ENDOMETRIAL SCRATCH: A GENE EXPRESSION STUDY OF HUMAN ENDOMETRIUM FOLLOWING LOCAL MECHANICAL INJURY

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INTRODUCTION

- Endometrial scratch is a procedure undertaken to induce mechanical injury to the endometrium using Pipelle endometrial biopsy or hysteroscopy and curette. It has been proposed as a procedure to improve endometrial receptivity in women with repeated IVF failure by provoking an inflammatory reaction and endometrial regeneration.
- It has been demonstrated by recent RCTs that endometrial scratch provides no benefit in the general IVF population, where the majority of women are not suffering from reduced uterine receptivity. However, evidence about the benefit of endometrial scratch in women suffering from recurrent implantation failure (RIF), especially those due to uterine receptivity issues, remains unclear.

Hypothesis:

- If endometrial scratch is an effective treatment for improving endometrial receptivity, differential endometrial gene expression would be detected in the cycle following scratch.

Aim for this study:

- To investigate whether any genes are differentially expressed in endometrium taken at the time of scratching compared to endometrium taken in the subsequent cycle

MATERIALS AND METHODS

- Women with history of subfertility were recruited from the Royal Women's Hospital, Parkville. Participants underwent endometrial Pipelle biopsies during the mid-luteal phase (LH surge +7 days) of two consecutive menstrual cycles.

Gene expression array

- Total RNA extracted from endometrial tissue was amplified and converted to biotinylated cRNA using Ambion Illumina TotalPrep RNA amplification kit (Ambion, Thermo Fisher Scientific, Scoresby). Expression profiles in endometrial tissue were generated by hybridizing 750ng of cRNA to Illumina Human HT-12 v4.0 Beadchips (Illumina, Inc., San Diego).

Statistical analyses

- Gene expression data were normalized using Illumina GenomeStudio software (Illumina Inc., San Diego).
- Empirical Bayes moderated t-statistics were used to assess if probes were significantly differentially expressed. The resulting p-values were corrected for multiple hypothesis testing using the Benjamini-Hochberg method to control the false discovery rate (FDR).
- A principal component analysis was performed to determine whether samples that originated from the same patient clustered together. Similarly, distance matrices were generated using Euclidean distance, and hierarchical clustering was performed to visualise which samples were most similar to each other.

RESULTS

- A total 10 endometrial samples (from 5 participants) were included in the final study. All women had had at least 3 (3 -10) unsuccessful embryo transfer cycles prior to participation in the study.
- Our analysis shows there was no evidence for differential gene expression (DGE) between endometrial samples collected in consecutive cycles from the same woman before and after endometrial injury (adjusted p-value = 0.97). (Table 1)
- Principal component analysis showed that each pair of the samples from the same women cluster together, with the exception of patient F. (Figure 1 & 2)
- Patient F conceived and had a live birth in her subsequent IVF cycle. The post-biopsy sample from patient F (F1) was the most different to all the other samples, including her own pre-biopsy sample. Subsequent histological examination of sample F2 identified presence of inflammatory cells, with the diagnosis of chronic endometritis (CE) given by two independent pathologists. This suggests that endometrial injury could have resulted in significant inflammatory changes in the endometrium for this individual, and that these changes did not exclude successful implantation and pregnancy in this case.

Table 1: Top 10 (all non-significant) up-regulated or down-regulated genes in endometrium before (B) and after (A) endometrial injury, ranked by p-value.

Genes	Log_2FC^a	$AveExpr^c$	p-value	Adj. p-value[d]	FC^a	Direction (in A)
MIEN1 (Migration And Invasion Enhancer 1)	-0.65	5.74	0.0005	0.97	1.56	Down
NEDD9 (Neural Precursor Cell Expressed, Developmentally Down-Regulated 9)	0.54	8.25	0.0006	0.97	1.45	Up
ZNF827 (Zinc Finger Protein 827)	0.68	6.47	0.0006	0.97	1.60	Up
AFF3 (AF4/FMR2 Family Member 3)	0.65	7.29	0.0007	0.97	1.57	Up
UNC13B (Unc-13 Homolog B)	-0.49	6.37	0.0010	0.97	1.40	Down
STXBP6 (Syntaxin Binding Protein 6)	0.86	7.11	0.0011	0.97	1.82	Up
CBLC (Cbl Proto-Oncogene C)	-0.49	5.78	0.0011	0.97	1.41	Down
TCTN3 (Tectonic Family Member 3)	-0.40	7.54	0.0011	0.97	1.32	Down
STMN3 (Stathmin 3)	0.44	10.41	0.0013	0.97	1.36	Up
LIFR (LIF Receptor Subunit Alpha)	0.47	6.75	0.0013	0.97	1.39	Up

Figure 1: Cluster Dendrogram

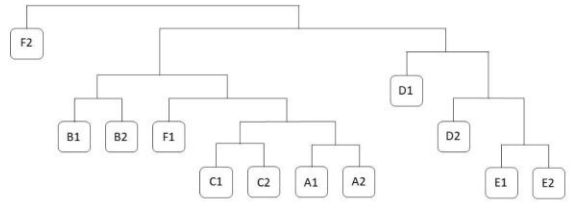
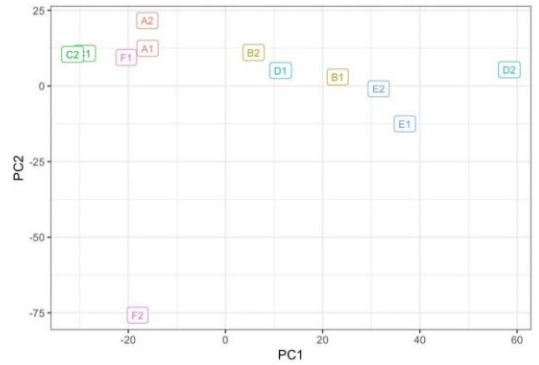


Figure 2: Principal components analysis plot



Each woman is represented by a letter (A to F). Endometrial samples collected before endometrial injury are represented by number 1, while samples collected after endometrial injury are represented by number 2.

KEY MESSAGES

- **Results from this study do not support the use of endometrial scratching as a general approach for all IVF patients.**
- **Endometrial gene expression profiles before and after endometrial injury were very similar in most of our women, and it was possible to show consistent differences between individual women. This suggests that endometrial gene expression remains consistent from cycle to cycle.**