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Gender proteomics II. Which proteins in sexual organs

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ABSTRACT

In continuity with the review dealing with differences by gender in non-sexual organs [1], this review collects data on the proteomes of the sexual organs as involved in human reproduction, under both physiological and pathological conditions. It also collects data on the tissue structures and biological fluids typical of pregnancy, such as placenta and amniotic fluid, as well as what may be tested on preimplantation embryos during medically assisted reproduction. The review includes as well mention to all fluids and secretions connected with sex organs and/or reproduction, including sperm and milk, to exemplify two distinctive items in male and female physiology.

Significance: The causes of infertility are only incompletely understood; the same holds for the causes, and even the early markers, of the most frequent complications of pregnancy. To these established medical challenges, present day practice adds new issues connected with medically assisted reproduction. Omics approaches, including proteomics, are building the database for basic knowledge to possibly translate into clinical testing and eventually into medical routine in this critical branch of health care.

1. Introduction

The subtitle of this review reads 'Which proteins in sexual organs'. Sexual organs, however, is a shorthand to mean not only the tissue structures of the gonads but also all of the fluids and secretions connected with them. Our writing also considers all structures and fluids involved in pregnancy and in its outcome *e.g.* placenta and amniotic fluid, but also colostrum and milk, as well as specimens connected with medically assisted reproduction. As in [1], data are taken as far as possible from human studies but information from work on animals is included when sample procurement from our species would be impractical or unethical.

The number of papers that we survey in this review, focusing on a *small number* of specialized organs, matches that in the first one in this thematic issue, dealing with *all* other parts of our body [1]. As we have already stressed, the interest for male/female differences in non-sexual organs is recent and the number of publications is comparatively small overall and even more so when individual topics are considered. Instead, understanding the physiology at the basis of human reproduction is an established research field: reference to these studies is being made when monitoring pregnancy progression as well as when addressing the issues of infertility and, more recently, of medically assisted reproduction.

We present in this review proteomics data for both, physiological and pathological conditions. For the latter, we specifically focus on infertility and the connected issue of medically assisted reproduction, with only some mention to a different and wider topic – cancer of uterus, breast and prostate.

Before proceeding with the writing, let's shortly frame the issue of infertility in either sex. As reviewed in [2], the most common conditions observed in infertile women (polycystic ovary syndrome, endometriosis, anomalies of the female reproductive system) are multifactorial; genetic infertility is instead connected with mutations in *FMR1*, *FOXL2*, *GLAT*, or *POLG*. In infertile men, the main gonosomal aneuploidy is represented by Klinefelter syndrome (47,XXY). Mutations in *CFTR* can result in congenital bilateral absence of the vas deferens, or obstructive azoospermia. Various Y chromosome microdeletions cause non-obstructive azoospermia or severe oligospermia; the number of chromosomal aberrations inversely correlates with sperm count.

Medically assisted reproduction involves a growing number of infertile individuals. The most recent data for Europe, reviewed in [3], show the average number of assisted reproductive technologies cycles in 2010 to have been around 8000 per million women 15–45 years of age, with peaks close to 15,000 per million in some countries. This situation poses new challenges to *research, clinical practice, ethics, legal issues and policy* as listed in the subtitle to the comprehensive review by

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Harper et al. [2]. Some of these challenges are open to contribution from omics technologies. For instance, screening on preimplantation embryos [4] has moved concern and diagnostic procedures to the phase that precedes the beginning of gestation. This adds to the routine monitoring of pregnancies and to the continuing search for predictors/early markers of congenital disease of the fetus as well as of pregnancy complications, including pre-eclampsia/eclampsia, pregnancy-induced hypertension, recurrent pregnancy loss and pre-term delivery.

The proteomics data on sex organs *et al.* in both physiological and pathological conditions along the above outline are thus listed and commented in the following. Ladies first.

2. Which proteins in sexual organs?

2.1. Female items

2.1.1. (Mainly) changes along the menstrual cycle

Hormonally-induced variations can be recognized in the composition of serum and urine along the menstrual cycle (dealt with in [1]): on this basis, it may be safely assumed that even deeper changes should be observed in the structures more closely connected with sexual functions.

In this perspective, the **nipple aspirate fluid** [5–7] was collected weekly from both breasts of a group (12) of premenopausal women during two months while measuring their serum levels of luteinizing hormone, follicle stimulating hormone and estradiol to determine the phase of each menstrual cycle. The individual samples were processed through SELDI; it was concluded that nipple aspirate fluid proteomic profile does not vary substantially during the menstrual cycle [8].

Another non-invasively collected sample, **endometrial fluid**, was so far analyzed only during the secretory phase of the menstrual cycle. The combination of three analytical strategies, gel-based and gel-free, led to the identification of > 800 different proteins [9]. The analysis of a coarser type of sample, **cervico-vaginal fluid** as a mixture of fluids originating from the vagina, cervix, endometrium, and oviduct, collected without control for subject's time point during the menstrual cycle led to the identification of a total of 685 proteins, mainly extracellular or membrane components, with several defense-related proteins (azurocidin, defensins, dermcidin, haptoglobin and lactoferrin) and many serine and cysteine proteases [10].

The **endocervical mucus** was studied before, during, and after ovulation. Among the 194 identified proteins, 3 gel-forming (MUC5B, MUC5AC, and MUC6) and 2 transmembrane mucins (MUC16 and MUC1) were detected. The analysis of mucin O-glycosylation showed an increase of GlcNAc-6GalNAcol core 2 structures and a relative decrease of NeuAc residues around ovulation, whereas NeuAc-6GalNAcol and NeuAc-3Gal- epitopes are typical for the non-ovulatory phases [11].

Evaluation of differentially regulated proteins between proliferative and secretory phase in the proteome of human **endometrium** [12] was carried out, with different procedures, on biopsies (*ca.*100 mg), resulting in the identification of 8 proteins [13] and on eutopic endometrium samples collected during routine surgical procedures, resulting in the identification of 49 proteins [14]. The differences in the narrow temporal interval between the pre-receptive and receptive phases were explored on bioptic material, resulting in the identification of 31 proteins [15]. Not only the proteomic protocols but also the procedures for phasing the endometrial samples and the sample timing itself differed from one report to the other. Likely as a result of these differences the overlap between the reported results is minimal, even when disregarding the difference between subunits of complex proteins or protein isoforms: as shown in Supplementary Table 1, not more than six proteins are found repeatedly in the three studies; opposite regulation is sometimes reported. The only common finding is with fibrinogen, a protein involved in hemostasis and wound repair but whose connection with hormonal status is well documented. Indeed, systematic cyclical variations of fibrinogen levels along the menstrual cycle have been reported, but individual cycling was small in comparison with intra-individual variability [16]. Morphology and viscoelastic properties of fibrin clots were found to depend on the levels of estrogen and progesterone [17]. In post-menopausal women without medication, fibrinogen correlates with endogenous estrogen levels, the association being stronger among women with body mass index over 25 kg/m^2 [18]. Conversely, the Postmenopausal Estrogen/Progestin Interventions (PEPI) trial demonstrated that hormone replacement therapy could limit the increase in fibrinogen in post menopausal women [19]. Maternal fibrinogen is essential for successful pregnancy [20]; in rats, fibrinogen gamma is specifically increased in uterine epithelial cells at the time of implantation, with higher dimer levels [21].

The proteins of the longest list may be classified according to their function as molecular chaperones (30%), structural proteins (27%), proteins involved in RNA biogenesis, protein biosynthesis and nuclear organization (14%), proteins involved in signal transduction (12%), immunity-related proteins (10%) and mitochondrial enzymes (4%) [14]: such a variety of aspects is to document the extensive remodeling occurring during this crucial biological stage.

In a previous shotgun investigation, only 2 proteins with unquestionable differential regulations in the secretory *vs* the proliferative endometrium could be identified (glutamate NMDA receptor submit zeta 1 and FRAT1, not in the above lists) [22].

When the endometrial tissue (*ca.* 60 nL in volume) was laser-microdissected into glandular epithelium and stroma, and the tryptic digests were analyzed by LC-MS/MS [23], the findings in the two compartments were vastly dissimilar. In the epithelial cells from the proliferative endometrium 318 proteins were identified as significantly altered *vs* the secretory endometrium; 145 of these proteins had a role in *cellular growth and proliferation* pathways. Conversely, only 19 proteins were significantly altered in the stromal samples harvested from the proliferative *vs* the secretory endometrium, and *tissue development* was identified as the most significantly enriched pathway.

From this summary, we must conclude that, unfortunately, the above investigations have failed to provide a consensus differential pattern; however, their number, together with the heterogeneity of the sampling procedures and of the analytical protocols reflect a strong interest on this specimen, in at least two perspectives – endometriosis and medically assisted reproduction.

Before closing this section, we have to mention one more type of specimen, **menstrual blood**, whose composition may be of interest both to pathology (infertility and uterine pathologies) and to forensic science (distinction between menstrual blood and circulating blood). The samples collected during the central days of the menstruation were analyzed along five protocols. More than 1000 proteins overall were identified, one third of which (361) by at least two methods. When the menstrual blood proteome was compared with those of circulating blood (1774 proteins) and vaginal fluid (823 proteins), 385 components – most of them involved in processes typical of the endometrial cycle – were found unique to menstrual blood.

2.1.2. Changes in pregnancy

As we did in 2.1.1, we first review the proteomic evidence on **biological fluids** – to conclude, once more, that data are all but systematic.

The closest approximation to an in-depth evaluation of the changes induced by pregnancy in **serum** may be found in a report assessing changes in relative protein abundance between paired serum samples, collected in the same pregnant women during first and third trimester and analyzed by 2DE-DIGE [24]. Significant differences were detected in *ca.* 10% of the resolved spots; the identified proteins were found associated with gestational age, cytoskeletal remodeling, blood pressure regulation, lipid and nutrient transport, and inflammation. Two proteins were detected for which there was either only transcriptional