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Introduction:

- Preeclampsia - a major pregnancy complication has higher incidence in twins (>9%) compared to singleton (2.5-7%) pregnancies
- There is an increase the past 30-40 years in conception by assisted reproduction technology (ART).
- 30% of twin pregnancies are conceived by in-vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI).

Objectives: to investigate if the mode of conception affects the rate of preeclampsia, the level of predictive markers and preeclampsia prediction efficacy.

Methods:

- A secondary analysis of the Pre-Twin Screen database of pregnancies with two live dichorionic (DC) or monochorionic di-amniotic (MCDA) twins.
- Study enrolled women from Bonn, T  bingen, Barcelona, Montreal, Rome, and Israel with two live fetuses at 11+0 to 13+6 weeks' gestation.
- The impact of the mode of conception on pregnancy outcomes was evaluated using Kruskal Wallis a-parametric statistics for continuous variables and Fischer Exact test or chi-square tests for categorical variables. Marker were converted to multiple of the medians (MoM).
- Preeclampsia screening efficacy was estimated by Box Plot, logistic regression models, and the area under the receiver operating characteristic (ROC) curves.

Figure 2: PE Rates by mode of conception

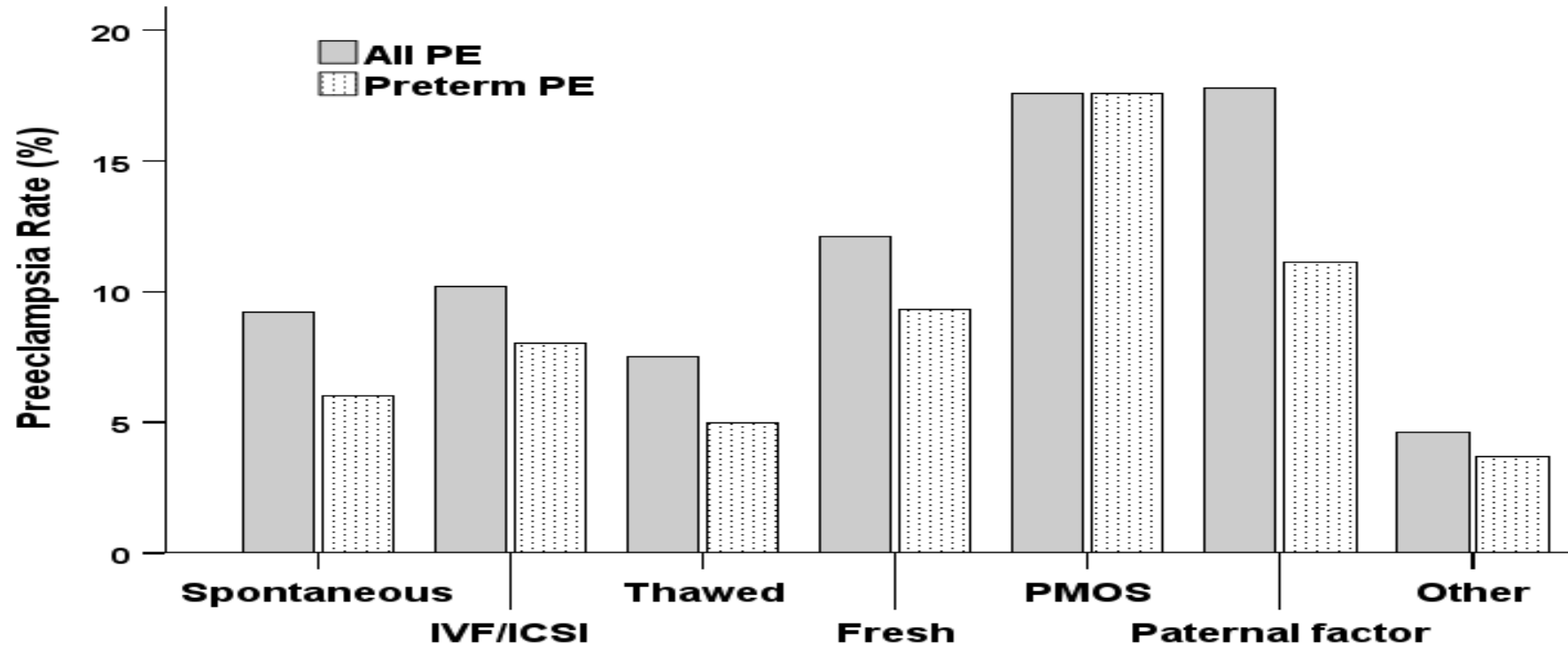


Figure 1: Study flow chart

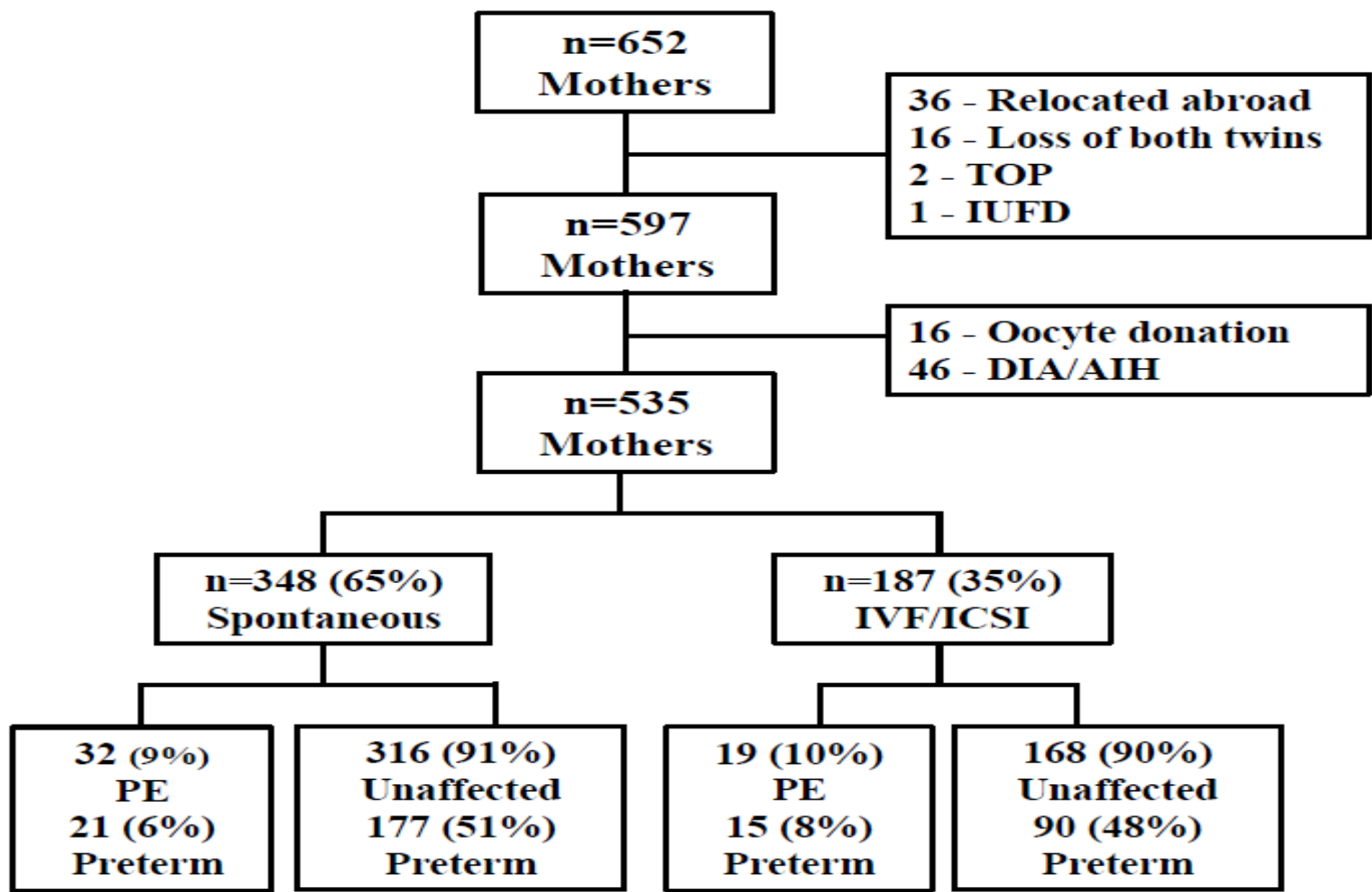


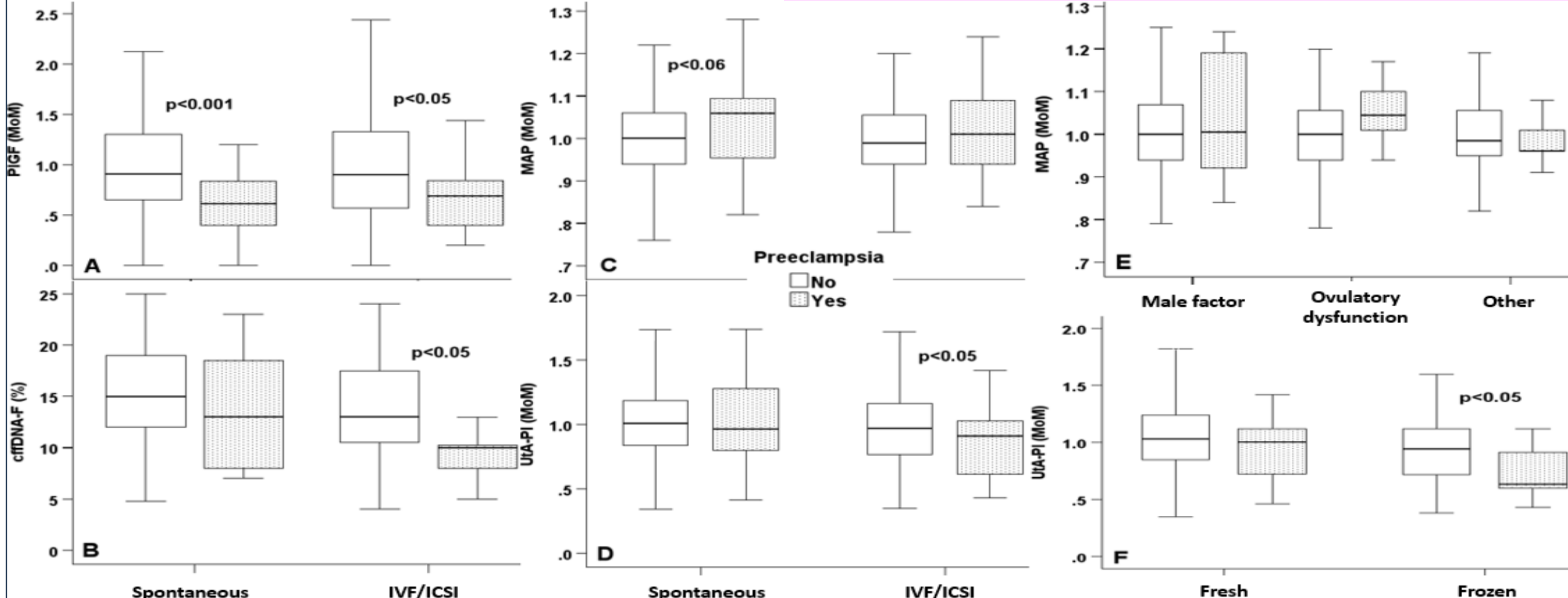
Table 1: Maternal characteristics at booking by mode of conception

Parameters	Spontaneous (n=348)	IVF / ICSI (n=187)	p
Maternal age y. Me (IQR)	33.5 (30.1-36.2)	35.6 (32.6-39.5)	<0.001
GA wk. at enrollment, Me (IQR)	12.7 (12.3-13.0)	12.6 (12.1-13.0)	0.584
BMI kg/m ² , Me (IQR)	24.5 (21.9-28.4)	24.9 (21.9-29.7)	0.313
Chorionicity, n (%)			
DCDA	227 (65.2)	151 (80.7)	<0.001
MCDA	121 (34.8)	36 (19.3)	
Nulliparous, n (%)	133 (38.2)	127 (67.9)	<0.001
Ethnicity, n (%)			
White	308 (88.5)	165 (88.2)	0.926
Other	40 (11.5)	22 (11.8)	
Maternal Chronic Diseases, n (%)			
DM	6 (1.7)	2 (1.1)	0.719
Thrombophilia	7 (2.5)	7 (4.9)	0.186
Hypothyroidism	37 (10.6)	20 (10.7)	0.982
CVD	11 (3.2)	2 (1.1)	0.154
Previous PE, n (%)	19 (5.5)	4 (2.1)	0.077
Pregnancy Outcome - Maternal			
GA wk. at delivery, Me (IQR)	36.5 (34.9-37.3)	36.9 (35.0-37.3)	0.495
Cesarean delivery, n (%)	225 (64.7)	148 (79.1)	<0.001
PTD, n (%)	(56.9)198	105 (56.1)	0.868
PE, n (%)	32 (9.2)	19 (10.2)	0.717
PE<37, n (%)	21(6)	15 (8)	0.075
Pregnancy outcome - Newborn	(n=696)	(n=374)	
Birth weight, All, g. Me (IQR)	2390 (2050-2682)	2415 (2080-2690)	0.216
Birthweight <1500 g. n (%)	52 (7.6)	17 (4.8)	0.089
NICU days, Me (IQR)	12 (4-27)	11 (3-20)	0.362
Respiratory Asphyxia, n (%)	116 (16.9)	52 (14.6)	0.347

Table 2: Multivariate analysis

Conception	Marker	AUC (95% CI)	P	Sensitivity at FPR		
				10%	15%	20%
Spontaneous All	PIGF	0.73(0.64-0.83)	<0.001	36	41	56
	PIGF+ cffDNA-F	0.81 (0.68-0.88)	<0.001	42	58	72
	PIGF+ cffDNA-F+ MAP	0.85(0.76-0.93)	<0.001	48	68	89
	PIGF+ cffDNA-F+ MAP+ Uta-PI	0.86(0.76-0.94)	<0.001	49	69	90
Spontaneous Preterm	PIGF	0.76(0.64-0.88)	<0.001	44	44	69
	PIGF+ cffDNA-F	0.88 (0.70-0.90)	<0.001	51	72	90
	PIGF+ cffDNA-F + MAP	0.92(0.76-0.96)	<0.001	60	83	98
	PIGF+ cffDNA-F+ MAP+ Uta-PI	0.93 (0.76-0.97)	<0.001	62	84	98
IVF/ICSI All	PIGF	0.71(0.60-0.82)	<0.001	28	33	45
	PIGF+ cffDNA-F	0.86(0.70.90)	<0.001	44	54	75
	PIGF+ cffDNA-F+ MAP	0.90(0.76-0.95)	<0.001	50	78	93
	PIGF+ cffDNA-F+ MAP+ Uta-PI	0.93(0.83-0.97)	<0.001	56	80	94
IVF/ICSI Preterm	PIGF	0.72(0.58-0.85)	<0.001	36	36	50
	PIGF+ cffDNA-F	0.87(0.72-0.91)	<0.001	51	72	90
	PIGF+ cffDNA-F+ MAP	0.90(0.76-0.96)	<0.001	60	83	96
	PIGF+ cffDNA-F+ MAP+ Uta-PI	0.95 (0.76-0.97)	<0.001	62	85	98
Frozen Embryos IVF	PIGF	(0.65-0.85)30.7	0.05	30	45	49
	PIGF+ Uta-PI	0.88 (0.69-0.94)	0.005	60	67	83
	PIGF+ Uta-PI+ cffDNA-F	0.89 (0.69-0.93)	0.002	61	74	84
	PIGF+ Uta-PI+ cffDNA-F+ MAP	0.89 (0.71-0.91)	0.002	62	75	85
Ovulatory Dysfunction	PIGF	0.65 (0.57-0.83)	0.03	30	47	52
	PIGF+ MAP	0.77 (0.62-0.84)	0.004	58	65	70
	PIGF+ MAP+ cffDNA-F	0.87 (0.69-0.91)	0.002	64	74	81
	PIGF+ MAP+ cffDNA-F+ Uta-PI	0.88 (0.71-0.91)	0.002	58	75	82

Figure 3: box-plot analysis



Conclusions: Preeclampsia risk in twins is influenced by the mode of conception (spontaneous versus IVF/ICSI), why IVF/ICSI was needed, and if fresh or frozen embryos were used. The findings enable tailoring personalized clinical management.

Figure 4: combined ROC curves

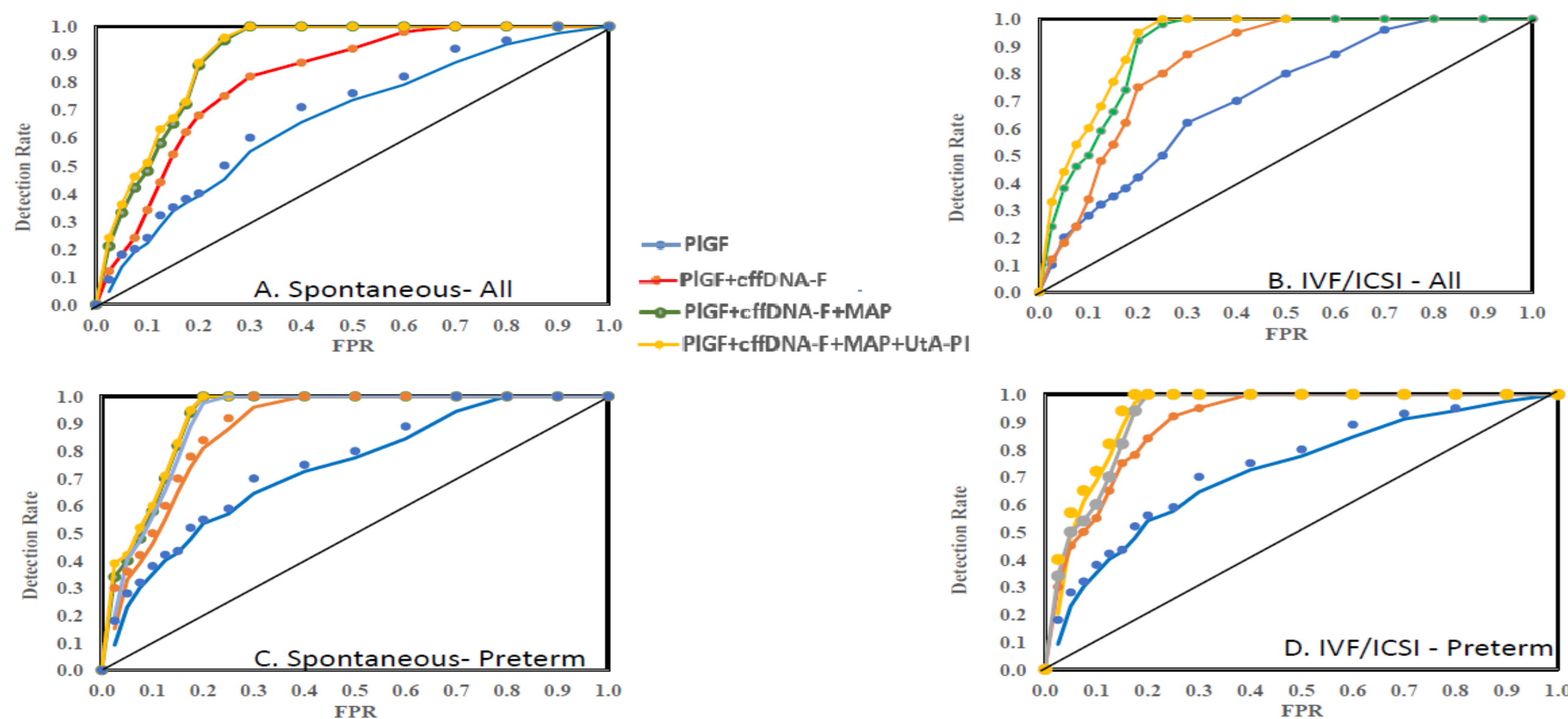


Table 3: Logistic regression models

Spontaneous Conception vs.	cffDNA,MAP, PIGF, Uta-PI History	# All Sample	# PE Samples	AUC	Coefficient Regression %	Odds Ratio
IVF/ICSI	+	535	52	0.87 [0.79-0.95]	50.2 [5.9-70.6]	18.25 [2.70-33.80]
FRESH	+	428	38	0.84 [0.77-0.91]	50.8 [34.4-63.1]	11.69 [3.79-19.59]
Thawed	+	455	45	0.86 [0.81-0.91]	50.2 [19.2-68.3]	11 [7.70-14.26]
PMOS	+	382	38	0.87 [0.81-0.93]	55.4 [38.1-68.4]	15.80 [10.52-10.99]
Paternal Factor	+	393	40	0.87 [0.79-0.95]	55.2 [27,5-73.0]	16.30 [10.20-32.42]
Others	+	457	40	0.85 [0.78-0.92]	45.7 [16.5-66.7]	15.2 [1.13-29.25]

Table 4: Summery of main findings:

Parameters	IVF/ICSI vs. Spontaneous	Frozen vs. Fresh	Ovulatory dysfunction vs. any other
Enrolment and delivery data			
Maternal age	�	-	�
Chorionicity, DCDA	�	-	-
Nulliparous	�	-	-
Thrombophilia	-	-	�
Cesarean delivery	�	-	-
GA at delivery	-	-	�
PE	-	-	�
Effective 1 st trimester Preeclampsia Screening Markers			
cffDNA	�	�	-
PLGF	�	�	�
MAP	�	-	�
Uta-PI	�	�	-
3 rd trimester Markers			
Uta-PI	�	�	-
sFLT-1/PIGF	�	-	-