The Added Value of Embryo Cryopreservation to Cumulative Ongoing Pregnancy Rates Per IVF Treatment: Is Cryopreservation Worth the Effort?

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Purpose: To calculate the added benefit of a cryopreservation program to the cumulative ongoing pregnancy rate over a maximum of three cycles of IVF.

Methods: A total of 1251 couples beginning their first IVF treatment between January 1995 and December 1999 were evaluated. Ongoing pregnancies from fresh and subsequent cryopreserved embryo transfer cycles were analyzed. Pregnancies arising from the cryopreservation cycle were considered to augment the cumulative pregnancy rate when no ongoing pregnancy arose from the fresh embryo transfer cycle.

Results: The ongoing pregnancy rate per cryopreserved embryo transfer was 11.7%. The cumulative ongoing pregnancy rate following three successive started fresh IVF cycles was 42.5%. When pregnancies arising from the transfer of thawed cryopreserved embryos were included, the cumulative ongoing pregnancy rate increased to 43.8%, rising to 44.8% when extrapolated data from as yet unfrozen embryos was included.

Conclusions: When analyzed in these terms, the supplementary benefit of cryopreserving supranumerical embryos appears limited.

KEY WORDS: Blastocyst; cryopreservation; embryo; ethics; health economics; IVF; minimal ovarian stimulation; pregnancy.

INTRODUCTION

The first reports of a clinical pregnancy (1) and birth (2) from frozen-thawed embryos introduced the clinical application of cryobiology into in-vitro fertilization (IVF) programs. Since the recognition of cryopreservation of preimplantation embryos as an established clinical procedure (3), its application in IVF programs has steadily increased, becoming

integral to the service provided by the majority of IVF centres (4).

Ovarian stimulation of multiple dominant follicle development has considerably improved the clinical outcome of assisted reproductive techniques (5,6). At present, the most commonly practised ovarian stimulation regimen for IVF includes pituitary down regulation with a gonadotrophin-releasing hormone (GnRH) agonist for at least 2 weeks followed by the coadministration of high dose exogenous follicle stimulating-hormone (FSH). This so-called long protocol aims to induce ongoing growth of large numbers of follicles in normo-ovulatory women, resulting in large quantities of oocytes (and resultant embryos) obtained from a single oocyte retrieval (7). The long protocol for ovarian hyperstimulation has been shown to be effective in providing large numbers of embryos for transfer or cryopreservation,

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improved pregnancy rates, and lower cancellation rates compared to other stimulation protocols for IVF (8).

The transfer of all available embryos dramatically increases the risk of high order multiple pregnancies and the associated obstetrical and perinatal complications. As a result, many IVF centres have adopted the policy of limiting the number of embryos transferred and of cryopreserving surplus embryos in an attempt to reduce the risk on multiple pregnancies and births (9). Indeed, the availability of cryopreservation programs is frequently cited as a justification for maximal stimulation of large quantities of follicles in a single IVF cycle (10). The risks associated with this approach has provoked increasing concern (11–13). The obligatory intensive conventional ovarian stimulation and extended oocyte retrieval procedures levy a high degree of physical and emotional stress on patients undergoing IVF (13). This is reflected by the high dropout rate seen in conventional IVF. If no pregnancy occurs in the first IVF attempt, many patients (around 25%) fail to attend second attempt, even in countries where IVF is covered by health insurance (14,15).

Cryopreservation of supranumerical embryos and transfer of thawed cryopreserved embryos in a subsequent cycle is now generally accepted. However, this approach is not free of legal, moral, and ethical issues (16,17). The added benefit of cryopreservation programs to IVF remains uncertain (18). A significant portion of frozen preimplantation embryos will not survive the thawing process (19). Moreover, embryo transfer of thawed cryopreserved preimplantation embryos is less successful in terms of pregnancy and implantation rates when compared to transfer of fresh embryos (20).

We carried out an observational longitudinal study to calculate the benefit of a cryopreservation program to the overall cumulative ongoing clinical pregnancy rate over a maximum of three consecutive IVF cycles.

METHODS

Study Population

All couples with an indication for IVF (21) who began their first IVF treatment, with or without intracytoplasmic sperm injection (ICSI), in the period between January 1995 to December 1999 were evaluated. In The Netherlands, the obligatory health insurance covers three IVF cycles, independent of the indication for IVF. In order to reduce possible bias

related to socioeconomic status a maximum of three IVF cycles, including subsequent cryopreservation of supranumerical embryos, were analyzed. Women who began pituitary down regulation in our routinely applied “long protocol” and agreed on cryopreservation of the surplus of preimplantation embryos were included in the study ($n = 1792$). Couples progressed to a second or third stimulation IVF cycle only when all cryopreserved embryos had been thawed for intended transfer. Couples undergoing oocyte donation, who had undergone previous IVF treatments or who had decided to cancel treatment, were excluded from analysis. The study was performed according to the standards outlined in the Declaration of Helsinki.

IVF Procedure

All patients underwent pituitary downregulation by daily s.c. injections of 0.1 mg of the GnRH agonist triptorelin (Decapeptyl, Ferring GmbH, Kiel, Germany) for at least 2 weeks prior to ovarian stimulation. Ovarian stimulation was initiated by daily injections (150–450 IU) of human menopausal gonadotrophin (HMG; Humegon, NV Organon, Oss, The Netherlands) i.m. or recombinant FSH (RecFSH; Puregon, NV Organon) s.c. When at least three follicles ≥ 18 mm were observed on transvaginal sonography, all medication was stopped and 10,000 IU human chorionic gonadotrophin (hCG; Pregnyl, NV Organon) was administered by i.m. injection in order to complete oocyte maturation. Oocyte retrieval took place 35 h after hCG administration. A maximum of two embryos were transferred on Day 3–5 after oocyte retrieval according to our previously published protocol (22,23). Surplus embryos of adequate quality were subsequently cryopreserved. Luteal phase support was initiated on the day of embryo transfer by daily vaginal administration of 600 mg progesterone (Progestan, NV Organon) and continued until menstruation or a positive urinary hCG test. Biochemical pregnancy was defined as a positive urinary hCG test 2 weeks after embryo transfer. Ongoing pregnancy was defined as a positive intrauterine fetal heart activity observed by transvaginal sonography 10 weeks after embryo transfer.

Cryopreservation and Thawing Procedure. Embryos remaining after transfer and meeting previously published quality criteria (22,24) were frozen. The cryopreservation procedure was performed using a slow freezing 1.5 M DMSO (British Drug House Ltd., Poole, United Kingdom) protocol (19) in a programmed freezer (Planer Kryo 10-III; Planer

Products, Sunbury-on-Thames, United Kingdom). The medium for cryopreservation consisted of Earle's balanced salt solution and Hams F10 medium (Life Technologies, Paisley, United Kingdom), supplemented with a pasteurized plasma protein solution and 1.5 M DMSO. Embryos were placed in medium for cryopreservation and subsequently transferred into cryostraws and equilibrated for 30 min at room temperature. Cryostraws were then placed in the freezing apparatus, preset at 0°C and frozen as follows: The embryos were cooled to -4°C at a rate of -2°C/min and subsequently cooled further at a rate of -1°C/min until a temperature of -6°C was reached. Seeding was performed manually with liquid nitrogen (LN₂)-cooled sponge forceps. Further cooling was performed at a rate of -0.3°C/min to -40°C and then at -40°C/min to -120°C, at which temperature the cryostraw was immersed in LN₂. Thawing was performed by removing the cryostraw from the LN₂, followed by placing in ice water. The embryos were removed from the cryostraw and placed in diluted freezing medium of consecutively 1.2 M, 0.9 M, 0.6 M, 0.3 M DMSO for 5 min, respectively. Embryos were subsequently rinsed in embryo culture medium and transferred to a 25 µL droplet in a culture dish, followed by culturing before transfer. Postthaw survival of cryopreserved embryos was defined as ≤50% blastomere loss. A maximum of two selected thawed embryos were transferred in the natural cycle after ovulation, timed by urinary LH tests or monitoring by transvaginal sonography. The same transfer technique was used as in fresh transfer cycles.

DATA ANALYSIS

For the purpose of measuring the ongoing pregnancy rate of IVF treatment from both fresh and cryopreserved embryos, pregnancies arising from cryopreservation cycles were considered to contribute to the pregnancy rate of the associated stimulation cycle. The "patient-specific concept" proposed by Jones *et al.* (18) is characterized by considering pregnancies arising from the cryopreservation cycle as augmentation only among patients who did not achieve an ongoing pregnancy from the fresh embryos transfer cycle. We applied this concept to evaluate the contribution of cryopreservation programs to the cumulative pregnancy rate of a maximum of three IVF cycles. The "projected cryoaugmented pregnancy rates," which assume that pregnancy rates of patients with unthawed cryopreserved embryos are comparable to

those who had an embryo transfer of thawed cryopreserved embryos, were calculated (25). Cumulative ongoing pregnancy rates calculated by life table analysis assume that patients who drop out from the study have the same probability of achieving an ongoing pregnancy as patients who continue treatment. In order to test this assumption, differences in patient characteristics, which may affect the probability of achieving an ongoing pregnancy, between patients who continue treatment and those who drop out from the study were compared using Mann-Whitney *U* test and Fisher's exact test. Clinical outcome in terms of ongoing pregnancy rates was analyzed by Markov chain analysis (26) to estimate the added value of the cryopreservation program. Differences were considered significant if $P < 0.05$.

RESULTS

Patients

In the period January 1995 to December 1999, 1251 couples met the inclusion criteria for analysis. The age of the women at the start of treatment ranged from 21 to 44 years. A total of 77.8% of the study population was treated for primary infertility. Indications for IVF were respectively male factor (44.4%), tubal factor (22.5%), idiopathic (21.6%), anovulation (4.8%), cervical hostility (4.0%), endometriosis (2.4%), and other (0.2%). Fertilization of the oocytes by ICSI was performed in 12.1% of the couples.

Couples who had children prior to IVF treatment and couples in which the IVF procedure did not result in an embryo transfer were more likely to stop treatment following an unsuccessful cycle (respective odds ratios 1.5 (95% CI 1.1–2.0) and 3.1 (95% CI 1.9–5.1)). These couples were considered as noninformative dropouts, since pregnancy rates in those patients who did continue were comparable to those of the rest of the study population.

Treatment Outcome

In the 1251 couples analyzed, a total of 2367 IVF cycles and 392 thawed cryopreserved embryo cycles were initiated. An embryo transfer of at least one embryo was performed in 71.8% of the started IVF cycles. In 25.0% of the initiated IVF cycles surplus embryos were available for cryopreservation. The postthaw survival rate of cryopreserved embryos was 24.9% per thawed embryo. An embryo transfer

Fig. 1. Results of 2367 IVF cycles initiated between January 1995 and December 2000 in 1251 patients. Overall pregnancy and ongoing pregnancy rates per initiated fresh cycle are given, together with results of the subsequent cryo-thaw transfer cycles.

was performed in 41.3% of the initiated thawed cryopreserved embryo cycles. Pregnancy and ongoing pregnancy rates per initiated cycle were 21.6 and 17.6% for the fresh embryo transfer cycles and 5.9 and 4.8% for thawed cryopreserved embryo cycles, respectively. Corresponding rates per embryo transfer were 30.0 and 24.5% for the fresh embryo transfer cycles and 14.2 and 11.7% for the thawed cryopreserved embryo cycles, respectively (Fig. 1).

Of the 1251 women beginning their first IVF stimulation cycle, 864 underwent fresh embryo transfer and 227 achieved an ongoing pregnancy. Of the 1024 women who did not conceive, 335 had embryos cryopreserved. Of these, 217 women had their embryos thawed, resulting in embryo transfer in 94 women, with 14 achieving an ongoing pregnancy. For 118 women unthawed embryos were still available for thawing and subsequent transfer. For these women a total of eight projected pregnancies was calculated (25) (Table I).

A total of 741 women proceeded to a second and 375 to a third IVF stimulation cycle, of whom respectively 557 and 280 underwent fresh embryo transfer, with 137 and 52 achieving an ongoing pregnancy,

respectively. In the second cycle, embryos from 167 nonconceiving women were cryopreserved, of whom 112 had their embryos thawed, resulting in an embryo transfer for 45 women and subsequently four ongoing pregnancies. In the third cycle, embryos of 90 nonconceiving women were cryopreserved, 63 had their embryos thawed, resulting in an embryo transfer for

Table I. Clinical Outcome of Three Consecutive IVF Cycles of 1251 Patients Starting IVF in the Period 1995–99

	Cycle 1	Cycle 2	Cycle 3
Number of patients who started IVF	1251	741	375
Fresh embryo transfer (fresh ET)	864	557	280
Pregnant after fresh ET	227	137	52
Not pregnant after fresh ET and no cryo embryos available	916	574	285
Not pregnant after fresh ET and cryo embryos available	335	167	90
Cryopreserved embryos thawed	217	112	63
Embryo transfer of thawed cryopreserved embryos (cryo ET)	94	45	23
Pregnant after cryo ET	14	4	1
Unthawed cryopreserved embryos	118	55	27
Projected pregnancies	8	3	0

Note. Values represent number of patients

23 women and subsequently one ongoing pregnancy. Of the nonconceiving women who had their embryos cryopreserved in the second and third cycle, a total of 55 and 27 women have cryopreserved embryos available for thawing, respectively. For these women a total of three ongoing pregnancies could be calculated (25) (Table I).

The cumulative ongoing pregnancy rate per started cycle for three successive fresh IVF cycles was 42.5%. When pregnancies arising from transfer of thawed cryopreserved embryos were added, the cumulative pregnancy rate of three consecutive IVF cycles was 43.8%. When projected pregnancies resulting from unthawed embryos were included, the cumulative ongoing pregnancy rate was 44.5% (Fig. 2).

In the 227 women achieving an ongoing pregnancy arising from the fresh ET in their first IVF attempt, 32 had a subsequent transfer of thawed cryopreserved embryos. This resulted in three ongoing pregnancies (9.4%). In the women achieving an ongoing pregnancy arising from the fresh ET in second and third IVF attempt, in both attempts, nine had a subsequent transfer of thawed cryopreserved embryos, none of which resulted in an ongoing pregnancy.

Fig. 2. Cumulative pregnancy rates in 1251 patients per started cycle of three consecutive fresh IVF cycles are shown, together with the cumulative rates when observed pregnancies and then projected pregnancies arising from transfer of frozen-thawed embryos were included.

DISCUSSION

The demographic and infertility characteristics of the study population were representative of those in other European centers (27,28). The ongoing pregnancy rate per initiated IVF cycle, excluding thawed cryopreserved embryos (16.7%), and the ongoing pregnancy rate per transfer of thawed cryopreserved embryos (11.8%) were also comparable with those reported by other European centers (10,29). Comparisons of outcomes from embryo cryopreservation are often complicated by difficulties in controlling for differences in the populations of embryos being assessed (30,31). This may partially explain the relatively lower pregnancy rates per thawed embryo transfer we observed when compared to those reported in the U.S. IVF registries during the same period (32).

By adopting the novel approach of analyzing outcomes in terms of cumulative pregnancy rate for three IVF cycles, we have revealed the limited added value of a representative embryo cryopreservation program. The additional contribution of embryo cryopreservation to cumulative pregnancy rates over three cycles of IVF was just 2% (42.5% vs. 44.5%). This meant that, of the 1251 women who started IVF treatment in the 5-year study period, only 19 had a direct benefit from the cryopreservation program in terms of the "patient-specific concept," with an additional 11 expected to benefit. Given the considerable day-to-day effort and commitment required to maintain a cryopreservation program, these results were disappointing.

The low postthaw survival rate of cryopreserved embryos may explain these findings. Despite the application of a widely used and recommended cryopreservation protocol utilizing DMSO (19,33), and plastic cryostraws as the freezing container (33), the postthaw survival rate of cryopreserved embryos in our study appeared to be lower (24.9%) when compared to cohorts described previously (10,34). This discrepancy may be due in part to differences in the definition of survival. When postthaw survival of a cryopreserved embryo is defined as $\leq 50\%$ blastomere loss, as in our center, the relatively low percentage survival we observed is comparable with other reports where similarly defined postthaw survival rates of 27% have been described (35). More recently, thus defined postthaw survival rates of up to 78% have been reported (36). However, these data were limited to early cleavage stage (Day 2) embryos. In this study, results from cryopreserved Day 3, 4, and 5 embryos were analyzed. The possible effect on outcome of the stage

of development at which embryos were cryopreserved was not delineated. Possible differences in postthaw survival rate between 8-cell, morula and blastocyst stage embryos, and their impact on the overall outcome cannot therefore be ruled out. The relatively complex structure of blastocysts and their fluid-filled cavity may render them more difficult to freeze successfully when compared to the morula or 8-cell embryo (37). Therefore, the blastocyst may require a different freezing procedure or freezing medium than those normally applied to less mature embryos. However, published data in this area remain contradictory (38,39).

The severe damage which can be sustained by an embryo during the cryopreservation and thawing procedure is well documented (37), and the chance of implanting appears to be directly related to the degree of blastomere loss following thawing (36). Ongoing improvements in cryopreservation techniques are suggested by recent data from the United States where a 5.9% increase in live birth rates arising from cryopreserved embryo cycles was observed in 1 year (40). Moreover, it has been recently demonstrated that when cryopreserved embryos show no blastomere loss, implantation rates are similar to fresh embryos (36). Further improvements in selection of embryos for cryopreservation and cryothaw techniques which reduce the rate of blastomere loss will be required in order to further improve outcomes of cryopreservation.

The legal, moral, and ethical issues surrounding the cryopreservation of supranumerical embryos (41,42) continue to command attention. The practice of holding embryos cryopreserved for many years, even when the couples to which the embryos belong are lost to contact, is also a matter of concern, as illustrated by the vocal public protests aroused when embryo banks are destroyed. Given this background, and the IVF-burden imposed on patients in order to produce sufficient embryos for cryopreservation, the benefits of cryopreservation services should be clear and substantial.

On the basis of the current findings we conclude that profound ovarian stimulation aimed at producing supranumerical embryos for cryostorage using present techniques is poorly justified.

The demands made on our patients by current stimulation protocols for IVF is illustrated by the substantial number of patients in our study who were unwilling to undergo a second ($n = 269$) or a third ($n = 225$) IVF attempt. This issue has been raised

previously (13) but received little attention. Apparently, a substantial proportion of patients refrain from subsequent IVF attempts, therefore, reducing the overall success rate per started IVF treatment. Those couples who already had children were more likely to halt their treatment, confirming a previously described phenomenon (14). The need to conceive at least one child may urge couples to accept the continued inconvenience and emotional and physical stress, and mask the effects of the "IVF-burden" from their clinicians (41,42).

Milder ovarian stimulation protocols aimed at producing a limited number of oocytes for fertilization in vitro with less burden for the patient and fewer risks have been proposed (43,44). There are indications that patients favor shorter, more convenient ovarian stimulation protocols even when the chance of conceiving in a given cycle is slightly reduced (45). However, providing milder stimulation protocols may actually increase cumulative pregnancy rates, since a higher proportion of patients may elect to continue treatment if the first cycle is unsuccessful. Our findings suggest that creating supernumery embryos for cryopreservation is a weak justification for profound ovarian stimulation. We conclude that any reduction in embryos available for cryopreservation arising from the institution of mild ovarian hyperstimulation protocols will have little negative impact on cumulative pregnancy rates.

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