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Differential Gene Expression during Placentation in Pregnancies Conceived with Different Fertility Treatments Compared to Spontaneous Pregnancies

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Abstract

Objective: To identify differences in the transcriptomic profiles during placentation from pregnancies conceived spontaneously to those with infertility, using non-in vitro fertilization (NIFT) or in vitro fertilization (IVF).

Design: Cohort study.

Setting: Academic Medical Center.

Patient(s): Women undergoing chorionic villus sampling at gestational age 11–13 weeks (n=141), with pregnancies that were conceived spontaneously (n=74), with NIFT (n=33), or IVF (n=34) resulting in the delivery of viable offspring.

Intervention(s): Collection of chorionic villus samples from women who conceived spontaneously, with NIFT or IVF for gene expression analysis using RNA sequencing.

Main Outcome Measure(s): Baseline maternal, paternal and fetal demographics, maternal medical conditions, pregnancy complications and outcomes. Differential gene expression of first trimester placenta.

Result(s): There were few differences in the transcriptome of first trimester placenta from NIFT, IVF, and spontaneous pregnancies. There was one protein-coding differentially expressed gene (DEG) between the spontaneous and infertility groups, *CACNA1I*, one protein-coding DEG between the spontaneous and IVF groups, *CACNA1I*, and five protein-coding DEGs between the NIFT and IVF groups, *SLC18A2*, *CCL21*, *FXYD2*, *PAEP*, and *DNER*.

Conclusion(s): This is the first and largest study looking at transcriptomic profiles of first trimester placenta demonstrating similar transcriptomic profiles in pregnancies conceived using NIFT or IVF and spontaneous conceptions. Gene expression differences found to be highest in the NIFT group suggest that the underlying infertility, in addition to treatmentrelated factors, may contribute to the observed gene expression profiles.

Capsule

The transcriptome during placentation of pregnancies conceived using infertility treatment are similar to that of spontaneously conceived pregnancies with gene expression differences found mainly in the non-IVF fertility treatment pregnancies.

Keywords

Non-IVF fertility treatment (NIFT); in vitro fertilization (IVF); placentation; RNA Sequencing; Transcriptome

Introduction

Infertility affects about 6.1 million people in the U.S., equivalent to ten percent of the reproductive age population (1). The use of assisted reproductive technologies (ART), including in vitro fertilization (IVF) contributes to 1.5% of live births in the United States and other non-IVF fertility treatments (NIFT) contribute to 4.6% (2, 3). Adverse pregnancy outcomes have been associated with ART, including low birth weight and small for gestational age babies, preeclampsia, retained placenta, placental abruption, placenta previa, preterm labor and delivery, and birth defects compared to pregnancies conceived spontaneously (2, 4–11). However, it is unclear whether these adverse outcomes are the result of the ART procedures, such as IVF or the underlying infertility, as pregnancies conceived by couples utilizing other types of fertility treatments, NIFT, are also at increased risk of adverse outcomes, including placental abruption, fetal loss, and gestational diabetes (12). Furthermore, pregnancies conceived in couples with infertility regardless of treatment are at increased risk of adverse outcomes, including placenta accreta (13), earlier gestational age at delivery, late preterm birth, greater neonatal intensive care unit admissions (11, 14) as well as birth defects (15–17).

As many of these adverse outcomes are related to placentation, a better understanding of placental function in pregnancies conceived in couples with infertility, utilizing fertility treatments, may uncover the underlying pathology leading to adverse outcomes. Previous studies examining the transcriptome of human placenta have been limited to term placentas with specific placenta pathologies, predominantly preeclampsia (18). Yet it is during the first trimester of a pregnancy, during placentation when trophoblast proliferation, differentiation, and invasion, as well as angiogenesis and vasculogenesis, crucial for laying the groundwork for successful placental function, are taking place and a necessary time point to study placental development (19). It is also the closest timepoint to conception, either spontaneously or through fertility treatments, that can be studied, in order to minimize placental changes due to placental pathology and not the mode of conception. This is the first and largest study looking at the first trimester placenta (chorionic villi) transcriptome in our SMAART (Spontaneously/Medically Assisted/ART) study cohort consisting of pregnancies that delivered from couples either with infertility, utilizing NIFT or IVF, and pregnancies conceived spontaneously.

Materials and Methods

Patient Population

Our SMAART Study cohort consisted of pregnancies conceived either spontaneously or in couples with infertility who conceived either through NIFT or IVF, that are in the late first trimester of pregnancy at the time of Chorionic Villus Sampling (CVS) and followed until

delivery. NIFT was defined as treatment using either medications for ovulation induction or controlled ovarian stimulation and intrauterine insemination (IUI). IVF pregnancies included fresh or frozen embryo transfers using either cleavage stage or blastocyst stage embryos. Our SMAART Study cohort consisted of 409 singleton pregnancies, of which 208 were spontaneous conceptions and 201 pregnancies conceived with a history of infertility. Of the infertility group, 90 were conceived with NIFT and 111 were conceived with IVF. All pregnancies had a normal karyotype and delivered (20).

Transcriptomic profiling was performed on a subset of 141 subjects that had chorionic villi available from the first trimester. The SMAART Transcriptome cohort consisted of 74 spontaneous conceptions and 67 pregnancies conceived with a history of infertility. Of the infertility group, 33 were conceived with NIFT and 34 were conceived with IVF.

Demographics Statistical Analysis

T-test and Analysis of Variance (ANOVA) were used for the baseline and pregnancy outcome demographics.

CVS collection

Chorionic villus samples were collected at 11–13 weeks gestation at the Cedars-Sinai Prenatal Diagnostic Center as previously described (21, 22). Left over tissue after clinical genetic testing was collected from consenting patients per institutional review board (IRB)-approved protocol and placed in RNAlater RNA Stabilization Reagent (Qiagen, Valencia, CA) and stored at −80°C in Cedars-Sinai Prenatal Biorepository until processing.

RNA Extraction for RNA-sequencing

CVS tissues were thawed on ice in 1% β-mercaptoethanol-containing lysis buffer using AllPrep DNA/RNA Extraction Mini kit (Qiagen). Cells were homogenized by repeatedly passing through single-use needles with decreasing gauge (22G, 25G, then 27G) attached to a 1ml sterile, RNase-free syringe before being loaded onto AllPrep spin columns (21, 22). DNA/RNA extraction was performed according to manufacturer's instructions and previously described. Purity of the extracted total RNA was evaluated using the NanoDrop 2000 spectrophotometer (Thermo Scientific, Wilmington, DE) and the concentration of the total RNA measured using the Qubit Fluorometer (Thermo Fisher Scientific, Carlsbad, CA). RNA integrity was assessed using the Fragment Analyzer (Advanced Analytical Technologies, Ankeny, IA) that analyzes the integrity of the total RNA by measuring the ratio between the 18S and 28S ribosomal peaks.

RNA-Sequencing and Statistical Analysis

RNA-Seq libraries were constructed from 200 ng of total RNA using Illumina TruSeq Stranded Total RNA with Ribo-Zero Gold sample prep kits (Illumina, Carlsbad, CA). Constructed libraries contained RNAs >200 nt and were depleted of cytoplasmic and mitochondrial ribosomal RNAs. RNA-seq reads were assessed for quality using FastQC (23). Transcript abundances were then quantified against the human reference genome, (Ensembl build GRCh38) using Kallisto (24), and read into the R statistical computing environment (25) as gene-level counts using the tximport package (26). We then used the

DESeq2 Bioconductor package (27, 28) to normalize for differences in sequencing depth between samples (using the default median-of-ratios method), estimate dispersion, and fit a negative binomial model for each gene. Datasets were adjusted for fetal sex and sequencing runs. The Benjamini-Hochberg False Discovery Rate (FDR) procedure (29) was then used to reestimate the adjusted p-values.

Pathway Analysis

To investigate biological significance of expressed transcripts, Ingenuity Pathway Analysis (IPA) software (Qiagen, Redwood City, CA, http://www.qiagen.com/ingenuity) was used to analyze enriched pathways. Differentially expressed genes were first filtered as significant at P-value < 0.05 and uploaded to IPA platform to perform Core Analyses, which rank pathways represented by the dataset and upstream regulators according to P-values calculated using Fisher's exact test.

Results

Clinical characteristics of SMAART Transcriptome cohort

From the SMAART cohort which consisted of 409 singleton pregnancies, all subjects underwent chorionic villus sampling for diagnostic testing. All subjects had a fetus with normal karyotype analysis and birth outcomes (20). Extra chorionic villi from the first trimester was available after tissue was removed for genetic testing for 141 subjects that was available for transcriptome profiling and analysis. This SMAART Transcriptome cohort, which was matched for maternal age, race, ethnicity and fetal sex, consisted of 74 spontaneous conceptions and 67 pregnancies conceived with a history of infertility. Of the infertility group, 33 were conceived with NIFT and 34 were conceived with IVF. When comparing pregnancies conceived spontaneously versus those from couples with infertility, there was no significant difference in maternal and paternal age and race. There was no significant difference in fetal sex distribution or fetal race. There was no difference in maternal underlying conditions but a significant difference in maternal BMI (22 ± 3.2 and 24 ± 5.5, respectively). When pregnancy outcomes were evaluated, there was a higher rate of Cesarean section and pregnancy-related diabetes in the infertility group (Table 1). When birth outcomes were examined, there was no difference in gestational age at delivery but a significant difference in birthweight (3430 ± 590 grams for spontaneous and 3192 ± 623 grams for infertility). When the infertility group was divided into pregnancies conceived with NIFT or IVF, the significant difference in maternal BMI was found to be between spontaneous and NIFT pregnancies (22 ± 3.2 versus 25 ± 6.3, respectively), rather than between spontaneous and IVF pregnancies. There was a higher rate of Cesarean section in the NIFT and IVF pregnancies, as was observed in the SMAART cohort. The difference in birthweight was found to be between spontaneous and IVF pregnancies (3430 ± 590 grams in spontaneous versus 3082 ± 586 grams in IVF) (Table 1).

Transcriptome

Two-Dimensional Principal component analysis (2D-PCA), which projects the RNA-seq dataset onto two dimensions to identify principal causes of variation in the dataset as represented by clustering of samples as data points (30), revealed that RNA sequencing

samples clustered together with similar data points regardless of pre-pregnancy maternal health conditions and medications, pregnancy complications, or in cases of IVF pregnancies, whether embryo transfer was performed with fresh versus frozen embryos or at cleavage versus blastocyst stages (data not shown) and therefore did not contribute to differences in outcomes. As there was clustering based on fetal sex, also demonstrated by other studies showing differences in the first trimester placenta due to sex (31), we corrected the dataset for fetal sex. In addition, there was clustering due to fetal race so we selected to use our largest populations that clustered together which included Caucasian and Caucasian-Asian fetuses.

All samples had at least 10 million uniquely mapped reads with an average of 20.8 million uniquely mapped reads per sample. All samples had at least 67% reads uniquely mapped with an average of 82% reads uniquely mapped per sample.

Overall, there were small differences in the transcriptome of first trimester placenta from spontaneous, NIFT, and IVF pregnancies (Figure 1) as the PCA plots of spontaneous versus infertility (Figure 1A) and spontaneous versus NIFT versus IVF pregnancies (Figure 1B) illustrate. Samples clustered according to sample subset (shown on the PC1 axis) and fetal sex (shown on the PC2 axis) (Figure 1), both of which were corrected in our analysis. When the transcriptome of spontaneous pregnancies was compared to that of pregnancies conceived with infertility treatment, of the 61799 genes examined, following adjustment for multiple comparisons, one protein-coding gene, *CACNA1I* was significantly differentially expressed at FDR of 5%, with 6.82-fold higher expression in the spontaneous group than in the infertility group (Table 2).

When we compared the transcriptome profiles among spontaneous, NIFT and IVF conceptions, *CACNA1I* was found to be the only significantly differentially expressed gene between spontaneous and IVF pregnancies again, with its expression 9.85-fold higher in the spontaneous group (Table 2). There were no protein-coding genes significantly differentially expressed between spontaneous and NIFT pregnancies at FDR of 5%. Comparing the transcriptome of NIFT versus IVF pregnancies, five protein-coding genes (*SLC18A2*, *CCL21*, *FXYD2*, *PAEP* and *DNER*) were significantly upregulated in NIFT pregnancies compared to IVF pregnancies, with fold changes ranging from 3.39 to 6.41 (Table 2). When expression levels of these DEGs represented by fragments per kilobase transcript per million (FPKM), a normalized version of RNA expression which accounts for gene length and sequencing depth (32), among the three groups were plotted and examined, the NIFT group had the highest expression of *SLC18A2*, *CCL21*, *FXYD2*, *PAEP* and *DNER,* with IVF pregnancies clustering with spontaneous pregnancies (Figure 2C).

Pathway Analysis

Due to the small number of differentially expressed genes, gene ontology studies were not possible for the DEGs that were identified following adjustment for multiple comparisons. However, in order to identify enriched pathways, differentially expressed genes with P-value < 0.05 were analyzed with IPA. When we used the p-value cutoff of 0.05 there were 966 genes that were significantly different between spontaneous and infertility, 729 genes between spontaneous and NIFT, 893 genes between spontaneous and IVF, and 482 between

NIFT and IVF pregnancies. IPA of these DEGs revealed similar canonical pathways in all comparisons with IVF subjects, including spontaneous versus infertility, spontaneous versus IVF, and NIFT versus IVF pregnancies (Supplemental Table 1). Pathogenesis of Multiple Sclerosis was the most represented in those comparison datasets (Supplemental Table 1), but this pathway was not represented in spontaneous versus NIFT pregnancies. Cytokines and cytokine receptors involved in Pathogenesis of Multiple Sclerosis were down-regulated in infertility and IVF pregnancies compared to spontaneous pregnancies; *CCR5*, *CXCL9*, and *CXCL10* were down-regulated in infertility compared to spontaneous pregnancies, *CCR5*, *CXCL9*, *CXCL10*, and *CXCL11* down-regulated in IVF compared to spontaneous pregnancies, and *CXCL9*, *CXCL10*, and *CXCL11* were down-regulated in IVF compared to NIFT pregnancies. None of these genes were differentially expressed in spontaneous versus NIFT (P-values ranged from 0.305 to 0.928). Another enriched canonical pathway, Neuroinflammatory Signaling Pathway, was significantly enriched in all three comparisons to spontaneous pregnancies, (Supplemental Table 1), but not significantly enriched in NIFT versus IVF (P-value = 0.11). Neuroinflammatory Signaling Pathway genes were more represented in spontaneous versus IVF pregnancies than other comparisons, and nine pathway genes overlapped in all comparisons to spontaneous pregnancies, five consistently downregulated in spontaneous pregnancies (*CALB1, GABRR1, HLA-A, IKBKG,* and *TNF*) and four consistently upregulated (*GABRA2*, *GABRG3*, *HLA-A*, *PIK3CA*, and *PPP3CB*). Antigen Presentation Pathway was significantly enriched in spontaneous versus IVF (P-value = 5.85×10^{-4}) and NIFT versus IVF (P-value = 1.07×10^{-3}) (Supplemental Table 1), and less significant in spontaneous versus NIFT (P-value = 0.0309). In general, all enriched canonical pathways were immune related, but spontaneous versus NIFT pregnancies were represented by a more diverse set of canonical pathways which included Crosstalk between Dendritic Cells and Natural Killer Cells and Production of Nitric Oxide and Reactive Oxygen Species in Macrophages (Supplemental Table 1).

Upstream analysis was performed to identify master regulators of DEGs with P-value < 0.05. In spontaneous versus IVF pregnancies, cytokines were abundantly represented among the most significant upstream regulators of DEGs, including interleukins such as IL27, various interferon-alpha subtypes, chemokine ligands such as CCL11, and other cytokine families (Supplemental Table 2). Notably, cytokines were rarely found among upstream regulators of spontaneous versus NIFT pregnancy DEGs (Supplemental Table 3).

Discussion

Transcriptomic profiling of the first trimester placenta (chorionic villi) from spontaneous, NIFT, and IVF pregnancies demonstrated little differences in global gene expression among the three groups. One protein-coding gene (*CACNA1I*) was significantly differentially expressed in infertility pregnancies when compared to spontaneous pregnancies and this differential expression of *CACNA1I* was significant between spontaneous and IVF pregnancies as well. There were no DEGs between spontaneous versus NIFT pregnancies. Five protein-coding genes (*SLC18A2*, *CCL21*, *FXYD2*, *PAEP*, and *DNER*) were significantly differentially expressed in NIFT versus IVF pregnancies.

A number of DEGs had higher expression levels in NIFT pregnancies with IVF pregnancies segregating more with spontaneous pregnancies (Figure 2C). This result complements our previous finding in a pilot study demonstrating differential methylation between NIFT and IVF pregnancies at select loci, with global methylation remaining unchanged among spontaneous, NIFT and IVF pregnancies (33). In addition, it may reflect the differences in the underlying cause of infertility, and not the IVF itself, where fertilization occurs in the laboratory and has been a source implicated in adverse outcomes. This is also consistent with characteristics of our cohort, where the NIFT group had a higher BMI, which may indicate a different etiology for infertility, including polycystic ovary syndrome (PCOS). As adverse outcomes exist, some likely due to an underlying placental pathology, it also remains to be determined if adverse outcomes are due to the underlying genetics of infertility or the intrauterine environment throughout gestation, as differences in methylation status in placenta at delivery have been reported in pregnancies conceived spontaneously versus those conceived using ART (33–37).

Since race did play a potential role in our transcriptome study, we opted to look at the Caucasian and Caucasian-Asian cohort as this group had the most similar global transcriptomic profile and would limit variability based on race, since race has been reported to play a role in reproductive and birth outcomes (38, 39).

Although global transcriptomic profiles were not found to differ, differences in specific gene expression and their potential roles in placental development may provide insight into long term regulation of placental function, as we did find differences in fetal growth with birth weight, with a lower birth weight in the IVF group among our transcriptomic cohort (Table 1).

The one gene found to be differentially expressed between pregnancies conceived spontaneously and those conceived with infertility treatment, specifically with IVF was *CACNA1I*. *CACNA1I* encodes the pore-forming alpha subunit of a voltage-gated calcium channel (VGCC) belonging to the low voltage-activated, T-type calcium channel subfamily. VGCCs mediate calcium (Ca^{2+}) signaling upon depolarization of membrane potential created by the Ca^{2+} gradient, and activates a variety of downstream events, including muscle contraction, excitation of neurons, regulation of gene expression, and release of hormones or neurotransmitters (40–42). In the placenta, Ca^{2+} signaling has been reported to be primarily mediated by the high voltage-activated, L-type VGCCs and transient receptor potential (TRP) channels (43, 44).

While the role of T-type VGCC in placentation remains to be elucidated, the presence of other calcium channels including L-type VGCCs has been identified in syncytiotrophoblasts (43, 44) and Ca^{2+} plays a role in hormone secretion regulation, including human chorionic gonadotropin (hCG) and human placental lactogen, which is important in fetal growth and development (45–52). Calcium channel-mediated Ca^{2+} influx regulates vasoconstriction of uterine radial arteries through L- and T-type VGCCs (53). Remodeling of uterine vasculature is critical for development of a healthy placenta to ensure normal fetal development. Impaired vascular remodeling has been associated with spontaneous abortion, intrauterine growth restriction, preeclampsia, and lower birth weights (54–59). Thus, decreased

expression of *CACNA1I* in IVF pregnancies may be impacting regulation of Ca^{2+} homeostasis in the placenta and leading to impaired vascular remodeling and altered hormone secretion necessary for normal placentation and fetal development.

Among the genes significantly differentially expressed between NIFT versus IVF pregnancies are *SLC18A2*, *CCL21*, *FXYD2* and *PAEP*, which may be involved in regulation of immune responses and transport of signaling molecules at the maternal-fetal interface. *SLC18A2* encodes a vesicular monoamine transporter which acts to accumulate cytosolic monoamines, such as norepinephrine, serotonin, dopamine and histamine, into synaptic vesicles. It has been reported that histamine plays a role in decidualization, implantation, as well as immune and blood flow regulation (60–63). There are many transporter proteins expressed in the placenta which normally keep extracellular monoamine concentrations low (64, 65). During decidualization, however, the accumulated histamine in intracellular vesicles is released and stimulates initial decidual formation (66, 67), as monoamines are potent vasoactive agents. In fact, an elevated concentration of monoamines was reported in preeclamptic pregnancies (68), possibly through monoamine transporters. Thus, *SLC18A2* may be important in regulating monoamines necessary for regulation of uterine and placental blood flow at the maternal fetal interface.

CCL21 is a small cytokine belonging to the CC chemokine family and is a ligand for chemokine receptor 7 (CCR7). As their function is to recruit immune cells in response to inflammation or homeostatic conditions, cytokines play an active role at the maternal-fetal interface by modulating interactions between the decidua and trophoblasts through its selective recruitment of leukocytes (69–71). Trophoblasts have been shown to actively recruit immune cells through chemokine production and expression of different chemokine-receptor profiles. For example, it has been shown that CXCL12 secreted by human first trimester trophoblast cells stimulates the decidualization process through increased production of CXCR4 (72). In other cell types, CCL21/CCR7 pair has been shown to promote growth and metastasis in tumors by stimulating angiogenesis and lymphangiogenesis (73, 74). Thus, CCL21 may be acting as a chemokine promoting decidualization at the maternal-fetal interface by stimulating angiogenesis/lymphangiogenesis necessary for proper placentation and placental function throughout gestation as it remains expressed in term placental mesenchymal stem cells (75). *FXYD2* encodes the sodium/potassium (Na^+/K^+)-transporting ATPase subunit gamma of the FXYD family of transmembrane protein. In the placenta, Na^+/K^+ ATPases are found in both the microvillous and basal membranes of syncytiotrophoblast (76), and play an important role in transport of nutrients. Inhibition of Na^+/K^+ ATPase, impairs first trimester cytotrophoblast cell proliferation, migration, and invasion (77) and reductions in Na^+/K^+ ATPase activity were seen in syncytiotrophoblast plasma membranes from intrauterine growth restricted placentas (76). Thus, misregulation of *FXYD2* may affect function of Na^+/K^+ transporters impairing nutrient transport to the fetus and resulting in growth restricted phenotypes.

Glycodelin-A, the protein encoded by *PAEP* is a glycoprotein found abundantly in the endometrium, decidua, and amniotic fluid and has been identified as a marker of endometrial receptivity (78). It plays an important role in regulation of immune responses at the maternal-fetal interface to help prevent immune rejection of the fetus. Specifically,

glycodelin-A facilitates the type 1 T helper cell (Th1)-type 2 T helper cell (Th2) shift of T lymphocytes in the decidua during pregnancy to increase Th2 and decrease Th1, balancing activity and cytokine secretion by the different T lymphocyte populations. Additionally, glycodelin-A regulates cytokine production by natural killer cells and dendritic cells to induce a tolerogenic phenotype (78–80). Glycodelin-A has also been suggested to induce differentiation of cytotrophoblast, as evidenced by an increase in the extravillous trophoblast (EVT) lineage population and stimulation of hCG production (81, 82). Thus, altered expression of *PAEP* may lead to impaired immune regulation at the maternal-fetal interface as well as a disrupted trophoblast differentiation, resulting in placental dysfunction.

DNER encodes a transmembrane protein that binds Notch1 (83). In early pregnancy, the Notch signaling pathway functions through cell-cell interactions to regulate stem cell renewal and differentiation, decidualization, implantation, placentation, and angiogenesis (84). Notch1 protein expression is higher in the mid-secretory (receptive phase) endometrium of fertile women, compared to infertile women (85). Thus, *DNER* may be important for maternal embryonic/fetal communication leading to appropriate placentation and downregulation of *DNER* in IVF pregnancies may impact this communication. Further functional studies are necessary to understand differences at the maternal- embryonic/fetal interface.

Enrichment analysis of top canonical pathways suggests that differences in fertility are largely linked to differences in inflammatory pathways during the late first trimester. Specifically, the C-X-C motif chemokine ligand family (including *CXCL9, CXCL10, CXCL11*) associated with autoimmunity (e.g. Multiple Sclerosis) seems to be lower in IVF pregnancies, driving differences in the infertility group. These C-X-C motif cytokines are important in immune cell migration at the maternal-fetal interface and increase implantation success (86, 87). Further, in upstream analysis, multiple cytokine families were among the top upstream regulators that predicted transcript differences in spontaneous versus IVF pregnancies, but not spontaneous versus NIFT pregnancies. These results suggest that IVF pregnancies may have slightly altered regulation of cytokine signaling pathways in late first trimester, compared to other pregnancies, which may be altering normal placentation. By contrast, spontaneous versus NIFT pregnancies had more diverse top canonical pathways and upstream regulators, which may reflect different etiology of infertility between NIFT and IVF pregnancies.

Limitations in our study include the racial distribution of our study population as the majority of our cohort was Caucasian. Future studies looking at the impact of race on transcriptomic differences of placenta will be important. Additionally, as CVS is more commonly performed in women of advanced reproductive age, we were limited by advanced maternal age in our cohort. However, multiple studies, including our own have identified increased maternal age with infertility (10, 11, 15). Although maternal age in our infertility cohort was greater, it was only slightly increased which may not be clinically significant and the spontaneous conceptions of advanced maternal age minimize differences that may be due to maternal age. Another limitation was that chorionic villi consist of multiple cell types. Future studies looking at specific cell types within the chorionic villus tree will be

necessary to determine their functional activity in overall fetal outcomes and how they relate to the underlying infertility or treatments utilized.

Conclusions

In conclusion, this is the first and largest study to demonstrate that the transcriptomic profiles of first trimester placenta, during placentation, in pregnancies conceived with infertility using NIFT or IVF and spontaneous conceptions are similar, which is reassuring, that overall pregnancies conceived as a result of infertility treatments are similar to spontaneous conceptions. However, there is a select group of genes that are differentially expressed, with the greatest differences found in the NIFT cohort, suggesting that it may be the underlying infertility, which may account for differences in overall outcomes. Further studies are needed to look at the functional role of these genes in placental development and function.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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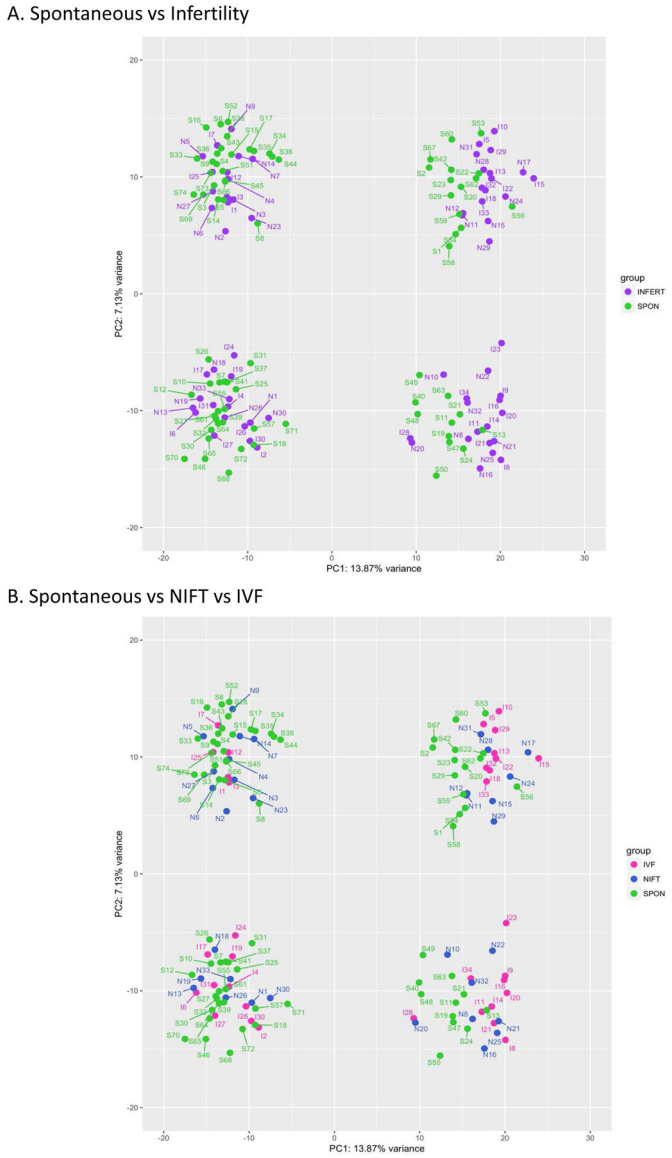

Figure 1.

Principal component analysis (PCA) plots of (A) spontaneous versus infertility pregnancies, (B) spontaneous versus NIFT versus IVF pregnancies. Samples clustered according to sample subset (shown on the PC1 axis) and fetal sex (shown on the PC2 axis).

(SPON: spontaneous, INFERT: infertility, Green: spontaneous, Purple: infertility, Blue: NIFT, Pink: IVF)

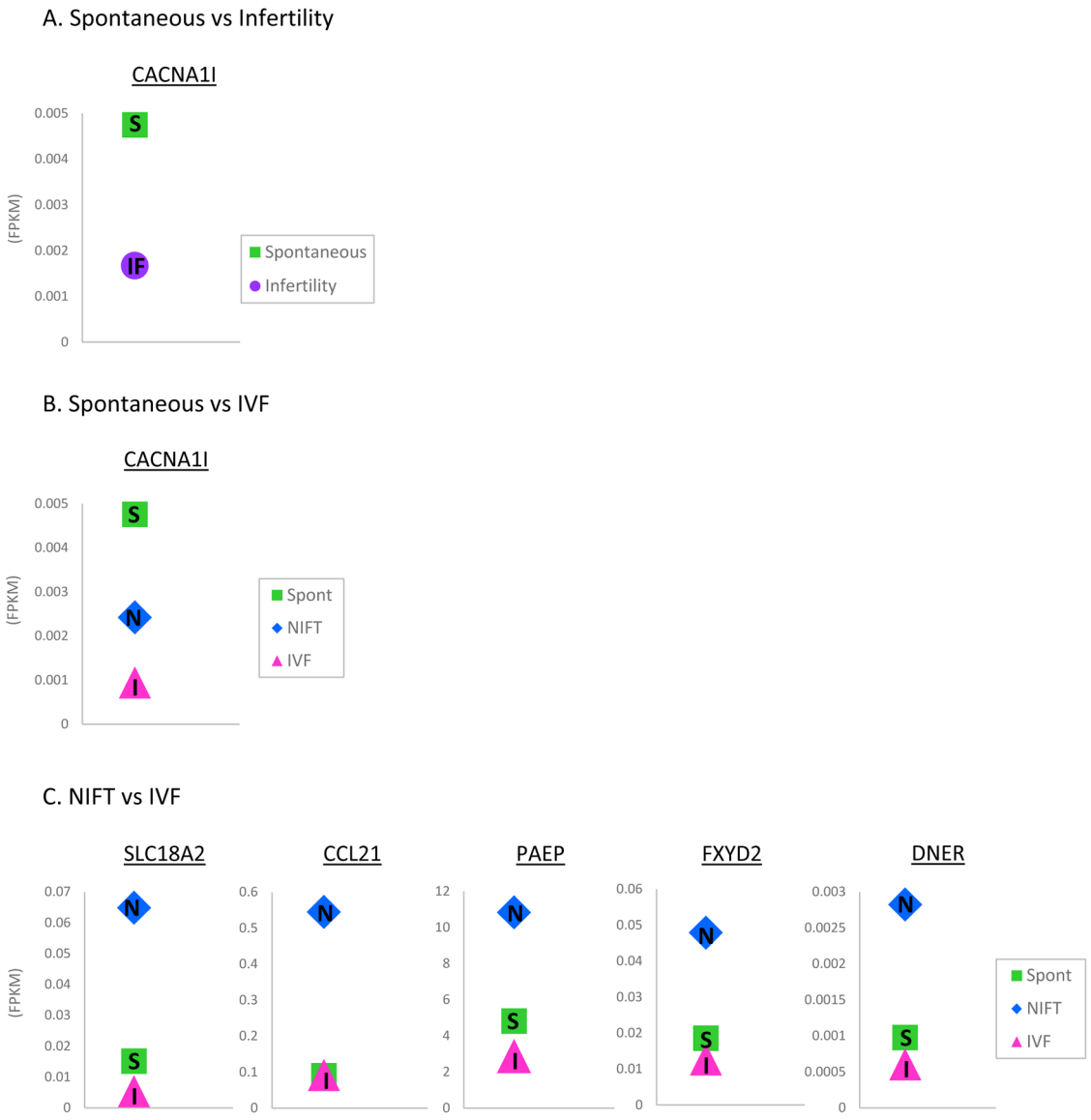

Figure 2.

Gene expression levels of differentially expressed genes in (A) *CACNA1I* is differentially expressed in spontaneous versus infertility pregnancies,(B) *CACNA1I* is differentially expressed in spontaneous versus IVF, and (C) *SLC18A2*, *CCL21*, *FXYD2*, *PAEP* and *DNER* are significantly upregulated in NIFT pregnancies compared to IVF pregnancies with spontaneous pregnancies segregating with IVF pregnancies.

(S: spontaneous, IF: infertility, N: NIFT, I: IVF, Green: spontaneous, Purple: infertility, Blue: NIFT, Pink: IVF, FPKM: fragments per kilobase of transcript per million mapped reads)

Table 1.

Baseline demographics and pregnancy outcomes for spontaneous versus infertility, and spontaneous versus NIFT versus IVF SMAART Transcriptome cohort.

	SPONT (N=74)	**INFERT (N=67)**	**P-value SPONT vs INFERT**	**NIFT (N=33)**	**IVF (N=34)**	**P-value SPONT vs NIFT vs IVF**	**P-value Differences among groups**
BASELINE DEMOGRAPHICS							
Maternal age (years)	39 ± 2.2	40 ± 3.0	0.2483	40 ± 2.7	40 ± 3.2	0.4985	
Paternal age (years)	41 ± 4.9	41 ± 6.9	0.6589	40 ± 6.3	43 ± 7.1	0.1092	
Maternal race			0.275			0.326	
Caucasian	71, 96%	60, 90%		30, 91%	30, 88%		
Asian	3, 4.1%	6, 9.0%		3, 9.1%	3, 8.8		
African American							
Biracial (Caucasian/Asian)	0	1, 1.5%		0	1, 2.9%		
Biracial (Other)							
Paternal race			0.308			0.546	
Sex of fetus			0.455			0.416	
Male	40, 54%	32, 48%		18, 55%	14, 41%		
Female	34, 46%	35, 52%		15, 45%	20, 59%		
Fetal race			0.130			0.181	
Caucasian	68, 92%	56, 84%		29, 88%	27, 79%		
Asian							
African American							
Biracial (Caucasian/Asian)	6, 8.1%	11, 16%		4, 12%	7, 21%		
Biracial (Other)							
Multiracial							
Maternal BMI	22 ± 3.2	24 ± 5.5	**0.0078**	25 ± 6.3	23 ± 4.5	**0.0108**	**0.009** (Spont vs NIFT)
Hypertension	2, 2.7%	2, 3.0%	0.92	1, 3.0%	1, 2.9%	0.995	
Diabetes	0	3, 4.5%	0.066	1, 3.0%	2, 5.9%	0.133	
Thyroid disease	16, 22%	14, 21%	0.916	7, 21%	7, 21%	0.993	
Other medical conditions	1, 1.4%	2, 3.0%	0.502	1, 3.0%	1, 2.9%	0.798	
Gestational age at CVS (days)	83 ± 6.1	82 ± 6.4	0.4572	82 ± 6.6	82 ± 6.4	0.7324	
Crown rump length (mm)	56 ± 11	53 ± 11	0.1701	54 ± 11	53 ± 12	0.3504	
PREGNANCY OUTCOMES							
Gestational age at delivery (days)	273 ± 13	270 ± 14	0.2004	272 ± 13	268 ± 15	0.1399	
Birthweight (grams)	3430 ± 590	3192 ± 623	**0.0317**	3294 ± 647	3082 ± 586	**0.04**	**0.035** (Spont vs IVF)
Mode of delivery						**0.007**	
Vaginal, Spontaneous	44, 64%	23, 37%		12, 39%	11, 34%		

	SPONT (N=74)	INFERT (N=67)	P-value SPONT vs INFERT	NIFT (N=33)	IVF (N=34)	P-value SPONT vs NIFT vs IVF	P-value Differences among groups
Cesarean Section	25, 36%	40, 63%	**0.002**	19, 61%	21, 66%		
Pregnancy complications	10, 14%	18, 27%	**0.047**	7, 21%	11, 32%	0.073	
Hypertension	3, 4.1%	4, 6.0%	0.601	1, 3.0%	3, 8.8%	0.481	
Diabetes	5, 6.8%	13, 19%	**0.025**	6, 18%	7, 21%	0.077	
Coagulation disorders	0	2, 3.0%	0.134	1, 3.0%	1, 2.9%	0.326	
Placenta previa	2, 2.7%	2, 3.0%	0.920	0	2, 5.9%	0.348	
Placental abruption	1, 1.4%	0	0.340	0	0	0.634	
Placenta other	0	0					

P-values < 0.05 are in bold. (SPONT: spontaneous, INFERT: infertility)

Table 2.

Differentially expressed genes in spontaneous versus infertility, spontaneous versus IVF, and NIFT versus IVF pregnancies.

Spontaneous vs Infertility			
SYMBOL	**DESCRIPTION**	**FC**	**P adj**
CACNA1I	Calcium voltage-gated channel subunit alphal	6.82	0.000779
Spontaneous vs IVF			
SYMBOL	**DESCRIPTION**	**FC**	**P adj**
CACNA1I	Calcium voltage-gated channel subunit alpha1	9.85	0.000568
NIFT vs IVF			
SYMBOL	**DESCRIPTION**	**FC**	**P adj**
SLC18A2	Solute carrier family 18 member A2	6.41	0.000283
CCL21	C-C motif chemokine ligand 21	6.02	0.0118
FXYD2	FXYD domain containing ion transport regulator 2	3.39	0.0235
PAEP	Progestagen associated endometrial protein	3.94	0.0137
DNER	Delta/notch like EGF repeat containing	3.73	0.0291

(FC: fold change, P adj: adjusted P-value)