How Infertility and Treatments Can Affect Human Placenta Function

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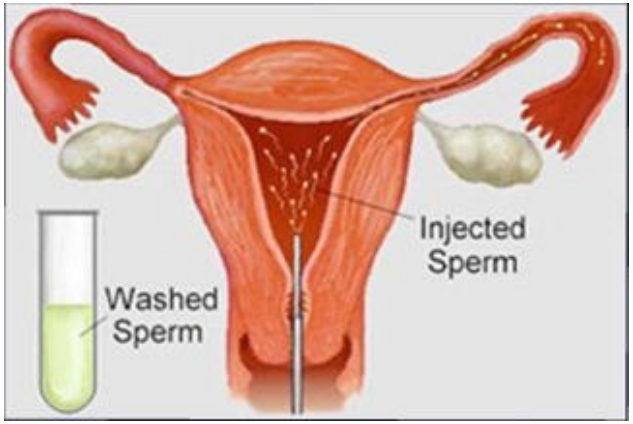
Disclosures

- *Ferring*
- *Natara*

Infertility And Treatment Options

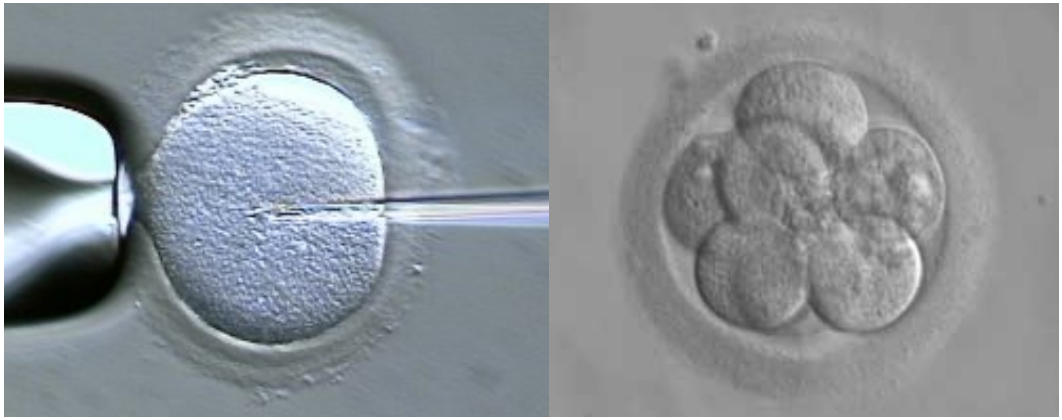
Infertility affects up to 15% of couples

Ovarian Stimulation/IUI



4.6% live births in US

In Vitro Fertilization



1.9% live births in US
8 million babies born worldwide

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Pisarska, et al. *J Clin Endocrinol Metab,* 2018
National Health Statistics Reports 2018
CDC, National Center for Health Statistics 2017

Outcomes based on fertility diagnosis

- *Significant difference in maternal age and race*
- *Infertility increases risk for cesarean section*
- *Conceptions from infertile couples deliver earlier*

	Infertile N=277	Fertile N=3016	P-value
Maternal Age, years	37.4±5.3	31.5±5.3	<0.0001
Maternal Race, n(%)			0.023
White	193 (69.9)	2066 (68.8)	
Black- or African-American	16 (15.8)	287 (9.6)	
Asian or Asian-American	49 (17.8)	391 (13.0)	
Other	18 (6.5)	260 (8.7)	
BMI, kg/m^2	23.3±4.6	23.0±4.6	0.3211
Mode of Conception, n(%)			<0.0001
IVF	136 (49.1)	4 (0.13)	
NIFT	73 (26.4)	4 (0.13)	
Presumed Spontaneous	68 (24.5)	3008 (99.7)	
Cesarean Delivery, n(%)	142 (51.8)	1078 (36.1)	<0.001
Gestational age, weeks	38.9±2.3	39.4±1.7	<0.0001
Birth weight, grams[c]	3268±634	3317±510	0.1378

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Wang, et al . J Matern Fetal Neonatal Med 2018

vAMA

- *ART were significantly older*
- *More likely to be white*
- *More likely to be nulliparous*
- *Significantly increased risk for cesarean delivery*
- *Risk of retained placenta was also significantly higher*

TABLE 1

Maternal characteristics and maternal and fetal outcomes in singleton gestations conceived either spontaneously or with assisted reproductive technology (ART).

Variable	Spontaneous (n = 193)	ART (n = 185)	*P* value
Maternal characteristic			
Age (y), mean	45.6 ± 0.1	47.0 ± 2.3	<.05
Race/ethnicity, % white	75.6	88.1	<.002
Parity	1.2 ± 1.8	0.4 ± 0.9	<.001
Maternal outcome			
Postpartum hemorrhage, %	3.1	5.9	NS
Estimated blood loss (mL)			
Vaginal delivery	303 ± 104	324 ± 116	NS
Cesarean delivery	730 ± 284	713 ± 137	NS
Retained placenta, %	0	2.7	<.02
Transfusion, %	2.1	1.1	NS
Hysterectomy, %	0	0.5	NS
Rate of ICU admission, %	0	1.1	NS
Length of stay (d), mean	3.2 ± 2.2	4.2 ± 3.9	<.01
Total CD, %	49.7	75.1	<.001
Primary CD	35.3	71.3	
Repeat CD	22.2	13.5	
Fetal outcome			
Gestational age, wk	38.9 ± 2.4	38.9 ± 2.4	NS
Birth weight, g	3,318 ± 527	3,284 ± 567	NS
NICU admission rate, %	1.5	4.3	NS
Apgar score at 5 min	8.8 ± 1	8.9 ± 0.7	NS

Note: CD = cesarean delivery; ICU = intensive care unit; NICU = neonatal intensive care unit; NS = not significant.

Jackson. Pregnancy in very advanced maternal age. Fertil Steril 2015.

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Jackson, et al Fertil Steril 2015;103:76-80.

Risks associated with infertility and fertility treatments

	spontaneous	NIFT	IVF
gestational diabetes		↑	↑
pregnancy induced hypertension		↑	↑
placenta previa			↑
placental abruption		↑	↑
postpartum hemorrhage			↑
preterm birth			↑
Low birth weight/SGA		↑	↑
perinatal mortality		↑	↑

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Shevell T, 2005 Obstet Gynecol 106:1039-1045
Qin J, 2016 Fertil and Steril 2016
Pisarska M, JCEM 2018

Significant maternal morbidity

Table 2. Rates of Most Commonly Reported and Statistically Significant Severe Maternal Morbidity Indicators and Overall Rate of Any Indicator During Delivery Hospitalizations or Postpartum Readmissions Per 10,000 Deliveries by Assisted Reproductive Technology Status, 2008–2012

	Singleton Pregnancies			Multiple Pregnancies		
Indicator	Non-ART	ART	*P**	Non-ART	ART	*P**
Blood transfusion	36	77	<.001	215	200	.567
Disseminated intravascular coagulation	20	46	<.001	68	98	.042
Mechanical ventilation	18	33	.001	105	143	.034
Adult respiratory distress syndrome	12	21	.009	49	48	1
Eclampsia	11	13	.656	34	41	.488
Heart failure during procedure or surgery	11	23	.001	26	25	1
Hysterectomy	9	27	<.001	38	34	.892
Sepsis	7	15	.004	22	32	.227
Acute renal failure	6	18	<.001	30	32	.881
Puerperal cerebrovascular disorders	6	9	.324	19	18	1
Operations on heart and pericardium	6	12	.041	21	23	.720
Internal injuries of thorax, abdomen, and pelvis	3	14	<.001	10	25	.018
Shock	4	14	<.001	22	16	.585
Overall	126	273	<.001	539	604	.089

ART, assisted reproductive technology.
Data are n unless otherwise specified.
* Holm-Bonferroni corrected $P \leq .001$ denotes statistical significance of Pearson χ^2 and Fisher exact tests.

Martin AS, Obstet Gynecol 2016;127:59-66

Significant Maternal Morbidity (SMM)

- **Using Gold Standard guidelines true SMM cases (Complications)**
  - hemorrhage
  - hypertension/neurologic
  - renal, sepsis
  - pulmonary, cardiac ICU/invasive monitoring
  - surgical, bladder, and bowel
  - Anesthesia
- **Higher rate of women utilizing fertility treatment that has significant maternal morbidity**
-

TABLE 1

Baseline characteristics of the maternal cohort.

Characteristic	SMM (n = 69)	No SMM (n = 6,474)	*P* value
Maternal age (y), n (SD)	34.0 (6.7)	32.9 (5.30)	.18
Maternal race			.001
White	36 (52.2)	4,541 (70.5)	
Black	14 (20.3)	590 (9.2)	
Asian	14 (20.3)	798 (12.4)	
Other	5 (7.3)	512 (8.0)	
Body mass index (kg/m^2)			.50
18.5–24.9	9 (13.6)	1,220 (18.9)	
25–29.9	29 (43.9)	3,021 (46.8)	
≥30	28 (42.4)	2,012 (34.3)	
Multifetal pregnancy	7 (10.1)	159 (2.5)	< .001
Mode of conception			.004
IVF	7 (10.1)	239 (3.7)	
NIFT	3 (4.4)	106 (1.6)	
Spontaneous	59 (85.5)	6,129 (94.7)	
Preterm delivery (<37 wk)	25 (36.8)	470 (7.4)	< .001
Cesarean delivery	55 (79.7)	2,338 (36.1)	< .001
Health insurance			< .001
Government	20 (29)	831 (13)	
Private	49 (71)	5,583 (87)	
Comorbidities			
Coronary heart disease	5 (7)	26 (0.4)	< .001
Diabetes mellitus	10 (15)	455 (7)	0.03
Hypertension	3 (4)	57 (1)	0.03

Note: Data presented as n (%), unless stated otherwise. IVF = in vitro fertilization; NIFT = non-IVF fertility treatment; SMM = severe maternal morbidity.

Wang. Fertility treatment and SMM. Fertil Steril 2016.

Wang et al. Fertil and Steril 2016

Infertility Diagnosis and Maternal Morbidity

- Insurance Claims Database
- *Fertile n=525,695*
- *Infertile n=19,658*
- ***Any severe maternal morbidity including the morbidities noted were associated with the diagnosis of infertility independent of treatment***

TABLE 3
Risk of severe maternal morbidity by fertility group[a]

	AOR (95% CI)			
	Infertile			
	Treatment vs fertile	Diagnosis vs fertile	Testing vs fertile	All infertile vs fertile
Severe maternal morbidity indicator				
Any severe maternal morbidity indicator	1.24 (1.12–1.37)	1.22 (1.13–1.33)	1.09 (0.81–1.45)	1.22 (1.14–1.31)
Acute myocardial infarction	1.68 (0.52–5.46)	0.90 (0.22–3.69)	[b]	1.33 (0.52–3.36)
Acute renal failure	1.03 (0.53–2.02)	0.86 (0.47–1.57)	[b]	0.84 (0.51–1.38)
Acute respiratory distress	1.57 (1.03–2.38)	1.14 (0.76–1.71)	[b]	1.26 (0.93–1.70)
Amniotic fluid embolism	1.61 (0.50–5.18)	1.10 (0.35–3.49)	[b]	1.31 (0.57–3.02)
Aneurysm	[b]	[b]	[b]	[b]
Cardiac arrest or ventricular fibrillation	1.22 (0.29–5.04)	2.68 (1.16–6.20)	[b]	1.94 (0.88–4.31)
Disseminated intravascular coagulation	1.67 (1.33–2.09)	1.34 (1.08–1.66)	1.57 (0.81–3.04)	1.48 (1.26–1.73)
Eclampsia	1.49 (1.02–2.17)	1.30 (0.95–1.79)	0.41 (0.06–2.91)	1.37 (1.05–1.79)
Heart failure during procedure or surgery	1.27 (0.85–1.91)	1.75 (1.3–2.36)	0.89 (0.22–3.57)	1.54 (1.21–1.97)
Internal injuries of the thorax, abdomen, or pelvis	1.61 (0.92–2.84)	1.52 (0.95–2.45)	0.99 (0.14–7.08)	1.77 (1.20–2.61)
Intracranial injuries	1.27 (0.31–5.28)	2.64 (1.14–6.10)	[b]	2.05 (0.97–4.32)
Puerperal cardiovascular disorders	1.05 (0.77–1.43)	1.41 (1.13–1.75)	1.65 (0.81–3.35)	0.94 (0.66–1.33)
Pulmonary edema	1.85 (1.09–3.14)	2.05 (1.36–3.08)	[b]	2.18 (1.54–3.10)
Severe anesthesia complications	0.33 (0.08–1.35)	0.85 (0.42–1.71)	[b]	1.13 (0.49–2.60)
Sepsis	1.04 (0.58–1.85)	0.70 (0.40–1.21)	1.37 (0.34–5.51)	0.90 (0.59–1.36)
Shock	1.76 (1.02–3.05)	1.06 (0.58–1.93)	[b]	1.14 (0.72–1.80)
Sickle cell anemia with crisis	[b]	[b]	[b]	[b]
Thrombotic embolism	1.35 (0.86–2.13)	1.77 (1.27–2.49)	1.21 (0.30–4.88)	1.58 (1.14–2.17)
Blood transfusion	1.69 (1.39–2.07)	1.30 (1.08–1.56)	1.44 (0.79–2.62)	1.50 (1.30–1.72)
Cardiology monitoring	1.01 (0.87–1.18)	1.14 (1.02–1.27)	0.83 (0.53–1.29)	1.09 (0.997–1.20)
Conversion of cardiac rhythm	0.72 (0.10–5.29)	0.95 (0.23–3.88)	[b]	0.83 (0.26–2.68)
Hysterectomy	1.61 (1.03–2.52)	1.10 (0.69–1.77)	1.53 (0.38–6.16)	1.35 (0.97–1.88)
Operations on the heart and pericardium	1.44 (0.86–2.39)	1.09 (0.67–1.77)	2.23 (0.72–6.96)	1.12 (0.77–1.64)
Temporary tracheostomy	[b]	[b]	[b]	[b]
Ventilation	0.95 (0.61–1.47)	1.08 (0.78–1.51)	[b]	0.91 (0.69–1.20)
Intubation	0.92 (0.29–2.92)	0.98 (0.40–2.39)	[b]	0.84 (0.39–1.80)

AOR, adjusted odds ratio; *CI*, confidence interval.

[a] A generalized estimating equation (GEE) model was used to estimate the odds ratios of the diseases between infertile and control groups, adjusted for maternal age, year of delivery, nulliparity, delivery mode, preterm birth, obesity, smoking, hypertension, diabetes, number of prenatal visits, race and ethnicity, and education, accounting for women who had more than 1 delivery of a singleton during the database enrollment period; [b] Calculation of AOR and 95% CI was not possible because of small numbers.

Murugappan et al. Maternal morbidity among infertile women. Am J Obstet Gynecol 2020.

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Murugappan, et al, AJOG 2020

Preterm, late preterm, early term, and term deliveries between infertile and fertile women

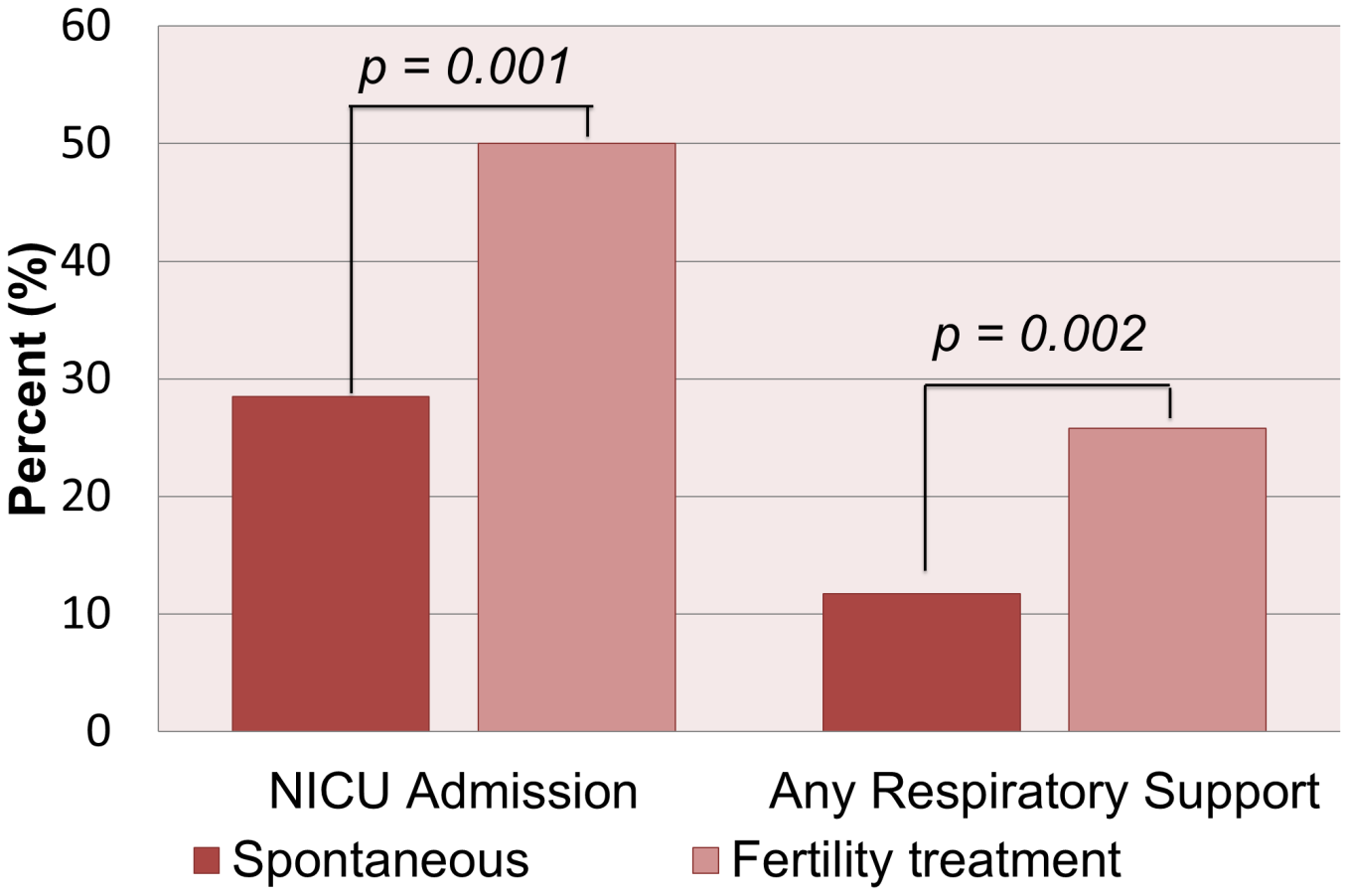
	Infertile N=277	Fertile N=3016	P value
<34 weeks	8 (2.9)	39 (1.3)	0.032[1]
34-36 6/7 weeks	23 (8.3)	130 (4.3)	0.003[2]
37-38 6/7 weeks	58 (20.9)	536 (17.8)	0.19[3]
≥39 weeks	188 (67.9)	2311(76.6)	0.001[4]

Adjusted for maternal age and race

Wang, et al . J Matern Fetal Neonatal Med 2018

Late preterm infants (34 0/7 to 36 6/7 weeks)

Figure 1: NICU Admission and Respiratory Outcomes



continuous positive airway pressure [CPAP] intermittent mechanical ventilation [IMV])

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Wang, Sundheimer et al. J Pediatrics 2017

Table 4. Odds Ratio for Any Birth Defects According to Type of Assisted Conception and Multiplicity.*

Type of Assisted Conception	Singleton Births		
	Defect	Unadjusted Odds Ratio	Adjusted Odds Ratio†
	no. of births with defect/ total no. of births		
Any	361/4333	1.45 (1.30–1.63)	1.28 (1.14–1.43)
IVF			
Fresh- or frozen-embryo cycles	105/1484	1.25 (1.02–1.52)	1.06 (0.87–1.30)
Fresh-embryo cycles	71/1005	1.25 (0.98–1.59)	1.05 (0.82–1.35)
Frozen-embryo cycles	34/479	1.24 (0.88–1.76)	1.08 (0.76–1.53)
ICSI			
Fresh- or frozen-embryo cycles	91/939	1.72 (1.38–2.15)	1.55 (1.24–1.94)
Fresh-embryo cycles	76/713	1.95 (1.53–2.48)	1.73 (1.35–2.21)
Frozen-embryo cycles	15/226	1.17 (0.70–1.97)	1.10 (0.65–1.85)
GIFT	34/319	1.98 (1.40–2.80)	1.73 (1.21–2.47)
Intrauterine insemination	54/580	1.67 (1.25–2.23)	1.46 (1.09–1.95)
Donor insemination	36/428	1.51 (1.08–2.11)	1.37 (0.98–1.92)
Ovulation induction	19/306	1.08 (0.68–1.74)	0.99 (0.62–1.59)
Clomiphene citrate at home	7/36	3.87 (1.58–9.51)	3.19 (1.32–7.69)
Other§	15/241	1.07 (0.63–1.82)	0.96 (0.56–1.63)
Spontaneous conception after previous birth from assisted reproductive technology	96/1306	1.27 (1.02–1.59)	1.26 (1.01–1.57)
Infertile but no history of treatment with assisted reproductive technology	52/600	1.54 (1.15–2.05)	1.37 (1.02–1.83)
No use of assisted reproductive technology and fertile	16,841/293,314	1.00	1.00

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Davies, et al, NEJM 2012; 366:1803-13

Risks of Birth Defects

Table III Risk of birth defects among singletons by maternal characteristics and mode of conception.*

		Major birth defect**		Blastogenesis		Cardiovascular		Musculoskeletal		Genitourinary-male		Chromosomal		Any birth defect	
		AOR	95% CI	AOR	95% CI	AOR	95% CI	AOR	95% CI	AOR	95% CI	AOR	95% CI	AOR	95% CI
Group***	Naturally conceived	1.00	Reference	1.00	Reference	1.00	Reference	1.00	Reference	1.00	Reference	1.00	Reference	1.00	Reference
	OI/IUI conceived	1.16	0.97, 1.38	1.11	0.66, 1.85	0.96	0.74, 1.24	1.29	0.86, 1.94	1.25	0.90, 1.73	1.00	0.60, 1.68	1.12	0.99, 1.26
	ART siblings	1.08	0.98, 1.19	1.19	0.90, 1.58	1.10	0.96, 1.26	**1.32**	**1.04, 1.67**	0.96	0.78, 1.19	0.94	0.69, 1.27	**1.15**	**1.08, 1.23**
	ART-auto-fresh, no ICSI	**1.18**	**1.05, 1.32**	0.99	0.69, 1.42	**1.20**	**1.03, 1.40**	1.19	0.89, 1.57	1.11	0.88, 1.41	0.65	0.44, 0.95	**1.18**	**1.09, 1.27**
	ART-auto-fresh, yes ICSI-no MF	**1.30**	**1.16, 1.45**	**1.49**	**1.08, 2.05**	**1.28**	**1.10, 1.48**	**1.34**	**1.01, 1.78**	1.09	0.85, 1.39	0.89	0.63, 1.26	**1.22**	**1.13, 1.32**
	ART-auto-fresh, yes ICSI-yes MF	**1.42**	**1.28, 1.57**	**1.56**	**1.17, 2.08**	**1.45**	**1.27, 1.66**	1.25	0.96, 1.64	**1.33**	**1.08, 1.65**	0.93	0.66, 1.33	**1.38**	**1.29, 1.48**
Maternal	18–29	1.00	Reference	1.00	Reference	1.00	Reference	1.00	Reference	1.00	Reference	1.00	Reference	1.00	Reference
Age (years)	30–34	**1.09**	**1.05, 1.12**	0.92	0.83, 1.02	**1.17**	**1.11, 1.23**	0.97	0.89, 1.06	1.08	1.00, 1.17	**1.76**	**1.52, 2.03**	**1.07**	**1.05, 1.10**
	35–37	**1.11**	**1.06, 1.16**	0.83	0.72, 0.96	**1.34**	**1.26, 1.43**	0.91	0.81, 1.04	1.11	1.00, 1.23	**3.46**	**2.95, 4.05**	**1.13**	**1.10, 1.17**
	38–40	**1.10**	**1.03, 1.17**	0.96	0.80, 1.14	**1.52**	**1.40, 1.64**	0.97	0.83, 1.14	1.03	0.90, 1.18	**6.79**	**5.800, 7.96**	**1.23**	**1.18, 1.28**
	41–43	**1.13**	**1.03, 1.24**	1.10	0.85, 1.43	**1.77**	**1.59, 1.97**	1.12	0.89, 1.42	1.19	0.98, 1.45	**15.4**	**12.99, 18.25**	**1.42**	**1.34, 1.51**
	≥44	**1.30**	**1.07, 1.59**	**1.80**	**1.12, 2.88**	**2.58**	**2.11, 3.16**	1.42	0.89, 2.26	1.32	0.87, 2.00	**28.7**	**22.47, 36.67**	**1.68**	**1.49, 1.90**
BMI (kg/m^2)	12–24	1.00	Reference	1.00	Reference	1.00	Reference	1.00	Reference	1.00	Reference	1.00	Reference	1.00	Reference
	25–29	1.01	0.97, 1.06	1.00	0.88, 1.14	1.03	0.96, 1.09	1.04	0.93, 1.17	0.99	0.89, 1.09	1.10	0.94, 1.30	1.00	0.97, 1.04
	30–59	**1.18**	**1.12, 1.24**	1.10	0.96, 1.26	**1.23**	**1.16, 1.31**	**1.25**	**1.11, 1.41**	0.96	0.86, 1.08	1.09	0.92, 1.29	**1.13**	**1.10, 1.17**
Diabetes	None	1.00	Reference	1.00	Reference	1.00	Reference	1.00	Reference	1.00	Reference	1.00	Reference	1.00	Reference
	Pre- or gestational	**1.34**	**1.27, 1.41**	**1.46**	**1.25, 1.69**	**1.47**	**1.37, 1.57**	1.05	0.90, 1.22	**1.14**	**1.01, 1.30**	1.11	0.93, 1.32	**1.26**	**1.21, 1.30**
Hypertension	None	1.00	Reference	1.00	Reference	1.00	Reference	1.00	Reference	1.00	Reference	1.00	Reference	1.00	Reference
	Pre- or gestational	**1.43**	**1.36, 1.51**	1.13	0.96, 1.33	**1.49**	**1.40, 1.60**	1.04	0.90, 1.21	**1.71**	**1.54, 1.91**	1.00	0.83, 1.21	**1.34**	**1.29, 1.39**
Infant sex	Female	1.00	Reference	1.00	Reference	1.00	Reference	1.00	Reference	–	–	1.00	Reference	1.00	Reference
	Male	**1.53**	**1.49, 1.58**	**1.17**	**1.08, 1.27**	0.96	0.92, 1.00	**1.44**	**1.34, 1.54**	–	–	1.01	0.92, 1.11	**1.55**	**1.52, 1.58**

*Models adjusted for all factors listed above, as well as maternal race and ethnicity, education, parity, and State and year of birth. ART births limited to autologous-fresh with partner ejaculated sperm. Bolded values are significantly increased.
**Major defects are limited to nonchromosomal only.
***Group (n, children): naturally conceived (1 066 652); OI/IUI conceived (6899); non-ART siblings (22 821); ART-auto-fresh, no ICSI (all infertility diagnoses, no ICSI: 16 433); ART-auto-fresh, yes ICSI-no MF (yes ICSI, no male factor diagnosis: 14 071); ART-auto-fresh, yes ICSI-yes MF (yes ICSI, yes male factor diagnosis: 16 629). AOR, adjusted odds ratio.
Major birth defects as defined by the National Birth Defects Prevention Network (NBDPN) (see Supplementary Table SI).
Any birth defect is any ICD-9 code with the first 3 digits 740–759, and any ICD-10 code inclusive of Q00.0–07.9, 10–18.9, 20–28.9, 30–45.9, 50–56.4, 60–87.89 and 89–99.9.

Cedars Sinai

Luke B, Brown ML, Wantman E… Hum Reprod 2021

Risk Assessment

- *Despite increased risk of adverse outcomes, the overall incidence and relative risk of these outcomes is low.*

Table 1. Risks Associated With In Vitro Fertilization–Conceived Pregnancies Compared With Naturally Conceived Counterparts—Singleton, Twin, and Nonstratified Gestations

Risk	Absolute Risk
Among singleton pregnancies	
Preterm delivery[50]	Half day earlier IVF–ICSI vs SC 9.7% IVF–ICSI vs 7.9% SC
Low birth weight delivery[50]	33 g less IVF–ICSI vs SC 6.8% IVF–ICSI vs 4.9% SC
Severe maternal morbidity (blood transfusion most common)[63]	273/10,000 IVF–ICSI vs 126/10,000 SC
Among twin pregnancies	
Monozygotic twins[31,32]	1.2–2.5% IVF–ICSI vs 0.4% SC
Preterm delivery[26–28]	Comparable
Low birth weight delivery[26–28]	Comparable
Not stratified	
DNA methylation[71,72]	Comparable
Imprinting disorder[71,72]	0.15% IVF–ICSI vs 0.02% SC
Any cardiac defect including ASD, VSD[70]	1.30% IVF–ICSI vs 0.68% SC

IVF, in vitro fertilization; ICSI, intracytoplasmic sperm injection; SC, spontaneous conception; ASD, atrial septal defect; VSD, ventricular septal defect.

Kawwass, et al Obstet Gynecol 2018;132:763–72

Infertility is the contributor to outcomes associated with placentation

- *Maternal morbidity is associated with diagnosis of infertility regardless of treatment*
- *Adverse outcomes are associated with both IVF and NIFT*
- *Congenital anomalies are associated with underlying infertility*
- *Time to pregnancy increases risk of congenital malformations*

- ***Models to study the effect of IVF need to include an infertile cohort***

- *Outcomes are related to placentation defects*
  - *Mother - diabetes, hypertension, preeclampsia, placenta previa and accreta, retained placenta and abruption as well as SMM*
  - *Child- prematurity, growth restriction, and birth defects*

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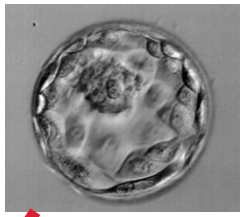
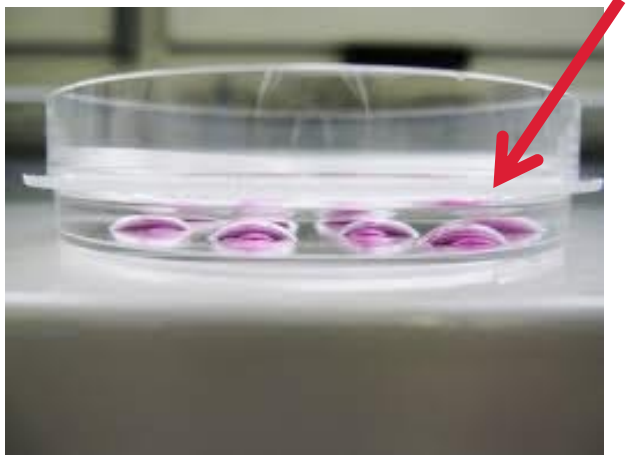
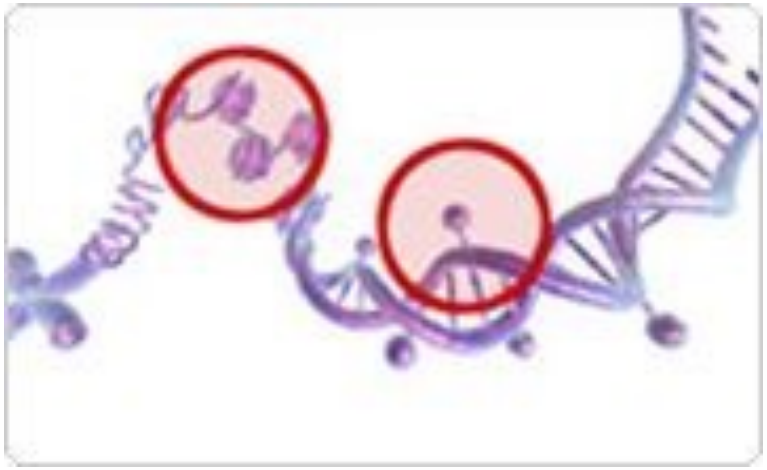
Murugappan AJOG 2020; Shevell T 2005 Obstet Gynecol ; Davies NEJM 2012; Zhu JL Br Med J 2006; Qin J Fertil and Steril 2016; Jackson Fertil Steril 2015; Wang Fertil and Steril 2016; Pisarska M, JCEM 2018

Are the adverse outcomes associated with ART due to the in vitro fertilization process, the treatments or the inherent infertility we are trying to overcome?





?



Prevalence of ART in patients with BWS

TABLE 3

Prevalence of ART in patients with BWS.

Reference no.	Type of study	No. of BWS cases	Prevalence of ART in BWS cohort (cases)	Prevalence of ART in reference population	Type of ART	Association between BWS and ART
62[a]	Case series	65[b]	4.6% (3[b])	0.8%	IVF/ICSI	Suggestive
63	Case series	149	4% (6[c])	1.2%	3 IVF/3 ICSI	Suggestive
64	Case series	149	4% (6[c])	1.3%	4 IVF/2 ICSI	Suggestive
65	Case-control	37	10.81%[d] (4)	0.67%[d]	3 IVF/1 ICSI	Suggestive
66[a]	Case-series	341	5.6%(19)	NA	5 IVF/5 ICSI[e]	NA
67	Survey	209	2.9% (6[c])	0.8%	1 IVF/5 ICSI	Suggestive
71	Survey	71	5.6% (4)	0.92%	IVF/ICSI	Suggestive

[a] Data from the same BWS registry (NCI BWS registry and Washington University BWS registry).
[b] Only BWS cohorts beginning in 2001 were used to calculate prevalence.
[c] The frequency of children born after ART in BWS cohort was significantly higher than the expected ART cases based on the ART prevalence in the general population.
[d] Fisher's exact test, two-sided, $P=0.006$.
[e] Data on type of ART obtained from 12 patients (two patients had only ovarian stimulation with intrauterine insemination).

Manipalviratn. Imprinting disorders and ART. Fertil Steril 2009.

Cohort studies of children

TABLE 4

Number of cases of BWS in a cohort study of children conceived naturally and after ART.

Reference no.	No. of cases of BWS in children born after ART	Number of children born after ART	No. of cases BWS in children conceived naturally	Number of children conceived naturally
68	0	6,052	0	442,349
69	0	16,280	NA	2,039,943
70	1	1,524	NA	NA

NA = Not available.

Manipalviratn. Imprinting disorders and ART. Fertil Steril 2009.

Adverse pregnancy outcomes: methylation

Differences in DNA methylation and gene expression in term placenta from children conceived in vitro versus in vivo

Term placenta and cord blood and may not reflect the early changes that occur as a direct result of the IVF conditions in ART.

Term placenta may reflect changes in the intrauterine environment, which has been associated with an altered fetal epigenome leading to altered gene expression.

Placental Weight, Fetal Weight and Fetal Weight to Placenta Weight Ratio

Figure 1: Placental and Birth Weight Parameters

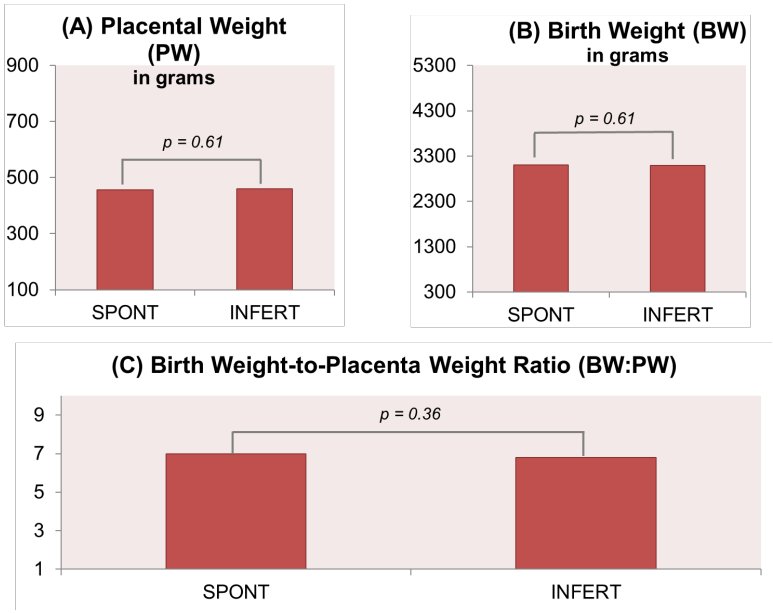


Figure 2: Placental Characteristics

	SPONT N=1333	INFERT (NIFT+IVF) N=110	p-value
Preeclampsia findings, n (%)	111 (8.3%)	9 (8.2%)	0.96
Chorioamnionitis, n (%)	392 (29.4%)	35 (31.8%)	0.59
Accreta, n (%)	16 (1.2%)	4 (3.6%)	0.036
Placental Shape, n (%)			0.23
Discoid	737 (55.3%)	58 (52.7%)	
Ellipsoid	202 (15.2%)	15 (13.6%)	
Ovoid	200 (15%)	14 (12.7%)	
Circular/Round	63 (4.7%)	5 (4.5%)	
Other	50 (3.8%)	9 (8.2%)	

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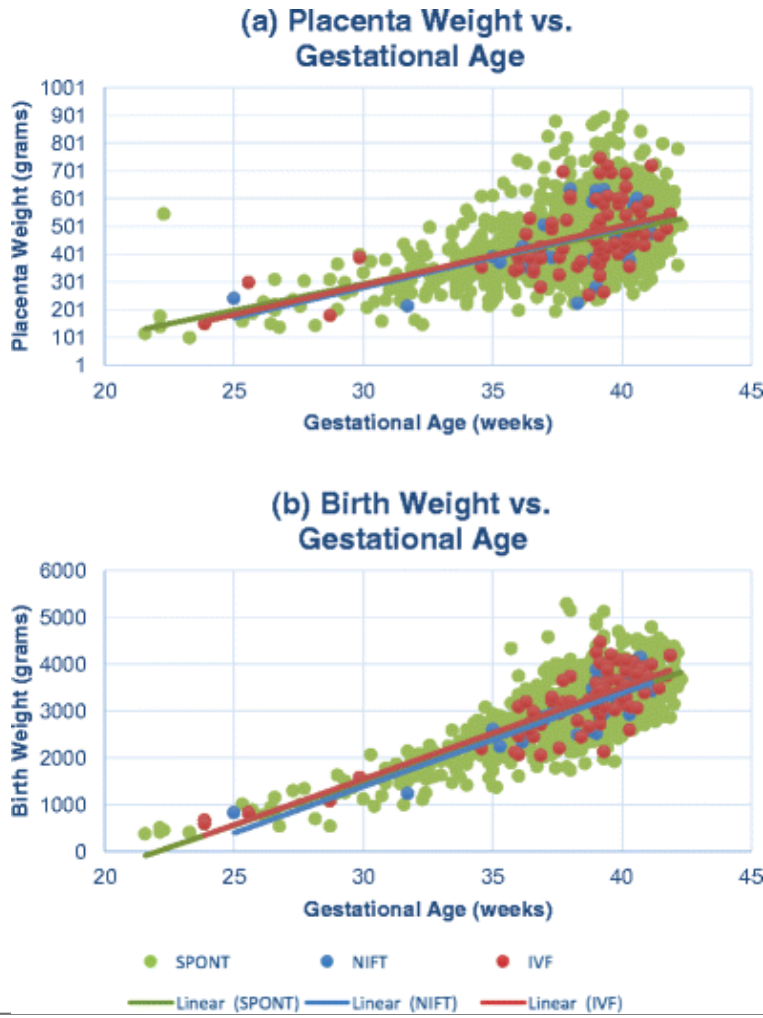
Sundheimer, et al. J Assist Reprod Genet. 2018

Placental Weight, Fetal Size and Fetal Size to Placenta Weight Ratio

- *Linear regression demonstrates that regardless of gestational age, the placenta weight, fetal weight and fetal size to placenta weight do not vary by mode of conception.*



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Sundheimer, et al. J Assist Reprod Genet. 2018

Placentation

Abnormal placentation is associated with adverse pregnancy outcomes - preeclampsia, PIH, gestational diabetes, previa, abruption, placental retention

Pregnancies conceived with infertility and treatments are at risk of:

abnormal placentation

abnormal placental morphology and cord insertion

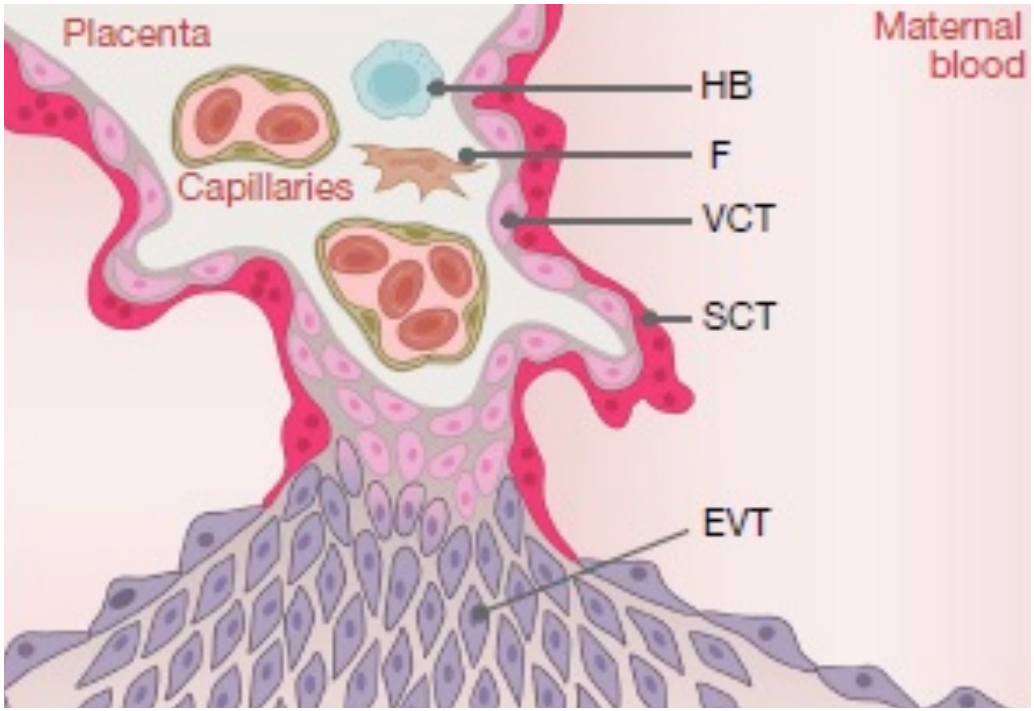
abnormal protein profiles

increased metabolism and clearance of steroids by the placenta

Small for gestational age babies

Gavriil P, et al 1993 Pediatr Pathol 13:453-462
Zhang Y, et al 2008 Proteomics 8:4344-4356
Collier AC, et al 2009 J Steroid Biochem Mol Biol 116:21-28
Delle Piane L,et al 2008 Reproductive Sciences 15:81A-81A

Placentation



The placenta is made up of important cell types

Villous cytotrophoblasts (VCT): undifferentiated precursor cells

Extravillous trophoblasts (EVT): Invade decidua and maternal blood vessels

Syncitiotrophoblasts (SCT): Facilitate nutrient exchange and produce hormones

Endothelial cells: Line fetal blood vessels

Immune cells: stromal fibroblasts (F), dendritic cells, macrophages or Hofbauer cells (HB)

Vento-Tormo et al (2019), *Nature*

Model of Placentation

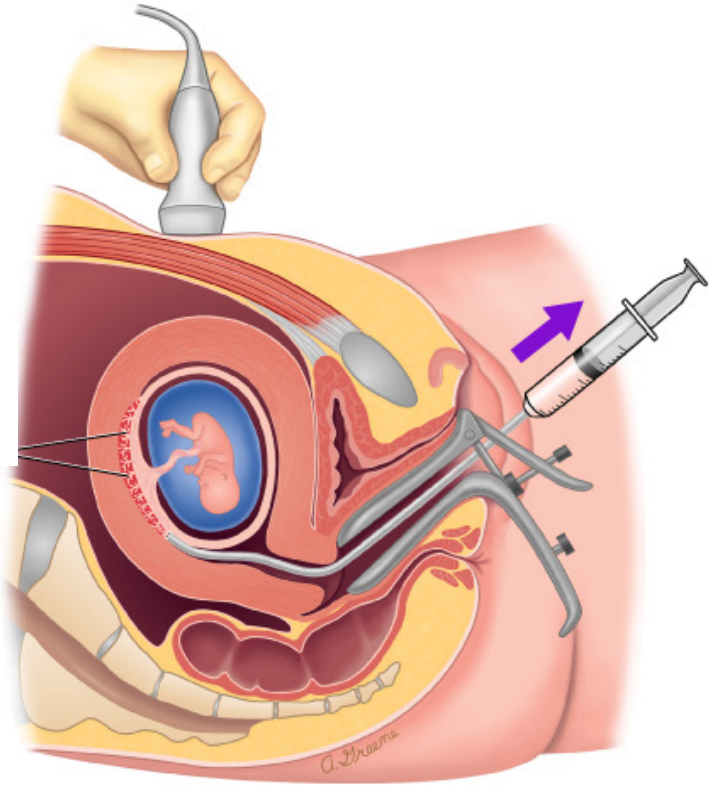
- **1st trimester placenta tissue**

Chorionic villus sampling

Prenatal diagnostic test at 11-13 weeks

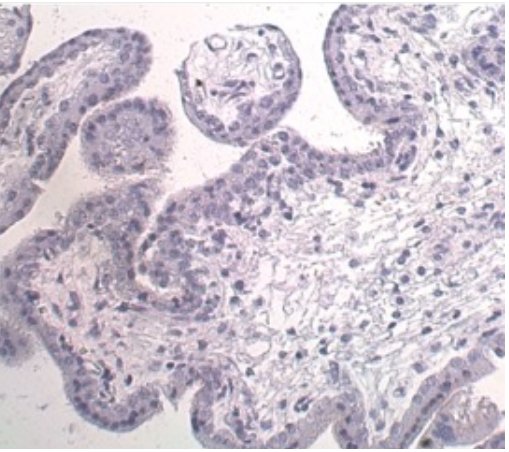
Ongoing pregnancies that deliver at term

Chorionic villi



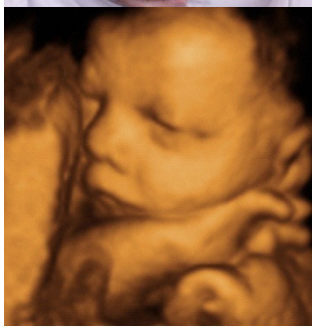
Are the outcomes associated with ART due to the in vitro fertilization process, the treatments or the inherent infertility we are trying to overcome?

Cells from early placentation

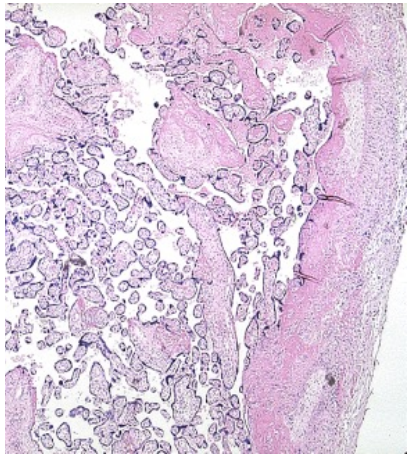


Earliest time point in ongoing pregnancy

Uterine environment



Cells from delivery



Outcomes

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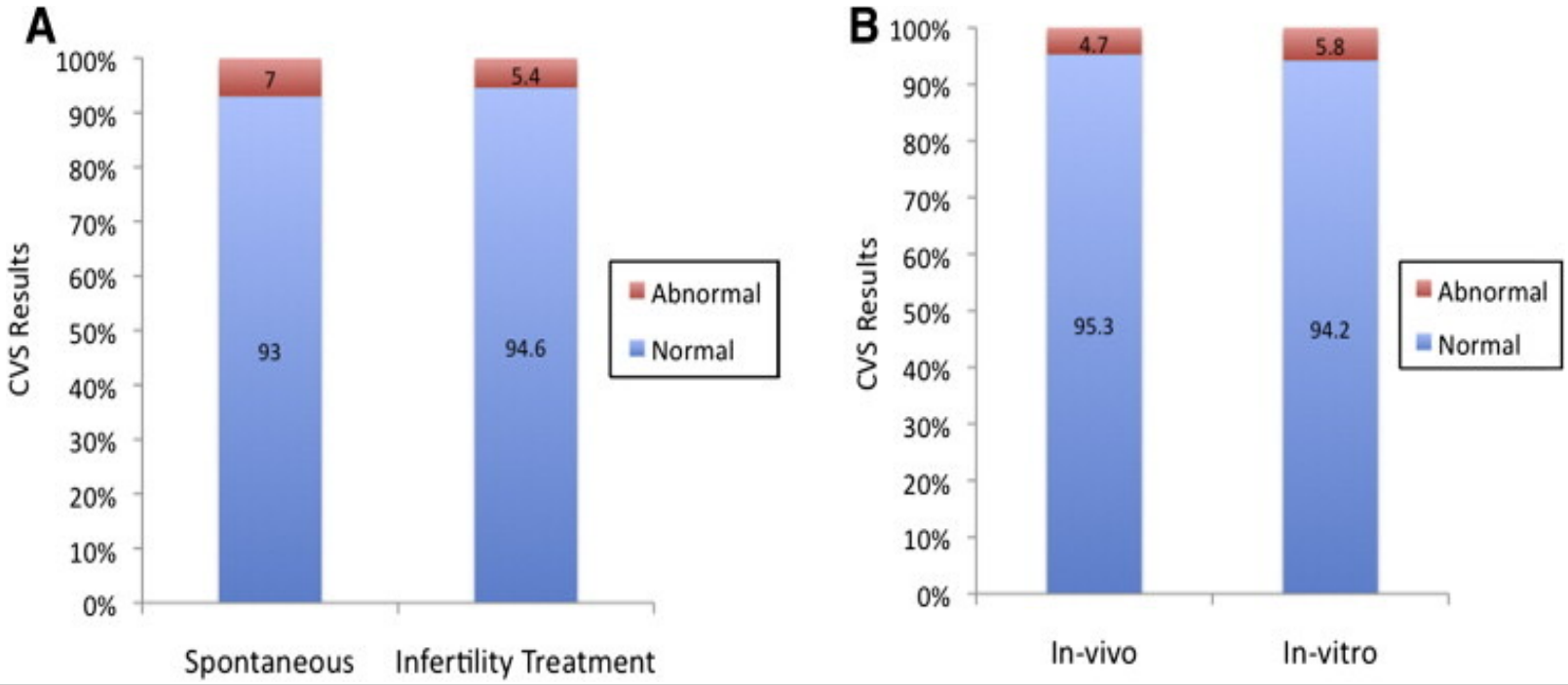
Cytogenetic Abnormalities assessed by CVS in Spontaneous vs. Infertile Patients

- 1,606 women conceived spontaneously
- 559 women conceived through infertility treatment
  - 233 conceived in vivo
  - 326 conceived in vitro



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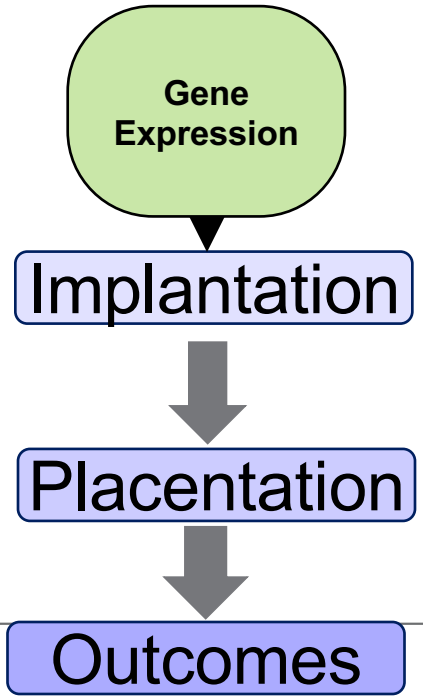
Conway Fertil Steril 2011 Feb;95(2):503-6

Spontaneous/ Medical Assisted/ART (SMAART) Cohort

- **a cohort of pregnancies conceived either spontaneously or in couples with infertility conceived either through non-IVF fertility treatment (NIFT) or IVF, that are enrolled in the late first trimester of pregnancy at the time of Chorionic Villus Sampling (CVS) and followed until delivery**
  - 208 spontaneous conceptions
  - 201 pregnancies conceived with a history of Infertility
    - 90 conceived with NIFT
    - 111 conceived with IVF

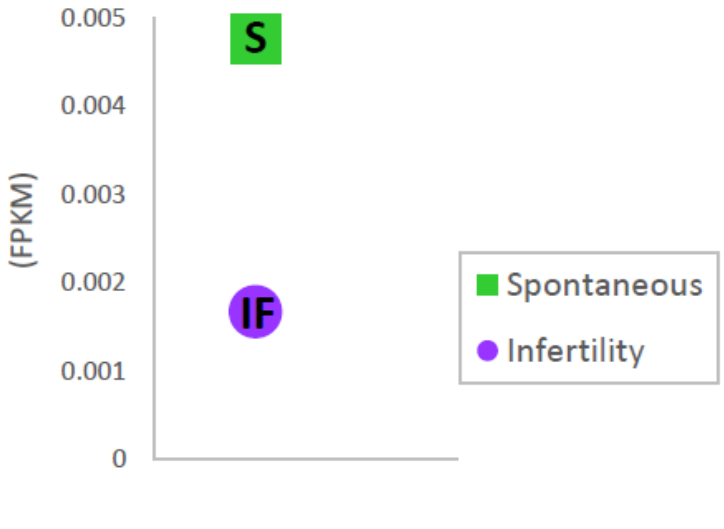
Sun, et al. JCEM. 2018

SMAART Cohort



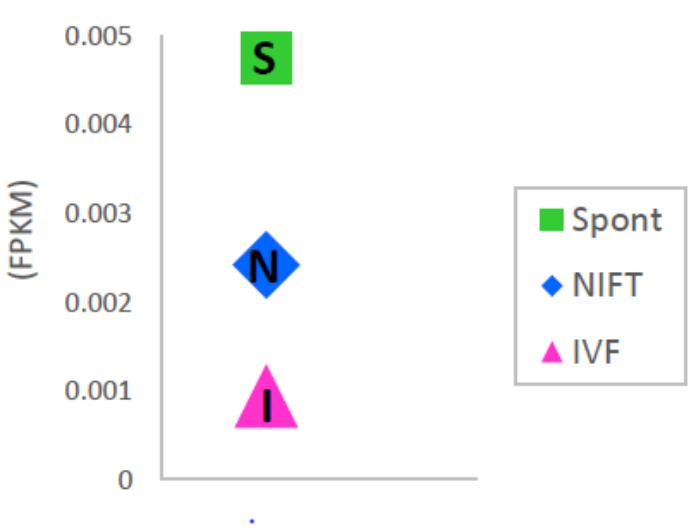
SMAART Transcriptome cohort

A. Spontaneous vs Infertility



Calcium voltage-gated channel subunit alpha1

B. Spontaneous vs IVF



Calcium voltage-gated channel subunit alpha1

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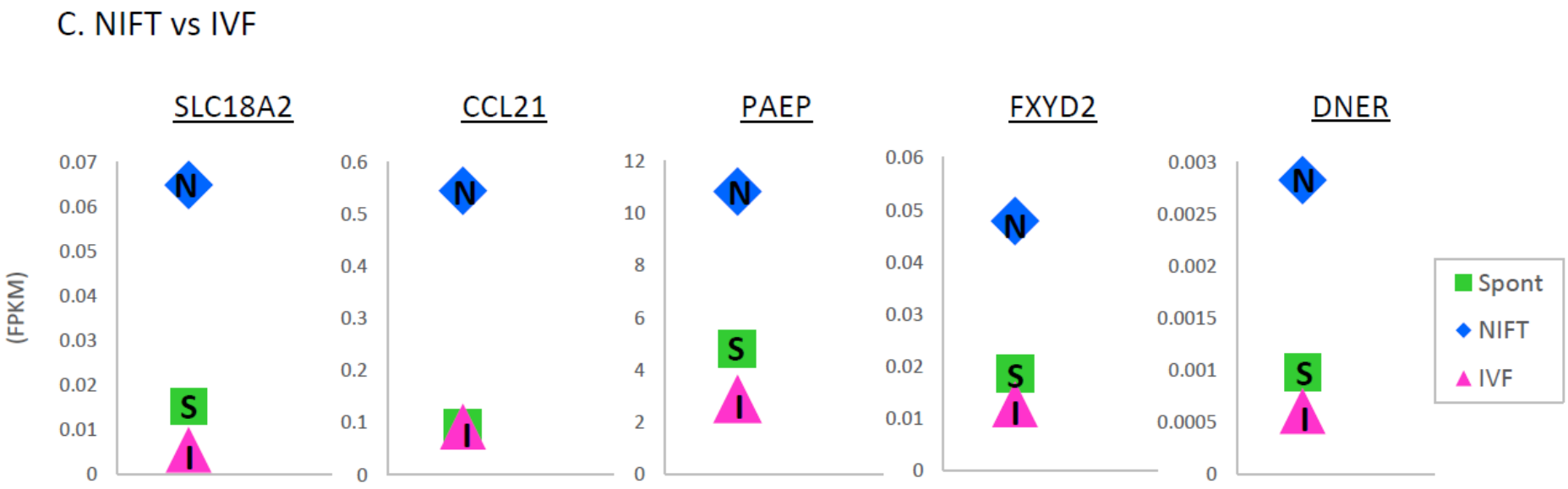
Lee et al, Fertil and Steril 2018

SMAART Transcriptome cohort



Solute carrier family 18 member A2

C-C motif chemokine ligand 21

Progestagen associated endometrial protein

FXYD domain containing ion transport regulator 2

Delta/notch like EGF repeat containing

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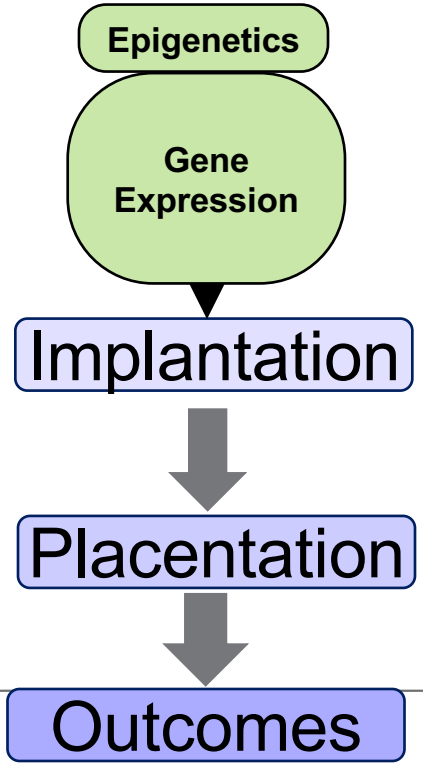
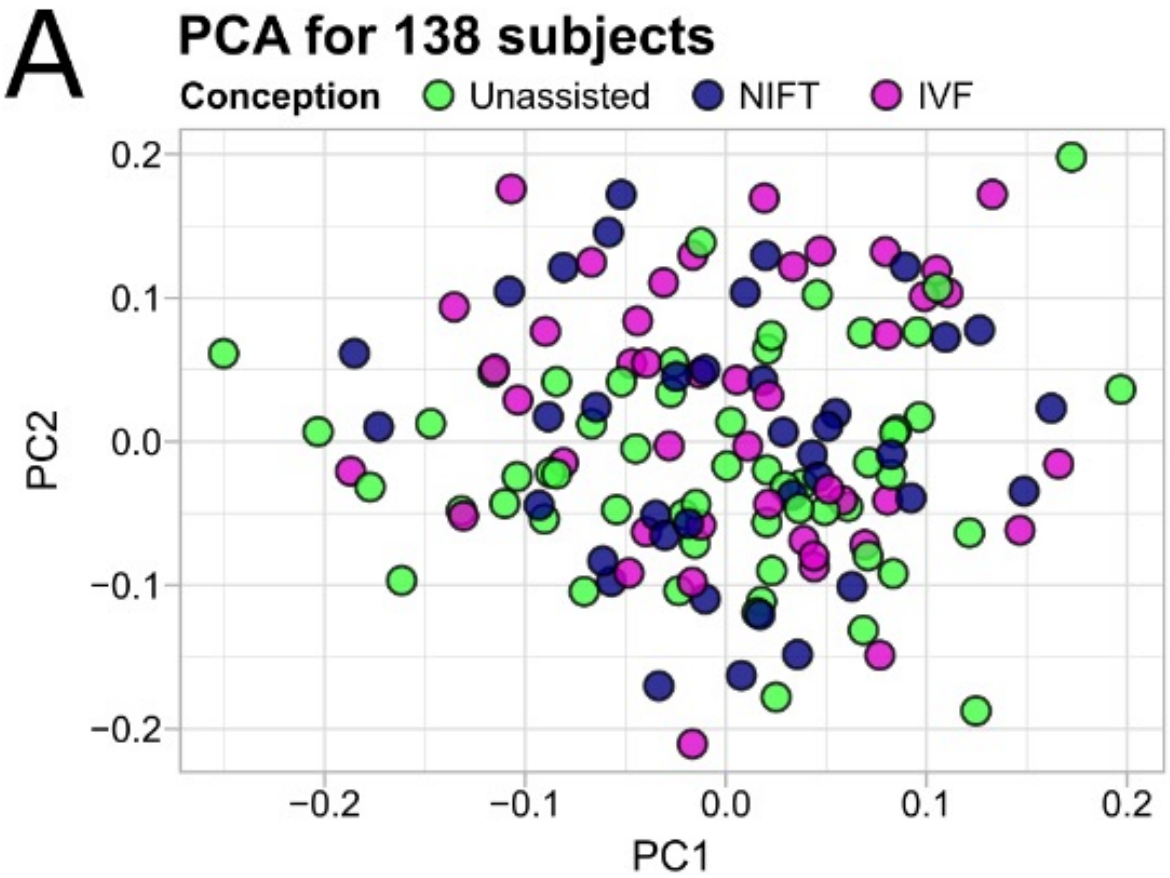
Lee et al, Fertil and Steril 2018

SMAART Cohort



Global methylation alterations due to infertility and treatments

- *Principle Component analysis does not demonstrate clustering*



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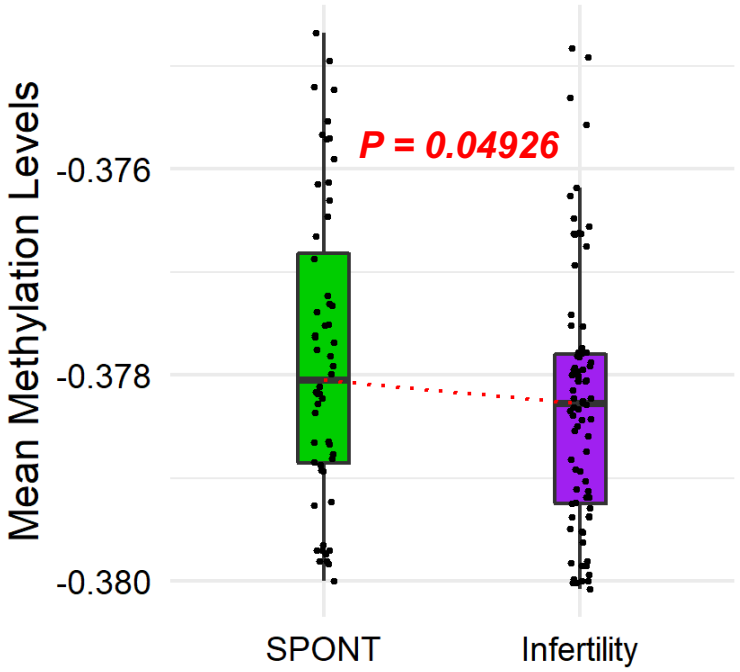
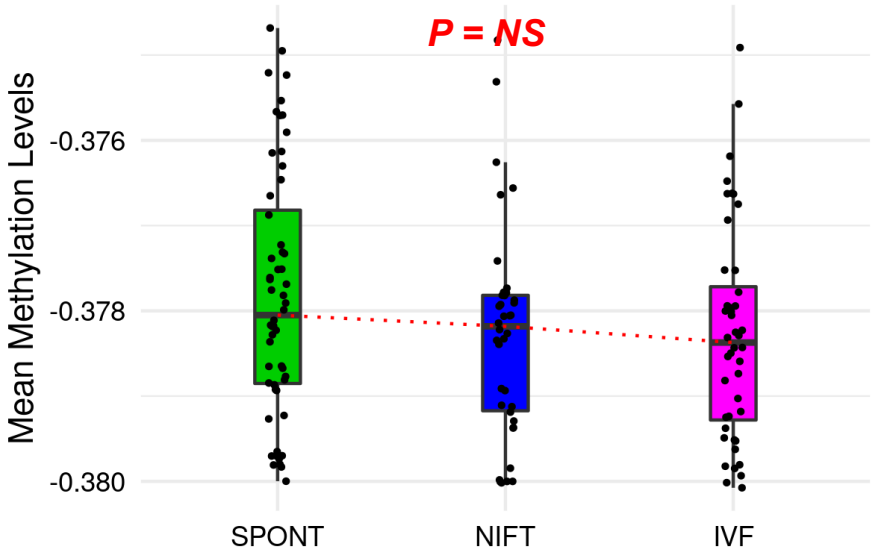
Gonzalez, et al. Fertil Steril 2023
Xu et al, Repro Sciences 2016

Global methylation alterations due to infertility and treatments

- *Median β values were significantly lower in the infertility cohort compared to the spontaneous cohort.*
- *Median β values were lower in the NIFT and IVF cohort compared to spontaneous cohort, but overall there was no significant difference among the groups.*
- *Infertility may be associated with global hypomethylation and not the specific treatment.*





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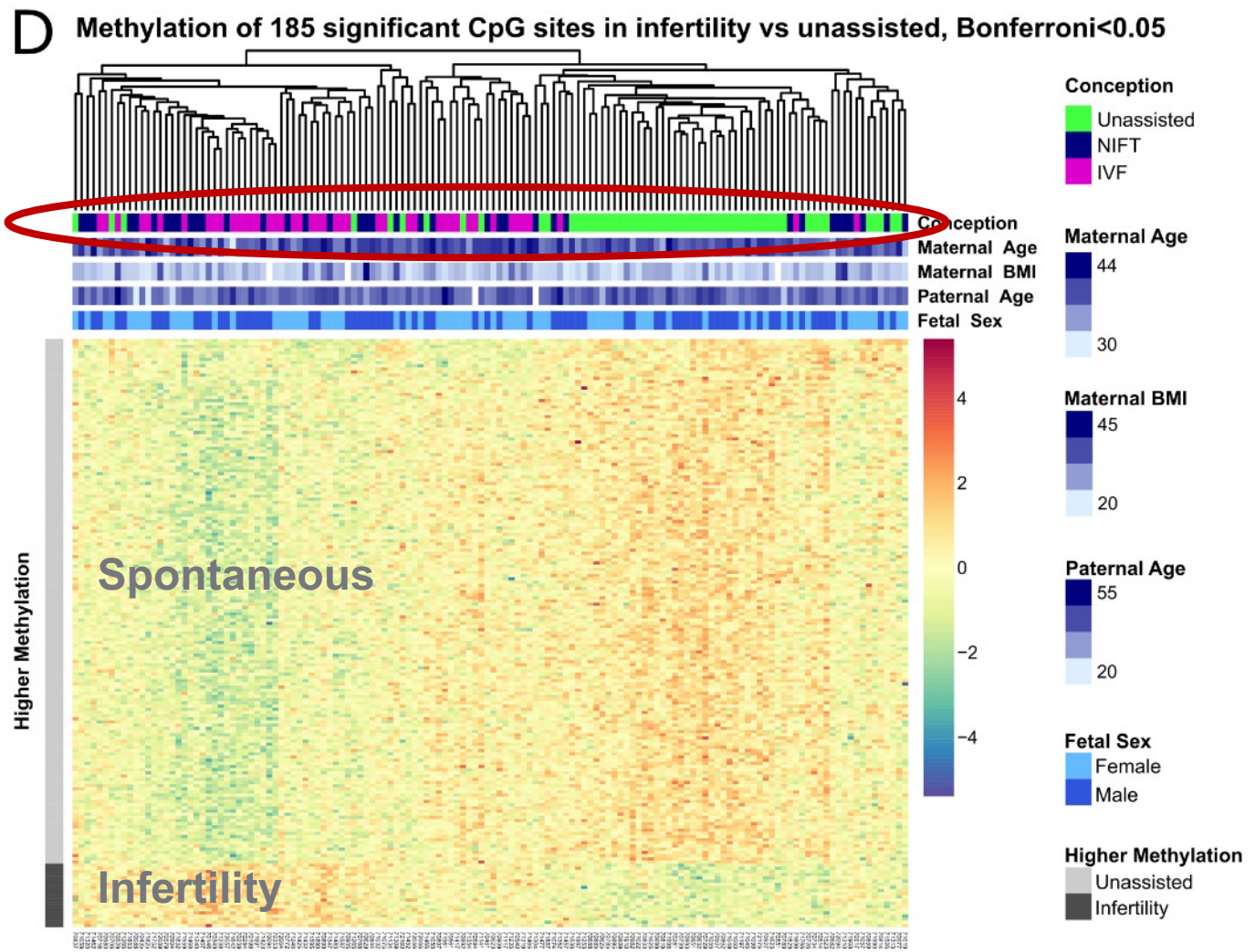
Gonzalez, et al. Fertil Steril 2023
Xu et al, Repro Sciences 2016

Global Differential Methylation due to infertility and treatments

- *Clustering is identified between the infertility and spontaneous cohort.*
- *Within the infertility cohort, there is no clustering of NIFT or IVF cohorts, suggesting diversity among the infertility group, independent of treatment utilized.*



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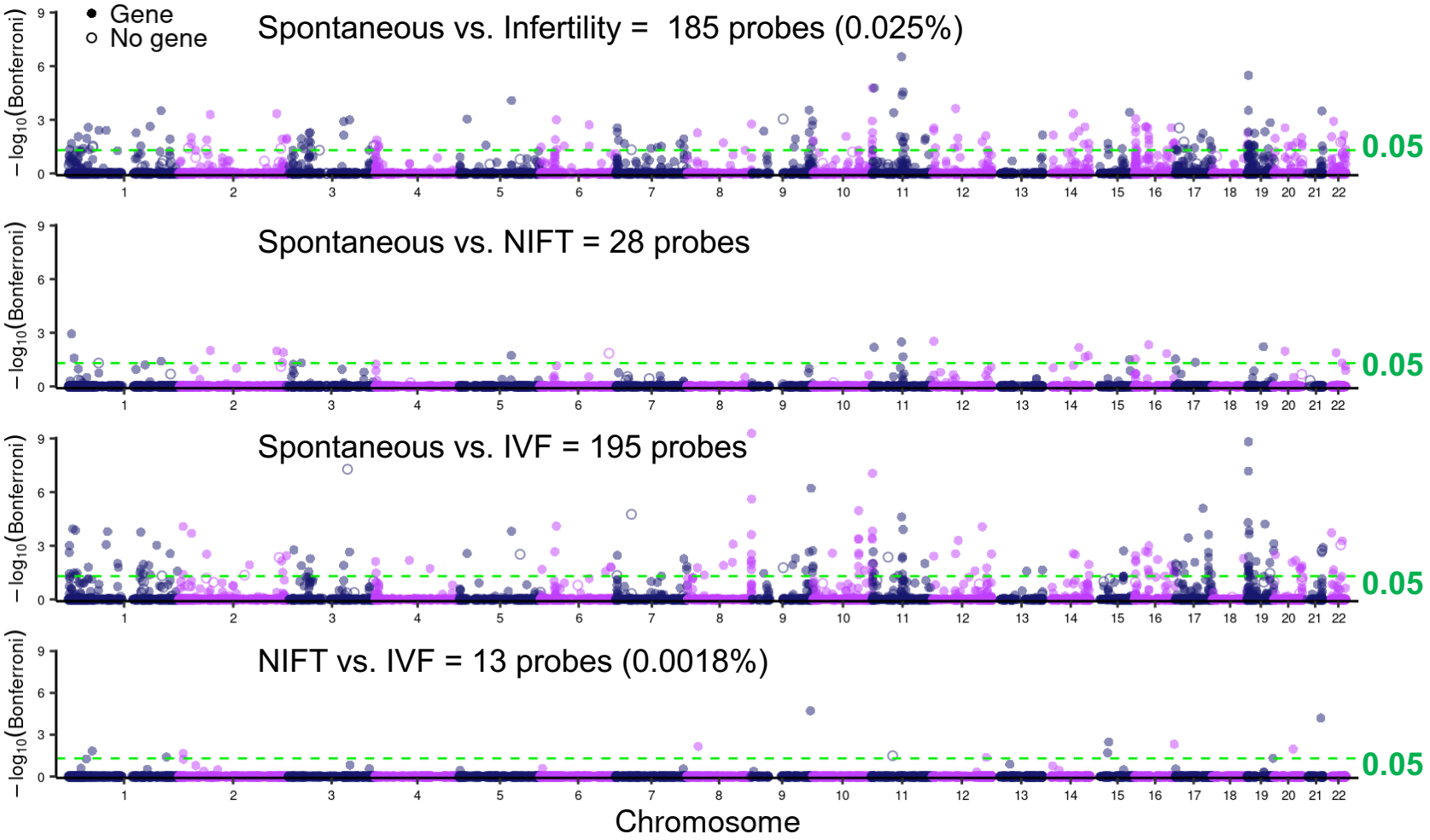
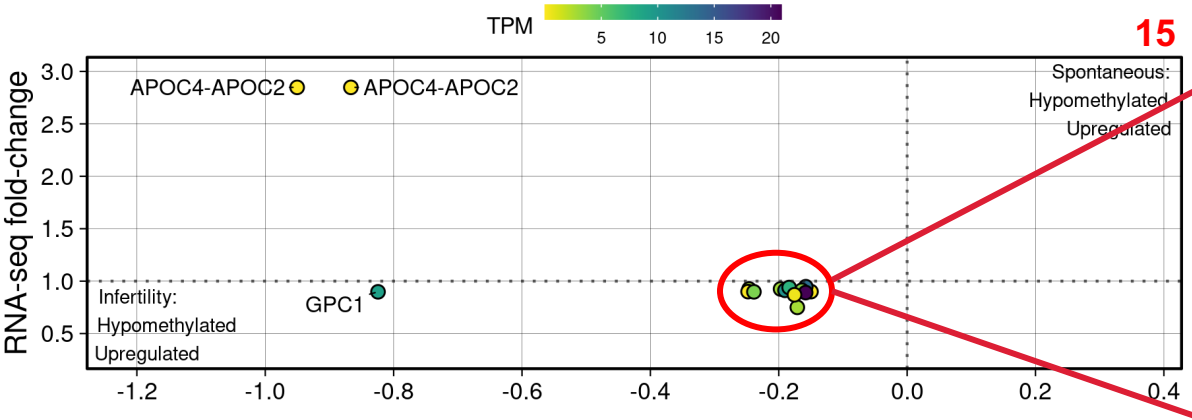
Gonzalez, et al. Fertil Steril 2023

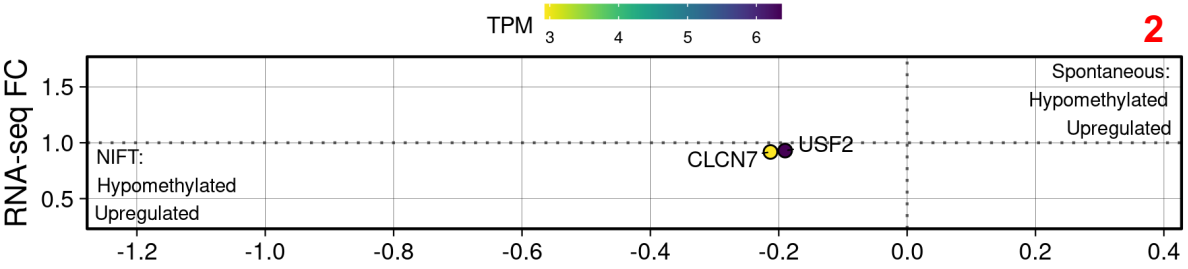
Chromosomal Distribution of Differentially Methylated Probes due to infertility and treatments

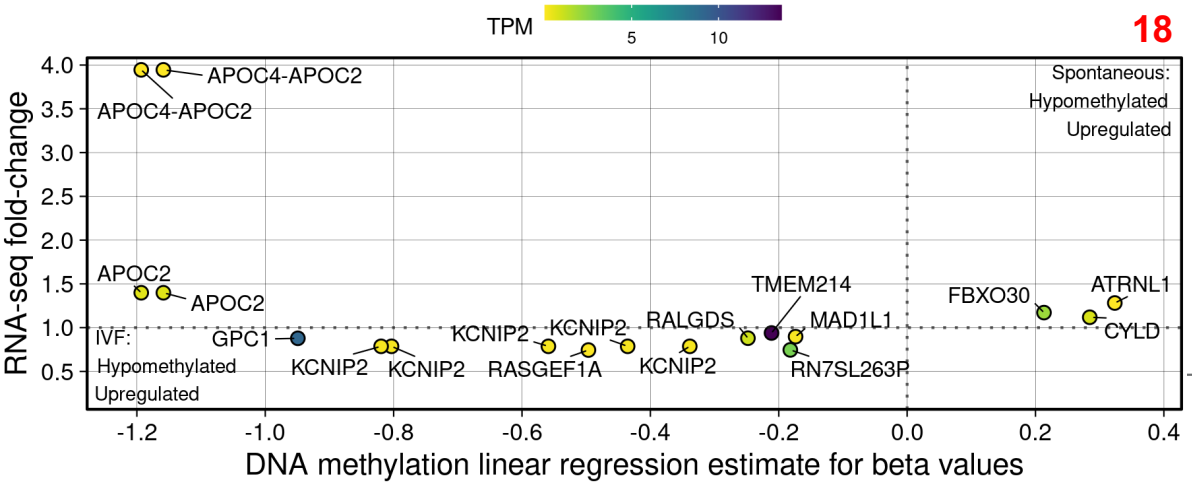


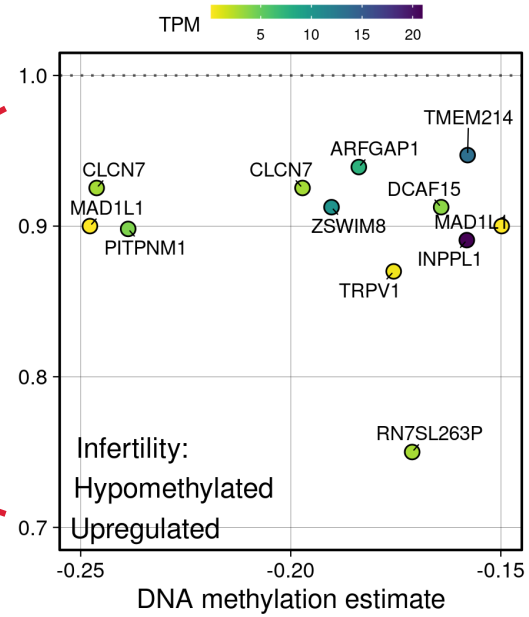
Gonzalez, et al Fertil Steril 2023

Differential methylation and gene expression in infertility and treatments









Differentially methylated probes and associated DEGs:

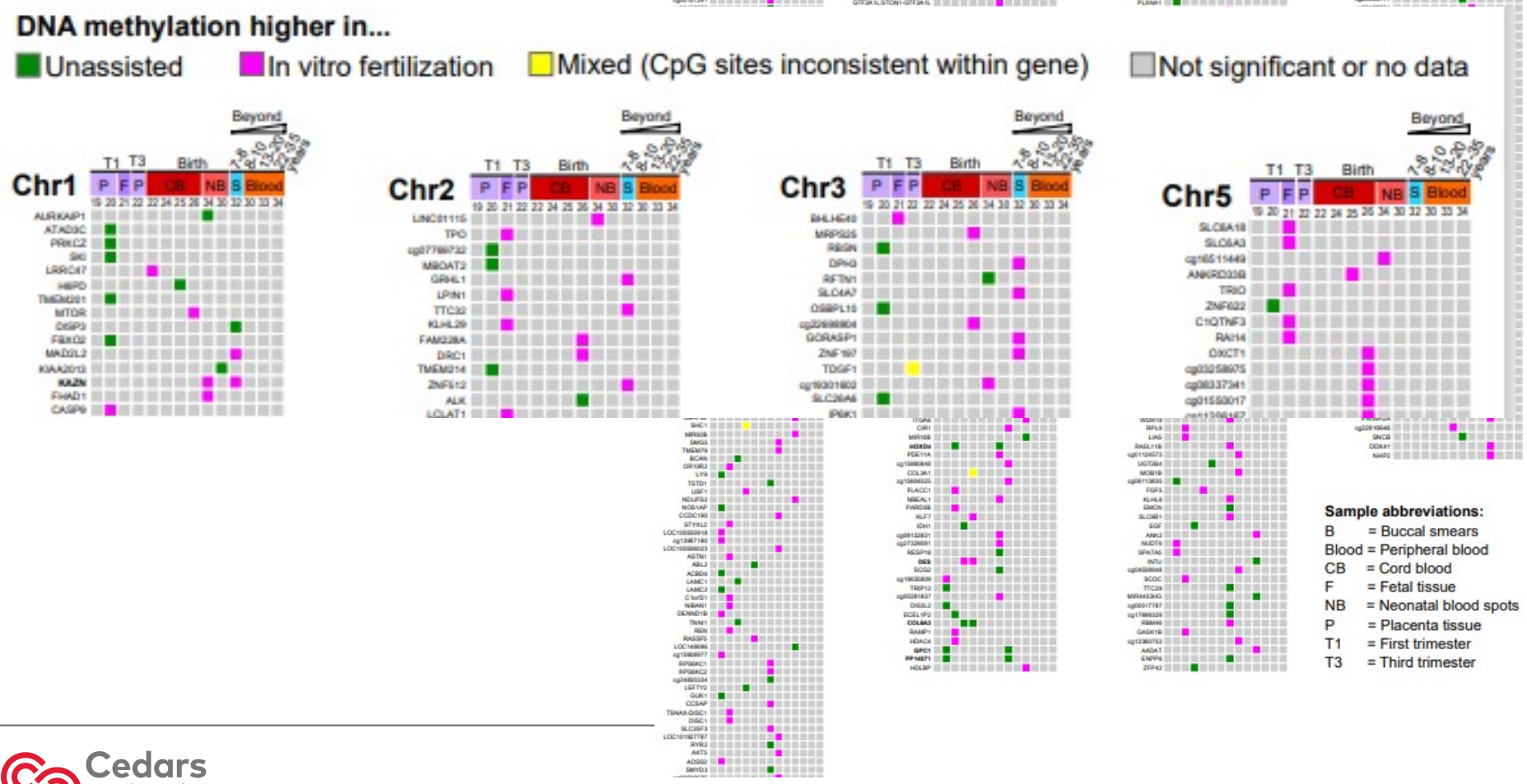
- **Spontaneous vs. Infertility =15**
- **NIFT vs. Spontaneous = 2**
- **IVF vs. Spontaneous = 18**
- **NIFT vs. IVF = 0**

Methylation Changes Across the Lifespan
A Systematic Review

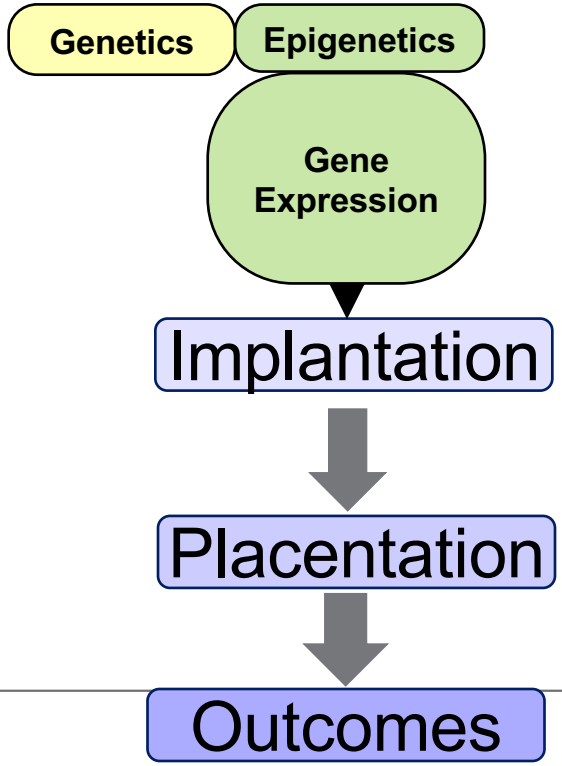
	Trophectoderm	Pregnancy			Newborn		Childhood		Adult
		1st Trimester Placenta	Fetal Tissue	Term Placenta	Cord Blood	Newborn Dried Blood Spot	Buccal Smears	Peripheral Blood	Peripheral Blood
Infertile vs Fertile	Denomme (2021)								
IVF (+/- ICSI) vs Unassisted		Xu (2017) Gonzalez (2022)	Liu (2021)	Katari (2009)	Katari (2009) Melamed (2015) Tobi (2020) Haberg (2022)	Novakovic (2019) ** Yeung (2021)	Ducreux (2021)	Yeung (2021)	Novakovic (2019)** Penova-Veselinovic (2021)
ICSI Only vs Unassisted					El Hajj (2017) Gentilini (2018)	Estill (2016) Yeung (2021)			
NIFT vs Unassisted		Xu (2017) Gonzalez (2022)				Estill (2016) Yeung (2021)		Yeung (2021)	
IVF vs NIFT		Xu (2017) Gonzalez (2022)		Choufani (2019) *		Estill (2016)			
Infertility (NIFT + IVF +/- unassisted with h/o infertility) vs Fertile		Gonzalez (2022)		Choufani (2019)	Caramaschi (2021)	Estill (2016) Yeung (2021)		Yeung (2021)	

*NIFT cohort contained those with history of infertility conceiving unassisted
** IVF cohort Also contained GIFT

Interpreting Data in a Larger Context: Systematic Review

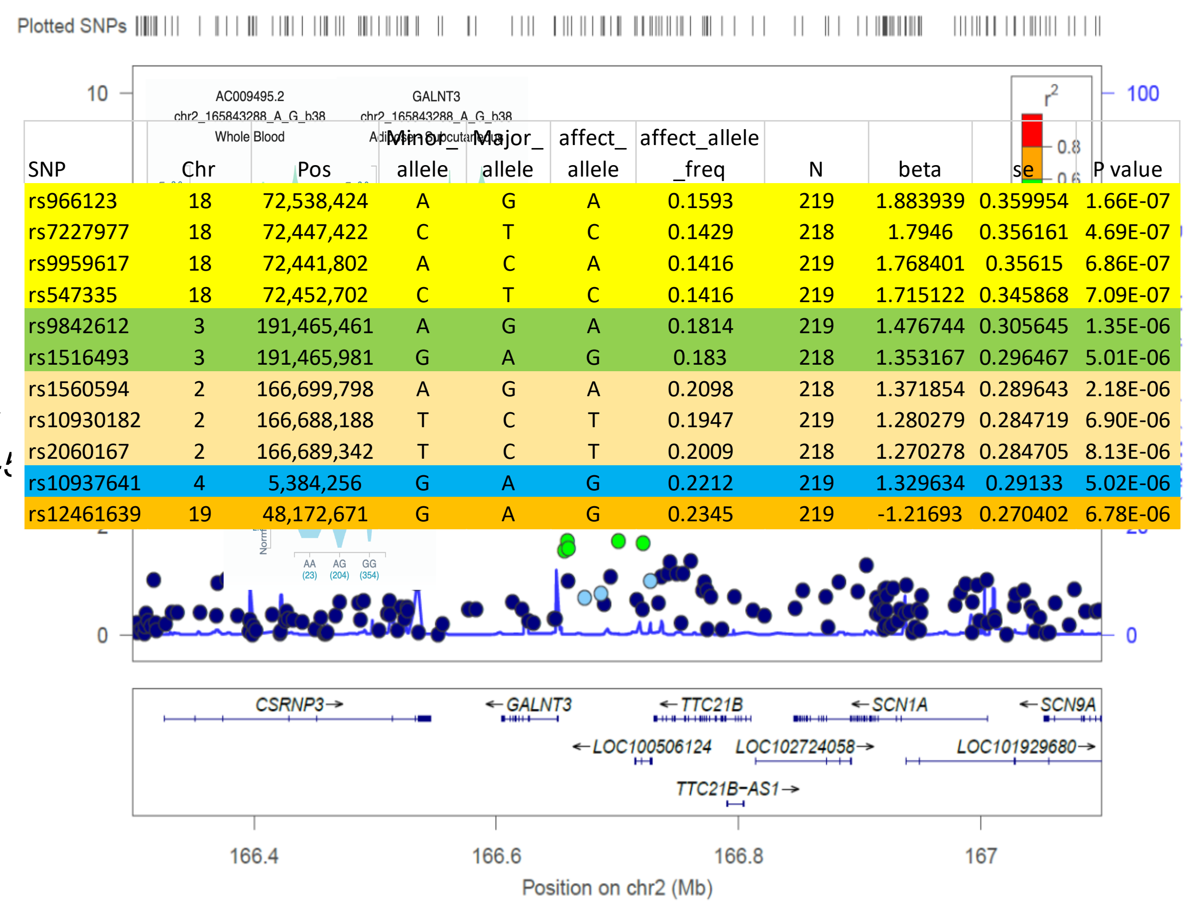


SMAART Cohort



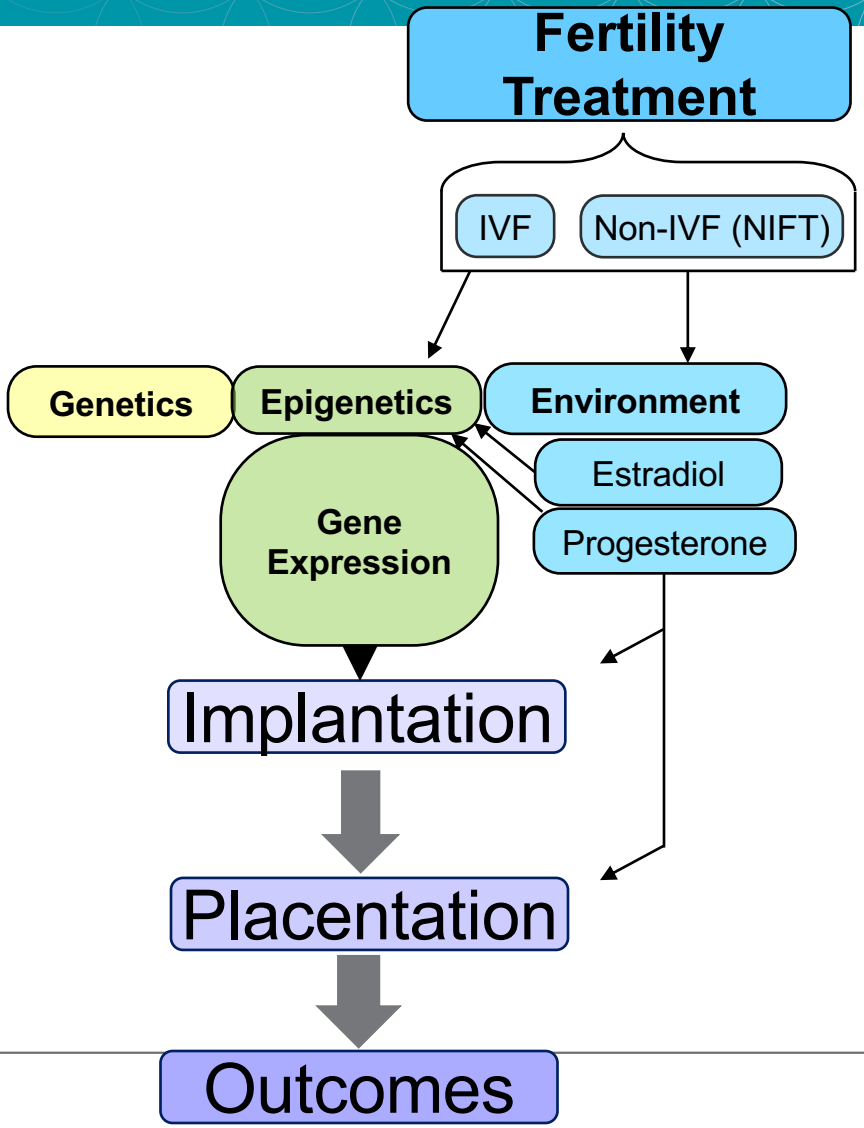
Single nucleotide variants (SNVs) that associate with infertility – Family associated GWAS

- At one of the 2 risk SNVs were associated with variation in gene expression. For the index risk SNV rs1560594, p=2.18 x 10^{-6}, eQTL plots demonstrate associations for the noncoding transcript AC009495.2, GALNT3 and TTC21B in whole blood and adipose.
- None of the SNVs were genome-wide significant at the p-value threshold of $5x10^{-8}$
- Of the 637,072 SNVs that passed quality control measures – 11 SNVs were identified with sub-genome-wide significance ($p < 1x10^{-5}$) representing 5 loci



SNP	Chr	Pos	Minor_ allele	Major_ allele	affect_ allele	affect_allele _freq	N	beta	se	P value
rs966123	18	72,538,424	A	G	A	0.1593	219	1.883939	0.359954	1.66E-07
rs7227977	18	72,447,422	C	T	C	0.1429	218	1.7946	0.356161	4.69E-07
rs9959617	18	72,441,802	A	C	A	0.1416	219	1.768401	0.35615	6.86E-07
rs547335	18	72,452,702	C	T	C	0.1416	219	1.715122	0.345868	7.09E-07
rs9842612	3	191,465,461	A	G	A	0.1814	219	1.476744	0.305645	1.35E-06
rs1516493	3	191,465,981	G	A	G	0.183	218	1.353167	0.296467	5.01E-06
rs1560594	2	166,699,798	A	G	A	0.2098	218	1.371854	0.289643	2.18E-06
rs10930182	2	166,688,188	T	C	T	0.1947	219	1.280279	0.284719	6.90E-06
rs2060167	2	166,689,342	T	C	T	0.2009	218	1.270278	0.284705	8.13E-06
rs10937641	4	5,384,256	G	A	G	0.2212	219	1.329634	0.29133	5.02E-06
rs12461639	19	48,172,671	G	A	G	0.2345	219	-1.21693	0.270402	6.78E-06

SMAART Cohort



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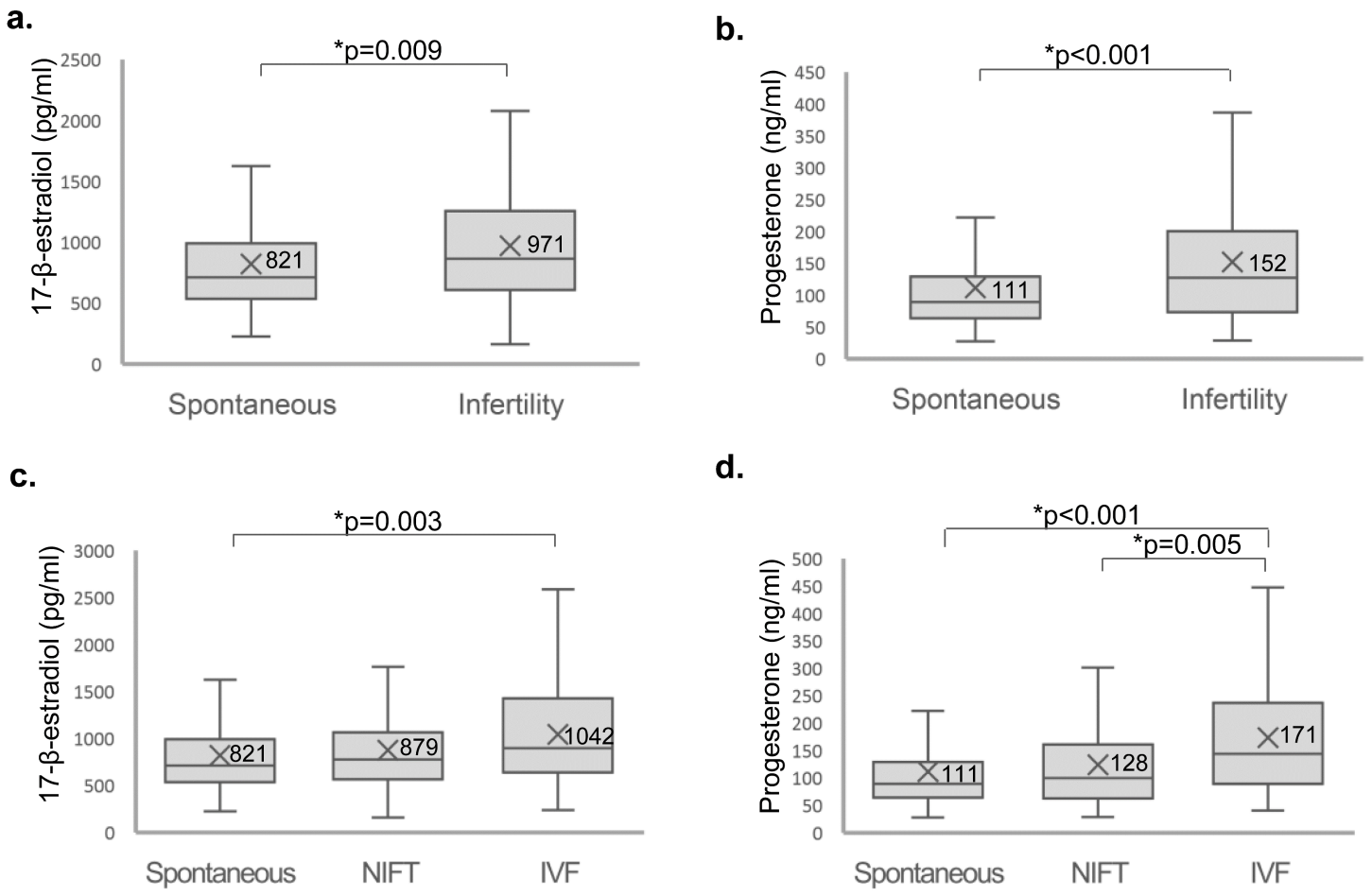
Supraphysiologic Hormones

- *Supraphysiologic hormone levels have been implicated in increased rates of low birth weight and small for gestational age babies.*
- *Since pregnancies conceived through fertility treatments are exposed to elevated estradiol and progesterone levels, either endogenously through treatments or exogenously to supplement the pregnancy, we wanted to determine whether previous treatments impact the hormonal milieu of an ongoing pregnancy.*

The Supraphysiologic Hormonal Milieu



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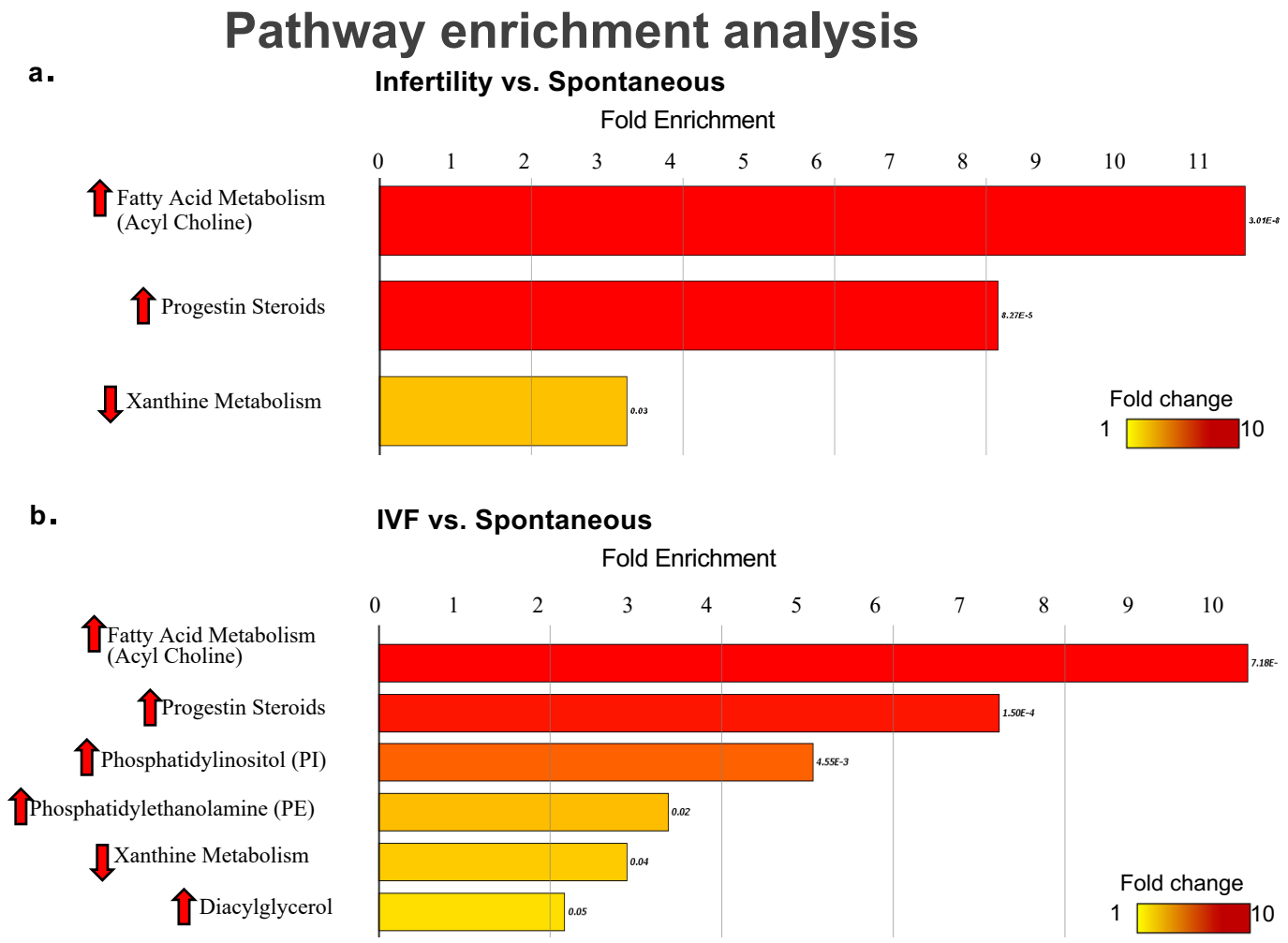
Sun, et al. JCEM. 2018

Differences in metabolomic profiles in late first trimester



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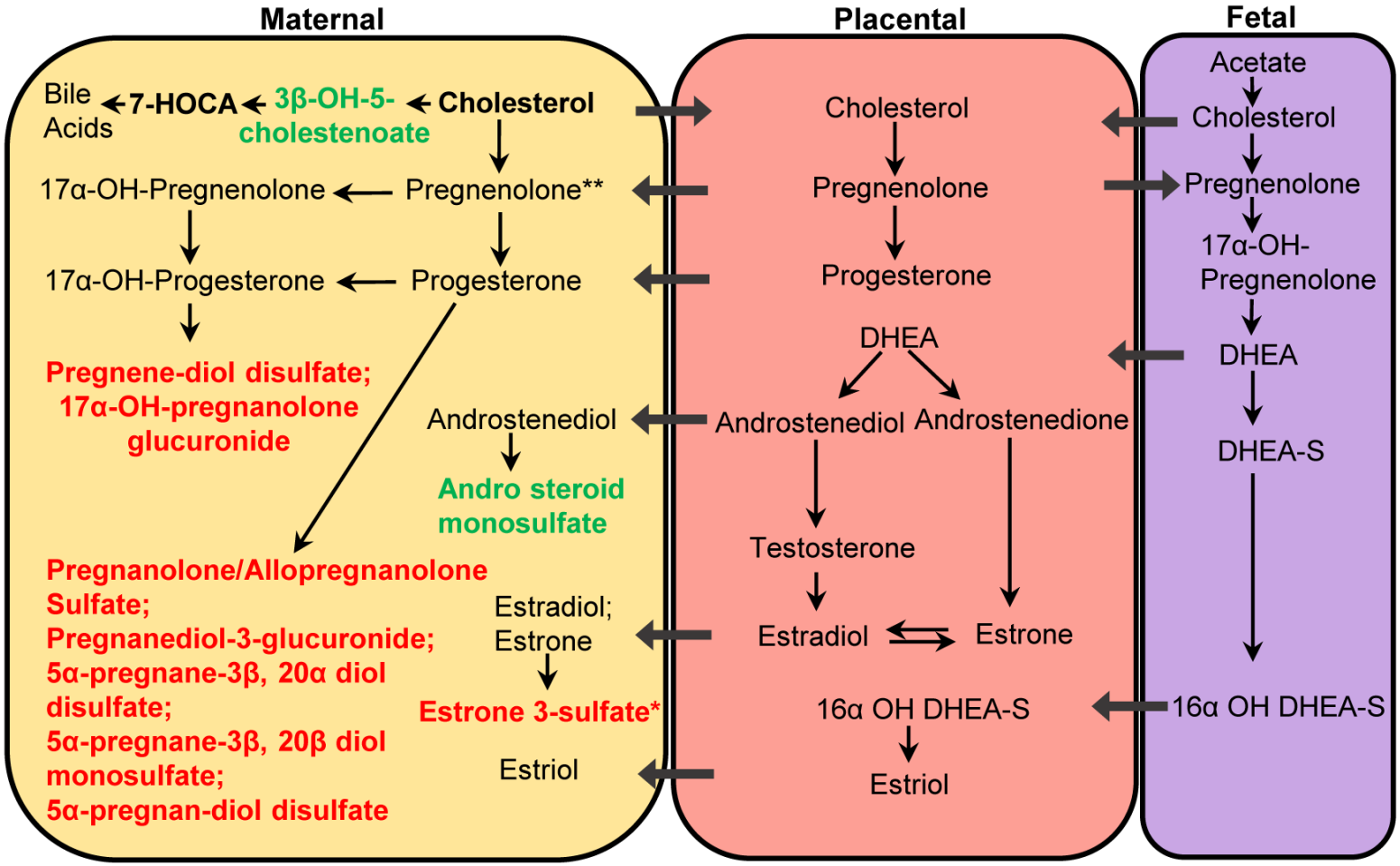
Sun, et al. JCEM. 2018

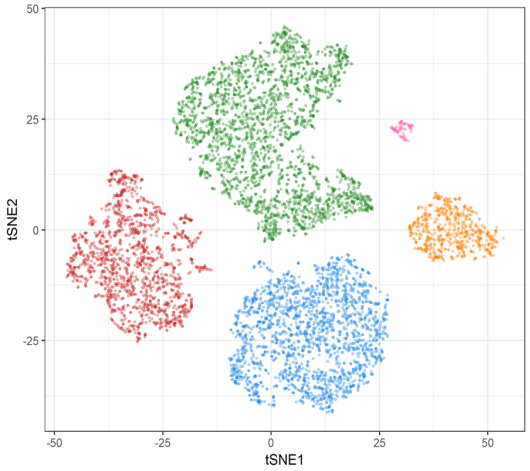
Model of steroid hormones and metabolites within the maternal-placental-fetal unit



Cedars Sinai

Sun, et al. JCEM. 2018

First Trimester Chorionic Villi- Single Cell Sequencing



P1 Trophoblast Cells
P2 Stromal Cells
P3 Macrophages
P4 Dendritic Cells
P5 Endothelial Cells

P1 (Trophoblast)	Molecule Type	p-value	Targets
BMP4	Growth factor	3.9E-21	26
beta-estradiol	Chemical – endogenous	9.34E-15	60
PGR	Ligand-dependent nuclear receptor	2.35E-13	22
ERBB2	Kinase	3.81E-12	33
ESR1	Ligand-dependent nuclear receptor	5.54E-12	44
TGFB1	Growth factor	9.98E-10	48
HRAS	Enzyme	1.98E-09	26
PTEN	Phosphatase	5.12E-09	22
TP53	Transcription regulator	9.39E-09	43
TNF	Cytokine	2.39E-08	45
P2 (Stromal)	**Molecule Type**	**p-value**	**Targets**
TGFB1	Growth factor	3.26E-29	98
beta-estradiol	Chemical – endogenous	5.06E-27	99
FGF2	Growth factor	8.5E-24	42
CTNNB1	Transcription regulator	1.52E-23	58
WNT3A	Cytokine	1.53E-21	34
AHR	Ligand-dependent nuclear receptor	5.98E-20	37
TGFB2	Growth factor	9.85E-19	22
TGFB3	Growth factor	2.83E-18	22
TWIST1	Transcription regulator	2.97E-18	25
HRAS	Enzyme	8.9E-18	45

P3 (macrophages)	Molecule Type	p-value	Targets
CSF2	Cytokine	5.32E-20	35
TNF	Cytokine	6.32E-20	66
leukotriene D4	Chemical - endogenous	3.48E-19	16
IL13	Cytokine	1.59E-15	28
IFNG	Cytokine	6.54E-15	50
beta-estradiol	Chemical – endogenous	2.19E-14	60
IL1B	Cytokine	5.7E-14	39
CSF1	Cytokine	3.34E-12	18
IL4	Cytokine	3.63E-12	36
IL2	Cytokine	8.48E-11	27
P4 (Dendritic-like)	**Molecule Type**	**p-value**	**Targets**
IFNG	Cytokine	4.82E-27	48
TGFB1	Growth factor	4.45E-20	46
TNF	Cytokine	1.91E-18	44
IL13	Cytokine	1.61E-17	23
IL27	Cytokine	6.75E-17	16
IL4	Cytokine	4.2E-16	30
CIITA	Transcription regulator	5.3E-15	10
IL1B	Cytokine	5.48E-15	29
CSF3	Cytokine	3.81E-14	15
beta-estradiol	Chemical - endogenous	5.37E-14	40
P5 (Endothelial)	**Molecule Type**	**p-value**	**Targets**
KLF2	Transcription regulator	4.38E-10	10
TNF	Cytokine	8.04E-09	25
TGFB1	Growth factor	8.61E-09	25
CAV1	Transmembrane receptor	7.25E-08	8
ENG	Transmembrane receptor	4.63E-07	5
ESR1	Ligand-dependent nuclear receptor	2.27E-06	18
SRC	Kinase	5.46E-06	6
TCF7L2	Transcription regulator	5.68E-06	9
ERBB2	Kinase	6.54E-06	13
miR-199a-5p	Mature microRNA	7.77E-06	5

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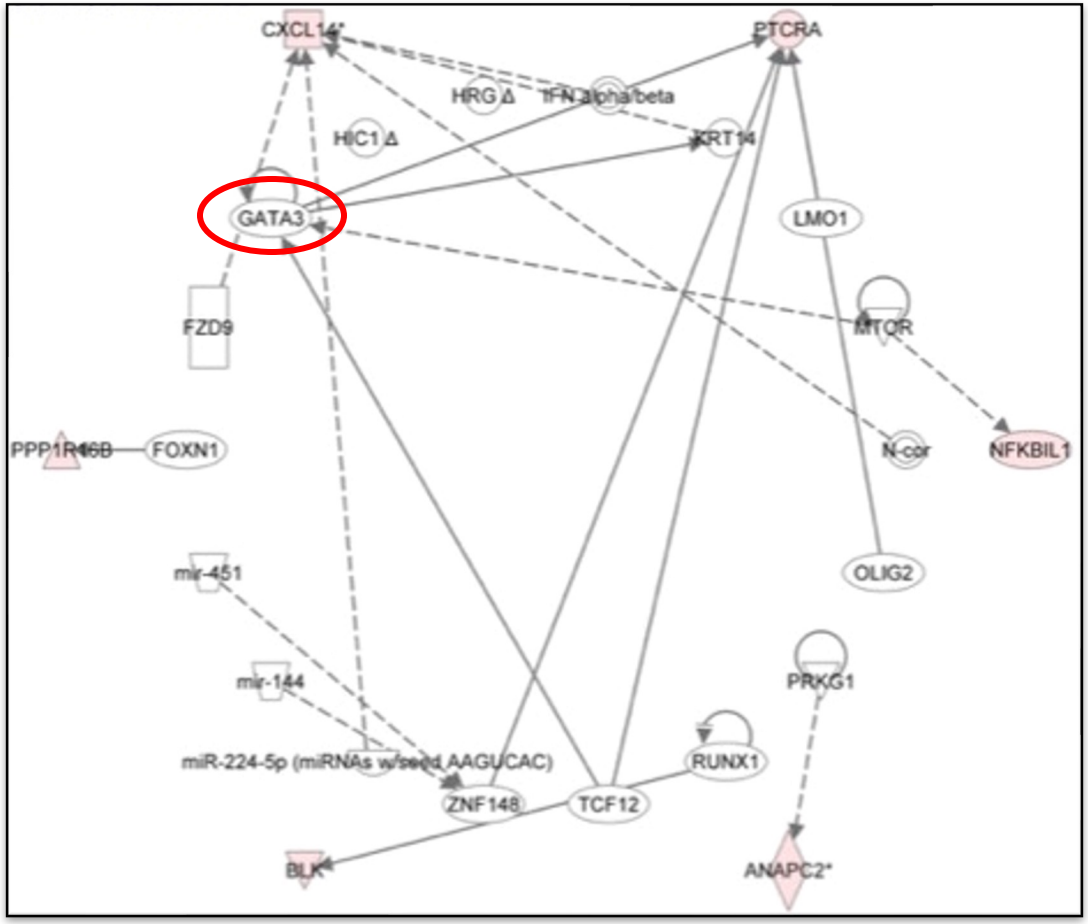
Sun, Gonzalez, Deng, Pisarska et al.
https://www.biorxiv.org/content/10.1101/641118v1

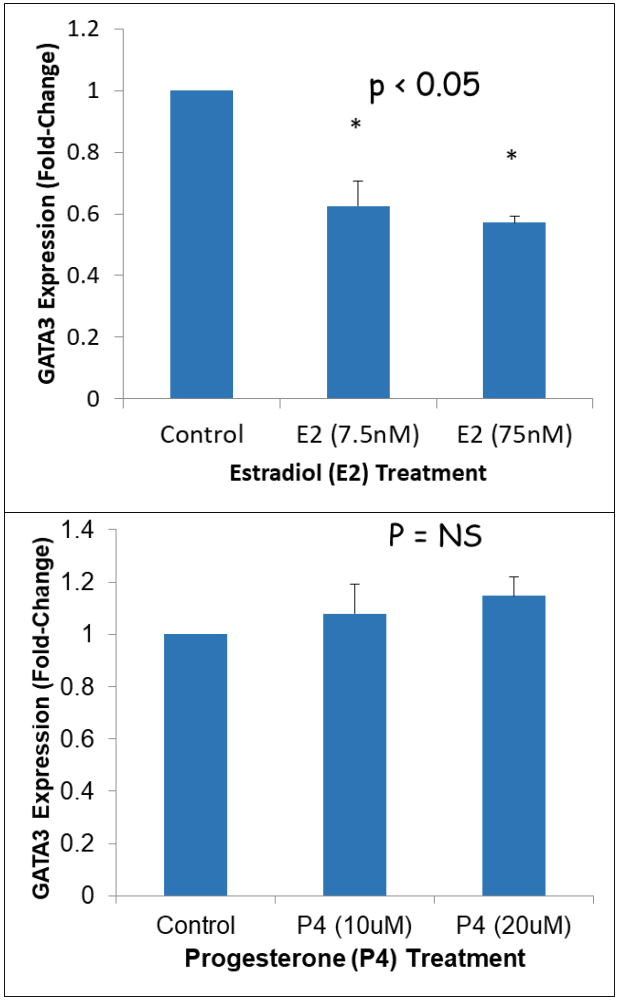
Upstream Analysis of Differentially Methylated Genes

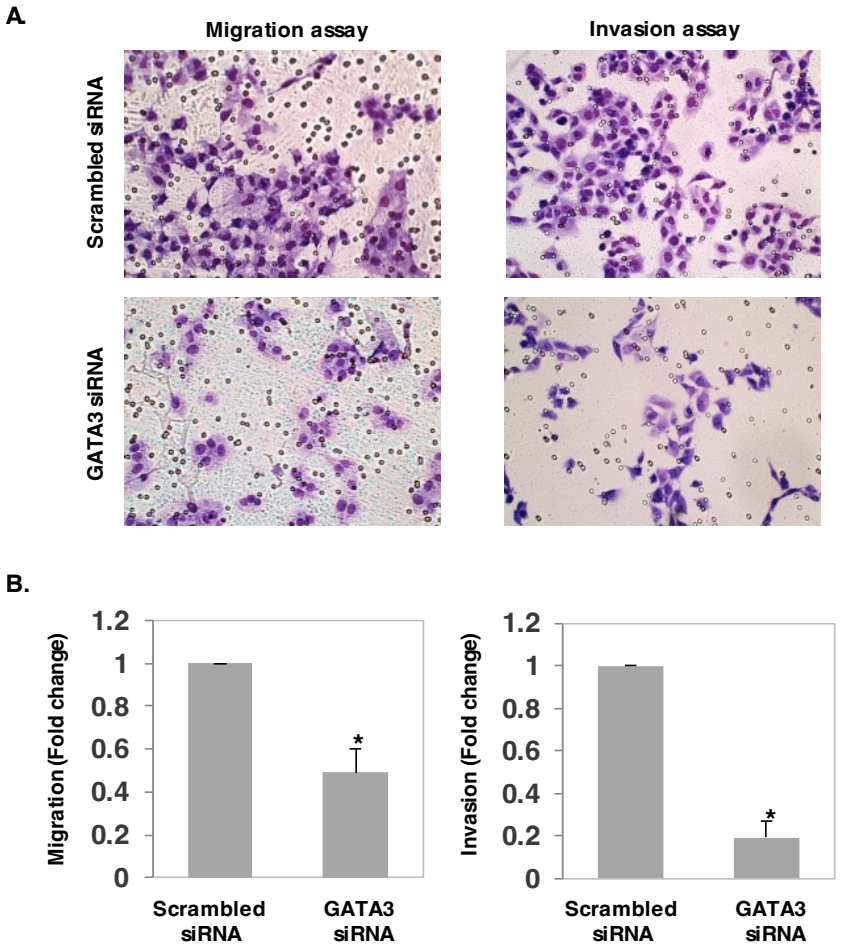


- Directs trophectoderm differentiation in the blastocyst
- *R*egulates trophoblast function important for:
- Placental vascularization
- Syncytiotrophoblast formation
- Reduced *GATA3* expression in mice shows decreased embryo hatching and implantation

Lee B. and Kroener L, et al. BOR 2016

Estradiol downregulates GATA3 and downregulation of GATA3 inhibits migration and invasion





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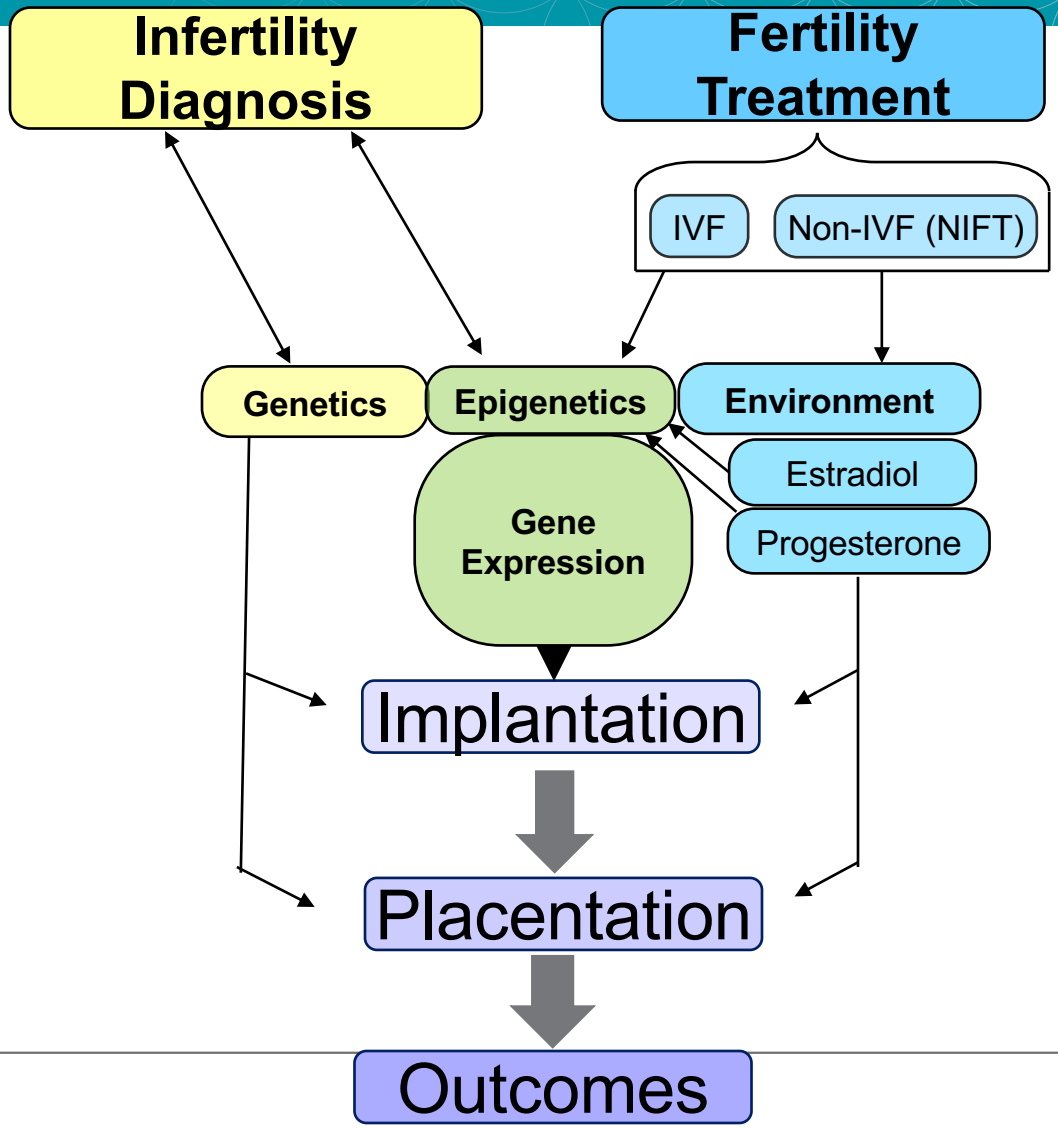
Lee B. and Kroener L, et al. BOR 2016

SMAART Cohort



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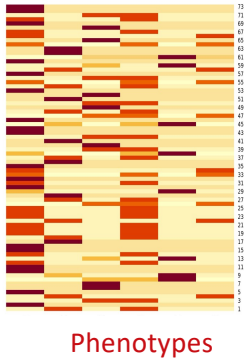
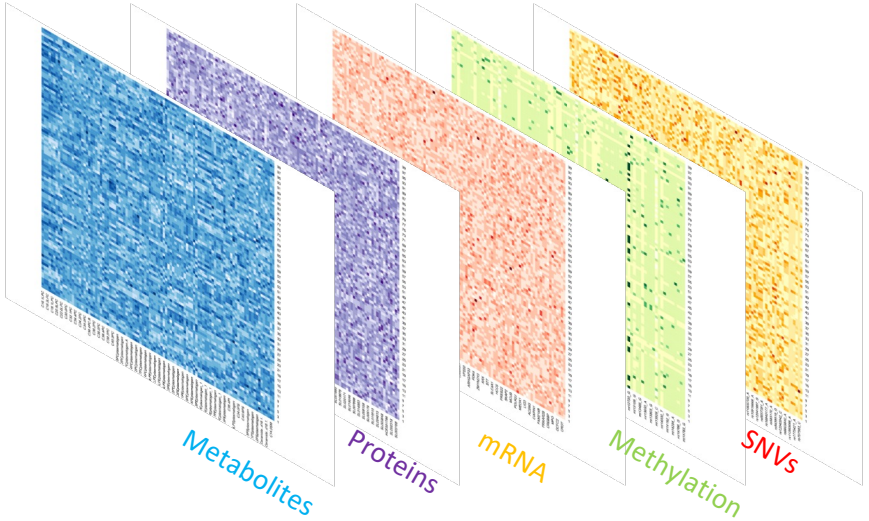
Impact of infertility on placentation through a multi-omics analysis

Regularized Canonical Correlation Analysis - Provides correlation across a large data landscape using small sample sizes





Data	Features
Phenotypes	7 traits
Genomics - OmniExpressExome Chip	688,534 SNVs
Methylomics - methylation EPIC Array (Illumina)	865,855 sites
Transcriptomics -Total RNA sequencing	61,801 genes
Metabolomics of mother's serum (Metabolon)	704 metabolites

Genetic/epigenetic impact of infertility on placentation through a multi-omics analysis

Correlation Component	Spont	Infertility	Sex	Mat Age	Race	CVS age
1	0.048				0.99	0.05
2				-0.11		
3	0.99		-0.11			
4			0.11	0.99		
5	0.11		0.99			
6	-0.034	0.002	-0.99			0.06

- *Component 3 contains the association of infertility with the rest of the data landscape*
- *Effect of sex, maternal age, race, and CVS age are separated from the effect of infertility*

- ***296 Features Identified in Component 3***

- *Genomics: 40 Features (SNVs)*
- *Methylomics: 40 Features (methylated regions)*
- *Metabolomics: 8 Features (metabolites)*
- *Transcriptomics: 209 Features (transcripts)*

- ***Central Theme – Mitochondrial Regulation***

- ***Mitochondrial Regulatory Genes***
  - *ARAF*
  - *MYOF*
  - *PRKCZ*
  - *DNAJC1*
  - *MTFR1*
- ***Mitochondrial small RNAs***
- *Nuclear encoded*
- *Regulators of mitochondrial transcription*
  - *MTATP6P9,23,31*
  - *MTCO2P7; MTCYBP42*
  - *MTND1P2,20,28,31*
  - *MTND2P15,20*
  - *MTND4P1,4,8*

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Conclusions

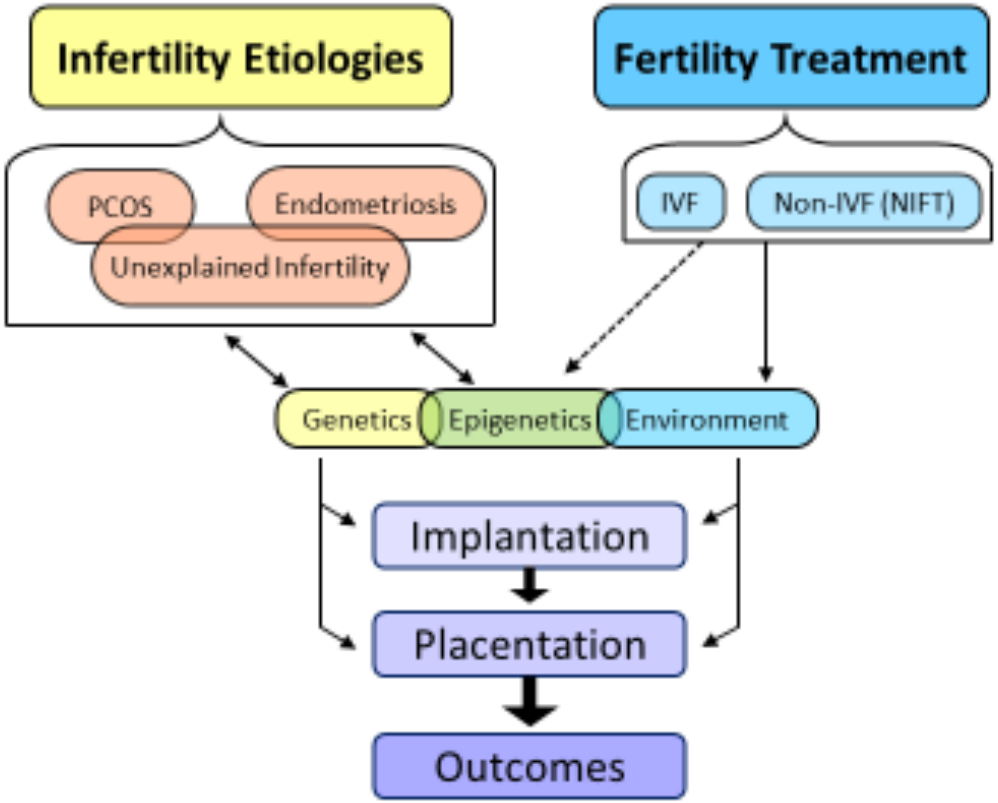
- *Infertility and/or the treatments are associated with some increased risks of adverse outcomes to mother and child including:*
  - *Mother -diabetes, pregnancy induced hypertension, placenta previa and abruption as well as SMM*
  - *Child- prematurity, growth restriction, and birth defects*

- *RISKS ARE SMALL*
- *Risks are independent of treatment utilized*
- *Outcomes are related to placentation*

Conclusions

- *Genetics of infertility appears to be a major contributor that may alter methylation and gene expression*
- *Supraphysiologic hormonal states may be a contributor*
  - *Altered methylation*
  - *Reprogramming the placenta to maintain a high hormonal state*
  - *Impacting trophoblast invasion and migration*
- *Multi-omics suggest genetics/epigenetics are impacting mitochondrial genes in the first trimester placenta*

Future Directions- Infertility Etiology



Maternal	Fetal/Childhood
Short term: Preeclampsia Gestational diabetes **Long term:** Cardiovascular disease Metabolic disease	**Short term:** Preterm birth Low birth weight **Long term:** Cardiovascular disease Metabolic

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