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Effect of hCG on the morphology of rat epididymal epithelial cells *in vitro*

Małgorzata Świder-Al-Amawi, Mariola Marchlewicz, Barbara Wiszniewska, Lidia Wenda-Różewicka

Department of Histology and Embryology, Pomeranian Medical University, Szczecin, Poland

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The epididymis is an androgen-dependent organ. The hormones regulate the morphology and secretory activity of the epididymal epithelial cells. The cells in vitro *resume their function as* in vivo *and also reveal features of steroidogenic cells. It can be expected that, as with Leydig cells, the morphology and function of the cells can be regulated by LH/hCG.*

The aim of the study was to assess the morphology of epididymal epithelial cells in vitro *after stimulation with hCG. The experiment was performed on cells isolated from sexually mature rats. The epididymal epithelial cells were cultured in a medium with the addition of dihydrotestosterone (DHT). Moreover, the cells were cultured in the medium with DHT and without DHT but enriched with hCG. The epididymal epithelial cells cultured with DHT formed a monolayer and accumulated glycogen, a PAS-positive substance and lipid droplets. The cells cultured without DHT were stellate in shape and low in glycogen and PAS-positive substance but they contained lipid droplets. The morphology of epididymal epithelial cells cultured without DHT but after stimulation with hCG was similar to the morphology of the cells cultured with DHT. This was the first sign that the morphology of the cells can be influenced by hCG.*

key words: epididymal epithelial cells, hCG, DHT, morphology, *in vitro*

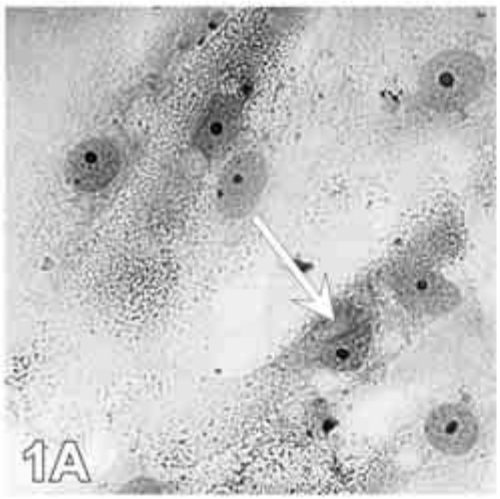
INTRODUCTION

Spermatozoa undergo morphological, biochemical, and functional modifications while traversing the epididymis. They require the ability to progress motility and egg fertilisation. The microenvironment for the maturation and storage of spermatozoa is created by the cells of the epididymal epithelium due to their secretory and reabsorptive activity [3]. Epididymal epithelial cells resume their function *in vitro* [8, 10]. They also appear as features of steroidogenic cells [9] and are able to produce and release androgens into the medium that are converted to 17β-estradiol [9]. Moreover, the cells express receptors for LH/hCG (LH/hCG-R) [7] and it may be supposed that epididymal steroidogenesis is regulated by gonadotropins. After stimulation with LH or hCG, steroidogenic cells e.g. Leydig cells response morphological changes following increase of steroidogenesis [6]. The aim of the work was to evaluate whether the morphology of the epididymal epithelial cells *in vitro* is influenced by stimulation with hCG.

MATERIAL AND METHODS

Epididymides were obtained from adult Wistar rats, each weighing 300–350 g. The rats were kept at a controlled temperature a photoperiod of 12 h light and 12 h dark. The animals were anaesthetized with sodium pentobarbitone (200 mg/kg body weight).

Address for correspondence: Barbara Wiszniewska, Department of Histology and Embryology, Pomeranian Medical University, Al. Powstańców Wlkp. 72, 70–111 Szczecin, Poland, tel/fax: +48 91 466 16 77, e-mail: barbwisz@sci.pam.szczecin.pl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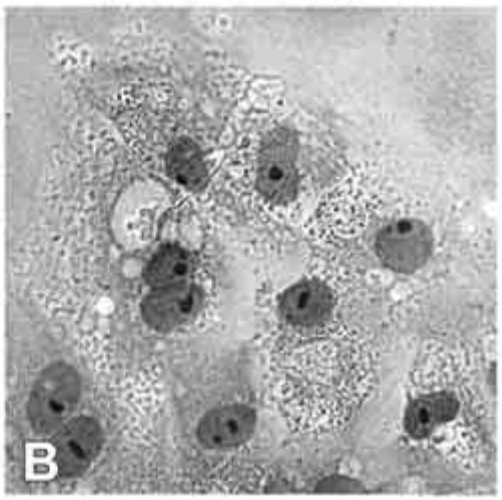
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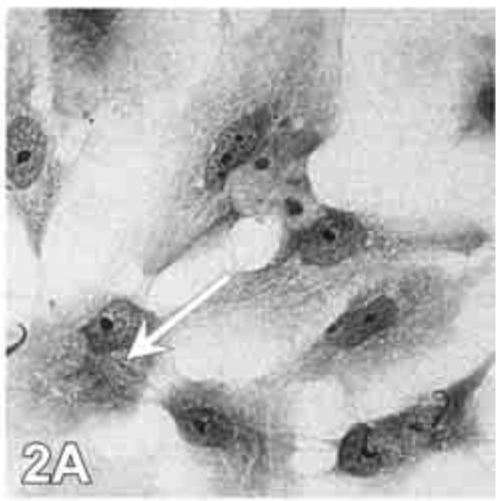
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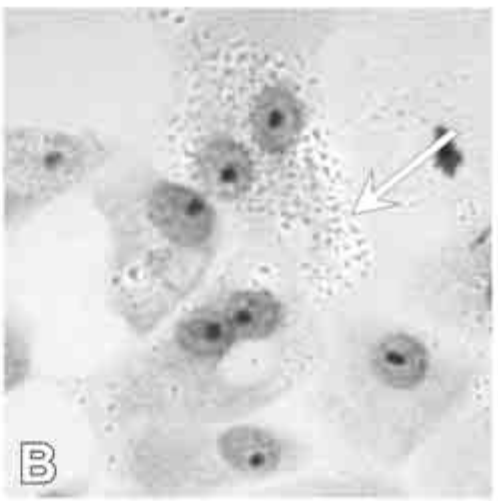
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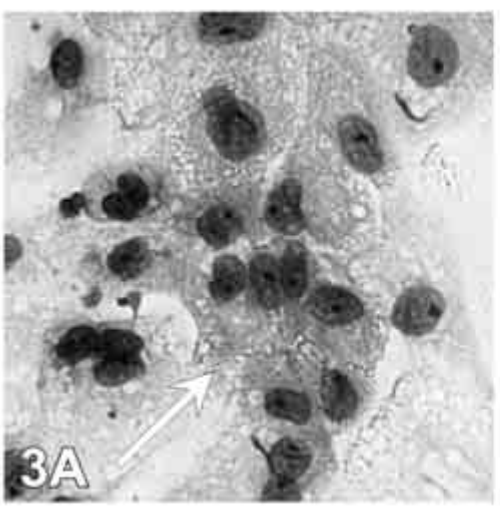
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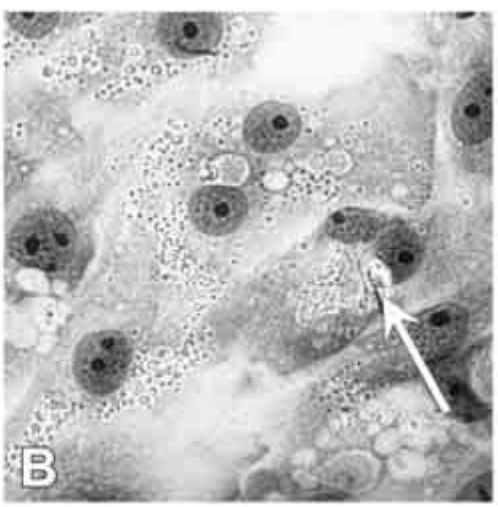
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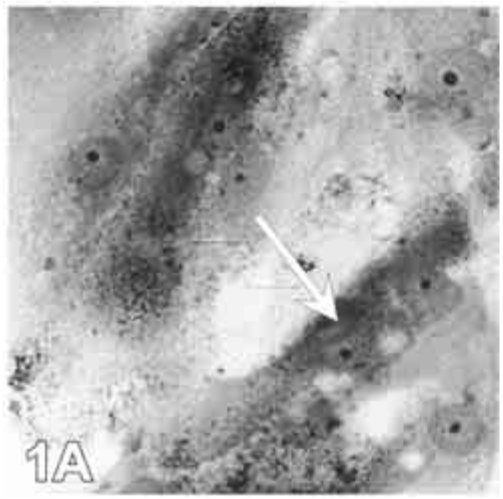
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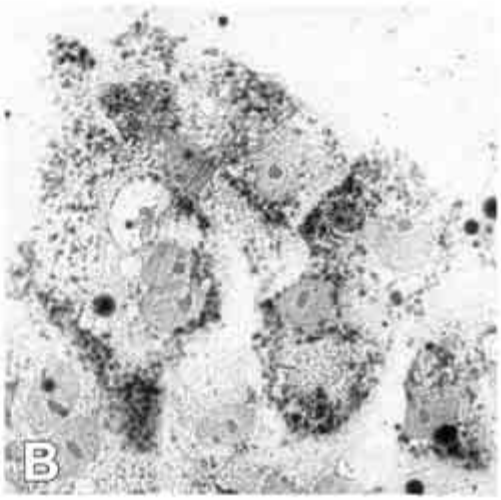
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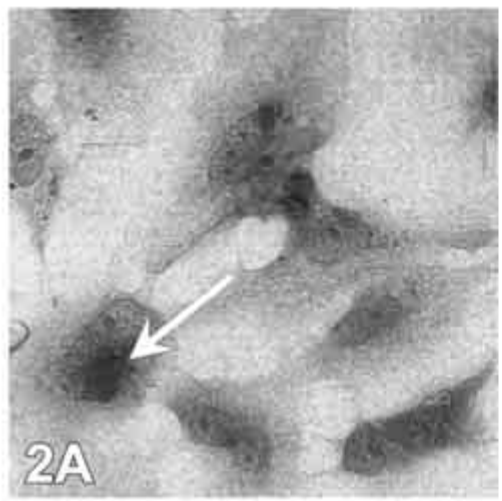
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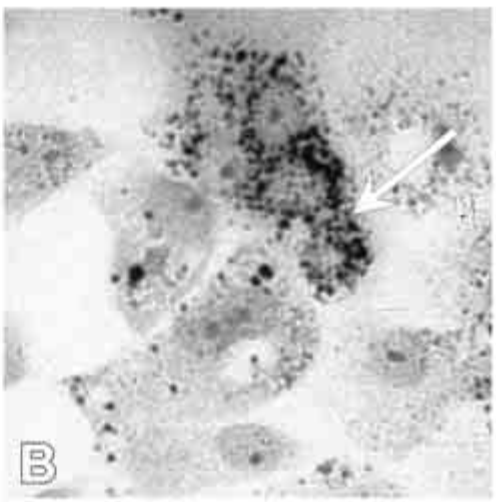
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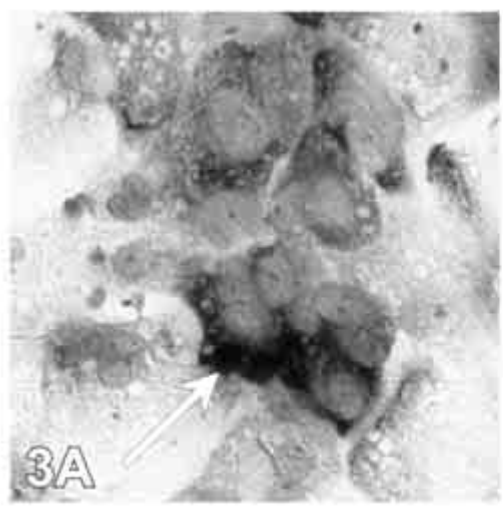
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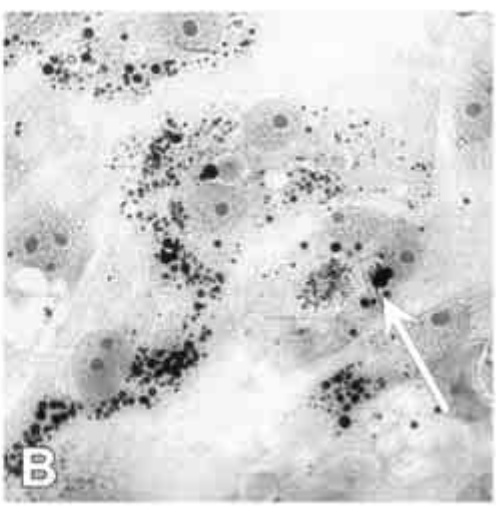
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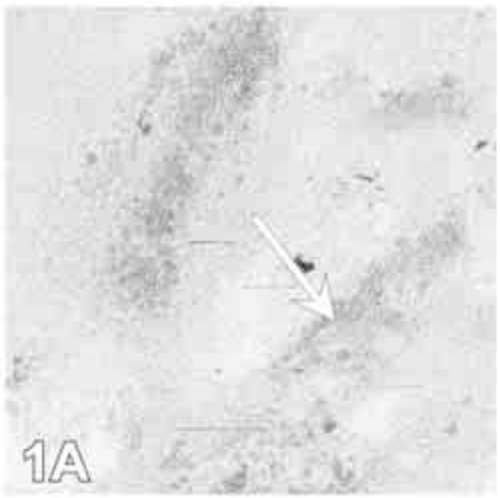
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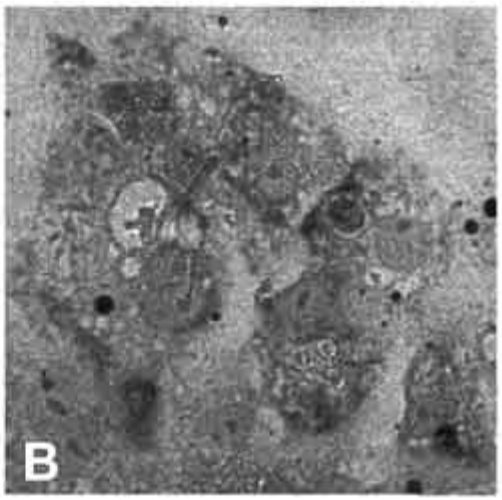
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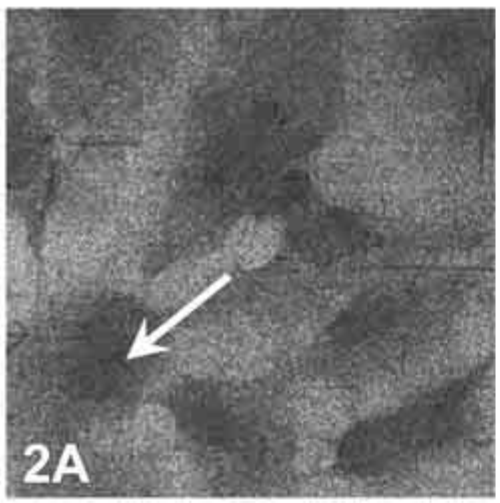
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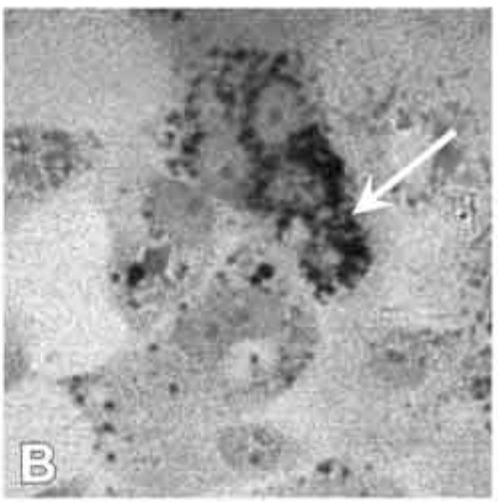
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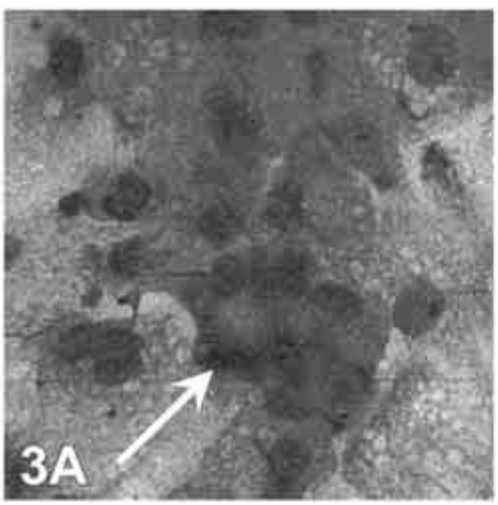
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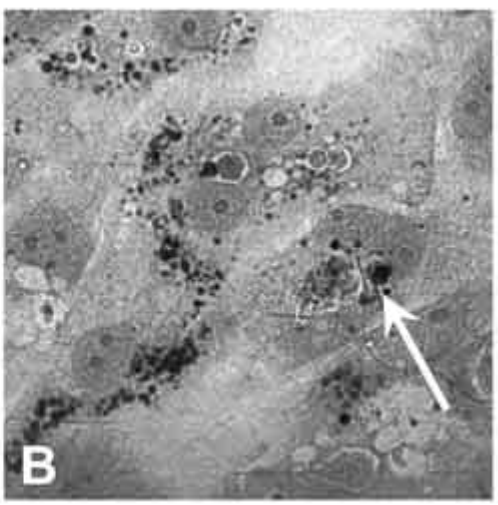
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Figure 1. Epididymal epithelial cells cultured with DHT. The cytoplasm rich in glycogen and PAS-positive substance (arrow) (**A**) and lipid droplets (**B**). A — PAS, B — ORO; Scale bar, 100 μm.

Figure 2. Epididymal epithelial cells cultured without DHT. The cytoplasm low in glycogen and PAS-positive substance (arrow) (**A**) and lipid droplets (arrow) (**B**). A — PAS, B — ORO; Scale bar, 100 μm.

Figure 3. Epididymal epithelial cells cultured without DHT and after stimulation with 12.5 U hCG. The cells form a monolayer. The cytoplasm rich in glycogen and PAS-positive substance (arrow) (**A**) and rich in lipid droplets (arrow) (**B**). A — PAS, B — ORO; Scale bar, 100 μm.

The procedure for the isolation and culture of epididymal epithelial cells has been described above [4]. The 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 foetal calf serum (FCS; Gibco BRL, Grand Island, USA), antibiotics, with and without the addition of 0.1 nmol/L dihytestosterone (DHT; Sigma Chemical Co, St Louis MO, USA). The epididymal epithelial cells cultured without DHT were supplemented by 12.5 U hCG (Chorulon — Intervet, Belgium). The cells were cultured at 34°C, in 5% CO_2 for 3 and 5 days. The cultures of the epididymal epithelial cells were stained with Oil Red O (ORO) for visualisation of lipid droplets and the PAS method for the presence of PAS-positive substance [1].

The experiment received the approval of the Local Ethical Committee.

RESULTS AND DISCUSSION

The epididymal epithelial cells *in vitro* resumed the function as *in vivo*. After 3 days of culture in the medium with DHT, the cells formed a monolayer (Fig. 1A, B) composed of adjoining cells, which were polygonal in the shape. The cytoplasm of the cells accumulated glycogen and PAS-positive substance (Fig. 1A). The cytoplasm of the cells was also rich in lipid droplets (Fig. 1B). It is well documented that to maintain their typical morphology and function, the epididymal epithelial cells *in vitro* require supplementation with androgens — testosterone (T) or dihydrotestosterone (DHT) [2, 10]. The morphology of the cells was changed when they were cultured in the medium without the addition of DHT. They took on a stellate shape and were connected by cytoplasmic processes (Fig. 2A, B). The cytoplasm of the epididymal epithelial cells was low in glycogen and PAS-positive substance (Fig. 2A) but contained lipid droplets (Fig. 2B). The cells synthesise and release androgens into the medium but in insufficient amounts to maintain their morphology [10]. The morphology of the cells was altered when the medium was supplemented with 12.5 U hCG. The cells appeared similar to the cells cultured in the medium with DHT. The cytoplasm of the cells was rich in glycogen and contained more PAS-positive substances (Fig. 3A) and lipid droplets (Fig. 3B). The observation is the first sign that hCG influences the morphology of the epididymal epithelial cells. It can be expected that LH/hCG can also influence the cells *in vivo*. In our former study we identified LH receptors in the epithelial cells of the rat epididymis [7]. Additionally, both LH and hCG demonstrate the same affinity towards receptors [5]. It seems that in the epididymal epithelial cells a similar situation exists as that in Leydig cells. After stimulation with LH/hCG ultrastructural changes in the cytoplasm of Leydig cells were observed. The area occupied by smooth endoplasmic reticulum was increased as well as the number of mitochondria [6]. Further morphological studies are required to assess whether LH/hCG exert a similar influence on the epididymal epithelial cells.

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