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Dockets Management Branch (HFA-305)

Food and Drug Administration

5360 Fishers Lane Room 1061

Rockville, MD 20852

Re: Docket #97N-484S, Suitability Determination for Donors of Human Cellular and Tissue-Based Products

Dear Sir or Madam:

This letter is to express our objection to proposed new rules regarding the quarantine of embryos derived from oocyte donor in vitro fertilization. Specifically we object to the proposal that embryos be cryopreserved and stored for 6 months to assess the suitability of the donor, prior to transfer of any embryos to the recipient patient. Our objection is based on several factors, the most important being a lack of scientific data or other evidence to suggest that embryos potentially transmit the diseases outlined in the proposal. Also, while it is a useful tool when necessary, the cryopreservation of embryos inevitably results in the deaths of otherwise healthy embryos; and severely decreases the chances of pregnancy after transfer, thus increasing the numbers of cycles required to achieve the desired outcome, with resultant increases in costs, anxiety to the patient and potential risks to reproductive health due to an age-related rise in infertility. There is also a potential increased risk for multiple births as the viability of cryopreserved embryos is more difficult to assess and assumed lower, thus potentially requiring the transfer of more embryos to achieve acceptable pregnancy rates.

Please review and reconsider these proposed changes. While we are always concerned for the health and well-being of our patients, there is no evidence that these rules would offer any added protection but there is clear indication they could be harmful.

Sincerely,

Shari E. Olson, PhD
Laboratory Director

Kevin E. Bachus, M.D.
Medical Director

97N 484S

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◆ Reproductive Endocrinology ◆ In Vitro Fertilization (IVF) ICSI ◆ Embryo Cryopreservation ◆ Oocyte Donation ◆ Artificial Insemination ◆
Tubal Reanastomosis ◆ Laser/Micro Surgery ◆ Male Factor Infertility ◆ Endometriosis ◆ Pelvic Pain

Diplomate of American Board of Obstetrics & Gynecology ◆ Diplomate of the Subspecialty Board
of Reproductive Endocrinology & Infertility ◆ Society of Reproductive Surgeons

Anonymous oocyte donation performed exclusively with embryos cryopreserved at the pronuclear stage

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Objective: To evaluate the clinical outcomes of patients who participated in an anonymous oocyte donation program that used embryos cryopreserved at the pronuclear stage.

Design: Observational study.

Setting: A tertiary care reproductive medicine unit.

Patient(s): Anonymous oocyte donors and their respective recipients.

Intervention(s): Oocyte donors underwent a standard controlled ovarian hyperstimulation protocol and transvaginal ultrasound-guided oocyte retrieval. Oocyte recipients underwent at least one programmed hormone replacement cycle with transcervical ET.

Main Outcome Measure(s): Thaw survival, implantation, clinical and ongoing pregnancy rates.

Result(s): Thirty-six oocyte retrievals resulted in one ET to date. The mean numbers of oocytes that were retrieved and normally fertilized were 18.2 and 11.6, respectively. Fifty-one embryo thaw-transfer cycles were performed, with an embryo thaw survival rate of 93.5%. The clinical and ongoing pregnancy rates per ET were 52.9% and 51%, respectively. The overall implantation rate was 28.7%. The percentage of oocyte retrievals that resulted in at least one ongoing pregnancy to date was 69.4%.

Conclusion(s): Anonymous oocyte donation can be conducted efficiently with the exclusive use of embryos cryopreserved at the pronuclear stage. This approach facilitates synchronization of the donor-recipient pair, eliminates the risk that recipients will begin hormonal therapy without embryo availability, and produces an acceptable ongoing pregnancy rate per oocyte donation. (Fertil Steril® 1999;71:830-5. ©1999 by American Society for Reproductive Medicine.)

Key Words: Oocyte donation, embryo cryopreservation, pronuclear stage, pregnancy rate, implantation rate

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The evolution of assisted reproductive technologies has made it possible to transfer in vitro fertilized donor oocytes to appropriately synchronized recipients. Since the first reported successful pregnancy after IVF of a donated oocyte in 1984 (1), the consistently excellent results in establishing pregnancies in women with or without ovarian function has led to the increasing popularity of oocyte donation.

At present, more than 2,500 oocyte donation procedures are performed annually in the United States and Canada (2). Candidates for oocyte donation include patients with premature ovarian failure, gonadal dysgenesis, or iatrogenically induced ovarian failure (e.g., irradiation, surgery, chemotherapy), carriers of genetic diseases, and patients in whom repeated IVF treatment attempts have failed (3).

Effective synchronization of the donor and

recipient such that embryonic and endometrial development coincide is vital to successful outcomes in oocyte donation. There is strong evidence that a temporal window of maximal endometrial receptivity exists. Implantation efficiency appears to decrease significantly when ET is performed outside this window (4, 5). For this reason, various strategies have evolved to aid in synchronization of the donor and recipient.

Several investigators have investigated the feasibility of artificially prolonging the follicular phase of the recipient (6, 7). The use of GnRH agonists to render cycling women agonal is another method to facilitate synchronization between donors and recipients (8). However, the donor's follicular phase can be shortened by gonadotropin therapy, and the timing of estrogen replacement in the recipient can be difficult to predict.

Embryo cryopreservation simplifies synchronization of the donor and recipient. After the cryopreservation of fertilized donor oocytes, the recipient is prepared with exogenous estrogen and progesterone therapy at a convenient time. The frozen embryos then are thawed at an appropriate interval after the initiation of progesterone therapy. Salat-Baroux et al. (9) reported success in 4 (31%) of 12 cycles in which cryopreserved donated embryos were transferred to recipients with ovarian failure who were undergoing hormone replacement cycles.

The generally poorer success rates achieved with cryopreserved embryos have led most practitioners to prefer the use of fresh ETs in oocyte donation. However, embryo cryopreservation techniques have improved significantly, and it may be worthwhile to reconsider the use of cryopreserved embryos for anonymous oocyte donation (AOD). In particular, embryo cryopreservation at the pronuclear stage appears to be associated with higher rates of embryo thaw survival and implantation (10).

MATERIALS AND METHODS

Oocyte Donors

Oocyte donors were recruited from two main sources: advertisements in Mayo Clinic monthly newsletters and direct contact with friends or relatives familiar with the program. All prospective donors underwent a comprehensive screening process. On initial screening, potential donors were required to be 21–32 years of age. Donors accepted into the program who wished to repeat donation were allowed to do so until 34 years of age.

All potential donors saw a medical geneticist for review of their family medical background and genetic risk. Presumed inheritable diseases or a history of a significant birth defect in a first-degree relative were grounds for exclusion. Potential donors saw a psychologist, who assessed the results of standardized psychological tests (Minnesota Multiphasic Personality Inventory-II; University of Minnesota Press, Minneapolis, MN) and performed a 30-minute interview. Potential donors then saw an IVF clinician for a complete history and physical examination.

A history of infertility, recurrent miscarriages, risk factors for human immunodeficiency virus (HIV) infection, and extensive smoking also were grounds for exclusion. Serum was tested for antibody to HIV type 1 and HIV type 2, hepatitis B surface antigen, hepatitis C antibody, reactive plasma reagent, and cystic fibrosis mutations. Donors were excluded if they showed evidence of infection or carrier status for cystic fibrosis. Hepatitis serology and reactive plasma reagent testing was repeated annually in active donors. Testing for antibody to HIV type 1 and HIV type 2 was performed again just before each oocyte retrieval on the day of hCG administration and then was performed once more after 6 months of embryo quarantine if requested by the recipient.

Oocyte Recipients

Potential oocyte recipients also underwent a comprehensive initial evaluation. Indications for oocyte donation included primary ovarian failure, premature ovarian failure, chromosomal abnormalities, prior bilateral oophorectomy, diminished ovarian reserve, prior IVF treatment failure, and advanced maternal age. Oocyte donation therapy was reserved for women <50 years of age.

Potential recipients saw an IVF clinician for a complete history and physical examination. Normalcy of the uterine cavity was determined by either hysterosalpingography or sonohysterography. A trial transfer procedure was performed to determine the depth and direction of the uterine cavity. Potential recipient couples also saw a psychologist, who assessed the results of standardized psychological tests (Minnesota Multiphasic Personality Inventory-II) and performed a 30-minute interview.

Sera from both partners were tested for antibody to HIV type 1 and HIV type 2, hepatitis B surface antigen, hepatitis C antibody, reactive plasma reagent, and blood type and Rh factor. A semen analysis was performed, including assessment of morphology with the use of strict criteria. Potential oocyte recipients did not undergo any mock hormone replacement cycles.

All recipients signed informed consent documents that transferred custody and decision-making regarding the disposition of oocytes to them once the oocyte donation was completed and fertilization was attempted. Advanced directives also were sought in the setting of potential prolonged intervals of embryo cryopreservation, death of the female and/or male partner, and divorce.

Treatment

Oocyte donors underwent down-regulation with the GnRH agonist leuprolide acetate (Lupron; TAP Pharmaceuticals, Deerfield, IL), which was administered beginning on cycle day 21 of the preceding menstrual cycle. In some instances, oral contraceptives (Ortho Novum 1/35; Ortho Pharmaceuticals, Raritan, NJ) were used before the GnRH agonist was administered (11). Leuprolide acetate initially was administered subcutaneously at a dosage of 1.0 mg/d. After withdrawal bleeding and confirmation of adequate ovarian down-regulation (no ovarian cysts of >18 mm in diameter and an E₂ level of <35 pg/mL), the dosage of leuprolide acetate was reduced to 0.5 mg/d.

Gonadotropin therapy was begun using either purified urinary FSH (Metrodin or Fertinex; Serono Laboratories, Norwell, MA) or recombinant FSH (Gonal-F; Serono Laboratories, or Follistim; Organon Inc., West Orange, NJ). In most cases, three ampules, each containing 75 IU of gonadotropins, were administered daily by either SC or IM injections. The daily dosage usually was decreased in a step-down fashion once follicular recruitment was established. Daily monitoring of serum E₂ levels and follicular growth was

initiated on the fifth or sixth day of gonadotropin therapy. Follicular monitoring was undertaken with a Corometrics Aloka 650 real-time ultrasound (Corometrics Medical Systems, Wallingford, CT) equipped with a 5-MHz transvaginal probe.

The timing of hCG administration was based on several parameters, including the mean diameter of the lead follicles, the serum E₂ level, the rate at which the serum E₂ level rose, and the quality of the oocytes or embryos in the patient's previous cycle(s), if applicable. In general, 10,000 IU of hCG was administered intramuscularly when the mean diameter of the lead follicles exceeded 19 mm. Oocyte retrievals were scheduled for 36 hours after hCG administration. Leuprolide acetate therapy was discontinued on the day of hCG administration.

All patients underwent transvaginal ultrasound-guided oocyte retrieval. Oocytes from a single donor were not shared between recipients. Oocytes were inseminated in organ culture dishes with 0.25–0.35 × 10⁶ motile sperm per milliliter when no male factor was present or with 0.5 × 10⁶ motile sperm per milliliter (high-concentration insemination) when a mild to moderate male factor was present. When a moderate to severe male factor was present, intracytoplasmic sperm injection was undertaken to facilitate fertilization, as previously reported (12). In all cases, sperm that previously had been cryopreserved was used.

We liberalized the indications for intracytoplasmic sperm injection only slightly compared with our standard IVF program, taking into account the decline in semen quality that is anticipated with cryopreserved sperm. Human tubal fluid supplemented with 10% synthetic serum substitute (Irvine Scientific, Santa Ana, CA) was used for oocyte culture and fertilization and for initial embryo culture. All normally fertilized zygotes were frozen at the pronuclear stage.

Embryo cryopreservation was performed according to previously published methods (13). Briefly, embryos were incubated for 15 minutes in phosphate-buffered saline with 1.5 M of propanediol and then for 10 minutes in 1.5 M of propanediol with 0.1 M of sucrose (Sigma, St. Louis, MO). Embryos were loaded into 250- μ L straws and frozen in a controlled-rate freezer at 2°C/min to -7°C and then at 0.3°C/min to -30°C, and then were plunged into liquid nitrogen. Straws were thawed at room temperature for 40 seconds and then in a 30°C water bath.

After thawing, embryos were incubated sequentially for 5 minutes in each of the following phosphate-buffered saline solutions: 1 M of propanediol with 0.2 M of sucrose, 0.5 M of propanediol with 0.2 M of sucrose, and 0.2 M of sucrose. Embryos were washed through two drops of preequilibrated human tubal fluid supplemented with 10% synthetic serum substitute and incubated at 37°C in 5% CO₂ until transfer. All embryo cryoprotectant and thaw media were supplemented with 20% synthetic serum substitute. Human tubal fluid supplemented with 10% synthetic serum substitute was used for further incubation before transfer.

All recipients underwent programmed hormone replacement cycles. Patients with ovarian function received GnRH agonist therapy with leuprolide acetate (Lupron), which was administered beginning in the midluteal phase at a dosage of 1 mg/d given subcutaneously. The dosage was reduced to 0.5 mg/d given subcutaneously after the occurrence of menses and confirmation of adequate down-regulation. Patients without ovarian function did not receive GnRH agonist therapy.

Estrogen replacement was accomplished with either oral micronized E₂ (Estrace; Bristol-Myers Squibb Co., Princeton, NJ) or transdermal E₂ (Estraderm or Vivelle; Ciba Pharmaceutical Co., Summit, NJ). The oral micronized E₂ protocol used a graduated dosage scheme, with 2 mg given daily on treatment days 1–4, 4 mg given daily on treatment days 5–8, and 6 mg given daily on treatment days 9–11 in divided doses.

A transvaginal sonogram was performed on cycle treatment day 11. Endometrial preparation was deemed appropriate if the endometrial thickness was \geq 8 mm, and the administration of oral micronized E₂ was continued at a dosage of 6 mg/d, with the administration of progesterone initiated on cycle day 14. If the endometrial thickness was <8 mm, the dosage of oral micronized E₂ was increased to 9 mg/d and transvaginal sonography was performed again on cycle day 14. If the endometrial thickness was adequate at that time (\geq 8 mm), the administration of oral micronized E₂ was continued at a dosage of 9 mg/d and the administration of progesterone was initiated. If the endometrial thickness was inadequate at that time (<8 mm), the cycle either was cancelled (<6 mm) or it was extended (6–7 mm) and transdermal E₂ patches were added to the regimen (0.1-mg patches changed twice weekly).

The transdermal E₂ protocol was used as a primary approach in patients with a history of suboptimal endometrial patterns. In this protocol, transdermal E₂ patches were applied to deliver a dose of 0.1 mg on days 1–4 (one patch applied every 48 hours), 0.2 mg on days 5–10 (two patches applied every 48 hours), and 0.4 mg on days 11–14 (four patches applied every 48 hours). Transvaginal sonography was performed on treatment cycle day 14, and the administration of progesterone was initiated if the endometrial thickness was adequate (\geq 8 mm).

In rare cases, transdermal E₂ patch therapy was prolonged to attain improved endometrial thickness and/or pattern before the administration of progesterone and thawing of the embryos. Transdermal E₂ therapy was monitored with serial serum E₂ levels obtained in the luteal phase and early pregnancy, and adjustments in therapy were made according to the results.

Embryos were evaluated just before ET for blastomere number and quality. Embryo quality was judged with the use of a scoring system that ranged from 0 (best) to 3 (worst), based on criteria related to blastomere symmetry and cellular fragmentation (14). The administration of progesterone-in-

oil (100 mg IM daily) was begun 48 hours before ET. Recipients who were ≥ 40 years of age also received progesterone vaginal suppositories (50 mg twice daily) in addition to IM progesterone (14). Embryos were thawed approximately 40 hours before transfer. In most instances, recipients chose to thaw the number of embryos they intended to have transferred. This policy on the number of embryos thawed made it possible for most recipients to undergo more than one ET cycle.

Data were gathered on donor and recipient age, duration of gonadotropin stimulation, type of gonadotropin used, number of ampules (75 IU) of gonadotropin administered, serum E₂ level on the day of hCG administration, number of oocytes retrieved, number of oocytes normally fertilized (two pronuclei), number of embryos frozen, number of embryos thawed and transferred, embryo quality, blastomere number, and endometrial thickness.

Biochemical pregnancies were defined as those pregnancies in which there was a transient elevation in the serum level of β -hCG (>10 mIU/mL), as defined by the Third International Reference Preparation, in the absence of a detectable intrauterine gestational sac by transvaginal ultrasonography or clinical evidence of an ectopic pregnancy. The definition of clinical pregnancies excluded biochemical and ectopic pregnancies and required that an intrauterine gestational sac be detected on ultrasound examination. Ongoing pregnancies were defined by the presence of intrauterine embryonic heart activity detected by transvaginal ultrasonography. The implantation rate was determined by dividing the number of sonographically visualized gestational sacs by the total number of embryos transferred. Continuous data were reported as means \pm SD.

Hormonal Assays

The E₂ assay performed in our laboratory consisted of an RIA conducted with the use of a commercial kit (Pantex, Santa Monica, CA) after hexane-ethylacetate extraction. The E₂ assay had an interassay coefficient of variation of 10% at a concentration of 40 pg/mL and an interassay coefficient of variation of 8% at a concentration of 1,000 pg/mL. The E₂ assays for all oocyte donors in our program were performed in our laboratory. A few of the E₂ assays for the oocyte recipients were performed elsewhere.

The hCG assay performed in our laboratory was an automated chemiluminescence assay that was performed on the ACS 180 instrument (Chiron Diagnostics, East Walpole, MA) and was standardized against the World Health Organization Third International Reference Preparation. The hCG assay had interassay and intra-assay coefficients of variation of $<6\%$ at usual working concentrations. Approximately half the serum hCG assays of the oocyte recipients were performed at an outside laboratory; each of these outside assays also was standardized against the World Health Organization Third International Reference Preparation.

TABLE 1

Characteristics of donor stimulation cycles and oocyte yields (44 oocyte retrievals, n = 21) to date.

Characteristic	Mean \pm SD (range)
Age (y)	31.1 \pm 3 (23.3–34.3)
Duration of stimulation (d)	9.7 \pm 1.4 (7–14)
No. of gonadotropin ampules (75 IU) administered	26.8 \pm 8.1 (14–46)
Serum E ₂ level on the day of hCG administration (pg/mL)	1,867 \pm 739 (980–4,464)
No. of oocytes retrieved	17.6 \pm 7.4 (5–37)

RESULTS

Of the 50 donor stimulations performed to date, there were 6 cancellations (12%). Twenty-one donors underwent 44 oocyte retrievals. Purified FSH (Metrodin), highly purified FSH (Fertinex), and recombinant FSH (Gonal-F or Follistim) were used in 21, 14, and 9 stimulations, respectively. Two donors were observed overnight for significant pain after oocyte retrieval. There were no hospitalizations for ovarian hyperstimulation syndrome or other significant complications. The stimulation characteristics of the donors are outlined in Table 1.

Six recipients have not yet undergone transfer of their cryopreserved embryos. Two recipients refused consent for use of their medical record for research purposes and cannot be reported in the present data by law (Minnesota statute 144.335). We therefore report the clinical outcomes of 36 donor oocyte retrieval procedures performed for 34 recipients.

The husband's sperm obtained by masturbation and frozen was used in 32 cases. The husband's sperm obtained by electroejaculation and frozen was used in 1 case. Frozen donor sperm was used in 3 cases. The insemination techniques used included standard insemination in 12 cases, high-concentration insemination in 9 cases, intracytoplasmic sperm injection in 14 cases, and a combination method in 1 case. The mean (\pm SD) number of pronuclear zygotes frozen was 11.6 \pm 5.4 (range, 2–26).

Fifty-one embryo thaw and transfer cycles were performed with cryopreserved pronuclear-stage embryos derived from the 36 donor oocyte retrievals. There were no instances in which embryos were unavailable for transfer. Recipients were prepared with oral micronized E₂ in 39 cycles, transdermal E₂ in 10 cycles, and a combination of both in 2 cycles. The mean (\pm SD) endometrial thickness was 9.6 \pm 2 mm. Three recipients had thawing of their embryos delayed because of suboptimal endometrial thickness. Only 4 recipients had more embryos thawed than they intended to have transferred (a total of 5 embryos). The rate of embryo survival after thawing (confirmed by cleavage) was 93.5%. Embryo characteristics are presented in Table 2.

TABLE 2

Embryo characteristics (51 embryo thaw-transfer cycles) to date.

Characteristic	Mean \pm SD (range)
No. of embryos thawed	3.61 \pm 0.85 (2-6)
No. of embryos that survived (cleaved)	3.37 \pm 0.92 (1-6)
No. of embryos transferred	3.28 \pm 0.80 (1-5)
Embryo quality*	1.17 \pm 0.56 (0.1-2.7)
No. of blastomeres	5 \pm 1.16 (2.8-8)

* Embryo grading was based on the system reported in reference 15.

Clinical outcomes included 27 clinical pregnancies, 2 biochemical pregnancies, and 1 ectopic pregnancy to date (Table 3). There were 26 ongoing pregnancies, for an ongoing pregnancy rate per transfer of 51%. The implantation rate was 28.7%. There were 15 multiple pregnancies (12 twins and 3 triplets), for a multiple pregnancy rate of 57.7%. The percentage of retrievals that resulted in at least one clinical pregnancy was 72.2%. The percentage of retrievals that resulted in at least one ongoing pregnancy was 69.4%.

DISCUSSION

There are many potential advantages to the use of embryo cryopreservation for AOD. Unfortunately, the generally poorer success rates achieved with frozen embryos has led most clinics to establish oocyte donation programs based on the use of fresh embryos despite their own disadvantages. To our knowledge, there has not been a prior published report from a clinic in the United States in which cryopreserved embryos exclusively were used for AOD.

Our refinement of embryo cryopreservation techniques allowed us to use cryopreserved embryos in this particular clinical setting. One of the main features of our cryopreservation method is our reliance on freezing exclusively at the pronuclear stage. Freezing at this stage was noted previously in the literature to be highly efficient and efficacious (10). In addition, with this method, there are no ambiguities about whether embryos survive thawing because subsequent embryo cleavage essentially proves cellular integrity.

Cleavage-stage embryos with blastomeres of differing viability after thawing are now believed to be of questionable quality because these embryos have a much lower implantation potential than those in which all blastomeres survive thawing (16). In our program, the use of presumably high-quality donor oocytes for pronuclear stage embryo cryopreservation resulted in a very high postthaw survival rate (93.5%) and implantation rate (28.7%).

The first advantage of using cryopreserved embryos solely for AOD may be the possibility of safeguarding patient confidentiality. The use of fresh embryos for AOD necessitates that both the donor and the recipient be treated

simultaneously. This presents a small, but identifiable, risk to patient confidentiality. In our system, because both the fertilized donor oocytes and the husband's sperm are initially cryopreserved and later are thawed for use, the donor and the recipient couple are not treated simultaneously. Neither the donor nor the recipient is aware of the actual date of treatment of the other party. This system may minimize the risk of intrusions into patient confidentiality.

Another advantage of using cryopreserved embryos for AOD is the simplification of donor-recipient synchronization. The treatment plan, particularly for recipients, is predictable and straightforward. In programs in which fresh embryos are used for AOD, there invariably is a certain amount of stress and anxiety for recipients because they do not know exactly when their transfer will be performed or how many embryos will be available. In our system, the recipients are notified after retrieval of the donor's oocytes and informed of the number of embryos cryopreserved. This gives them the opportunity to make thoughtful, informed decisions regarding the number of embryos they want to thaw and transfer. In addition, at that time, frozen ETs are scheduled for a specific date. This method optimizes the efficiency of our embryology laboratory. In addition, there appears to be little risk that recipients will undergo hormone treatment when no embryos are available; we have not had a cycle in which no embryos survived thawing.

Our use of cryopreserved embryos also allows us some flexibility in optimizing the hormonal preparation of the recipient. The recipient is not "locked into proceeding" in cycles in which there is a suboptimal endometrial pattern on ultrasound examination or late discovery of other pathologic findings, such as an endometrial polyp or fluid within the endometrial cavity. Under these conditions, we have the option of correcting the pathologic abnormality or attempting to improve the endometrial echogenic pattern.

As we noted, three of our recipients had their hormonal

TABLE 3

Clinical outcomes to date.

Variable	Value
No. of clinical pregnancies (%)	27 (52.9)
No. of ongoing pregnancies (%)	26 (51)
No. of biochemical pregnancies (%)	2 (3.9)
No. of ectopic pregnancies (%)	1 (2)
Multiple pregnancy rate	57.7
Implantation rate	28.7
Percentage of retrievals that resulted in at least one clinical pregnancy	72.2
Percentage of retrievals that resulted in at least one ongoing pregnancy	69.4

Note: Pregnancy rates are expressed as the number of pregnancies achieved per ET.

therapy prolonged and embryo thawing delayed in an attempt to improve their endometrial echogenic patterns. This system also allows the recipient to proceed in a reasonable fashion without requiring "mock treatment cycles." The use of ultrasonography rather than endometrial histology to judge proper endometrial preparation in hormone replacement cycles reduces patient inconvenience in a cost-effective manner.

Last, an additional important benefit of using cryopreserved embryos for AOD is the ability to retest the donor for infectious diseases, including HIV infection, before embryo thawing and transfer. It has been argued that quarantining embryos for 6 months to retest the oocyte donor, as is the present standard in sperm donation, would be ideal (17). In fact, there are European clinics that conduct oocyte donation programs using cryopreserved and quarantined embryos solely for this reason (18).

We retested all our donors at the time of oocyte retrieval for HIV infection and awaited a negative HIV infection report before notifying the recipient. We then offered all recipients the option of further embryo quarantining for 6 months. Some of our recipients chose further quarantining, whereas others did not. Because the 6-month quarantine period often is not favored by our recipients, we are reexamining this issue. It appears that it may be safe to recommend a shorter quarantine period based on the increased sensitivity of second- and third-generation viral antibody detection methods (19).

It is important to note that our report of the percentage of retrievals in which clinical pregnancies (72.2%) and ongoing pregnancies (69.4%) were achieved to date may be underestimates of the full treatment potential. Of the 11 patients who have not achieved an ongoing pregnancy, 6 do not have any remaining cryopreserved embryos. The other 5 patients have remaining cryopreserved embryos (mean embryo number, 10.6; range, 4–15). Therefore, the final percentage of retrievals that result in an ongoing pregnancy may be higher than the percentage reported here.

We achieved a high rate of multiple pregnancy (57%) using a mean of 3.3 embryos per transfer. The embryo thaw survival and implantation potential of these cryopreserved embryos exceeded our initial expectations. There was a 77% rate of multiple pregnancy for the 18 four-embryo and 2 five-embryo transfers performed. Since the beginning of the program, we have decreased the number of embryos that we recommend be transferred. We now usually recommend that 3 embryos be thawed and transferred.

We hope that our efficient embryo cryopreservation system may play a vital role in our efforts to reduce further the rate of multiple gestation because it allows for additional opportunities for the transfer of embryos with high thaw survival and implantation potential. This may allow patients to undergo transfer procedures with fewer embryos without significantly compromising their ultimate chance for pregnancy with each oocyte donation.

In summary, AOD can be performed efficiently and successfully with the exclusive use of cryopreserved embryos. Cryopreserving embryos solely at the pronuclear stage results in high rates of postthaw survival and implantation. The use of cryopreserved embryos for AOD also has many potential advantages, such as protection of patient confidentiality, ease of donor-recipient synchronization, optimization of the recipient's uterine environment, and the possibility of retesting donors for the presence of infectious agents.

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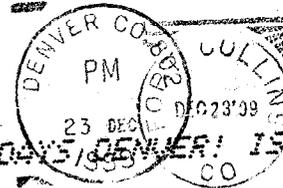
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