Comparison of effects of thawing entire donor sperm vial vs. partial thawing (shaving) on sperm quality

Micha Baum^{1,2} · Raoul Orvieto^{1,2} · Sapir Kon^{1,2} · Ronit Machtinger^{1,2} · Gil M. Yerushalmi^{1,2} · Ariel Hourvitz^{1,2}

Received: 6 November 2017 / Accepted: 3 January 2018 / Published online: 15 January 2018
© Springer Science+Business Media, LLC, part of Springer Nature 2018

Abstract

Purpose Partial thawing of a vial of cryopreserved sperm (shaving) is sometimes applied as a measure to preserve sperm for further use, particularly in cases of very restricted sperm quantity. However, mechanical violence may disrupt the sperm-wall and lead to impaired in-vitro fertilization (IVF) outcomes.

Material and methods In a retrospective case-control study at a tertiary, university-affiliated medical center, we compared the IVF/intracytoplasmic sperm injection (ICSI) outcomes of patients who used donor sperm following partial thawing (shaving) of the vial of cryopreserved sperm ($n = 99$) to a control group consisting of patients for whom the vial of sperm was completely thawed before use ($n = 99$).

Results While no differences were observed in the rates of oocyte fertilization, the mean number of top-quality embryos (TQE) was significantly lower in the shaving group than in the complete thawing group (1.33 ± 0.17 vs. 1.87 ± 0.17 , $p < 0.02$). Experimental analysis of aliquots from the same donors revealed significantly reduced motility in sperm samples that were shaved vs. fully thawed (6.5 vs. 37.1%, $p < 0.001$).

Conclusions In cases in which available cryopreserved sperm samples are limited, shaving of the vial without thawing can be used but with caution and only when absolutely necessary. Further, large prospective studies are needed to better clarify whether there is post-thawing sperm damage and to compare IVF outcomes after these two thawing methods.

Keywords Sperm · Cryopreservation · Complete thaw · Partial thaw · Shaving · IVF outcome

Introduction

Cryopreservation is a widely practiced procedure for storing sperm prior to intrauterine insemination (IUI) or in-vitro fertilization (IVF) that uses donor sperm, in cases of male surgical infertility treatment, or prior to treatment for malignancies. However, compared to the original sperm quality, marked reductions in viability and overall motility have been reported after freezing and thawing [1].

The introduction of intracytoplasmic sperm injection (ICSI), which enables the use of a small number of sperm, initiated considerable interest in the potential refreezing and later re-use of sperm when there is a limited number of sample vials or low concentration of cryopreserved sperm available. In an attempt to address the consequences of refreezing of cryopreserved human sperm, Polcz et al. [2] examined the effects of repeated freeze-thaw cycles on the viability and motility of human spermatozoa. They found that human spermatozoa were able to maintain their vitality (defined by ration between motile and non-motile viable sperm) mainly for the first thawing cycle. Repeated freeze-thaw cycles resulted in very low sperm motility recovery. While pre-freeze motility was 70.1%, sperm motilities after each of the five freeze/thaw cycles were 24.4, 8.0, 3.5, 1.5, and 1.8%, respectively. Bandularatne and Bongso [3] further demonstrated the drop in the percentage of sperm motility and vitality recovery after three freeze-thaw cycles, although they showed that according to the sperm parameters the ability to fertilize following ICSI was maintained.

Drs. Baum and Orvieto should be considered “similar in author order.”

✉ Ronit Machtinger
mmachtin@gmail.com

¹ Department of Obstetrics and Gynecology, Chaim Sheba Medical Center, Tel-Hashomer, 52621 Ramat Gan, Israel

² Sackler School of Medicine, Tel-Aviv University, Tel-Aviv, Israel

Another method clinically applied in attempts to conserve sperm for later use, especially in cases of very limited sperm, is shaving a small portion of the vial of cryopreserved sperm without complete thawing. While it is well established that shaving is feasible and able to obtain a tiny volume of sperm that contains sufficient sperm for ICSI, the literature contains no data on the clinical outcomes of IVF/ICSI cycles that use shaving compared to those that use complete thawing of the vial of sperm. Prompted by the aforementioned observations, we sought to examine whether the outcomes of IVF/ICSI using cryopreserved donor sperm differed depending on whether the vial of sperm was shaved or completely thawed.

Materials and methods

To validate findings, the present study used both collection of retrospective data and experimental analysis. First, the computerized files for all IVF ovum pick-up (OPU) cycles over an 8-year period in a tertiary, university-affiliated medical center were reviewed. The study group included all patients who used donor sperm following shaving of the vial of cryopreserved sperm, and the control group included 99 patients, matched by age, who underwent IVF/ICSI treatment during the same period and used donor sperm from a sperm vial that was completely thawed before use. Exclusion criteria were the use of donor oocytes or the transfer of frozen-thawed embryos. The study was approved by the institution's review board (approval no. SMC-15-2317).

Patients' files provided data on their ages and the variables related to their infertility treatments, including the ovarian-stimulation protocols, the number of oocytes retrieved, and the number of embryos transferred per cycle.

Sperm freezing and thawing protocols

Sperm was aliquoted into freezing tubes (0.5–1 ml per tube) according to sperm concentration. Sperm volume for each tube was calculated to achieve minimum of 8×10^6 total motile sperm, after thawing, estimating that 50% of the sperm would not survive freezing and thawing. Freezing medium (Irving scientific, USA) was added to each tube (1:1 medium:sperm volume). Sperm was frozen gradually (10 min at a temperature of 4 °C and then for another 10 min at –20 °C) until complete freezing. Frozen sperm was kept in liquid nitrogen until thawing.

In cases of complete thawing, vials were placed in warming block at 37 °C for 10 min while specimen thaws. Thawed sperm was separated using density gradient centrifugation (PureCception 40% Upper Phase Gradient, PureCception 80% Lower Phase Gradient, SAGE, USA) at 1800 RPM for

20 min. After that, 2 ml of Multipurpose Handling Medium-Complete (MHM-C, Irvine Scientific, CA, USA) was added to the vial, and sperm was centrifuged at 1800 RPM for 5 min. Shaving was performed by scratching a small sample of sperm (100–200 µl) from a vial of frozen sperm using a scalpel to a new vial and refreezing the remained volume of the sample. The shaved sperm was warmed at room temperature and suspended with 2 ml of MHM-C medium. The vial was centrifuged 5 min at 1800 RPM. The supernatant was removed and the pellet was re-suspended with MHM and centrifuged for 5 min at 1800 RPM. The specimens were kept at 37 °C till IVF/ICSI was performed.

Embryos were classified based on individual embryo-scoring parameters according to previously established definitions [4]. Top-quality embryos (TQE) were defined as having six or more blastomeres on day 3, equal-sized blastomeres, and $\leq 10\%$ fragmentation. All other embryos were termed as being of poor quality [5]. Clinical pregnancy was defined as visualization of a gestational sac and fetal cardiac activity on transvaginal ultrasound 3 weeks after embryo transfer.

Experimental warming of vials

To further explore whether there was any qualitative difference in sperm samples after shaving as opposed to complete thawing, we performed an experiment to assess the sperm concentration and motility in 10 donors attending the Sheba Medical Center sperm bank.

Two equal volume samples were frozen at the same time from each donor. Later, one of each couple was shaved and the other was completely thawed. We compared the sperm quality in between each couple sperm sample. Shaving was performed 4 times and tested whether there was any difference in sperm parameters after the subsequent thawing. After shaving the sample, the vial with the sperm was replaced in the liquid nitrogen tank until the next shave. The volumes of all shaved samples were similar: between 160 and 225 µl.

Statistical analysis

Analysis was performed using the Student's *t* test, the Fischer's exact test, and analysis of variance (ANOVA), as appropriate. Results are presented as means $\pm$ standard error. A *p* value of less than 0.05 was considered statistically significant. All analyses were performed using SPSS version 21 software.

Results

The study retrospectively evaluated 198 IVF/ICSI cycles, 99 in the shaving group and 99 in the complete thaw group. There were no differences between the groups in patient age, length

Table 1 Patients' characteristics in the shaving and complete thaw groups*

Parameter	Shaving (<i>n</i> = 99)	Complete thaw (<i>n</i> = 99)	<i>p</i> value
Age (years)	41.5 ± 0.3	40.9 ± 0.4	0.20
Length of stimulation	10.3 ± 0.2	9.95 ± 0.263	0.53
Total gonadotropin dose (intrauterine (IU))	3757 ± 190	3536 ± 212	0.44
E ₂ on day of hCG (pmol/mL)	3871.2 ± 318.4	4238.1 ± 352.8	0.44
Endometrium (mm) on day of hCG	8.89 ± 0.21	9.45 ± 0.26	0.17
No. of retrieved oocytes (per patient)	498 (5.0 ± 0.4)	557 (5.6 ± 0.4)	NS
No. of MII oocytes vitrified (per patient)	377 (3.8 ± 0.3)	417 (4.2 ± 0.3)	NS
No. of fertilized eggs (per patient)	268 (2.7 ± 0.2)	313 (3.2 ± 0.2)	NS
No. of TQE (per patient)	132 (1.3 ± 0.2)	185 (1.9 ± 0.2)	0.02
No. of transferred embryos (ET per patient)	174 (1.8 ± 0.1)	211 (2.1 ± 0.1)	0.02
CPR/transfer, <i>n</i> (%)	9/99 (9.1)	13/99 (13.1)	NS

*Data are shown in means ± standard error

TQE top-quality embryos, CPR clinical pregnancy rates

of ovarian stimulation, total dose of gonadotropins used, peak estradiol (E₂) levels, number of oocytes retrieved, or fertilization rate (Table 1). Interestingly, the number of TQE was significantly lower in the shaving group than in the control group (1.33 ± 0.17 vs. 1.87 ± 0.17 , $p = 0.02$).

Experimental warming of the vials

In the aliquots from the same donors, sperm concentrations were similar between the samples that used shaving and those that were completely thawed (Table 2). Sperm motility in the shaving group was significantly lower as compared to sperm motility of the completely thawed group (6.5 vs. 37.1%, $p < 0.001$). The difference in sperm motility between shaving and completely thawing remained significant after controlling for the volume of the thawed samples. Interestingly, samples that underwent further shaving (1–4 times) showed no further impairment in sperm motility (Table 2).

Discussion

This preliminary study found that patients undergoing IVF/ICSI treatment using donor sperm yielded a significantly lower number of TQEs when the shaving method was used on the

sperm sample than did those who used sperm from vials that were completely thawed before use. In the laboratory analysis, shaving resulted in decreased motility as compared to fully thawing the sample vials.

In cases of cryopreserving sperm samples before treatment for cancer or another disease, our sperm bank often offers reuse of the cryopreserved sperm vial to maximize the use of limited samples. Likewise, in cases of donor-insemination, sperm shaving offers reuse to increase the cost-effectiveness of pre-purchasing vials of donor sperm.

While vials that were completely thawed were warmed at 37 °C, specimens that were shaved were warmed at room temperature. Also, the two techniques, shaving and complete thawing, differed as complete thawing included also the use of gradient and swim up that enables to choose the best sperm in the sample for IVF/ICSI.

Thawing cryopreserved human sperm has been found to impair sperm motility and to decrease its fertilization rate by detrimentally affecting its membranes, acrosomal structure, and acrosin activity [6]. The process of freezing and thawing may also detrimentally affect its chromatin structure [7], potentially risking the decondensation of its nucleus following ICSI [8]. In an experiment that repeatedly frozen and thawed sperm samples, Vutyavanich et al. [9] demonstrated that fragmentation of sperm DNA increased after the second cycle of

Table 2 Sperm analysis of scratch compared to complete-thaw groups*

Parameter	Complete thaw (<i>n</i> = 10)	First shaving (<i>n</i> = 10)	Second shaving (<i>n</i> = 10)	Third shaving (<i>n</i> = 10)	Fourth shaving (<i>n</i> = 10)	<i>p</i> value
Volume (microliters)	765.0 ± 43.6	164.0 ± 12.8	188.5 ± 16.0	183.5 ± 16.7	221.67 ± 11.2	
Concentration (million/ml)	46.3 ± 7.17	44.8 ± 5.5	44.9 ± 5.7	54.6 ± 11.8	48.8 ± 2.5	0.018
Motility (%)	37.1 ± 6.5	7.8 ± 3.2	6.1 ± 2.1	7.1 ± 2.4	4.8 ± 1.2	< 0.001

*Data are shown in means ± standard error

freezing and thawing. However, these changes were not clinically significant. Furthermore, it was not until the seventh freezing-thawing cycle that no motile sperm at all were observed after thawing. The present study did not compare DNA fragmentation between the groups, but it found significantly impaired sperm motility in samples that were shaved compared with those that were fully thawed. Recurrent shaving (up to four times) did not result in further impairment of sperm motility.

The shaving of frozen sperm may disrupt the sperm-wall as a result of mechanical violence applied to frozen sperm cells that are full of cryoprotectant (usually, glycerol). As shaving has neither been described in the World Health Organization (WHO) laboratory manual for the examination and processing of human sperm nor approved for donor-sperm cycles, the technique is not routinely offered to patients using donor sperm. However, in rare cases when patients insist on using only a limited sample of sperm, they are offered the possibility of shaving. In those cases, embryologists inform the patients that shaving of frozen sperm may detrimentally alter its performance after thawing.

The present study was limited because retrospective studies are not an optimal design for use in reproductive medicine and because a prospective randomized study design would be needed to replicate the results obtained. However, to better understand the effect of shaving on sperm quality, we performed an in-vitro analysis using two aliquots of sperm from the same donor, and the results showed that although sperm motility was impaired after shaving, the use of ICSI maintains the ability of the sperm to fertilize.

Conclusions

There are some justifications for using shaving. A patient who has a child from a particular donor may not be able to locate an adequate number of sperm vials to produce a future sibling. In addition, cancer survivors who cryopreserved sperm prior to therapy may later become azoospermic and discover that they have a limited number of vials of frozen sperm. This preliminary study found that shaving sperm samples seems to result

in decreased sperm motility and impaired embryo development. However, the shaving strategy should be further evaluated in clinical trials, and freezing sperm in lower volumes seems to overcome the problem of re-freezing and re-thawing. Further studies are required to elucidate the mechanisms affecting sperm viability after thawing and possible sperm damage and to prospectively compare the IVF/ICSI outcomes of patients who used cryopreserved donor sperm after shaving as opposed to after complete thawing of the vial of sperm.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Nijs M, Ombelet W. Cryopreservation of human sperm. *Hum Fertil (Camb)*. 2001;4:158–63.
2. Polcz TE, Stronk J, Xiong C, Jones EE, Olive DL, Huszar G. Optimal utilization of cryopreserved human semen for assisted reproduction: recovery and maintenance of sperm motility and viability. *J Assist Reprod Genet*. 1998;15:504–12.
3. Bandularatne E, Bongso A. Evaluation of human sperm function after repeated freezing and thawing. *J Androl*. 2002;23:242–9.
4. Ziebe S, Lundin K, Janssens R, Helmgaard L, Arce JC, M. Group. Influence of ovarian stimulation with HP-hMG or recombinant FSH on embryo quality parameters in patients undergoing IVF. *Hum Reprod*. 2007;22:2404–13.
5. Racowsky C, Vernon M, Mayer J, Ball GD, Behr B, Pomeroy KO, et al. Standardization of grading embryo morphology. *Fertil Steril*. 2010;94:1152–3.
6. Cross NL, Hanks SE. Effects of cryopreservation on human sperm acrosomes. *Hum Reprod*. 1991;6:1279–83.
7. Royere D, Hamamah S, Nicolle JC, Lansac J. Chromatin alterations induced by freeze-thawing influence the fertilizing ability of human sperm. *Int J Androl*. 1991;14:328–32.
8. Sakkas D, Urner F, Bianchi PG, Bizzaro D, Wagner I, Jaquenoud N, et al. Sperm chromatin anomalies can influence decondensation after intracytoplasmic sperm injection. *Hum Reprod*. 1996;11:837–43.
9. Vutyavanich T, Lattiwongsakorn W, Piromlertamorn W, Samchinchom S. Repeated vitrification/warming of human sperm gives better results than repeated slow programmable freezing. *Asian J Androl*. 2012;14:850–4.