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Rat epididymal epithelial cells and 17β-estradiol synthesis under hCG stimulation in vitro

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Abstract: Epithelial cells of human and animal epididymis display features of steroidogenic cells. Rat epididymal epithelial cells *in vitro* produce androgens which are converted to 17β-estradiol, and released into the medium. The regulation of the epididymal steroidogenesis is not fully understood but it could be expected that it remains under LH influence. In previous study we observed that the morphology of rat epididymal epithelial cells *in vitro* was affected by hCG and the increase of amount of lipid droplets, glycogen and PAS-positive substances was observed. The present studies show the organelles which take part in synthesis of steroids in rat epididymal epithelial cells *in vitro* and the effect of hCG on E2 synthesis. The cells were cultured in the medium with/without DHT and without DHT in supplementation with hCG. After hCG stimulation the amount of an active mitochondria were increased when compared to the amount of mitochondria in the epididymal epithelial cells cultured without DHT. Ultrastructure of the cells was similar to the cells cultured with DHT, while the cytoplasm of the cells cultured without DHT was disorganized. The synthesis of 17β-estradiol was stimulated by hCG, that exerted its effect through LH/hCG receptors, localized in the epididymal epithelial cells.

Key words: Cell culture - Epididymal epithelium - hCG - Mitochondria - SER - Ultrastructure

Introduction

The main function of the epididymal epithelium is to create the luminal microenvironment necessary for maturation, storage and survival of spermatozoa [1,2]. The expression of genes in the epididymal epithelial cells encoding proteins released into the epididymal lumen is region-specific and is regulated mainly by androgens - T and DHT [3-7]. DHT is the main androgen in epididymis, formed via the conversion of testosterone to 5α-dihydrotestosterone in an irreversible reaction catalyzed by 5α-reductase (5α-red) [8]. There are two isoforms of the enzyme: 5α-red 1 and 2. The other form is preferentially expressed in the epididymis, and its activity can be inhibited by finasteride [8].

However, epididymal epithelial cells of rat *in vitro* apart from features for cells producing proteins display features of steroidogenic cells [9]. They contained lipid droplets located near mitochondria with lamellar cristae and well developed smooth endoplasmic reticulum (SER), the structures involved in steroidogenesis [9]. The cells produce and release androgens into the culture medium that are converted to 17β-estradiol due to the activity of cytochrome P450 aromatase [10]. It is probable that steroidogenesis in epididymal epithelial cells of rat is regulated by luteinizing hormone (LH). It was reported that epididymis contains functional LH receptor [11], and the immunoexpression of LH receptors (LH-R) was observed in our former studies in the man and rat cells of epididymal epithelium *in vivo* [12,13]. Luteinizing hormone is known to produce morphological changes in the cytoplasm of Leydig cells both, *in vitro* and *in vivo* [14,15]. The lack of LH receptor in knockout mice results in the morphological changes of epididymis [16]. They include a decrease in luminal diameter of epididymis, the absence of clear and halo cells in the epithelium, and decrease in the height of principal cells [16]. Moreover, a decrease in periodic acid-Schiff reaction products synthesis, the glycogen and glycoproteins, was observed [16]. In our former studies we noted the increase in the accumulation of lipid droplets, PAS-positive substances and glycogen in the cytoplasm of cells cultured in the medium without addition of dihydrotestosterone (DHT) but supplemented with hCG

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[12,13]. It was the first sign of LH/hCG effect in rat epididymal epithelial cells *in vitro*. The aim of the present study was to estimate the influence of hCG on the organelles included in process of steroidogenesis and synthesis of 17β-estradiol in rat epididymal epithelial cells.

Materials and methods

The experiment was carried out on adult Wistar rats, weighing 300 - 350 g each. The rats were maintained at controlled temperature (22-24°C) and photoperiod of LD 12:12 h. The rats were randomly divided into control (10 animals) and experimental (5 animals) groups. The animals in the experimental group received *per os* 5mg/kg body weight/day finasteride (Proscar®, MSD Sweden), steroid-based inhibitor of *5α-red2* for 56 days (the total duration of spermatogenesis). Epididymides were obtained from control and experimental rats. The animals were anaesthetized with Thiopental (Biochemie GmbH, Kundl-Austria) (90 mg/kg body weight). The epididymides intact and finasteride-treated animals were assigned for immunohistochemical studies and animals of control group for isolation of epididymal epithelial cells. The caput and cauda of epididymis for immunostaining were fixed in 4% formaldehyde freshly prepared from paraformaldehyde and embedded in paraffin. The experiment received the approval of the Local Ethics Committee.

Isolation and culture of epididymal epithelial cells. The procedure of isolation and culture of epididymal epithelial cells was described previously by Dominiak and Rozewicka [6] as a modified procedure of Kierszenbaum *et al.* [18]. Viability of the cells was detected by the trypan blue exclusion test. The isolated cells were transferred to plastic Petri culture dishes (Nunc Inc., Naperville, Il., USA) and cultured in Dulbecco's modified Eagle's medium (Gibco BRL, Grand Island, USA) supplemented with 5% inactivated fetal calf serum (FCS; Gibco BRL, Grand Island, USA) without or with 1 nmol/L dihydrotestosterone (DHT; Sigma Chemical Co, St Louis MO, USA), and without DHT but in supplementation with hCG (Chorulon - Intervet, Holland) in finally concentration 12.5 U hCG/mL. The cells were cultured at 34°C, in 5% CO_2 for 3 days, until they formed a monolayer. Thereafter, the medium was changed and the cells were cultured for the next two days. After that time, the cultures of epididymal epithelial cells were used for morphological evaluation.

For hormone assay the epididymal epithelial cells were placed into 24-multi cell culture dishes (Corning, USA) - 500.000 cell/well. The cells were cultured in Dulbecco's modified Eagle's medium without phenol red (Gibco BRL, Grand Island, USA), without DHT and in supplementation with hCG.

Visualization of mitochondria in epithelial cells of the epididymis. Epithelial cells of the caput and cauda epididymis cultured in medium with and without addition of DHT, and without DHT but supplemented with 12.5 U/mL hCG, were incubated in medium containing specific mitochondrial probe Mito-Tracker Green FM (Molecular Probes, Europe BV, Leiden, The Netherlands). A stock solution contained 20 μg fluorochrome dissolved in 1 mL DMSO. Incubating medium was prepared from the stock and contained 0.2 μg fluorochrome dissolved in 1 mL Dulbecco's medium. The cells were incubated in the medium at 34°C, in 5% CO_2 for 25 minutes. Next, they were examined in fluorescence microscopy (Axioscop Zeiss, Germany), using filter set 09-487909-0000; Zeiss, Germany).

Simultaneously, cryostat slides of the caput and cauda epididymis of control rats were incubated in the medium with the fluorochrome. Cryostat sections used for visualization of mitochondria were prepared from fresh tissues.

Transmission electron microscopy [TEM]. For electron microscopic studies, cultures of the epiddidymal epithelial cells were fixed in 0.25 mol/L glutaraldehyde in 0.1mol/L cacodylate buffer, pH=7.4 for 2 h at 4°C, post-fixed in 0.04 mol/L OsO_4. Next, the monolayer of the cells was mechanically retrieved from Petri dishes. Then the cells were transferred to Eppendorf tubes, dehydrated in ethyl alcohol and acetone, embedded in a Spurr low-viscosity embedding kit (Polysciences, Inc. Warrington, PA). Ultrathin sections were prepared with Reichert OmU2 ultramicrotome (Leica Aktiengesellschaft, Vienna, Austria), contrasted with uranyl acetate and lead citrate (Sigma), and analysed under JEM-1200 EX (JEOL Ltd, Tokyo, Japan) transmission electron microscopy at 80 kV.

Immunohistochemical studies. Paraffin-embedded sections of caput and cauda epididymides were stained for LH/hCG receptors visualization. Moreover, epithelial cells of caput and cauda epididymis cultured in medium with DHT were used to estimate the expression of these receptors. The immunohistochemical reaction was carried out using rabbit polyclonal antibody anti-rat Luteinising Hormone Receptor (BIOTREND Chemikalien GmbH, Köln, Germany)(1:100).

Briefly, specimens were cut in 5 μm sections, mounted on poly-L-lysine-coated microscope slides, deparaffinized in xylene, passed through graded ethanol solution, and washed with water. To reduce non-specific staining, the slides were immersed in 3% hydrogen peroxide in methanol for 30 min at room temperature to quench endogenous peroxidase activity. After rinsing in Tris-buffered saline (TBS) twice for 5 min, sections were submerged in 10 nM citric buffer, pH=6.0, and microwaved at high power two times for 5 min each time. Slides were then left to cool to room temperature in the buffer solution (20 min). Slides were again washed in TBS twice for 5 min, and non-specific binding was blocked by incubation with 3% normal goat serum for 30 min at room temperature followed by incubation with primary antibody overnight at 4°C. Slides were subsequently washed twice with TBS for 5 min and incubated with secondary antibody. LH/hCG-receptors were localized using reagent provided in the ABComplex/HRP (DakoCytomation, Denmark) by following the manufacturer's instruction. To avoid a non-specific reactivity, control sections were incubated without primary antibody.

Hormone assays. Centrifuged media from epididymal epithelial cells cultured for 3 and 5 days were designed for 17β-estradiol (E2) assays. The concentration of E2 was estimated in the culture media enriched with 5% FCS before initiation of the cell culture (control media) and in media on the 3rd and 5th day of the culture. Levels of E2 were estimated by Enzyme Linked Fluorescent Assay (Bio Merieux, France) VIDAS Estradiol II (E2II).

Statistical analysis. Hormone concentration data were analysed statistically. Results are expressed as median (M), lower and upper quartile (Q1-Q3). Nonparametric Mann-Whitney U-test for variables with distributions different from normal (Shapiro-Wilk's test) and Student-t test for normally distributed variables were used to check significance of differences between study and control groups. The value of $p<0.05$ was considered to indicate statistically significant differences. Calculations were done using the software package Statistica 6.1.

Results

Mito Tracker Green FM staining

Active mitochondria in the cytoplasm of epithelial cells of the caput and cauda epididymis cultured in a medium with DHT displayed intensive yellow-green

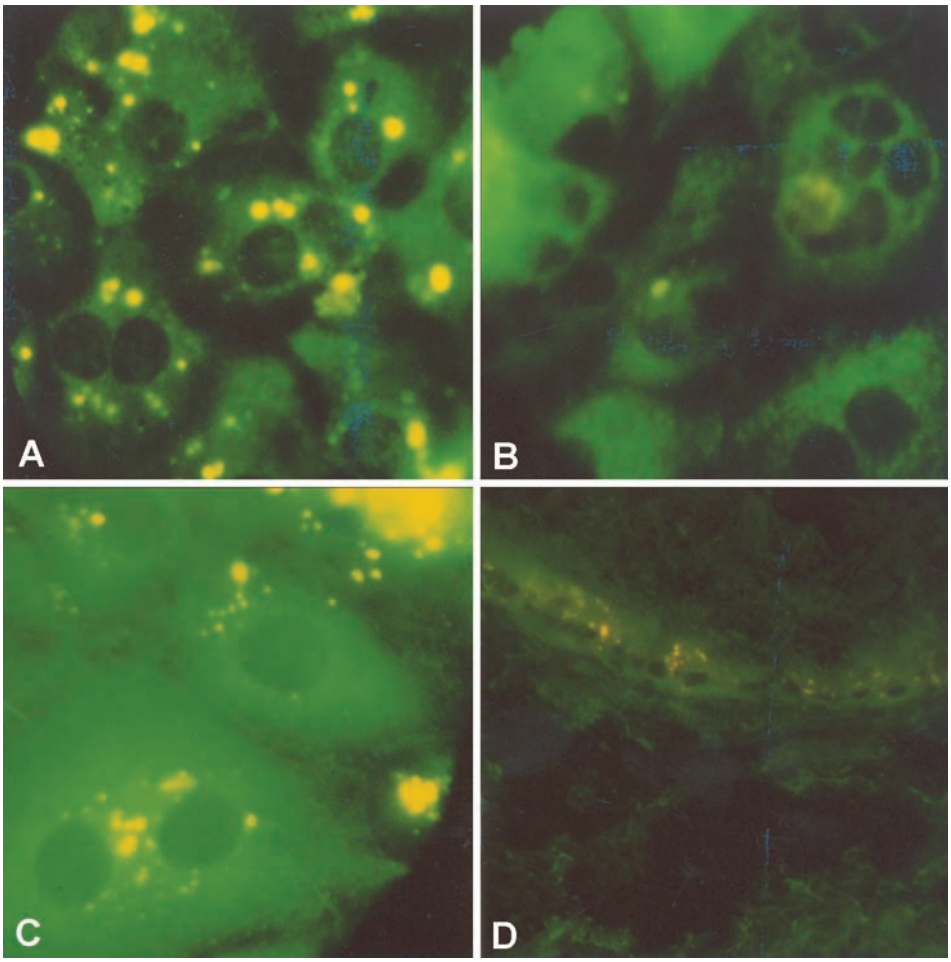

Fig. 1. Mitochondria in the cytoplasm of the caput epididymal epithelial cells cultured in the medium with DHT (**A**), without DHT (**B**), without DHT in supplementation with hCG (**C**), and in cryostat sections in the epithelium of the caput epididymis of control rat (**D**). The yellow-green fluorescence indicates localization of the active mitochondria. Non-specific yellow aggregates of fluorochrome are visible in the cell cytoplasm (A and C). Mito Tracker Green FM (magnification ×670 A,B,C; magnification ×330 - D).

fluorescence. They were dispersed within the cytoplasm (Fig. 1A). The epithelial cells of the caput and cauda epididymis cultured in the medium without DHT contained significantly lower amount of active mitochondria (Fig. 1B). The epithelial cells of the caput and cauda epididymis cultured in the medium without DHT but supplemented with 12.5U hCG contained more numerous mitochondria with intensive yellow-green fluorescence (Fig. 1C) than the cells cultured in the medium without DHT. Frequently, non-specific yellow aggregates of fluorochrome were visible in the cytoplasm (Fig. 1A and C).

In cryostat slides, the epithelial cells of the caput and cauda epididymis of control rats showed active mitochondria (Fig. 1D).

Transmission electron microscopic studies (TEM)

The cytoplasm of the epididymal epithelial cells cultured with DHT was rich in mitochondria with lamellar cristae, osmophilic lipid droplets (Fig. 2A) and well developed smooth endoplasmic reticulum (Fig. 2B). The cytoplasm of the cells cultured in the medium without DHT was disorganized, however the osmophilic lipid droplets and spherical shape mitochondria were visible (Fig. 2C and D). The ultrastructure of epididymal epithelial cells cultured in the medium without DHT, supplemented with hCG was similar to the ultrastructure of the cells cultured in the medium with DHT. They cytoplasm contained mitochondria with lamellar cristae (Fig. 2E) and numerous osmophilic lipid droplets (Fig. 2F).

Hormone assays

The concentration of 17β-estradiol (E2) detected in the medium on day 3 of cell culture was considered basal. The concentration of E2 in the medium on day 5 of cell culture was considered to result from *de novo* synthesis. The concentration of E2 in the culture media where the cells were cultured with DHT was compared to the concentration of E2 in the media of cells cultured with DHT and supplemented with hCG (Table 1). The concentration of E2 in media of cells cultured without DHT was compared to the concentration of E2 in

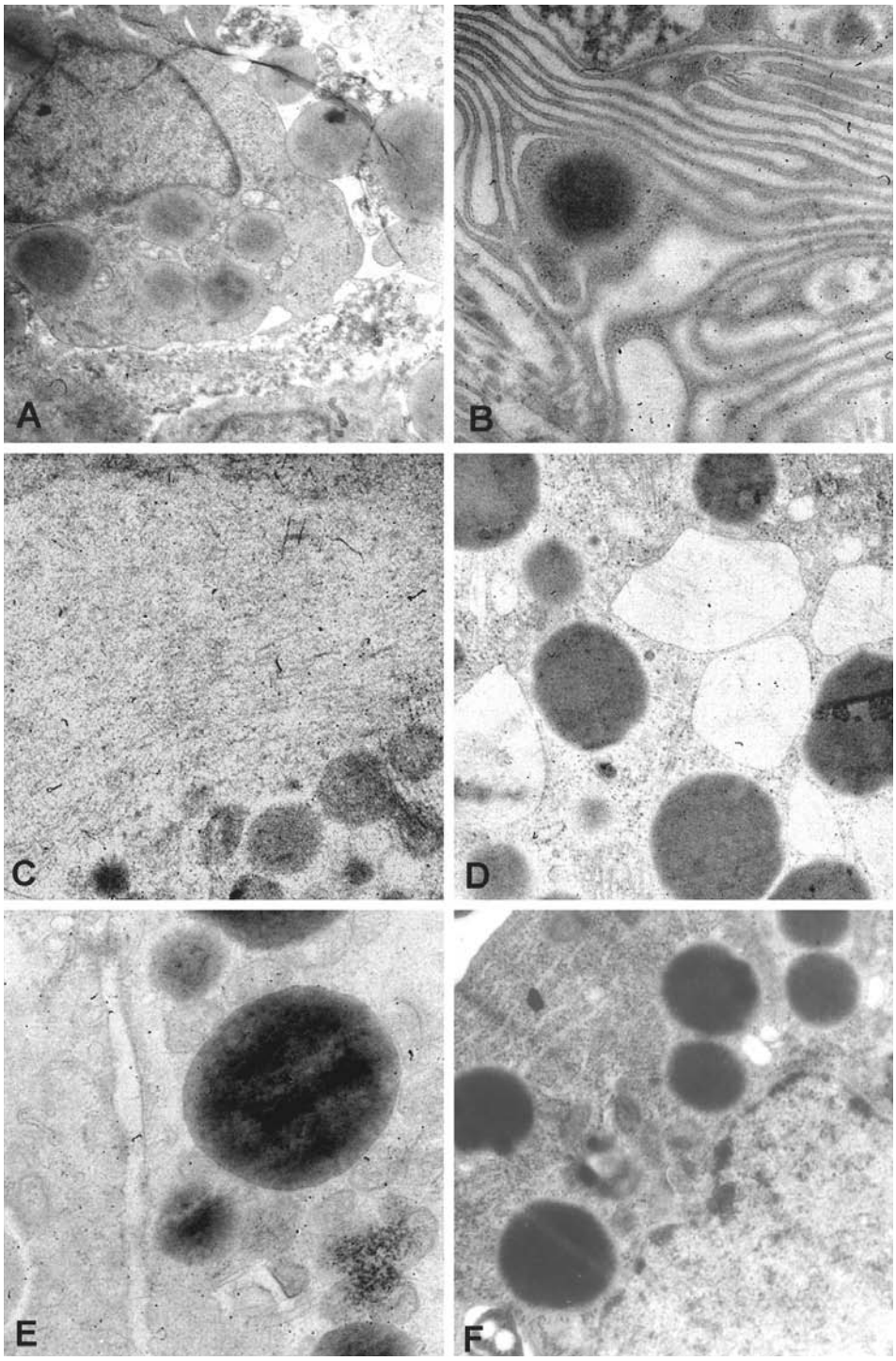

Fig. 2. Ultrastructure of the epididymal epithelial cells. **A, B**. Numerous mitochondria (M) with lamellar cristae (A), lipid droplets (L) and well developed smooth endoplasmic reticulum (SER) (B) in the cytoplasm of the cells cultured in the medium with DHT. **C, D**. The cytoplasm disorganization in the cells cultured without DHT (C). The large of lipid droplets (L) and round mitochondria (M) (D). **E, F**. Mitochondria (M) with lamellar cristae (arrows) and lipid droplets (L) in the cytoplasm of the cells cultured in the medium without DHT but in supplementation with hCG (magnification ×30000 - A, magnification ×22000 - B; magnification ×40000 - C; magnification ×30000 - D; magnification ×25000 - E; magnification ×10700 - F).

media of cells cultured without DHT in supplementation with hCG (Table 1).

There were no significant differences between concentration of E2 in medium of caput epididymal epithelial cells cultured with DHT on day 3. and 5., and in the medium of caput epididymal epithelial cells cultured with DHT and supplemented with hCG on day 3. and 5. The concentration of E2 in media of caput epididymal epithelial cells cultured with DHT on day 5. was significantly lower than on day 3. Concentrations of E2 in medium of caput epididymal epithelial cells cultured with DHT and with supplementation of 12.5 U/mL hCG on day 5. was significantly lower than on day 3 (Table 1).

The addition of 12.5 U/mL hCG significantly increased the concentration of 17β-estradiol in the medium of epithelial cells of cauda epididymis cultured with DHT on days 3. and 5. After 5 days of

Table 1. Concentration of estradiol (E2) in the media containing cells from caput and cauda epididymis cultured with /without DHT, and in supplementation with 12.5 U/mL hCG

Cell culture n=5		Epididymal caput E2 (pg/mL) 3 days of culture	Epididymal caput E2 (pg/mL) 5 days of culture	Epididymal cauda E2 (pg/mL) 3 days of culture	Epididymal cauda E2 (pg/mL) 5 days of culture
DHT	M $Q_1 – Q_3$ X ± SD	252.1 237.3 – 296.7 259.6 ± 36.2	161.4 157.8 – 172.7 164.4 ± 7.6 vs DHTcaput3**	253.2 199.8 – 390.2 284.5 ± 104.0	372.9 281.5 – 372.9 342.5 ± 61.8 vs DHTcaput5**
DHT + 12.5U/mL hCG	M $Q_1 – Q_3$ X ± SD	315.7 308.5 – 347.4 304.4 ± 59.3	189.8 149.1- 198.8 174.2 ± 31.4 vs DHT+ hCGcaput3**	560.2 560.2 – 604.1 548.1 ± 79.0 vs DHTcauda3** vs DHT+ hCGcaput3**	815.1 709.5 – 865.3 813.9 ± 115.3 vs DHTcauda5** vs DHT+ hCGcaput5** vs DHT+ hCGcauda3**
–DHT	M $Q_1 – Q_3$ X ± SD	221.4 178.3 – 244.4 210.8 ± 37.5	151.6 131.6 – 165.4 149.6 ±18.8 vs – DHTcaput3*	381.4 274.1 – 570.6 404.3 ± 161.9 vs – DHTcaput3*	320.8 307.4 – 464.4 372.8 ± 83.7 vs –DHTcaput5**
–DHT + 12.5U/mL hCG	M $Q_1 – Q_3$ X ± SD	280.5 231.6 – 343.3 285.4±57.7	215.3 211.2 – 218.2 215.0 ± 4.2 vs –DHT + hCGcaput3** vs –DHTcaput5**	425.1 399.5 – 430.8 413.5 ± 22.0 vs –DHT + hCGcaput3**	526.1 522.7 – 526.1 525.7 ± 3.4 vs –DHT cauda5** vs –DHT + hCGcaput5** vs –DHT + hCGcauda3**
Control medium	X ± SD	32.5 ± 8.5			

M - median, Q1-Q3 -lower-upper quartiles, X ± S.D. - mean ± SD, n = 5 animals per group, vs - versus, asterisks indicate statistically significant differences in Mann-Whitney U test: *p 0.05, **p<0.01

incubation of cauda epididymal epithelial cells with DHT and 12.5 U/mL hCG, the concentration of E2 in medium was significantly higher than with DHT only.

The concentrations of E2 in media of cauda epididymal epithelial cells cultured with DHT and with DHT with supplementation of hCG on day 3. and 5. were significantly higher than in media of respective caput epididymal groups.

The concentrations of E2 in media of caput and cauda epididymal epithelial cells cultured without DHT but with supplementation of 12.5 U/mL hCG on day 5. were significantly higher than in media of cells cultured without DHT and without 12.5 U/mL hCG (Table 1).

The concentrations of E2 in media of cauda epididymal epithelial cells cultured without DHT in supplementation with 12.5 U/mL hCG on day 3. and 5. were significantly higher than in media of respective caput epididymal epithelial cells. The level of E2 in media before the initiation of cell culture (control media) was many fold lower then in media of cultured cells (Table 1).

Immunostaining

Immunohistochemical product of reaction, localising of LH-R protein, was observed in the cytoplasm of epithelial cells of epididymis in intact and finasteride-treated rats as well as in the cytoplasm of epithelial cells of caput and cauda epididymis *in vitro*. There was no immunoexpression in nuclei of the cells.

In the epithelium of caput epididymis in control rats, the immunoexpression of LH-R was detected in the basal and apical part of principal cells cytoplasm (Fig. 3A). In the cauda epididymis, LH-R protein was located in the same pattern, however lower amount of cells in the epithelium showed immunoexpression (Fig. 3B). Treatment of rats with finasteride during time of one spermatogenesis resulted in the overexpression of LH-R in both caput and cauda epididymis epithelium (Fig. 3 C and D).

The intensity of LH-R expression in the cytoplasm of epididymal epithelial cells cultured in media with DHT (Fig. 4A) and without DHT in supplementation with hCG (Fig. 4B) was similar. The increase of intensity of LH-R expression was observed in the cyto-

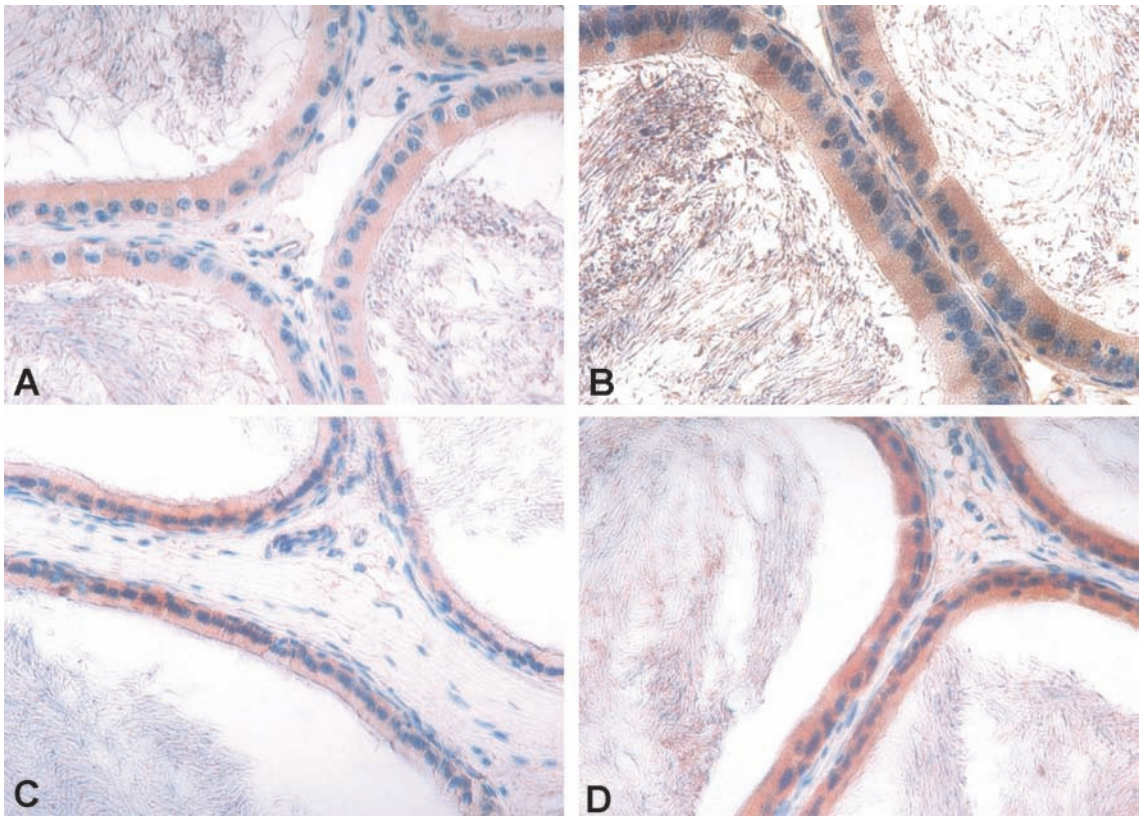

Fig. 3. Immunoexpression of LH receptors in the cytoplasm of epithelial cells in caput (**A**) and cauda epididymis (**B**) of intact rats and in the cytoplasm of caput (**C**) and cauda (**D**) epididymis of finasteride-treated rats (magnification ×670 - A,B,C,D)

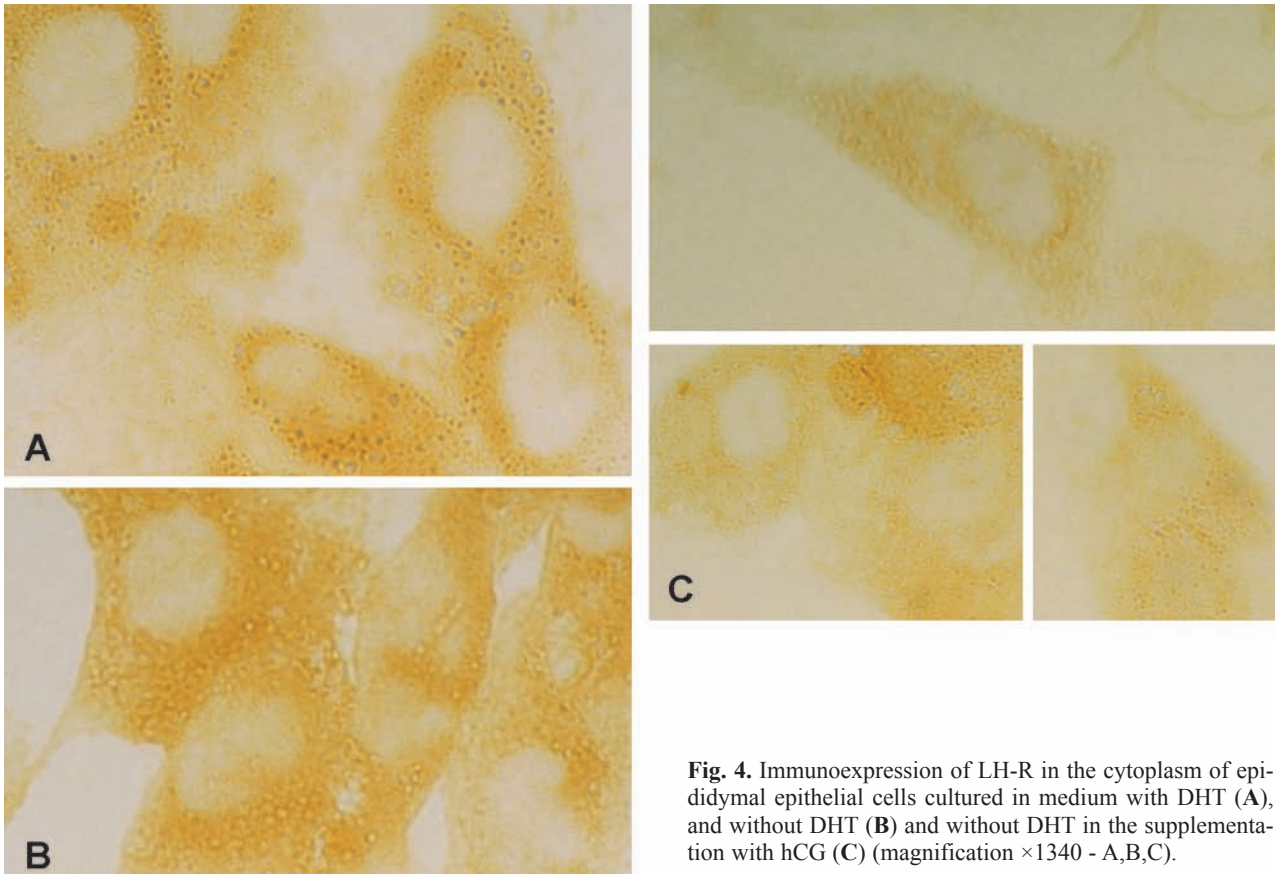

Fig. 4. Immunoexpression of LH-R in the cytoplasm of epididymal epithelial cells cultured in medium with DHT (**A**), and without DHT (**B**) and without DHT in the supplementation with hCG (**C**) (magnification ×1340 - A,B,C).

plasm of cells cultured in media without addition of DHT (Fig. 4C). There was no immunoreaction in specimens of the caput and cauda epididymis of control and experimental rats incubated without primary antibody.

Discussion

The main function of the epididymal epithelial cells is the synthesis of components of the epididymal fluid to create a specific microenvironment for sperm maturation, storage and survival [19-21]. However, apart from the secretory and reabsorptive activity, the epididymal epithelial cells are involved in epididymal steroidogenesis. Results of our former study [9,10] showed that principal cells of rat epididymal epithelium *in vitro* displayed the typical ultrastructure of cells included in synthesis of proteins as well as in process of steroidogenesis. Therefore, the cells in culture produce androgens which are converted to 17β-estradiol (E2), released into a medium in high concentration [9,10].

While the regulation of protein synthesis in epididymal epithelial cells is well characterized, the regulation of epididymal steroidogenesis is poor understood. It seems probable that disorganization of cytoskeletal elements (microtubules and microfilaments) caused by the lack of androgens in the culture medium promoted synthesis of E2 [22]. The phenomenon can reflect the effect of LH stimulation of estrogen synthesis in Leydig cells followed by the disorganization of cytoskeletal elements [23-25]. It is well documented that testicular Leydig cells contain receptor for luteinizing hormone (LH) and are responsive for LH or its analogue hCG (human chorionic gonadotropin). Chorionic gonadotropin is structurally similar to LH and these hormones showed the same affinity for LH/hCG receptors on the plasma membrane [26,27]. In many studies hCG is used instead of LH to exert LH activity. It is known, that acute and chronic LH/hCG stimulation of Leydig cells causes changes in their morphology [14,15], and prompts trafficking of intracellular cholesterol in which peroxisomes are included [28]. Both, LH and hCG are known to stimulate the steroidogenesis in the cells [14,15,25,29].

To study whether organelles in the cytoplasm of epididymal epithelial cells and E2 synthesis is influenced by LH/hCG, we cultured rat's epididymal epithelial cells in a medium without addition of DHT, but enriched in hCG. The gonadotropin was introduced into the medium on day 0. of cell culture and epididymal epithelial cells were exposed on the influence of hCG over the culture time. Therefore, we assented that the cells were chronically stimulated by hCG. Moreover, we tested the influence of finasteride, inhibitor 5α-reductase type 2, on the LH-R expression in the epididymis of rats.

As it was presented in our former studies, after spreading on the bottom of culture dishes, the epididymal epithelial cells cultured in the medium with DHT resumed their function [9,10,30]. The cytoplasm of the cells was rich in lipid droplets and PAS-positive substances, while the cytoplasm of the cells cultured in the medium deprived of androgens was poor in lipid droplets and PAS-positive substances. The first sign of stimulatory effect of hCG on the epididymal epithelial cells was the increase in the accumulation of PAS-positive substances and lipid droplets when the cells were cultured in the medium without exogenous DHT but in supplementation with hCG [13]. It can be expected that hCG stimulated androgen production by the cells in amount to maintain their morphology. To confirm the stimulatory effect of hCG on organelle in the epididymal epithelial cells cultured in the medium without DHT, we used a mitochondrion-selective reagent enable to probe mitochondrial activity, localization and abundance. The Mito Tracker Green FM passively diffuses across the plasma membrane, accumulates in the lipid environment of active mitochondria of living cells and becomes fluorescent [31].

There were numerous active mitochondria in the cytoplasm of epididymal epithelial cells cultured without DHT but under stimulation with hCG. They were more numerous than in the cytoplasm of the cells cultured in the medium without DHT.

Electron microscopy studies confirmed our observation in fluorescent microscope. The addition of hCG into the medium of the cells resulted in the increase amount large mitochondria with tubular cristae located close to lipid droplets. The changes observed in the cytoplasm of epididymal epithelial cells cultured without DHT but during chronic hCG stimulation are similar to changes in morphology of the Leydig cells. The changes observed in Leydig cells under LH stimulation, included increase in the cellular organelle, mainly smooth endoplasmic reticulum and mitochondria [14,15]. According to the similarities, we decided to control the influence of hCG on the synthesis of 17β-estradiol. Human chorionic gonadotropin stimulated the E2 synthesis by the epididymal epithelial cells *in vitro*, as it did in Leydig cells [23-25]. The cells under stimulation of hCG released E2 into the culture medium in concentration frequently statistically significant higher than the cells which were cultured without addition of hCG.

The organelle in the cytoplasm of epididymal epithelial cells and E2 synthesis can be influenced by hCG, because the cells of epithelium both *in vivo* and *in vitro* display the expression of LH/hCG receptors. In caput epididymis *in vivo* the immunostaining was visible in the most of epithelial cells while in the cauda

epididymis low number of cells expressed LH/hCG receptors. The differences can be connected with different function of the part of epididymis. The regional-specific expression of epididymal proteins is regulated mainly by androgens and/or testicular factors but some of proteins are under the influence of estrogens. The acquisition of fertilizing potential the spermatozoa occurs in the caput epididymis. This region is a very active in protein synthesis and secretion and many of the reproductive and somatic genes are expressed in this part [3,32]. The functions of cauda epididymis are to storage and maintain vitality of spermatozoa. Other genes are expressed in the region of epididymis and they need different regulation [3,33].

In epididymis of rats treated with finasteride we observed overexpression of LH-R in both studied segments of epididymis. It is reported that finasteride effectively inhibits DHT formation and reduced the level of the hormone in serum of rats [34,35]. Our earlier study showed that treatment of rats with finasteride for 56 days resulted in sloughing of germinal cells in testis, without changes in morphology of epididymal epithelium [36]. However, the immunoexpression of AR was altered in epididymis of finasteride-treated rats [37]. It could be suggested that the pronounced expression of LH-R in our experiment is connected with necessity of the stimulation of epididymal epithelial cells to the formation of the optimal microenvironment for sperm maturation in changed hormonal condition.

The study documents that the epididymis is a target tissue for LH/hCG. The gonadotropin produces changes in the morphology of epididymal epithelial cells *in vitro* similar to the those observed in Leydig cells. Moreover, hCG stimulates synthesis of E2 in the cells.

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