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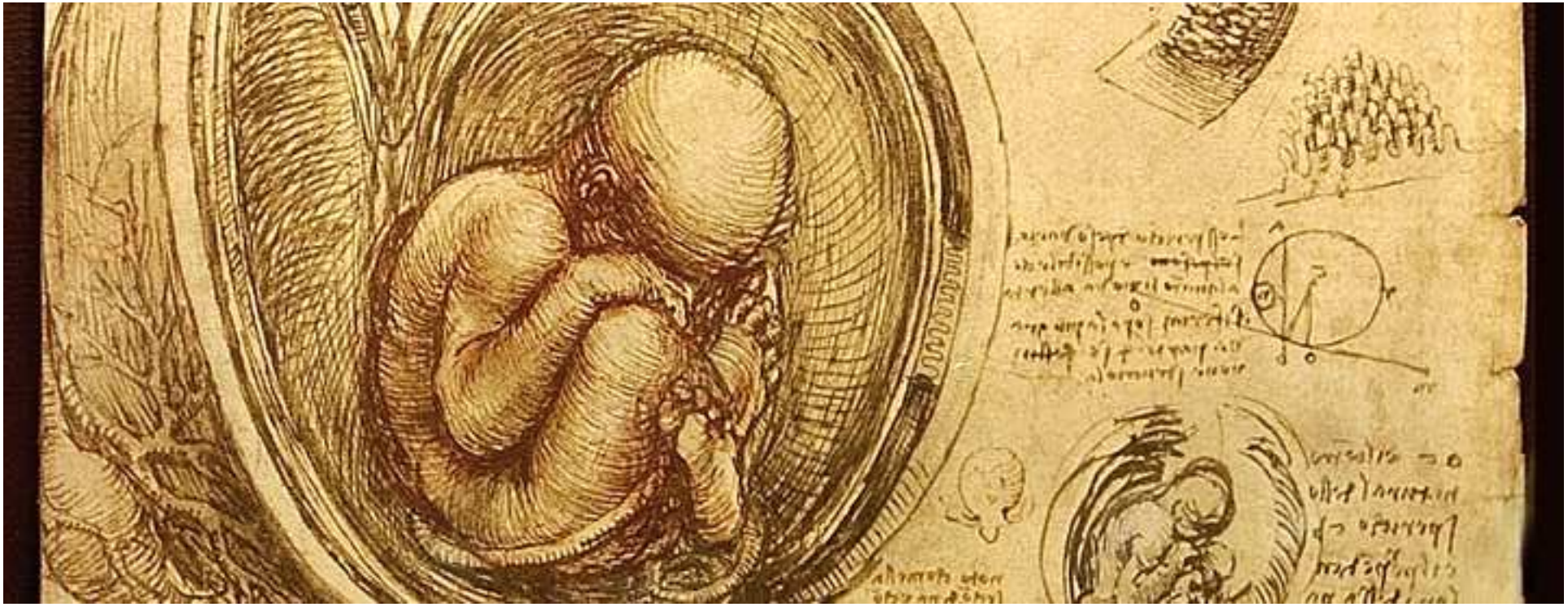

Highlights

- The review lists proteomic data on sexual apparatuses and reproductive function
- Focus is on our species and both tissues and biological fluids are dealt with
- Both physiological and pathological conditions are analyzed
- Pregnancy and its complications are dealt with in detail

REVIEW ARTICLE

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.

Highlights

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- Focus is on our species and both tissues and biological fluids are dealt with
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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 8,000 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 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 protein (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 1,000 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 evidence of existence (pregnancy-specific beta-1-glycoprotein 4) or no information had been available about differential abundance along pregnancy (gelsolin). Changes in abundance of proteins in serum that are associated with syncytiotrophoblasts (beta-2-glycoprotein I, gelsolin, and pregnancy-specific beta-1-glycoprotein 1) probably reflect dynamics of the proportion of placental proteome shed into maternal circulation.

In contrast to the lack of overall evaluations on serum, focused studies in the course of gestational time have been devoted to subsets of serum proteins. One of these distinctive classes is that of lipoproteins, in keeping with the metabolic shift from the glycolytic to the lipolytic pathway in pregnant women. Apolipoprotein C-II is mainly distributed in the HDL of normolipidemic individuals, whereas it is predominantly found in the VLDL and LDL of hypertriglyceridemic individuals; both the mature proteolytically cleaved protein and its proform can activate lipoprotein lipase. Mature apo C-II represents around 1/15 of total apo C-II in early and 1/3 in late gestation (7x increase); pro-apo C-II only increases in the third trimester (by 40% *vs* first trimester). Singly and doubly sialylated apo C-III subtypes linearly increase with gestational age (apo C-III_2, 3x, and apo C-III_3, 2x). None of the other apos changes during normal pregnancy but modified apo A-II forms are significantly elevated in plasma from mothers who delivered prematurely relative to term controls [25]. In a mother/fetus comparison, maternal HDLs appear to mainly belong to the dense HDL_3, fetal HDL to the light HDL_2 sub-fraction. Some proteins are statistically elevated in fetal HDL (apoE, proteins involved in coagulation and in transport processes), some are decreased or absent (apoA-I, apoL and paraoxonase 1); the quantity of CETP is similar but its enzymatic activity is reduced to ½ in fetal HDL. Overall, with their distinct composition, fetal HDLs are associated with decreased anti-oxidative and innate immunity properties [26].

A second focused study deals with glycoproteins, or better with their N-linked glycans, which means with the serum N-glycome. Changes in the glycosylation during pregnancy were first reported for a model protein, transferrin, as early as in 1988 [27]. It is known that *specific glycoforms are involved in recognition events; this is true also of the key molecules involved*

in the innate and adaptive immune response [28]. Pregnancy requires partial suppression of the immune system to ensure maternal-fetal tolerance. Protein glycosylation, and especially terminal sialic acid linkages, are of prime importance in regulating the pro- and anti-inflammatory immune responses. A survey on all the serum proteins shows that the levels of extensively sialylated bi-, tri-, and tetra-antennary glycans increase during pregnancy, while biantennary glycans, with no more than one sialic acid, decrease [29]. In a more detailed investigation, a very large number of glycans (77) was followed through 6 time points, during and after pregnancy. An increase during pregnancy and a decrease after delivery were observed for both alpha-2,3- and alpha-2,6-linked sialylation, with a different time evolution after delivery [30]. When aiming at Igs, marked differences in glycosylation exist between Fab and Fc (higher levels in Fab of galactosylation and sialylation, incidence of bisecting GlcNAc, and presence of high mannose structures, lower levels of fucosylation). During pregnancy Fab N-glycan sialylation is higher and bisection is lower relative to postpartum time points, and nearly complete galactosylation of Fab glycans may be observed throughout. Fc undergoes similar changes in glycosylation [31].

Urine was investigated at a single time point (by comparing the pattern one day before and 30 days after vaginal delivery). Analysis through 1-DE and LC-MS/MS lead to the identification of > 800 proteins common to both conditions *vs* ca. 600 proteins unique to pregnancy and twice as many unique to non-pregnancy samples. Of the 105 identified phosphoproteins, 14 were up-regulated and 2 were down-regulated in urine samples from women just before vaginal delivery [32].

Two types of secretions have also been investigated. One is **cervico-vaginal fluid** in healthy, pregnant women at term. After 2-DE, 15 proteins could be identified with various functions (transport and calcium binding, fatty acid metabolism, proteinase inhibitors, defense against inflammation and oxidative stress) [33]. Another is **cervical mucus** plug, the sealant of the uterine cavity during pregnancy, which was obtained from women in labor at term and analyzed by LC-MS/MS. Several proteins, which have been described neither in the cervical mucus of non-pregnant women nor in cervicovaginal fluids, were identified, such as CD81 antigen and pregnancy zone protein. Gene ontology analysis of identified proteins showed significant enrichment of several biological processes including *activation of plasma proteins involved in acute inflammatory response* and *positive regulation of cholesterol esterification* [34].

As for tissues, it is all but surprising that the number of human samples that could ever be assessed is very small. One notable exception is **myometrium** (uterine smooth muscle), whose S-nitrosoproteome was on focus trying to understand the regulation of uterine contraction-relaxation in view of a possible treatment for preterm labor. The study was based *on the hypothesis that myometrial NO-mediated relaxation is dependent on the S-nitrosation of specific and critical proteins.* Myometrium biopsies were obtained from three groups of mothers undergoing elective cesarean section (laboring preterm, laboring at term, at term & not laboring) and then processed through a

protocol starting with incubation with S-nitrosoglutathione. More than 100 proteins were found to be S-nitrosated in one or more states of human pregnancy; as shown by Figure 1, some of them are present at higher concentration in samples from laboring, some in samples from non-laboring mothers [35].

Other studies have involved instead material from animal species: rat for corpus luteum [36], sheep for endometrium (aglandular caruncular *vs* glandular intercaruncular areas) [37] and extracellular vesicles (exosomes and microvesicles, of endosomal and plasma membrane origin, that mediate conceptus-maternal interactions during early pregnancy) [38].

Back to our species, a number of investigations have addressed the issue of fertility *vs* infertility. 2DE-DIGE on **uterine lavages** collected from fertile and infertile women during the mid-secretory phase, and from fertile women during the mid-proliferative phase, detected 18 differential spots between fertile and infertile women: all of them, however, correspond to major serum proteins [39]. A more specific picture of the events occurring at the point of the first mother-embryo contact may be provided by an *in vitro* system. ECC1 is an endometrial adenocarcinoma epithelial cell line that may be taken as model of the **endometrial luminal epithelium**. ECC1 cells were treated with estrogen in the absence/presence of progesterone to mimic the proliferative and secretory phases of the menstrual cycle, and hCG was used to mimic the embryonic signal at the time of conception. Proteomic analysis (1-DE and LC-MS/MS) on cell lysates identified ca.150 progesterone-regulated cellular proteins, with further ca.200 significantly altered in response to hCG; cellular changes were associated with metabolism, basement membrane and cell connectivity, proliferation and differentiation. In the shotgun analysis of soluble **secretome** 123 proteins were found significantly altered by progesterone, and 43 proteins altered by hCG, including proteins associated with cellular adhesion, extracellular-matrix organization, developmental growth, growth factor regulation, and cell signaling [40].
In a symmetric approach, with *in vitro* fertilization through intracytoplasmic sperm injection cycles and culture of the zygotes before in utero transfer, the **secretome of pre-implantation embryos** could be evaluated from the spent media. Separation by nano-HPLC and identification via tandem nano-ESI MS led to the identification of unique proteins in both the positive- and negative-implantation groups [41]. A more recent investigation focused on the quantitation of few cytokines combined with time-lapse morphokinetic analysis: higher implantation rates were found associated with the presence of IL-6 and a cell cycle duration of 5-12 h [42]. As already mentioned in [1], during the earliest phases of their existence, female embryos have two active X chromosomes, which entails a different proteome *vs* male embryos. Direct analysis of the secretome as above, or indirect quantitation of cellular functions under the influence of X-linked genes/proteins, may be a means of non-invasively determining the sex of an embryo (as an alternative to blastomere biopsy followed by the identification of the sex chromosomes through either FISH, PCR, SNP arrays or comparative genomic hybridization) [43]. **Blastocoel fluid**, isolated by micromanipulation from surplus blastocysts, was found to contain several heat shock proteins as well as proteins that

regulate ciliary assembly and function, together with zona pellucida proteins, vitamin D-binding protein, and retinol-binding protein 4 [42]. Knowledge on the composition of this liquid could possibly assist defining optimal culture conditions of human embryonic stem cells for possible applications in regenerative medicine. Similar investigations on preimplantation embryos have been carried out also on bovine specimens: blastocoel fluid and blastocyst cells, around day 6 [44], but also ovoid and elongated embryos, containing the primitive yolk sac fluid, as late as at day 13, after *in vitro* produced embryos had been transferred into a recipient heifer and cultured *in vivo* during 7 further days (embryos were recovered by flushing both uterine horns after slaughter) [45].

Most reports dealing with **placenta** address the differences between physiological and pathological conditions, as detailed in the following; a few refer instead to baseline conditions. As an example, Mushahary *et al.* performed 2DE-MS/MS on tissue from full term delivery and then integrated their identifications with results from earlier analyses, compiling a comprehensive dataset that also included function and clinical relevance of each item [46]. There are obvious ethical and practical limitations to the procurement of material along the progression of human pregnancy. One investigation addressing the difference between early and late weeks compared late first-trimester placentas from pregnant women referring for legal abortion due to mother indication (such as heart disease) to normal full-term placentas from normotensive mothers who underwent an elective-term cesarean section around the gestational age of 38 weeks. The functions of the differential proteins (4 more and 7 less abundant in late first-trimester placentas) range between energy metabolism, redox regulation, chaperoning, and muscle contraction [47]. Using a mouse model, it was instead possible to carry out at two time points (embryonic day 7.5 and 10.5) an in-depth comparative proteomic analysis of extraembryonic tissues and placentas after either *in vivo* fertilization and development or *in vitro* fertilization and culture. More than 150 differential proteins were identified in each type of specimen; many were functionally associated with genetic information processing, such as impaired *de novo* DNA methylation, as well as post-transcriptional, translational and post-translational dysregulation; such findings were confirmed by hypomethylation of the genetic material and by a lower level of correlation between transcriptome and proteome in the *in vitro* samples. Other differential proteins were involved in energy and amino acid metabolism, cytoskeleton organization and transport, vasculogenesis and angiogenesis: disturbance of these processes and pathways is likely to be associated with embryonic intrauterine growth restriction, an enlarged placenta, and impaired labyrinth morphogenesis observed in the *in vitro* samples [48].
Two regions may be recognized in the placenta: the chorionic plate, composed primarily of fetal cells, and the basal plate, consisting of a mixture of fetal (trophoblast) and maternal cells. In an investigation, portions of both plates were excised and homogenized; after removal of high M_r proteins by precipitation with acetonitrile, the supernatant, containing small proteins, peptides, lipids, and potentially other metabolites, was processed through LC-MS analysis. Statistically significant differences were observed between the

two placental regions for 16 molecular species: of these, four were peptides, the remainders lipids [49].
The apical plasma membrane of the multinuclear syncytiotrophoblast features the human feto-maternal barrier and is the site of initial transport processes across the placenta. More than 170 integral and peripheral proteins were identified in isolated membranes, containing between 1 and 12 trans-membrane domains or lipid anchors, and with functions as transporters or receptors, or involved in signal transduction processes and vesicular trafficking [50]. A follow-up investigation addressing the same subproteome (and using highly enriched preparations) lead to the identification of some more proteins, expanding the current proteomic database of the apical plasma membrane of the syncytiotrophoblast [51].
Another specialized material analyzed by 2DE-MS/MS was the culture of cells from chorionic villi, sampled during prenatal diagnosis of various fetal disorders: almost 300 proteins were identified, including enzymes, structural, signalling and carrier molecules [52].

Our group was involved in one of the first investigations on the proteome of **amniotic fluid** collected in gestational weeks 16-18. We concentrated our analysis on the M_r fraction < 50 kDa, obtained by gel filtration (and as such extensively depleted in albumin) [53]. The 2-DE map shown in Figure 2 was obtained under non-reducing denaturing conditions, which provide the best resolution among the sample components [54]. In this fraction we identified 33 full-length proteins: 23 had never been previously detected in the amniotic fluid and, of these, 22 are not present in the human plasma proteome under physiological conditions. We also identified fragments of 16 larger proteins; from the sequence coverage data, several correspond to autonomous domains that may have biological roles on their own. Many of the detected proteins and peptides appear to be involved in critical regulatory processes associated with placentation and early development.
In that same year (2007), amniotic fluid was investigated with different technical approaches. A free-flow technique based on isoelectric electrophoresis was used by Michel *et al.* [55], resulting in the identification of 69 proteins: 26 of them were exclusively present in amniotic fluid and not in plasma of the same mother. Two types of 2D LC-MS/MS as well as LC-SDS-PAGE-LC-MS/MS were instead used by Cho *et al.* [56], leading to the identification of a much larger number of proteins (as many as 842 non-redundant proteins). A time course analysis of amniotic liquid shows changes in protein abundance with gestational age, the largest occurring between the first and second trimesters (572 out of 755 protein spots); among the proteins increasing in level: apo A-I, apo A-II, gamma-glutamyl transferase 4, IGFBPs, and pigment epithelial derived factor; among those decreasing in level: alpha2-HS-glycoprotein, angiotensinogen, ceruloplasmin, kininogen, orosomucoid, and ubiquitin; in addition to IGFBPs, transthyretin and vitamin D binding protein are present at very high levels throughout gestation [57].

It is known that the secretion of mammary glands changes with time. In late pregnancy and in the first days after childbirth it has the composition of **colostrum**: richer in proteins, vitamin A, and sodium, but poorer in carbohydrates, lipids, and potassium than mature milk. Bioactive components

in colostrum are growth factors, which stimulate the development of the gut, and antimicrobial factors - components of the adaptive (antibodies = IgA, IgG, IgM) and of the innate immune system (complement, lactoperoxidase, lactoferrin, lysozyme, and proline-rich polypeptides), together providing passive immunity against pathogens. Minor proteins in the aqueous phase of colostrum were identified after immunodepletion of the major components, to a total of ca.150 hits [58]; colostral fat globule membrane proteins were also investigated, to a total of ca.100 hits [59]. Comprehensive time-course surveys of the dynamic changes in **milk** protein abundance over a twelve-month lactation period involved both whey [60] and fat globule membrane [61] fractions.

The analysis of whey focused on low-abundance proteins, enriched by adsorption on ProteoMiner beads (reviewed in [62]), and identified by LC-MS/MS after in-solution digestion [60]. Most identified proteins were involved in growth/maintenance, immunity support, lipid metabolism – the known key functions of breast feeding. As seen in Figure 3, some proteins (alpha-1-antitrypsin, carbonic anhydrase, chordin-like protein 2, galectin-3-binding protein, lactadherin, lipoprotein lipase, and tenascin) are expressed at higher concentrations during early than during late lactation; others (fatty-acid binding protein, lysozyme C, monocyte differentiation antigen, proactivator polypeptide, transcobalamin-1, and zinc-R-2-glycoprotein) have an opposite trend. An investigation that also includes the major whey proteins but concentrates on the first six months after childbirth (weeks 1, 2, 3, 4, 8, 16, 24) draws similar conclusions, with ca.10% of the identified proteins significantly changing in abundance with time. This includes serum albumin and fatty acid binding protein, which are involved in transporting nutrients to the infant; conversely, the decrease of the enzyme bile salt-activated lipase as well as the immunity proteins immunoglobulins and lactoferrin coincide with the gradual maturation of the digestive and immune system of the infants [63].

In addition to full-length proteins, milk contains large amounts of peptides. A comprehensive peptidomic investigation led to the identification of over 300 of them, most deriving from beta-casein (and none from lactoferrin, alpha-lactalbumin, and IgA). From sequence data, several peptides with known antimicrobial or immunomodulatory functions could be recognized. Proteolytic events seem thus to provide substances able to protect both the mother's mammary gland and her nursing offspring from infection [64]. A systematic survey suggests a mechanistic perspective: proteolysis is selectively favored when able to release bioactive peptides and selectively prevented when it would hamper the protein’s function; presence of specific proteases, position and concentration of cleavage sites, and intrinsic disorder of segments of the protein are the factors contributing to the differential susceptibility to proteolysis [65].

Proteolytic cleavage in milk occurs after K and R residues (characteristic of plasmin or trypsin cleavage), after F, L, W or Y (pepsin- or chymotrypsin-like enzymes), after A, I, L, P, and V (cathepsin D or elastase) and at the termini of peptides (proline endopeptidase). In stomach aspirates sampled 2 h after feeding through a naso-gastric tube from infants who were hospitalized due to health problems unrelated to the gastrointestinal tract, a largely similar assortment of peptides was observed, with enrichment, however, in cleavage

after F, L, W, and Y and low frequency of further cleavage after K and R residues [66].
Other specific components analyzed from human milk are the proteins in extracellular vesicles (submicron-sized formations released by cells for intercellular communication) [67], and the N-glycoproteome of the milk fat globule (in a cross-species comparison involving six farm animals) [68]. An earlier application of proteomics to this field had been in the evaluation of proteins from different mammalian species looking for suitable substitutes for breast milk for infants allergic to cow's milk-based formulas [69].

2.1.3 Pathological aspects of pregnancy

Due to their frequent occurrence [70] and the severity of the sequels, hypertensive disorders of pregnancy have been extensively investigated, including proteomic tools.

There is little doubt that the dysregulation of systemic and local blood flow typical of **pre-eclampsia/eclampsia** originates from placenta. In all pathological cases, placental tissue is available in large amounts soon after symptoms develop due to the need of emergency delivery of the babies of affected mothers. Accordingly, much of the current evidence comes from the analysis of placentas; the final goal of this area of investigation, however, remains the identification of one or a few biomarker(s) that may be easily evaluated in the blood of pregnant women and that predict(s) the development of the pathological condition in advance of overt, life-threatening symptoms.
Having gone through the lists of differential proteins, the conclusion is – as for many other sample types – that no obvious overlap exists between the findings of different investigations. The likely reasons are connected with the different analytical procedures used in either case (1-DE [71], 2-DE [72, 73], 2D LC [74]), but also with the alternatives in sample procurement and processing. Indeed, whole placental tissue implies a heavy burden of blood (a prominent hemoglobin spot dominates the proteomic pattern in [72]) and the finding of serum components among the differential proteins. This problem is much reduced focusing the analysis on trophoblast tissue, microdissected by laser capture [71, 74]: this technique, associated with 2D LC-MS/MS after ICAT labeling, resulted in the so far most exhaustive list of differentially regulated proteins. Trophoblast proteins detected at higher or lower concentrations in pre-eclampsia placentas are listed in Table 1 [74]. N-glycoproteome phosphoproteome and nitroso proteome of pre-eclamptic *vs* normotensive placentas have also been studied [75, 76].

Table 1 – Proteins detected at significantly different concentrations in pre-eclamptic *vs* normotensive placentas *

proteins at higher concentrations in pre-eclampsia placentas		proteins at lower concentrations in pre-eclampsia placentas	
protein name	*fold change*	*protein name*	*fold change*

Fraser syndrome 1 isoform 2	13.7	lamin B1	100.0
pancreatic tumor-related protein	13.7	clathrin heavy chain	100.0
KIAA0226 protein	9.3	laminin β-2 chain	12.5
anti-pneumococcal antibody A7 light chain variable region	8.7	fibronectin 1 isoform 3 preproprotein	9.1
DNA-dependent protein kinase catalytic subunit	8.6	laminin β-2 chain precursor	9.1
ADAMTS-like 3	8.0	fibrillin	8.3
osteonidogen	8.0	laminin M chain	7.7
low density lipoprotein-related protein 1	7.3	fibrillin 1	6.7
immunoglobulin κ light chain	5.9	laminin α-5 chain	6.3
ENO1P protein	5.8	laminin γ-1 chain	5.6
coagulation factor XIIIb	5.8	laminin M chain	5.6
fibulin-1 A	5.7	Cd-7 Metallothionein-2 (β-domain)	4.8
mitogen-activated protein kinase kinase kinase 5	5.7	heparin sulfate proteoglycan	3.7
human elongation factor 2	5.3	migration stimulation factor FN70	2.9
nidogen	4.8	EGF-like domain	2.3
porin 31HM	4.6	integrin α-6 subunit	2.2
tenascin XB 1	4.5	annexin VII isoform I	2.1
myosin heavy chain	4.2		
plasminogen	3.6		
chaperonin (HSP60)	3.5		
collagen type XIV	3.2		
fibulin-1 A	2.9		

* on laser-capture microdissected trophoblast tissue processed through iTRAQ 2D LC-MS/MS [74]

A very interesting perspective in terms of both, scientific insight and diagnostic applications of the proteomics findings, is provided by a prospective study addressing the high-to-medium-abundance serum proteins at 20 weeks of gestation in women who later developed pre-eclampsia; the group was further sorted into women giving birth to babies with either an appropriate or a small birth weight for gestational age, and compared to healthy controls with uncomplicated pregnancies [77]. The technical approach was DIGE on

samples immunodepleted of the most abundant components. The differential proteins are involved in lipid metabolism, complement cascade, coagulation, inflammation, extracellular matrix remodeling, protease inhibition and heme scavenging. The authors postulate that many of the identified proteins may be mediators or regulators of the maternal vascular, inflammatory and coagulation responses to placenta-derived triggers and may reflect a susceptibility to the condition. One half of the differential items overlap with proteins found in complex with high-density lipoproteins and linked to cardiovascular disease, confirming a connection between the different types of vascular dysfunction.
A more recent investigation took a different approach: during the discovery phase, samples drawn at the 15th week of pregnancy and eventually pooled depending on later development of pre-eclampsia or absence of health problems, were immunodepleted of the top-abundance proteins, then trypsin digested and iTRAQ labeled; the resulting peptides were processed through 2D LC and MALDI-TOF/TOF. Based on the results of the discovery phase, a label-free selected reaction monitoring workflow was defined, with the identification of some peptides, derived from platelet basic protein (also known as CXCL7) and pregnancy-specific glycoprotein, as potential predictive markers of early-onset pre-eclampsia [78].

Other investigations addressed instead **pregnancy-induced hypertension** [79] and could identify a few proteins, among the differential signals they detected (alpha-2-HS-glycoprotein, fibrinogen alpha, kininogen-1, some – undefined – complement component). Of some relevance, the authors of this study warn that the levels of most of the differential peptides were significantly decreased by albumin+IgG depletion, a standard procedure before proteomics analysis of serum.
Finally, an investigation compared pregnancy-induced hypertension and eclampsia for their effects on the urinary proteome [80]. The differential proteins were connected with such biological processes as blood coagulation, cell adhesion and differentiation, immune response and cytoskeleton development.

Recurrent pregnancy loss is defined by the occurrence of at least 3 pregnancy losses in series prior to 20 weeks from the last menstrual period; it affects 1% to 2% of women. A recent investigation compared placental villi from women who have undergone early pregnancy loss and from age-matched women undergoing intentional terminations of pregnancy at the same gestational age. About 10% of the ca. 6,000 identified proteins were found differentially regulated; the differential proteins participated in a variety of signaling pathways, including focal adhesion and ribosome pathway [81].
An earlier work applied 2-DE to decidual cells and recognized sustained endoplasmic reticulum stress in pathological samples, including decreased levels of glucose-regulated protein 78 and valosin-containing protein, and a high burden of ubiquitinated proteins [82]. In an investigation by 2-DE on serum samples from women facing recurrent pregnancy loss, 3 proteins (alpha-1-antichymotrypsin, alpha-2-macroglobulin, and insulin-like growth factor-binding protein 1) were found at increased levels whereas inter-alpha trypsin inhibitor-heavy chain 4 was found at reduced concentrations in the

native 120 kDa form and at increased levels in the form of a 36 kDa fragment [83].

Finally, **preterm birth**, defined as delivery before 37 weeks of gestation, affects 15 million infants born each year, varying from ca. 5% to 18% of all births across different countries worldwide. In the US, it is the leading cause of neonatal death and the second-leading cause of death in children before 5 years of age. Preterm birth is also a major source of long-term health problems, including chronic lung disease, hearing and visual impairments, and neurodevelopmental disabilities, such as cerebral palsy. Prior history of spontaneous preterm delivery is currently the single strongest predictor of subsequent events: after a prior preterm delivery, the probability of a second is 30 to 50%. Other maternal risk factors include black race, low maternal body mass index, and short cervical length.
In a proteomic investigation, preterm birth was found associated with serpin B7 [84]. In another it was found to correlate with insulin-like growth factor-binding protein 4 and sex hormone-binding globulin: the former being found at higher, the latter at lower circulating levels in the time span between the beginning of the 19^{th} and the end of the 21^{th} week of gestation, in women who incurred preterm delivery [85]. The ratio between the two biomarkers was assessed as predictor of preterm birth: Figure 4 shows the Kaplan-Meyer plot for mothers diagnosed at low *vs* high risk for preterm delivery as well as the receiver operating characteristic curve for the predictor.

A connected investigation explored instead cervical-vaginal fluid looking for proteomic markers of preterm labor. 2D LC-MS/MS and 2D-DIGE identified 28 and 17 proteins, respectively, as potential biomarkers for the development of new tests for the early, noninvasive positive prediction of preterm birth. The short list of overlapping findings between the two approaches includes: annexin A3; calgranulin A and B; cystatin A; fatty acid-binding protein, epidermal; heat-shock protein beta-1; 14-3-3 protein sigma [86].

2.2 Male items

No investigations have been devoted to tissue specimens if not in the context of comparative analyses of health *vs* disease samples – most often of samples from fertile *vs* infertile subjects.

Current evidence suggests that, in addition to deliver the packaged male DNA to the oocyte, sperm provides a specific epigenetically marked genome together with a complex population of RNAs as well as proteins that are crucial for early embryogenesis. The seminal fluid itself appears to serve multiple roles, providing a series of supplementary components that allow the sperm to successfully reach and fertilize the oocyte and prepare the female immune system to tolerate the semiallogenic embryo [87].

The components of **seminal plasma** have been individually identified [88, 89] and then analyzed in association with sperm functional alterations. In a detailed investigation, three such parameters were monitored - mitochondrial activity alterations, acrosome damage and DNA fragmentation. Proteomic

investigation was carried out through LC-MS and the results were statistically evaluated through univariate and multivariate analysis. This led to the identification of a small number of markers, positively or negatively correlated with each of the three conditions (Table 2) [90].

Table 2 – Seminal plasma biomarkers of sperm functional traits (from Table 2 in [90])

trait	*protein name*	*protein function(s)*	*vari-co-cele*[a]
mitochondrial activity alteration	annexin A7	- regulation of exocytosis - anti-apoptotic effect	
	endoplasmic reticulum resident protein 44	- protein disulfide bonds formation and rearrangement - cell redox homeostasis	
	glutathione S-transferase mu 3	- acrosome action and fertilization - reactive oxygen species detoxification	↑
acrosome damage	phospholipid transfer protein	- binding to vitamin E - regulation of phospholipid exchange in lipoproteins	
DNA fragmentation	proteasome subunit alpha type-5	- protein degradation - removal of sperm with damaged DNA	↑[b]
DNA integrity	cysteine-rich secretory protein LCCL domain-containing 1	- cellular adhesion - fertilization	
	cysteine-rich secretory protein LCCL domain-containing 2	- cellular adhesion - fertilization - anti-inflammatory effect	uni-late-ral
	retinoic acid receptor responder protein 1	- tumor suppression	

[a] from Table 1 in [91]
[b] proteasome subunit alpha type-7-like

Unfortunately, none of the proposed markers coincides with those listed in [92] as differentiating the seminal plasma from fertile and infertile males when the samples were processed through SELDI-TOF MS after adsorption on a strong anion exchanger. Conversely, three at least partial matches are observed with the proteins associated with reproductive function differentially expressed in men with unilateral *vs* bilateral varicocele (rightmost column in Table 1) [91]. In this latter case, the analytical procedure was 1-DE followed by LC-MS; technical replicates were run on pooled samples. It is all too obvious from the above that individual exploratory tests based on different inclusion/exclusion criteria for control and/or patient selection, on different protocols for sample handling, on different proteomics approaches can at most hint to the definition of useful biomarkers. Only a systematic review on the findings may eventually lead to a consensus practice and to the development of suitable diagnostic tests.

Seminal plasma collects secretions from different districts of the male genital tract. The contribution from the **prostate** has been singled out in some

reports; for instance, after in solution digestion, prostasomes, membrane-bound storage vesicles found in prostate epithelial cells, have been analyzed by μLC-MS/MS [93] whereas expressed prostatic secretions have been by MudPIT [94].

Spermatozoa are transcriptionally and translationally silent, and may be characterized only via a proteomic approach. The most relevant conditions to contrast in this respect are normo- and asthenozoospermia, as defined with reference to sperm motility. Shotgun analysis of samples from a few fertile and infertile subjects led to the identification of hundreds of proteins. Principal component analysis on differential proteins (Anova p value < 0.05 and fold change > 2) resulted in a complete separation of the samples into two clusters, normozoospermic and asthenozoozpermic. No such separation could be obtained using proteomic data collected in parallel on seminal plasma samples from the same subjects. The most relevant differential proteins are the following: dynein light chain 1, cytoplasmic; fascin-3; leucine-rich repeat-containing protein 37B; ninein; PHD finger protein 3; plexin-B2; protein PROCA1; putative beta-actin-like protein 3. Some of the enriched pathways are then: axoneme activation and focal adhesion assembly, glycolysis, gluconeogenesis, cellular response to stress and nucleosome assembly [95]. Clusters found in sperm data by Self-Organizing Maps (SOM) [96] are shown in Figure 5 in the form of Venn's diagram.
A complementary approach to the above evaluation comes from the comparison between sperm subpopulations fractionated from normozoospermic samples on the basis of differential motility (non-migrated *vs* migrated spermatozoa in a swim-up setting). Similar proteomic alterations were detected in the two experiments (80 proteins differentially abundant in the two groups of samples and 93 differentially abundant in the two groups of subpopulations); involved proteins were associated with energetic metabolism, protein folding/degradation, vesicle trafficking, or were cytoskeletal components [97].
Consistently with the focus on motion, the comparison between normo- and asthenozoospermic semen samples was later narrowed to an investigation on sperm tails [98]. Differences were observed for 21 spots, corresponding to 14 proteins – none overlapping with items in the above list. An earlier study had analyzed the two compartments of heads and flagella, identifying > 500 proteins as unique to the former and > 700 proteins as unique to the latter [99]. Another specialized survey had focused on sperm glycocalyx, assessed with the use of a lectin microarray (among 91 tested lectins, 16, 13, 24 and 37 showed strong, medium, weak and negative binding, respectively) [100]. A parallel investigation had been carried out on the N-linked glycoproteome of human seminal plasma, using glycosylated peptide enrichment, combined with LC-MS/MS analysis [101]. Glycosylation is just one of the several PTMs occurring in spermatozoa. Protein synthesis is switched off long before the sperm is mature and spermatozoa may only rely on PTMs of existing proteins as a way to modulate their proteome [102, 103]. Freshly ejaculated sperm acquires the fertilizing potential by a continuing process, termed capacitation, that occurs during sperm transport through the female genital tract, and is physiologically not complete until the spermatozoon reaches the oocyte. From the above, quali- and quantitative differences observed between the 2DE

maps of freshly ejaculated *vs* 3-h *in vitro* capacitated sperm should only involve re-assortment among different protein species; this includes the effects of proteolysis, as flagellar organization proteins were found decreased during capacitation whereas their cleaved fragments increased [104]. Different PTM seem to serve different functions: N-terminally acetylated proteins and non-modified proteins appear to be more associated with the basic cellular functions, whereas acetylated, phosphorylated, and glycosylated proteins are more directly involved in specialized sperm functions [105]. Lysine acetylation is essential for sperm motility and fertilization as shown by the interference in such processes by histone acetylase inhibitors and anti-acetyllysine antibodies [106]. Lysine acetylproteome was compared between uncapacitated and capacitated sperm [107, 108]; different acetylation profiles were observed for functional proteins involved in sperm capacitation, sperm-egg recognition, sperm-egg plasma fusion, and fertilization. Phosphorylation, as resulting from the balance between the catalytic action of kinases [109] and phosphatases [110], is also of key importance in the mechanisms of sperm maturation and capacitation. Among the over 200 sites with increased phosphorylation levels during sperm capacitation, PTM of insulin growth factor 1 receptor seems to play a pivotal role [111].

Another mechanism to modify the properties of spermatozoa, and chiefly their ability to interact with zona pellucida as the first step towards oocyte fertilization, is for existing proteins to organize into multimeric complexes. This hypothesis was investigated by blue native polyacrylamide gel electrophoresis (BN-PAGE) and led to the identification of the 20S proteasome and chaperonin-containing TCP-1 (CCT) complexes [112].

2.3 Cancer of the sexual organs

As we state in the Introduction, among the physiology/pathology issues this review on the proteomics data for sexual organs aims to focus on fertility/infertility rather than on health/disease at large. However, cancer of the sexual organs deserves a mention, both because of its relevance as medical and social issue and because proteomic investigations are especially active in the field (we could retrieve a total of > 3400 original publications and >700 reviews overall; source: https://www.ncbi.nlm.nih.gov/pubmed/). Available evidence is being consolidated in a Cancer Proteomics database [113]; other repositories of relevant information are The Cancer Genome Atlas, for ovarian and endometrial cancer [114], and BCCTBbp: the Breast Cancer Campaign Tissue Bank bioinformatics portal [115].

While the number of publications per cancer type reflects the incidence of the various forms (new cases per year in the US, source: https://www.cancer.gov/), the lines along which the research is moving are similar irrespective of the organ being affected by the disease. Of course the identification of the proteins and protein species differing in concentration between physiological and pathological tissues, and among different subtypes of the pathological tissues, is one essential step; proteomic evidence is sometimes supported by transcriptomic and/or genomic data. More and more often, however, the analysis targets specific subproteomes, e.g. glycoproteins (with altered glycan composition) and/or membrane proteins, or exosomes.

Specific protocols are being devised for the study of archival samples. Due to the difficulties of assessing disease markers in the general circulation, investigations are being devoted to fluids collected in closer proximity to the affected organ, trading ease of procedures for specificity of protein content; urine is also investigated as an accessible alternative to serum. Table 3 categorizes along these lines a selection of papers published in the most recent years on four cancer types: cancer of cervix, endometrium, breast and prostate.

Table 3 – Recent publications on cancer in sexual organs

organ	*research area*	*specimen*	*procedure*	*references*
cervical cancer	overall proteome	tissue	iTRAQ & LC-MS/MS	[116]
	overall proteome	tissue	2D-DIGE & MS	[117]
	alternative biological fluids	cervical smear	iTRAQ & LC-MS/MS	[118]
	alternative biological fluids	cervicovaginal fluid	label-free shotgun 2D-LC/MS	[119]
endometrial cancer	overall proteome	tissue	integrated genomics, transcriptomics and proteomics	[120]
	subtyping	aneuploid vs diplod cells	2DE & MS	[121]
	archival samples	tissue	laser microdissection & mTRAQ LC-MS/MS	[122]
	archival samples	tissue	laser microdissection & shotgun LC-MS/MS	[123]
	subproteomes	membranes	iTRAQ & LC-MS/MS	[124]
	subproteomes	exosomes	functional proteomics	[125]
	alternative biological fluids	uterine aspirate	data mining & LC-MS/MS	[126]
	alternative biological fluids	fluid fraction of uterine aspirate	targeted proteomics	[127]
	alternative biological fluids	urine	2DE & MS	[128]
	alternative biological fluids	urine	N-glycopeptide profiling by SELDI-TOF	[129]
breast cancer	subtyping	secreted proteins	transcriptomics & MS proteomics	[130]
	subtyping	tissue	label-free shotgun 2D-LC/MS	[131]
	subtyping archival samples	archive samples (and cultured cells)	SILAC cell culture, shotgun LC-MS/MS	[132]
	archival samples		label free shotgun LC-MS/MS	[133]
	subproteome	membranes		[134]
	subproteome	membranes	label free LC-MS/MS	[135]
	subproteome	secreted proteins	2DE & antibodies	[136]

	subproteome	exosomes	tandem-mass-tag proteomics	[137]
	circulating markers	serum	antibodies & label-free LC-MS/MS	[138]
	alternative biological fluids	urine	label free LC-MS/MS	[139]
prostate	overall proteome	tissue	2D-DIGE & MS	[140]
	overall proteome	tissue	transcriptomics & Ab-profiling	[141]
	overall proteome	archive samples (and cultured cells)	SILAC cell culture, shotgun LC-MS/MS	[142]
	subtyping	tissue	immunofluorescence	[143]
	subtyping	cell lines	label free shotgun, CITP and CZE, LC-MS/MS	[144]
	subtyping archival samples	biopsies	multiplex proteomics imaging	[145]
	subtyping	tissue cell lines	2D-DIGE & MS	[146]
	subproteome	glycoproteins	iTRAQ & LC-MS/MS	[147]
	subproteome	exosomes	label-free shotgun LC-MS/MS	[148]
	subproteome	exosomes	aptamer-based array platform	[149]
	subproteome alternative biological fluids	exosomes urine	label-free shotgun LC-MS/MS	[150]
	circulating markers	serum	antibody microarrays	[151]
	circulating markers	serum	depletion-free iTRAQ 3D LC/MS	[152]
	alternative biological fluids	seminal plasma	capillary electrophoresis & MS	[153]

3 Conclusions

We can repeat here one statement from the Conclusions of the accompanying review [1]: In most cases evidence gathered thus far may only be regarded as preliminary. A leap forward, from proof-of-concept to steady knowledge, requires statistically robust data collected under highly standardized conditions. Collaborative efforts shared by institutions and convergence among disciplinary approaches may be regarded as the rational route to problem solving.

Figure legends

Figure 1 – Myometrium proteins that show a statistically significant increase (left) or decrease in S-nitrosation (middle and right) during preterm labor. Legend: ALBU, serum albumin; ANXA6, annexin A6; CLIC1, chloride intracellular channel protein 1; CNN1, calponin-1; GSTP1, glutathione S-transferase P; HBB, hemoglobin subunit beta; HBD, hemoglobin subunit

delta; LEG1, galectin-1; LPP, lipoma-preferred partner; MYL6, myosin light polypeptide 6; MYL9, myosin regulatory light polypeptide 9; MYLK, myosin light chain kinase, smooth muscle; PALLD, palladin; PDLI1, PDZ and LIM domain protein 1; PGR, progesterone receptor; PROF1, profilin-1; TAGL, transgelin-1; THIO, thioredoxin; VINC, vinculin. Redrawn from Figures 4 and 5 in [35].

Figure 2 – Reference map of the low molecular mass proteins of amniotic fluid run under non-reducing, denaturing conditions. Main identifications are marked with protein full names or with UniProt codes. Experimental: 1d on 4-10 NL IPG [154] in 8 M urea without reduction, 2d on 10-17%T PAA according to Schägger and von Jagow [9]; 300 μg protein with molecular mass lower than albumin; Coomassie stain. Modified from Figure 2 in [53].

Figure 3 – Heat map presentation of spectral counting data. Colors represent the scaled fold-change of spectrum counts between samples within a row (same protein at different time points). Modified from Figure 4 in [60].

Figure 4 – Assessment of a predictor of preterm birth based on insulin-like growth factor-binding protein 4 and sex hormone-binding globulin concentrations in serum. Top panel: Kaplan-Meier estimator of high- and low-risk groups. Subjects at or above 2× the background risk (14.6%) are considered high-, those below 2× low-risk. Bottom panel: ROC plot of sensitivity *vs* (1 – specificity), in which preterm delivery cases are defined as delivery ≥ 37 weeks and term controls as delivery ≤37 weeks gestational age. The AUROC corresponds to 0.72. Redrawn form Figures 4 and 5 in [85].

Figure 5 – Venn's diagram summarizing the relationships among sperm proteins. Clusters found by Self-Organizing Maps (SOM). From Figure 9 in [95].

Bibliography

[1] Gianazza E, Miller I, Guerrini U, Palazzolo L, Parravicini C, Eberini I. Gender proteomics - I. Which proteins in non-sexual organs. Journal of Proteomics. under evaluation.
[2] Harper J, Geraedts J, Borry P, Cornel MC, Dondorp WJ, Gianaroli L, et al. Current issues in medically assisted reproduction and genetics in Europe: research, clinical practice, ethics, legal issues and policy. Human reproduction. 2014;29:1603-9.
[3] Präg P, Mills MC. Assisted reproductive technology in Europe. Usage and regulation in the context of cross-border reproductive care. In: Kreyenfeld M, Konietzka D, editors. Childlessness in Europe Patterns, Causes, and Contexts.
[4] Brezina PR, Kutteh WH. Clinical applications of preimplantation genetic testing. BMJ. 2015;350:g7611.
[5] Paweletz CP, Trock B, Pennanen M, Tsangaris T, Magnant C, Liotta LA, et al. Proteomic patterns of nipple aspirate fluids obtained by SELDI-TOF: potential for new biomarkers to aid in the diagnosis of breast cancer. Dis Markers. 2001;17:301-7.
[6] Ruhlen RL, Sauter ER. Proteomics of nipple aspirate fluid, breast cyst fluid, milk, and colostrum. Proteomics Clin Appl. 2007;1:845-52.
[7] Brunoro GV, Ferreira AT, Trugilho MR, Oliveira TS, Amêndola LC, Perales J, et al. Potential correlation between tumor aggressiveness and protein expression patterns of nipple aspirate fluid (NAF) revealed by gel-based proteomic analysis. Curr Top Med Chem. 2014;14:359-68.

879 [8] Noble J, Dua RS, Locke I, Eeles R, Gui GP, Isacke CM. Proteomic analysis of nipple
880 aspirate fluid throughout the menstrual cycle in healthy pre-menopausal women. Breast
881 Cancer Res Treat. 2007;104:191-6.
882 [9] Casado-Vela J, Rodriguez-Suarez E, Iloro I, Ametzazurra A, Alkorta N, García-Velasco JA,
883 et al. Comprehensive proteomic analysis of human endometrial fluid aspirate. J Proteome
884 Res. 2009;8:4622-32.
885 [10] Shaw JL, Smith CR, Diamandis EP. Proteomic analysis of human cervico-vaginal fluid. J
886 Proteome Res. 2007;6:2859-65.
887 [11] Andersch-Björkman Y, Thomsson KA, Holmén Larsson JM, Ekerhovd E, Hansson GC.
888 Large scale identification of proteins, mucins, and their O-glycosylation in the endocervical
889 mucus during the menstrual cycle. Mol Cell Proteomics. 2007;6:708-16.
890 [12] Zieba A, Sjöstedt E, Olovsson M, Fagerberg L, Hallström BM, Oskarsson L, et al. The
891 Human Endometrium-Specific Proteome Defined by Transcriptomics and Antibody-Based
892 Profiling. OMICS. 2015;19:659-68.
893 [13] Parmar T, Gadkar-Sable S, Savardekar L, Katkam R, Dharma S, Meherji P, et al. Protein
894 profiling of human endometrial tissues in the midsecretory and proliferative phases of the
895 menstrual cycle. Fertil Steril. 2009;92:1091-103.
896 [14] Rai P, Kota V, Sundaram CS, Deendayal M, Shivaji S. Proteome of human endometrium:
897 Identification of differentially expressed proteins in proliferative and secretory phase
898 endometrium. Proteomics Clin Appl. 2010;4:48-59.
899 [15] Li J, Tan Z, Li M, Xia T, Liu P, Yu W. Proteomic analysis of endometrium in fertile women
900 during the prereceptive and receptive phases after luteinizing hormone surge. Fertil Steril.
901 2011;95:1161-3.
902 [16] Hill AM, Stewart PW, Fung MK, Kris-Etherton PM, Ginsberg HN, Tracy RP, et al. Monthly
903 haemostatic factor variability in women and men. Eur J Clin Invest. 2014;44:309-18.
904 [17] Swanepoel AC, Visagie A, de Lange Z, Emmerson O, Nielsen VG, Pretorius E. The
905 clinical relevance of altered fibrinogen packaging in the presence of 17beta-estradiol and
906 progesterone. Thromb Res. 2016;146:23-34.
907 [18] Canonico M, Brailly-Tabard S, Gaussem P, Setiao J, Rouaud O, Ryan J, et al.
908 Endogenous oestradiol as a positive correlate of plasma fibrinogen among older
909 postmenopausal women: a population-based study (the Three-City cohort study). Clinical
910 endocrinology. 2012;77:905-10.
911 [19] Effects of estrogen or estrogen/progestin regimens on heart disease risk factors in
912 postmenopausal women. The Postmenopausal Estrogen/Progestin Interventions (PEPI) Trial.
913 The Writing Group for the PEPI Trial. JAMA. 1995;273:199-208.
914 [20] Inbal A, Muszbek L. Coagulation factor deficiencies and pregnancy loss. Semin Thromb
915 Hemost. 2003;29:171-4.
916 [21] Lecce L, Kaneko Y, Madawala RJ, Murphy CR. ICAM1 and fibrinogen-gamma are
917 increased in uterine epithelial cells at the time of implantation in rats. Mol Reprod Dev.
918 2011;78:318-27.
919 [22] DeSouza L, Diehl G, Yang EC, Guo J, Rodrigues MJ, Romaschin AD, et al. Proteomic
920 analysis of the proliferative and secretory phases of the human endometrium: protein
921 identification and differential protein expression. Proteomics. 2005;5: 270-81.
922 [23] Hood BL, Liu B, Alkhas A, Shoji Y, Challa R, Wang G, et al. Proteomics of the Human
923 Endometrial Glandular Epithelium and Stroma from the Proliferative and Secretory Phases of
924 the Menstrual Cycle. Biol Reprod. 2015;92:106, 1-8.
925 [24] Scholl PF, Cole RN, Ruczinski I, Gucek M, Diez R, Rennie A, et al. Maternal serum
926 proteome changes between the first and third trimester of pregnancy in rural southern Nepal.
927 Placenta. 2012;33:424-32.
928 [25] Flood-Nichols SK, Tinnemore D, Wingerd MA, Abu-Alya AI, Napolitano PG, Stallings JD,
929 et al. Longitudinal analysis of maternal plasma apolipoproteins in pregnancy: a targeted
930 proteomics approach. Mol Cell Proteomics. 2013;12:55-64.
931 [26] Sreckovic I, Birner-Gruenberger R, Obrist B, Stojakovic T, Scharnagl H, Holzer M, et al.
932 Distinct composition of human fetal HDL attenuates its anti-oxidative capacity. Biochim
933 Biophys Acta. 2013;1831:737-46.
934 [27] de Jong G, van Eijk HG. Microheterogeneity of human serum transferrin: A biological
935 phenomenon studied by isoelectric focusing in immobilized pH gradients. Electrophoresis.
936 1988;9:589-98.
937 [28] Rudd PM, Elliott T, Cresswell P, Wilson IA, Dwek RA. Glycosylation and the immune
938 system. Science. 2001;291:2370-6.

[29] Ruhaak LR, Uh HW, Deelder AM, Dolhain RE, Wuhrer M. Total plasma N-glycome changes during pregnancy. J Proteome Res. 2014;13:1657-68.
[30] Jansen BC, Bondt A, Reiding KR, Lonardi E, de Jong CJ, Falck D, et al. Pregnancy-associated serum N-glycome changes studied by high-throughput MALDI-TOF-MS. Scientific reports. 2016;6:23296.
[31] Bondt A, Rombouts Y, Selman MH, Hensbergen PJ, Reiding KR, Hazes JM, et al. Immunoglobulin G (IgG) Fab glycosylation analysis using a new mass spectrometric high-throughput profiling method reveals pregnancy-associated changes. Mol Cell Proteomics. 2014;13:3029-39.
[32] Zheng J, Liu L, Wang J, Jin Q. Urinary proteomic and non-prefractionation quantitative phosphoproteomic analysis during pregnancy and non-pregnancy. BMC Genomics. 2013;14:777.
[33] Di Quinzio MK, Oliva K, Holdsworth SJ, Ayhan M, Walker SP, Rice GE, et al. Proteomic analysis and characterisation of human cervico-vaginal fluid proteins. Aust N Z J Obstet Gynaecol. 2007;47:9-15.
[34] Lee DC, Hassan SS, Romero R, Tarca AL, Bhatti G, Gervasi MT, et al. Protein profiling underscores immunological functions of uterine cervical mucus plug in human pregnancy. J Proteomics. 2011;74:817-28.
[35] Ulrich C, Quilici DR, Schlauch KA, Buxton IL. The human uterine smooth muscle S-nitrosoproteome fingerprint in pregnancy, labor, and preterm labor. Am J Physiol Cell Physiol. 2013;305:C803-16.
[36] Gonzalez-Fernandez R, Martinez-Galisteo E, Gaytan F, Barcena JA, Sanchez-Criado JE. Changes in the proteome of functional and regressing corpus luteum during pregnancy and lactation in the rat. Biology of reproduction. 2008;79:100-14.
[37] Al-Gubory KH, Arianmanesh M, Garrel C, Bhattacharya S, Cash P, Fowler PA. Proteomic analysis of the sheep caruncular and intercaruncular endometrium reveals changes in functional proteins crucial for the establishment of pregnancy. Reproduction. 2014;147:599-614.
[38] Burns GW, Brooks KE, Spencer TE. Extracellular Vesicles Originate from the Conceptus and Uterus During Early Pregnancy in Sheep. Biology of reproduction. 2016;94:56.
[39] Hannan NJ, Stephens AN, Rainczuk A, Hincks C, Rombauts LJ, Salamonsen LA. 2D-DiGE analysis of the human endometrial secretome reveals differences between receptive and nonreceptive states in fertile and infertile women. J Proteome Res. 2010;9:6256-64.
[40] Greening DW, Nguyen HP, Evans J, Simpson RJ, Salamonsen LA. Modulating the endometrial epithelial proteome and secretome in preparation for pregnancy: The role of ovarian steroid and pregnancy hormones. J Proteomics. 2016;144:99-112.
[41] Cortezzi SS, Garcia JS, Ferreira CR, Braga DP, Figueira RC, Iaconelli AJ, et al. Secretome of the preimplantation human embryo by bottom-up label-free proteomics. Anal Bioanal Chem. 2011;401:1331-9.
[42] Dominguez F, Meseguer M, Aparicio-Ruiz B, Piqueras P, Quiñonero A, Simón C. New strategy for diagnosing embryo implantation potential by combining proteomics and time-lapse technologies. Fertil Steril. 2015;104:908-14.
[43] Gardner DK, Larman MG, Thouas GA. Sex-related physiology of the preimplantation embryo. Molecular human reproduction. 2010;16:539-47.
[44] Jensen PL, Grøndahl ML, Beck HC, Petersen J, Stroebech L, Christensen ST, et al. Proteomic analysis of bovine blastocoel fluid and blastocyst cells. Syst Biol Reprod Med. 2014;60:127-35.
[45] Jensen PL, Beck HC, Petersen J, Hreinsson J, Wånggren K, Laursen SB, et al. Proteomic analysis of human blastocoel fluid and blastocyst cells. Stem Cells Dev. 2013;22:1126-35.
[46] Mushahary D, Gautam P, Sundaram CS, Sirdeshmukh R. Expanded protein expression profile of human placenta using two-dimensional gel electrophoresis. Placenta. 2013;34:193-6.
[47] Gharesi-Fard B, Zolghadri J, Kamali-Sarvestani E. Proteome differences in the first- and third-trimester human placentas. Reprod Sci. 2015;22:462-8.
[48] Sui L, An L, Tan K, Wang Z, Wang S, Miao K, et al. Dynamic proteomic profiles of in vivo- and in vitro-produced mouse postimplantation extraembryonic tissues and placentas. Biol Reprod. 2014;91.
[49] Kedia K, Nichols CA, Thulin CD, Graves SW. Novel "omics" approach for study of low-abundance, low-molecular-weight components of a complex biological tissue: regional

998 differences between chorionic and basal plates of the human placenta. Anal Bioanal Chem.
999 2015;407:8543-56.
1000 [50] Zhang Q, Schulenborg T, Tan T, Lang B, Friauf E, Fecher-Trost C. Proteome analysis of
1001 a plasma membrane-enriched fraction at the placental feto-maternal barrier. Proteomics Clin
1002 Appl. 2010;4:538-49.
1003 [51] Vandré DD, Ackerman WEt, Tewari A, Kniss DA, Robinson JM. A placental sub-
1004 proteome: the apical plasma membrane of the syncytiotrophoblast. Placenta. 2012;33:207-13.
1005 [52] Xanthopoulou AG, Anagnostopoulos AK, Thanasopoulou A, Anastasiadou E, Sifakis S,
1006 Siafaka-Kapadai A, et al. The proteome of normal human chorionic villus sampling cells. In
1007 Vivo. 2011;25:945-61.
1008 [53] Gianazza E, Wait R, Begum S, Eberini I, Campagnoli M, Labò S, et al. Mapping the 5-50
1009 kDa fraction of human amniotic fluid proteins by 2DE and ESI-MS. Proteomics Clin Appl.
1010 2007;1:167-75.
1011 [54] Wait R, Begum S, Brambilla D, Carabelli AM, Conserva F, Rocco Guerini A, et al. Redox
1012 options in two-dimensional electrophoresis. Amino Acids. 2005;28:239-72.
1013 [55] Michel PE, Crettaz D, Morier P, Heller M, Gallot D, Tissot JD, et al. Proteome analysis of
1014 human plasma and amniotic fluid by Off-Gel isoelectric focusing followed by nano-LC-MS/MS.
1015 Electrophoresis. 2007;27:1169-81.
1016 [56] Cho CK, Shan SJ, Winsor EJ, Diamandis EP. Proteomics analysis of human amniotic
1017 fluid. Mol Cell Proteomics. 2007;6:1406-15.
1018 [57] Michaels JE, Dasari S, Pereira L, Reddy AP, Lapidus JA, Lu X, et al. Comprehensive
1019 proteomic analysis of the human amniotic fluid proteome: gestational age-dependent changes.
1020 J Proteome Res. 2007;6:1277-85.
1021 [58] Palmer DJ, Kelly VC, Smit AM, Kuy S, Knight CG, Cooper GJ. Human colostrum:
1022 identification of minor proteins in the aqueous phase by proteomics. Proteomics.
1023 2006;6:2208-16.
1024 [59] Fortunato D, Giuffrida MG, Cavaletto M, Garoffo LP, Dellavalle G, Napolitano L, et al.
1025 Structural proteome of human colostral fat globule membrane proteins. Proteomics.
1026 2003;3:897-905.
1027 [60] Liao Y, Alvarado R, Phinney B, Lönnerdal B. Proteomic characterization of human milk
1028 whey proteins during a twelve-month lactation period. J Proteome Res. 2011;10:1746-54.
1029 [61] Liao Y, Alvarado R, Phinney B, Lönnerdal B. Proteomic characterization of human milk
1030 fat globule membrane proteins during a 12 month lactation period. J Proteome Res.
1031 2011;10:3530-41.
1032 [62] Gianazza E, Miller I, Palazzolo L, Parravicini C, Eberini I. With or without you -
1033 Proteomics with or without major plasma/serum proteins. J Proteomics. 2016;140:62-80.
1034 [63] Zhang L, de Waard M, Verheijen H, Boeren S, Hageman JA, van Hooijdonk T, et al.
1035 Changes over lactation in breast milk serum proteins involved in the maturation of immune
1036 and digestive system of the infant. J Proteomics. 2016;147:40-7.
1037 [64] Dallas DC, Guerrero A, Khaldi N, Castillo PA, Martin WF, Smilowitz JT, et al. Extensive in
1038 vivo human milk peptidomics reveals specific proteolysis yielding protective antimicrobial
1039 peptides. J Proteome Res. 2013;12: 2295-304.
1040 [65] Guerrero A, Dallas DC, Contreras S, Chee S, Parker EA, Sun X, et al. Mechanistic
1041 peptidomics: factors that dictate specificity in the formation of endogenous peptides in human
1042 milk. Mol Cell Proteomics. 2014;13:3343-51.
1043 [66] Holton TA, Vijayakumar V, Dallas DC, Guerrero A, Borghese RA, Lebrilla CB, et al.
1044 Following the digestion of milk proteins from mother to baby. J Proteome Res. 2014;13:5777-
1045 83.
1046 [67] van Herwijnen MJ, Zonneveld MI, Goerdayal S, Nolte-'t Hoen EN, Garssen J, Stahl B, et
1047 al. Comprehensive Proteomic Analysis of Human Milk-derived Extracellular Vesicles Unveils
1048 a Novel Functional Proteome Distinct from Other Milk Components. Mol Cell Proteomics.
1049 2016;15:3412-23.
1050 [68] Yang Y, Zheng N, Wang W, Zhao X, Zhang Y, Han R, et al. N-glycosylation proteomic
1051 characterization and cross-species comparison of milk fat globule membrane proteins from
1052 mammals. Proteomics. 2016;16:2792-800.
1053 [69] D'Auria E, Agostoni C, Giovannini M, Riva E, Zetterström R, Fortin R, et al. Proteomic
1054 evaluation of milk from different mammalian species as a substitute for breast milk. Acta
1055 Paediatr. 2005;94:1708-13.

[70] Abalos E, Cuesta C, Grosso AL, Chou D, Say L. Global and regional estimates of preeclampsia and eclampsia: a systematic review. European journal of obstetrics, gynecology, and reproductive biology. 2013;170:1–7.
[71] Jin H, Ma KD, Hu, R., Chen Y, Yang F, Yao J, et al. Analysis of expression and comparative profile of normal placental tissue proteins and those in preeclampsia patients using proteomic approaches. Anal Chim Acta. 2008;629:158-64.
[72] Kim YN, Kim HK, Warda M, Kim N, Park WS, Prince Adel B, et al. Toward a better understanding of preeclampsia: Comparative proteomic analysis of preeclamptic placentas. Proteomics Clin Appl. 2007;1:1625-36.
[73] Gharesi-Fard B, Zolghadri J, Kamali-Sarvestani E. Proteome differences of placenta between pre-eclampsia and normal pregnancy. Placenta. 2010;31:121-5.
[74] Ma K, Jin H, Hu R, Xiong Y, Zhou S, Ting P, et al. A proteomic analysis of placental trophoblastic cells in preeclampsia-eclampsia. Cell Biochem Biophys. 2014;69:247-58.
[75] Wang F, Wang L, Shi Z, Liang G. Comparative N-glycoproteomic and phosphoproteomic profiling of human placental plasma membrane between normal and preeclampsia pregnancies with high-resolution mass spectrometry. PLoS One. 2013;8:e80480.
[76] Zhang HH, Wang YP, Chen DB. Analysis of nitroso-proteomes in normotensive and severe preeclamptic human placentas. Biol Reprod. 2011;84:966-75.
[77] Blumenstein M, McMaster MT, Black MA, Wu S, Prakash R, Cooney J, et al. A proteomic approach identifies early pregnancy biomarkers for preeclampsia: novel linkages between a predisposition to preeclampsia and cardiovascular disease. Proteomics. 2009;9:2929-45.
[78] Blankley RT, Fisher C, Westwood M, North R, Baker PN, Walker MJ, et al. A label-free selected reaction monitoring workflow identifies a subset of pregnancy specific glycoproteins as potential predictive markers of early-onset pre-eclampsia. Mol Cell Proteomics. 2013;12:3148-59.
[79] Araki Y, Nonaka D, Tajima A, Maruyama M, Nitto T, Ishikawa H, et al. Quantitative peptidomic analysis by a newly developed one-step direct transfer technology without depletion of major blood proteins: its potential utility for monitoring of pathophysiological status in pregnancy-induced hypertension. Proteomics. 2011;11:2727-37.
[80] Chen G, Zhang Y, Jin X, Zhang L, Zhou Y, Niu J, et al. Urinary proteomics analysis for renal injury in hypertensive disorders of pregnancy with iTRAQ labeling and LC-MS/MS. Proteomics Clin Appl. 2011;5:300-10.
[81] Xin L, Xu B, Ma L, Hou Q, Ye M, Meng S, et al. Proteomics study reveals that the dysregulation of focal adhesion and ribosome contribute to early pregnancy loss. Proteomics Clin Appl. 2016;10:554-63.
[82] Liu AX, He WH, Yin LJ, Lv PP, Zhang Y, Sheng JZ, et al. Sustained endoplasmic reticulum stress as a cofactor of oxidative stress in decidual cells from patients with early pregnancy loss. J Clin Endocrinol Metab. 2011;96:E493-7.
[83] Kim MS, Gu BH, Song S, Choi BC, Cha DH, Baek KH. ITI-H4, as a biomarker in the serum of recurrent pregnancy loss (RPL) patients. Mol Biosyst. 2011;7:1430-40.
[84] Parry S, Zhang H, Biggio J, Bukowski R, Varner M, Xu Y, et al. Maternal serum serpin B7 is associated with early spontaneous preterm birth. Am J Obstet Gynecol. 2014;211:678.e1-12.
[85] Saade GR, Boggess KA, Sullivan SA, Markenson GR, Iams JD, Coonrod DV, et al. Development and validation of a spontaneous preterm delivery predictor in asymptomatic women. American journal of obstetrics and gynecology. 2016;214:633 e1- e24.
[86] Pereira L, Reddy A, Jacob T, Thomas A, Schneider K, Dasari S, et al. Identification of novel protein biomarkers of preterm birth in human cervical-vaginal fluid. J Proteome Res. 2007;6:1269-76.
[87] Jodar M, Sendler E, Krawetz SA. The protein and transcript profiles of human semen. Cell Tissue Res. 2016;363:85-96.
[88] Fung KY, Glode LM, Green S, Duncan MW. A comprehensive characterization of the peptide and protein constituents of human seminal fluid. Prostate. 2004;61:171–81.
[89] Pilch B, Mann M. Large-scale and high-confidence proteomic analysis of human seminal plasma. Genome Biol. 2006;7:R40.
[90] Intasqui P, Camargo M, Antoniassi MP, Cedenho AP, Carvalho VM, Cardozo K, et al. Association between the seminal plasma proteome and sperm functional traits. Fertil Steril. 2016;105:617-28.

[91] Agarwal A, Sharma R, Durairajanayagam D, Cui Z, Ayaz A, Gupta S, et al. Differential proteomic profiling of spermatozoal proteins of infertile men with unilateral or bilateral varicocele. Urology. 2015;85: 580-8.
[92] Cadavid JAP, Alvarez A, Markert UR, Cardona Maya W. Differential protein expression in seminal plasma from fertile and infertile males. J Hum Reprod Sci. 2014;7:206-11.
[93] Utleg AG, Yi EC, Xie T, Shannon P, White JT, Goodlett DR, et al. Proteomic analysis of human prostasomes. Prostate. 2003;56:150–61.
[94] Drake RR, Elschenbroich S, Lopez-Perez O, Kim Y, Ignatchenko V, Ignatchenko A, et al. In-depth proteomic analyses of direct expressed prostatic secretions. J Proteome Res. 2010;9:2109-16.
[95] Saraswat M, Joenväärä S, Jain T, Tomar AK, Sinha A, Singh S, et al. Human spermatozoa quantitative proteomic signature classifies normo- and asthenozoospermia. Mol Cell Proteomics. 2016:mcp.M116.061028.
[96] Kohonen T. Self-organizing Maps: Springer; 1995.
[97] Amaral A, Paiva C, Attardo Parrinello C, Estanyol JM, Ballescà JL, Ramalho-Santos J, et al. Identification of proteins involved in human sperm motility using high-throughput differential proteomics. J Proteome Res. 2014;13:5670-84.
[98] Hashemitabar M, Sabbagh S, Orazizadeh M, Ghadiri A, Bahmanzadeh M. A proteomic analysis on human sperm tail: comparison between normozoospermia and asthenozoospermia. J Assist Reprod Genet. 2015;32:853-63.
[99] Baker MA, Naumovski N, Hetherington L, Weinberg A, Velkov T, Aitken RJ. Head and flagella subcompartmental proteomic analysis of human spermatozoa. Proteomics. 2013;13:61-74.
[100] Xin AJ, Cheng L, Diao H, Wang P, Gu YH, Wu B, et al. Comprehensive profiling of accessible surface glycans of mammalian sperm using a lectin microarray. Clin Proteomics. 2014;11:10.
[101] Yang X, Liu F, Yan Y, Zhou T, Guo Y, Sun G, et al. Proteomic analysis of N-glycosylation of human seminal plasma. Proteomics. 2015;15:1255-8.
[102] Baker MA. Proteomics of post-translational modifications of mammalian spermatozoa. Cell Tissue Res. 2016;363:279-87.
[103] Samanta L, Swain N, Ayaz A, Venugopal V, Agarwal A. Post-Translational Modifications in sperm Proteome: The Chemistry of Proteome diversifications in the Pathophysiology of male factor infertility. Biochim Biophys Acta. 2016;1860:1450-65.
[104] Secciani F, Bianchi L, Ermini L, Cianti R, Armin iA, La Sala GB, et al. Protein profile of capacitated versus ejaculated human sperm. J Proteome Res. 2009;8:3377-89.
[105] Wang Y, Wan J, Ling X, Liu M, Zhou T. The human sperm proteome 2.0: An integrated resource for studying sperm functions at the level of posttranslational modification. Proteomics. 2016;16:2597-601.
[106] Sun G, Jiang M, Zhou T, Guo Y, Cui Y, Guo X, et al. Insights into the lysine acetylproteome of human sperm. J Proteomics. 2014;109:199-211.
[107] Baker MA, Nixon B, Naumovski N, Aitken RJ. Proteomic insights into the maturation and capacitation of mammalian spermatozoa. Syst Biol Reprod Med. 2012;58:211-7.
[108] Yu H, Diao H, Wang C, Lin Y, Yu F, Lu H, et al. Acetylproteomic analysis reveals functional implications of lysine acetylation in human spermatozoa (sperm). Mol Cell Proteomics. 2015;14:1009-23.
[109] Ickowicz D, Finkelstein M, Breitbart H. Mechanism of sperm capacitation and the acrosome reaction: role of protein kinases. Asian J Androl. 2012;14:816-21.
[110] Fardilha M, Ferreira M, Pelech S, Vieira S, Rebelo S, Korrodi-Gregorio L, et al. "Omics" of human sperm: profiling protein phosphatases. OMICS. 2013;17:460-72.
[111] Wang J, Qi L, Huang S, Zhou T, Guo Y, Wang G, et al. Quantitative phosphoproteomics analysis reveals a key role of insulin growth factor 1 receptor (IGF1R) tyrosine kinase in human sperm capacitation. Mol Cell Proteomics. 2015;14:1104-12.
[112] Redgrove KA, Anderson AL, Dun MD, McLaughlin EA, O'Bryan MK, Aitken RJ, et al. Involvement of multimeric protein complexes in mediating the capacitation-dependent binding of human spermatozoa to homologous zonae pellucidae. Dev Biol. 2011;356:460-74.
[113] Arntzen MO, Boddie P, Frick R, Koehler CJ, Thiede B. Consolidation of proteomics data in the Cancer Proteomics database. Proteomics. 2015;15:3765-71.
[114] Iijima M, Banno K, Okawa R, Yanokura M, Iida M, Takeda T, et al. Genome-wide analysis of gynecologic cancer: The Cancer Genome Atlas in ovarian and endometrial cancer. Oncology letters. 2017;13:1063-70.

[115] Cutts RJ, Guerra-Assuncao JA, Gadaleta E, Dayem Ullah AZ, Chelala C. BCCTBbp: the Breast Cancer Campaign Tissue Bank bioinformatics portal. Nucleic Acids Res. 2015;43:D831-6.
[116] Ding Y, Yang M, She S, Min H, Xv X, Ran X, et al. iTRAQ-based quantitative proteomic analysis of cervical cancer. Int J Oncol. 2015;46:1748-58.
[117] Serafin-Higuera I, Garibay-Cerdenares OL, Illades-Aguiar B, Flores-Alfaro E, Jimenez-Lopez MA, Sierra-Martinez P, et al. Differential proteins among normal cervix cells and cervical cancer cells with HPV-16 infection, through mass spectrometry-based Proteomics (2D-DIGE) in women from Southern Mexico. Proteome science. 2016;14:10.
[118] Papachristou EK, Roumeliotis TI, Chrysagi A, Trigoni C, Charvalos E, Townsend PA, et al. The shotgun proteomic study of the human ThinPrep cervical smear using iTRAQ mass-tagging and 2D LC-FT-Orbitrap-MS: the detection of the human papillomavirus at the protein level. J Proteome Res. 2013;12:2078-89.
[119] Van Raemdonck GA, Tjalma WA, Coen EP, Depuydt CE, Van Ostade XW. Identification of protein biomarkers for cervical cancer using human cervicovaginal fluid. PLoS ONE. 2014 9:e106488.
[120] Cancer Genome Atlas Research N, Kandoth C, Schultz N, Cherniack AD, Akbani R, Liu Y, et al. Integrated genomic characterization of endometrial carcinoma. Nature. 2013;497:67-73.
[121] Lomnytska MI, Becker S, Gemoll T, Lundgren C, Habermann J, Olsson A, et al. Impact of genomic stability on protein expression in endometrioid endometrial cancer. Br J Cancer. 2012;106:1297-305.
[122] DeSouza LV, Krakovska O, Darfler MM, Krizman DB, Romaschin AD, Colgan TJ, et al. mTRAQ-based quantification of potential endometrial carcinoma biomarkers from archived formalin-fixed paraffin-embedded tissues. Proteomics. 2010;10:3108-16.
[123] Alkhas A, Hood BL, Oliver K, Teng PN, Oliver J, Mitchell D, et al. Standardization of a sample preparation and analytical workflow for proteomics of archival endometrial cancer tissue. J Proteome Res. 2011;10:5264-71.
[124] Yokoyama T, Enomoto T, Serada S, Morimoto A, Matsuzaki S, Ueda Y, et al. Plasma membrane proteomics identifies bone marrow stromal antigen 2 as a potential therapeutic target in endometrial cancer. Int J Cancer. 2013;132:472-84.
[125] Liang H, Cheung LW, Li J, Ju Z, Yu S, Stemke-Hale K, et al. Whole-exome sequencing combined with functional genomics reveals novel candidate driver cancer genes in endometrial cancer. Genome research. 2012;22:2120-9.
[126] Martinez-Garcia E, Lesur A, Devis L, Campos A, Cabrera S, van Oostrum J, et al. Development of a sequential workflow based on LC-PRM for the verification of endometrial cancer protein biomarkers in uterine aspirate samples. Oncotarget. 2016;7:53102-15.
[127] Martinez E, Lesur A, Devis L, Cabrera S, Matias-Guiu X, Hirschfeld M, et al. Targeted proteomics identifies proteomic signatures in liquid-biopsies of the endometrium to diagnose endometrial cancer and assist in the prediction of the optimal surgical treatment. Clin Cancer Res. 2017.
[128] Mu AK, Lim BK, Hashim OH, Shuib AS. Detection of differential levels of proteins in the urine of patients with endometrial cancer: analysis using two-dimensional gel electrophoresis and o-glycan binding lectin. International journal of molecular sciences. 2012;13:9489-501.
[129] Mu AK, Lim BK, Aminudin N, Hashim OH, Shuib AS. Application of SELDI-TOF in N-glycopeptides profiling of the urine from patients with endometrial, ovarian and cervical cancer. Archives of physiology and biochemistry. 2016;122:111-6.
[130] Pavlou MP, Dimitromanolakis A, Diamandis EP. Coupling proteomics and transcriptomics in the quest of subtype-specific proteins in breast cancer. Proteomics. 2013;13:1083-95.
[131] Panis C, Pizzatti L, Herrera AC, Correa S, Binato R, Abdelhay E. Label-free proteomic analysis of breast cancer molecular subtypes. J Proteome Res. 2014;13:4752-72.
[132] Tyanova S, Albrechtsen R, Kronqvist P, Cox J, Mann M, Geiger T. Proteomic maps of breast cancer subtypes. Nature communications. 2016;7:10259.
[133] Gamez-Pozo A, Ferrer NI, Ciruelos E, Lopez-Vacas R, Martinez FG, Espinosa E, et al. Shotgun proteomics of archival triple-negative breast cancer samples. Proteomics Clin Appl. 2013;7:283-91.
[134] Muraoka S, Kume H, Adachi J, Shiromizu T, Watanabe S, Masuda T, et al. In-depth membrane proteomic study of breast cancer tissues for the generation of a chromosome-based protein list. J Proteome Res. 2013;12:208-13.

[135] Ziegler YS, Moresco JJ, Tu PG, Yates JR, 3rd, Nardulli AM. Plasma membrane proteomics of human breast cancer cell lines identifies potential targets for breast cancer diagnosis and treatment. PLoS ONE. 2014;9:e102341.
[136] Netsirisawan P, Chokchaichamnankit D, Srisomsap C, Svasti J, Champattanachai V. Proteomic Analysis Reveals Aberrant O-GlcNAcylation of Extracellular Proteins from Breast Cancer Cell Secretion. Cancer genomics & proteomics. 2015;12:201-9.
[137] Clark DJ, Fondrie WE, Liao Z, Hanson PI, Fulton A, Mao L, et al. Redefining the Breast Cancer Exosome Proteome by Tandem Mass Tag Quantitative Proteomics and Multivariate Cluster Analysis. Anal Chem. 2015;87:10462-9.
[138] Olsson N, Carlsson P, James P, Hansson K, Waldemarson S, Malmstrom P, et al. Grading breast cancer tissues using molecular portraits. Mol Cell Proteomics. 2013;12:3612-23.
[139] Beretov J, Wasinger VC, Millar EK, Schwartz P, Graham PH, Li Y. Proteomic Analysis of Urine to Identify Breast Cancer Biomarker Candidates Using a Label-Free LC-MS/MS Approach. PLoS ONE. 2015;10:e0141876.
[140] Davalieva K, Kostovska IM, Kiprijanovska S, Markoska K, Kubelka-Sabit K, Filipovski V, et al. Proteomics analysis of malignant and benign prostate tissue by 2D DIGE/MS reveals new insights into proteins involved in prostate cancer. Prostate. 2015;75:1586-600.
[141] O'Hurley G, Busch C, Fagerberg L, Hallstrom BM, Stadler C, Tolf A, et al. Analysis of the Human Prostate-Specific Proteome Defined by Transcriptomics and Antibody-Based Profiling Identifies TMEM79 and ACOXL as Two Putative, Diagnostic Markers in Prostate Cancer. PLoS ONE. 2015;10:e0133449.
[142] Iglesias-Gato D, Wikstrom P, Tyanova S, Lavallee C, Thysell E, Carlsson J, et al. The Proteome of Primary Prostate Cancer. European urology. 2016;69:942-52.
[143] Shipitsin M, Small C, Choudhury S, Giladi E, Friedlander S, Nardone J, et al. Identification of proteomic biomarkers predicting prostate cancer aggressiveness and lethality despite biopsy-sampling error. Br J Cancer. 2014;111:1201-12.
[144] Tan SH, Furusato B, Fang X, He F, Mohamed AA, Griner NB, et al. Evaluation of ERG responsive proteome in prostate cancer. Prostate. 2014;74:70-89.
[145] Blume-Jensen P, Berman DM, Rimm DL, Shipitsin M, Putzi M, Nifong TP, et al. Development and clinical validation of an in situ biopsy-based multimarker assay for risk stratification in prostate cancer. Clin Cancer Res. 2015;21:2591-600.
[146] Li Q, Li Y, Wang Y, Cui Z, Gong L, Qu Z, et al. Quantitative proteomic study of human prostate cancer cells with different metastatic potentials. Int J Oncol. 2016;48:1437-46.
[147] Shah P, Wang X, Yang W, Toghi Eshghi S, Sun S, Hoti N, et al. Integrated Proteomic and Glycoproteomic Analyses of Prostate Cancer Cells Reveal Glycoprotein Alteration in Protein Abundance and Glycosylation. Mol Cell Proteomics. 2015;14:2753-63.
[148] Duijvesz D, Burnum-Johnson KE, Gritsenko MA, Hoogland AM, Vredenbregt-van den Berg MS, Willemsen R, et al. Proteomic profiling of exosomes leads to the identification of novel biomarkers for prostate cancer. PLoS ONE. 2013;8:e82589.
[149] Webber J, Stone TC, Katilius E, Smith BC, Gordon B, Mason MD, et al. Proteomics analysis of cancer exosomes using a novel modified aptamer-based array (SOMAscan) platform. Mol Cell Proteomics. 2014;13:1050-64.
[150] Overbye A, Skotland T, Koehler CJ, Thiede B, Seierstad T, Berge V, et al. Identification of prostate cancer biomarkers in urinary exosomes. Oncotarget. 2015;6:30357-76.
[151] Nordstrom M, Wingren C, Rose C, Bjartell A, Becker C, Lilja H, et al. Identification of plasma protein profiles associated with risk groups of prostate cancer patients. Proteomics Clin Appl. 2014;8:951-62.
[152] Larkin SE, Johnston HE, Jackson TR, Jamieson DG, Roumeliotis TI, Mockridge CI, et al. Detection of candidate biomarkers of prostate cancer progression in serum: a depletion-free 3D LC/MS quantitative proteomics pilot study. Br J Cancer. 2016;115:1078-86.
[153] Neuhaus J, Schiffer E, von Wilcke P, Bauer HW, Leung H, Siwy J, et al. Seminal plasma as a source of prostate cancer peptide biomarker candidates for detection of indolent and advanced disease. PLoS ONE. 2013;8:e67514.
[154] Gianazza E, Giacon P, Sahlin B, Righetti PG. Non-linear pH courses with immobilized pH gradients. Electrophoresis. 1985;6:53-6.

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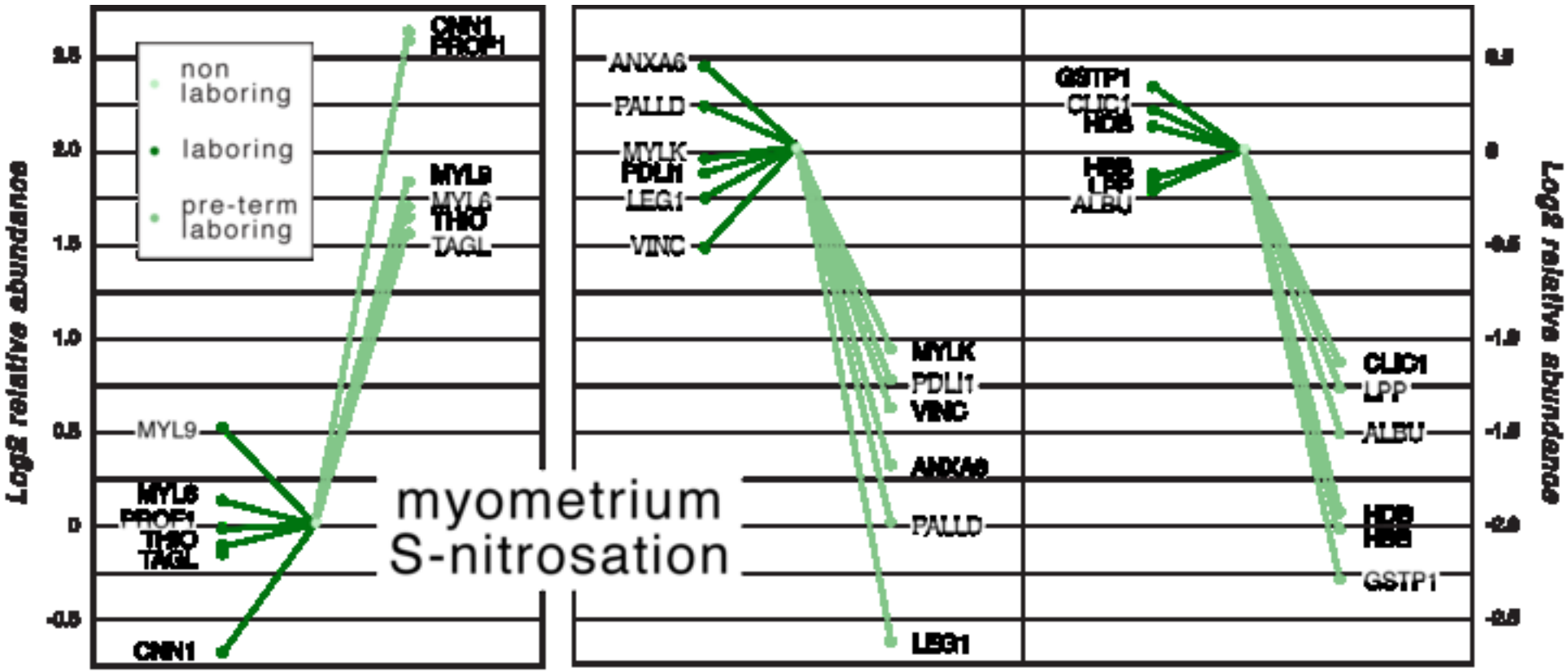

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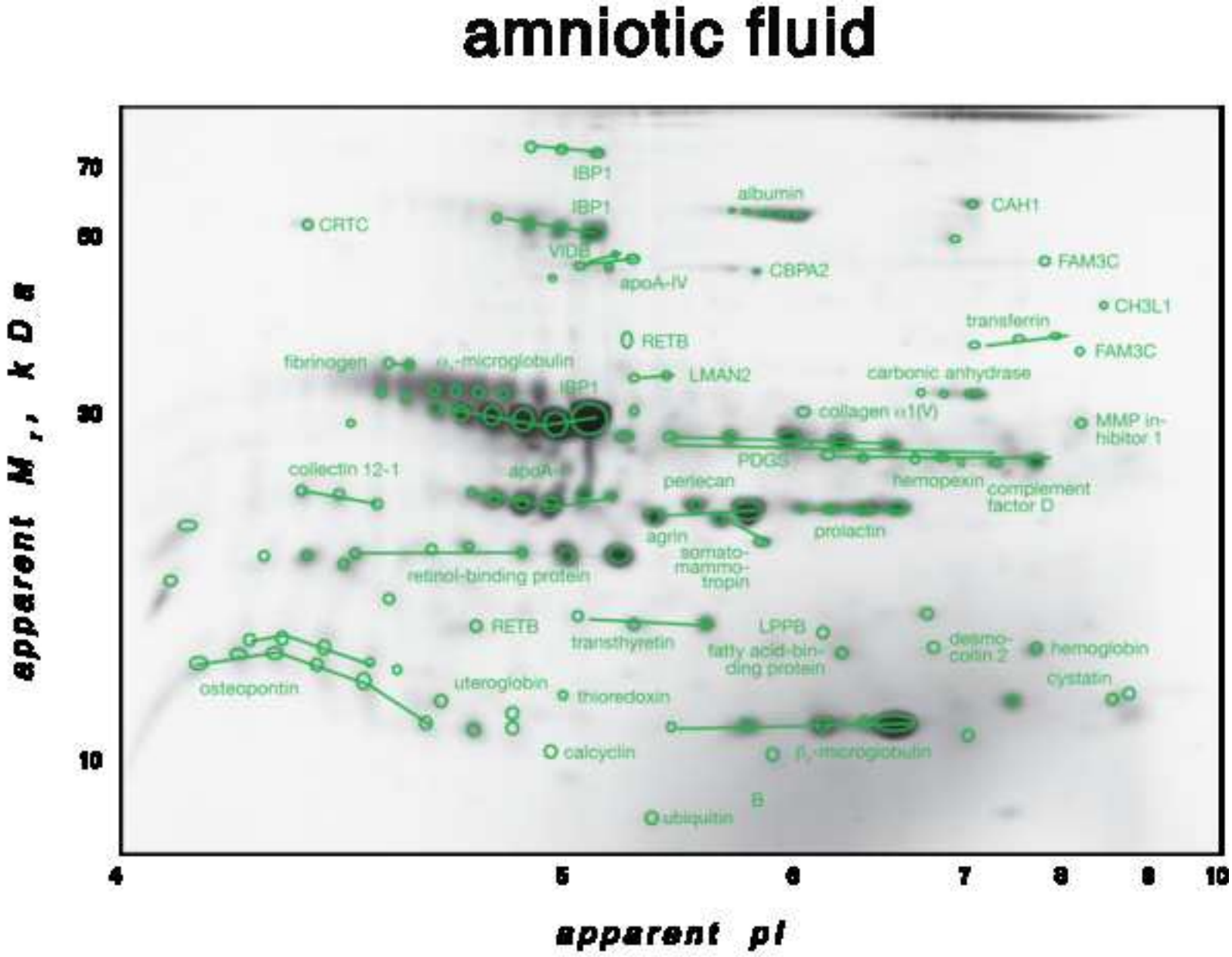

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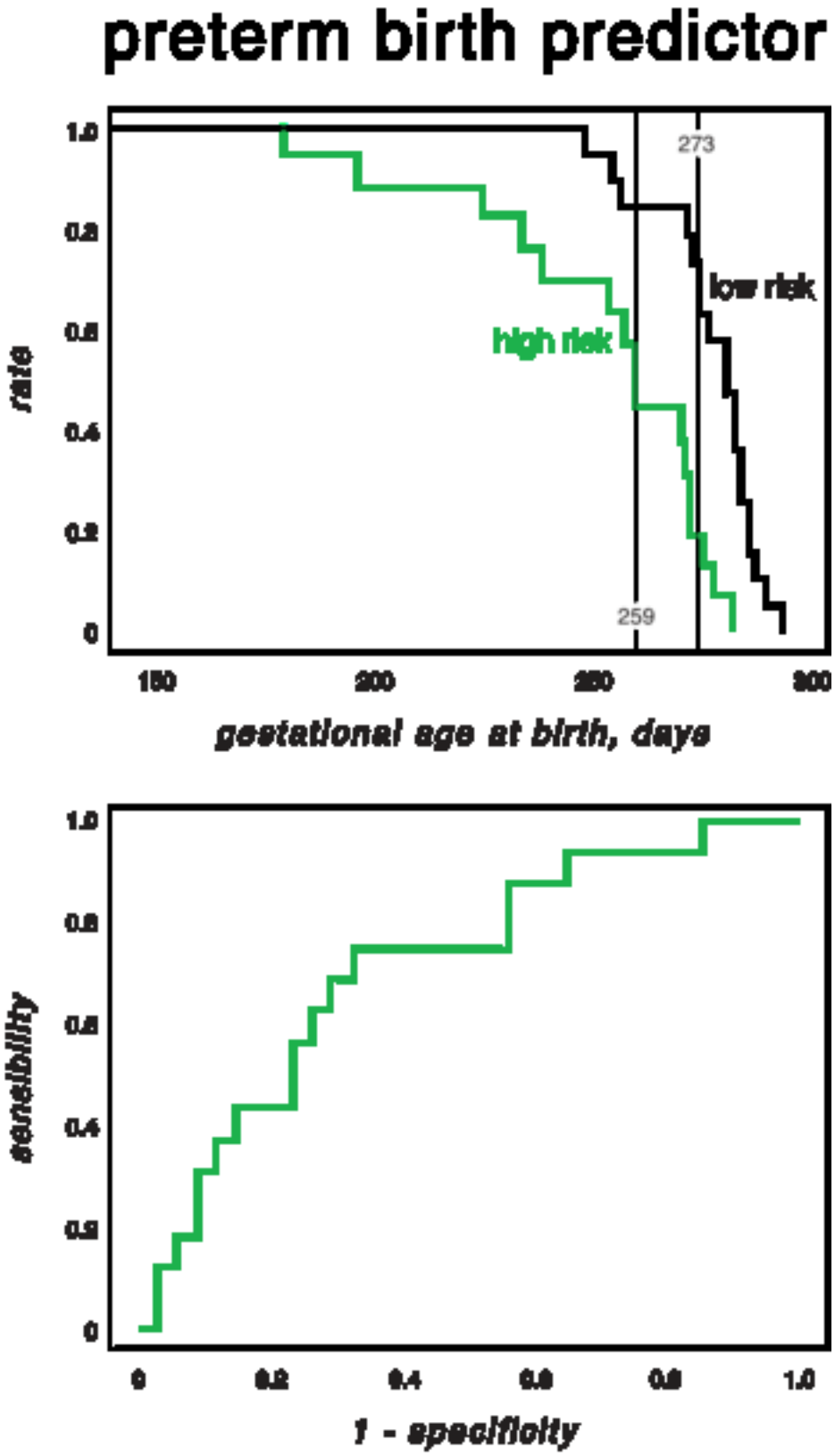

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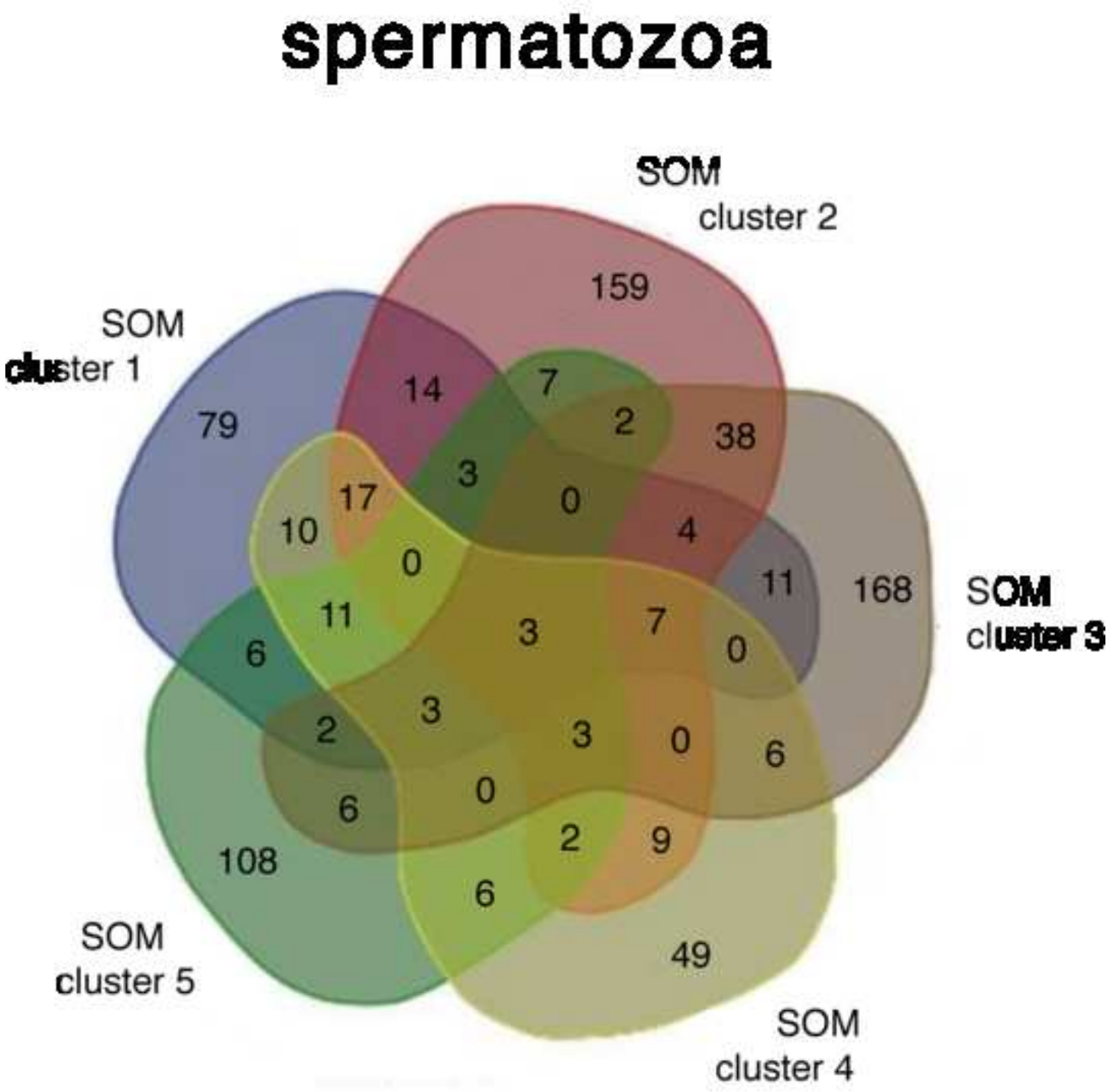

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