

# Intrauterine insemination–ready versus conventional semen cryopreservation for donor insemination: a comparison of retrospective results and a prospective, randomized trial

Don P. Wolf, Ph.D.,<sup>a,b,c</sup> Phillip E. Patton, M.D.,<sup>a</sup> Kenneth A. Burry, M.D.,<sup>a</sup> and Paul F. Kaplan, M.D.<sup>d</sup>

University Fertility Consultants, Portland, and Women's Care Fertility Center, Eugene, Oregon

**Objective:** To compare fecundity rates following intrauterine insemination (IUI) with donor sperm frozen conventionally versus an IUI-ready preparation.

**Design:** Both retrospective results and a prospective, randomized study where recipients were assigned to one of two sperm cryopreservation methods in each cycle of intrauterine insemination are reported.

**Setting:** University-based infertility practice, affiliated private practices, and andrology laboratory.

**Patient(s):** Women desiring therapeutic insemination in an effort to establish pregnancy.

**Intervention(s):** Intrauterine insemination with donor sperm frozen conventionally or by an IUI-ready protocol.

**Main Outcome Measure(s):** Cycle fecundity in donor IUI recipients.

**Result(s):** In a retrospective analysis involving 642 inseminations in 209 recipients, 79 pregnancies were recorded for an overall pregnancy rate of 12.3% per insemination (or cycle): 11.3% with IUI-ready sperm and 13.9% with conventionally preserved sperm. In a follow-up prospective, randomized study, the pregnancy rate for IUI-ready sperm preparations was 36% per cycle (14 of 39) whereas that for conventionally preserved sperm was 19.6% per cycle (9 of 46). Thirteen of the 23 pregnancies occurred in the first study cycle of insemination; only two pregnancies were observed in patients undergoing more than four cycles of insemination.

**Conclusion(s):** Cycle fecundity for IUI-ready donor sperm is equivalent to conventional cryopreserved sperm based on both prospective and retrospective assessments. (*Fertil Steril*® 2001;76:181–5. ©2001 by American Society for Reproductive Medicine.)

**Key Words:** Donor insemination, intrauterine insemination, sperm cryopreservation, prospective clinical trial, IUI-ready

Therapeutic intrauterine insemination with donor sperm (TID) is a common treatment alternative for human infertility secondary to uncorrectable semen deficiencies, for a desired pregnancy by single or lesbian women, or as an alternative source of semen during cycles of assisted reproductive technology when the original source of semen is unsuitable. A continued role for intrauterine insemination (IUI) with donor sperm seems secure, based on considerations of cost effectiveness and the universal availability of the technology.

The recognized risk of infectious disease

transmission, especially of human immunodeficiency virus (HIV), that is associated with therapeutic IUI with donor sperm has led to the exclusive use of cryopreserved semen. Using a quarantine protocol, semen collected from a screened donor must be cryopreserved and stored for 6 months, at which time the donor is rescreened and confirmed seronegative before the sample is released for clinical use (1–3). Unfortunately, cryopreservation is associated with irreversible sperm damage impacting both the recovery of motile, morphologically normal cells and the ensuing pregnancy rates (4–6).

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Reprint requests: Don P. Wolf, Ph.D., University Fertility Consultants, 1750 S.W. Harbor Way, Suite 100, Portland, Oregon 97201-5133 (FAX: 503-418-3757).

<sup>a</sup> Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, Oregon Health Sciences University, University Fertility Consultants.

<sup>b</sup> Department of Physiology and Pharmacology, Oregon Health Sciences University, University Fertility Consultants.

<sup>c</sup> Division of Reproductive Sciences, Oregon Regional Primate Research Center, Beaverton, Oregon.

<sup>d</sup> Women's Care Fertility Center.

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At least two approaches to sperm cryopreservation are now in common use: conventional semen freezing involving sample dilution with a cryoprotectant, usually glycerol, and post-thaw processing to remove seminal plasma along with the cryoprotectant before IUI. The second approach is an IUI-ready protocol that involves sperm washing to remove seminal plasma before cryopreservation. With the latter, the sample can be thawed and used without the need for further processing, thereby providing improved convenience and cost effectiveness as well as a potentially greater number of motile sperm, especially for inseminations performed outside regular laboratory hours or where laboratory services are unavailable.

We have previously described an IUI-ready protocol that supports the recovery of equivalent or improved post-thaw sperm parameters (7) and have reported promising preliminary results from a retrospective study of cycle fecundity comparing IUI-ready sperm preparations with conventionally cryopreserved sperm (8). Our objectives in this report are to present details of the retrospective study and the results of a controlled, prospective, randomized clinical trial comparing cycle fecundity of donor samples cryopreserved by the conventional semen technique with those preserved by the new IUI-ready sperm protocol.

## MATERIALS AND METHODS

### Sperm Cryopreservation

Sperm donors were recruited from the medical and university community and were screened by established guidelines (3). Semen samples were obtained by masturbation after 24 to 48 hours of sexual abstinence. All donors had normal semen parameters according to World Health Organization guidelines (9) before freezing, and there was evidence of recovery of at least 50% of the initial motile sperm after freezing and thawing. After determining sperm count and motility, semen samples were cryopreserved within 2 hours of collection by one of two methods.

For conventional semen cryopreservation, a standard cryoprotectant (TES, Tris yolk freezing medium; Irvine Scientific, Santa Ana, CA) enhanced with glycerol to a final concentration of 29.6% was added in a dropwise fashion to aliquots of semen at a ratio of three parts semen to one part cryoprotectant to provide a final glycerol concentration of 7.4%. After mixing by repeated aspiration in and out of a 1-mL graduated pipette, the samples were transferred to 1-mL cryotubes (Nunc, Kamstrup, Denmark) and cooled by immersion for 90 minutes in a 25°C water bath placed in a 4°C refrigerator. Samples were then exposed to liquid nitrogen vapors for 30 minutes followed by immersion into and storage in liquid nitrogen.

IUI-ready samples were processed as described previously (7). Briefly, semen samples were subjected to a three-layer Percoll gradient centrifugation. The sperm-rich fraction

was resuspended in TH3 (HEPES-buffered Tyrode's solution with 0.3% human serum albumin, lactate, and pyruvate) and centrifuged to remove Percoll. The final pellet was resuspended in a volume of IUI-ready cryoprotectant to ensure an adequate number of motile sperm per vial and was distributed in 0.4 mL aliquots in cryovials. The IUI-ready cryoprotectant consisted of HTF-HEPES with 1% HSA, 4% sucrose, and 6% glycerol. Washed sperm aliquots were then cryopreserved as described above with a 4°C cooling step preceding their exposure to liquid nitrogen vapors and subsequent immersion in liquid nitrogen.

### Thawing and Post-thaw Processing of Samples and Insemination

Samples were thawed at room temperature for 5 minutes followed by 10 to 15 minutes at 37°C. Semen, cryopreserved by the conventional freeze technique, was processed by either centrifugation/resuspension or Percoll density gradient centrifugation as described elsewhere (7) and resuspended in 0.4 mL of sperm wash medium in preparation for use. Intrauterine insemination-ready specimens were thawed and used directly. For both methods the minimum motile sperm requirement per insemination was 15 million.

All intrauterine inseminations were performed by personnel at the Division of Reproductive Endocrinology or at Women's Care Fertility Center in Eugene, Oregon. The woman's cervix was gently swabbed with a cotton tip applicator to remove excess cervical mucus and secretions, and the sample was then delivered with a syringe-cannula system (17 g; Intracath-Deseret, Sandy, UT) by inserting the flexible cannula through the cervical os into the upper fundal area of the uterine cavity. The suspension of processed sperm was injected over 30 to 60 seconds, and the patient remained recumbent for 10 to 15 minutes. All pregnancies were confirmed by standard serum assays of rising hCG levels and ultrasound evidence of an intrauterine gestational sac.

### Patient Screening and Characteristics

In a retrospective analysis, carried out between April 1996 and May 1998, the study population consisted of a diverse, unselected group of single and married women including women with a variety of diagnoses, ages, and preinsemination screening procedures. A total of 642 inseminations were conducted in 209 women. The decision to use conventionally frozen sperm (post-processed sperm) or IUI-ready sperm was made on the day of insemination on the basis of sample availability and convenience. Semen from 50 different donors was employed.

Candidates for the prospective study were women who desired therapeutic insemination from donor semen by the physicians of University Fertility Consultants in Portland or Women's Care Fertility Center in Eugene, Oregon between 1996 and 1999. The protocol and consent forms were approved by the OHSU institutional review board. Each insemination cycle, a few days prior to sample thawing, was

randomly assigned to either insemination with cryopreserved sperm prepared by the conventional method or by the IUI-ready method. A computer generated list was used to direct the assignments.

The preinsemination screening criteria consisted of the following: an analysis of ovulatory function by basal body temperature; age less than 40; a negative *Chlamydia* titer; a normal Papanicolaou smear result; no history of pelvic inflammatory disease, gonococcal infection, or other sexually transmitted disease; and less than three previous donor IUIs. A hysterosalpingogram was performed in selected cases where indicated for both the retrospective and prospective study.

Each woman received a single IUI on the day after detection of the LH surge by conventional urine kits (Ovu-Quick or OvuKit, Quidel Corporation, San Diego, CA). Testing was initiated approximately 4 days before the predicted time of ovulation, based on review of previous basal body temperature records.

Cycle adequacy was evaluated retrospectively after each insemination by the supervising physician with the use of body basal temperature records, measurement of LH surge levels, and midluteal progesterone assay results, as indicated. Basal body temperature records were considered adequate when there was evidence of a biphasic curve with at least 12 days of temperature elevation. Abnormalities in ovulatory function were treated with clomiphene citrate (50–150 mg), depending on the review of the body basal temperature records.

### Statistical Analysis

The results were analyzed by chi square or Student's *t*-test with a statistical significance level set at .05.

## RESULTS

### Retrospective Study

A total of 79 pregnancies was reported in 642 cycles of insemination in 209 recipients for an overall pregnancy rate of 12.3% per cycle and 38% per patient. In 334 of these cycles, IUI-ready sperm was used resulting in 42 pregnancies and an overall rate of 11.3%. In 229 cycles of donor insemination with conventionally frozen sperm, 37 pregnancies were reported for a rate of 13.9% ( $P=.33$ ).

There was no difference in the mean number of sperm inseminated between IUI-ready preparations (21.5 million) and the conventionally frozen preparations (21.9 million). Additionally there was no difference in the total motile sperm inseminated when pregnant cycles were compared to nonpregnant cycles. A significant difference was found between the average age of the recipients inseminated with sperm prepared by the two methods, with a mean of 36.5 years for the group receiving IUI-ready sperm versus 35.2 years for patients receiving conventionally frozen sperm ( $P$

$<.05$ ). A significant difference was also found between the mean age of the recipients achieving pregnancy (34.6 years) and those not achieving pregnancy (36.1 years). On average, those achieving pregnancy were 1.5 years younger.

### Prospective Randomized Trial

Donor insemination was initiated in 33 different women (36 total with two women having more than one pregnancy) with a total of 85 inseminations or 2.4 inseminations per patient. The mean patient age of the study population was 33 ( $\pm 3.4$ ) years and the indications for insemination were 66% male factor infertility and 33% were lesbian or single women. The total motile sperm per insemination did not vary between the conventional ( $25.6 \pm 9.3$  million) and IUI-ready ( $25.2 \pm 13.8$  million) protocols.

The overall pregnancy rate per cycle was 23 out of 85 or 27%, and per patient was 23 out of 33 (70%). Of the 46 cycles of insemination with conventionally cryopreserved sperm, nine pregnancies were recorded, for 19.6% per cycle. There were 39 cycles of insemination with IUI-ready sperm and 14 pregnancies, for 36% per cycle. Thirteen of the 23 pregnancies occurred in the first study cycle of insemination (Table 1) and only two pregnancies were conceived in the fifth and sixth study cycles combined.

## DISCUSSION

The primary indications for therapeutic donor insemination include severe male factor infertility and pregnancy initiation in single women. When quarantined frozen donor sperm is used, pregnancy rates per cycle of insemination are highly variable, ranging from 9% to 26% (10, 11). However, pregnancy rates in donor programs depend on multiple variables such as timing, number and technique of insemination, the assessment and treatment of female infertility factors, recipient age, medical diagnosis and indication for insemination, and the number and quality of inseminated sperm (11–16). Individual differences in donor fecundability represent an additional important variable (17). Unfortunately, routine screening tests are unable to predict donor fecundability and more sophisticated testing is necessary (18). In all, differences in reported pregnancy rates and inconsistencies in studies could reflect the impact of one or more of the aforementioned variables.

The conventional approach to sperm cryopreservation is to simply dilute semen with cryoprotectant and cryopreserve in glycerol at liquid nitrogen temperatures, leaving the processing step until the sample is thawed and prepared for use. More recently, the advantages of storing preprocessed sperm have become apparent and IUI-ready sperm samples have become available through several commercial sperm banks. However, few studies have directly compared the efficacy of one processing technique against another.

Based on these considerations, we conducted a carefully designed prospective clinical trial designed to assess the

**TABLE 1**

Pregnancies in women undergoing prospective, randomized intrauterine insemination with IUI-ready or conventionally frozen sperm as a function of the number of cycles of insemination. A total of 85 inseminations was conducted with 23 pregnancies.

Cycle number	Number of patients	Number pregnant	Number nonpregnant	Dropouts (pregnant or gone)	Patients pregnant this cycle
1	36	13	23	0	13/36 (36%)
2	21	1	20	15	1/21 (5%)
3	16	4	12	5	4/16 (25%)
4	8	3	5	8	3/8 (38%)
5	3	1	2	5	1/3 (33%)
6	1	1	0	2	1/1 (100%)

Wolf. IUI-ready or conventional sperm freezing. *Fertil Steril* 2001.

efficacy of a new sperm processing technique against a standard or conventional technique. In this report both retrospective and randomized prospective study results were included for women undergoing IUI with cryopreserved donor sperm using a conventional post-thaw processing method to remove seminal fluids and cryoprotectants and a new protocol using frozen-thawed samples where seminal plasma is removed prior to cryopreservation (IUI-ready).

Our prospective, randomized study results indicate that suitable pregnancy rates can be established with either sperm processing method in a carefully screened recipient population. In our study, 36% of insemination cycles resulted in pregnancy in women who received IUI-ready sperm preparations whereas the corresponding value was 19.6% following insemination with conventionally cryopreserved semen. Overall, 70% of patients became pregnant within 6 cycles of insemination.

Pregnancy rates in our prospective study are higher than those generally reported in trials of IUI. In part, the higher pregnancy rates may reflect the vigorous selection criteria and limited number of cycles (6) conducted in our study. Slightly lower fecundity rates might be expected in centers using less rigid entry requirements, or where IUI is performed over an extended number of cycles. In centers where the mean age of the recipient population is significantly higher than ours (mean 33 years), average fecundity rates may also be lower.

In fact, such considerations presumably account for the differences in pregnancy rates between our prospective and retrospective studies. For example, the mean recipient age was 33 years for the prospective study and 36 years for the retrospective assessment. Second, the mean, total motile sperm per insemination (25 million) for the prospective study was higher than found in the retrospective review (21.5 million). Third, there was no limit on the number of cycles of IUI in the retrospective study, which could decrease

average fecundity rates for these patients due to the well-recognized decrease in cycle fecundity over time.

Given the limited number of cycles completed in both groups in our prospective study over a 3-year study period, we were unable to prove that the techniques have equivalent efficacy. To provide a power of 0.8, a total of 284 completed cycles would be necessary. Nevertheless, the assumption that the use of IUI-ready samples offers no obvious disadvantages is supported by both the retrospective and prospective results. In these study populations, no significant differences in pregnancy rates were observed in the women who received IUI-ready or those who received conventionally processed IUI samples. Based on our cumulative data, we feel that the use of the IUI-ready protocol represents an alternative to the conventional semen processing methods for women who are undergoing donor insemination.

In summary, our study supports the use of IUI-ready processed donor semen in centers offering donor insemination. The use of IUI-ready samples offers several advantages for clinics using cryopreserved donor semen. The processing technique appears safe, simple, and efficacious. Although the preparation of donor semen for IUI-ready samples requires experienced laboratory personnel, the post-thaw processing of IUI-ready samples is relatively simple. The use of IUI-ready semen appears ideal for centers that have a limited number of laboratory technicians, and particularly for those centers with a large weekend or off-hours workload.

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