ANDROLOGY

Effects of Cryopreserved Semen Quality and Timed Intrauterine Insemination on Pregnancy Rate and Gender of Offspring in a Donor Insemination Program

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Purpose: Our purpose was to study the relationship among cryopreserved donor semen quality, pregnancy rates, and preconception sex selection after intrauterine insemination.

Methods: We reviewed the records of the 203 women in our donor insemination program from 1987 to 1994 who became pregnant after more than one insemination cycle and had no female-factor infertility. They were categorized according to the number of cycles required for pregnancy. Semen samples from 54 donors were analyzed before freezing and after thawing. Specimens resulting in pregnancy were compared to specimens from the same donor that did not. Semen characteristics were compared to gender of the child.

Results: Two hundred fifty two-women became pregnant of the 422 who were enrolled. The pregnancy rate per cycle was 13%. Semen quality was not related to pregnancy outcome or offspring gender. However, more male children (101 vs 83) were born.

Conclusions: Semen characteristics in good-quality cryopreserved donor semen do not affect pregnancy rate or offspring gender.

KEY WORDS: artificial insemination; computer-assisted semen analysis; donor sperm; pregnancy; semen characteristics.

INTRODUCTION

Infertility is a common problem in the United States, and almost 16% of couples are infertile (1). Artificial

insemination with frozen donor semen is offered to couples when the man's semen quality is inadequate and, especially, when a couple has undergone extensive medical and surgical treatment to improve semen quality without success. An estimated 30,000 pregnancies occur each year from donor insemination in the United States (2). Recent developments in assisted reproductive techniques such as intracytoplasmic sperm injection have allowed most infertile men to regain fertility. However, these new treatments are too costly for many couples, and until less expensive treatments for male infertility are developed, donor insemination will remain important in the treatment of infertility (3). Others may choose donor insemination due to the genetic risks involved with the advanced micromanipulation procedures such as intracytoplasmic sperm injection (4).

Previous studies demonstrate that the sex ratio cannot be altered by the timing of intercourse (5,6) or by selecting the day of conception (7,8). It is unclear whether the occasional pregnancies occurring 2 or 3 days after ovulation produce a slightly higher proportion of male births (9). Insemination timed to coincide with ovulation may itself account for most, if not all, of the increase in male births reported by Ericsson *et al.* (10). Unfortunately, the evidence has been contradictory because of the inadequacy of the methods for detecting ovulation, estimating the timing of conception, diagnosing early pregnancy, and assessing outcomes.

The purpose of this study was to determine (a) whether spermatozoal characteristics are related to pregnancy after timed IUI, (b) whether prefreeze or

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postthaw semen analysis results would provide better prediction for subsequent pregnancy after timed IUI, and (c) whether any semen characteristic or the timing of insemination corresponding with ovulation is related to the gender of the offspring.

MATERIALS AND METHODS

Patient Selection

This study was approved by our institutional review board. We reviewed the medical records of 422 women who enrolled in our donor insemination program from 1987 to 1994. Women enrolled in our study were screened for endometriosis, pelvic adhesions, tubal disease, or ovulatory dysfunction. Women with bilateral tubal obstruction or severe pelvic adhesions were eliminated. Patients with normal ovulatory cycles received no treatment. Ovulation was documented by midluteal phase serum progesterone and/or secretory endometrial biopsy. Those patients who had a history of sexually transmitted disease were found to have at least one patent fallopian tube by hysterosalpingogram and/or laparoscopy. Pregnancy outcome was used as the measure of success for patients in this study. A quantitative β -hCG unit was performed approximately 2 weeks after insemination and repeated if positive. If both determinations demonstrated normal increases, ultrasound confirmation of pregnancy was obtained 6 weeks after insemination and positive fetal heart motion observed by endovaginal ultrasound detection.

Frozen semen specimen from 54 donors was used, a single donor specimen was used for insemination during various IUI cycles in the same as well as in other patients. Inseminations were always timed by basal body temperature and lutenizing hormone testing using the LH predictor kits to define more precisely the time of ovulation. Intrauterine insemination was timed to correspond to the patient's time of ovulation.

Donor Selection

Donors were selected from healthy volunteers who were tested for the presence of immunodeficiency virus, hepatitis, and other sexually transmitted disease according to American Society for Reproductive Medicine guidelines (11). These donors were matched to the patients racial, ethnic, and physical characteristics. In addition to the abstinence period and semen volume, minimal requirements for an acceptable semen analysis were a sperm concentration of 50×10^6 sperm/ml

with $>60\%$ initial motility as per World Health Organization criteria (12). Several ejaculates were cryopreserved from the same donor and then tested for postthaw motility. The donor was rejected if $<50\%$ of motile sperm survived the freeze-thaw cycle.

Semen Analysis

Standard semen analysis was performed on all samples by computer-assisted semen analyzer. All subjects abstained from ejaculation for 48 hr. The samples were obtained by masturbation into a sterile specimen cup. The ejaculate was allowed to liquefy at 37°C , and the semen characteristics were analyzed on a computer-assisted semen analyzer (Cell-Trak, Model VP 110, Version 4.22B; Motion Analysis, Santa Rosa, CA) for the following criteria: sperm concentration ($\times 10^6/\text{ml}$), motility (%), curvilinear velocity ($\mu\text{m}/\text{sec}$), linearity (%), and amplitude of lateral head displacement (μm).

Donor Cryopreservation, Thawing, and Preparation for Insemination

Semen specimens were cryopreserved by standard protocol (13). TEST-yolk buffer with glycerol (freezing medium) was used as a cryopreservative. An aliquot of the freezing medium equal to 25% of the original semen volume was added to the specimen and it was gently mixed for 5 min using Hema-Tek aliquot mixer (Miles, Elkhart, IN). This was repeated to give a final 1:1 (v/v) ratio of freezing medium and the sperm sample. Cryovials containing the specimens were placed in the freezer at -20°C for 8 min and thereafter in liquid nitrogen vapors at -100°C for 2 hr. The vials were finally transferred to liquid nitrogen at -196°C for long-term storage.

On the day of insemination, cryovials were thawed at room temperature for 5 min and then placed in a 37°C incubator for 20 min. The cryomedium was removed by a wash and resuspend technique. The thawed specimens were washed by mixing with human tubal fluid (HTF) containing 5% human serum albumin (HSA) at a 1:3 ratio. The suspension was washed by centrifuging at 300g for 7 min, resuspended in 1 ml of HTF, and centrifuged again for 7 min at 300g. For insemination, the sperm pellet was resuspended in a volume of 0.5 ml to give a motile sperm count of 20×10^6 .

The timing of insemination for all patients in this study was determined by daily quantitative measurements of urinary luteinizing hormone (LH) beginning 2 to 3 days before the expected time of ovulation.

Inseminations were performed on the day of the LH peak and the following morning. Samples prepared for insemination were transferred approximately 30 min after preparation. The sperm preparation was aspirated into a 3.5-in. Teflon catheter (French Tomcat catheter, Richmond Veterinarian Supply, Richmond, VA) that was connected to a 1-ml syringe (Becton Dickinson, Rutherford, NJ). The catheter was gently passed through the cervical canal to within 1 cm of the fundal extend of the uterine cavity. The patient resumed normal activities after resting for 15 min in supine position.

Prefreeze and postthaw semen characteristics were compared to pregnancy outcome and gender of the offspring. In addition, all women were placed in one of four groups based on the number of inseminations performed to achieve conception. Group I consisted of those women who became pregnant within 2 or 3 cycles; group II, 4 or 5 cycles; group III, 6 to 10 cycles; and group IV, more than 10 cycles. Semen specimens from the same donor that resulted in pregnancy were compared to specimens that did not in the same woman during the previous insemination cycle. These two inseminations were done within a 3-month period.

Statistical Analysis

The Kaplan–Meier estimates test was used to determine the pregnancy rate based on the number of insemination cycles each patient underwent. The ratio estimate method was also used to determine the percentage of pregnancies per cycle. A paired Student's *t* test and the signed rank test were used to compare the preefreeze and postthaw semen characteristics of the semen specimen that resulted in pregnancy to the previous specimen that did not. Semen analysis results from the donor specimen that resulted in the birth of a boy or a girl were compared to analyze the relationship between the sperm characteristics and gender. To observe the effect of IUI timed with ovulation on pre-conception sex selection, the incidence of the birth of a male child was compared to that of a female child and with the natural sex ratio by Student's *t* test. The SAS statistical software package (SAS Institute Inc., Cary, NC) was used for analyzing the data.

RESULTS

Of the 422 women who underwent artificial insemination with donor semen, 60% (252/422) became pregnant. After the first insemination, 19% (49/422) of the

Table I. Pregnancy Rate and Number of Insemination Cycles^a

No. of cycles	Pregnancy rate (%)	Confidence interval (95%)	
		Lower	Upper
1	18.01	14.34	21.68
2	31.25	26.74	35.76
3	40.66	35.76	45.57
4	45.89	40.83	50.95
5	52.57	47.31	57.82
6	57.39	52.01	62.77
7	62.12	56.60	67.65
8	67.31	61.69	72.94
9	70.69	65.04	76.35
10	71.33	65.66	77.00
15	83.72	77.27	90.18
20	85.53	78.89	92.17
25	90.35	81.46	99.25
30	95.18	87.15	100.00

^a Pregnancy rates calculated according to the Kaplan–Meier estimates.

women became pregnant, while 203 women achieved pregnancies after more than one cycle of IUI (Table I, Fig. 1).

The pregnancy rate increased with the increase in the number of insemination cycles a woman underwent (Fig. 2). However, at the same time, the number of women available for these inseminations was very small, especially in those cases where there were more than 15 insemination cycles (Table II).

In our study, the patients underwent a total of 1881 insemination cycles. A total of 252 women in the study became pregnant. Using the ratio estimator method, the pregnancy rate per cycle was 13.4% (0.134, with a lower limit of 0.117 and an upper limit of 0.151 at the 95% confidence interval).

When sperm characteristics were compared with the pregnancies, in group I postthaw velocity ($P = 0.03$) and linearity ($P = 0.004$) were significantly higher in specimens that resulted in pregnancy (Table II). Prefreeze and postthaw semen characteristics of the specimens in groups II, III, and IV were analyzed for comparison between the groups that resulted in pregnancy and those that did not. No significant differences were seen among the three groups. We also compared the semen characteristics to determine an association between the postthaw semen characteristics and the sex ratio of the child. Although a higher number of male children were born (55%; 101/184) compared to the number of girls (45%; 83/184), these differences were not significant (Table III).

Fig. 1. Pregnancy data for 422 women enrolled in the donor insemination program.

DISCUSSION

Our study differs from earlier reports in that normal semen from healthy donors was used for inseminations rather than semen from subfertile men (14,15). Furthermore, all women included in this study were without any interfering female factors. This prevented false negative observation of failure to conceive. Finally, a follow-up on all insemination cycles was done in all women to evaluate more accurately the relationship between semen characteristics and conception.

The monthly fecundity rates range from 5 to about 15% for cryopreserved semen (16–19). The probability of pregnancy is 5.4% after one cycle and 48.6% after seven cycles (20). The pregnancy rate per cycle has been reported to drop significantly after the third insemination cycle (21,22). An overall pregnancy rate of 9.9% per treatment cycle has been reported (16,23,24). In our study a pregnancy rate of 13% per cycle was seen. The pregnancy rate increased with an

increasing number of insemination cycles, however, the number of women undergoing subsequent inseminations dropped significantly after the tenth cycle ($n = 39$).

Compared to fresh semen, frozen–thawed semen has a diminished capacity to achieve pregnancy after donor insemination (17,19,23). Fertility outcome can be better predicted with sperm motion characteristics of a frozen–thawed specimen following insemination (24,25). The relationship between sperm motion characteristics and pregnancy rates using fresh and frozen sperm is conflicting (16–19,22,26–30). In subfertile patients, pregnancy rates are highly correlated with velocity, linearity, motility, or sperm concentration (14,15,31). In contrast to other reports, we did not find significant differences in the sperm motion characteristics and the number of pregnancies in the different groups of inseminations. This lack of correspondence between normal donor sperm characteristics and preg-

Table II. Comparison of Cryopreserved Donor Specimens that Resulted in Pregnancy Compared with Specimens that Did Not Result in Pregnancy in the Same Patient^a

Number of cycles	Volume (ml)	Motility (%)		Velocity ($\mu\text{m}/\text{sec}$)		Linearity (%)		ALH (μm)	
		Prefreeze	Posthaw	Prefreeze	Posthaw	Prefreeze	Posthaw	Prefreeze	Posthaw
2-3 cycles (group I; $n = 85$)									
No pregnancy specimen	3.1 $\pm$ 1.6	64.9 $\pm$ 14.9	36.1 $\pm$ 12.0	46.9 $\pm$ 6.0	38.6 $\pm$ 6.0	5.5 $\pm$ 0.6	5.4 $\pm$ 0.8	2.1 $\pm$ 1.3	1.7 $\pm$ 1.1
Pregnancy specimen	3.2 $\pm$ 1.5	66.1 $\pm$ 13.7	37.1 $\pm$ 12.1	46.6 $\pm$ 9.9	40.0 $\pm$ 6.1	5.5 $\pm$ 0.7	5.6 $\pm$ 0.9	2.1 $\pm$ 1.3	1.7 $\pm$ 1.1
Difference	0.1 $\pm$ 1.0	1.3 $\pm$ 10.8	0.98 $\pm$ 9.7	0.33 $\pm$ 8.2	1.4 $\pm$ 5.7	0.02 $\pm$ 0.6	0.18 $\pm$ 0.6	0.03 $\pm$ 0.6	-0.2 $\pm$ 0.6
<i>P</i> value	0.26	0.29	0.35	0.71	0.025	0.74	0.004	0.58	0.80
4-6 cycles (group II; $n = 53$)									
No pregnancy specimen	3.2 $\pm$ 1.3	65.3 $\pm$ 14.9	36.8 $\pm$ 10.3	49.1 $\pm$ 9.6	39.9 $\pm$ 4.7	5.6 $\pm$ 0.7	5.7 $\pm$ 0.8	2.4 $\pm$ 1.2	1.9 $\pm$ 0.8
Pregnancy specimen	3.3 $\pm$ 1.4	68.3 $\pm$ 12.0	36.4 $\pm$ 9.0	49.4 $\pm$ 9.9	40.2 $\pm$ 4.5	5.7 $\pm$ 0.7	5.7 $\pm$ 0.8	2.4 $\pm$ 1.2	2.0 $\pm$ 0.8
Difference	0.1 $\pm$ 0.8	3.1 $\pm$ 11.3	-0.4 $\pm$ 10.8	0.32 $\pm$ 6.8	0.37 $\pm$ 3.8	0.7 $\pm$ 0.54	-0.04 $\pm$ 0.5	0.01 $\pm$ 0.8	0.05 $\pm$ 0.5
<i>P</i> value	0.35	0.051	0.78	0.73	0.48	0.32	0.60	0.91	0.53
6-10 cycles (group III; $n = 46$)									
No pregnancy specimen	3.3 $\pm$ 1.7	63.5 $\pm$ 12.8	36.7 $\pm$ 11.4	47.5 $\pm$ 8.6	38.7 $\pm$ 4.4	5.5 $\pm$ 1.2	5.7 $\pm$ 0.7	2.3 $\pm$ 1.2	1.8 $\pm$ 0.9
Pregnancy specimen	3.3 $\pm$ 1.5	64.9 $\pm$ 11.2	36.9 $\pm$ 12.2	48.2 $\pm$ 8.5	39.0 $\pm$ 5.4	5.5 $\pm$ 0.8	5.7 $\pm$ 0.7	2.4 $\pm$ 1.1	1.9 $\pm$ 0.8
Difference	0.06 $\pm$ 0.8	1.4 $\pm$ 11.1	0.23 $\pm$ 10.6	0.72 $\pm$ 7.4	0.34 $\pm$ 5.0	0.07 $\pm$ 1.2	-0.02 $\pm$ 0.5	0.05 $\pm$ 0.6	0.03 $\pm$ 0.4
<i>P</i> -value	0.42	0.38	0.88	0.52	-0.64	0.71	0.78	0.57	0.61
>10 cycles (group IV; $n = 19$)									
No pregnancy specimen	3.4 $\pm$ 1.3	61.6 $\pm$ 13.0	38.0 $\pm$ 10.3	52.3 $\pm$ 7.6	38.1 $\pm$ 3.6	5.6 $\pm$ 0.7	5.9 $\pm$ 0.8	2.8 $\pm$ 0.9	2.0 $\pm$ 0.6
Pregnancy specimen	3.5 $\pm$ 1.3	65.7 $\pm$ 11.2	38.8 $\pm$ 9.4	49.8 $\pm$ 7.0	39.6 $\pm$ 4.2	5.8 $\pm$ 0.8	5.9 $\pm$ 0.6	2.6 $\pm$ 0.8	2.1 $\pm$ 0.6
Difference	0.1 $\pm$ 0.6	4.2 $\pm$ 13.2	0.9 $\pm$ 9.6	1.5 $\pm$ 3.9	1.5 $\pm$ 3.9	0.2 $\pm$ 0.5	0.03 $\pm$ 0.5	-0.14 $\pm$ 0.5	0.11 $\pm$ 0.3
<i>P</i> value	0.42	0.19	0.7	0.11	0.11	0.17	0.8	0.24	0.11

^a Values are expressed as mean $\pm$ SD. *P* values of less than 0.05 considered significant.

Fig. 2. Life table analysis of pregnancy rates and number of insemination cycles.

nancy outcome indicates that factors other than semen quality may be important (21,23,32–35).

Some investigators have reported that the timing of intercourse or insemination affects the sex ratio (36,37), but others have found no relationship (7,37,38). Similarly, reports of other preconception sex selection methods are also conflicting (39,40). The percentage of male births observed in our study is similar to that reported by Dickey *et al.* (36). The reported increase in the birth ratio by “Y-sperm” enrichment procedures (10) may be explained partly by the timing of insemination.

In summary, our study suggests that donor sperm characteristics from normozoospermic men do not play a major role in initiating a pregnancy using intrauterine insemination. The quality of donor semen specimen is not related to the gender of the offspring. Insemination corresponding with ovulation may be a factor in preconception sex selection.

Table III. Comparison of Postthaw Semen Characteristics of Donor Specimens and the Gender of the Offspring

Semen characteristic	Child delivered ^a		P value ^b
	Girl (n = 83)	Boy (n = 101)	
Volume (ml)	3.2 ± 1.6	3.3 ± 1.3	0.77
Concentration (10 ⁶ /ml)	133.4 ± 67.1	122.4 ± 60.2	0.24
Postthaw characteristics			
Motility (%)	41.2 ± 13.3	38.2 ± 12.4	0.11
Velocity (μ/sec)	39.4 ± 5.0	40.3 ± 7.0	0.34
Linearity (%)	5.6 ± 0.76	5.63 ± 0.86	0.63
ALH (μm)	1.7 ± 1.0	1.84 ± 1.0	0.40

^a Values are mean ± SD.

^b P < 0.05 considered significant.

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