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ScienceDirect
European Journal of Obstetrics & Gynecology and Reproductive Biology 135 (2007) 116–122
ejog European Journal of Obstetrics & Gynecology and Reproductive Biology
www.elsevier.com/locate/ejogrb

Crystallographic investigation of the dried exudate of the major vestibular (Bartholin's) glands in women

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Received 22 November 2005; received in revised form 1 June 2006; accepted 13 June 2006

Abstract

Objective: The aim of this study was to investigate the dried secretion of human major vestibular glands in order to establish its crystallographic pattern and to compare the data with those obtained for other human genital biopolymers.
Study design: After air drying, samples were examined comparatively under transmitted and polarized light. At first sight, dehydrated vestibular fluid exhibits a fern-like crystallographic pattern very similar in appearance to those described in mid-cycle cervical mucus and bulbo-urethral fluid.
Results: Dendritic structures fill the central space of all preparations, prolonged by apparently amorphous peripheral fucus-like expansions. Spherulitic interdendritic crystalline microstructures (ICMs) can be considered a constant feature of dried vestibular exudate. In contrast with dendritic formations, fucus-like expansions and isolated spherulites are anisotropic under polarized light. Anisotropy appears to be the guise of a luminescent border lining the dendrites or bright nodules shining on a dark background.
Conclusion: The study confirms the close physico-chemical proximity of vestibular secretion, mid-cycle cervical mucus and bulbo-urethral fluid. However, if isotropic dendritic formations and anisotropic structures are grossly similar, the number and size of the anisotropic ICMs are typical of human vestibular secretion. The different patterns of ICMs observed in these three human biological hydrogels demonstrate differences in the salt concentrations.
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Keywords: Human major vestibular secretion; Dehydration; Fern-like pattern; Polarized light; Anisotropic crystalline microstructures

1. Introduction

The celebrity of Caspar II Bartholin, who gave his name to the so-called Bartholin's glands, could be the result of good fortune mixed with unscrupulous ambition [1]. Although many physicians of the day had suspected their existence, the major vestibular glands (MVG) were first discovered in the cow by Joseph Du Verney [2]. They were described thereafter in women by ''his friend'' Caspar Bartholin on his return to Copenhagen in 1676–1677 [3,4]. Later on, MVG were described in many species of mammals including opossum, bat, cat, cow, horse and monkey [5,6]. Curiously, more than 300 years after their discovery, very little and incomplete information is available regarding these glandular formations and their exudates. Bartholin's glands are considered to be homologous organs of the bulbo-urethral (Cowper's) glands in males [7,8], which secrete during the early ejaculatory phase and therefore contribute to the first fraction of the seminal plasma. In animals, vestibular fluid appears to play a role not only in the lubrication of the reproductive tract during mating, but also in the stimulation of males before copulation.

In adult women, MVG are small glands situated at each side of the vaginal orifice and can be palpated easily only if they are enlarged by inflammation. They are composed of lobules of acinar epithelium discharging through a network of secondary ductules.

The secretory material is emitted at the opening of the major excretory ducts during the late excitement and early plateau stages in response to sexual stimulation [9,10].

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0301-2115/$ – see front matter © 2006 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.ejogrb.2006.06.031

The understanding of the nature and function of the compounds secreted by human Bartholin's glands is still scarce and incomplete. Most studies have been devoted to the structure and the carbohydrate chemistry of the glands rather than to their secretory product. These studies revealed both periodic acid Schiff (PAS) and alcianophilic reactive in the secretory cells of the fetal and newborn human [11,12] and in the cow [13]. In normal female calves and animals treated by anabolic drugs, acinar cells produce sialomucins, whereas tubular cells develop neutral mucins [14]. In the cat, the amount of sulphated glycoconjugates in the cytoplasm of the secretory cells is larger than that in the calf [15].

Like other human mucoid productions, such as secretions of the nasal, salivary, cervical and bulbo-urethral glands, human vestibular secretion is a clear, viscous, somewhat sticky substance. From a physico-chemical point of view, these various exudates consist of a very complex hydrogel mainly composed of a liquid phase including various salts in solution and trapped in a glycoproteic solid phase [16]. After air drying, saliva [17], nasal mucus [17] and bulbo-urethral fluid [18] exhibit an amazing crystallographic pattern resembling fern leaves, which was originally described by Papanicolaou in human ovulatory cervical mucus [19].

Although physico-chemical affinities exist between Bartholin's exudate and other secretions of the male and female genital tract, and in spite of their clinical interest, very little has been added to our global knowledge; its physical structure and rheological properties are still unknown. Spurred on by the little information available, we have carried out this study in order to obtain further insights into the secretions of the human major vestibular glands by means of crystallographic techniques combining air drying with light and polarizing microscopy.

2. Materials and methods

2.1. Sampling

Normal human Bartholin's gland secretions were obtained from five women of proven fertility, with at least one offspring, with a mean age of 29.6 years (range 23–37). Pertaining to hospital staff, the volunteers were fully aware of the medical interest of the projected study. They were instructed with regard to the technical requirements and readily accepted the necessary constraints. In consideration of the dependency of the vestibular glands on radiation and ovarian hormones [20], volunteers had no X-rays or hormonal treatment nor had they used oral contraceptives for at least three months. In addition, they agreed to avoid washing of the vulva with soap 24 h prior to the sampling and to abstain from sexual intercourse for 48 h.

Volunteers consented to supply at least eight different samples spread over two cycles in the following three periods: 7–10th day; 12–15th day; 20–25th day (Table 1).

Table 1
Number of samples

Volunteers	7–10th day	12–15th day	20–25th day
A	1	3	1
	1	2	1
B	1	3	1
		3	
C	1	3	1
		2	1
E	1	2	1
	1	2	1
F	1	3	1
	1	3	1
Total	8	26	9

A total of 43 specimens of vestibular fluid were obtained by subdued masturbation after traces of vulvar secretions and uric acid were removed by means of a sterile gauze impregnated with sterile de-ionized water. In order to avoid the formation of air columns or bubbles that are detrimental to normal crystal genesis and distribution, vestibular fluid was aspirated very carefully by means of a sterile 1-ml disposable syringe. In order to avoid desiccation, syringes were closed by a plug of modelling clay and then immediately stored at 4 °C in a Petri dish humidified by a damp spongy paper until drying.

2.2. Dehydration procedure

All specimens were prepared for experimental purposes within 6 h of collection. In order to focus upon optimal samples with standard criteria for crystallization, only very clear samples were analysed. Obvious cellular specimens were systematically rejected. Vestibular fluid was gently extruded onto a dry glass cover slip previously scoured and degreased by means of a mixture of potassium dichromate and sulphuric acid and then rinsed copiously in de-ionized water. Small droplets of approximately 20 μl were carefully deposited onto the cover slip in a circle of nearly 8 mm in diameter whose depth did not exceed 2 mm, or stretched by means of Dumont tweezers in the form of a thin ribbon approximately 3 mm in breadth.

To allow the drying procedure to begin under physiological conditions, each syringe was warmed to 37 °C directly before drying. To prevent dust contamination, vestibular fluid samples were allowed to dry for a week at room temperature in Petri dishes and were then kept in sealed flasks containing a desiccant (silicagel) in order to avoid rehydration of very hygroscopic materials.

2.3. Microscopic analysis

No sooner had the dried vestibular fluid samples been taken out from the sealed flask, were they observed successively under transmitted and polarized light by

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