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Morphology and function of rooster efferent ductule epithelial cells in culture

S. J. Janssen', D. Bunick 2 , C. Finnigan-Bunick 2, Y. C. Chen 21, R. Hess2, J. M. Bahr 3

Abstract. Regulation of the excurrent ducts of the testis is not well understood, particularly in avian species. **To investigate the role of steroid hormones** in the male reproductive tract, we developed a primary cell culture of epithelia isolated from rooster ductuli efferentes (efferent ductules). Efferent ductules of the avian testis comprise 77% of the epididymal region and form a mass of tubules containing a heavily folded epithelium enmeshed in connective tissue. The epididymal region was separated by microdissection and small epithelial plaques isolated by serial digestion with collagenase, elastase and repeated pipetting. Isolated cell plaques were cultured in a bicameral chamber on Millicell-CM inserts coated with two layers of basement membrane matrix, consisting primarily of laminin and Types I and IV collagen. Active ciliary beat was observed before plating and this activity was maintained for 14 days in culture. Cell plaques attached within 24 h and outgrowths formed a confluent monolayer by 5-6 days. The epithelial nature of cultured cells was demonstrated by immunocytochernical staining for cytokeratin. Light and electron microscopy confirmed that morphology and polarity of the original epithelial cells were maintained in culture. Cultured efferent ductal epithelium was cuboidal in shape and maintained many of the cytoplasmic organelles typical of these cells in vivo. The uptake of cationic ferritin indicated the endocytotic activity of these cultured cells was maintained. Estrogen receptor mRNA expression was maintained in cultured cells. These data demonstrate avian efferent ductal epithelium can be isolated and grown in defined culture medium for the purpose of determining the role of hormones and other factors in regulating the function of the epididymal region in the bird.

Keywords: Efferent ductule, cell culture, estrogen

Introduction

Production of fertile sperm is dependent on proper functioning of the testis and excurrent duct system. Sperm produced in the become concentrated in the excurrent ducts of the male reproductive tract. The excurrent ducts of the avian species consist of the rete testis, proximal and distal efferent ductules (ED), a short connecting tubule, ductus epididymis and the ductus deferens (Hess et al., 1976; Tingari, 1971). The rete testis conduct sperm rapidly from seminiferous tubules of the testis to the ED. Avian ED consist of a highly convoluted network of tubules intertwined with the aqjacent rete testis and epididymal duct. The epithelium of ED is heavily folded and has a large luminal surface area (Fig. 1). There are two cell types found in ED, ciliated and non-ciliated cells. Abundant connective tissue is present throughout the rooster reproductive tract.

ED play an essential role in male fertility. In quail, ED comprise 77% of the epididymal region, suggesting ED of the avian species represent a more important component of

'Department of Molecular and Integrative Physiology 2Department of Veterinary Biosciences 3Department of Animal Sciences, University of Illinois, Urbana, IL 61801, USA §Current address: Department of Radiation Oncology and Pathology, Wayne State University, Detroit, MI 48202, USA.
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Correspondence to: Janice M. Bahr, Department of Animal Sciences, 326 Animal Sciences Laboratory, 1207 W. Gregory Dr., University of Illinois, Urbana, IL 61801. Tel.: +1 (217) 333-2900; Fax: +1 (217) 333-8286; E-mail: j-bahr@uiuc.edu

'Department of Molecular and Integrative Physiology 2Department of Veterinary

become concentrated in the excurrent ducts of the male

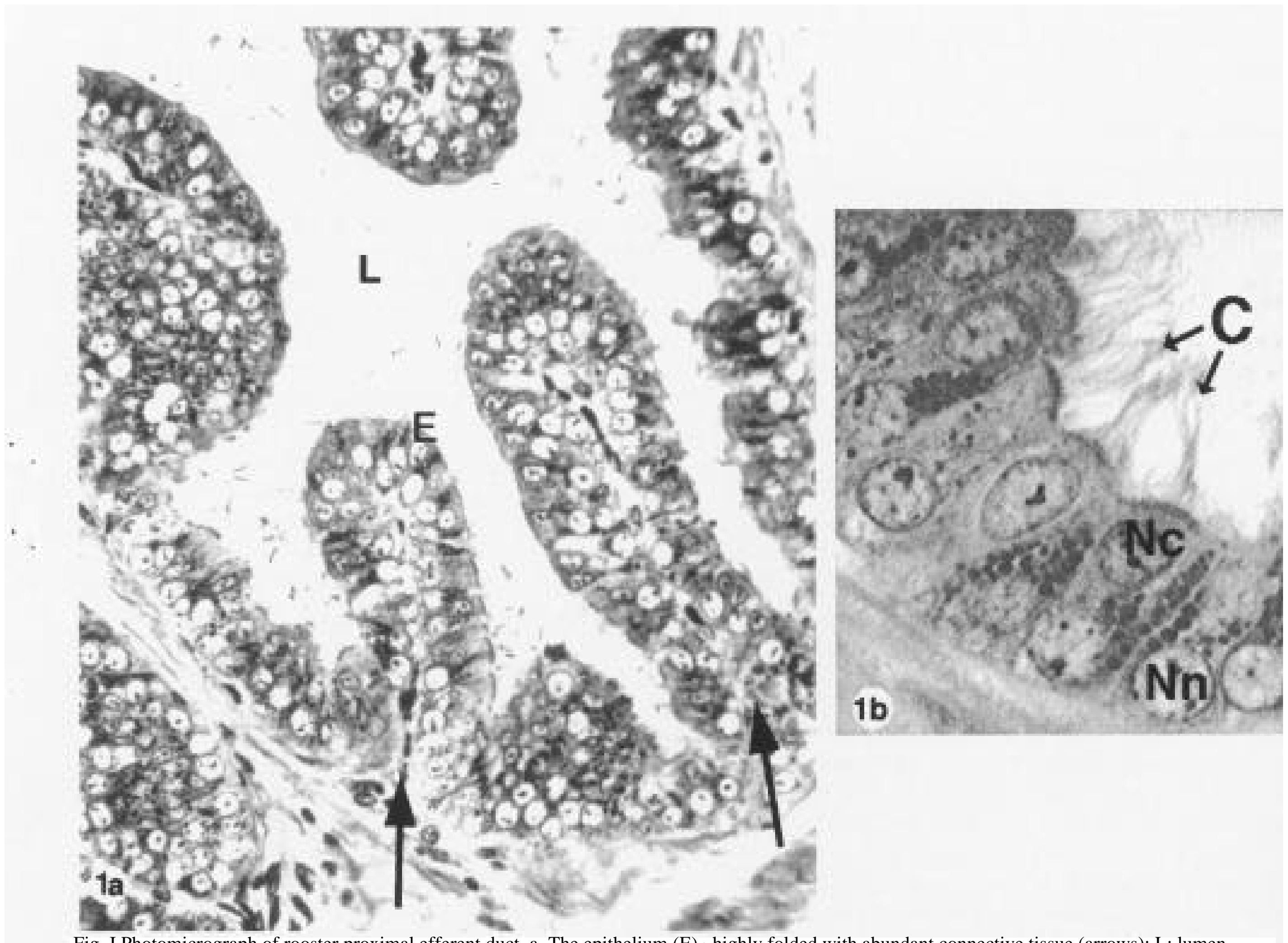

Fig. I Photomicrograph of rooster proximal efferent duct. a. The epithelium (E) is highly folded with abundant connective tissue (arrows); L: lumen. x570. b) Two cells types are present in this region: ciliated and non-ciliated cells. The basally located nucleus of a non-ciliated cell (Nn) is shown adjacent to the more apically located nucleus of a ciliated cell (Nc). Long cilia project into the lumen (C). x 1850

the excurrent duct system than does the rete testis or epididymal duct (Clulow & Jones, 1988). Primary functions of ED are reabsorption of -90% of rete testis fluid, concentration and transport of sperm, protein reabsorption and secretion, and phagocytosis of stagnant sperm (Clulow & Jones, 1988; Hess & Thurston, 1977; Nakai et al., 1989; Tingari, 1972). Dysfunction of the ED region in avian species results in decreased fertility (Hess et al., 1982; K irby et al., 1990)_ Despite the importance of the ED in male fertility, little is known about the regulation of its necessary functions. In fact, few studies have been published on rooster reproduction and the importance of ED in fertility.

To investigate the potential role of steroid hormones in the regulation of ED, we have developed a primary epithelial cell culture system. In vitro studies are a better approach for studying hormonal effects in ED epithelium because in vivo approaches would require long-term administration of hormones which disrupt the hypothalamic-pituitary-gonadal axis. Also, anatomical complexity ligation plus hormone replacement or micropuncture to investigate steroid regulation of ED. In vivo approaches are further complicated by the proluminal orientation of the ED epithelium, resulting in a greater response to luminal hormones and other factors, than to circulating hormones (Turner, 1991). The use of a primary cell culture system provides a direct method of determining the roles of steroid hormones in the function of avian ED epithelium. The present study describes establishment of an in vitro culture of ED epithelium and procedures employed to confirm that the epithelial characteristics maintained in vitro are analogous to those in vivo.

Materials and Methods

Tissue culture

Epithelial culture preparations consisted of two steps: preparation of extracellular matrix (ECM) and isolation of epithelial cell plaques. The preparation of collagen/matrix

of the avian epididymal region complicates the use of ED

coated Millicell-CM insert and the procedure of cell culture

were based on protocols established by Klinefelter (Klinefelter, 1992).

Preparation of ECM

The extracellular matrix for cell culture inserts consisted of two layers. The first layer contained Types I and IV collagen and laminin. The second layer contained a commercially prepared extracellular matrix, Matrigel (Collaborative Biomed. Products, Lexington, MA), derived from Engelbreth-Holm-Swarm tumor cells and contained laminin, Type IV collagen, entactin and heparin sulfate proteoglycan.

Rat tail (Type 1) collagen was isolated as described by Klinefelter (Klinefelter, 1992). Two days prior to cell culture, 1.8 ml of isolated rat tail collagen was mixed with 0.32 ml of 1OX Hank's Balanced Salt Solution (HBSS) (GIBCO BRL Products, Grand Island, NY), buffered with 260 μM sodium bicarbonate (GIBCO BRL, Grand Island, NY), pH 7, and 0.1 ml fetal bovine serum (FBS) (GIBCO BRL, Grand Island, NY). After thorough mixing, I ml was removed and added to another culture tube containing -133 μg laminin (Collaborative Biomed. Products, Lexington, MA) and Type IV collagen (Collaborative Biomed. Products, Lexington, MA). After thorough mixing, 0.05 ml of the gel mix was pipetted onto several Millicell-CM semipermeable inserts (12 mm diameter, 0.4 μm pore) (Millipore, Bedford, MA) placed within individual wells of a multi-well culture dish (Costar, Cambridge, MA). The matrix mixture was spread evenly over the entire surface by gentle tapping on adjacent sides of the culture dish. Inserts were allowed to dry overnight in an incubator set at 37°C in a 5% CO_2:95% air atmosphere. The following day inserts were overlaid with 0.05 ml Matrigel diluted 1:10 in sterile PBS and again dried overnight. On the day of culture, inserts were pre-wet with Dulbecco's Modified Eagles Medium (DMEM) (Sigma, St. Louis, MO) plus 10% FBS. Smaller inserts (6.4 min diameter, 0.4 μm pore) (Becton Dickinson Labware, Franklin Lakes, NJ) were also used and coated with 0.025 ml Matrigel only.

Buffer and Media formulations

A 1X HBSS buffer was prepared from I OX HBSS concentrate buffered with 15 mM HEPES and 4 mM sodium bicarbonate, and formulated to contain 0.2% BSA (Fisher, Fair Lawn, NJ), 5 mM D-glucose (Sigma, St. Louis, MO), I mM sodium pyruvate (GIBCO BRL, Grand Island, NY) and 25 mg/ml soybean trypsin inhibitor (Sigma, St. Louis, MO) pH 7.4. DMEM contained L-glutamine and 1000 mg/L glucose and was phenol-red free. DMEM was buffered with 44 mM sodium bicarbonate and formulated to contain 10% tryptose phosphate broth (GIBCO BRL, Grand Island, NY), 1000 U-μg/L penicillin-streptomycin (GIBCO BRL, Grand Island, NY), 5 mg/L transferrin (Sigma, St. Louis, MO), 100 nM hydrocortisone (Steraloids, Inc., Wilton, NJ) and 200 nM dihydrotestosterone (DHT) (Sigma, St. Louis, MO). For estrogen-treated culture groups, medium was formulated to contain I nM 17p-estradiol (Sigma, St. Louis, MO). Steroids were prepared in 100% ethanol and the fi concentration of ethanol in the medium was <0.0001%.

Mature, white Leghorn roosters, ages 18-52 weeks, hous at the poultry farm at University of Illinois under a 15L: lighting schedule and provided feed and water ad libitum, w killed by cervical dislocation. The testes containing epididymal region were removed, quickly rinsed in 70% etha and then immersed in prewarmed HBSS. The number of roost used depended on the experimental design. Typically, rooster yielded enough cells to plate two, 12 mm diame Millicell-CM inserts. The epididymal region was isolated i Petri dish containing sterile HBSS using an Olympus SZH-IL dissecting microscope, No. 5 jewelers forceps and fine spr scissors. The surrounding capsule and connective tissue w removed before dividing the epididymal tissue into sections. Sections were transferred to a sterile 50 ml centrif tube and tubules uncoiled using 2.0 mg/mI collagenase (Boehringer-Mannheim, Indianapolis, IN) and 0.33 mg Type I elastase (Sigma, St. Louis, MO) in HBSS at 37°C i shaking water bath for 30 min. Fragments of tubules w formed during this digestion. The contents of the centrif tube were then transferred to a clean Petri dish for removal loosened connective tissue with sterile forceps under dissecting microscope.

A second digestion was performed with 2.0 mg collagenase, 2.0 mg/mI Type IV hyaluronidase (Sigma, Louis, MO) and 0.33 mg/ml elastase in HBSS for 15 min. A 15 min, the tubular fragments were rapidly pipetted 15 tin through a 10 ml sterile pipette, and returned to the water b for an additional 15 min. After a second rapid pipetting of times, 35 ml of HBSS were added to the tube to st digestion. Fragments were allowed to settle by gravity for 10 min. The supernatant, containing connective tissue a sperm, was aspirated until 1.5-2 ml of fluid was left in t tube. The pellet and remaining fluid were then transferred t 15 ml conical tube. After pipetting 20 times with a sterile in Pasteur pipette, 10 ml of HBSS was added and the soluti allowed to settle by gravity again. The supernatant v removed and the pellet transferred to a Petri dish for furt removal of remaining connective tissue with sterile forc under the dissecting microscope. By this time, smaller tub fragments, <1 mm', and some smaller cell aggregates h formed.

A third digestion was performed after transferring the tub fragments to a clean 15 ml tube. Tubules were suspended DMEM plus 10% FBS and digested with I mg/mI of collagen for 3 min in the shaking water bath. After pipetting 20 tin with a Pasteur pipette, 10 ml of DMEM were added and solution allowed to settle for 5 min before removing supernatant. The pellet was transferred a final time to a P dish for sterile removal of any remaining connective tis under the hood. At this point, as a result of the repea digestions and pipettings. epithelial cell plaques had forn from the tubular fragments and aggregates. These cell plaq were 100-300 μm in diameter and contained 50-2 cells/plaque. The

epithelial isolate was then transferred to a 15 ml tube and after several washings and pipettings the pellet resuspended in DMEM plus 10% FBS. Final cell plaque concentration was - 1000 cell plaques/ml medium. Added to each Millicell insert were 300 µl of the final cell suspension and the plaques were dispersed evenly across the ECM by gently tapping adjacent sides of the culture dish. Finally, 200 µl of DMEM + 10% FBS were added to the basal chamber and cultures incubated overnight at 37˚C in a 5% CO_2:95% air atmosphere.

The next day, medium from the basal chamber was aspirated and medium from the apical chamber allowed to drain to facilitate attachment of epithelial cell plaques to the basement membrane matrix. After removing drained medium, 350 µl and 200 µl of DMEM (without FBS) containing 0. 18 mg/mI bovine lipoprotein (Sigma, St. Louis, MO) were added to the apical and basal chambers, respectively. Epithelial cell plaques attached to the basement membrane matrix and began to form a single layer of cells after several hours. Cultures were maintained in serum-free medium. Some cultures were treated with I nM 17B-estradiol beginning on day 2 of culture. Medium was subsequently changed daily. Medium without phenol red was used to preclude any potential estrogenic activity.

Morphology

Cell cultures were observed daily using an Olympus T041 inverted microscope. Photographs were taken using a Sony DKC-5000 digital camera and printed on a Kodak dyesublimation printer.

Immunofluorescence

Indirect immunofluorescence staining of cytokeratin was done as a modification of the procedure of Mostov and Deitcher (Mostov & Deitcher, 1986). After 5 days of culture, monolayers were washed twice with PBS and fixed in PBS solution (pH 7.4) containing 1.5% paraformaldehyde and 0.2% Triton xI00 at 4˚C for 1 It. After two PBS rinses, non-specific binding was blocked with a PBS solution containing 0.2% gelatin (PBS-G). Cells were then incubated at 4˚C for 30 min with a mouse monoclonal anti-Pan cytokeratin antibody (Sigma, St. Louis, MO) diluted 50-100 times in PBS-G. This antibody recognizes cytokeratins 1,4,5,6,8,10,18 and 19 found in human epithelial cells and has a wide range of species cross-reactivity. Controls were incubated with mouse preimmune ascites fluid. After two PBS-G rinses, samples were labeled for 30 min at 25˚C with a fluorescein isothiocyanate (FITC) conjugated goat anti-mouse IgG (Sigma, St. Louis, MO) diluted 1:100 in PBS-G. Cell monolayers were washed two times with PBS and mounted on a slide in SlowFade™ solution (Molecular Probes Inc., Eugene, OR). Samples were photographed on Kodak T-400 film (Eastman Kodak, Rochester, NY) at 400 ASA using a Zeiss Fluorescent microscope.

Electron microscopy

(Tousimis, Rockville, MD), 0. 1 M sucrose (pH 7.4) at 4˚C for I h. After glutaraldehyde fixation, cells were washed three times in a collidine/sucrose buffer and post-fixed in a solution of 1.5% potassium ferrocyanide and 2% osmium tetroxide (pH 7.4) at 4˚C for I h. Samples were washed three times with deionized water and dehydrated through a series of ethanol solutions and propylene oxide. Cell monolayers were embedded in epoxy resin and polymerized at 90˚C for 2 h. Sections I µm thick were stained with toluidine blue in 1% sodium tetraborate and observed by bright field microscopy. Selected ultrathin sections of cell monolayers were stained in uranyl acetate and lead acetate. Cells were photographed with a JEOL 100CX electron microscope at 80KV.

To evaluate endocytotic function in the cultured cells, 5-day monolayers were rinsed with fresh DMEM medium twice and then incubated with I mg/ml of cationic ferritin (Sigma, St. Louis, MO) at 34˚C for 10 min. Ferritin uptake was stopped by pipetting glutaraldehyde fixative over the cultures. Samples were then processed according to the above description for electron microscopy.

RNA isolation

Fresh cultured efferent ductal epithelium was collected from 5-day cultures. Total cellular RNA was isolated according to the method described by Sambrook et al. (1989) with a slight modification. Fresh cell cultures were homogenized in 0.5 ml of buffer containing 4 M guanidine

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thiocyanate (GT), 25 mM sodium citrate (pH 7.0), 0.5% N-lauroylsarcosine and 0. 1 M B-mercaptoethanol. Total nucleic acids were extracted with phenol/chloroform/isoamyl alcohol (v:v:v/25:24:1) and precipitated with 2 M sodium acetate (pH 4) on ice for 15 min. Samples were centrifuged at 10 000 x g for 30 min at 4˚C. Supernatants were transferred to new microfuge tubes and incubated with 1v of isopropyl alcohol at -20˚C for 30 min. After centrifugation at 10 000 x g for 30 min at 4˚C, supernatants were discarded and pellets were suspended in 200 µI of GT buffer and isopropanol (v:v/1:1) and incubated at -20˚C for 30 min. RNA samples were centrifuged at 10 000 x g for 30 min at 4˚C, pellets were washed with 70% ethanol and resuspended in diethylpyrocarbonate (DEPC)-treated water. RNA sample concentration was determined by photometric quantitation at A260 and stored at -70˚C until further use. Each culture insert yielded -2 µg of total RNA.

Reverse transcription polymerase chain reaction

Gene specific primers, 11 15L, 1484R and 1808R (numbered according to GENEBANK sequence, accession #X03805, Krust et al., 1986) were selected to amplify a conserved region of the chicken estrogen receptor. For this experiment, first strand cDNA was synthesized by annealing I µg RNA sample, 9.5 µl of DEPC-H_20 and I µl of 10 µM primer 1808R in a 65˚C water bath for 10 min. Samples were then immediately chilled in an

For electron microscopy, 5-day-old cell monolayers were fixed ice bath for 2 min, and quickly centrifuged at 10 000 x g for 10

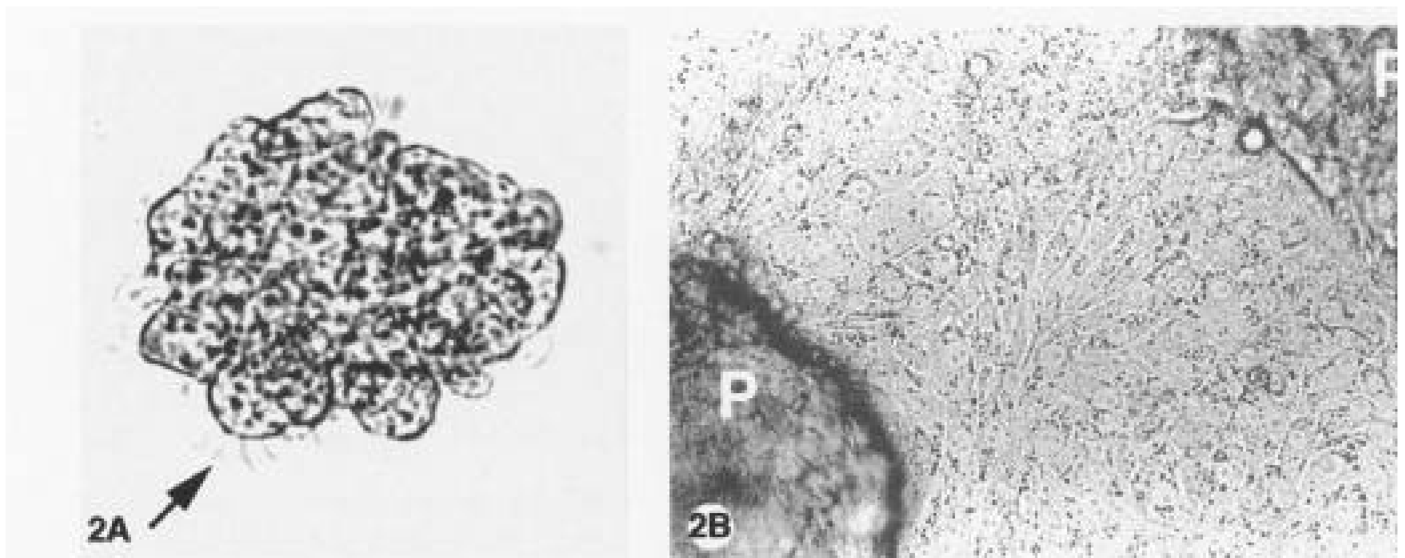

Fig. 2 Cells of rooster efferent ductules as viewed with a phase-contrast microscope. A Fresh isolated cell plaques with active ciiary beat (arrow) before attachment to basement membrane; arrow: cilia. x 540 (B). B 5-day cell monolayer; P: original cell plaque. x 514.

benchtop centrifuge. Samples were mixed with 4 µl of 5X reverse transcription (RT) buffer, I µl of 10 mM deoxyribonucleoside triphosphates (dNTPs), 2 µl of 0. 1 M dithiothreitol (DTT), 0.5 µl of RNase inhibitor, and incubated in a 42˚C water bath for 2 min. Added to the reaction mixture and incubated at 42˚C for 30 min was I µl of SuperScript 11 reverse transcriptase (200 units/µI, GIBCO BRL, Grand Island, NY). The reaction was terminated by incubating at 70˚C for 15 min and chilling on ice for 5 min. After reverse transcription, DNA samples were incubated with I µl of Rnase H (GIBCO BRL, Grand Island, NY) at 42˚C for 10 min to digest excess RNAs and purified using a GlassMax DNA Clean Cartridge system (GIBCO BRL, Grand Island, NY) to remove excess primers and small RNA products.

To amplify the DNA, 2 pi of first strand DNA, 5 µl of lOx PCR buffer, 37 µl of DEPC-H2O, 2.5 µl of 50 mM MgCl2, I µl of 10 mM dNTPs, I µl of 10 µM 180SR primer and I µl of 11 15R primer were mixed and overlaid with 75 µl of mineral oil. After preheating for 5 min at 94˚C, 0.5 µl of Taq DNA polymerase (5 units/µl, GIBCO BRL, Grand Island, NY) was added to each tube. Reaction mixtures were subjected to thermal cycles as follows: 35 thermal cycles of 94˚C for 45 s, 57˚C for 25 s, and 72˚C for 2 min, followed by 72˚C for 15 min and storage at 4˚C. PCR products were purified using a GlassMax DNA Clean Cartridge to remove excess primers. PCR products were diluted 1:20 with deionized water, and I [l of diluted PCR product was subjected to hemi-nested amplification with downstream primer 1484R and upstream primer I I 15L as above. The PCR samples were electrophoresed in a 1.5% agarose gel and stained with ethidium bromide. The DNA bands were visualized on a transilluminator and photographed with Polaroid film.

Results

Cultures

Primary epithelial cell cultures of rooster ED were established. Isolated ED epithelial cell plaques contained both ciliated and non-ciliated cells. Freshly isolated plaques were observed to have vigorously beating cilia which propelled the plaques throughout the medium prior to seeding (Fig. 2A).

Cell plaques attached to the basement membrane matrix within 24 h. This process was facilitated by placing cell plaques in a minimal amount of medium to limit plaque movement owing to vigorous ciliary beat. After the Ist day in culture, cells plaques began to spread and covered 70-80% of the surface area of an insert after 5-6 days in culture. When smaller, 6.4 turn diameter inserts were used, 100% confluent monolayers were obtained after 5 days. The original cell plaque exhibited a raised appearance compared to the peripheral, spreading epithelial cells (Fig. 2B). Beating cilia were observed in the cultured cells from ED for 14 days. A physiological concentration of 200 nM DHT in the culture medium was required for proper cell growth and viability.

Light microscopy

Near the original cell plaque, the ED epithelial cells were cuboidal in shape and contained many cytoplasmic organelles characteristic of epithelial cells in vivo. Cell polarity was maintained with apically located cilia and microvilli (Fig. 3). Indirect immunofluorescence for cytokeratin revealed that cells retained epithelial characteristics with cytokeratin stain localized to the cytoplasm (Fig. 4).

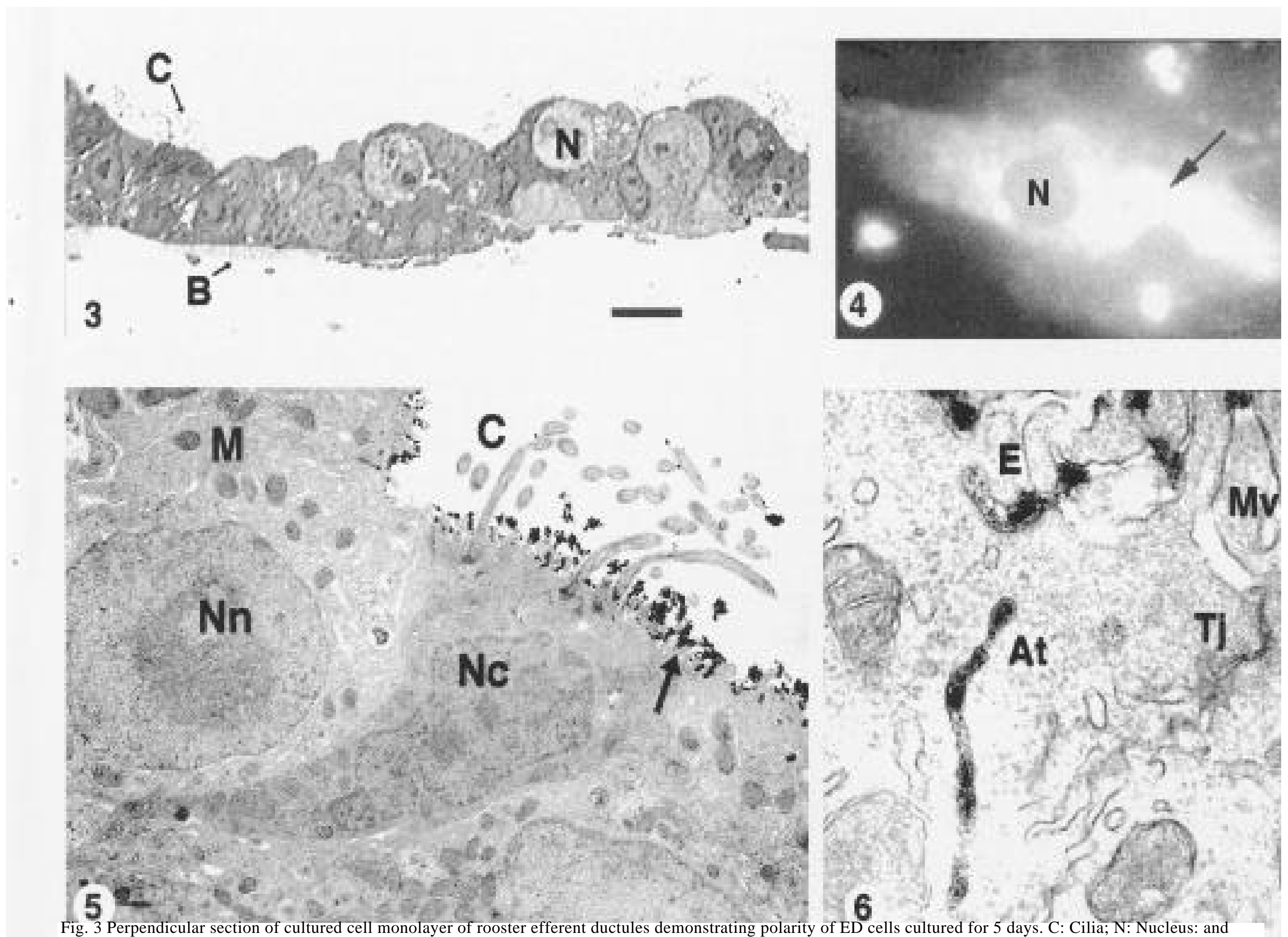

Fig. 3 Perpendicular section of cultured cell monolayer of rooster efferent ductules demonstrating polarity of ED cells cultured for 5 days. C: Cilia; N: Nucleus: and B: basement membrane. Bar = 50 µm.
Fig.4 Immunofluorescence of the cytokeratin network (arrow), localized in the cytoplasm of the 5-day cultured efferent ductule epithelial cell; N: Nucleus. x 6000
Fig.5 Electron micrograph of 5-day cultured efferent ductule epithelium. The epithelial cells contain numerous mitochondria (M). Cilia (C) are present on the apical surface of the cell. Ferritin (arrow) is present at the apical surface. Nn: Nucleus of non-ciliated cell; Nc: nucleus of ciliated cell. x 10 000
Fig. 6 Endocytosis of cationic ferritin by 5-day cultured efferent ductule epithelial cells. The ferritin particles are localized between microvilli (Mv) and within the endocytotic apparatus (E) and apical tubules (At). Tj: tight junction. x 26 000

Electron microscopy

Ultrastructural observations further demonstrated that the polarity and morphology of the epithelial cells were maintained in culture (Fig. 5). Apical tight junctions and numerous mitochondria were observed in both cell types. Ciliated cells were normal in appearance with long cilia, apical elongated nuclei, and vesicles. Non-ciliated cells also maintained normal morphology with dark granules, endocytotic vesicles and basally located nuclei. Endocytotic activity of the non-ciliated cells was maintained as evidenced by the uptake of cationic ferritin. Ferritin particles were localized to the endocytotic apparatus, apical tubules and endocytotic vesicles after a 10 min incubation with cationic ferritin (Fig. 6). As noted from light and electron microscopy, there was minimal contamination with fibroblasts and smooth muscle cells present at the basal aspects of the cells (data not

Expression of ER mRNA

Cultured cells expressed ER mRNA (Fig. 7). RT-PCR designed to amplify a 369 bp fragment for ER mRNA, detected ER mRNA in cultures treated for 5 consecutive days with 200 nM DHT or 200 nM DHT + I nM E2. No dramatic differences were noted in ER expression between the two treatment groups using standard PCR amplification conditions.

Discussion

This is the first report that ciliated and non-ciliated epithelial cells can be isolated from the rooster ED, maintained in a defined culture medium and grown to confluency after 5-6 days. Confluent monolayers were achieved by using smaller diameter culture inserts (6.4 min) and a single layer of extracellular matrix (Matrigel). This differs from the culture methods required to achieve confluent monolayers

shown).

Expression of ER mRNA

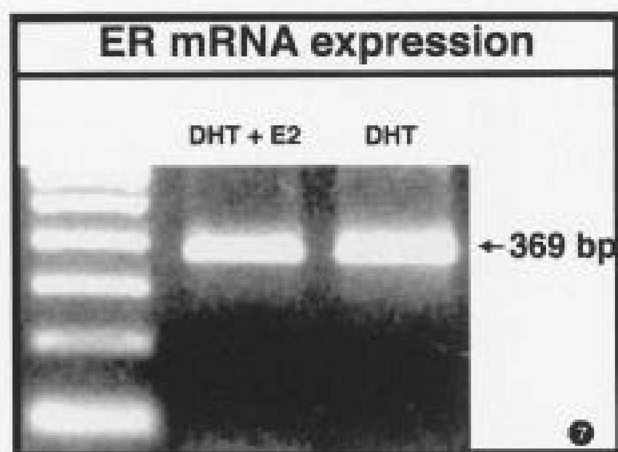

Fig.7 Expression of estrogen receptor mRNA in cultured efferent ductule epithelial cells after 5 days. A 369 bp fragment for estrogen receptor mRNA was detected in cell cultures treated either with 200 nM DHT plus I nM E2 (Lane 1) and 200 nM DHT only (Lane 2) for 5 consecutive days.

of ED epithelial cells in the rat (Chen, et al., 1998). A physiological concentration of DHT was also required for proper cell growth in culture. It is possible some other factor absent in our culture medium would alleviate the need for DHT. However, under our present culture conditions, future experiments investigating the role of steroids in the functioning of rooster ED will require DHT in all treatment groups.

Cultured ED cells displayed several characteristics analogous to those of ED epithelia in vivo. Indirect immunofluorescence demonstrated epithelial characteristics were preserved with uniform staining for cytokeratin throughout the monolayer. A Pan-cytokeratin antibody was used for immunocytochemical staining, because this cytoskeletal element is detected in epithelial cells, but is not found in the connective tissue of ED and epididymis in humans, dogs, and rats (Dinges et al., 1991; Kasper and Stosiek, 1989; Wakui et al., 1994). Minimal contamination with smooth muscle cells or fibroblasts was observed indicating cell cultures consisted primarily of epithelial cells.

Ultrastructurally, cells maintained normal morphology, polarity and endocytotic function as described previously for epithelial cells of avian ED (Clulow & Jones, 1988; Hess & Thurston, 1977; Tingari, 1972). Both ciliated and nonciliated cells were present in cultures. Both cell types contained apical tight junctions and numerous mitochondria, typical of these cell types in vivo. Ciliated cells were further characterized by apically located nuclei and vesicles. Ciliary beat was maintained in culture for 14 days. Non-ciliated cells contained apical microvilli, basally located nuclei and an endocytotic apparatus with apical vesicles, apical tubules and numerous lysosomes. Because the efferent ductules are the major site of fluid reabsorption (Clulow & Jones, 1988), it was important to document endocytotic function was maintained in cell culture. Cationic ferritin was taken up by efferent ductule cells cultured in a defined medium for 5 days. After a 10 min incubation, ferritin particles were found between microvilli, in the endocytotic apparatus and in apical tubules. This observation is in agreement with previous reports of cultured cells from efferent ductules (Chen et al., 1998; Heiniger et al., 1996) and in vivo ED epithelium (Hermo & Morales, 1984; Morales & Hermo, 1983) from mammals.

Previous work in our laboratory has demonstrated that the rooster reproductive tract contains ER, and the greatest abundance of ER was present in the ED (Kwon et al., 1997). Therefore, it was of interest to determine whether estrogen receptor mRNA expression was maintained in ED cultured cells. In this study, we have demonstrated cultured epithelial cells maintained ER mRNA expression for 5 days.

We are interested in investigating the role of E_2 in regulating the function of epithelial cells of the ED because previous studies in our laboratory have shown the enzyme P450 aromatase is localized to the tails of sperm in this region of the epididymis in many species (Janulis et al., 1996; Kwon et al., 1995; Nitta et al., 1993; Tsubota et al., 1993). The ED have been shown to contain high concentrations of ER in birds (Kwon et al., 1997), rodents (Fisher et al., 1997; Hess et al., 1997b), large mammals (Goyal et al., 1997), and humans (Ergun et al., 1997). Studies with the ER knockout mouse have established that estrogen is required for proper development and fertility in male mice (Eddy et al., 1996; Korach et al., 1996). A recent study in our laboratory (Hess et al., 1997a), has shown that estrogen is required for proper fluid reabsorption from the ED. Disruption of this function results in a low sperm concentration and infertility. Thus, it appears that estrogen plays an important role in male fertility.

This study and the previously described results demonstrate our in vitro culture system is appropriate for studying the role of estrogen in ED epithelial cells of the rooster. This study will be important because little is known about endocrine regulation of the avian male reproductive tract and its role in production of fertile sperm. Yet, as poultry production becomes more commercialized, artificial insemination is more widely used. Therefore, it will be essential that poultry producers use males with high fertility. We are focusing specifically on ED because they comprise 77% of the epididymal region and are the primary site of fluid reabsorption. Furthermore, dysfunction of ED results in decreased fertility in turkeys and chickens. Our confluent cell culture system will be necessary in future studies to test the hypothesis that estrogen regulates fluid reabsorption in the ED by modulation of ion transport. Furthermore, this cell culture system can be used to study the effects of estrogen at the level of gene expression and gene product synthesis in the rooster ED.

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