IS THERE A ROLE FOR VITRIFICATION OF ALL EMBRYOS IN CYCLES OF ASSISTED REPRODUCTIVE TECHNOLOGY (ART) ?

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INTRODUCTION

Elective cryopreservation of all embryos in programs of assisted reproduction techniques with subsequent frozen-thawed embryo replacement has been advocated as a method to avert iatrogenic complications of gonadotropin ovarian stimulation such as severe ovarian hyperstimulation syndrome. (1) Additionally, it has been suggested that supraphysiological levels of estradiol during ovarian stimulation may adversely affect embryo implantation.(2) Elective vitrification of all embryos (EVAE) could provide an effective clinical approach to improve implantation in assisted reproductive techniques if frozen-thawed embryo replacement can take place into a spontaneous, non-hyperstimulated, more physiological cycle.(3) 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 73 patients (age range 24 to 43 years) who underwent vitrification of all fresh embryos in a controlled ovarian hyperstimulation (COH) cycle because of either the risk of severe ovarian hyperstimulation syndrome (OHSS)(n=54) or the presence of a uterine factor (UF) (inadequate endometrium or complicated embryo transfer) (n=19).Ovarian stimulation was carried out with recombinant or urinary FSH (Gonal F®-Serono or Menopur®-Ferring)and GnRH antagonist(Cetrotide®- Serono); ovulation was triggered with recombinant hCG (Ovidrel®- Serono) or Leuprolide acetate (Lupron®- Abott) for high responders. 560 embryos were vitrified according to the technique described by Kuwayama at 8 blastomeres or blastocyst stage.(4) After thawing 363 embryos, 320 embryos were subsequently transferred into hormone replacement cycle as previously described (5) in all 73 patients in a total of 126 cycles (range 1 to 4 cycles per patient). The average number of embryos transferred per cycle was 2.5. Embryo survival rate, defined as at least 80% of intact blastomeres after thawing, was 98%. There was not any hospital admission for OHSS. At the time of the calculations for this presentation 197 embryos remain vitrified.

Results

Table 1: Pregnancy Outcome Overall

Patients	73
Replacement cycles	126
Vitrified embryos	560
Embryos thawed	363 (56%)
Frozen embryos remaining	161 (44%)
Embryos transferred (ET)	320
ET per cycle (X- range)	2,5 (1-3)
Pregnancy rate per cycle (%)	40,5
Pregnancy rate per patient (%)	70
Multiple pregnancy rate (%)	21 -Seven sets of twins and four set of triplet
Clinical abortion rate (%)	17
Implantation rate (%)	20

Table 2: Pregnancy outcome per group

	Group OHSS*	Group UF**	statistics
Patients	54	19	
Age (years)	32,5	34,9	NS
Oocytes retrieval :X (range)	17 (9-35)	6,3 (2-11)	p=0.0002
Replacement cycles (n)	103	23	NS
Transferred embryos (n)	261	59	NS
Vitrified embryos remaining (n)	185	12	NS
Clínical pregnancies (n)	42	9	NS
Abortion rate (%) (n)	19(8/42)	11,11(1/9)	NS
Implantación rate (%)	20,3	15,25	NS
Pregnancy rate per cycle (%)	41	39,1	NS
Pregnancy rate per patient (%)	78	47	NS

**OHSS risk was defined previously described by Asch et al in 1991 (6)*

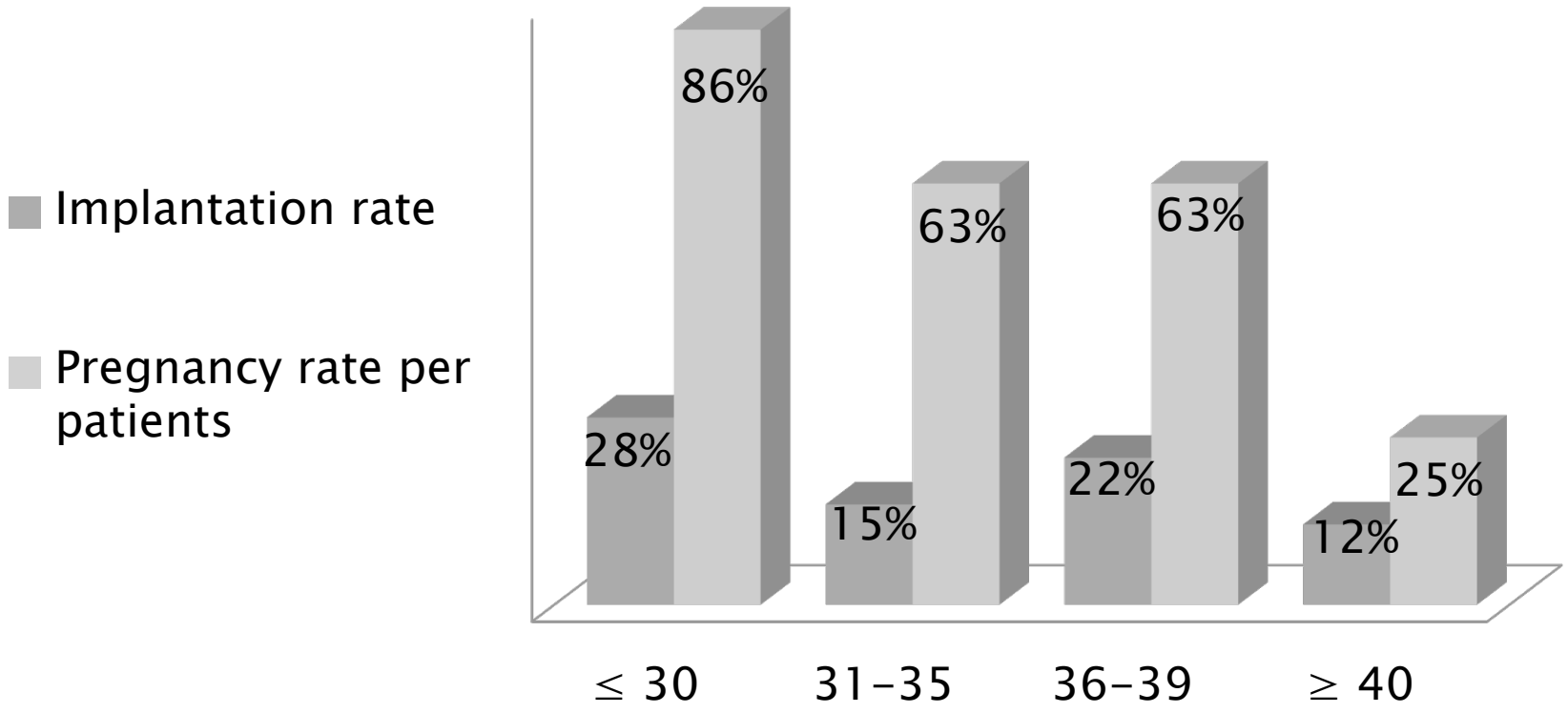
***Uterine Factor was defined as suboptimal endometrial thickness on ultrasound at the time of oocyte recovery or difficulty embryo transfer.*

Table 3: Distribution of pregnancies by transfer cycle

	Cycle 1	Cycle 2	Cycle 3	Cycle 4
Nº of Completed cycles	**73**	**37**	**12**	**4**
Nº of pregnancies	**30**	**13**	**6**	**2**
Pregnancy rate per cycle (%)	**41**	**35,13**	**50**	**50**

Statistical comparisson among all cycles: NS

Figure 1: Pregnancy and implantation rate according to age



No statistical significance for clinical rate and implantation rate was observed among groups.

Tabla 4: Current status of all patients according to occurrence of pregnancy and disponibility of vitrified embryos

	Remaining vitrified embryos	
PREGNANCY	YES	NO
YES	28*	14*
NO	13*	18+
TOTAL	41	32

(*) 76 % of all patients became pregnant and/ or have the potential of conceiving from vitrified embryos.

(+)24% of all patients did not become pregnant and have no further chance of conceiving due to exhaustion of all vitrified embryos.

Table 5: Theoretical Calculation for cumulative pregnancies rate when all embryos are transferred

N° current existing pregnancies (n)	51
N° of patients with vitrified embryos (n)	41
N° of embryos remaining (n)	161
N° of embryos available for transfer(n) (98% survival)	158
N° of transfers (n) (2,5 embryo per transfer)	63
N° of additional pregnancies (n) (assumption 40% overall PR per cycle)	25
N° of total possible pregnancies (n)	76

Conclusions

These results suggest that elective vitrification of all embryos (EVAE) in a cleaved stage offers patients an excellent chance of pregnancy when embryo transfers are performed in a gonadotropin non-stimulated cycle. In our patient population, 76% of all participants either have become pregnant or still have a chance of conceiving from vitrified embryos in storage. On the other hand, 24% of the patients did not become pregnant and don't have any further chance of conceiving due to exhaustion of frozen embryos. This successful pregnancy outcome could be due to the temporal dissociation of the ovarian stimulation and the embryo transfer to avoid a negative influence of supraphysiological steroid concentration on embryo implantation(7). It has been proposed that an asynchrony between the endometrial development and embryonic age with delayed glandular maturation and advanced stromal secretion as a possible mechanism that may cause detriment of the implantation process(8). More recently, a trial that investigated endometrial profile of gene expression in luteal phase of natural and stimulated cycles in high responders found that high serum estradiol levels caused aberrant expression of genes affecting the regulation of a number of biologic pathways that could play important roles on human implantation.(9) Other investigators have proposed that increasing levels of estradiol are deleterious to embryonic implantation because they directly adversely affect the embryo development to the blastocyst stage (10, 11).

Vitrification of human cleavage stage embryos in an ART program is performed with increasing frequency, providing higher survival rates and minimal deleterious effects on post-thawing embryo morphology and improving clinical outcome compared with conventional slow freezing. Recently, a great interest in embryo cryopreservation by vitrification to avoid the incidence of multiple embryo transfer has been reported(12). Based on the results of the present study obtained in patients that underwent IVF who were at risk for OHSS and those with a suboptimal endometrium, we can theorize that the option of vitrification and storing ALL embryos with a subsequent transfer in a gonadotropin non-stimulated cycle as a potential successful alternative to fresh transfers and could be expanded virtually to almost all cycles in an ART program.

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