Improvement of sperm motility by short-interval sequential ejaculation in oligoasthenozoospermic patients

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Abstract

Introduction: The aim of this study was to evaluate the effect of a consecutive second ejaculate, i.e, to determine if it contains an equal, or even higher, number of motile sperm, and whether it can improve the fertilization rate in IVF-ET treatment.

Material and methods: From July 1996 through April 2007, 32 cycles in 22 couples, who had previously exhibited oligoasthenozoospermia, were recruited in this study. The first ejaculate was obtained after 3-5 days following sexual ejaculate abstinence. The second ejaculate was obtained 30-60 min after the first one. Several parameters concerning sperm quality and fertilization rate in IVF were compared between the first and second ejaculation.

Results: The mean duration between the first and second ejaculation was 34.2 min (mean ± SEM). The second ejaculate showed a significant reduction in the volume of ejaculate. The sperm motility (35.2±13.3) and motile sperm concentration (5.4±7.3) of the second ejaculate were significantly higher than those of the first (24.3±13.2 and 2.2±1.8, respectively; $P<0.05$). The fertilization rate of sperm from the second ejaculate (53.3%) was significantly higher than that from the first one (28.9%, $P<0.05$), but cleavage rates and embryo scores were similar.

Conclusions: Therefore, this sequential ejaculation might be useful for treatment of male infertility at these hospital and clinics.

Key words: IVF, male infertility, motility, sperm concentration, sequential ejaculate.

Introduction

It is a commonly held belief that sperm count is inversely related to frequency of intercourse. In normozoospermic men, sperm counts were shown to decrease significantly with sequential ejaculation. A nearly 60% decrease in the total motile sperm count of the second ejaculate compared with the first has been reported [1]. A previous study showed a correlation between sperm count and frequency of ejaculation and confirmed that sperm concentration, volume and total sperm count decreased with frequent ejaculation; the conclusion being that to increase the chance of fertilization, it might be more efficient to have intercourse every other day rather than daily [2].

Intrauterine insemination (IUI), *in vitro* fertilization and embryo transfer (IVF-ET) have become suitable treatments for male infertility. Since

the introduction of intracytoplasmic sperm injection (ICSI) by Palermo et al, in 1992 [3], the outcome for these patients has improved dramatically. In IUI and IVF-ET, a higher number of sperm appears to improve fertilization rates [4, 5]. Sperm preparation procedures for selecting motile sperm may significantly decrease the number of sperm available for insemination [6]. Consequently, the final motile sperm fraction of oligozoospermic males may, at times, be insufficient for adequate insemination and/or re-insemination of oocytes.

Recently, it was reported that the second consecutive ejaculate of oligozoospermic men might contain an equal, or even higher, number of motile sperm than the first ejaculate in contrast with normozoospermic males [7-12]. The aim of this study was to evaluate the effect of the second ejaculate collected from infertile men as to sperm motility and fertilization in IVF-ET treatment.

Material and methods

Patients

Thirty-two cycles in 22 couples who previously exhibited oligoasthenozoospermia at the IVF-ET unit of the Department of Obstetrics and Gynecology at Tokushima University Hospital and at the Division of Reproductive Medicine, Sugiyama Clinic from July 1996 through April 2007 were recruited in this study. The inclusion criteria were as follows: for preprocessing semen, sperm concentration was less than 20 million/ml and sperm motility was less than 50%. The mean age of the men and women were 33.2±2.5 and 39.1±3.1 years of age, respectively (mean ± SEM).

Ovarian hyperstimulation

To 32 cycles in 22 infertile couples, intrauterine insemination and IVF-ET were performed in 7 cycles and 25 cycles, respectively. All patients receiving IVF-ET were stimulated according to the GnRH-a long protocol using the human menopausal gonadotrophin (hMG) protocol [13]. Our conventional ovarian hyperstimulation protocol with GnRH-agonist (GnRH-a) was as follows: 600 µg of buserelin acetate (Suprecur; Mochida, Tokyo) was intranasally administered daily from the midluteal phase of the pretreatment cycle to the day of hCG injection. After menstrual flow, 225 IU of follicle-stimulating hormone (FSH, Fertinom P; Merk-Serono, Tokyo) was administered daily from the third day of the treatment cycle for 4 days, and, consequently, 150 IU of human menopausal gonadotropin (hMG, Humegon; Organon, Tokyo) was given daily until a dominant follicle reached 18 mm in diameter. Human chorionic gonadotropin (5000 IU, Gonatropin®; Teikoku-Zoki, Tokyo) was injected 35 hours before oocyte retrieval. The scheduled ovarian hyperstimulation protocol was the almost the same as the conventional method except that 4 days of FSH administration was started 11 days before scheduled oocyte retrieval day, followed by hMG administration for 5 days. Details of the protocol were described in a previous report [13, 14].

The first ejaculate was obtained after 3-5 days of sexual ejaculate abstinence. The second ejaculate was obtained 30-60 min after the first. The ejaculates were assessed using light microscopy and swim-up studies. The sperm quality was analyzed blindly.

The oocytes were assigned equally and randomly for exposure to sperm of either the first or the second ejaculate. In every case, 50,000 motile sperm were added to a tube containing one preincubated oocytes. Fertilization rates were assessed after 24 h of incubation. Fertilization was defined as the presence of two pronuclei with the extraction of the second polar body. Pregnancy was defined as the development of the gestational sac visualized by transvaginal ultrasonography within 18-20 days after embryo transfer.

Sperm analysis

After liquefaction at room temperature for 30 min, an aliquot of the sperm sample was diluted and counted using a Nitrin hemocytometer (Niippon Rinsho Kikaikogyou, Tokyo) to determine total sperm count, sperm motility and anomaly by light microscopy. Microcephalic, macrocephalic, bicephalous, and bicaudal spermatozoa or cells processing a coiled tail or a deformed or anomalous small acrosome were all classified as abnormal. Total motile sperm counts were calculated from the motile sperm concentrations and the volumes of the ejaculates, and the first and second ejaculates were compared for several parameters. processing

Swim-up studies

After liquefaction, all sperm samples were washed two times using human tubal fluid (HTF) medium (Irvin Scientific, Santa Ana, Calif) supplemented with 7.5% of heat-inactivated patient's serum. After washing twice, aliquots of the sperm samples were transferred to 5 ml Falcon tubes (Falcon 2058, Becton Dickinson Labware, Franklin Lakes, NJ) containing 0.5 ml of HTF medium supplemented with 7.5% patient's serum. The semen was injected carefully under the medium to avoid bubbles. The tubes were incubated at 37°C in an atmosphere of 5% CO_2 in air for 60 min. Therefore, an aliquot of overlying medium was aspirated. A portion of the medium was removed to assess count and motility, and he rest was used for fertilization of oocytes. In this particular

Table I. Comparison of semen parameters between two sequential ejaculates

	First ejaculate	Second ejaculate
Volume [ml]	3.4±1.4	1.9±7.3*
Sperm concentration [million/ml]	8.9±5.0	11.7±7.3
Motility [%]	24.3±13.2	35.2±13.3*
Motile sperm concentration [million/ml]	2.2±1.8	5.4±7.3*
Total motile sperm count [million]**	6.9	6.8

Values are mean ± SD
**P<0.05, **values are mean*

swim-up technique a sperm pellet is not produced: instead, a liquid-to-liquid interface is created.

IVF procedure

Oocytes were retrieved transvaginally using a needle-guided technique with ultrasound imaging. HTF medium supplemented with 7.5% or 15% heat-inactivated patient's serum (v/v) was used for insemination and embryo culture *in vitro*, respectively, in non-immunological cases; in immunological cases, donors' sera were used. Donors' sera were separated from the blood of non-immunologically infertile women who underwent successful IVF-ET treatment. The donors had no history of any known deleterious diseases, and no laboratory evidence of infection such as syphilis, hepatitis and adult T cell leukemia. All donors' and patients' sera were heated at 56°C for 30 min to inactivate human immunodeficiency virus and complement and used as mixtures with T6 medium or HTF medium at 7.5% (v/v) for insemination and 15% for embryo culture. Semen was produced by masturbation and, after washing, sperm were allowed a 30-60 min swim-up. *In vitro* insemination was performed by incubating each oocyte with 5×10^4 motile sperm within 5-6 h after collection.

Inseminated oocytes were examined under a dissecting microscope 16-18 h after insemination. The presence of two pronuclei with extrusion of the second polar body was taken as evidence of successful fertilization. Fertilized oocytes were transferred to cleavage medium, and examined for cleavage 24 h after fertilization.

Oocytes from IVF-ET patients with a post-swim-up motile sperm count of 50,000 sperm per one oocyte in the first and second ejaculate were randomly allocated to two groups. The motile sperm after swim up obtained from the first and second ejaculates were inseminated. The embryo derived from the first and second ejaculates were incubated and scored individually according to the embryo score previously described [14].

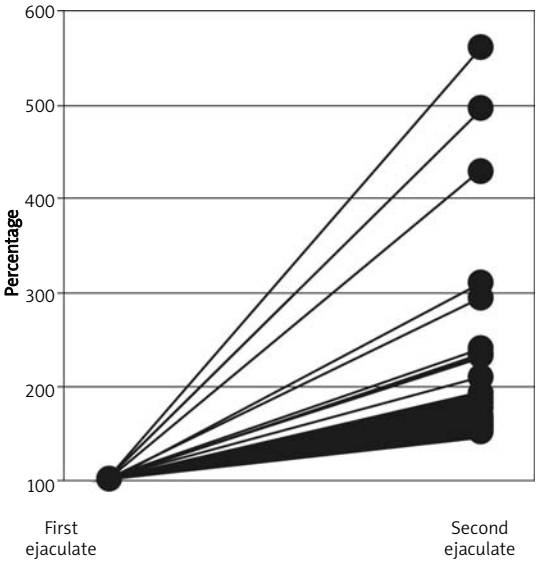

Figure 1. The change on rate of the sum of the total motile sperm count of the second ejaculates
This graph indicates the rate of the sum of total motile sperm count in both the first and second ejaculates to the sum of total motile sperm count in the first ejaculate. The sum of total motile sperm count in the first and second ejaculates increased about 2.5 times the total motile sperm counts in the first one alone.

Statistical analysis

All data are presented as real numbers, percentages, or the mean ± SD (standard deviation). The significance of the differences was determined using a Mann-Whitney U test and a χ^2 test.

Results

The results of the sperm analysis for the first and second ejaculates of 32 cycles in 22 oligo-asthenozoospermic men are summarized in Table I. The mean duration between the first and second ejaculation was 34.2 min. The second ejaculate showed a significant reduction in the volume of ejaculates (3.4±1.4 in the first one and 1.9±0.9 in the second one, respectively, P<0.05). The sperm concentration of the first ejaculate was 8.9±5.0 million/ml, and that of the second was 11.7±11.2 million/ml, which equates to no significant difference. However, the sperm motility (35.2±13.3) and motile sperm concentration (5.4±7.3) of the second ejaculate were significantly higher than those of the first one (24.3±13.2 and 2.2±1.8, respectively, P<0.05). The motile sperm count in the first and second ejaculates were 6.9 and 6.8 million, respectively, but there was no significant difference. However, the sum of the total motile sperm count of the first and second ejaculates increased by about 2.5 times the total motile sperm count found in only the first ejaculate (Figure 1).

In all 68 oocytes, 38 were inseminated by the first ejaculates and 30 by the second. The IVF outcomes are summarized in Table II. The number of fertilized oocytes inseminated by the first ejaculates was 11, and fertilization and cleavage rates were 28.9 and 90.9%, respectively. The number of fertilized oocytes inseminated by the second ejaculates was 16, and fertilization and cleavage rates were 53.3 and 93.8%, respectively. The fertilization rate for the second ejaculate was significantly higher than that of the first (P<0.05). The mean fertilization rates per patient from the first and second ejaculates were 28.8±33.0 and 56.1±42.0, respectively, which represents a significant difference (P<0.05). The embryo score from the first and second ejaculates were 5.9±1.4 and 6.1±6.1, respectively.

Table II. Comparison of IVF outcomes between two sequential ejaculates

	First ejaculate	Second ejaculate
Number of inseminated oocytes	38	30
Number of fertilized oocytes	11	16
Fertilization rate [%]	28.9	53.3**
Mean fertilization rate [%]*	28.8±33.0	56.1±42.0**
Cleavage rate [%]	90.1	92.3
Score of embryos*	5.9±1.4	6.1±6.1

**Values are mean ±SD, **P<0.05*

Disccusion

This study demonstrated that the second consecutive ejaculate collected a short interval after the first showed an improved sperm quality in oligoasthenozoospermic patients. The improvement was mainly in motility and motile sperm concentration, but no improvement was shown in sperm cell count. The volume of the second ejaculate was significantly decreased in comparison with the first. These results coincide with previous reports by Barash et al. [7] and Tur-Kaspa et al. [8]. The fertilization rate using the second ejaculated sperm tended to be higher than that using the first, but there was no significant difference. Total motile sperm count in the first ejaculate was not improved by means of sequential ejaculation.

Recently, Barash et al. [7] demonstrated that the fertilization, cleavage and pregnancy rates were increased when oocytes were exposed to sperm from the second ejaculate. Therefore, to evaluate these findings we tried to perform the insemination using either the first or second ejaculated sperm. The fertilization rate with the second ejaculated sperm was significantly higher than that with the first, but the cleavage rate and embryo quality was similar. The pregnancy rate could not be compared because two or three embryos were transferred to the patients according to embryo quality rather than by ejaculation groups. However, 68% of IVF treatment cycles could be performed by embryo transfer and clinical pregnancy rate per embryo transfer was 17.6% in spite of due to male infertility. From these results, it is believed that sequential ejaculation might be useful for male infertility patients.

The mechanism of this improvement in sperm quality for the second consecutive ejaculate collected in a short interval is unclear. Barash et al. [7] speculated that improvement in certain semen parameters of the second ejaculate also showed improved *in vivo* reproductive function with frequent intra-vaginal ejaculation. *In vivo*, sperm ascendance in the female genital tract probably depends on the total number of motile sperm ejaculated into the vagina. From our data, we presumed that frequent intra-vaginal ejaculation might make up for the reproductive function *in vivo*.

In this manner, the data presented in our study coalesce with previous data [7, 8] in raising an important question regarding the value of sexual abstinence in fertile treatment. If the second consecutive ejaculate on the same day is of improved quality in subfertile men, then perhaps infertile couples should not be instructed to copulate only once every 36 hours. In many cases, women actually do conceive with coital frequency of more than once a day.

Now, intracytoplasmic sperm injection (ICSI) is widespread the world over, and the outcomes of male infertility patients have improved dramatically. Although ICSI has already become a common procedure for the male infertility, some infertile couples in this study were hesitant to undergo ICSI. There was no particular reason for this hesitancy, but couples, especially men, were unlikely to undergo ART due to vague anxiety. Therefore, a regimen of sequential ejaculation might be useful for male infertility couples at hospital and clinics.

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