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RESEARCH ARTICLE

Lymphatic lacunae of the mucosal folds of human uterine tubes — A rediscovery of forgotten structures and their possible role in reproduction

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ABSTRACT

The mucosa of uterine tube forms multiple and branched longitudinal mucosal folds and takes part in many reproduction events, such as oocyte pick-up, gamete transport, sperm capacitation, fertilization, and early embryonic development. In the habilitation thesis of German physician Paul Kroemer (1904) was the first to describe the lymphatic lacunae inside the tubal folds (by injection of Indian ink), which the author named the öLymphbahnen" (ölymphatic channels"). Despite the fact that this first description has existed for 110 years, there is no mention of these lacunae in most of the current literature. In this article we present a rediscovery of completely overlooked morphological structures of uterine tubes — the lymphatic lacunae in their mucosal folds. The specimens from the uterine tubes were taken from 72 women (mean age 46.25 years) who underwent transabdominal or laparoscopic salpingectomy. The tissue samples from anatomically different parts of the uterine tubes were used for hematoxylin and eosin staining and for immunohistochemistry. Primary antibodies were used to label and detect podoplanin D2-40, a selective marker of lymphatic endothelia, CD34 antigen, and von Willebrand factor (Factor VIII). In the histological slides of the uterine tubes, there were noticeable slits or gaps within the loose connective tissue of the lamina propria of the mucosal folds. They were lined with one layer of squamous endothelial cells. These öempty spaces" were most prominent in the fimbriae, but were still well recognizable in mucosal folds of the ampulla. They always run through the central part of the fold. As a results of immunohistochemistry, we confirmed that in the centre of every mucosal fold, as well as in the fimbriae of the uterine tubes, dilated lymphatic spaces were situated and were lined with a simple layer of lymphatic endothelial cells (positive for podoplanin and CD34, and negative for Factor VIII). As there is no mention on them in the current *Terminologia Histologica*, we proposed the term ölymphatic lacunae of tubal mucosal folds and fimbriae" in English and ö*lacunae lymphaticae plicae mucosae et fimbriae*" in Latin. According to our hypothesis, these lymphatic lacunae may be responsible for the thickening of the fimbriae during the oocyte pick-up and the maintenance of the tubal fluid.

1. Introduction

Uterine tubes, oviducts, or Fallopian tubes (according to Italian Gabriele Falloppio, a well-known Italian anatomist of the 16th century) form a passageway between the ovaries and uterus, not only for the oocytes or early embryos but also for sperms (heading in the opposite direction). The general belief, as well as that of professional medical circles, that this organ is a passive tube could not be further from the truth (Kölle et al., 2009, 2010; Hunter, 2012). The complex and sophisticated histological composition and diverse and exquisite patterns and regulatory chemical cooperation between transported cells and the tissue of uterine tubes provide many physiological actions and functions needed for fertilisation, early development and transport of the embryo (Croxatto, 2002). Currently, the question of human fertility is widely discussed and becoming a major concern in society (Kulu and Washbrook, 2014; Gnoth, 2013). It is important to understand the physiology and function of uterine tubes, as tubal infertility accounts for a large portion of female factor infertility (Dun and Nezhat, 2012; Abrao et al., 2013) — around 30% of infertile women worldwide have

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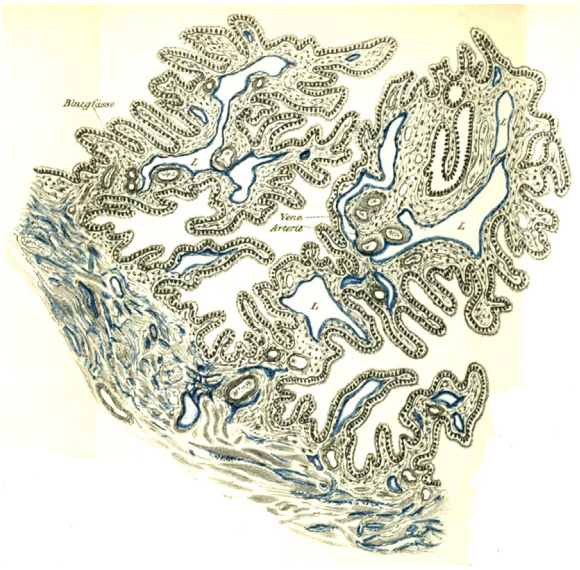

Fig. 1. The original drawing of lymphatic lacunae (bordered in blue and labelled with the letter L) from a pregnant woman's uterine tube found in the habilitation thesis of Kroemer (1904). These areas were called "Lymphbahnen" and were visualised by injecting them with Indian ink. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

associated uterine tube pathology (Briceag et al., 2015). To bypass this problem, assisted reproductive techniques are increasingly being used for the treatment of tubal factor infertility (Gomel and McComb, 2006). However, increased attention should be paid to the techniques of *in vitro* fertilization and resultant embryo transfer (IVF-ET), as new studies are bringing disconcerting data about the significantly increased risk of undesirable ectopic tubal pregnancy after IVF-ET techniques (Refaat et al., 2015; Santos-Ribeiro et al., 2016).

Human uterine tubes are paired, muscular tubes of great mobility that measure about 12 cm in length. The wall of the uterine tube is composed of a mucosa, a muscular layer, and a serosa. The mucosa forms multiple and branched longitudinal mucosal folds and is lined with a simple columnar epithelium consisting of morphologically and functionally different epithelial cells (FICAT, 2008). The *lamina propria* of the mucosa is composed of loose connective tissue and predominantly contains spindle-shaped stromal cells with ultrastructural and immunohistochemical features of myofibroblasts (Hagiwara et al., 2008). The degree of folding is most pronounced in the infundibulum and is progressively lower and less complex towards the isthmus. The mucosa of the uterine tube takes part in many reproduction events, such as oocyte pick-up, gamete transport, sperm capacitation, fertilization, and early embryonic development (Yániz et al., 2000, 2014; Marettová and Maretta, 2014). Therefore, a detailed description of the histological structure of the human tubal mucosa may be useful for understanding some of the events that determine reproductive success.

Due to the facts stated above about the important physiological functions of the tubal mucosa, we have focused on this part of the uterine tube wall. After concentrated work, we present in the following text a rediscovery of completely overlooked and yet important structures — the lymphatic lacunae inside the mucosal folds and fimbriae of uterine tubes. In the scientific literature, they are first mentioned, to the best of our knowledge, in the habilitation thesis (which relates to the achievement of a scientific title "*Venia Legendi in Arte Obstetricia et Gynaecologia*") of German physician Paul Kroemer from the University of Giessen, dated in 1904 (Fig. 1, Kroemer, 1904). Despite the fact that this first description has existed for 110 years, there is no mention of these lacunae in most of the current literature. Such atypical lymphatic drainage of mucosal folds should be revisited, as it might play a crucial role in the proper function of the uterine tube, and its dysfunction could be an important element in reproductive pathology.

2. Material and methods

2.1. Patients

The specimens from the uterine tubes were taken from 72 women (mean age 46.25 ± 10.55 years) who underwent transabdominal or laparoscopic surgery – salpingectomy with or without hysterectomy – at the Department of Gynecology and Obstetrics in General Hospital in Komárno, Slovak Republic, with diagnoses of uterine fibroids, ectopic tubal pregnancy or inflammatory diseases of the pelvis. The study protocol was approved by the Ethical Committee of the Komárno hospital, and informed consent was obtained from all patients.

2.2. Histological processing

The tissue samples from anatomically different parts of the uterine tubes (infundibulum with fimbriae, ampulla and isthmus) were immediately after the surgical removing fixed in formalin for 24 h in room temperature, embedded in paraffin, and 5-μm-thick sections were used for hematoxylin and eosin staining and for immunohistochemistry. Primary antibodies (Table 1) were used to label and detect podoplanin D2-40, a relatively new and selective marker of lymphatic endothelia, CD34 antigen, a universal marker of endothelial cells of blood and lymphatic vessels, and von Willebrand factor (Factor VIII), a selective marker of blood endothelia. The primary antibodies (DAKO, Denmark) were used according to the manufacturer's protocol. For visualization, we used an EnVision™ FLEX Detection system (DAKO, Denmark) with diaminobenzidine as a brown chromogen. For better orientation within the slide, the cell nuclei were stained with Mayer's hematoxylin dark blue. For visualization of the histological sections by means of light microscopy, the LEICA DM 2500 microscope was used, and images were captured using the LEICA DFC290HD digital camera.

2.3. Archive and recent library research

As we were not able to find any information about the lymphatic spaces in the tubal mucosa in the PubMed/Medline database for the appropriate discussion, we decided to study the contents of current as well as historical histological textbooks. We wanted the set of textbooks to be as broad as possible, so we went through the texts of different origins and different dates. Subsequently, our investigation consisted in searching for possible descriptions of these lymphatic spaces in the tubal mucosa in historical textbooks and scientific monographies — whether in the form of verbal

Table 1
Specification of used antibodies.

Antibodies against	Manufacturer, code no.	Identified cells
CD34	DAKO, M7165	Capillary endothelial cells, but also hematopoietic progenitor cell, embryonic fibroblasts, and others
Von Willebrand factor (Factor VIII)	DAKO, A0082	Blood endothelial cells, but also megakaryocytes
Podoplanin, clone D2-40	DAKO, M3619	Endothelium of lymphatic capillaries, but not the blood vasculature (Kahn and Marks, 2002)