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### ABSTRACT

**Objective:** To assess the seminal characteristics of multiple ejaculates collected sequentially via masturbation or at intercourse using a semen collection device (Male Factor Pak™, MFP™).

**Design:** Three ejaculates were collected by patients over a 3 day period (1 semen specimen/day) via masturbation or at intercourse by using the MFP™. Semen specimens were assessed for volume (ml), sperm count ( $\times 10^6/\text{mL}$ ), percentage and grade of motility, percentage of normal morphology and for the total functional sperm fraction (TFSF;  $\times 10^6$ ).

**Setting:** Clinical and research environment.

**Patient(s):** Forty couples participating in an intrauterine insemination (IUI) program.

**Main Outcome Measure(s):** Differences in semen quantitative and qualitative characteristics of ejaculates produced via masturbation or intercourse.

**Result(s):** The sperm count of the first ejaculate collected at intercourse or via masturbation yielded  $170.0 \pm 19.0$  and  $150.0 \pm 21.0 \times 10^6$  spermatozoa, respectively. The sperm count decreased by 53% and 32% on day 2 and 3 of semen collection via masturbation. The sperm count decreased by 71% and 49% on day 2 and 3 of semen collection via the use of the MFP™ at intercourse ( $P < 0.05$ ). The semen volume decreased by 45% and 50% after the third collection day in specimens collected at intercourse or via masturbation, respectively. Semen qualitative characteristics increased as a function of collection frequency, regardless of collection method. Those characteristics tended to be higher, but not significantly different ( $P > 0.05$ ), in specimens collected at intercourse.

**Conclusion(s):** Using the semen collection device, there was a tendency to have better quantitative and qualitative semen characteristics as well as the advantage that the method of collection will closely resemble the semen produced naturally when deposited in the vagina during intercourse and subsequent ejaculation.

**Key words:** semen, spermatozoa, masturbation, intercourse

Harvesting the maximum quantity and quality of spermatozoa is of extreme importance in an artificial insemination intrauterine insemination (IUI) program using husband (AIH) or donor (AID) semen for infertility treatment purposes (1-3).

The collected specimen should, as closely as possible, resemble the ejaculate delivered during intercourse, if the male infertility factor is to be properly identified and treated (1-10). Seminal characteristics in the various animal species can be influenced by factors such as the frequency of collection, degree of stabilization of epididymal sperm reserves and extent of sexual stimulation (11).

Precoital sexual stimulation affects both the composition of the ejaculate and androgen secretion as observed in various animal models.

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Restraining or false mounts prior to semen collection significantly increases the number of motile spermatozoa in bulls and boars (12, 13). Meanwhile, the most widely accepted method of semen collection in humans for the purpose of semen analysis or artificial insemination is via masturbation (1, 2, 4-9, 14, 15). It has been shown that precoital sexual stimulation (PSS) in conjunction with the use of a semen collection device at intercourse significantly improves the quantity and quality of collected spermatozoa (15). The semen collection device consists of a nonspermicidal condom generally made of materials such as polyurethane or silicone (1-10, 15-17). Due to its acceptability by patients, lack of negative effects on sperm viability and assistance in the improvement of collected specimens to closely resemble the ejaculates obtained at intercourse, those devices may be used routinely for semen collection, and specially for the improvement of semen specimens with various dysfunctions and subsequent use in an IUI program or various forms of assisted reproductive technologies (ART; 1-10, 15-18).

The objective of this study was to compare the semen characteristics in ejaculates collected sequentially by patients via masturbation or at intercourse by using a semen collection device during a 3 day collection period.

## MATERIALS AND METHODS

### Semen Collection and Assessment

Forty couples who were referred to our laboratory for andrological work-up and

subsequent IUI procedures participated in this study. The male patients were instructed to produce one semen specimen every 24 hours during a 3 day collection period via either masturbation or at intercourse using a semen collection device. Fifteen men were instructed to produce semen specimens via masturbation and the other twenty-five men were instructed to produce semen specimens using a semen collection device at intercourse (1-10). Men that could not produced specimens in the required manner, from either group, were eliminated from the study. The semen collection device consisted of a nonspermicidal condom made of polyurethane (Male Factor Pak™ MFP™; ZDL, Inc., Lexington, KY, USA). Specimens were assessed according to the World Health Organization (19) standards for volume (ml), count ( $\times 10^6/\text{mL}$ ), percentage and grade of motility (0 to 4), and morphological (% normal) characteristics. Specimens were evaluated by the same technician under blind conditions. The total functional sperm fraction (TFSF;  $\times 10^6$ ), an inclusive term that includes sperm quantitative and qualitative factors, was calculated as the product of sperm count ( $\times 10^6$ ) by motility (%) by normal morphology (%) characteristics (20, 21).

### Statistical Analysis

The results were reported as means $\pm$ SD. The seminal specimen and subject data was analyzed according to General Linear Model procedures (22). The statistical model employed included the analysis of method of collection and semen characteristics. A difference of  $P < 0.05$  was considered statistically significant.

**Table 1.** Clinical data of patient groups studied\* (means $\pm$ SD)

Semen collection method	Men's Age	Women's age	Years married	Years trying to conceive	Sexual frequency (times/month)
Masturbation	30.0 $\pm$ 1.3	29.011.1	5.4 $\pm$ 1.6	2.7 $\pm$ 0.4	9.6 $\pm$ 0.6
Intercourse	31.0 $\pm$ 0.8	30.0 $\pm$ 1.3	5.1 $\pm$ 1.3	2.3 $\pm$ 0.3	8.4 $\pm$ 0.4

\*No significant differences were noted in all parameters studied ( $P > 0.05$ ).

Table 2. Semen characteristics in specimens (n=25) produced via masturbation or at intercourse via the use of a semen collection device (means±SD)

Semen collection method	Seminal characteristics	Sequential ejaculates (days)			Mean*
		1	2	3	
Masturbation	Volume (ml)	2.9±0.5	2.2±0.3	1.5±0.3	2.2±0.3
Intercourse	Volume (ml)	3.7±0.6 <sup>a</sup>	2.4±0.4	1.7±0.3	2.6±0.4
Masturbation	Total Count (x10 <sup>6</sup> )	150.0±21.0	80.0±15.0	47.0±13.0	92.3±16.1
Intercourse	Total Count (x10 <sup>6</sup> )	170.0±19.0 <sup>a</sup>	121.0±13.0 <sup>a</sup>	84.0±11.0 <sup>a</sup>	125.0±15.2 <sup>a</sup>
Masturbation	Motility (%)	61.0±9.0	63.0±12.0	67.0±14.0	63.7±12.2
Intercourse	Motility (%)	65.0±8.0	69.0±11.0	74.0±12.0	69.3±10.8
Masturbation	Grade (0 to 4)	3.3±0.2	3.3±0.3	3.4±0.3	3.3±0.3
Intercourse	Grade (0 to 4)	3.4±0.2	3.5±0.2	3.5±0.3	3.4±0.3
Masturbation	Morphology (% normal)	63.0±8.0	65.0±8.0	68.0±9.0	65.3±8.3
Intercourse	Morphology (% normal)	67.0±8.0	71.0±9.0	74.0±10.0	70.7±9.2
Masturbation	TFSF (x10 <sup>6</sup> )	58.0±9.0	33.0±7.0	21.0±8.0	37.3±8.1
Intercourse	TFSF (x10 <sup>6</sup> )	74.0±11.0 <sup>a</sup>	59.0±9.0 <sup>a</sup>	46.0±9.0 <sup>a</sup>	59.7±9.7 <sup>a</sup>

\*Mean values represent average measurement of semen characteristics over a 3 day semen collection period.

<sup>a</sup>Significant differences in semen characteristics noted between specimens collected via masturbation or at intercourse by using the Male Factor Pak<sup>TM</sup>

## RESULTS

The results obtained in this study are summarized in Tables 1 and 2. The clinical profiles of couples participating in the study were similar and did not affect the outcome of the patient response in regards to semen collection via masturbation or intercourse (Table 1). Semen volume, the number of spermatozoa and TFSF values decreased each day during the 3 day collection period, regardless of the collection method (Table 2). Sperm qualitative characteristics such as motility, grade and normal morphology increased each day during the 3 day collection period, regardless of collection method (Table 2). Significant differences in semen volume between specimens collected via masturbation Vs intercourse were noted on day 1 of semen collection (P<0.05) and tended to stabilize on day 2 and 3. Semen specimens collected by using the MFP<sup>TM</sup> at intercourse were superior in regards to sperm count and TFSF values than specimens collected via masturbation when compared between and among collection intervals (P<0.05). Sperm qualitative characteristics were higher, but

not significantly different (P>0.05), in specimens collected via intercourse vs. masturbation on three consecutive collection days.

## DISCUSSION

Various methods of semen collection have been employed for semen analysis or for IUI or ART purposes. Those methods include masturbation, incomplete coitus or *coitus interruptus*, and complete intercourse (1-10, 14, 16, 17, 23). The most widely accepted method of semen collection in humans for the purpose of semen analysis or artificial insemination is via masturbation (1, 2, 4-9, 14, 15). Increasing the frequency of ejaculation via masturbation generally results in reduction of semen volume and sperm numbers while qualitative characteristics such as motility and normal morphology remain relatively unaltered (24-30). Sperm quantity tends to decrease for approximately 5 days of consecutive collection followed by stabilization of sperm numbers (29). The quantity of sperm usually returns to its original value after 3 to 4 days of abstinence (29). It has

been previously shown that seminal characteristics can be significantly improved by the extent of sexual stimulation (1, 2, 4, 5, g-10). With current advancements in the ART market, the desire to use, in those technologies (especially for ICSI), higher quality spermatozoa has significantly increased. Therefore, the desire to use different methods for semen collection, to yield higher quality spermatozoa, should be of equal importance and should be appropriately employed and adopted.

In a study by Sotikitis et al. (15), the markers of the secretory function of the prostate and the outcome of various sperm functional tests such as the hypoosmotic swelling (HOS) test, acrosin, and sperm penetration assay, were significantly higher for sperm samples collected at intercourse than for those collected via masturbation (1.5). The findings of a subsequent study indicated that ejaculates produced via the complete coitus method are of larger volume and contain larger numbers of spermatozoa that have greater motility when compared with ejaculates obtained via the coitus *interruptus* method, although the sperm concentration did not differ between the two semen collection methods (10). It was suggested that during the production of ejaculates via the complete coitus method, the apparent sexual stimulation of the males by their partners during the later part of the ejaculation process was actively involved in the completion of the ejaculatory process, which also could have produced the ejaculate improvements observed (10). It has been suggested that PSS can be responsible for the greater loading of the vas deferens during sexual stimulation and subsequent unloading of the vas deferens during the duration of the ejaculatory process achieved via the longer stimulatory period (9, 3 1).

Precoital stimulation affects both composition of the ejaculate and androgen secretion as observed in various animal models. Restraint of bulls or false mounts prior to semen collection could increase numbers of motile spermatozoa by 50% at first attempt (13). Allowing the bulls three false mounts over a 10 minute restraining period was shown to increase total sperm numbers threefold in

bulls (12). Large increases in total (261%) and motile (278%) spermatozoa were also found in the ejaculates of boars restrained up to 10 minutes compared to unrestrained boars. Data in humans have shown that ejaculates collected at intercourse using semen collection devices are superior in volume, total number of sperm in the ejaculate and grade of motility (1, 5). It seems that during the production of ejaculates via intercourse using the MFP<sup>TM</sup> method, the apparent sexual stimulation of the males by their partners was positively involved in quantitative and qualitative improvements of the ejaculate. Although the direct cause of such stimulation is not completely understood, it has been suggested that oxytocin and prostaglandin F<sub>2α</sub> may be, at least in part, responsible for the improvement in ejaculates following sexual stimulation in bulls (32). Similar observations were made in humans implicating masturbation and subsequent prolactin levels and/or other hormones may be involved (23, 33).

Further studies investigating the involvement of sexual stimulation and ejaculate production in humans are currently in progress in our facilities at the Andrology Institute of America. The results obtained in this and previous studies tend to suggest that masturbation in humans may not be the method of choice for collection of semen specimens, especially in patients with spermatogenic dysfunction such as oligozoospermia and asthenozoospermia (I, 3, 14, 30). It is suggested that the specimen collected should resemble the ejaculate delivered during intercourse to assess the infertility factor properly (I-10, 15-18). In addition, this method of semen collection could be of some assistance to male infertility patients with ejaculatory and spermatogenic dysfunction, as well as, to scientists and clinicians involved in the assessment and treatment of male and female infertility.

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