What is the Molecular Link Between Infertility and Cancer?

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Abstract

Female unexplained infertility is a multifactorial disorder, and its molecular underpinnings remain poorly understood. Approximately 15-20% of infertility cases are unexplained, posing a significant challenge for reproductive medicine. The endometrial epithelium's molecular network and signalling pathways play a crucial role in unexplained infertility, where the preservation of structural and functional integrity of maternal uterine tissue is essential for successful implantation and conception. The molecular environment within the uterus influences pregnancy establishment and development, with key components such as extracellular matrix (ECM) components, adhesion molecules, cytokines, and growth factors contributing to structural support and cellular processes. However, the intricate nature of these molecular networks necessitates further investigation. In our study, we employed proteome profiling techniques to analyse uterine tissue samples from women with unexplained infertility, aiming to identify potential alterations in specific proteins and pathways associated with infertility. Interestingly, emerging evidence suggests a molecular link between infertility and cancer in women. Infertility has been associated with an increased risk of certain cancers, including ovarian, endometrial, and breast cancer. Dysregulations in the uterine molecular environment may contribute to the development of cancer by promoting abnormal cell growth, impairing DNA repair mechanisms, and disrupting hormonal balance. Understanding the shared molecular mechanisms between infertility and cancer could provide valuable insights into both conditions. Using high-resolution two-dimensional gel electrophoresis and mass spectrometry, we identified twenty-four differentially expressed proteins, including ANKRD36, ZNF658B, MALRD1, and PRRC2A. Additionally, enrichment analysis revealed numerous pathways associated with infertility morbidity, highlighting their potential relevance to cancer development. These findings underscore the significance of investigating the molecular link between infertility and cancer, paving the way for future research aiming to unravel shared pathways and develop targeted interventions.

Keywords: Female Infertility; Endometrial epithelium; Signalling pathways; Structural integrity; Extracellular matrix (ECM) components; Infertility-associated cancers; Ovarian cancer; Endometrial cancer; Breast cancer

Abbreviations: DEPs: Differentially expressed proteins; 2-DE: Two-dimensional Gel Electrophoresis; MALDI-TOF MS: Matrix-Assisted Laser Desorption/Ionisation Time-Of-Flight Mass Spectrometry; KEGG: Kyoto Encyclopedia of Genes and Genomes; STRING: Search Tool for the Retrieval of Interacting Genes/Proteins; PANTHER: Protein Analysis Through Evolutionary Relationships

Introduction

Approximately 17% of infertility cases remain unexplained without a clearly identified cause [1]. Infertility, a distressing experience particularly affecting women, has been extensively studied in the literature due to its profound impact on various aspects of mental-emotional, social, and cultural well-being [2]. Successful implantation of the fertilised egg, or blastocyst, into the uterine lining is contingent upon a healthy and receptive uterine environment [3]. The integrity of the uterine tissue plays a vital role in facilitating proper attachment and embedding of the blastocyst, establishing crucial connections with the maternal blood supply. Disruptions in maternal uterine integrity, resulting from structural abnormalities, inflammation, or hormonal imbalances, can detrimentally affect the processes of implantation and conception [4].

The intricate nature of embryo implantation and placentation is underscored by the involvement of numerous cytokines and growth factors that exert significant regulatory roles in these processes [5]. Both in mice and humans, deviations from normal

expression and functioning of these cytokines and interferons at the tissue level can lead to complete or partial failure of implantation, as well as the occurrence of abnormal placenta formation [6].

Female infertility can be brought about by idiopathic infertility, tubal factor infertility, ovulatory problems, and endometriosis, among other conditions [7]. Approximately 50% of cases of female infertility are attributable to hereditary factors, many of which are yet unknown [8]. Primary infertility refers to females who have never been pregnant, while secondary infertility pertains to those who have had previous pregnancies [9]. In both the cases, gaining a better understanding of the underlying causes and mechanistic pathways is crucial for comprehensive disease comprehension and management.

Intriguingly, emerging evidence suggests a complex molecular link between infertility and cancer in women. Infertility has been associated with an increased risk of certain cancers, including ovarian, endometrial, and breast cancer [10]. Dysregulations in the uterine molecular environment may contribute to the development of cancer by promoting abnormal cell growth, impairing DNA repair mechanisms, and disrupting hormonal balance. Unraveling the shared molecular mechanisms between infertility and cancer holds the potential to provide valuable insights into both conditions, enhancing diagnostic and therapeutic approaches [11].

While microarray studies have identified genomic regions associated with female infertility over the past 20 years, the molecular mechanisms underlying human infertility resulting from these changes remain largely unclear, even in current studies. The advent of high-throughput sequencing technology has facilitated the detection of gene abnormalities related to infertility [12]. Among the omic studies, proteomics has emerged as a valuable tool in the field of human reproduction. Recent advancements in proteomic methods have significantly enhanced the literature on the proteome database, particularly in relation to various reproductive tissues in males and females [13]. Furthermore, network and pathway analysis utilising bioinformatic tools offer a more comprehensive understanding of the potential pathways associated with differentially expressed proteins (DEPs) and their relevance to specific infertility conditions [14].

Proteomics, a study of the temporal and spatial expression of proteins in cells or tissues, enables the assessment of quantitative and qualitative cellular responses related to specific proteins [15,16]. High-resolution two-dimensional gel electrophoresis (2-DE) analysis provides a powerful technique for comparing protein expression patterns, while matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS) aids in identifying protein spots on 2-DE gels [17]. Our study involved the acquisition of paired samples from six endometrial tissue specimens obtained from both infertile and fertile women during the mid-secretory phase (LH +7). These samples underwent two-dimensional gel electrophoresis and mass spectrometry to identify upregulated proteins (Figure 1 provides an overview of our study's workflow). Subsequently, we examined the physiological pathways associated with the identified proteins to assess the impact of specific genes on the protein expression profile observed in patients with unexplained female infertility and those without infertility.
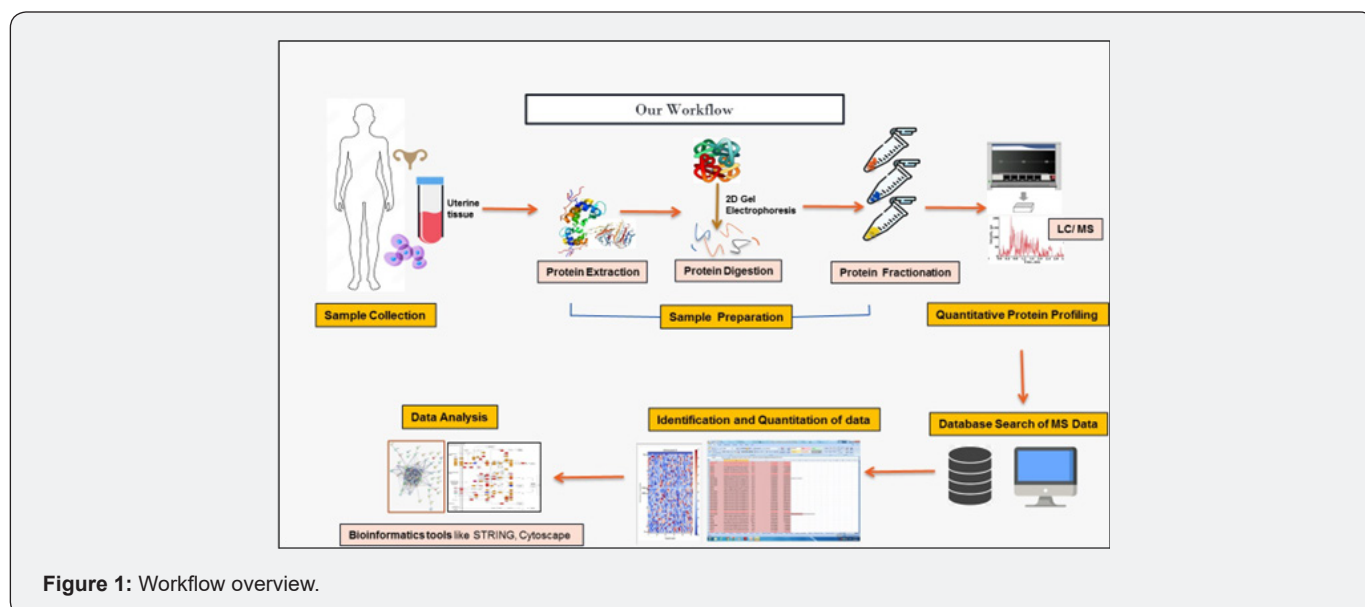

Figure 1: Workflow overview.

Our goal is to delve into the molecular pathways associated with unexplained infertility through comprehensive analysis of uterine tissue proteomes. By identifying potential alterations and dysregulations in specific proteins and pathways related to this condition, we aim to shed light on the complex molecular networks contributing to its pathogenesis ultimately aiming

to reduce embryo wastage and enhance implantation rates. Furthermore, understanding the shared molecular mechanisms between infertility and cancer will provide crucial insights into their interconnected nature, potentially leading to improved strategies for both diagnosis and treatment [18].

Results

Quantitative Protein Profiling Using 2D Gel Electrophoresis and MALDI-TOF

We performed a comparative proteomic analysis of uterine tissues from both groups to generate 2-DE reference maps and identify upregulated proteins. The quantitative analysis utilising Spectrum Mill and Mascot search engines resulted in the identification of over 55 proteins. Among them, 24 proteins exhibited an upregulation of >1.5-fold in cases of unexplained infertility. On average, 1200 spots were detected in the gels for both proteomes. Comparative analysis with normal endometrium revealed a significant elevation of 24 protein locations in the infertile samples (p<0.05). All 24 proteins exhibited a fold change of up to 1.5-fold in terms of expression. Additionally, we identified four down regulated proteins (Haemoglobin beta isoforms), although the differences were not significant and were not further investigated. Protein spots were identified using MALDI-TOF/TOF and LTQ-Orbitrap XL, with MS/MS data searched against the human section of the UniProt database (Version 20140709, 88,993 sequences) (Table 1).

Bioinformatic Analysis

To elucidate the biological function, pathways, and interaction networks associated with the collected data, we employed various bioinformatic tools and software, including UniProt, Genecards (v.4.8.2), KEGG, Reactome, and STRING (version 10.5). The genes corresponding to differentially expressed proteins were mapped to multiple gene annotation data and biological pathways using PANTHERS 14.0. The categorization of genes was based on molecular function, biological process, route, cellular location, and protein class.

Protein–Protein Interaction and Co-Expression Study

In our study, we utilised the web-based visualisation tool STRING to construct and explore the protein-protein interaction (PPI) network, while Cytoscape (v3.8.2) was employed for additional assessment. The PPI network was represented as an undirected graph, with proteins as nodes and their connections as edges (Figure 2a). Coexpression analysis revealed a strong association among the genes of three metabolic enzymes, including Zinc finger CCCH domain-containing protein 13 and Putative RNA-binding protein 15 (combined score: 0.840), as well as between homologues of Hemoglobin subunit beta- HBB and HBA1 (combined score: 0.781), HBB and HBA2 (combined score: 0.560), and HBA1 and HBA2 (combined score: 0.800). We further analyzed co-expression mapping using the STRING database (Figure 2b) and found the most significant co-expression between PRRC2A and MAP4. Additionally, PRRC2B co-expressed with ERCC6, CNTLN co-expressed with ANKRD36, ZC3H1D co-expressed with EIF2AK2, and CAMSAP2 co-expressed with EIF2AK2. Notably, there is a potential link between EIF2AK2, CAMSAP2, LAMA3, ANKRD36, and unexplained female infertility.
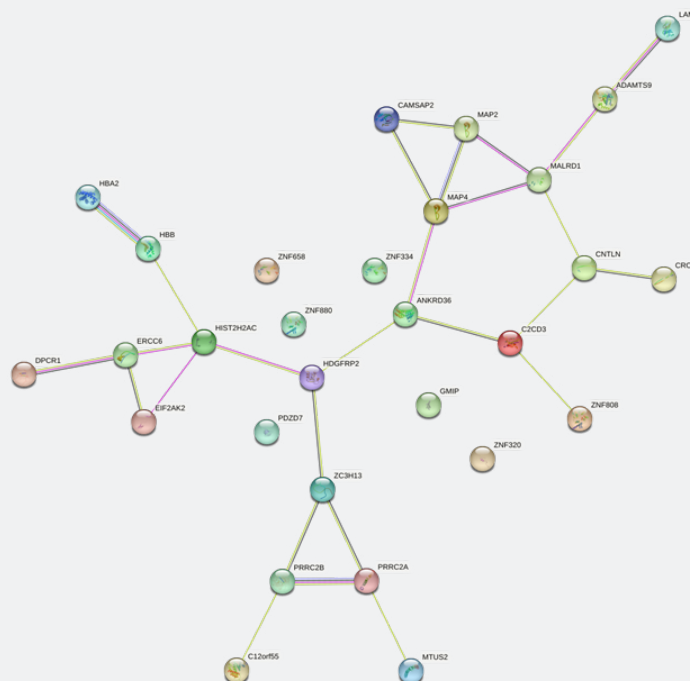

Figure 2a: Protein-Protein Interaction network between the annotated genes using STRING Database based on confidence prediction.
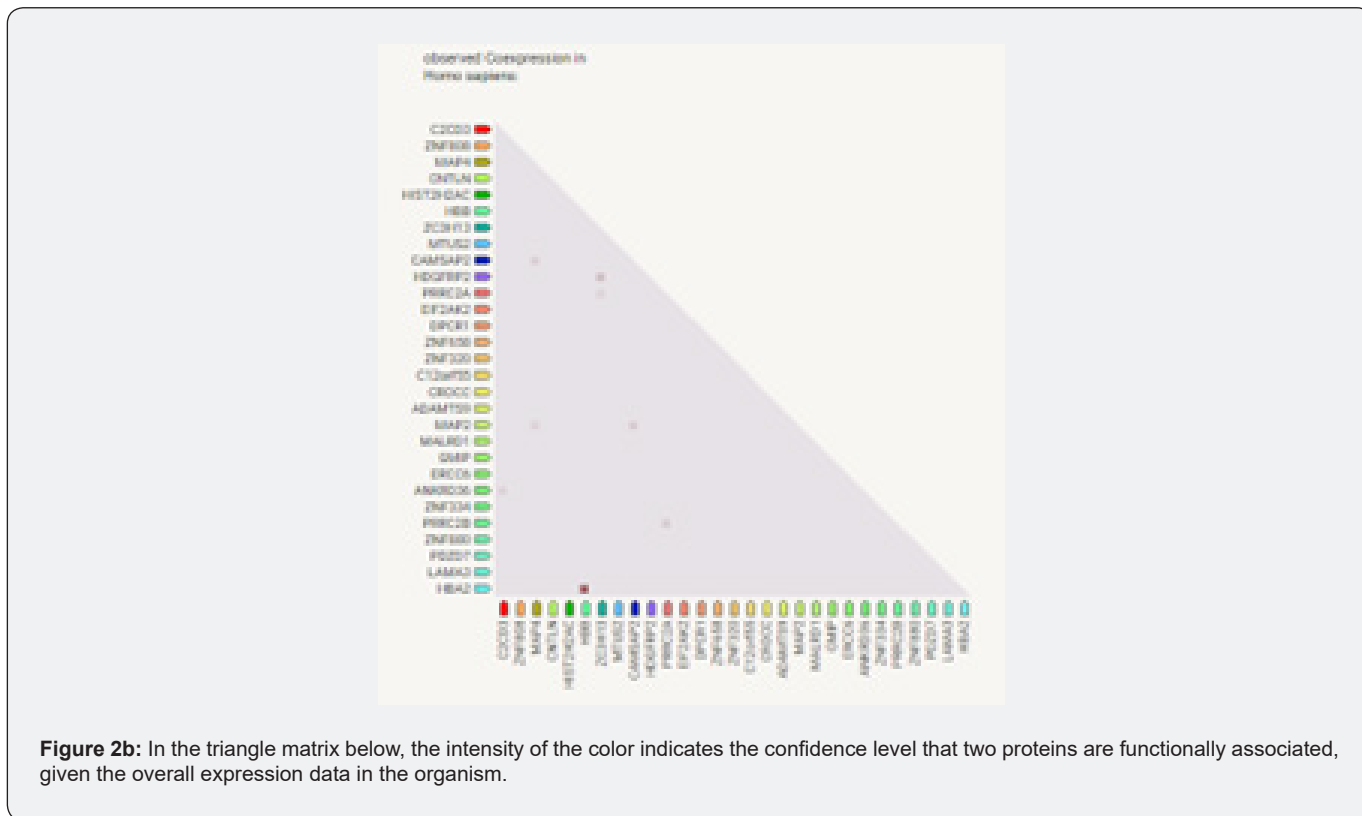
Figure 2b: In the triangle matrix below, the intensity of the color indicates the confidence level that two proteins are functionally associated, given the overall expression data in the organism.

Table 1: Protein expression levels measured in the unexplained infertile and the fertile proteome as identified by MALDI-TOF/TOF or LTQ-Orbitrap XL mass. Only the most expressed proteins are reported.

Accession Number	Protein Description	Gene Symbol	Subcellular localisation	Protein Score*	Fold Change**	Ratio	p value
A0A0K2BMD8	Mutant hemoglobin alpha 2 globin chain	HBA2	Cytosol	258	1.6	0.32	0.012
A6QL64	Ankyrin repeat domain-containing protein 36A	ANKRD36	-	385.14	7.2	144	0.05
A0A804HKE7	Microtubule-associated protein	MAP4	Cytoplasm, Cytoskeleton	53.85	5.6	50.4	0.012
Q5T200	Zinc finger CCCH domain-containing protein 13	ZC3H13	Nucleus	45.13	4.4	22.1	0.016
F8W9U4	Microtubule-associated protein	MAP4	Cytoplasm, Cytoskeleton	48.8	2.9	4.3	0.039
A0A024R7N1	GEM interacting protein, isoform CRA_b	GMIP	Cytosol, Nucleoplasm, Plasma Membrane	168.45	3.3	10	0.047
Q59FK4	Tyrosine-protein kinase	EIF2AK2	Cytoplasm	149.27	2.2	15.2	0.026
Q6PDB4	Zinc finger protein 880	ZNF880	Nucleus	138.16	5.2	9.2	0.037
P48634	Protein PRRC2A	PRRC2A	Cytoplasm, Nucleus	76.99	5	27.9	0.025
Q9H5P4	PDZ domain-containing protein 7	PDZD7	ell projection (cilium), Nucleus	66.9	2.9	3.2	0.021
Q5VYJ5	MAM and LDL-receptor class A domain-containing protein 1	MALRD1	Cytoplasmic vesicle membrane	56.21	5.3	41.2	0.038
Q9NXG0	Centlein	CNTLN	Cytoplasm, Cytoskeleton, microtubule organising center, centrosome, centriole	51.24	3.5	11.5	0.021
A0A3B3ITG1	Laminin subunit alpha 3	LAMA3	extracellular membrane, extracellular matrix, basement membrane	45.84	3.8	14.7	0.025
Q5JSZ5	Protein PRRC2B	PRRC2B	-	76.99	1.8	0.5	0.038
A0A7P0T9G4	ERCC excision repair 6, chromatin remodeling factor	ERCC6	-	32.61	3.9	15.4	0.021

Q7Z4V5	Hepatoma-derived growth factor-related protein 2	HDGFL2	Nucleus, Cytoplasm	32.61	3.9	15.4	0.05
Q16777	Histone H2A type 2-C	H2AC20	Nucleus	124.9	2.2	13.3	0.012
Q5TZA2	Rootletin	CROCC	-	87.35	1.9	0.71	0.05
A0A7P0Z4H1	C2 domain containing 3 centriole elongation regulator	C2CD3	-	177.9	1.7	2.5	0.05
A0A1B0GV46	Mucin like 3	MUCL3	Membrane	118.3	2.1	0.93	0.026
Q08AD1	Calmodulin-regulated spectrin-associated protein 2	CAMSAP2	Cytoplasm, Cytoskeleton, Gogi Complex	116	1.8	1.2	0.037
A0A0J9YWL9	Putative testis-expressed protein 13C	TEX13C	Cytoplasm	18.3	1.9	0.73	0.054
Q9P2N4	A disintegrin and metalloproteinase with thrombospondin motifs 9	ADAMTS9	Extracellular space, Extracellular matrix, Endoplasmic Reticulum	117	1.7	4.5	0.021
A0A1B0GU80	Cilia and flagella associated protein 54	CFAP54	-	296	2.1	3.4	0.021

* The protein score is the sum of the Mascot ion scores of all of the peptides that were identified for a given protein; ** the fold change is the ratio of the mean percentage relative volume (%V) (%V = V(single spot)/V(total spot)) of two groups.

Pathway and Functional Enrichment Analysis

We conducted pathway and functional enrichment analysis using the web-based gene set enrichment analysis program EnrichR, which utilises all differentially expressed common genes to identify significant molecular pathways. We employed KEGG, WiKi, and the Reactome pathway databases to analyse the signalling pathways and gene ontology of the differentially expressed genes in our cohort of unexplained female infertility. Signalling pathways and gene ontology (GO) categories, including biological processes, molecular function, and cellular components, were used as annotation sources. Statistical significance was determined based on the adjusted p-value to obtain enhanced results.

Using the PANTHER Database, we characterised the upregulated proteins based on their biological processes, cellular components, and protein classes. We represented the gene ontology of the upregulated proteins using a pie chart (Figure 3a), which revealed that the majority (32.4%) of these genes belong to the category of Basement Membrane, closely followed by the Ribosome category (25.4%). In terms of the influenced biological processes, we observed derangements in microtubule minus-end binding, protein tyrosine kinase activator activity, and kinesin binding activities of the cells (Figure 3b). The majority of the proteins (35.5%) did not fall into any specific Panther class, indicating a lack of research in this area (Figure 3c). Among the known classes, the Gene-specific transcriptional regulator protein class (Panther Category ID: PC00264) exhibited the highest upregulation in our study.

The enrichment study identified 25, 20, and 114 pathways in the KEGG, WiKi, and Reactome databases, respectively. Notably, we considered only the top ten significant pathways from each pathway database. We found several significant pathways, including Proteoglycans in cancer (hsa05205), Notch Signalling (hsa04330), PPAR signalling pathway (hsa03320), Pathways in cancer (hsa05200), Diseases of signal transduction (R-HSA-5663202), CD28 dependent PI3K/Akt signalling (R-HSA-389357), ABC transporters in lipid homeostasis (R-HSA-1369062), VEGF-VEGFR2 Signalling Pathway (hsa04370), and Thyroid hormone signalling pathway (hsa04919). These pathways, along with other significant pathways, are depicted in Figure 4a-4c.

Materials and Methods

Patients

We retrospectively recruited twelve infertile women undergoing IVF–ET, hysterectomy, or dilatation and curettage at the Medical Health and Research Institute from February 2021 to February 2023. Informed consent was obtained from all participants after the Institutional Review Board of the study institute authorised the protocol. The study included female subjects under 40 years of age with regular menstrual cycles (27–35 days), a typical double-phase basal body temperature, normal serum prolactin levels, and a male partner with ≥5 million motile sperms, as recruited in our study.

The cause of infertility was investigated following the minimum propaedeutic procedure for infertile couples. This included hormone and biochemistry profiling, testing for sexually transmitted diseases, imaging examinations, investigation of genetic and/or immunological abnormalities, semen analysis of the partner, hysterosalpingography, hysteroscopy, and laparoscopy (performed in all women up to 36 years old and in patients over 36 years old whenever there were symptoms or abnormalities on imaging examinations). If none of these examinations revealed an abnormality, infertility was considered unexplained. Women who did not achieve pregnancy after at least six natural or induced cycles following laparoscopy were considered infertile. The control group consisted of fertile women with a history of parity and no uterine abnormalities. None of the participants had

used any steroidal medications in the past six months. Women with hyperprolactinemia, polycystic ovarian syndrome (PCOS), or moderate-severe endometriosis were excluded. Age, basal serum follicle-stimulating hormone (FSH) levels, and body mass index (BMI) did not significantly differ between the groups.

Clinical data and tissue samples were collected after providing a detailed explanation of the study's objectives. All participants signed an informed consent form approved by the Research Ethics Committee of the Medical Health and Research Institute (MHRI).
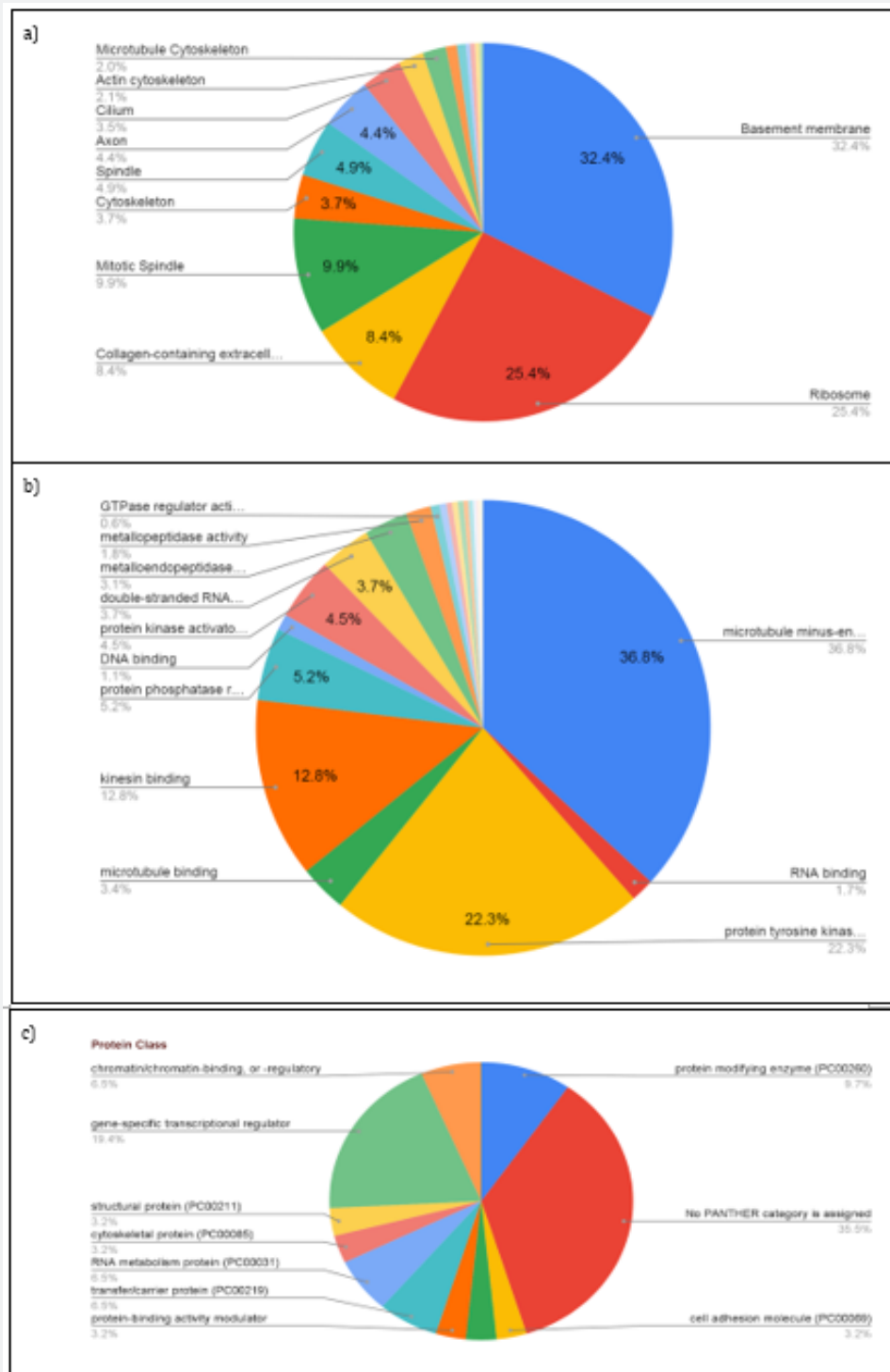

Figure 3 (a-c): Gene Ontology using PANTHER database. Pie diagrams representing the a)Cellular Component, b) Biological Process and c) Protein Class of the annotated proteins.
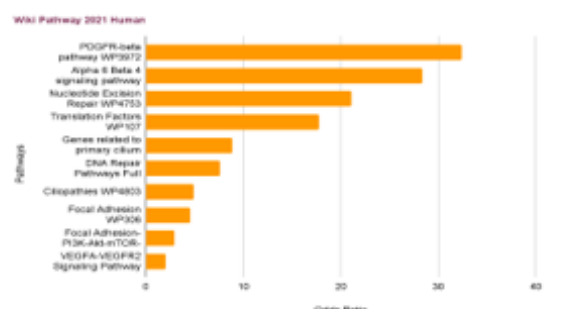
a) The top ten signalling pathways from KEGG enrichment analysis are shown by the bar diagram with p-value

KEGG Pathways	Genes in the Pathway	Adjusted P-value
Necroptosis	H2AC20; EIF2AK2	0.26
Nucleotide excision repair	ERCC6	0.26
ECM-receptor interaction	LAMA3	0.26
Protein processing in endoplasmic reticulum	EIF2AK2	0.26
Neutrophil extracellular trap formation	H2AC20	0.26
Focal adhesion	LAMA3	0.26
Viral carcinogenesis	EIF2AK2	0.26
PI3K-Akt signalling pathway	LAMA3	0.37
Pathways in cancer	LAMA3	0.46
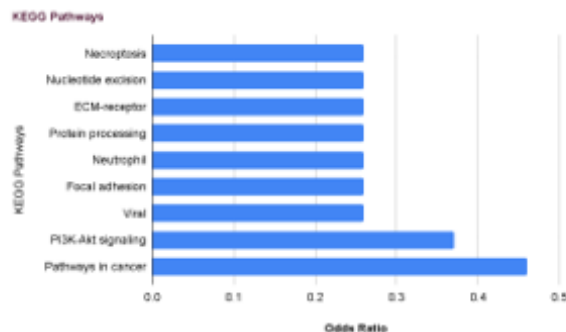
b: The top ten signaling pathways from WiKi enrichment analysis are shown by the bar diagram with p-values.

Wiki Pathway	Genes in the Pathway	Adjusted P-value
PDGFR-beta pathway WP3972	EIF2AK2	0.11
Alpha 6 Beta 4 signalling pathway WP244	LAMA3	0.12
Nucleotide Excision Repair WP4753	ERCC6	0.12
Translation Factors WP107	EIF2AK2	0.12
Genes related to primary cilium development (based on CRISPR) WP4536	C2CD3	0.17
DNA Repair Pathways Full Network WP4946	ERCC6	0.184
Ciliopathies WP4803	C2CD3	0.24
Focal Adhesion WP306	LAMA3	0.24
Focal Adhesion-PI3K-Akt-mTOR-signaling pathway WP3932	LAMA3	0.33
VEGFA-VEGFR2 Signaling Pathway WP3888	ADAMTS9	0.39

c: The top 20 signaling pathways from Reactome enrichment analysis are shown by the bar diagram with p-values.

| Reactome Pathway	Genes in the pathway	Adjusted P-value
ERCC6 (CSB) And EHMT2 (G9a) Positively Regulate rRNA Expression R-HSA-427389	H2AC20;ERCC6	0.08
Positive Epigenetic Regulation Of rRNA Expression R-HSA-5250913	H2AC20;ERCC6	0.08
RNA Polymerase I Promoter Clearance R-HSA-73854	H2AC20;ERCC6	0.08
Epigenetic Regulation Of Gene Expression R-HSA-212165	H2AC20;ERCC6	0.14
Metalloprotease DUBs R-HSA-5689901	H2AC20	0.19
RNA Polymerase I Promoter Opening R-HSA-73728	H2AC20	0.19
Packaging Of Telomere Ends R-HSA-171306	H2AC20	0.19
DNA Methylation R-HSA-5334118	H2AC20	0.19
Activated PKN1 Stimulates Transcription Of Androgen Receptor Regulated KLK2 And KLK3 R-HSA-5625886	H2AC20	0.19
Cleavage Of Damaged Purine R-HSA-110331	H2AC20	0.19
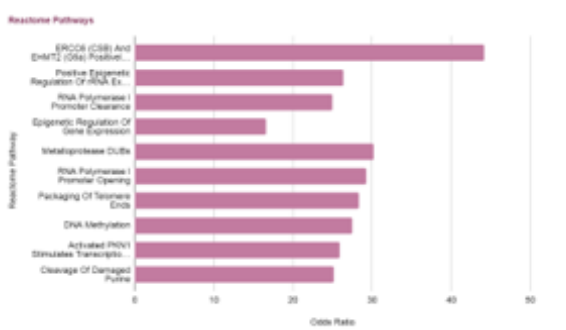
Figure 4 (a-c): The top ten signalling pathways from GO biological process enrichment analysis are shown by the bar diagram with p-values.

Sample Preparation

Biopsies of endometrial tissue were obtained during the mid-secretory phase of the menstrual cycle (LH +7). Endometrial samples were collected under sterile conditions using a pipette catheter and stored at -80 °C for proteome analysis. To deplete serum samples, the ProteoPrep Blue Albumin and IgG Depletion Kit (SIGMA-ALDRICH) was employed, and protein content was measured using the Bradford test. Frozen endometrial tissue

samples (40mg) were homogenised using a handheld homogenizer (POLYTRON® System PT 1200E) with 400μl of urea lysis solution (8M urea, 65mM CHAPS, 2 M thiourea, 33 mM Tris, 6 mM PMSF, 65 mM DTT, and 1% protease cocktail inhibitor). The homogenate was then vortexed for 30 seconds at 10-minute intervals (three times) and sonicated on ice using an ultrasonic cleaner in 10-second bursts at 10-second intervals ten times. Subsequently, the sample was centrifuged at 16,000 g for 10 minutes using an Eppendorf centrifuge, and the clear supernatant was collected and stored at -80 °C for further analysis.

DE and Image Analysis

For the first dimension, pH 3-10 13cm IPG strips from GE Healthcare, Uppsala, Sweden, were utilised, and active/passive rehydration was performed. The proteins were focused on an IPGPhor III (GE Healthcare, Uppsala, Sweden) apparatus using the following IEF conditions: 100 V gradient for 1 hour, 300 V gradient for 2 hours, 1000V gradient for 1 hour, 5000V gradient for 5 hours, and 5000 V step, followed by a 5000 V step held for 7 hours at a constant temperature of 20 °C. Each IPG strip underwent isoelectric focusing (IEF), followed by equilibration with 2% DTT and incubation with a different buffer containing 2.5% iodoacetamide in place of DTT. The second dimension PAGE (12.5%) was performed using the SE600 system (GE Healthcare, Uppsala, Sweden) at 1W/gel for 1 hour and 13W/gel for 3 hours. The protein spots were stained with colloidal Coomassie blue G-250 and scanned using a high-precision scanner (ScanMaker 9700XL, Microtek). The gel images were analysed using the gel image analysis tool PDQuest 8.01 (Bio-Rad) [19]. Mass spectrometric analysis was performed on the target protein spots excised from the gel after trypsin digestion. Gel fragments were rinsed with Milli-Q water and treated with a decolorizing solution containing 50% acetonitrile and 25% ammonium bicarbonate. After dehydration in 100% acetonitrile (ACN) for 10 minutes, the decolorized gel particles were vacuum dried for 30 minutes.

Protein Identification and Data Analysis

Protein spots that were excised from the gel were decolorized, digested, and extracted following the protocol described by Liu AX et al. [20]. The peptide samples were analysed using Matrix-assisted Laser Desorption Ionization (MALDI-TOF/TOF) Mass Spectrometry and LTQ-Orbitrap XL (Bruker Daltonics, Bremen, Germany). Protein identification was performed using the Bio Tools 3.0 software on MASCOT (V2.1, Matrix Science, UK) based on the peptide mass fingerprint data. Proteins with a MASCOT score greater than 64 and more than four peptide matches were considered significant (P<0.05). Proteins exhibiting a minimum 1.5-fold change between groups and a P-value of 0.05 were subjected to further bioinformatic analysis.

Statistical Analysis

Clinical and experimental data were analysed using Student's t-tests conducted in SPSS for IBM Statistics version 20 (IBM Corp., Armonk, NY, USA). Only proteins that showed consistent and significant changes (either increased or decreased) were included for subsequent bioinformatics analysis. P-values were calculated using right-tailed Fisher's exact tests, when appropriate, to identify statistically significant pathways and networks associated with the identified proteins. A significance level of P<0.05 was used for all analyses [21].

Discussion

Infertility, a specific disease entity with a high prevalence, has been considered a social disease [22,23]. While infertility primarily affects reproductive capabilities, it also has far-reaching implications for mental-emotional, social, and cultural well-being. Couples dealing with unexplained infertility often exhibit intrinsic gamete and uterine abnormalities that hinder sperm-oocyte interaction and fertilization, leading to decreased fertility or even complete fertilization failure, even with assisted reproductive techniques [24].

In contrast to males, who generate fresh sperm cells continuously during their lifespan, females possess their complete stock of germ cells at birth, which remains unchanged throughout their lifetime. Many genes, proteins, metabolites, and RNA molecules are all included in the female reproductive interactome, a highly complex network of functionally interconnected biological components. Many diseases are now thought to arise due to biological cascade disruption by changed interactions between different network components.

The endometrial epithelium, which lines the innermost layer of the uterus, plays a pivotal role in establishing a receptive environment for conception. This phase is tightly regulated and occurs approximately 6-10 days after ovulation when the endometrium becomes receptive to embryo attachment and invasion. The uterine luminal epithelium (LE), serving as the first maternal contact for an implanting embryo, undergoes specific changes to facilitate embryo implantation. These changes include intrauterine fluid resorption, cessation of LE proliferation and apoptosis, and conducive LE structural modifications, all of which are essential for establishing transient uterine receptivity for successful implantation [25]. The molecular network and signalling pathways involved in this process are orchestrated by a variety of factors, including hormones, cytokines, growth factors, and cell adhesion molecules.

In contrast to conventional biochemical approaches that monitor only one or a few specific proteins at a time, proteomics is a valuable method for identifying altered protein expression and proteins involved in disease pathogenesis. MS-based proteomics has the potential to detect and quantify any protein within a given sample, making it a more powerful tool compared to microarrays, which are inherently limited to studying the expression of genes with probes spotted on their surface [26]. Therefore, proteomics has emerged as an indispensable tool for evaluating potential pathomechanisms of diseases, both for diagnostic and therapeutic

purposes. A recent proteomic analysis of proliferative phase uterine fluid in fertile and infertile women revealed significant differences in secreted proteins, including ECM1 [27]. Although a few studies have investigated the association between genes such as PAI-14G/5G (rs1799889) [28-31] and the risk of infertility, the study of genes associated with infertility in rodent models has expanded the field of translational genetics in identifying the underlying causes of human infertility problems. However, many intriguing aspects of the molecular basis of infertility in humans, including the pathophysiology of unexplained infertility, remain poorly understood even today.

Among the overexpressed proteins in our study, we found few proteins that have been previously described confirming the validity of the quantitative proteomic approach undertaken by us in this study. Among the proteins previously shown to be upregulated in infertility were the Zinc Finger proteins, Claudins and Ankyrin Repeat Domain proteins which were significantly upregulated compared with the endometrial tissue of the control group [31-33]. The cadherin superfamily encompasses an impressive and exhaustive investigation of different categories of molecules engaged in cellular adhesion. These include tight junctional molecules, desmosomal molecules, adhesion molecules from the Ig superfamily, integrins and their ligands, receptors that bind to carbohydrates, galactins, and selectins, which are implicated to also play a role in abnormal cell behaviour leading to development of cancers.

In 1998, Greenhouse et al. embarked on the exploration of the genetic underpinnings of unexplained female infertility, utilising animal models. They hypothesised that abnormalities in the expression or structure of Zfp36l2 could potentially account for approximately 10% of cases of unexplained female infertility in humans [24]. Zfp36l2, also known as zinc finger protein 36-like 2 or TIS11D, ERF2, and BRF2, belongs to a unique family of zinc finger proteins characterised by tandem zinc-binding motifs distinguished by three cysteines followed by one histidine (CCCH) [32]. Zinc finger proteins play pivotal roles in various cellular processes, including gene expression and DNA repair. Concerning female infertility, studies have indicated that specific mutations in zinc finger proteins may impede ovary and follicle development, compromise oocyte quality and viability [33], and disrupt crucial hormonal signalling pathways required for successful reproductive function [34]. In addition to zinc finger proteins, a study by Yu et al. [35] discovered the significance of ankyrin repeat protein ANK6 in fertilisation, particularly in gamete recognition. ANK6, also referred to as At5g61230, exhibited strong expression in both male and female gametophytes before and during fertilisation, but not afterward. Furthermore, a genetic study involving a T-DNA insertional mutant of Ankyrin Repeat proteins demonstrated that alterations in ANK6 expression could result in embryo developmental arrest [36], further emphasising the critical role these proteins play in the infertility process.

Alterations in cadherin expression and function in the uterine endometrium have been implicated in unexplained infertility, affecting cell-cell adhesion and communication. Cadherins like E-cadherin and N-cadherin are crucial for maintaining the structural integrity of the endometrial epithelium and supporting successful embryo implantation. Studies have linked aberrant cadherin expression to impaired embryo attachment and invasion, compromising pregnancy establishment. Notably, E-cadherin dysfunction is associated with endometrial hyperplasia, cancer development, and increased invasion and metastasis of endometrial cancer cells. These findings suggest that cadherin dysfunction may play a role in various endometrial conditions, including infertility. In our study, we observed dysregulation of the cadherin signalling pathway, along with upregulation of other pathways such as Notch Signalling, PPAR signalling pathway, and Pathways in cancer (hsa04330, hsa03320, hsa05200), highlighting their potential relevance. [37-40]. However, it's important to note that the overall risk of developing cancer as a result of infertility or its treatments remains relatively low, and more research is needed to fully understand the complex relationship between infertility and cancer.

Understanding the potential link between infertility and cancer is crucial for comprehensive disease comprehension and management. Further research is warranted to investigate the underlying molecular mechanisms and identify specific factors and pathways that contribute to both infertility and cancer development. By gaining a better understanding of these shared molecular mechanisms, we can potentially develop more effective strategies for both diagnosing and treating infertility and associated cancers, ultimately improving patient outcomes.

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