Supplemental material for "Pregnancy achievement by medical assisted reproduction is correlated to the G protein-coupled receptor 30 mRNA abundance in human spermatozoa" by Pereira SC *et al,* 2022

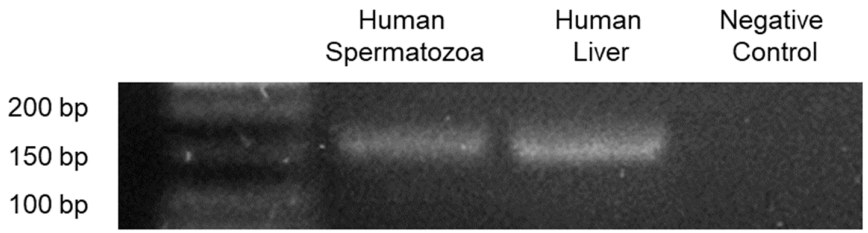

Figure S1: Identification of the *GPR30* transcript (~156 bp) in human spermatozoa through conventional polymerase chain reaction. A cDNA-free sample was used as a negative control. A human liver sample was used as a positive control.

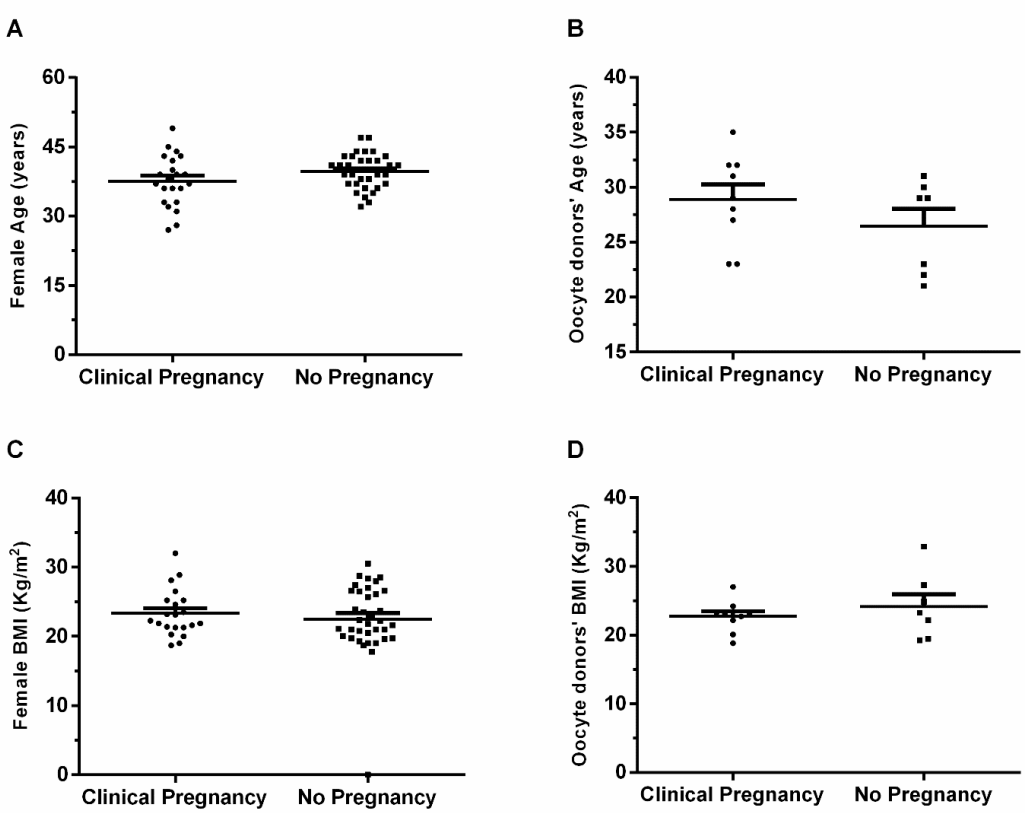

Figure S2: Association between the Age and BMI of females and oocyte donors with the outcome of ART. (A) From the total 60 embryo transfers, 22 women became clinically pregnant. The mean maternal age is represented in panel A, Clinical Pregnancy: 38 ± 2 years old, *n* = 22; No Pregnancy: 40 ± 1 years old, *n* = 32. (B) 16 embryos from oocyte donations were transferred. 9 women became pregnant. The mean age of oocyte donors is represented in panel B, Clinical Pregnancy: 29 ± 1 years old, *n* = 9; No Pregnancy: 26 ± 2 years old, *n* = 7. (C) The mean maternal BMI is represented in panel C, Clinical Pregnancy: 23.3 ± 0.7 Kg/m^2, *n* = 21; No Pregnancy: 22.5 ± 0.9 Kg/m^2, *n* = 32. The mean BMI of oocyte donors is represented in panel D, Clinical Pregnancy: 22.7 ± 0.7 Kg/m2, *n* = 9; No Pregnancy: 24.1 ± 1.8 Kg/m2, *n* = 7. All results are expressed as mean ± standard error mean. Statistical analysis was performed by two-tailed Student's t-teste for parametric data (confidence interval of 95%). No results were considered statistically significant.

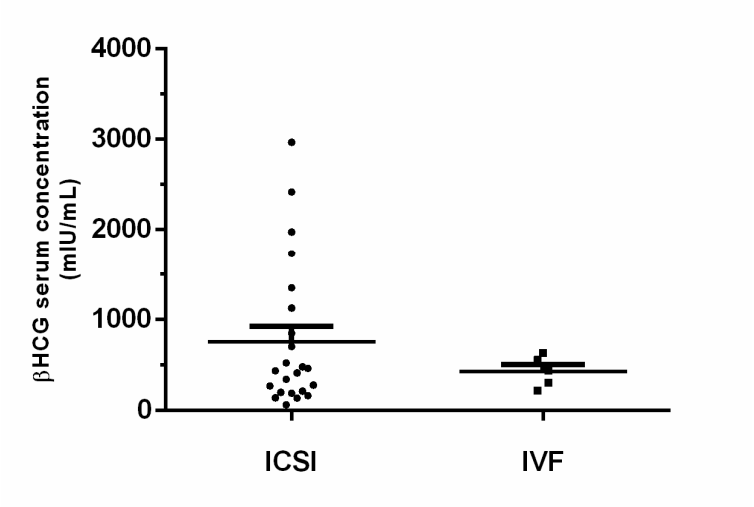

Figure S3: Association between fertilization procedures (ICSI and IVF) with the outcome of ART. From the total 60 embryo transfers, 28 women were reported to be biochemically pregnant after βHCG serum levels surpassed the value of 20 mIU/mL. ICSI was performed in 23 clinical cases, βHCG serum levels after embryo transfer: 759.2 ± 167.3 mIU/mL, *n* = 23. IVF was performed in 5 cases, βHCG serum levels after embryo transfer: 432.0 ± 76.2 mIU/mL, *n* = 5. The results are expressed as mean ± standard error mean. Statistical analysis was performed by Two-tailed Student's t-test (for nonparametric data, Kolmogorov-Smirnov test). No results were considered statistically significant.

Table S1: Clinical data from the participants of this study, Age and body mass index (BMI) of the cohort of couples that underwent ART (n=81), including the data of 16 females who donated oocytes used in 22 couples. Data are represented as mean ± standard deviation.

81 couples	Male Age (Years)	Male BMI (Kg/m²)	Female Age (Years)	Female BMI (Kg/m²)	22 oocyte donations from 16 different women	Oocyte donors Age (Years)	Oocyte donors BMI (Kg/m²)
	40 ± 5	26 ± 3	39 ± 4	23 ± 4		27 ± 4	23 ± 2

Table S2: Cut-of-values analysis of several parameters (male BMI, male Age, and sperm-quality parameters) in relation to pregnancy (biochemical and clinical) and no pregnancy groups. Statistical analysis was performed by two-tailed Student's t-teste for parametric data (confidence interval of 95%). Values of *P<0.05 were considered as statistically significant.

	Biochemical Pregnancy			**No Pregnancy**			***p***
	n	**MEAN**	**SEM**	*n*	**MEAN**	**SEM**	
Male BMI	28	26.43	0.5356	31	25.67	0.6421	0.3701
Male Age	28	37.32	0.9841	32	42.56	1.085	0.0008*
Total sperm count	28	272.6	51.82	32	253.0	34.71	0.7502
Total Motility	24	60.08	3.922	31	60.71	3.389	0.9040
Vitality	26	67.31	3.226	29	65.55	3.140	0.6985
Normal Morphology	28	5.679	0.8684	31	6.161	1.551	0.7929
	Clinical Pregnancy			**No Pregnancy**			***p***
	n	MEAN	SEM	*n*	MEAN	SEM	
Male BMI	22	26.27	0.5518	35	25.79	0.5929	0.5792
Male Age	22	37.18	1.135	36	41.86	1.032	0.0048*
Total sperm count	22	292.0	59.28	35	261.4	34.78	0.6354
Total Motility	19	62.63	3.567	35	58.77	3.474	0.4784
Vitality	20	69.35	2.109	32	66.00	2.901	0.4108
Normal Morphology	21	6.190	1.079	36	5.722	1.356	0.8122

Table S3: The association between *GPR30* mRNA abundance, and paternal age in couples that proceeded with embryo implantation (N=60). Statistical analysis was performed by computing Pearson correlation coefficients (r) assuming a Gaussian distribution (confidence interval of 95%). No correlations between the studied parameters were found to be statistically significant.

	Male Age (Age)		
	p	r	*n*
***GPR30* mRNA abundance**	0.0780	-0.2293	60