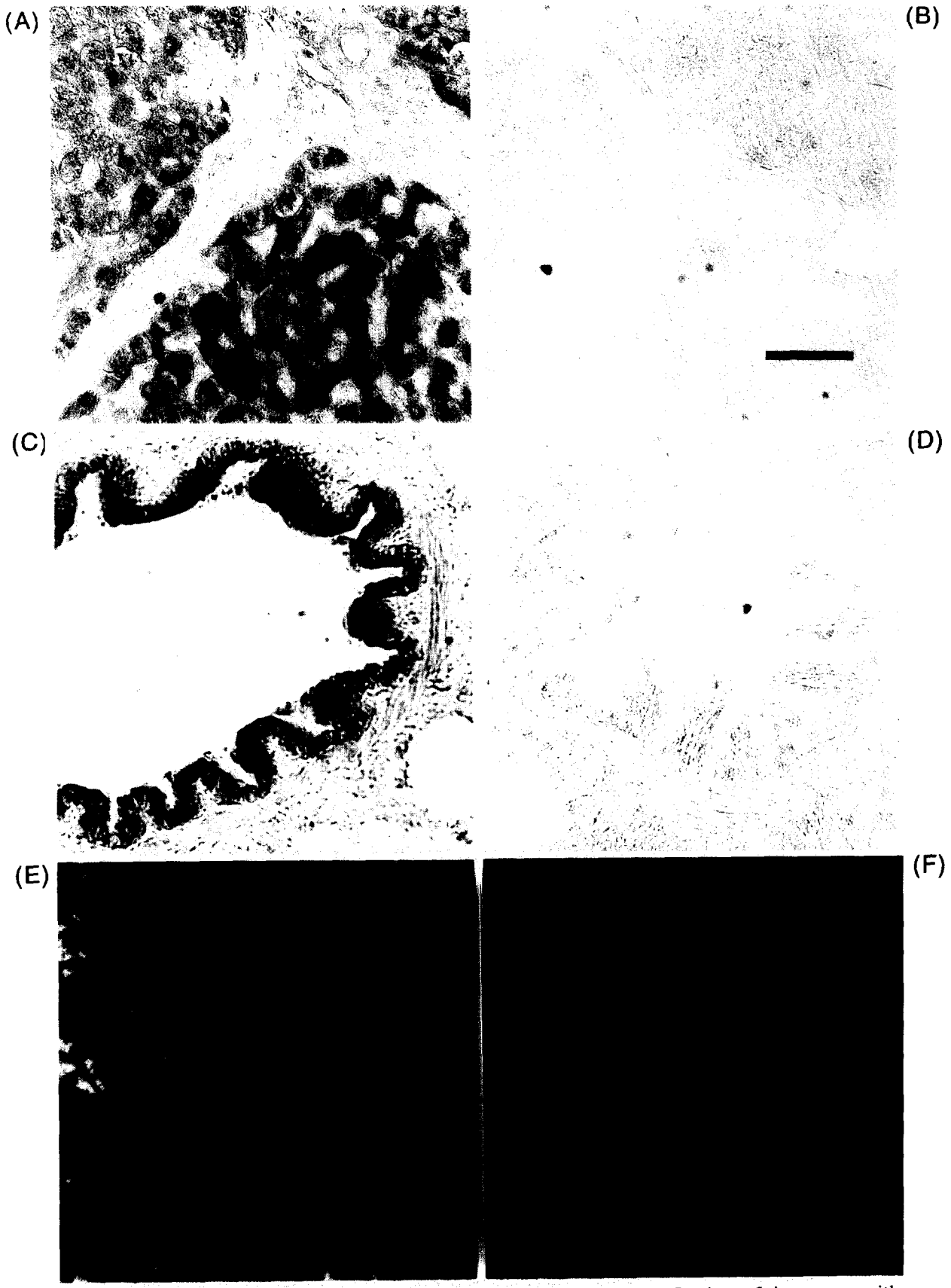


**Fig. 3. Immunocytochemical assignment of $E_2$ dehydrogenase in intact gilts. Sections of tissues were either incubated with antibody F1 conjugated with peroxidase (4 μg/ml) and color reaction developed with diaminobenzidine/$H_2O_2$ or incubated with F1 (15 μg/ml) followed by goat-anti-mouse-F(ab')$_2$-Cy3 (200-fold diluted) fluorescent secondary antibody. (A) liver; (C) lung; (E), kidney. Controls for liver (B) and lung (D) were performed by preadsorption of primary F1 antibody with antigen and for kidney (F) by the omission of primary antibody and by the observation of autofluorescence. Bar 20 μm.**

concentrations of the enzyme in the porcine gonads. Whether a concerted action of the three domains allows for metabolic advantages remains to be proven.

Differential regulations of oxidative and reductive pathways have been observed in T47D cells. The retinoic acid is without effect on the oxidation of $E_2$ while it enhances the reduction of $E_1$ [21]. Progesterone, as described here, increases porcine EDH but not that of estrone reductase. These two enzymes have been separated by chromatography on Blue Sepharose [2]. Porcine tissues apparently express the EDH as a predominant dehydrogenase as judged by kinetic analyses of steroid metabolism. Other porcine 17β-hydroxysteroid dehydrogenases similar to human enzymes type 1, 2 or 3 [22–26] must be present at much lower levels.

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