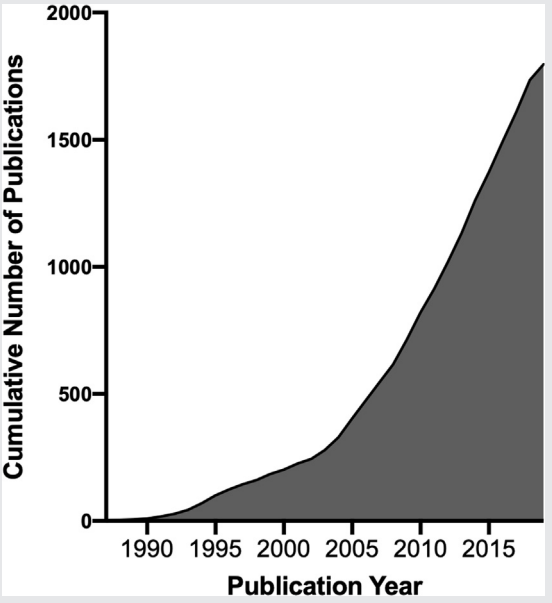
Bioinformatic jujutsu to defeat an endometrial transcriptomic foe

The literature describing the changes in the endometrial transcriptome that are associated with reproductive failure is becoming quite large (Figure 1) and quite confusing! Differentially expressed genes (DEGs) in one study often demonstrate little to no overlap with DEGs in another study, despite similar patient populations. There are many likely reasons for these discrepancies, including technical considerations, such as different methods of transcriptome assessment, such as different microarray platforms or different types/methods of RNA sequencing. Biological sources of variability are also important, and this is particularly true for endometriosis or fertility studies. For endometriosis studies, for example, study populations recruited from a pain clinic versus an infertility clinic are likely to have different comorbidities that bias results; furthermore, endometriosis is sometimes optimally treated in the disease group, which might affect the eutopic endometrial gene expression. Moreover, occult endometriosis is common in the infertility population and cannot be rigorously excluded without laparoscopy, which few studies uniformly employ. Fertility studies can also suffer from lack of rigorous exclusion or correction for other comorbidities, including obesity, endometritis, and small polyps. The choice of a control group is another potential source of variability, as many studies use populations that have not had proven fertility (egg donors), have other gynecological disorders (surgical population), or those that have demonstrated normal fertility only in the distant past. Given the enormous patient variability and potential confounders, how can we possibly interpret these data to understand the endometrial contributions to disorders affecting our patients?

In some martial arts, especially jujutsu (from Japanese: *Ju*, meaning soft or pliable and *Jutsu*, meaning art), success in combat can be achieved by cleverly turning an opponent's greater strength and weight into an advantage, allowing a smaller, weaker individual to defeat a stronger, bigger foe (1). In an application analogous to bioinformatic jujutsu, the paper by Devesa-Peiro et al. seeks to turn the weakness (profound heterogeneity and diversity of the transcriptomic literature), into a strength (2). The authors apply a type of meta-analysis to the transcriptomic literature to search for final common endometrial dysfunction pathways in samples from a diverse set of disorders: endometrial adenocarcinoma (ADC), recurrent implantation failure (RIF), recurrent pregnancy loss (RPL), and stage II–IV endometriosis.

The authors evaluated 163 unique endometrial transcriptomic studies, but only eight datasets, encompassing four disorders, could be included by the defined inclusion and exclusion criteria. Each dataset was reanalyzed separately for DEGs in a standardized fashion, with attempted correction for various cycle phases, using linear models. The DEGs for each study underwent a standardized functional assessment, using KEGG and GO functional annotations. The functional analyses were combined by clinical disorder, with weighting to avoid overcounting changes in databases with higher variability. Study heterogeneity was corrected using linear effect modeling. Despite the rigorous analytical approach, the data

FIGURE 1



Estimated cumulative numbers of published primary papers using human endometrial transcriptomics by year, through 2019. Data obtained from PubMed using the following search query in February 2020: ((((((endometri*) AND (microarray OR transcriptome OR RNA-sequencing))) NOT (review[Publication Type]))) AND (humans[Filter])). No further filtering was done to remove any possible non-relevant papers or those using duplicate or overlapping data.

Young. Meta-analysis of endometrial transcriptomics. Fertil Steril 2020.

are derived from only eight studies, based solely on mRNA patterns, and have undergone functional analyses based on gene functions largely inferred from other, nonreproductive tissues. Despite all of these potential concerns, Devesa-Peiro et al. found commonalities within each disorder and, remarkably, similar categories across multiple disorders.

Among the most immediately interesting, novel transcriptional findings in a single disease group are those regarding ciliary function in endometriosis, which the authors speculate might help to reframe some previous cellular observations. Another novel result is the larger-scale groupings of the disorders. Between disorders, the authors found similar and opposing changes in direction of specific functional categories, resulting in grouping the endometrial functional changes of those with RIF and ADC as opposite in some ways to those with endometriosis and RPL. Even more frequently, the direction of functional changes in RPL oppose those of RIF, highlighting a previous hypothesis that unexplained RIF and unexplained RPL are the result of opposite changes in endometrial receptivity.

These analyses comprise the first rigorous and systematic meta-analysis of endometrial transcriptomic information, and suggest novel and interesting conceptional models of these diseases. Overall the findings also point out the need for more primary data along with more large-scale meta-analyses. The era of single-cell transcriptomics is upon us, and soon we will have even better in vitro implantation models using combinations of stem cell and organoid cultures from

healthy fertile women and those with specific disorders. These developments will produce more complex transcriptomic and cellular data to use for further meta-analysis. If successful, this may be the decade in which endometrial disorders finally find a firm pathophysiological foundation for the development of targeted diagnostics and therapeutics.

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