Abstract title: Functional genomic meta-analysis identifies similarities between endometrial-related subfertilities


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Study question:
Are there relevant enriched functions shared both within and between endometrial-related subfertilities as endometrial adenocarcinoma, endometriosis, Recurrent Implantation Failure (RIF) and Recurrent Pregnancy Loss (RPL)?

Summary answer:
Specific enriched functions were common between all considered endometrial-related subfertilities. RIF and endometriosis were the most similar, and RPL differed from all others.

What is known already:
Endometrial-related pathologies are complex, multifactorial conditions affecting female fertility. Prior studies described endometrial adenocarcinoma, endometriosis, RIF, and RPL through transcriptomics analysis using case vs control approaches to identify altered genes and functions. However, the underlying mechanisms linking these conditions to endometrial subfertility remain controversial due to small sample sizes and differing experimental designs. Functional meta-analysis techniques provide a more robust method to highlight the most important functions associated with a set of individual studies. The objective of this research was to identify enriched shared functions within and between endometrial-related subfertilities studies for robustly underlaying the common molecular basis.

Study design, size, duration:
An in-silico study involving systematic review, transcriptomic analysis, and functionally integrative meta-analysis was applied to selected case vs control experiments associated with endometrial-related pathologies. From 613 datasets, we included 3 from endometrial adenocarcinoma, 2 from RIF, 2 from RPL, and 2 from eutopic endometrium of endometriosis. Functional meta-analysis techniques were employed to a) separately identify shared functions and pathways related to each single condition; and b) to determine altered functions common between endometrial-related conditions.

Participants/materials, setting, methods:
Raw data were downloaded from Gene Expression Omnibus (GEO). Selected datasets were preprocessed, normalized using quantile method (Limma R-package), and explored through principal component and clustering analysis. Gene Set Enrichment (mdgsa R-package) was performed on differential expression analysis results (Limma R-package). Functional meta-analysis was performed using Der-Simonian & Laird random-effects model to identify significant shared functions (False Discovery Rate (FDR) < 0.05). Functional databases consulted were Gene Ontology and Kyoto Encyclopedia of Genes and Genomes.

Main results and the role of chance:
Functional meta-analysis allowed us to identify functional connections within studies related to each endometrial-related pathology. Functions such as cell junctions, p53 signaling pathway, endoplasmic reticulum, and cell adhesions \((3.9412\times 10^{-07} \leq \text{FDR} \leq 0.00016)\) were associated with adenocarcinoma; proteasome complex, amine transport, apical part of cell, and chromosome-related functions \((7.2107\times 10^{-06} \leq \text{FDR} \leq 0.04849)\) with RIF; DNA replication and ribonucleoprotein complex \((0.00035 \leq \text{FDR} \leq 0.04281)\) with RPL; and proteasome, mitochondria, and microtubule-related processes \((0.00434 \leq \text{FDR} \leq 0.04823)\) with endometriosis. Results from integrating all endometrial subfertilities (adenocarcinoma, RIF, RPL, endometriosis) identified 8 shared functions related to cell projections \((0.00282 \leq \text{FDR} \leq 0.03690)\), chromatin DNA binding (FDR=0.0440), and organelle assembly \((0.00053 \leq \text{FDR} \leq 0.0336)\). Furthermore, when RPL, RIF, and endometriosis were integrated by pairs, RPL was the least similar, having only one common function with RIF (FDR=0.0484) and 8 with endometriosis \((1.069e-05 \leq \text{FDR} \leq 0.04390)\). RIF and endometriosis were the most similar, sharing 21 functions \((1.1262\times 10^{-08} \leq \text{FDR} \leq 0.04823)\). Finally, those pathologies involving tissue growth (endometrial adenocarcinoma and endometriosis) were found to have a higher number of altered genes and functions than RIF or RPL.

**Limitations, reasons for caution:**
Despite including good-quality GEO datasets, this study is limited by available endometrial-related subfertilities and the heterogeneity between studies. However, all datasets were transcriptomically analyzed using the same methodology and the applied meta-analysis incorporates the variability of each study, robustly integrating them at a functional rather than at a gene level.

**Wider implications of the findings:**
Due to functional alteration similarities between endometriosis and RIF, there is evidence that eutopic endometrium is affected, supporting the controversial idea that endometriosis affects endometrial function. Shared detected functions between all considered endometrial subfertilities could enable development of a common method to diagnose all of them.

**Trial registration number:**
not applicable

**Keywords:**
Endometrial adenocarcinoma
Recurrent Implantation Failure
recurrent pregnancy loss
eutopic endometriosis
functional genomic meta-analysis