

# *Efficiency and efficacy of vitrification in 35 654 sibling oocytes from donation cycles*

*HUMAN REPRODUCTION, VOL.00, NO.0, PP. 1–10, 2020*

*SUBMITTED ON APRIL 2, 2019; RESUBMITTED ON MAY 6, 2020; EDITORIAL DECISION  
ON JUNE 17, 2020*

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*PUBLISHED BY OXFORD UNIVERSITY PRESS ON BEHALF OF EUROPEAN SOCIETY OF  
HUMAN REPRODUCTION AND EMBRYOLOGY*

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**STUDY QUESTION:** *Is oocyte vitrification/warming as efficient and effective as using fresh oocytes in donation cycles?*

**STUDY DESIGN, SIZE, DURATION:** *Retrospective cohort analysis of 1844 cycles of oocyte donation (37 520 oocytes), each donor in the study provided enough oocytes for at least one reception cycle with fresh oocytes (2561 cycles) and one reception cycle with vitrified oocytes (2471 cycles) from the same ovarian stimulation (sibling oocytes). Overall, 35 654 oocytes were considered in the analysis. All embryo transfers (n ¼ 5032) were carried out between 2011 and 2017.*

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## *Introduction*

*OOCYTE VITRIFICATION IS AN EFFECTIVE PROCEDURE TO STORE THE REPRODUCTIVE POTENTIAL OF OOCYTES; IT IS USED TO PRESERVE FERTILITY IN WOMEN UNDERGOING GONADOTOXIC PROCEDURE, AFFECTED BY CERTAIN GENETIC CONDITIONS(E.G. FRAGILE X AND TURNER'S SYNDROME) OR WISHING TO PRESERVE THEIRREPRODUCTIVE POTENTIAL IN THE FACE OF OVARIAN EXHAUSTION DUE TO AGE.*

*IT HAS ALLOWED THE DEVELOPMENT OF OOCYTE BANKS,WHICH IN TURN HAVE CHANGED PATIENTS' ACCESS TO THIRD-PARTY REPRODUCTION IN TIME AND SPACE.*

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***Cobo et al., 2008; Rienzi et al., 2010; Cobo and Diaz, 2011; Parmegiani et al., 2011:***

*EARLY RANDOMIZED CONTROLLED TRIALS (RCTS) HAVE SHOWN THAT VITRIFIED OOCYTES FROM YOUNG DONORS PROVIDE REPRODUCTIVE OUTCOMES THAT ARE COMPARABLE TO THE USE OF FRESH OOCYTES.*

*ALL THESE RCTS WERE CARRIED OUT BY HIGHLY TRAINED GROUPS WITH SIGNIFICANT CRYOPRESERVATION EXPERIENCE; HOWEVER, VITRIFICATION IS PER SE A DELICATE TECHNIQUE WHICH REQUIRES EXACT ADHERENCE TO PROTOCOL, AND IT IS PRONE TO INEFFICIENCIES, ESPECIALLY AMONG LESS EXPERIENCED TEAMS. MOREOVER, THERE IS SIGNIFICANT OPERATOR TO OPERATOR VARIATION IN ITS EFFICIENCY, AND THE ACHIEVEMENT OF >90% SURVIVAL RATE IS CONSIDERED AN INDICATOR OF GOOD COMMAND OF THE TECHNIQUE.*

***Kushnir et al., 2015; Crawford et al., 2017; Kushnir et al., 2018:***

*THE ANALYSIS OF LARGE REGISTRY-BASED COHORTS PERFORMED IN USA HAS INDICATED LOWER REPRODUCTIVE OUTCOMES OVERALL WITH THE USE OF VITRIFIED DONOR OOCYTES.*

***SHIRAZI ET AL., 2016; AMOUSHAHI ET AL., 2017; AZARI ET AL., 2017:***

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*MOREOVER, CAREFUL ANALYSIS OF VITRIFIED/ THAWED OOCYTES INDICATE ALTERATIONS IN GENE EXPRESSION PATTERNS AND REDUCED MITOCHONDRIAL DNA (MTDNA) CONTENT.*

***THIS STUDY:*** WE ASSESSED VITRIFICATION EFFICIENCY BY COMPARING REPRODUCTIVE OUTCOMES FROM AN OPTIMAL AND LARGE COHORT OF VITRIFIED OOCYTES IN RELATION TO A COHORT OF FRESH OOCYTES OBTAINED FROM THE SAME DONORS.

WE FURTHER TEST THE HYPOTHESIS THAT POST-THAWING OOCYTE LOSS WILL HAVE A NEGATIVE IMPACT ON REPRODUCTIVE OUTCOMES BUT NOT CARRY OVER EFFECTS COMPROMISING EMBRYO DEVELOPMENT COMPETENCE. TO TEST THIS, WE COMPARED ONLY THE VITRIFIED OOCYTES AVAILABLE TO ICSI WITH THEIR SIBLING FRESH COUNTERPARTS.

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## ***Materials and methods***

*THIS STUDY INCLUDES 35 654 METAPHASE II (MII) OOCYTES FROM 1844 CYCLES OF OOCYTE DONATION, WITH EACH DONOR PROVIDING OOCYTES FOR AT LEAST ONE RECIPIENT OF FRESH OOCYTES (2561 CYCLES) AND ONE RECIPIENT OF VITRIFIED OOCYTES (2471 CYCLES) FROM THE SAME OVARIAN STIMULATION CYCLE (SIBLING OOCYTES).*

***OOCYTE DONORS:*** 18 AND 35 YEARS OLD, NORMAL KARYOTYPE, GOOD GENERAL HEALTH AND A BMI BETWEEN 18 AND 30 KG/M<sup>2</sup> .

***RECIPIENTS:*** 23 AND 51 YEARS OLD.

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***MAIN INDICATIONS FOR OOCYTE DONATION:*** OVARIAN FAILURE (32.6%), ADVANCED AGE (29.6%) , PREVIOUS IVF FAILURE (21.1%).

***SPERM SAMPLES:*** NORMOZOOSPERMIC AND INCLUDED DONOR FROZEN SAMPLES AND FROZEN OR FRESH PARTNER SAMPLES. CASES OF TESTICULAR BIOPSY WERE EXCLUDED.

## ***Donor ovarian stimulation***

*RECOMBINANT FSH (GONAL-F VR , MERCK)*

*HIGHLY PURIFIED HMG (MENOPURVR , FERRING S.A.U., SPAIN)*

*PITUITARY SUPPRESSION: GNRH ANTAGONIST (CETROTIDEVR , 0.25 MG ,MERCK)*

*TRIGGER: THREE OR MORE FOLLICLES 18 MM, OVULATION WAS TRIGGERED WITH 0.3 MG OF GNRH AGONIST (DECAPEPTYLVR , IPSEN PHARMA S.A., SPAIN).*

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# *Oocyte manipulation*

*CUMULUS-OOCYTE COMPLEXES (COCS) WERE COLLECTED BY TRANSVAGINAL OPU 36 H AFTER TRIGGER.*

*DONOR OOCYTES WERE ALLOCATED TO MULTIPLE PATIENTS, WITH A MINIMUM OF FOUR MII ATTRIBUTED TO EACH RECIPIENT.*

*OOCYTES WERE VITRIFIED FOR LOGISTIC REASONS RELATED TO EMBRYO TRANSFER (ET) SCHEDULE OR IN THE CASE THAT A DONOR PRODUCED MORE OOCYTES THAN NEEDED FOR THE NUMBER OF RECIPIENTS PLANNED. THE OOCYTES COLLECTED AT OPU WERE ARBITRARILY ASSIGNED TO BE USED FRESH OR TO BE VITRIFIED.*

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*VITRIFICATION WAS PERFORMED USING KUWAYAMA'S METHOD 2 H AFTER OVUM PICK-UP*

SEMEN FREEZING WAS PERFORMED IN CRYOPROTECTANT AND THAWED ON THE DAY OF ICSI. ALL SEMEN SAMPLES UNDERWENT SELECTION BY SWIM-UP PRIOR TO ICSI.

ALL OOCYTES WERE INSEMINATED BY ICSI. FERTILIZATION WAS ASSESSED 16–19 H POST-ICSI BY VISUALIZATION OF TWO PRONUCLEI AND TWO POLAR BODIES. EMBRYO'S MORPHOLOGICAL SCORE WAS ASSESSED ON DAY 3 OF DEVELOPMENT BASED ON ITS DEVELOPMENTAL TIMING, THE NUMBER AND SYMMETRY OF THE BLASTOMERES AND THEIR FRAGMENTATION.

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THE BEST QUALITY EMBRYOS WERE TRANSFERRED FRESH TO THE UTERUS OF THE RECIPIENT AFTER 3–5 DAYS OF EMBRYO CULTURE.

## *Endometrial preparation*

*THE RECIPIENT ENDOMETRIUM WAS PREPARED WITH AN INJECTION OF GNRH AGONIST DEPOT IN THE MESOLUTEAL PHASE OF THE CYCLE.*

*A CONTROL ULTRASOUND: ON WOMEN WITHOUT MENSTRUAL CYCLES AND THOSE AT RISK OF A THIN ENDOMETRIUM (E.G. AFTER REPEATED CURETTAGE, UTERINE FIBROID EMBOLIZATION, PELVIC RADIOTHERAPY OR HISTORY OF UTERINE SYNECHIA).*

*ON THE FIRST DAY OF MENSTRUATION OF THE CYCLE: ESTROGEN 6 MG/DAY OF ORAL ESTRADIOL VALERATE OR 150 MG/DAY TRANSDERMAL ESTRADIOL HEMIHYDRATE.*

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*ON THE NIGHT OF THE RETRIEVAL RECIPIENTS: 400 MG/12 H OF MICRONIZED VAGINAL PROGESTERONE, CONTINUED UNTIL THE FIRST ASSAY OF BETA-HCG IN BLOOD 14 DAYS AFTER ET. IN CASE OF A POSITIVE PREGNANCY TEST, THE TREATMENT WAS PROLONGED UNTIL WEEK 12 OF PREGNANCY*

*A BIOCHEMICAL PREGNANCY: BETA-HCG LEVELS HIGHER THAN 5 IU/ML 15 DAYS AFTER THE ET.*

*CLINICAL PREGNANCY: A SAC WITH A VISIBLE EMBRYO WITH BEATING HEART 7 WEEKS AFTER LAST MENSTRUAL PERIOD WAS OBSERVED.*

*ONGOING PREGNANCY: A NORMALLY PROGRESSING PREGNANCY BY ULTRASOUND 12 WEEKS AFTER LAST MENSTRUAL PERIOD.*

*PREGNANCY RATES WERE CALCULATED PER ET.*

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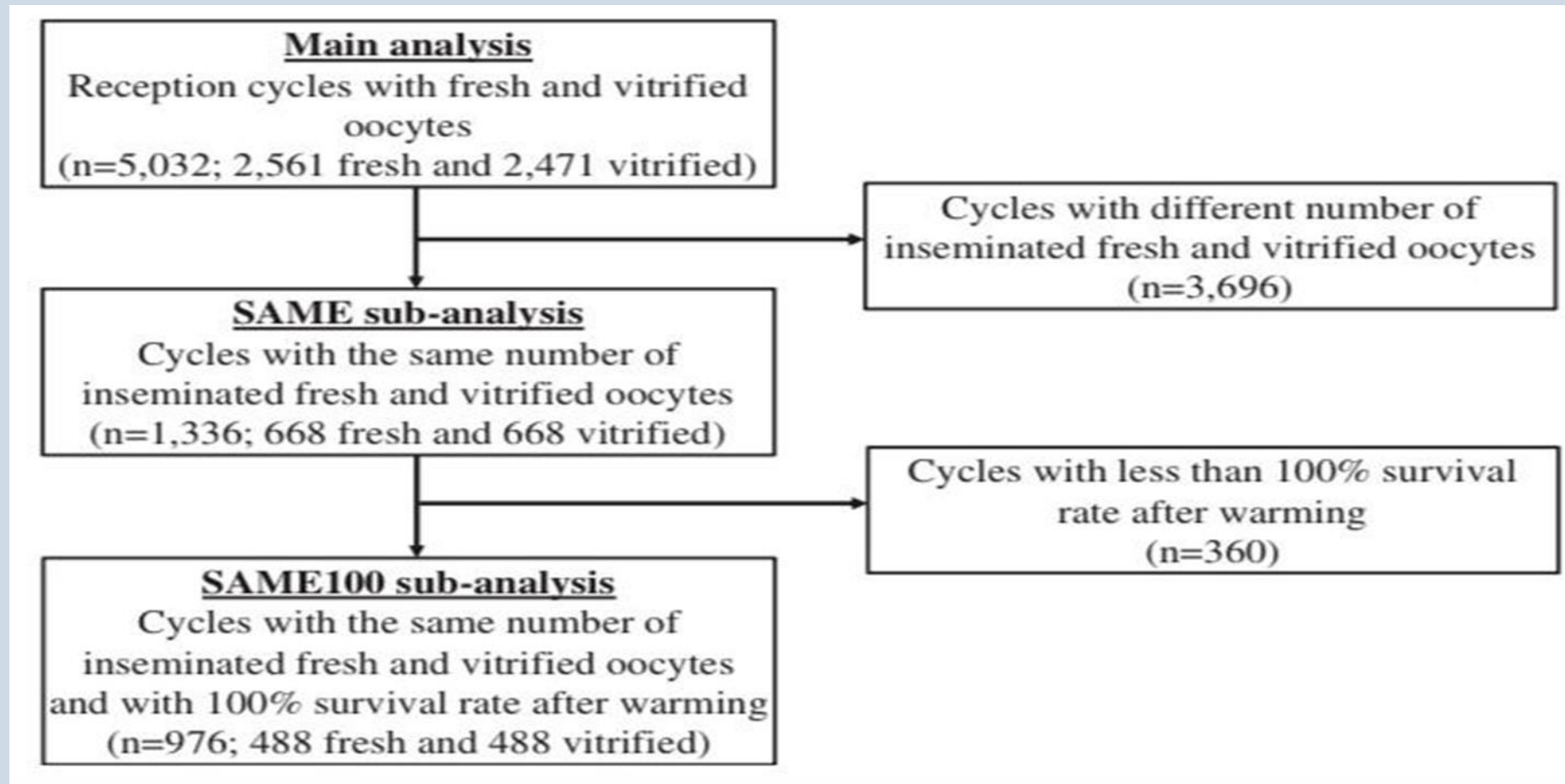
## *Statistical analysis*

*THE MAIN ANALYSIS : PERFORMED ON A TOTAL OF 5032 RECEPTION CYCLES, CONSISTING OF 2561 (50.9%) CYCLES WITH FRESH OOCYTES AND 2471 (49.1%) CYCLES WITH VITRIFIED OOCYTES. WE CALCULATED THE SURVIVAL RATES OF VITRIFIED OOCYTES AFTER THAWING ON THIS POPULATION.*

*A FIRST SUB-ANALYSIS (SAME): CONDUCTED SELECTING THE CYCLES WHERE THE SAME NUMBER OF PAIRED OOCYTES, EITHER FRESH OR VITRIFIED, WAS AVAILABLE FOR ICSI (I.E. AFTER THE LOSS OF OOCYTES DUE TO VITRIFICATION/WARMING).THE SAME ANALYSIS INCLUDED 1336 CYCLES: 668 WITH FRESH AND 668 WITH VITRIFIED OOCYTES.*

*A SECOND SUB-ANALYSIS (SAME100): PERFORMED SELECTING ONLY THE CYCLES THAT, BESIDES HAVING THE SAME NUMBER OF PAIRED OOCYTES AVAILABLE FOR ICSI, ALSO HAD 100% SURVIVAL RATE AFTER WARMING (I.E. OPTIMAL TECHNIQUE). THE SAME100 ANALYSIS INCLUDED 976 CYCLES: 488 WITH FRESH AND 488 WITH VITRIFIED OOCYTES.*

**FIGURE 1:** *THE NUMBER OF CYCLES INCLUDED IN EACH ANALYSIS AND NUMBER OF CYCLES EXCLUDED BECAUSE THEY DID NOT MEET THE INCLUSION CRITERIA FOR EACH ANALYSIS.*



**Figure 1.** Flow chart of the process followed to design the main analysis and the two sub-analyses (SAME and SAME100)

*DIFFERENCES IN FERTILIZATION RATE AND EMBRYO QUALITY: EVALUATED WITH A STUDENT'S T-TEST.*

*DIFFERENCES IN BIOCHEMICAL, CLINICAL, ONGOING PREGNANCY RATES AND LIVE BIRTH RATES (LBRS) AFTER THE FIRST ET: EVALUATED WITH A PEARSON'S CHI-SQUARE TEST.*

*IMPLANTATION RATES AND TWIN PREGNANCY RATE: COMPARED WITH A STUDENT'S T-TEST AND PEARSON'S CHI-SQUARE TEST, RESPECTIVELY.*

*THE EFFECT OF OOCYTE VITRIFICATION ON PREGNANCY AND LBRS: EVALUATED BY LOGISTIC REGRESSION ANALYSIS ADJUSTED FOR RECIPIENT'S AGE AND BMI, SPERM STATUS (FRESH OR FROZEN) AND ORIGIN (DONOR OR PATIENT), NUMBER OF EMBRYOS TRANSFERRED AND EMBRYO MORPHOLOGICAL SCORE.*

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*ALL ANALYSES WERE PERFORMED USING SPSS VERSION 22.0 (NEW YORK, USA). A P-VALUE*

## *Results*

*BASELINE AND CYCLE CHARACTERISTICS OF STUDY POPULATION ARE SHOWN IN **TABLE I** AND WERE COMPARABLE IN THE MAIN ANALYSIS, AS WELL AS IN THE SAME AND SAME100 ANALYSES.*

*MEAN (SD) DONOR'S AGE AND BMI WERE 25.9 (4.6) AND 22.5 (3.2).*

*DISTRIBUTION OF OOCYTE DONATION INDICATION WAS SIMILAR IN BOTH STUDY GROUPS.*

*PARTNER'S FROZEN SPERM WAS USED IN MOST CYCLES (69.7% OVERALL).*

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**Table 1** Descriptive statistics of the studied population in the main analysis.

	Overall n = 5032	Cycles with vitrified oocytes n = 2471	Cycles with fresh oocytes n = 2561
Recipients' age, Mean (SD)	41.6 (4.7)	41.6 (4.7)	41.5 (4.6)
Recipients' BMI, Mean (SD)	23.9 (4.4)	23.7 (4.2)	24 (4.6)
Recipient cycle number, Mean (SD)	1.3 (0.6)	1.3 (0.6)	1.3 (0.6)
First reception cycle, n (%)	3953 (78.6%)	1897 (76.8%)	2056 (80.3%)
Endometrial preparation length in days, Mean (SD)	37.2 (8.9)	39.3 (8.8)	35.1 (8.5)
Sperm used for ICSI, n (%)			
Donor frozen	790 (15.7%)	383 (15.5%)	407 (15.9%)
Partner frozen	3504 (69.7%)	1690 (68.4%)	1814 (70.9%)
Partner fresh	736 (14.6%)	398 (16.1%)	338 (13.2%)
Sperm quality (only for partners')			
Volume	1.0 (1.3)	1.0 (1.4)	0.9 (1.3)
Concentration	39.4 (41.0)	39.3 (43.6)	39.5 (37.8)
A+B%	16.6 (19.0)	16.8 (16.2)	16.5 (21.9)
Day of embryo transfer			
2-3, n (%)	4671 (92.8%)	2299 (93%)	2372 (92.6%)
5, n (%)	361 (7.2%)	172 (7%)	189 (7.4%)
Number of transferred embryos			
1, n (%)	610 (12.1%)	354 (14.3%)	256 (10%)
2, n (%)	4396 (87.4%)	2103 (85.1%)	2993 (89.5%)
3, n (%)	26 (0.5%)	14 (0.6%)	12 (0.5%)

## **Laboratory results**

DETAILED LABORATORY RESULTS PER STUDY COHORT ARE PRESENTED IN **TABLE II**.

OOCYTE SURVIVAL RATE AFTER VITRIFICATION/WARMING WAS 90.9%, 92.4% AND 100% IN THE MAIN, SAME AND SAME100 ANALYSES.

SURVIVAL RATES WERE ALSO SIMILAR BETWEEN YOUNGER (<30) AND OLDER (30) DONORS: 91.0% VS. 90.6% (P  $\frac{1}{4}$  0.37).

DESPITE A SIMILAR NUMBER OF INSEMINATED OOCYTES, VITRIFIED/THAWED OOCYTES SHOWED SIGNIFICANTLY LOWER FERTILIZATION RATE COMPARED TO FRESH OOCYTES IN THE THREE ANALYSES.

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THE MORPHOLOGICAL SCORE OF THE EMBRYO COHORT IN GENERAL AND OF TRANSFERRED EMBRYOS IN PARTICULAR WAS ALSO LOWER IN VITRIFIED OOCYTES THAN IN FRESH OOCYTES IN THE THREE ANALYSES, ALTHOUGH NOT CLINICALLY SIGNIFICANT.

**Table II** Laboratory results of the three analyses performed.

<b>Main analysis</b>	<b>Overall cycles n = 5032</b>	<b>Cycles with vitrified oocytes n = 2471</b>	<b>Cycles with fresh oocytes n = 2561</b>	<b>P-value</b>
Number of oocytes attributed to recipients	35 654	18 296	17 358	NA
Inseminated oocytes number (n)	33 993	16 635	17 358	NA
Inseminated MII, Mean (SD)	6.8 (1.4)	6.7 (1.5)	6.8 (1.2)	0.63
Fertilized MII (2PN), number, Mean (SD)	24 425 4.9 (1.7)	11 498 4.7 (1.7)	12 927 5 (1.6)	<0.001
Mean Fertilization rate (%)	72%	69.2%	74.6%	<0.001
Number of viable embryos, Mean (SD)	4.2 (1.6)	4 (1.6)	4.4 (1.6)	<0.001
Morphological score of embryo cohort, Mean (SD)*	7.1 (1.0)	7.0 (1.0)	7.2 (1.0)	<0.001
Morphological score of transferred embryos, Mean (SD)*	7.8 (1.2)	7.6 (1.2)	8.0 (1.1)	<0.001
<b>SAME sub-analysis</b>	<b>Overall n = 1336</b>	<b>Cycles with vitrified oocytes n = 668</b>	<b>Cycles with fresh oocytes n = 668</b>	<b>P-value</b>
Number of oocytes attributed to recipients	8905	4628	4277	NA
Inseminated MII, number, Mean (SD)	8554 6.4 (0.9)	4277 6.4 (0.9)	4277 6.4 (0.9)	I
Fertilized MII (2PN), number, Mean (SD)	6109 4.6 (1.5)	2940 4.4 (1.5)	3169 4.7 (1.4)	<0.001
Fertilization rate (%)	71.4%	68.7%	74.2%	<0.001
Number of viable embryos, Mean (SD)	4 (1.5)	3.9 (1.5)	4.2 (1.5)	<0.001
Morphological score of embryo cohort, Mean (SD)*	6.9 (1.6)	6.7 (1.6)	7 (1.6)	<0.001
Morphological score of transferred embryos, Mean (SD)*	7.8 (1.2)	7.7 (1.2)	8.0 (1.2)	<0.001
<b>SAME100 sub-analysis</b>	<b>Overall n = 976</b>	<b>Cycles with fresh oocytes n = 488</b>	<b>Cycles with fresh oocytes n = 488</b>	<b>P-value</b>
Number of oocytes attributed to recipients	6220	3110	3110	NA
Inseminated MII, number, Mean (SD)	6220 6.4 (0.9)	3110 6.4 (0.9)	3110 6.4 (0.9)	I
Fertilized MII (2PN), number, Mean (SD)	4430 4.5 (1.5)	2116 4.3 (1.5)	2314 4.7 (1.5)	<0.001
Fertilization rate (%)	71.3%	68.2%	74.4%	<0.001
Number of viable embryos, Mean (SD)	4 (1.5)	3.9 (1.5)	4.3 (1.5)	<0.001
Morphological score of embryo cohort, Mean (SD)*	7.1 (1.0)	7.0 (1.0)	7.2 (1.0)	<0.001
Morphological score of transferred embryos, Mean (SD)*	7.8 (1.2)	7.7 (1.2)	8.0 (1.2)	<0.001

# ***Reproductive outcomes***

## **MAIN ANALYSIS**

*SIGNIFICANTLY LOWER REPRODUCTIVE OUTCOMES RATES PER ET WERE OBSERVED FOR VITRIFIED OOCYTES COMPARED TO FRESH SIBLING OOCYTES IN THE MAIN UNIVARIABLE ANALYSIS:*

**BIOCHEMICAL PREGNANCY RATE:** 1031/2471 (41.7%) IN VITRIFIED VERSUS 1229/2561 (48.0%) IN FRESH OOCYTES ( $P < 0.001$ ).

**CLINICAL PREGNANCY:** WAS 905/2471 (36.6%) VERSUS 1102/2561 (43.0%) OOCYTES ( $P < 0.001$ ).

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**ONGOING PREGNANCY:** 794/2471 (32.1%) VERSUS 960/2561 (37.5%) OOCYTES ( $P < 0.001$ ).

**LIVE BIRTH:** 761/2454 (31.0%) VERSUS 918/2544 (36.1%),  $P < 0.001$  (LIVE BIRTH RESULTS WERE NOT AVAILABLE IN 34 CASES).

**IMPLANTATION RATE AT WEEK 7 OF PREGNANCY: SIGNIFICANTLY HIGHER WITH FRESH OOCYTES: 28.9% VS. 23.7% (P < 0.001).**

**TWIN PREGNANCIES: A HIGHER RATE OF FRESH OOCYTES(26.7% VS 20.8%, P¼ 0.027).**

**THE LOGISTIC REGRESSION ANALYSIS CONFIRMED THAT USING VITRIFIED OOCYTES HAD A SIGNIFICANT NEGATIVE EFFECT ON BIOCHEMICAL AND CLINICAL PREGNANCY, AND A TREND TO SIGNIFICANCE FOR ONGOING PREGNANCY AND LIVE BIRTH (TABLE III).**

**THE ADJUSTED ODDS RATIO (OR) AND 95% CI OF VITRIFIED VERSUS FRESH SIBLING OOCYTES FOR EACH REPRODUCTIVE OUTCOME: 0.84 (95% CI 0.74, 0.95) FOR BIOCHEMICAL, 0.85 (95% CI 0.75, 0.97) FOR CLINICAL, 0.88 (95% CI 0.77, 1.00) FOR ONGOING PREGNANCY AND 0.88 (95% CI 0.77, 1.01) FOR LIVE BIRTH.**

**Table III Multivariable analysis of sibling fresh and vitrified oocytes from the same stimulation cycle.**

	OR	95% CI		P-value	
		Lower	Upper		
Biochemical pregnancy	Vitrified versus fresh oocytes	0.84	0.74	0.95	0.005
	Recipients' age	0.98	0.97	1.00	0.011
	Recipients' BMI	0.98	0.97	0.99	0.002
	Partner frozen versus donors'	0.88	0.74	1.05	0.15
	Partner fresh versus donors'	0.91	0.73	1.14	0.40
	1 embryo versus 2	0.45	0.36	0.57	<0.001
	3 embryos versus 2	1.17	0.53	2.59	0.70
	Embryo quality	1.24	1.17	1.31	<0.001
Clinical pregnancy	Vitrified versus fresh oocytes	0.85	0.75	0.97	0.013
	Recipients' age	0.99	0.97	1.000	0.037
	Recipients' BMI	0.98	0.96	0.99	0.002
	Partner frozen versus donors'	0.91	0.76	1.08	0.27
	Partner fresh versus donors'	0.88	0.70	1.11	0.78
	1 embryo versus 2	0.45	0.35	0.57	<0.001
	3 embryos versus 2	1.24	0.55	2.75	0.61
	Embryo quality	1.28	1.21	1.35	<0.001
Ongoing pregnancy	Vitrified versus fresh oocytes	0.88	0.77	1.00	0.050
	Recipients' age	0.98	0.97	1.00	0.011
	Recipients' BMI	0.98	0.96	0.99	0.004
	Partner frozen versus donors'	1.02	0.85	1.22	0.87
	Partner fresh versus donors'	1.01	0.80	1.28	0.93
	1 embryo versus 2	0.47	0.36	0.60	<0.001
	3 embryos versus 2	0.74	0.31	1.79	0.50
	Embryo quality	1.30	1.23	1.38	<0.001
Live birth	Vitrified versus fresh oocytes	0.88	0.77	1.01	0.07
	Recipients' age	0.98	0.97	1.00	0.007
	Recipients' BMI	0.98	0.96	0.99	0.001
	Partner frozen versus donors'	1.02	0.85	1.22	0.84
	Partner fresh versus donors'	0.99	0.78	1.26	0.94
	1 embryo versus 2	0.47	0.37	0.60	<0.001
	3 embryos versus 2	0.77	0.32	1.87	0.57
	Embryo quality	1.30	1.23	1.38	<0.001

## **SAME ANALYSIS**

*THE NUMBER OF FERTILIZED OOCYTES WAS THE SAME FOR FRESH AND VITRIFIED OOCYTES (I.E. WHEN THE EFFICIENCY OF THE WARMING PROCESS WAS TAKEN INTO ACCOUNT).*

**REPRODUCTIVE OUTCOMES RATES: COMPARABLE IN THE UNIVARIABLE ANALYSIS:**

**BIOCHEMICAL PREGNANCY:** 292/668 (43.7%) IN VITRIFIED VERSUS 302/668 (45.2%) IN FRESH SIBLING OOCYTES,  $P \frac{1}{4}$  0.58.

**CLINICAL PREGNANCY:** 258/668 (38.6%) VERSUS 265/668 (39.7%),  $P \frac{1}{4}$  0.70.

**ONGOING PREGNANCY:** 224/668 (33.5%) IN BOTH GROUPS,  $P \frac{1}{4}$  1.

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**LIVE BIRTH:** 213/663 (32.1%) VERSUS 212/665 (31.9%),  $P \frac{1}{4}$  0.92 (LIVE BIRTH RESULTS WERE NOT AVAILABLE IN EIGHT CASES).

**IMPLANTATION RATE AT WEEK 7 OF PREGNANCY: SIMILAR BETWEEN GROUPS, 25.9% IN FRESH VERSUS 24.9% IN VITRIFIED OOCYTES (P¼ 0.59).**

**TWIN PREGNANCY RATE: FRESH 23.0% AND VITRIFIED 20.4%, (P¼ 0.49).**

THE LOGISTIC REGRESSION ANALYSIS ALSO SHOWED NO NEGATIVE EFFECT OF VITRIFICATION ON PREGNANCY RATES (**TABLE IV**).

**ADJUSTED ORS OF VITRIFIED VERSUS FRESH SIBLING OOCYTES: 1.06 (95% CI 0.84, 1.34) FOR BIOCHEMICAL PREGNANCY, 1.11 (95% CI 0.87, 1.42) FOR CLINICAL PREGNANCY, 1.16 (95% CI 0.90, 1.49) FOR ONGOING PREGNANCY AND 1.16 (95% CI 0.90, 1.50) FOR LIVE BIRTH.**

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**Table IV** Multivariable analysis of the **SAME** sub-analysis: cycles where the same number of paired oocytes, either fresh or vitrified, was available for ICSI.

		OR	95% CI		P-value
			Lower	Upper	
Biochemical pregnancy	Vitrified versus fresh oocytes	1.06	0.84	1.34	0.63
	Recipients' age	1.00	0.97	1.03	0.94
	Recipients' BMI	0.98	0.95	1.00	0.047
	Partner frozen versus donors'	0.71	0.52	0.97	0.030
	Partner fresh versus donors'	0.62	0.40	0.98	0.040
	1 embryo versus 2	0.40	0.26	0.62	<0.001
	3 embryos versus 2	6.6	0.47	14.5	0.27
	Embryo quality	1.23	1.11	1.36	<0.001
Clinical pregnancy	Vitrified versus fresh oocytes	1.11	0.87	1.42	0.39
	Recipients' age	1.00	0.97	1.02	0.79
	Recipients' BMI	0.98	0.95	1.00	0.07
	Partner frozen versus donors'	0.65	0.47	0.89	0.007
	Partner fresh versus donors'	0.55	0.35	0.88	0.012
	1 embryo versus 2	0.40	0.25	0.63	<0.001
	3 embryos versus 2	1.63	0.32	8.23	0.56
	Embryo quality	1.28	1.15	1.42	<0.001
Ongoing pregnancy	Vitrified versus fresh oocytes	1.16	0.90	1.49	0.26
	Recipients' age	0.99	0.97	1.02	0.69
	Recipients' BMI	0.98	0.95	1.00	0.09
	Partner frozen versus donors'	0.74	0.54	1.03	0.08
	Partner fresh versus donors'	0.71	0.44	1.13	0.15
	1 embryo versus 2	0.44	0.27	0.72	0.001
	3 embryos versus 2	1.07	0.19	5.97	0.94
	Embryo quality	1.31	1.18	1.46	<0.001
Live birth	Vitrified versus fresh oocytes	1.16	0.90	1.50	0.26
	Recipients' age	0.99	0.97	1.02	0.63
	Recipients' BMI	0.97	0.94	0.99	0.016
	Partner frozen versus donors'	0.75	0.54	1.04	0.09
	Partner fresh versus donors'	0.69	0.43	1.11	0.13
	1 embryo versus 2	0.41	0.25	0.68	0.001
	3 embryos versus 2	1.14	0.20	6.34	0.88
	Embryo quality	1.31	1.17	1.46	<0.001

## **SAME 100 ANALYSIS**

*WHEN CYCLES WITH THE SAME NUMBER OF INSEMINATED FRESH AND VITRIFIED SIBLING OOCYTES THAT ALSO HAD 100% SURVIVAL RATE AFTER WARMING WERE SELECTED, REPRODUCTIVE RESULTS WERE SIMILAR BETWEEN GROUPS:*

**BIOCHEMICAL PREGNANCY:** 220/488 (43.4%) IN VITRIFIED VERSUS 212/488 (45.1%) IN FRESH OOCYTES, P  $\frac{1}{4}$  0.61.

**CLINICAL PREGNANCY:** 195/488 (40.0%) VERSUS 180/488 (36.9%), P  $\frac{1}{4}$  0.32.

**ONGOING PREGNANCY:** 170/488 (34.8%) VERSUS 159/488 (32.6%), P  $\frac{1}{4}$  0.46.

**LIVE BIRTH:** 159/483 (32.9%) VERSUS 151/487 (31.0%), P  $\frac{1}{4}$  0.52 (LIVE BIRTH RESULTS WERE NOT AVAILABLE IN SIX CASES).

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**IMPLANTATION RATE:** SIMILAR BETWEEN GROUPS: 25.0% IN VITRIFIED VS. 24.6% IN FRESH OOCYTES (P  $\frac{1}{4}$  0.85).

**TWIN PREGNANCY RATE AT WEEK 7: 22.5% AND 17.3%, (P = 0.22).**

**THE LOGISTIC REGRESSION ANALYSIS CONFIRMED THESE RESULTS (TABLE V).**

**ADJUSTED ORS OF VITRIFIED VERSUS FRESH SIBLING OOCYTES: 1.24 (95% CI 0.94, 1.64) FOR BIOCHEMICAL PREGNANCY, 1.36 (95% CI 1.02, 1.8) FOR CLINICAL PREGNANCY, 1.32 (95% CI 0.98, 1.77) FOR ONGOING PREGNANCY AND 1.27 (95% CI 0.95, 1.71) FOR LIVE BIRTH**

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**Table V** Multivariable analysis of the **SAME100** sub-analysis: cycles with the same number of inseminated fresh and vitrified oocytes and with a 100% survival rate after warming.

		OR	95% CI		P-value
			Lower	Upper	
Biochemical pregnancy	Vitrified versus fresh oocytes	1.24	0.94	1.64	0.13
	Recipients' age	1.00	0.97	1.03	0.88
	Recipients' BMI	0.98	0.95	1.01	0.19
	Partner frozen versus donors'	0.70	0.48	1.02	0.07
	Partner fresh versus donors'	0.62	0.36	1.07	0.08
	1 embryo versus 2	0.31	0.18	0.51	<0.001
	3 embryos versus 2	1.97	0.32	12.0	0.46
	Embryo quality	1.22	1.11	1.37	0.001
Clinical pregnancy	Vitrified versus fresh oocytes	1.36	1.02	1.80	0.035
	Recipients' age	1.00	0.97	1.03	0.94
	Recipients' BMI	0.98	0.95	1.01	0.18
	Partner frozen versus donors'	0.64	0.44	0.93	0.020
	Partner fresh versus donors'	0.49	0.28	0.86	0.012
	1 embryo versus 2	0.31	0.18	0.53	<0.001
	3 embryos versus 2	1.11	0.18	6.85	0.91
	Embryo quality	1.23	1.13	1.44	<0.001
Ongoing pregnancy	Vitrified versus fresh oocytes	1.32	0.98	1.77	0.06
	Recipients' age	1.00	0.97	1.03	0.97
	Recipients' BMI	0.98	0.95	1.01	0.16
	Partner frozen versus donors'	0.62	0.43	0.92	0.016
	Partner fresh versus donors'	0.53	0.30	0.93	0.028
	1 embryo versus 2	0.37	0.21	0.64	<0.001
	3 embryos versus 2	1.43	0.23	8.81	0.70
	Embryo quality	1.27	1.11	1.44	<0.001
Live birth	Vitrified versus fresh oocytes	1.27	0.95	1.71	0.11
	Recipients' age	1.00	0.97	1.04	0.91
	Recipients' BMI	0.97	0.94	1.00	0.030
	Partner frozen versus donors'	0.65	0.44	0.96	0.031
	Partner fresh versus donors'	0.51	0.29	0.92	0.024
	1 embryo versus 2	0.33	0.18	0.59	<0.001
	3 embryos versus 2	1.52	0.25	9.39	0.65
	Embryo quality	1.24	1.09	1.41	0.001

## ***Discussion***

*THIS IS THE LARGEST STUDY COMPARING LABORATORY AND REPRODUCTIVE RESULTS OF SIBLING DONOR OOCYTES (VITRIFIED AND FRESH)*

***THE AIM OF OUR STUDY:*** *COMPARE EFFICIENCY AND EFFICACY OF VITRIFIED/WARMED AND FRESH DONOR OOCYTES IN RECIPIENT CYCLES.*

***A STRENGTH OF OUR STUDY:*** *USE OF SIBLING DONOR OOCYTES.*

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***CONTROLS FOR POTENTIAL CONFOUNDERS RELATED TO SELECTION AND OVARIAN STIMULATION:*** *WHILE CLINICAL OUTCOMES ARE NOT RELATED TO PATIENT'S INDICATION FOR OO-CYTE DONATION.*

**REPRODUCTIVE OUTCOMES:** LOWER BIO- CHEMICAL, CLINICAL AND ONGOING PREGNANCIES, AS WELL AS LOWER LBR IN VIT- RIFIED VERSUS FRESH OOCYTES, DESPITE THE HIGH SURVIVAL RATE AFTER WARMING (93%).

THESE RESULTS ARE IN AGREEMENT WITH ONE PREVIOUS REPORT

(**KUSHNIR ET AL., 2018**), BUT CONTRADICTS THE FINDINGS OBSERVED IN THE EARLY RCTS (**COBO ET AL.,2008; RIENZI ET AL.,2010; COBO AND DIAZ, 2011;**

**PARMEGIANI ET AL.,2011**) AND ALSO PREVIOUS STUDIES COMPARING FRESH AND VITRIFIED SIBLING OOCYTES (**TROKOUDES ET AL.,2011**).

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**OTHER STUDIES:**

- 1. REPORTS UPLOADED BY DIFFERENT CLINICS*
- 2. PROTOCOL FOR CRYOPRESERVATION USED IN EACH CLINIC IS NOT KNOWN.*
- 3. DIFFERENT CLINICS HAVE DIFFERENT EXPERTISE*

**THIS STUDY:**

*TO TEST THE HYPOTHESIS THAT THE EFFICIENCY OF VITRIFICATION/WARMING DRIVES REPRODUCTIVE OUTCOMES IN THESE CYCLES, WE PAIRED FRESH CYCLES WITH SIBLING VITRIFIED CYCLES WHERE THE SAME NUMBER OF OOCYTES WERE AVAILABLE AT ICSI.*

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***WE FOUND:*** *REPRODUC-TIVE OUTCOMES ARE SIMILAR IN THOSE TWO GROUPS, EFFICIENCY PLAYS A SIGNIFICANT ROLE IN REPRODUCTIVE OUTCOMES OF THESE CYCLES.*

## **OUR RESULTS:**

**1.** THAT CLINICS SHOULD ASSIGN PROPORTIONALLY MORE OOCYTES TO VITRIFIED/WARMED CYCLES THAN FRESH ONES, IN ORDER TO ACHIEVE SIMILAR REPRODUCTIVE RESULTS

**2.** THAT CYCLES WITH VITRIFIED OOCYTES MIGHT BE AFFECTED BY THE TECHNIQUE IN TWO DIFFERENT WAYS.

**FIRST:** THE AVAILABILITY OF A CERTAIN NUMBER OF OOCYTES DIRECTLY AFFECTS REPRODUCTIVE OUTCOMES BUT CAN BE MITIGATED BY INCREASING THE NUMBER OF ASSIGNED OOCYTES

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**SECOND:** VITRIFICATION MIGHT ALSO AFFECT THE DEVELOPMENTAL COMPETENCE OF THE OOCYTES THAT SURVIVED THE PROCESS, AS SUGGESTED BY SIGNIFICANT LOWER REPRODUCTIVE OUTCOMES FOUND IN OUR THREE ANALYSES IN TERMS OF BOTH FERTILIZATION RATE AND EMBRYO SCORES IN CYCLES WITH VITRIFIED OOCYTES



VITRIFICATION HAS BEEN REPORTED TO RESULT IN ALTERED GENE EXPRESSION, IMPAIRED ABILITY TO RESPOND TO A CALCIUM IONOPHORE, DIFFERENT CALCIUM OSCILLATION PATTERN AFTER ICSI, ALTERED LIPID STRUCTURE AND PROPERTIES, REDUCED MTDNA COPY NUMBER AND INCREASED REACTIVE OXYGEN SPECIES LEVELS IN VITRIFIED MAMMALIAN OOCYTES ([SHIRAZI ET AL., 2016](#); [AMOUSHAHI ET AL., 2017](#); [AZARI ET AL., 2017](#)).

BY ANALYZING CYCLES WITH 100 % SURVIVAL RATES, BIOLOGICAL EFFECT OF VITRIFICATION SHOULD HAVE THE LOWEST IMPACT.

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EFFICIENCY WAS THE MAJOR VARIABILITY, SIMILAR REPROD UCTIVE OUT COME IN THE SAME AND SAME100 ANALYSIS.

THE BIOLOGICAL EFFECT OF VITRIFICATION (EFFICACY) WAS MAJOR VARIABILITY ,HIGHER REPRODUCTIVE OUTCOMES IN THE SAME100 ANALY-SIS.

*THE RESULTS OF THE SAME AND SAME100 ANALYSIS OVERLAP ALMOST PERFECTLY, WHEN EXECUTED HIGH-QUALITY OOCYTES.*

*IN OUR STUDY, OOCYTES ARE ASSUMED TO BE OF GOOD QUALITY BE-CAUSE ALL DONORS WERE YOUNGER THAN 35, AND WE DID NOT FIND DIFFERENCES IN SURVIVAL RATES AFTER WARMING BETWEEN YOUNGER AND OLDER DONORS.*

*OVERALL, OUR RESULTS INDICATE THAT OOCYTE VITRIFICATION PER SE MAINTAINS THE DEVELOPMENTAL POTENTIAL COMPARABLE TO FRESH OOCYTES.*

*CONSEQUENTLY, CLINICS AND OOCYTE BANKS SHOULD ENFORCE STRICT QUALITY CONTROLS ON THEIR VITRIFICATION PROCESSES AND MAXIMIZE SURVIVAL RATES TO KEEP TREATMENT BOTH EFFECTIVE AND EFFICIENT.*

*TO IVF UNITS WITH SURVIVAL RATES LOWER THAN 90%. THESE RESULTS MIGHT NOT BE APPLICABLE TO PATIENT'S OOCYTE VITRIFICATION.*

*Thanks for attention*

***VRHRC.TUMS.AC.AR***

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