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Why Human Prepuce is a Valuable Resource in Science? Histological, Ultrastructural, Immunohistochemical and Statistical Analysis

¿Por qué el Prepucio Humano es un Recurso Valioso en la Ciencia? Análisis Histológicos, Ultraestructurales, Inmunohistoquímicos y Estadísticos

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SUMMARY: This research was to examine the histological and ultrastructural characteristics of prepuce samples, as well as vimentin and S100 protein localization and statistical analysis. Urologists have long struggled with the prepuce, which is used to treat a variety of urethral problems. Skin biopsies were collected from the prepuce at the moment of circumcision and processed for light microscopy, electron microscope examination, immunohistochemical techniques, and statistical analysis in a total of six boys. Histologically, the prepuce epidermis displayed focal spiky ridges, which are saw-toothed interspersed with sulci, slight hyperpigmentation, looser connective tissue and plentiful vascular components. Immunohistochemically, the existence of melanocytes and Langerhans cells in the epidermis, as well as smooth muscles in the dermis, was stained positively for vimentin. Also, there was a positive reactivity of the Langerhans cells in the epidermis and around Meissner's corpuscles in the dermis for S100 protein staining. Ultrastructurally, the prepuce's intercellular gaps were widened, melanocytes rested on a folded basement membrane, and desmosomal content was reduced, with a prominent active euchromatic nucleus. Cytoplasmic projections were distended and elongated, and the interstitial blood vessels were surrounded by endothelial cells and rested on a basement membrane. There were also minimal collagen fibers in the interstitium. The prepuce's histological and ultrastructural features, as well as immunohistological studies using vimentin and S100 protein as intermediate filaments and statistical analysis, all demonstrated that it is a useful scientific resource.

KEY WORDS: Prepuce; Light Microscopy; Vimentin; S100 Protein; Electron Microscopy; Statistical Analysis.

INTRODUCTION

The foreskin is a unique mucocutaneous tissue that covers the glans penis and serves to shield them from the irritating effects of urine. It is extensively vascularized and innervated (Cold & Taylor, 1999). It has been used to treat difficult wounds and to test novel drugs. In the future, they could be employed to aid in the repair of organs (Glick, 2021). Collagen and elastic fibers are fibrotic components of the cellular matrix that have been linked to pathological changes in various tissues, including the foreskin (Ushiki, 2002; Favorito *et al*., 2012). Also, it contains free nerve endings, Meissner's corpuscles, and Pacinian corpuscles, all of which have distinct staining behaviors (Martín-Alguacil *et al*., 2015).

Male urethral reconfiguration is one of the oldest reconstructive surgery problems and has always presented urologists with a significant challenge (Olajide *et al*., 2010). Surgical repair of severe hypospadias (one of the most

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common genital anomalies in male newborns) may require multiple procedures and mucosal grafting of the foreskin (Perovic & Basting, 1999).

Intermediate filaments make composed one or more members of a large extended family of cytoskeletal proteins (IFs). In tasks ranging from cell motility to signal transmission, their relevance in regulating cells' physiological features is becoming well acknowledged (Lowery *et al*., 2015). All IF proteins are synthesized under strict developmental regulation, Vimentin (VIM) is a type III, IF protein that is the primary cytoskeletal component of mesenchymal cells. Assume that all animal cells contain proteins, as well as tubulin-based microtubules and actin-based microfilaments, which constitute the cytoskeleton. VIM is frequently used as a marker for cells derived from the mesenchyme (Cabeen & Jacobs-Wagner, 2010).

S100 proteins are a family of proteins with a common basic structure that allows them to carry out a variety of intracellular functions related to the regulation of proliferation, energy metabolism, calcium homeostasis, enzyme activities, cell growth and differentiation, and extracellular tasks including such participation in innate and adaptive immune responses, tissue regeneration, and remodeling (Halawi *et al*., 2014). They additionally act as chemotactic agents and may have a role in the etiology of epidermal disorders like psoriasis. They're overexpressed in wound healing, skin cancer, inflammation, and cellular stress, among other aspects (Eckert *et al*., 2004).

Transmission electron microscopy (TEM) is a remarkable technology in cell biology that permits viewing of cells from the ultrastructure to the molecular level (Koster & Klumperman, 2003), and it's a good opportunity to know more about how they behave. TEM is being used to characterize every cellular component, including organelles, membrane systems, and specialized structures. The loose aspect of the dermal structure adds to high elastin content, which likely contributes to contractility, according to a TEM investigation comparing human foreskin (Tuncali *et al*., 2005).

The purpose of this study was to look into the unique histologic structure of the human foreskin, with a focus on the histologic structures, immunohistological studies using vimentin and S100 protein as intermediate filaments, transmission electron microscopy to evaluate the fine structures and statistical analysis was performed.

MATERIAL AND METHOD

Collection of human skin tissue. Skin slices measuring 1–2 cm^2 were inserted in a sterile tube filled with phosphate-buffered saline (PBS) without Ca^{2+} Mg^{2+} containing 1 % penicillin/streptomycin (Invitrogen, USA) and transferred to the lab on ice for rapid processing and storage at 4 °C.

Light microscope procedure. For light microscopy, the specimens were maintained in 10 % neutral buffered formalin for 24–48 hours before being dehydrated in a graded alcohol series, cleaned in xylene, and embedded in paraffin wax. Hematoxylin and eosin (H&E) were used to stain paraffinized specimens, which were subsequently viewed under a light microscope (Olympus CX31, Japan) and photographed with a digital camera (Olympus, Camedia-5060, Japan) (Bancroft & Gamble, 2008).

Immunohistochemistry procedure (IHC). The ready-to-use IHC kit was used on paraffin-embedded, formalin-fixed skin slices (4 μm). Using a streptavidin-biotin peroxidase system and 1:100 rabbit anti-(rat S100) serum (Biogenex, San Ramon, Calif., USA) and anti-Vimentin antibody, Dako Clone V9, the sections were immune-stained. Diaminobenzidine (DAB) was used as the peroxide-sensitive chromogen, which was counterstained with Mayer's hematoxylin, dehydrated in escalating degrees of alcohol, cleaned in xylol, and mounted with Canada balsam (Nadji & Morales, 1986).

Transmission electron microscope procedure (TEM). Foreskin samples were preserved in 2.5 percent glutaraldehyde for two hours, then trimmed and diced into one cubic millimeter pieces, fixed in glutaraldehyde solution in 0.1 M sodium cacodylate buffer, pH 7.2, and stored in a thermal box cooled to 4°C. They were post-fixed in a sodium cacodylate buffer containing 1 % osmium tetroxide, then dehydrated in an escalating series of ethyl alcohol before being embedded in Spur's resin. The Electron Microscopy Unit, Pathology Department, College of Medicine, King Khalid University evaluated ultrathin sections stained with uranyl acetate and lead citrate using a TEM (JEM-1011, Jeol Co., Japan) operating at 80 kV (Eid *et al*., 2021).

Statistical Analysis. The arithmetic mean, median and standard deviation were used to present data. Mann-Whitney non-parametric test of significance was used to detect data of foreskin. The chosen level of significance was 5 %.

RESULTS

Histological findings: The epidermis of the human foreskin and the connective tissue beneath it were shown. The succession of epidermal layers with a higher content of keratinocyte layers and ridges associated with sulci was visible in histological

cross-sections of foreskin specimens (Fig. 1A). Meissner's corpuscles were more abundant at the crests of ridges, typically in little bunches extending the terminals of papillae that appeared as elongated bodies linked to the basement membranes. With looser connective tissue, the substantial vascular component may be appreciated (Fig. 1B).

Immunohistochemistry

Vimentin Figures: In foreskin samples, VIM labeling demonstrated strong positivity in epidermal (melanocytes and Langerhans cells) and mesenchymal (such as smooth muscle cells) cells throughout the dermis (Fig. 2A).

S100 protein Figures: Langerhans cells were discovered in all strata of the epidermis but are particularly prominent in the stratum spinosum, as demonstrated by S100 protein staining of foreskin specimens. The dermal papillae, which are near to Meissner's corpuscles, also showed a strong reactivity (Fig. 2B).

Transmission electron microscope (TEM): Different layers of the epidermal skin with keratinocytes dispersed among them, melanocytes were resting on a folded basement membrane with its lamina densa of the dermo-epidermal junction. Minimal collagen fibrils in its dermis were also seen (Fig. 3A).

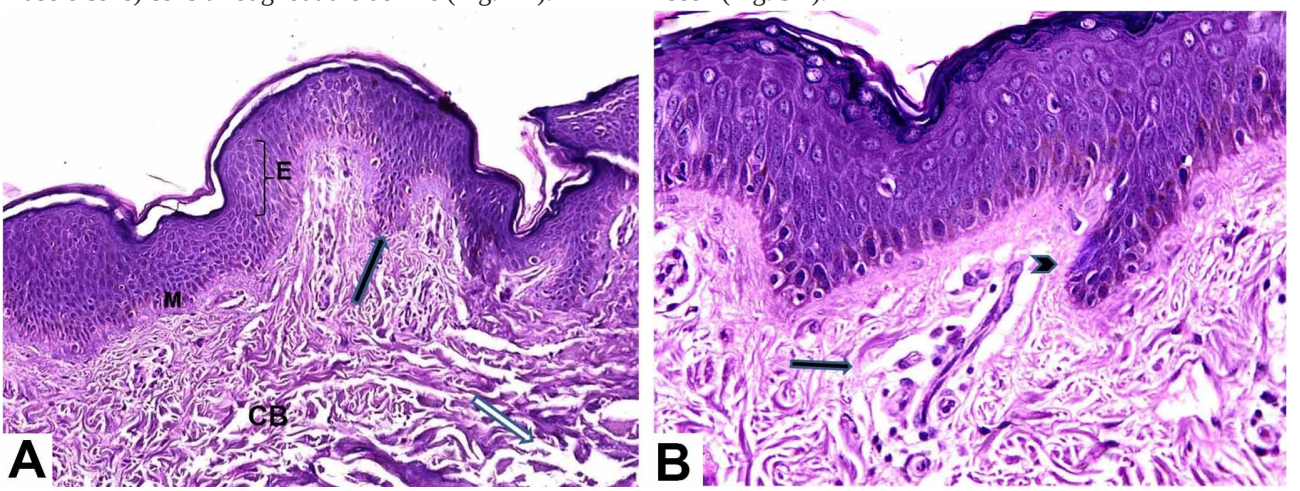

Fig. 1. Histological micrographs obtained from the human foreskin stained by H&E. **A.** The section showing some rete ridges are saw-toothed (black arrows). The abundant vascular component (white arrows) can be seen with a wide range of lumenal caliber. The dense collagenous bundle (CB) zone observed in most parts of the dermis is absent from the deeper zone of the foreskin. In the epidermis's basal layer, melanocytes (M) are less visible (E). X200. **B**. The distinct layers of epidermis (E) with focal ridges (arrowhead) alternated with sulci are seen at a higher magnification in the top image. Meissner's corpuscles (arrow) are more abundant on ridge crests and in little clumping that expanded the tips of papillae, which look as large oval or elongated bodies. X400.

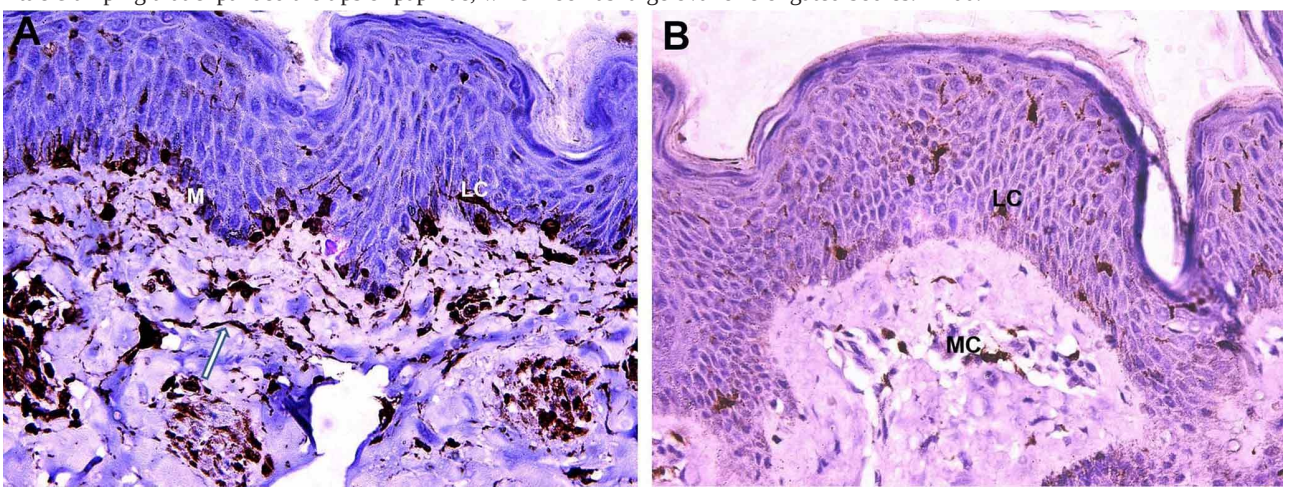

Fig. 2. Immunohistochemical micrographs obtained from the human of foreskin stained by vimentin (A) and S100 protein (B) counterstained with hematoxylin. (400X). **A.** The micrograph showing strong positivity of vimentin in the epidermis (melanocytes (M) and Langerhans cells (LC)) and in mesenchymal cells throughout the dermis (arrow), such as smooth muscle cells. **B.** The epidermis is made up of Langerhans cells (LC), which can be found in all levels of the epidermis but are most predominant in the stratum spinosum (arrows). The positive reaction also found in the dermal papillae, especially near Meissner's corpuscles.

Keratinocytes with an active euochromatic nucleus and a pronounced nucleolus terminated at the desmosomes that joined the cells and formed the vast cellular extensions (Fig. 3B). Melanocytes had melanin granules filled the majority of its cytoplasm, a prominent euochromatic nucleus, nucleolus, abundant mitochondria, widened intercellular gaps and fewer desmosomes (Fig. 3C). A lot of interstitial blood vessels was surrounded by endothelial cells and a basement membrane were also seen (Fig. 3D).

Statistical Results: These table show the foreskin melanocytes (median=17.00), collagen bundles (median=2.00), keratinocytes (median=8.00), Langerhans cells (median=23.00), vimentin antibodies (median=4.00), Meissner's corpuscles (median=6.00), papillary ridges number (median=6.00) and vascularity (median=48.00) (Fig. 4 and Table I).

DISCUSSION

The foreskin serves as a donor source for childhood autologous keratinocyte grafting (Rosado *et al*., 2012) and as mucosal grafting for surgical reconstruction of serious hypospadias (Stein, 2012). This research simulated the

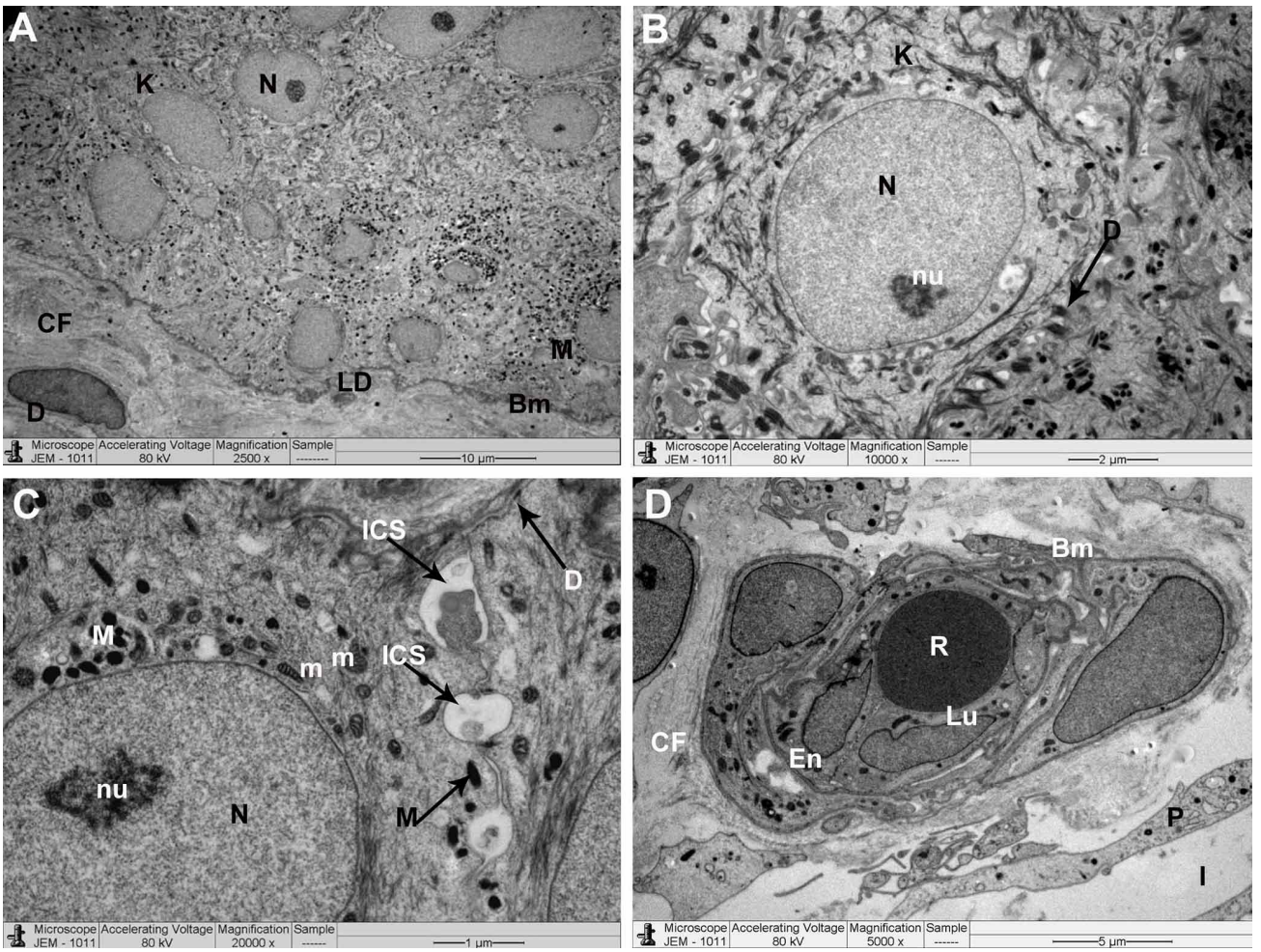

Fig. 3. TEM images of the foreskin of a human dyed with uranyl acetate and lead citrate. **A**. The photomicrograph showing different layers of the epidermal skin with keratinocytes (K) dispersed among them, melanocytes (M) that rest on a folded basement membrane (BM) with its lamina densa (LD) of the dermo-epidermal junction. Note the presence of minimal collagen fibrils (CF) in the graph's corner in its dermis (D). (X2500). **B.** The photomicrograph showing a single keratinocyte (K) with an active nucleus (N) with prominent nucleolus (nu). Keratinocytes terminate at the desmosomes (D), linking the cells (arrows) and forming wide cellular extensions. (X10000). **C.** The photomicrograph showing a melanocyte with intercellular spaces (ICS) that are widened and reduced desmosomal content with prominent active euochromatic nucleus (N) and nucleolus (nu). Note the presence of melanin granules (M) filling most cytoplasm and prominent mitochondria (m). (X20000). **D.** The photomicrograph showing interstitial blood vessel showing lumen (Lu) surrounded by endothelial cells (En) and basement membrane (Bm). Note, pericyte (P) and minimal collagen fibers (CF) in the interstitium (I). (X5000).

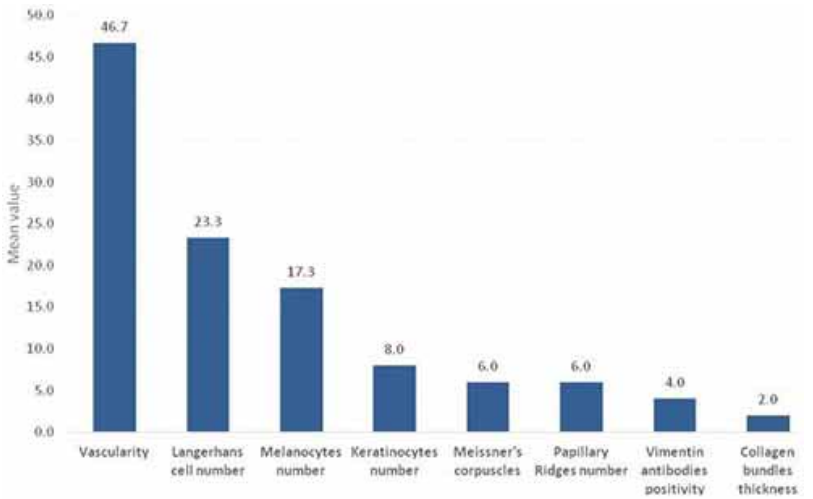

Fig. 4. Histological profile among foreskin samples.

Table I. Histological mean, median, standard deviations of skin profile among foreskin samples.

Parameter	Foreskin (n=6)			*P*
	Mean	Median	SD	
Melanocytes	17.33	17.00	1.52	0.05
Keratinocytes	8.00	8.00	1.00	0.05
Langerhans cell	23.33	23.00	3.51	0.05
Vimentin	4.00	4.00	1.00	0.05
Collagen bundles	2.00	2.00	1.00	0.05
Meissner's	6.00	6.00	1.00	0.05
Papillary Ridges	6.00	6.00	1.00	0.05
Vascularity	46.67	48.00	5.13	0.05

microstructural study of human foreskin that is clinically important. The foreskin is densely innervated with sensory receptors, including Meissner's corpuscles, which is remarkable (Cold & Taylor, 1999).

The current foreskin specimens indicated a succession of epidermal layers with a greater content of keratinocyte layers and localized, ridge crests. Skin resistance, cornified envelope formation, and desmosome/tight junction integrity are all aided by the suprabasal skin layers (Simpson *et al*., 2011; Wallace *et al*., 2012).

Meissner's corpuscles were more frequent in this study, sometimes clumped together to extend the terminals of papillae that looked as elongated entities linked to the basement membranes. Some researchers assume that the ridged band, with its distinct structure, tactile corpuscles, and other nerves, is primarily sensory tissue that collaborates with other foreskin components (Lakshmanan & Prakash, 1980). The limited proportion of melanocytes in this foreskin sample resulted in little darkening, which can be explained by the fact that melanin synthesis continues, as evidenced by ultrastructural criteria in this and other DOPA incorporation assays (Wilkins *et al*., 1985).

The connective tissue in the currently reported foreskin structure had a lower content of collagen fibrils and a higher proportion of vascular parts. There is no dense collagenous region, both in density and thickness, indicating that the profuse vascular component might be appreciated with looser connective tissue (Halata & Munger, 1986).

VIM antibody positivity was observed in epidermal Langerhans cells (ELCs) and melanocytes in the foreskin tissues, as well as smooth muscle cells in the dermis, according to immunohistochemistry. VIM is found in all animal cells, including the cytoskeleton, and is expressed in mesenchymal cells (Eriksson *et al*., 2009).

In the current study, the ELCs of the epidermal skin's stratum spinosum and dermal papillae, particularly those were surrounding the Meissner's corpuscles, reacted strongly to S100 protein staining. ELCs secrete cytokines, which are low-molecular-weight hormone-like molecules that control the strength and duration of immune responses (Weiss *et al*.,1993).

The stratum spinosum keratinocytes were bound to each other by cell junctions, or desmosomes, according to TEM analysis of the foreskin samples from the current investigation. To provide structural support for tight junctions, involucrum is generated in the stratum spinosum as a terminal indication of keratinocyte maturation (Murthy *et al*., 1993). Proteins like claudins and occludin generate homotypic interactions between nearby cells and regulate water, ions, and huge molecules transit across paracellular channels (Furuse *et al*., 2002; Shen *et al*., 2011).

The intercellular gaps between the keratinocytes were also broadened using TEM, and the cytoplasmic projections of the foreskin in this investigation were distended and elongated. Desmosome internalization in cytoplasmic vesicles has been observed in fresh skin specimens (Iwatsuki *et al*., 1989). Endocytosis of split desmosomes was identified, leading to the conclusion that antigen-antibody complexes are internalized through this method, according to these scientists.

The TEM images of the foreskin samples in this study revealed different layers of epidermal skin with keratinocytes dispersed among them. Melanocytes are supported by a folded basement membrane with a dermo-epidermal junction lamina densa. Epithelial and mesenchymal components work together to generate organized basement membranes and control normalized epithelial proliferation and tissue architecture in human skin (Andriani *et al*., 2003). When human neonatal keratinocytes were plated on top of a human dermal fibroblast, the lamina densa was disrupted and there were no anchoring fibrils.

The histological and ultrastructural characteristics of the foreskin, as well as immunohistological experiments employing vimentin and S100 protein as intermediate filaments and statistical analysis, all revealed that it could be a useful scientific resource in the future for a variety of difficulties.

ETHICAL APPROVED AND INFORMED CONSENT. The Department of Plastic Surgery, College of Medicine, King Khalid University of Abha, Saudi Arabia, generously contributed tissue samples from the foreskin (from six boys ages 1, 2, and 3 years, respectively). Human tissue research was carried out in accordance with Saudi Arabia's Medical University's regulations. Surgical procedures were performed by a urological surgeon who was trained and licensed. Before surgery, all patients signed a written informed consent form.

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RESUMEN: El presente trabajo de investigación se realizó para examinar las características histológicas y ultraestructurales de las muestras de prepucio, así como la localización y el análisis estadístico de la vimentina y la proteína S100. Los urólogos han intentado trabajar durante mucho tiempo con el prepucio, que se usa para tratar una variedad de problemas uretrales. Se recolectaron biopsias de piel del prepucio de seis niños en el momento de la circuncisión y se procesaron para microscopía óptica, examen con microscopio electrónico, técnicas inmunohistoquímicas y análisis estadístico. Histológicamente, la epidermis del prepucio mostraba crestas puntiagudas focales, intercaladas con surcos, hiperpigmentación leve, tejido conectivo más laxo y abundantes componentes vasculares. Inmunohistoquímicamente, la existencia de melanocitos y células dendríticas epidérmicas (células de Langerhans), así como músculo liso en la dermis, se tiñeron positivamente para vimentina. Además, hubo una reactividad positiva de las células dendríticas epidérmicas en la epidermis y alrededor de los corpúsculos del tacto (de Meissner) en la dermis para la tinción de la proteína S100. Ultraestructuralmente, los espacios intercelulares del prepucio se ensancharon, los melanocitos descansaban sobre una membrana basal plegada y el contenido desmosómico se redujo, con un núcleo eucromático activo prominente. Las proyecciones citoplasmáticas estaban distendidas y alargadas, y los vasos sanguíneos intersticiales estaban rodeados por células endoteliales y descansaban sobre una membrana basal. También había fibras de colágeno mínimas en el intersticio. Las características histológicas y ultraestructurales del prepucio, así como los estudios inmunohistológicos utilizando vimentina y proteína S100 como filamentos intermedios y el análisis estadístico, demostraron que es un recurso científico útil.

PALABRAS CLAVE: Prepucio; Microscopía de luz; Vimentina; Proteína S100, Microscopía electronica; Análisis estadístico.

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