

Optimizing success with donor insemination

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1. ABSTRACT

Artificial insemination with donor sperm yields pregnancy rates similar to the general fertile population with the woman's age being the best predictor for success. This article reviews the indications for donor insemination and the current American Society for Reproductive Medicine guidelines for screening both the donors and recipients. For most women, timing the insemination the day after detecting the LH surge with a urinary ovulation predictor kit gives the best results. The addition of clomiphene or letrozole provided no benefit in women with regular menstrual cycles. Superovulation with FSH or hMG did significantly increase the fecundity rate but at a much greater cost and risk of multiple pregnancy and ovarian hyperstimulation syndrome. Intrauterine insemination has been shown to be superior to intracervical insemination in most studies. Adding a second insemination doesn't appear to significantly improve upon the pregnancy rates to justify the additional cost and inconvenience. Fallopian sperm perfusion has shown promise in preliminary studies. The different techniques of sperm processing are reviewed but no technique was clearly better.

2. INDICATIONS FOR DONOR INSEMINATION

The standard indications for considering donor insemination (DI) have been azoospermia, severe oligozoospermia, or other significant sperm or seminal fluid abnormalities, ejaculatory dysfunction or women without a male partner. Additional indications include cases where the male partner has a known significant genetic defect or the couple has had a child affected by a condition for which carrier status cannot be determined, the male partner has a sexually transmissible viral infection and the female partner is negative, or an Rh-negative female partner who is severely Rh isoimmunized and the male partner is Rh-positive. Requests for DI decreased significantly since 1992 due to the introduction of intracytoplasmic sperm injection (ICSI) in clinical *in vitro* fertilization (IVF) (1).

3. DONOR SCREENING

The British Andrology Society published its guidelines for donor screening in 1999 (2). The American Society for Reproductive Medicine (ASRM) Practice

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Committee expanded upon these guidelines in their 2006 publication (3). The following are condensed from their recommendations.

The donors should be in good health and free of genetic abnormalities. The donor should be at least legal age but less than 40 years old. Proven fertility is desirable but is not required. The ASRM guidelines acknowledge that there are no uniformly accepted standards. Both the ASRM and British Andrology Society recommend adhering to the minimal criteria established by the World Health Organization in 1999 (4). These include a sperm concentration of 20 million per ml, 50 percent motility and 30 percent normal morphology. An American College of Obstetricians and Gynecologists (ACOG) committee opinion in 1994 stated the specimen should have at least 20 million motile sperm per insemination (5). Our sperm bank requires a minimum of 50 million sperm per ml with over 60 percent motility prewash and over 50 percent of the motile sperm must survive the cryopreservation and thawing (6).

A complete medical history is obtained with particular attention to risk factors for transmissible infections. The following is a summary of the ASRM exclusion criteria (3):

- Sex with another man in the preceding 5 years
 - Frequent sexual relations with multiple partners
 - Intravenous, intramuscular, or subcutaneous injections of drugs for non-medical reasons in the preceding 5 years
 - Received human-derived clotting factor concentrates for hemophilia or other coagulation disorders
 - Sex in exchange for money or drugs in the preceding 5 years
 - Sex in the preceding 12 months with any person meeting any of the above criteria or with any person suspected of having HIV, hepatitis B, or hepatitis C infection
 - Exposure, through percutaneous inoculation or contact with an open wound, non-intact skin, or mucous membrane, to blood that is known or suspected to be infected with HIV, hepatitis B, and/or hepatitis C virus within the last 12 months
 - Close contact (e.g., living in the same household wherein sharing of kitchen and bathroom facilities occurs regularly) with another person who has viral hepatitis within 12 months preceding the donation
 - Incarcerated in jail (for more than 72 hours) within the previous 12 months
 - Treated for syphilis, gonorrhea, or chlamydia, within the preceding 12 months
 - Undergone acupuncture, body piercing, and/or tattooing procedures within the preceding 12 months in which sterile procedures were not used or it is unclear whether sterile procedures were used
 - Received smallpox vaccination (vaccinia virus) until 21 days after vaccination or until the scab separates spontaneously and physical examination confirms the absence of a scab at the vaccination site (whichever is later)
 - At risk for, or family history of, transmissible spongiform encephalopathy (TSE), such as Creutzfeldt-Jakob disease (CJD); a history of changes in cognition, speech, or gait; or history of exposure to tissues (e.g., dura mater grafts, corneal transplants) suspected of harboring a TSE
 - Fever and headache or a diagnosis of West Nile virus (WNV) infection should be deferred for at least 28 days after the onset of symptoms or diagnosis or for 14 days after resolution of such symptoms (whichever is later)
 - Suspected of having, or received treatment for, sudden acute respiratory syndrome (SARS) during the preceding 28 days, have had close contact with a person known or suspected to have SARS in the preceding 14 days, or traveled to or resided in an area affected by SARS in the preceding 14 days
 - Received xenotransplants (live cells, tissues, or organs from a nonhuman animal source or human body fluids, cells, tissues, or organs that have had ex-vivo contact with live nonhuman animal cells, tissues, or organs) or have been in close contact with a xenotransplant recipient
 - Received human organ or tissue transplants or treatment with human extracts
- A complete physical examination, including evaluation for urethral discharge, genital warts, and genital ulcers is performed on donor candidates with follow-up examinations every 6 months if they remain active donors. Donors should not be used if any such findings are present. Further, the FDA requires that the following tests for infectious disease are negative before the donor can be considered. The donors must be retested at 6 month intervals as long as they remain active.
- HIV-1 and -2
 - HTLV-1 and -2
 - Hepatitis C antibody
 - Hepatitis B surface antigen
 - Hepatitis B core antibody (IgG and IgM)
 - Serologic test for syphilis
 - CMV (IgG and IgM). Men who test positive for active infection (positive urine or throat culture or paired serum samples demonstrating a fourfold rise in IgG antibody and IgM antibody at least 30 percent of the IgG level should be excluded. Because CMV is so common, insemination with semen from a CMV-seropositive man (without active infection) is permissible when the female partner is also CMV seropositive. Although the practice is not entirely without risk, because there are many strains of CMV and superinfection is possible, the associated risk of newborn CMV infection is approximately 1 percent, and such infants appear to have no significant illness or other abnormality.
 - Semen, urinary, or urethral tests should be obtained initially for *Neisseria gonorrhoeae*. Either urethral or

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urinary testing for *Chlamydia trachomatis* should be performed. These tests should be repeated if clinically indicated. Donors found to be positive should be treated and retested before being reconsidered.

The potential donor should be referred for appropriate counseling and management if any positive test is confirmed. If testing is negative, semen samples may be cryopreserved and quarantined for a minimum of 180 days and the donor retested. The cryopreserved specimens may be used for insemination if repeat testing is negative.

All potential donors should be screened for cystic fibrosis and other genetic testing obtained based on the donor's ethnicity and family history. A karyotype is recommended but not required. Psychological assessment and counseling by a qualified mental health professional are strongly recommended for all sperm donors. This is to confirm that the donors are giving full informed consent and that there is no evidence of financial or emotional coercion. The donors should be counseled about the screening process and how the results will be used and shared with others. Also, the donors should be informed as to what personal information might be disclosed to potential recipients as well as how confidential information will be maintained. Finally, the donor should be aware of plans regarding future contact with the offspring in addition to the handling and disposition of any potential embryos. The donor may be excluded for any of the issues listed below. Potential donors who are excluded should be counseled regarding the reasons for their exclusion and, if appropriate, offered referral.

- Presence of significant psychopathology
- Positive family history of heritable psychiatric disorders
- Substance abuse
- Two or more first-degree relatives with substance abuse
- Current use of psychoactive medications
- History of sexual or physical abuse with no professional treatment
- Excessive stress
- Marital instability
- Impaired cognitive functioning
- Mental incompetence
- High-risk sexual practices

Only 13 to 30 percent of prospective donors pass the screening and are suitable for use (7). Using a known donor is acceptable if all parties are in agreement. Confidentiality and the potential effects on the relationships between the donor, the recipient and the child should be discussed. Known donors must undergo the same screening and testing as anonymous donors.

4. RECIPIENT SCREENING

The female recipients should have a complete history and physical examination and must undergo the same laboratory evaluation as the donors with the exception

of genetic testing. In addition, blood type with Rh factor and antibody screening, as well as rubella and varicella titers should be obtained. If rubella and varicella titers indicate susceptibility, vaccination prior to DI is recommended. It is recommended that the male partner of the recipient be tested for the same sexually transmitted infections as the recipient to avoid potential medico-legal problems if the recipient or partner seroconverts following DI treatments. The recipient, and partner if applicable, should be counseled regarding the positive and negative aspects of disclosure and nondisclosure with the offspring in addition to nonbiological parenting issues.

Women with regular 21-35 day menstrual cycles not varying by more than a week are assumed to be ovulating normally. Confirmatory tests such as basal body temperature charting, urinary luteinizing hormone ovulation predictor kits, serum progesterone level or serial ultrasound for follicular monitoring may be obtained if desired. Further fertility testing to document a normal uterine cavity and bilateral tubal patency, by hysterosalpingography or diagnostic laparoscopy and hysteroscopy, is recommended for patients who fail to conceive after four to six DI cycles.

5. TIMING OF INSEMINATION

Based on the 1988 recommendation of the American Fertility Society and the Centers for Disease Control, cryopreserved donor sperm has been used almost exclusively to prevent the transmission of infectious diseases (8,9). Cryopreservation and thawing decreases sperm viability and motility by as much as 50 percent (7). DI with cryopreserved sperm required twice as many cycles as fresh sperm to achieve similar pregnancy rates (10). A randomized trial of 198 cycles in 57 DI patients reported a monthly fecundity rate (MFR) of 20.6 percent for fresh and 9.4 percent for cryopreserved sperm (p greater than 0.003) (11). Therefore, timing of insemination may be more critical with cryopreserved sperm.

Two randomized studies compared DI with cryopreserved sperm using basal body temperature charts versus urinary luteinizing hormone (LH) predictor kits and found the monthly fecundity rate was about double with the LH kits, though statistical significance was not reached (12,13). Zreik *et al* advised against relying on cycle length, symptoms of ovulation, cervical mucus changes and basal body temperature charts due to their inherent inaccuracy (14). Currently, women with regular menstrual cycles, or those on clomiphene citrate (CC), may time DI with urinary ovulation predictor kits to detect the spontaneous LH surge or by administering a human chorionic gonadotropin (hCG) injection when transvaginal ultrasonography reveals a preovulatory follicle. Insemination is usually performed 24 hours after the LH kit tests positive and 36 hours after delivering hCG. The mean interval from hCG to ovulation is 37 to 38 hours with a range of 34 to 46 hours (15). A retrospective study of 90 patients undergoing 182 cycles of husband IUI on CC at 24 or 36 hours post hCG noted a MFR of 7 percent vs. 15.9 percent, respectively (p equals .06) (16).

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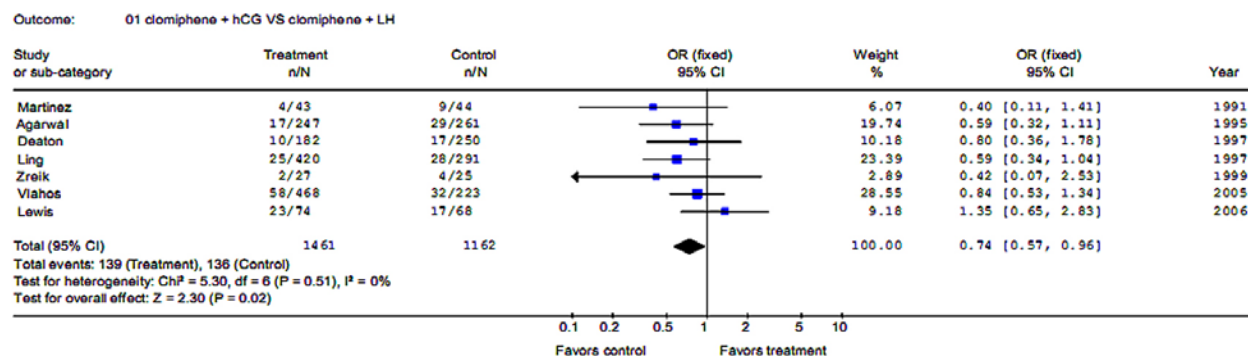


Figure 1. Urinary hCG testing versus administration of hCG for DI timing. Reproduced with permission from (17).

A meta-analysis of seven studies comparing hCG with urinary LH testing for timing intrauterine insemination (IUI) with husband sperm for ovulatory, male factor and unexplained infertility in women on CC reported that hCG had a lower MFR, OR 0.74 (95 percent CI 0.57-0.96), Figure 1. The authors attribute this to the fact that the LH surge can occur at different follicle sizes and that suboptimal timing of hCG carries the theoretical risks of ovulating an immature oocyte or luteinized unruptured follicle syndrome (17). The mean follicle size at ovulation for women on CC was 24 mm with a range of 18 to 30 mm (18). Two studies comparing IUI timed by LH testing or hCG noted that IUI was performed one day later using LH testing than with hCG (18,19). Since women failed to detect the LH surge 23 percent of the time, difficulties with ovulation predictor kits, as well as patient preference, are reasons to select ultrasound monitoring and hCG timing (19).

6. ONE VERSUS TWO INSEMINATIONS PER CYCLE

Recognizing that insemination with cryopreserved sperm is less efficient, many centers perform two inseminations per cycle to try to assure that viable sperm will be present at the time of ovulation. A meta-analysis of eight randomized controlled studies of IUI with husbands' sperm using CC or controlled ovarian hyperstimulation with human menopausal gonadotropins (hMG) and hCG for timing found no difference in MFR, Figure 2 (20). The analysis suffered from substantial heterogeneity regarding the indication for treatment, ovulation stimulation protocols, timing of insemination and sperm preparation. Only one study reported a significantly higher pregnancy rate with two inseminations but it was very small (37 cycles total) and the pregnancy rate in the two insemination group of 52 percent was more than double the highest rates published (21). The third largest study in the analysis performed single IUI 14 to 18 hours after hCG which could explain the poor MFR rate in the single IUI group (22).

Two quasi-randomized studies of DI by IUI yielded conflicting results. Matilsky *et al.* found a significantly higher pregnancy rate with double insemination for women treated with CC or hMG timed by hCG (23). Bissonnette reported no significant difference between one or two inseminations for women on CC timed

with hCG (24). A second IUI only adds to the cost and inconvenience without appreciably improving the treatment outcome (25).

7. SPERM PROCESSING TECHNIQUES

Within the female reproductive tract, motile spermatozoa are separated from the other components of the ejaculate by propelling themselves through cervical mucus. This process is important not only to physically gain access to the upper reproductive tract, but to undergo chemical reactions such as capacitation and the acrosomal reaction which are necessary for fertilization to occur (26,27). Sperm separation must be accomplished artificially in order to perform intrauterine insemination. This is not only to enable the above sperm physiologic processes to occur, but to avoid the introduction of the seminal fluid into the uterine cavity. The high concentrations of prostaglandins in the seminal fluid can induce intense uterine contractions and may even cause anaphylactic reactions (27).

Several techniques of artificial sperm separation are available to choose from. All current techniques have advantages and disadvantages. The simplest technique is the wash and resuspend method. In this technique, the liquefied ejaculate is mixed with culture media and centrifuged. The resulting sperm pellet is resuspended in a small volume of media. This technique accomplishes the goals of removing the seminal plasma and initiating sperm capacitation. While it yields the highest number of sperm, the percentages of normal morphological sperm is the lowest (28). Other methods of sperm separation actually enhance the quality of the sperm sample, though at the expense of lower sperm numbers.

The other techniques of sperm separation are migration, density gradient centrifugation and filtration. Comparative data to aid in selecting one technique over another are sorely lacking. A Cochrane review comparing swim-up and density gradient centrifugation shows no evidence to support one method over another (29). However, this was based on only two small randomized trials, neither of which was double blinded or included live birth as an outcome (28,30). In order to accurately evaluate the different methods, large double blinded randomized trials need to be performed.

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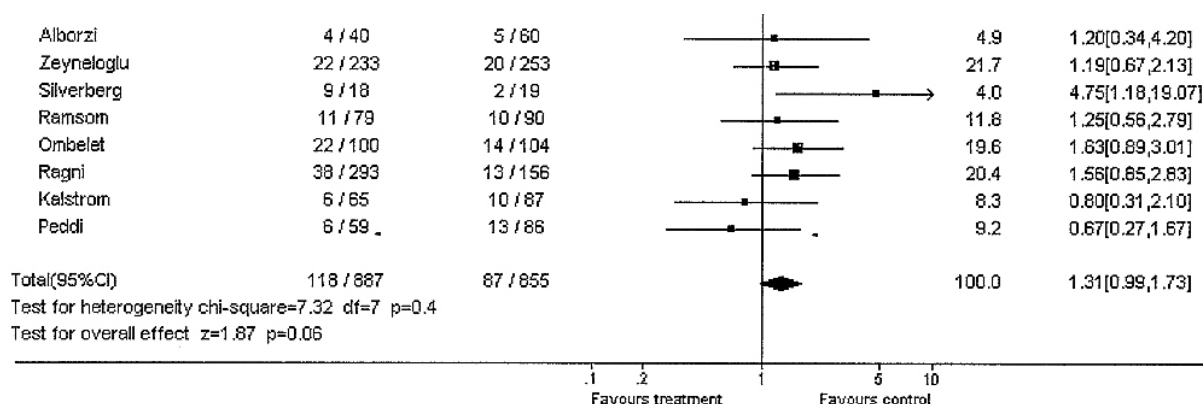


Figure 2. 1 versus 2 inseminations per DI cycle. Reproduced with permission from (20).

7.1. Migration

Migration techniques rely on the ability of the sperm to move through a media (31). The traditional swim-up method was first described in 1984 (32). The ejaculate is centrifuged into a pellet as in the wash and resuspend method. The pellet is then overlaid with media and the sperm are allowed to “swim-up” into the media solution over an hour. This very common method is mostly used in patients with female infertility (27).

This method is easy with recovery of a high percentage of motile sperm. While the percentage of motile sperm is high, the yield of total number of sperm from the ejaculate is low compared to other techniques (28). It requires that ejaculates have high sperm counts. If the pellet is too thick, motile sperm at the bottom may not have time to reach the media. Even with prewashing, the pellet can contain high levels of reactive oxygen species which can damage spermatozoa leading to decreased mobility (33,34). These damaged spermatozoa are then unable to ‘swim-up’ into the media.

Several modifications have been suggested to overcome the problems with the swim-up technique. Different shaped tubes, differing concentrations of media and pellet are among a few (27). A media of hyaluronic acid has been shown to be more effective in separating motile spermatozoa but also can initiate the acrosome reaction prematurely (35-37). The migration-sedimentation is a variation of the migration technique. Media is layered over the unprocessed ejaculate and the sperm swim-up directly from semen with concurrent sedimentation. This technique has advantages over conventional swim-up such as decreased reactive oxygen species and no need for centrifugation. It does however decrease the total number of spermatozoa isolated.

7.2. Density gradient centrifugation

With this method, the ejaculate is layered on top of one or more media of different densities then centrifuged. Motile sperm reach the bottom of the tube faster. The lowest layer is removed and washed as with the wash and resuspend method. There are several density gradient media available for both continuous and step-wise centrifugation. Most of these media contain silane-coated

silica particles with polysucrose (38-42). Advantages of this technique include the ability to use oligozoospermic samples, it decreases the number of leukocytes which can produce reactive oxygen species, and it enriches the proportion of morphologically normal motile sperm. However, it is more expensive and takes longer to perform than the other techniques.

7.3. Filtration

In this technique, the ejaculate is filtered through a substance such as glass wool or Sephadex beads. After filtration to separate the motile spermatozoa from debris and leukocytes, centrifugation is performed. This technique can also be applied to patients with oligozoospermia. By removing leukocytes, reactive oxygen species are reduced (43,44). This technique also has been shown to isolate spermatozoa with normal chromatin (45). Sperm with normal chromatin condensation are thought to yield better IVF success rates (46). One study reported that magnetic cell separation, following sperm washing, can remove apoptotic spermatozoa from the sample (47).

7.4. Chemical treatment of sperm

In addition to above separation techniques, several chemical treatments have been proposed in an attempt to stimulate sperm motility and thus improve its fertilization potential and ultimately, pregnancy rates. The most common treatment is with methylxanthine derivatives such as caffeine. Caffeine increases intracellular cAMP concentrations. Studies on the effects of treating sperm with caffeine showed an increase in motility, but no increase in fertility (48,49). Several studies correlated caffeine consumption and fertility and found that it was largely detrimental (50-52). Pentoxifylline, a xanthine derivative, like caffeine, also increases intracellular cAMP concentrations. There are conflicting data regarding its effects on fertility (53,54). Pentoxifylline is also thought to decrease reactive oxygen species concentrations. Spermatozoa are especially sensitive to reactive oxygen species and seminal fluid contains a high concentration of molecules that neutralize the reactive oxygen species.

Platelet-activating factor (PAF) is found naturally in human sperm its concentration seems to correlate with

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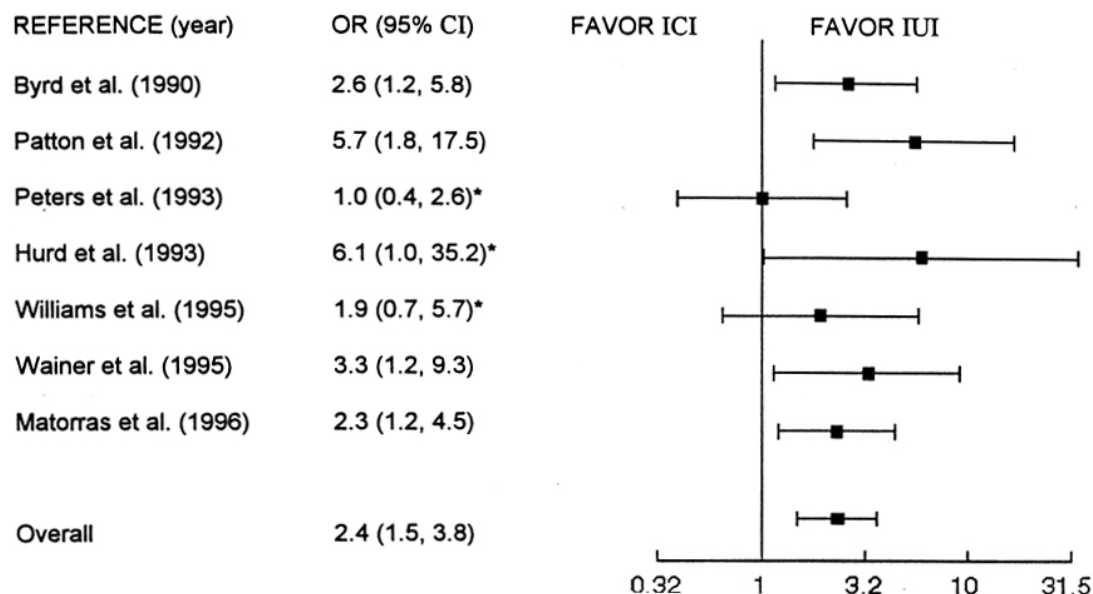


Figure 3. Intrauterine versus intracervical DI. Reproduced with permission from (57).

improved motility and pregnancy rates (55). One randomized controlled study of 52 couples undergoing IUI with clomiphene for unexplained infertility showed an increase in the clinical pregnancy rate from 8 percent to 23 percent with the addition of PAF to the sperm prep media (56). The exact mechanism by which PAF may improve sperm fertilization capacity is unknown and further studies are required before its routine use in clinical IUI can be recommended.

8. INSEMINATION TECHNIQUES

8.1. Intrauterine versus intracervical insemination

Intracervical insemination (ICI) simply places the semen in the cervical canal. A cervical cup may be placed over the cervix for several hours. ICI has the advantages of being less expensive and not requiring a laboratory to prepare the specimen. IUI requires sperm washing by one of a variety of techniques to remove the seminal plasma which can induce severe uterine contractions, and even anaphylaxis, if placed in the uterine cavity. IUI delivers several-fold more motile sperm to the uterine fundus and may be advantageous, especially with the compromised viability of cryopreserved sperm.

A randomized study of DI with fresh versus frozen sperm showed that the MFR with ICI was 20.3 and 7.8 percent respectively, p less than 0.03. However, the rates for IUI were not significantly different, 21.2 and 15.8 percent (p equals 0.6) (11). Therefore, IUI overcomes the adverse effects of the freeze/thaw process. A meta-analysis of seven randomized studies comparing DI with cryopreserved sperm using IUI versus ICI demonstrated that IUI had a significantly higher MFR, OR 2.4 (CI 1.5 – 3.8) (Figure 3) (57). A subsequent randomized comparison of IUI and ICI found that the 15 percent MFR with IUI was not significantly different from the 9 percent with ICI. If the data were limited to one DI per cycle, IUI was

significantly better than ICI, 14 versus 5 percent, p equals 0.04. Adding a second DI per cycle was only of benefit with ICI. There was no difference between one and two inseminations with IUI (58).

The technique of intrauterine insemination is fairly standardized with only slight variations. The catheter should be gently inserted to the top of the fundus and the insemination fluid is then injected slowly. For patients with cervical stenosis or acute angulation of the cervical canal, a tenaculum and/or a catheter with a malleable stylet may facilitate entry past the internal os. Patients are instructed to remain on the table for at least ten minutes as a randomized controlled trial demonstrated significantly higher pregnancy rates per IUI cycle when patients rested for ten minutes versus getting up immediately (59).

There are several types of insemination catheters currently approved for use in the United States, though none have been shown to be superior in several prospective randomized trials evaluating catheter type and clinical pregnancy rates (60-62). A meta-analysis also confirmed this finding (63). The volume of insemination fluid deposited into the uterine cavity also has not shown to influence clinical pregnancy rates (64).

8.2. Fallopian sperm perfusion

Another technique that may improve donor insemination rates is fallopian sperm perfusion (FSP). Following sperm washing, the pellet is resuspended in a larger volume of fluid (4ml) compared with 0.5 ml with conventional IUI. A catheter with a small balloon to occlude the cervix is used and the fluid is slowly injected to perfuse the fallopian tubes. FSP has been shown to be effective in achieving higher pregnancy rates only in patients with idiopathic infertility in two meta-analyses.(65-66) One retrospective trial of FSP for donor insemination utilized frozen donor sperm and

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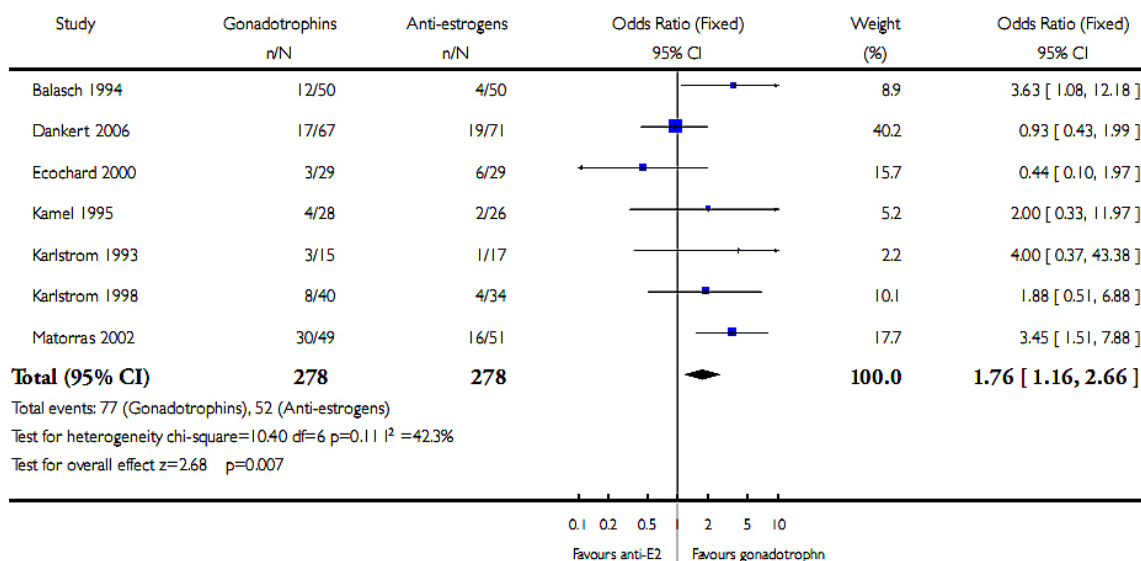


Figure 4. CC vs gonadotrophins with partner IUI. Reproduced with permission from (69).

superovulation. Ninety-six couples underwent 172 cycles with a pregnancy rate of 28 percent per cycle. Overall, 50 percent of patients achieved a pregnancy. (67) Large randomized controlled trials are needed to assess the effect of FSP for donor insemination.

9. OVULATION INDUCTION VS SPONTANEOUS CYCLES

Most women with regular monthly ovulatory menstrual cycles undergo DI with their natural cycle initially. Medical therapies for ovulation induction have been utilized in an attempt to improve pregnancy rates with partner IUI and DI. These include clomiphene citrate (CC), letrozole, and parenteral human menopausal gonadotropins (hMG) or follicle stimulating hormone (FSH) with or without gonadotropin releasing hormone (GnRH) agonists or antagonists.

A meta-analysis of 3106 partner IUI cycles found no difference in pregnancy rates with CC compared to natural cycles, 7 versus 6 percent respectively, whereas FSH doubled the success rate to 15 percent with an OR 2.35 (95 percent CI 1.87 – 2.94) (68). A Cochrane review comparing ovulation stimulation protocols for partner IUI found no difference between CC and letrozole. Gonadotropins (hMG and FSH) were significantly better than CC with a OR of 1.76 (95 percent CI 1.16 – 2.66), (Figure 4). There was no difference between hMG and FSH (urinary or recombinant) nor did the addition of GnRH agonists or antagonists affect pregnancy rates (69).

A retrospective trial of over one thousand IUI cycles with frozen donor sperm noted no difference in pregnancy rates using hMG or CC (70). However, a prospective trial of forty-nine patients randomized to either FSH or CC with DI reported that pregnancy rates were 14 percent per cycle with FSH compared to 6 percent with CC, almost identical to the Hughes meta-analysis with partner

IUI (71). Lashen *et al* treated DI patients with three cycles each of natural cycle followed by CC then FSH with GnRH agonist. The pregnancy rates per cycle and per patient were 13 and 35 percent for natural cycle, 10 and 18 percent for CC, and 21 and 53 percent for FSH with GnRH analog (72). The above studies failed to find a benefit to adding CC for women with regular ovulatory cycles. Letrozole and CC were comparable. However, hMG and FSH both resulted in a doubling of the pregnancy rate per insemination cycle. Unfortunately, these treatments are associated with greater cost, inconvenience and risks of multiple pregnancy and ovarian hyperstimulation syndrome. The addition of GnRH analogs to these treatments only seems to increase cost.

10. PREDICTORS OF SUCCESS

Women treated with DI conceive at the same rate as women who discontinue oral contraceptives (73). Also, a French study of 21,597 DI cycles reported that birth weight, sex ratio, premature delivery, intrauterine growth retardation, chromosomal anomalies and birth defects, ectopic pregnancies and spontaneous abortions were all similar to the general population (74). The age of the women is a strong predictive factor for pregnancy with DI just as it is with spontaneous conceptions and other infertility treatments. In a study of 2998 DI cycles in 443 women, the pregnancy rates at 3, 6 and 12 months in women under 30 years of age was significantly higher than for women 30 years or older; 21 percent, 40 percent and 62 percent versus 17 percent, 26 percent and 44 percent respectively, p equals 0.008 (75). Another study reported that the DI pregnancy rate in women under 35 was five times higher than for women 35 and older, p less than 0.0001 (76). Similarly, ovarian reserve also predicted success with DI. Cycle day three estradiol over 45 pg/ml and FSH IU over 16 were associated with significantly lower pregnancy rates (Table 1) (77).

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Table 1. Correlation of serum estradiol and FSH on DI pregnancy rates (77)

E2	Pregnant	FSH	Pregnant
less than or equal to 45	12/27 (44 percent)	less than or equal to 16	11/23 (48 percent)
		greater than 16	1/4 (25 percent)
greater than 45	3/21 (14 percent)	less than or equal to 16	3/18 (17 percent)
		greater than 16	0/3 (0 percent)

Reproduced with permission from Witt B. R., D.H. Barad, P. Barg, B.L. Cohen, S. R. Lindheim, L. Testaiuti, H. K. Amin: Basal serum follicle stimulating hormone (FSH) and estradiol levels as predictors of pregnancy in unstimulated donor insemination cycles. Reproduced with permission from (77)

Other female factors that were predictive of poorer DI outcomes were primary infertility (76), unilateral tubal occlusion (76,78,79), mild endometriosis (76,80,81) and pelvic adhesions (76). Women at the extremes of body weight have lower success rates with DI. Pregnancy rates were 28 and 21 percent with a body mass index (BMI) of 16 to 19 and 28 to 36 respectively compared with pregnancy rates of 42 and 33 percent with BMIs of 20 to 24 and 25 to 27 percent respectively. (82). Increasing waist to hip ratio was also negatively associated with pregnancy (83). Finally, women with azoospermic partners have higher pregnancy rates than those with oligospermic partners (76,79,80,84). This is presumably because fertile women may conceive with subfertile partners whereas subfertile women can't compensate for the poorer sperm quality.

The donor's age may also influence treatment success. Semen volume decreases 3-22 percent with age, motility decreases 3-27 percent and morphology 4-18 percent while concentration did not appear to be affected. Studies that controlled for the women's age noted a 23-38 percent decrease in pregnancy rates when the partner was over 50 years of age compared with men under 30. (85) Paternal age of 40 or older is considered a risk factor for infertility (86,87). In addition, the risk of spontaneous abortions and late fetal death were higher with men older than 35 when compared to men less than 35 (87). The risk of autosomal dominant diseases such as Alpert's, Marfan's and Waardenbrug syndromes, achondroplasia and neurofibromatosis are increased with greater paternal age (87-89). For the above reasons, the British Andrology Society and the American Society of Reproductive Medicine recommend that donors should be under 40 years of age (2,3).

Studies which evaluated the predictability of the donor's semen profile on pregnancy rates yielded inconsistent results. Thyer *et al.* noted that postwash volume, motility, and morphology were not predictive of fecundability. However, the percentage of normal motile sperm in the postwash specimen directly correlated with the MFR (7). Johnson *et al.* reported that morphology was the most significant predictive factor followed by post-thaw motility (90). Another study, using logistic regression analysis, found that post-thaw motility (p less than 0.001) and morphology (p less than 0.05) were the only variables that correlated with pregnancy rate (1). Macleod *et al.* claimed that conventional semen analysis using World Health Organization criteria was not useful, whereas computer assisted semen analysis (CASA) predicted cycle outcome in 86.9 percent based on faster average path

velocity and lower elongation in specimens resulting in pregnancy (91). Sidhu *et al.* found that semen characteristics in good quality cryopreserved donor semen did not affect pregnancy rates as the rates were similar in pregnant versus nonpregnant cycles with the same donors and recipients (6).

Despite apparently good semen characteristics, some donors have limited or no success (90). The donors' performance at 15 cycles was predictive of the next 25 cycles and it was recommended that performance be calculated every 15 cycles and 5-25 percent of those in the lowest quartile discarded from the donor pool (7). The clinical outcome should be recorded for each insemination cycle and the medical records detailing the donation should be maintained as stipulated by federal and local requirements (3). The pregnancies should also be followed for abnormal offspring (2). A record of delivery is necessary to limit the number of offspring per donor to prevent inadvertent consanguineous conception. The Human Fertilization and Embryology Authority set a maximum of 10 recipient births plus siblings (92). A Dutch study calculated that a donor should be limited to 25 births for a population of 800,000 (93).

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