



Prospective trial of vitrification of all embryos in cycles of ART: implantation and pregnancy rate.

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ABSTRACT

Study question: To determine the pregnancy outcome after vitrification of all fresh embryos produced in stimulated assisted reproduction technique cycles (ART) and replacing them in subsequent non-gonadotropin stimulated cycles.

Summary answer: Vitrification of all fresh embryos produced in stimulated ART cycles and replacing them in subsequent non-gonadotropin stimulated cycles resulted in highly successful implantation rate and individual and cumulative pregnancy rates.

What is known already: It has been proposed that supraphysiological hormone levels during ovarian stimulation may adversely affect embryo implantation in assisted reproductive treatments. Very few studies have advocated the elective cryopreservation of all embryos as a method to avert the undesirable effect of gonadotropin ovarian stimulation on implantation and pregnancy rate, as well as to prevent the occurrence of iatrogenic events such as ovarian hyperstimulation syndrome (OHSS).

Study design, size, duration: A prospective trial of series of cases in a private fertility center-ART program. 135 patients (average age 35 years) with risk to develop OHSS or with uterine factor who underwent vitrification of all fresh embryos from January 2010 to May 2013.

Participants/materials, setting, methods: 1106 embryos were vitrified. Embryo survival rate was 98%. After thawing, 609 embryos were transferred into hormone replacement cycles in a total of 249 cycles. The average number of embryos transferred per cycle was 2.3.

Main results and the role of chance: 103 pregnancies were achieved for a cumulative clinical Pregnancy Rate (CCPR) of 84% per patient and 48% per cycle. These results are higher than fresh embryos transfer cycles for the center and study period (48% vs 31%). Implantation Rate (IR) was 22%. Pregnancies were achieved 57% in the first, 29% in the second, 11% in the third and 3% in the fourth cycle of thawing per patient, respectively. Of those patients that did not achieved successful clinical pregnancies, 57 % still have embryos vitrified (4,5 embryos/ patient).

Limitations, reason for caution: Descriptive measure of prospective trial.

Wider implications of the findings: These results reassure the role of embryo vitrification in an IVF program, and could also be a possible approach to prevent the alleged adverse effects of ovarian hyperstimulation on the implantation process, and it is tempting to propose its use routinely in all ART cycles in the future.

INTRODUCTION

It is well known that supraphysiological hormone levels during controlled ovarian stimulation (COS) for assisted reproduction techniques (ART) may adversely affect implantation in fresh embryos IVF cycles (1,2).

Several studies have advocated the elective vitrification of all embryos (EVAE) as a method to avert the undesirable effect of gonadotropin ovarian stimulation as well as prevent the occurrence of the ovarian hyperstimulation syndrome (OHSS) but very few have assessed pregnancy outcome in such cases. (3,4)

The aim of this study is to determine the pregnancy outcome after vitrification of ALL fresh embryos produced in a stimulated ART cycle and replacing them in subsequent non-stimulated cycles.

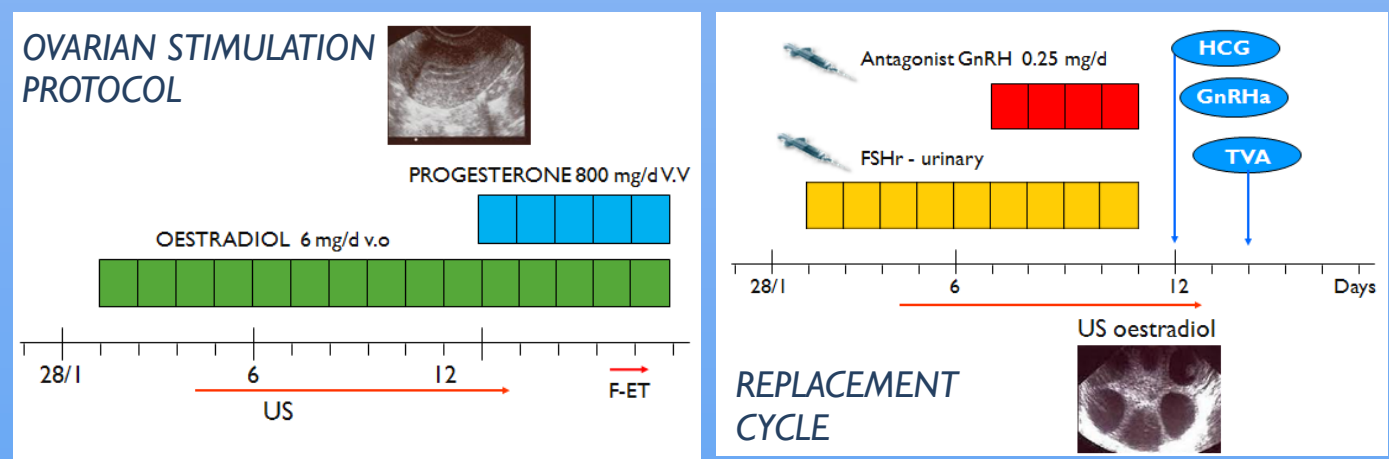
MATERIALS AND METHODS

We studied 135 patients (age range 24 to 43) who underwent vitrification of all fresh embryos in a controlled ovarian hyperstimulation (COH) cycle due to either a risk of severe OHSS or a uterine factor from January 2010 to May 2013. Ovarian stimulation was carried out with recombinant or urinary FSH and GnRH antagonist; ovulation was triggered with recombinant hCG or Leuprolide acetate for high responders (5). A total of 1106 embryos were vitrified with Kuwayama's technique at cleavage-stage embryos (72 h) or blastocyst stage (120 h) (6).

After thawing 693 embryos, 609 were subsequently transferred into a hormone replacement cycle in all 135 patients, in a total of 249 cycles (range 1 to 4 cycles per patient)(7). The average number of embryos transferred per cycle was 2.3 (range 1-3) Embryo survival rate, defined as at least 80% of intact blastomeres after thawing, was 98%. At the time of the assessment for this presentation 413 embryos remained vitrified.

✗ Patients (n)	135
✗ Replacement cycles (n)	249
✗ Vitrified embryos (n)	1106
✗ Embryos thawed (n)	693
✗ Embryo survival rate (%)	98
✗ Frozen embryos remaning (n)	413
✗ Embryos transferred (n)	609
✗ ET per cycles X (range)	2.3 (1-3)

Design: Prospective study of a series of cases in a University affiliated private fertility center- ART program.



RESULTS

✗ Patients (n)	135
✗ Replacement cycles (n)	249
✗ Clinical pregnancies (n)	105
✗ Pregnancy rate per cycle (%)	42,1 (105/249)
✗ Cumulative Pregnancy rate per patient (%)	77,7 (105/135)
✗ Implantation rate (%)	22,3 (136/609)*
✗ Multiple pregnancy rate (%)	14,2 (15/105)*
✗ Clinical abortion rate (%)	23,8 (25/105)

DISTRIBUTION OF PREGNANCIES BY TRANSFER CYCLE

	Cycle			
	1	2	3	4
N° of completed cycles	135	78	27	9
N° of pregnancies	64	28	10	3
Pregnancy rate per cycles (%)	47.4	35.8	37	33.3

Statistical comparison among all cycles: NS

DISCUSSION

Embryocryopreservation by vitrification is performed with increasing frequency, providing higher survival rates and minimal deleterious effects on post-thawing embryo quality, improving clinical outcome compared with conventional slow freezing.

Vitrification of all embryos offers patients an excellent chance of pregnancy when embryo transfer is performed in a non-gonadotropin stimulated cycle to avoid a negative influence of supraphysiological steroid level in embryo implantation as well as a strategy to prevent iatrogenic complication (3,4).

Several mechanisms for this impairment on implantation for supraphysiological steroid level have been proposed: -an asynchrony between endometrial development and embryonic age with delayed glandular maturation and advanced stromal secretion (8), -altered expression of genes and cytokines (9,10) -direct negative effect on embryo development (11).

Schoolcraft et al has found successful results when all embryos were vitrified after embryo biopsy and genetic screening with delayed transfer. The author claims that such results could be due to both strategies, delayed transfer and genetic screening (12).

Also, recent studies suggested that pregnancies arising from the transfer of frozen thawed embryos seem to have better obstetric and perinatal outcome as another beneficial effect to delayed transfer (13).

We compared the results in this study group of patients with all patients treated for ART in the same Institution that fulfilled similar criteria as population (age, duration and etiology of infertility) that received fresh embryo transfers and have additional embryos for vitrification. The results are shown in the following table:

PREGNANCY OUTCOME PER GROUP

	Group Vitri-Warm	Group Fresh	P value
Patients (n)	135	593	
Age X (range)	33.6 (24-43)	35.9 (26-43)	(NS)
Clinical pregnancies (n)	105	168	
Pregnancy rate per cycle (%)	42.1 (105/249)	28.3 (168/593)	0.006
Implantation rate (%)	22.3 (136/609)	16.2 (206/1274)	0.007
Multiple pregnancy rate (%)	23.8 (15/105)	12.5 (21/168)	0.711(NS)
Abortion rate (%)	23.8 (25/105)	22.6 (38/168)	(NS)

CONCLUSION

Based on the literature review it is tempting to speculate that the results of this study are the consequence of the delay in embryo transfer into the uterus in a state of more receptive milieu.

Successful outcome as high pregnancy rate and implantation rate makes us consider the option of EVAE with a subsequent transfer in a non-gonadotropin stimulated cycle as a strategy to apply in all cases, changing traditional thinking, improving pregnancy outcome and preventing iatrogenic complications of ART.

The high incidence of multiple pregnancies makes us rethink diminishing the number of embryos to transfer per cycle in the future.

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