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ASSESSMENT OF TWO DEVICES FOR IN VITRO PREPARATION OF HUMAN SPERM

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The aim of the study was to assess and compare the efficiency of the ZSC-II versus the Sperm Select techniques in preparing human sperm from normozoospermic patients for use in intrauterine insemination and other assisted reproductive technologies. Twenty-five patients were included in the study. Semen was collected at intercourse and processed via 2 sperm preparation methods. Recovery of motile sperm and other sperm qualitative measurements were evaluated before and after ZSC-II and Sperm Select sperm preparation. Sperm qualitative measurements were not significantly different after the ZSC-II and Sperm Select procedures ($p > .05$). Differences ($p < .05$) were noted between the 2 procedures in the number of sperm recovered. A higher survival rate (longevity test, 72 h) was observed after ZSC-II sperm preparation and recovery. The ZSC-II procedure yielded higher total motile sperm than the Sperm Select. The superiority in longevity may suggest possible advantages in obtaining higher fertilization and pregnancy rates.

Keywords sperm, sperm preparation techniques, Sperm Select, ZSC-II

Sperm used for in vitro fertilization (IVF) and intrauterine insemination (IUI) could yield higher rates of conception if the sperm are selected on the basis of their motility, progressive motility, and morphological characteristics [9]. The removal of all debris and background, as well as antifertility factors present in the seminal plasma, may also increase sperm fertilizability [14]. Several techniques have been developed to remove the undesirable sperm, debris, and

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other factors, and increase sperm quality: swim up or sperm rise [6, 14], swim down or sedimentation methods [3, 5], Ficoll centrifugation [7], Percoll density gradient [4, 7, 11] and Sephadex [1, 10, 12, 15] or glass wool fiber filtration [8, 13].

Many of these manipulative techniques often increase sperm quality at the expense of numbers of recovered sperm, which may not be especially advantageous for men with seminal deficiencies such as oligozoospermia, teratozoospermia, and/or asthenozoospermia. Two new techniques have been introduced for use in IUI and assisted reproductive technologies (ART): (1) the ZSC-II (device 1), a 1-step process that combines a swim-up/swim-down sperm-self migration [2], and (2) the Sperm Select (device 2), a 2-phase swim-up system [22]. They are both easy to use, do not require centrifugation, encompass an acceptable degree of sperm recovery, yield reproducible results, and could assist in the recovery of high-quality sperm from infertility patients [2, 17, 19, 20, 22]. The unique design and standardization in the mode of semen–media layering within these devices makes them very attractive for physician office use and for other settings where standardized techniques for semen preparation may be necessary.

This study was designed to evaluate the effectiveness of the 2 methods for sperm preparation from normozoospermic patients. The concentrations of sperm along with various qualitative characteristics of the sperm recovered via the 2 methods were assessed and compared. To assess the possible long-term physiological effects on the sperm, the survival of sperm recovered via the 2 methods was assessed utilizing a longevity test.

MATERIALS AND METHODS

Semen Collection, Evaluation, and Preparation

Ejaculates were collected from 25 normozoospermic men of known fertility (accomplished conception during the last 3 years) who were referred to the Andrology Institute of America laboratory facilities for male infertility examination. Ejaculates were collected with exactly 4 days of abstinence each time. All patients collected their own ejaculates using the Male Factor Pak (MFP, ZDL, Lexington, KY) at intercourse [18, 21]. After semen was completely liquefied (within 15 to 30 min), each specimen was evaluated according to WHO procedures [16]: volume, sperm concentration, percentage motility, grade of motility (scale of 0 to 4), normal sperm morphology, and percentage of sperm reactive to the hypoosmotic swelling (HOS) test. All seminal parameters were evaluated under blind conditions by the same technician.

Two aliquots were used from each ejaculate: one aliquot was used for device 1 (0.5 mL) and the other for device 2 (1.0 mL). A modified Ham's F-10 medium (SpermPrep medium: ZDL) containing 3% (v/v) bovine serum albumin (BSA) was used for device 1 and a phosphate buffer (0.5 mL) containing 1.0 mg hyaluronate and phosphate-buffered saline (0.5 mL) containing magnesium sulfate and glucose (Select Medical Systems, Williston, VT) was used for device 2.

Use of the Two Devices

The 2 semen preparation devices were used according to the manufacturer's instructions. An aliquot of semen (2.0 mL) was placed into the conical cylinder of device 1 (Figure 1) and 0.7 mL of medium was placed into the periconical area [2]. For device 2 (Figure 1), 0.5 mL of the phosphate-buffered saline was added to the hyaluronate solution (0.5 mL) vial, and 1.0 mL of

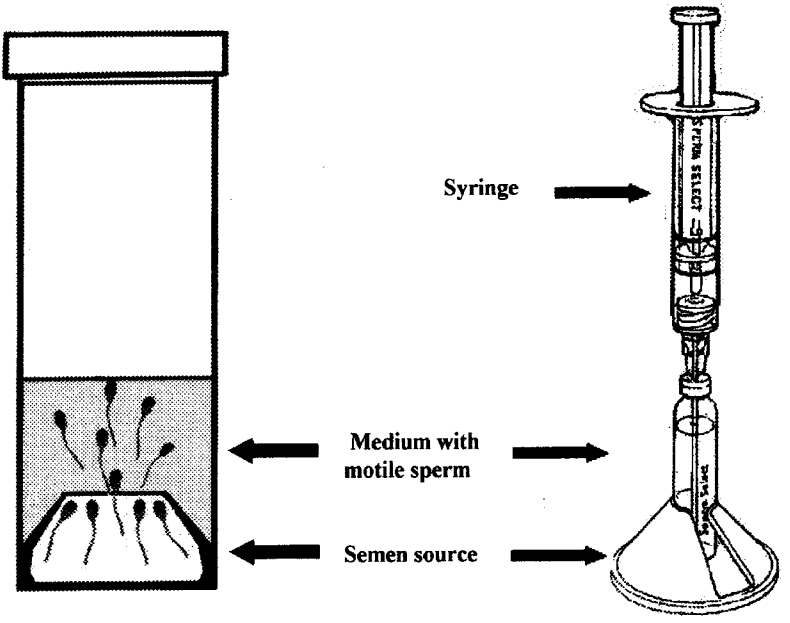

Figure 1. The 2 semen preparation methods employed in this study: device 1, ZSC-II column (left) and device 2, Sperm Select (right). Device 1 uses 2 mL of semen overlaid with 0.7 mL of medium, whereas device 2 requires 1.0 mL of semen and 1.0 mL of medium. After 1 h of incubation, the supernatants were removed and evaluated.

semen was injected into the bottom of the vial. Both devices were incubated at 37°C for 1 h, after which the overlayered medium was aspirated. The recovered sperm were evaluated as previously described.

RESULTS

The semen specimens used in this study were considered normozoospermic according to WHO standards. The mean seminal characteristics (unprocessed) were 2.7 ± 0.4 mL, 115 ± 17.8 × 10^6 sperm/mL, 61 ± 6.8% motility, 2.9 ± 0.3 progressive motility, 57 ± 8.1% normal morphology, and 63 ± 7.1% sperm responding to the HOS test. Total sperm recovery from specimens processed via device 1 and device 2 represented 15.8 and 10.0% of the initial number of motile sperm in the unprocessed specimens, respectively. Sperm specimens recovered via both tested methods were qualitatively similar ($p > .05$). However, significant differences were noted in the numbers of motile sperm recovered and total functional sperm fraction (TFSF) values ($p < .05$). The TFSF for device 1 and device 2 specimens represented approximately 23 and 14% of the initial values noted in the unprocessed sperm specimens, respectively (Table 1). Furthermore, the longevity test showed quite clearly (Table 2) that the sperm recovered via device 1 were superior ($p < .05$) after the first 24 h of incubation and beyond until the completion of the test (72 h).

Table 1. Sperm characteristics of specimens assessed before and after processing via the two devices (means ± SD)

Sperm parameters assessed (N = 25)	Semen treatments (N = 25)		
	Unprocessed	Device 1[a]	Device 2[b]
Volume recovered (mL)	2.7 ± 0.4	0.7 ± 0.0	0.5 ± 0.0
Sperm concentration (×10⁶/mL)	116 ± 17.8	12 ± 1.3	7 ± 1.8[c]
Motility (%)	61 ± 6.8	95 ± 4.3	96 ± 3.6
Grade (0 to 4)	2.9 ± 0.3	3.6 ± 0.2	3.6 ± 0.2
Morphology (% normal)	57 ± 8.1	81 ± 6.7	83 ± 5.8
HOS (%)	63 ± 7.1	95 ± 3.7	96 ± 2.8
TFSF (×10⁶/mL)	41 ± 4.3	9 ± 1.1	6 ± 0.9[c]

[a]ZSC-II.

[b]Sperm Select.

[c]Significant differences noted between device 1 and device 2 values within each sperm parameter ($p < .05$).

Note. HOS, hypoosmotic swelling test (% swollen sperm); TFSF, total functional sperm fraction (sperm concentration × % motiliuty × % normal morphology).

DISCUSSION

Semen manipulative techniques have as their main objective the selection of the group of sperm that have the highest fertilizing capacity among the overall sperm present in the original ejaculate for use in IUI and ART [17, 19]. The data presented in this study indicated that both sperm selection methods yielded significant improvements in the assessed qualitative characteristics when compared to the unprocessed specimens ($p < .05$; Table 1). Device 1 yielded sperm of similar qualitative characteristics ($p > .05$) but significantly higher numbers of total sperm ($p < .05$) than those recovered via device 2 (11.2 vs. 7.1 × 10^6 sperm/mL, respectively). Significant differences ($p < .05$) were also noted in the TFSF value, an inclusive parameter that takes into consideration the total sperm count, percent motility, and percent morphologically normal sperm, between the two methods (9 ± 1.1 vs. 6 ± 0.9 × 10^6 sperm, respectively). Also, the longevity of the sperm after processing via device 1 was superior to that of those recovered

Table 2. Longevity of sperm processed via the 2 devices, expressed as percentage motility (mean ± SD)

Incubation time (h)	Semen treatments (N = 25)		
	Unprocessed	Device 1[a]	Device 2[b]
0	62 ± 6.1	62 ± 6.1	62 ± 6.1
1	61 ± 7.0	95 ± 4.3	96 ± 3.6
72	0	31 ± 5.3	18 ± 6.0[c]

[a]ZSC-II.

[b]Sperm Select.

[c]Significant differences noted between device 1 and device 2 values ($p < .05$).

via device 2 ($p < .05$; Table 2), and yielded a higher percentage sperm recovery in both the total motile sperm and the TFSF (16% vs. 10% and 23% vs. 14%, respectively).

Although the percentage yield of sperm may be low, these methods have been designed and directed for in-office use without the need for expensive or elaborate laboratory equipment and/or experienced personnel. Device 1 (ZSC-II) was less cumbersome and easier to use than device 2 (Sperm Select). Because of the unique design of the newly introduced device 1, it enables the user to layer the semen and the media with much greater ease along with a lower margin of error. The same seems to be true during the recovery of the overlayered media that contains the motile, healthy population of sperm. This device also enables the harvesting of almost 100% of the overlayered medium, and most importantly the medium closest to the underlayered semen, which maximizes the number of sperm recovered. This technique could be extremely beneficial for patients with spermatogenic deficiencies and also could be time saving for normozoospermic patients. Furthermore, device 1 is a standardized technique, which could enable the clinician and the researcher to draw various inferences and conclusions during comparisons between different specimens from the same patient or from different patients with various spermatogenic parameters [17, 19].

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