Virchows Arch A (1987) 411:409–414

Virchows Archiv A

Effects of estrogens on the testis of transsexuals: a pathological and immunocytochemical study

Anna Sapino [1], Alberto Pagani [1], Adriana Godano [2], and Gianni Bussolati [1]

[1] Department of Biomedical Sciences and Human Oncology, University of Turin, Italy
[2] Endocrinology Service, Mauriziano Hospital, Turin, Italy

Summary. Testes from five male-to-female transsexuals removed during sex-reversal surgery after administration of estrogens were studied histologically and with immunoperoxidase procedures to locate estrogen receptors (ER) and related antigens. Atrophy of the seminiferous tubules was observed in all cases; its degree, and a marked decrease in Leydig cells, correlated with low plasma gonadotropin levels (rather than with the duration of treatment). In all cases, the rete testis appeared hyperplastic and the other components (efferent ductules, epididymus) well preserved. The efferent ductules proved to be the only structure where ER can be located by the ER-ICA procedure, whereas staining for the p 29 ER-associated antigen is strongly positive both here and in the rete testis. The immunocytochemical data, in parallel with the observed biological effects, indicate that the rete testis and the efferent ductules and, to a minor degree, the epididymus and Leydig cells are the main direct targets of estrogens in the human testis.

Key words: Estrogens – Testis – Transsexualism – Histology – Immunoenzyme techniques

Introduction

The role of estrogens in the physiological and pathological modulation of growth and function of the different tissue components of the human testis is poorly understood. Estrogen treatment has been shown to induce testicular atrophy in elderly patients with carcinoma of the prostate (Oshima et al. 1974). Furthermore, estrogen receptors have been detected by Murphy et al. (1980) in the epididymus but not in the testis proper by means of biochemical assay; their immunocytochemical location has not yet been described, however.

New information on the hormone targets and related changes can be obtained by studying the morphology and function of the testis of male-to-female transsexuals subjected for variable months or years to a heavy regimen of estrogen administration prior to plastic surgery.

In this paper, we report a histological and immunohistochemical comparison of male-to-female transsexuals and normal testes. Specific monoclonal antibodies and an immunoperoxidase procedure were used to detect estrogen receptors (ER) and related antigens. These techniques provide the information on target cells and tissues needed for interpretation of the effects of estrogen on the testis.

Material and methods

The testes (didymus and epididymus) of 5 transsexuals aged 25–59 removed during sex-reversal were examined.

All patients had received 40–50 mg/week of polyestradiol phosphate, for varying periods (see Table 1). The patients were asked to withdraw the treatment 10 days before surgery.

Both gonads were put on ice and immediately brought to the laboratory. After careful dissection, they were measured. Selected blocks were then a) frozen in liquid N_2 for cryostat sectioning or b) fixed in methacarn (Puchtler et al. 1970) for histology and immunohistochemistry (see below).

The following normal testes served as controls:
1. from 2 patients aged 67–76 who underwent orchiectomy for prostatic carcinoma not preceded by estrogen management
2. from 2 subjects aged 56–62 autopsied 24 h after road accidents.

Histology and immunohistochemistry. Methacarn-fixed, paraffin embedded tissue sections were stained with haematoxylin-eosin. Other sections were immunostained with the D5 monoclonal antibody (kindly supplied by Dr. Roger King, Imperial Cancer Research Found of London, UK) recognizing a 29 kd phospho-

Offprint requests to: G. Bussolati, Dipartimento di Scienze Biomediche e Oncologia Umana, Sezione di Anatomia e Istologia Patologica, Via Santena 7, I-10126 Torino, Italy

Table 1. Clinical and pathological data

Case	Age	Estrogen treatment (months)	Testis dimensions (mm)	Plasma hormone levels			
				FSH mU/ml	LH mU/ml	E pg/ml	T ng/ml
1	25	60	25 × 20 × 15	1.2	5.2	376.3	0.34
2	59	6	30 × 25 × 15	53.9	42.9	8.6	2.8
3	28	96	30 × 25 × 15	47	66.1	13.2	8.12
4	29	18	30 × 25 × 15	1.6	3.5	101.9	0.89
5	22	24	30 × 20 × 16	20.6	27.3	31.8	4.2

Normal testis dimensions: 42 × 38 × 25 mm
FSH: Follicle Stimulating Hormone, normal value 4.5–20 mU/ml
LH: Luteinizing Hormone, normal value 4.5–20 mU/ml
E: Estradiol, normal value 10–57 pg/ml
T: Testosterone, normal value 3–11 ng/ml

protein (p 29) associated with estrogen receptor (ER) (King et al. 1985). They were brought through graded alcohols to phosphate-buffered saline (PBS) and were treated with 3% hydrogen peroxide in PBS to remove endogenous peroxidase activity, treated with non-immune goat serum diluted 1:50 in PBS, and then with D5 monoclonal antibody 1:50 in PBS overnight at room temperature. Parallel control sections were treated with normal mouse serum diluted 1:50. The test and the control sections were treated with horse anti-mouse biotinylated antibody diluted 1:500 in PBS (Vector, CA, USA) and then with the ABC (avidin-biotin peroxidase complex) reagent (Hsu et al. 1981). The reaction was developed with diaminobenzidine; the nuclei were counterstained with haemalum and the slides were mounted in balsam.

A human breast carcinoma strongly reactive with the D5 antibody and with a high ER level as detected by the dextran-charcoal method (see Pietribiasi et al. 1986) was stained in parallel, as a positive control.

The ER-ICA procedure (King and Green 1984) was employed to locate ER with the specific monoclonal antibodies produced by Abbott Laboratories (Wiesbaden, FRG). Fresh sections were collected on slides and immediately fixed in 3.7% phosphate-formaldehyde for 10–15 min and then in methanol and acetone at −20° C (5 min and 5 s, respectively), according to the manifacturer's instructions. The binding of the rat antibody was revealed by peroxidase conjugated anti-rat immunoglobulin antiserum. After DAB-H_2O_2 development, the nuclei were counterstained with methyl green.

Smears of an estrogen-dependent mammary carcinoma cell line (MCF-7) were used as control.

Results

All the transsexual testes were atrophic and smaller than normal. No relationship was found between the degree of atrophy (gross and microscopic – see below) and the duration of estrogen treatment, however. A correlation was established between extent of atrophy and the plasma levels of gonadotropins (FSH and LH), estradiol and testosterone (tested at the time of operation) (see Table 1).

The seminiferous epithelium shows similar alterations in all subjects, irrespective of serum hormone values or duration of treatment: the germ cell maturation is blocked at spermatogonium stage; the Sertoli cells appear hyperplastic and the only type detectable in some areas. Hyalinosis and thickening of the basal membrane of the tubules appears more prominent in cases 1 and 4, which are also devoid of Leydig cells (Fig. 1). In the other patients, and especially in case n. 5 (Fig. 2) a moderate degree of tubular sclerosis correlates with the presence of Leydig cells arranged in relatively large groups. Their structure is preserved with distinct Reinke crystalloids, but focal cytoplasmic vacuolation. Their gonadotropin levels were slightly increased, whereas their testosterone and estrogen values were normal.

In all patients, the rete testis and the efferent ductules of the epididymus appear not only preserved, but display various degrees of hyperplasia. The epithelium of the rete testis is thickened and the cells are cuboid or columnar, not flattened as in controls (Figs. 3 and 4). Pluristratification and inter-anastomosing cristae are observed in the epithelium.

The epididymal ciliated cells are well preserved and similar to those of the controls; in case 5, the basal cells are prominent and the epithelium is of increased thickness.

The two monoclonal antibodies, one directed against estrogen receptors and one (D5) against the 29 kd estrogen receptor associated phosphoprotein, give a different staining distribution; the results obtained in various testicular tissues are reported in Table 2. D5 monoclonal is reactive on fixed and embedded tissue sections and even on autopsy material, whereas the ER-ICA method can only be used on fresh-frozen tissues. A similar distribution is obtained in the control and transsexual testis. D5 gives a uniform cytoplasmic staining of the smooth muscular components of the blood

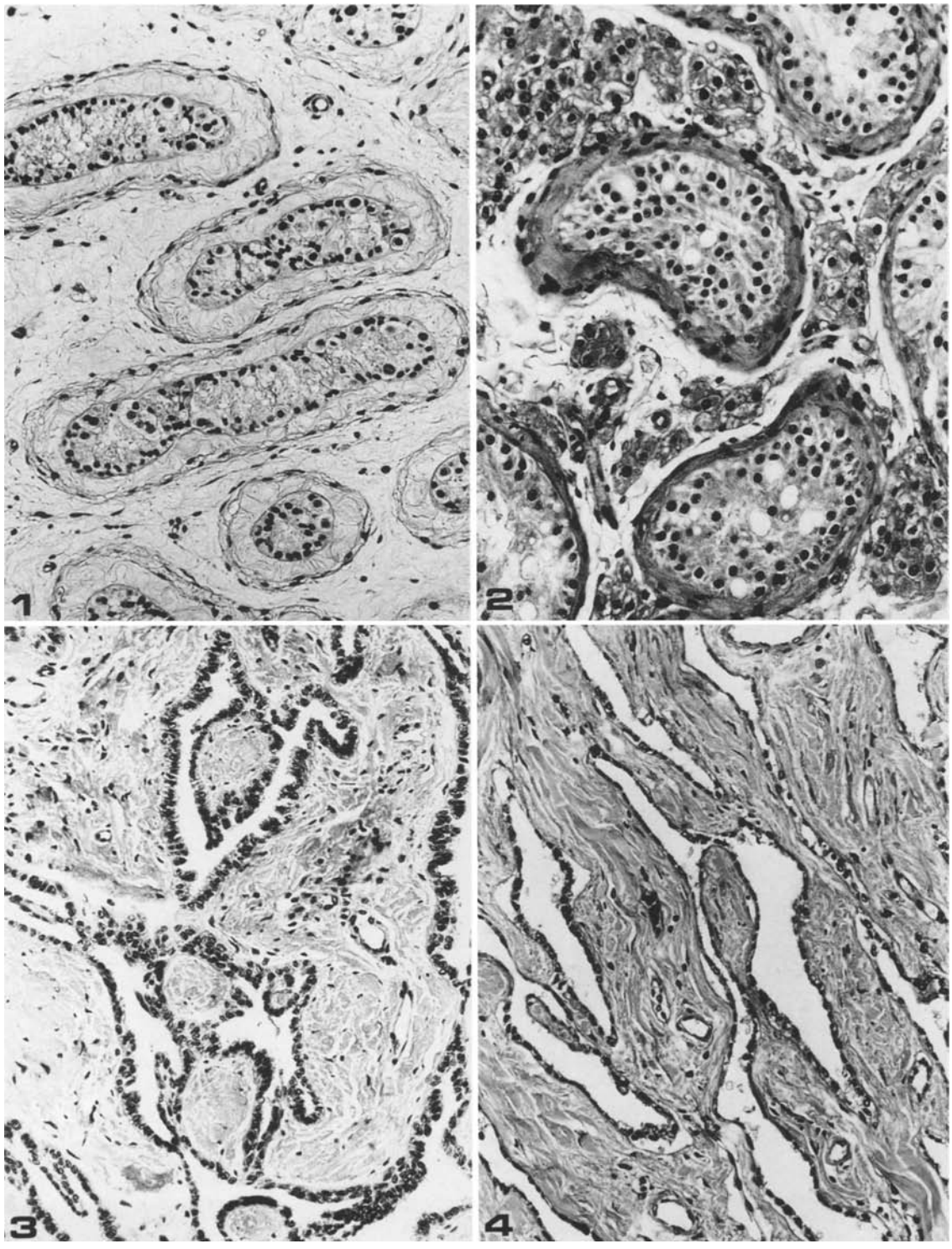
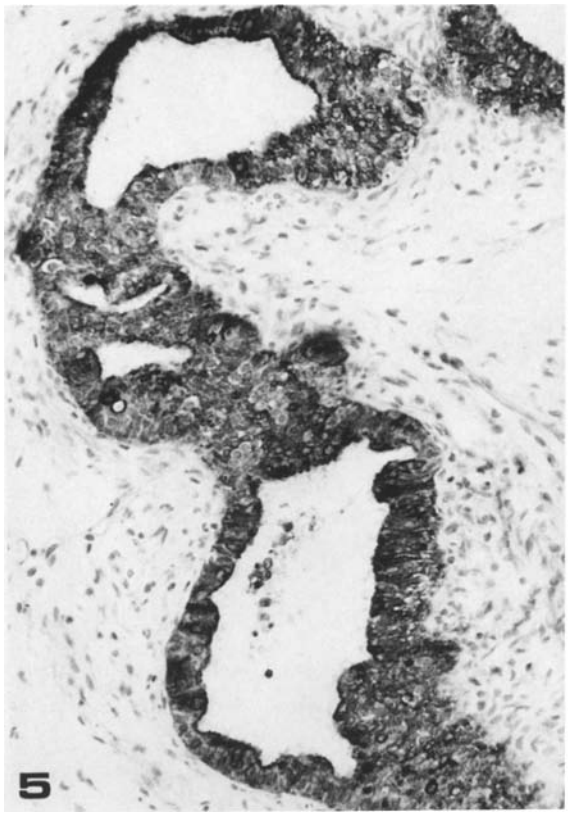
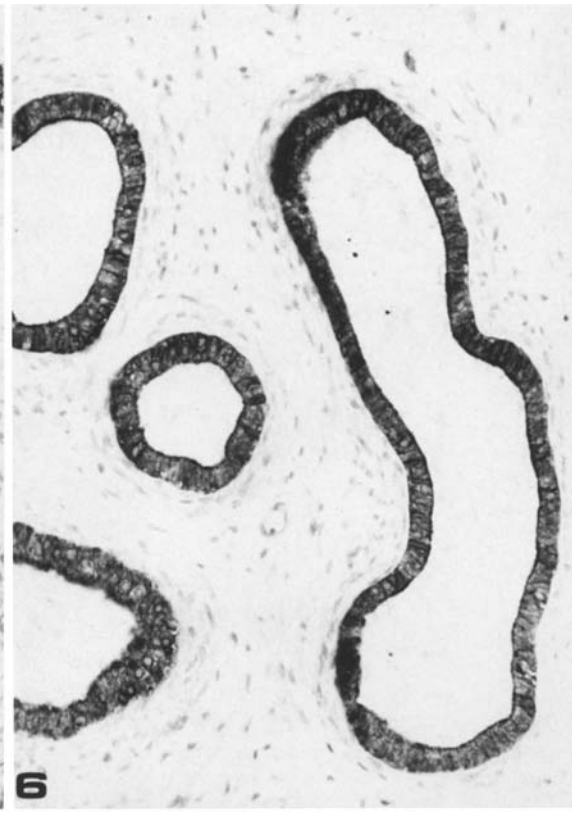


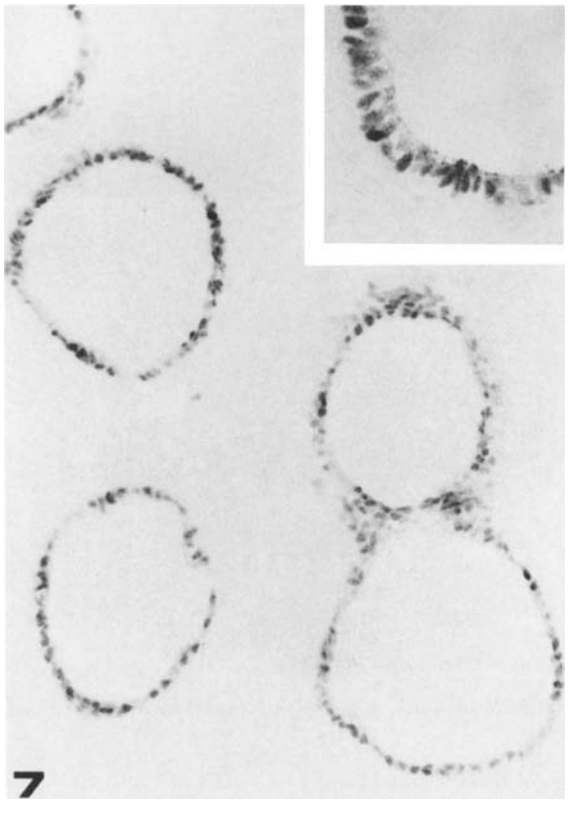
Fig. 1. Testis of a subject treated for 60 months with estrogens. Disappearance of the Leydig cells and marked atrophy of the seminiferous tubules; a few spermatogonia are still present. (H and E, × 198)

Fig. 2. Case 5. Moderate degree of tubular sclerosis and relative hyperplasia of the interstitial Leydig cells. (H and E, × 198)

Figs. 3 and 4. The rete testis more prominent in estrogen-treated subjects (Fig. 3) as opposed to controls (Fig. 4). In the former, the cells are columnar and the epithelium pluristratified and with inter-anastomosing cristae. (H and E, × 198)







Figs. 5 and 6. Testis of a transsexual subject. Immunocytochemical staining with the D5 antibody detecting the p 29 ER-associated antigen is strongly positive on the cytoplasm of cells of the rete testis (Fig. 5) and of the efferent tubules (Fig. 6). Nuclei counterstained with Haemalum (× 198)

Fig. 7. Case 5. Estrogen receptors, located by the ER-ICA procedure, are only detectable in the nuclei of the columnar cells of the efferent ductules. (× 220; inset: × 331)

Table 2. Immunocytochemical staining of estrogen receptor-related-antigens in testicular tissues

	D5	ER-ICA
Seminiferous tubules	–	–
Interstitial vessels	+	–
Interstitial cells	+ –	–
Rete testis	+ +	–
Efferent ductules	+ +	+
Epididymus	(*)	–

(*) Weak staining of the basal cells

vessels and of the epididymus, a rather weak reaction on the Leydig cells (visible in controls and in cases 2, 4 and 5) and in the basal cells of the epididymus; a strong reaction, instead, is observed in the rete testis cells and in the efferent ductules (Figs. 5 and 6). D5 is completely negative in the seminiferous tubules. A positive reaction with the anti-estrogen receptor antibody (ER-ICA procedure) is only found in the nuclei of the columnar cells of the efferent ductules (Fig. 7).

Discussion

Tubular atrophy (inhibition of spermatogenesis and marked thickening of the basement membrane) and disappearance of the Leydig cells is commonly described in the literature dealing with the testicular alterations in estrogen-treated patients (Oshima et al. 1974).

In all our transsexuals, the epithelium of the seminiferous tubules was formed mainly of Sertoli cells and only a few spermatogonia were still recognizable. Marked hyalinization, stromal sclerosis and disappearance of Leydig cells was only found in cases 1 and 4 irrespective of the reported duration of estrogen treatment. The effect of estrogen on the testis may, however, be direct or mediated through induced changes in gonadotropin levels. The Leydig cells of our patients with increased gonadotropin levels (nos 2, 3, 5) showed features interpretable as hyperplasia, similar to those described by Hatakeyama (1965). A direct influence of estrogens on Leydig cells may, never the less, be suggested in the light of experimental studies in mice on the presence of ER (Tarakawa et al. 1982) and on the uptake of ^{3}H-estradiol by these cells (Stumpf 1969), as well as by our immunocytochemical observation of their positive staining with the D5 antibody. This is known to reveal an ER-related protein (King et al. 1985; Cano et al. 1986). Individual differences in the type of LH response to estrogen administration in heterosexual, homosexual and transsexual males have been reported by Gooren (1986).

We cannot explain the discrepancies observed in the effects of reportedly similar estrogen treatments in our cases of transsexuals, irrespective the duration of treatment. Congenital or acquired differences in reaction to estrogen seem the only explanation.

The present study indicates that the main target of estrogen stimulation in the testis is the epididymus and rete testis. Biochemical studies had already shown that ERs are located in the epididymus (Murphy et al. 1980): our immunocytochemical investigation with an anti-ER monoclonal antibody (ER-ICA procedure; King and Green 1984) has indicated that the ductuli efferentes are the only positive area in the testes of both controls and estrogen-treated subjects. Staining with D5 antibody gave a less restricted location since the rete testis and epididymus were also strongly positive. The smooth muscle component is also known to stain positively with this monoclonal (King et al. 1985).

The exact significance of the p 29 protein is unknown (Coffer et al. 1985). In our opinion, its location points to a topographic distribution of estrogen-dependent areas in the testis more in keeping with the observed biological effects than that provided by biochemical analysis or ER-ICA staining.

A common finding in our transsexuals was cellular hypertrophy and hyperplasia of the rete testis and, to a minor degree, of the ductuli efferentes and of epididymal ductal epithelium. In the only previous investigation of histology of the testis in transsexuals (Rodriguez-Rigau et al. 1977) and in studies on elderly estrogen-treated patients with prostatic carcinoma (Oshima et al. 1974), no mention was made of the structure of the rete testis and of epididymus. Our suggestion that the rete testis is an estrogen-dependent area seems to fit in with Newbold et al.'s observations (1985) of a high frequency of rete testis hyperplasia and even adenocarcinoma-like lesions in mice exposed to diethylstilbestrol prenatally. The embryology of the rete testis is still controversial (Carlon and Stahl 1973); Byskow (1978) suggests a mesonephric tubular origin for the system, which agrees with the estrogen-dependence observed. The possible estrogen dependency of the rare adenocarcinomas of the rete testis (Schoen and Rush 1959; Yoshitomi and Morii 1984) and of testicular tumors occurring in subjects exposed in utero to diethylstilbestrol (Depue et al. 1983) requires investigation in this connection.

Acknowledgements. Work supported by grants from the Italian M.P.I. (Rome) and the Regione Piemonte.

References

Byskov AG (1978) The anatomy and ultrastructure of the rete system in the fetal mouse ovary. Biol Rep 19:720–735

Cano A, Coffer A, Adatia R, Millis R, Rubens R, King RJB (1986) Histochemical studies on oestrogen receptor-related protein in human breast tumors. Cancer Res 46:6475–6480

Carlon N, Stahl A (1973) Les premiers stades dy développement des gonadés chez l'homme et les vertébrés supérieurs. Pathol Biol 21:903–914

Coffer AI, Spiller GM, Lewis KM, King RJB (1985) Immunoradiometric studies with monoclonal antibody against a component related to human estrogen receptor. Cancer Res 45:3694–3698

Depue RH, Pike MC, Henderson BE (1983) Estrogen exposure during gestation and risk of testicular cancer. J Natl Cancer Inst 71:1151–1155

Gooren L (1986) The neuroendocrine response of luteinizing-hormone to estrogen administration in heterosexual, homosexual, and transsexual subjects. J Clin Endocrinol Metabol 63:583–588

Hatakeyama S (1965) A study on the interstitial cells of the human testis. Especially on their fine-structural pathology. Acta Pathol Jap 15:155–197

Hsu SM, Raine L, Fanger H (1981) Use of avidin-biotin peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabeled antibody (PAP) procedures. J Histochem Cytochem 29:577–580

King RJB, Coffer AL, Gilbert J, Lewis K, Nash R, Millis R, Raju S, Taylor RW (1985) Histological studies with a monoclonal antibody raised against a partially purified soluble estradiol receptor preparation from human myometrium. Cancer Res 45:5728–5733

King WJ, Green GL (1984) Monoclonal antibodies localize estrogen receptor in the nuclei of target cells. Nature 307:745–747

Murphy JB, Emmott RC, Hicks LL, Walsh PC (1980) Estrogen receptors in the human prostate, seminal vesicle, epididymis, testis, and genital skin: a marker for estrogen-responsive tissues? J Clin Endocrinol Metabol 50:938–948

Newbold RR, Bullock BC, McLachlan JA (1985) Lesions of the rete testis in mice exposed prenatally to diethylstilbestrol. Cancer Res 45:5145–5150

Oshima H, Sarada T, Ochiai OK, Tamoaki B (1974) Effects of synthetic estrogen upon steroid bioconversation in vitro in testis of patients with prostatic cancer. Invest Urol 12:43–49

Pietribiasi F, Gugliotta P, Racca S, Di Carlo F, Bussolati G (1986) A combined histological, immunocytochemical and biochemical approach in the evaluation of estrogen receptors in breast carcinomas. Int J Biol Markers 1:9–14

Puchtler H, Waldrop ES, Meloan SM, Terry MS, Conner HM (1970) Methacarn (methanol-carnoy) fixation. Histochemie 21:97

Rodriguez-Rigau L, Tcholakian RK, Smith K, Steinberger E (1977) Effect of in vivo estrogen administration on in vitro steroid bioconversation in human testis. In: Troen P, Nankin HR (eds) The testis in normal and infertile men. Raven Press, New York, pp 457–468

Schoen SS, Rush BF Jr (1959) Adenocarcinoma of the rete testis. J Urol 82:356–363

Stumpf WE (1969) Nuclear concentration of ^{3}H-estradiol in target tissues. Dry-mount auto radiography of vagina, oviduct, ovary, testis, mammary tumor, liver and adrenal. Endocrinology 85:31–37

Tarakawa N, Huseby RA, Fang SM, Samuels LT (1982) Quantitative changes in estrogen receptor produced by chronic DES treatment of two mouse strains differing in susceptibility to Leydig cell tumor induction. J Steroid Biochem 16:643–652

Yoshitomi K, Morii S (1984) Benign and malignant epithelial tumors of the rete testis in mice. Vet Pathol 21:300–303

Accepted May 22, 1987