

Improvement in the Quality and Fertilization Potential of a Human Sperm Population Using the Rise Technique

LONNIE D. RUSSELL AND B. JANE ROGERS*

Semen from 63 individuals participating in an *in vitro* fertilization program was processed using a modified rise technique. Overall normal morphology was significantly improved in the rise (79.2%) as compared with the unprocessed sample (57.8%), and six of seven specific morphologic abnormalities were significantly reduced. Motility was significantly enhanced from 51.8% in the unprocessed samples to 89.1% in the rise samples. Spermatozoa recovered in the rise portion of the sample represented 5.9% of the total sample. Ultrastructural morphometry revealed that the rise was relatively free of abnormal sperm forms, acellular debris and non-sperm cellular elements as compared with the non-rise portion of the sample or a typical unprocessed sample. Volume density measurements demonstrated that only 18.1% of the volume of the non-rise sample was composed of normal spermatozoa compared with 83.4% of the volume of the rise. In a separate set of experiments utilizing 21 samples, the penetration of hamster eggs was significantly enhanced from 37.9% to 67.2% using spermatozoa from the initial washed sample and those from the rise, respectively. These data demonstrate the qualitative and quantitative improvements, as well as the increase in fertilizing potential, of the rise portion of the semen sample.

Key words: sperm rise, fertilization potential, sperm penetration assay (SPA), human fertility, semen evaluation.

J Androl 1987; 8:25-33.

Treatment of infertility in recent years has greatly increased the number of options available for infertile couples. The technical advances accompanying fertility treatment generally have placed more emphasis on "exteriorization" of the reproductive

*From the Department of Physiology, Southern Illinois University, School of Medicine, Carbondale, IL and the *Department of Obstetrics & Gynecology, Vanderbilt University, School of Medicine, Nashville, Tennessee.*

process. For *in vitro* fertilization, the reproduction process has been largely bypassed since gametes are obtained outside of the normal means for conception. Egg quality and egg numbers, as obtained by artificial stimulation and laparoscopy, have improved in recent years. Washing and storage of spermatozoa in media have eliminated the detrimental properties of seminal plasma on sperm fertilizing ability and have allowed capacitation (Kanway et al, 1979; Rogers et al, 1983). Further improvements in sperm quality have involved use of a technique that takes advantage of the swimming abilities of a small percentage of spermatozoa within a population (Drevius, 1971; Overstreet et al, 1980; Makler et al, 1984; Yovich and Stanger 1984; Cohen et al, 1985). This technique, variously referred to as the "rise" or "swim-up" procedure, is frequently used in *in vitro* fertilization programs and, to a lesser extent, in intrauterine insemination and the sperm penetration assay.

The rise procedure has been recently examined (Rogers and Russell, 1985; McDowell et al, 1985) for its ability to improve a population of spermatozoa in a semen sample. Our preliminary report first indicated an enhancement in both normal morphologic forms as well as an increase in motile forms, the latter of which can be expected since the procedure takes advantage of sperm swimming ability. The present

Reprint requests: Lonnie Russell, Ph.D., Department of Physiology, Southern Illinois University, School of Medicine, Carbondale, Illinois 62901.

Submitted for publication January 28, 1986; revised version received April 11, 1986; accepted for publication May 28, 1986.

study examined the enhancement in fertilizing potential of the rise sample compared with the unprocessed sample, as well as the enhancement of the routine semen parameters of motility and morphology. Ultrastructural morphometric techniques were used to examine sperm morphology and the sperm penetration assay was used to evaluate the fertilizing potential.

Materials and Methods

The Rise Procedure

Sixty-three males participating in an *in vitro* fertilization program were utilized to establish the morphology and motility comparisons. Three of these individuals were considered as male-factor patients. Semen obtained by masturbation was allowed to liquify for 30 minutes prior to performing a routine semen analysis. Counts were performed using a hemocytometer and the motility percentage was evaluated subjectively using at least 100 spermatozoa. Morphology determinations were made on 100 spermatozoa from stained smears.

A diagrammatic representation of the rise procedure is shown in Fig. 1. Initially, the liquified semen sample (unprocessed sample) is diluted with an equal volume of BWW (Biggers, Whitten and Whittingham) or insemination media composed of Ham's F-10 media (Gibco) with 7.5% heat-treated fetal cord serum. After centrifugation of the sample at $600 \times g$ for 10 minutes, the supernatant is removed. The sample is washed by resuspension in 2 ml of BWW or insemination media. A second centrifugation is followed by resuspension to a final volume of 0.3 ml. This small volume, containing a large number of spermatozoa, is underlayered beneath 2 ml of BWW or insemination media in a Falcon tube. Special care is taken to avoid introduction of air bubbles that would float to the top and agitate the two fluids. The tube is loosely capped and placed in a 37 C incubator (5% CO₂ and air) at a 45 to 60 degree angle for 1½ to 2 hours. During this period, motile spermatozoa migrate or "rise" from the underlayered sperm suspension to the upper layer. Subsequently, the top 1.6 to 1.8 ml is removed while the test tube remains at the 45 to 60 degree angle. Extreme care is exercised in removing the fluid to avoid disturbing the interface of the two layered fluids and including immotile spermatozoa in the sample. The fluid removed contains the actively motile spermatozoa and is termed the "rise." The remaining spermatozoa are termed the "non-rise" (Fig. 1). The rise sample was analyzed for sperm count, morphology and motility as described above. For 25 of these individuals, the non-rise portion of the sample was similarly analyzed. Motility and morphology were compared using a paired t-test.

Electron Microscopy and Morphometry

The pellet from the rise and the non-rise portion of the same sample was processed for electron microscopy after centrifugation at $600 \times g$. The nine individuals whose samples were analyzed with the electron microscope were

randomly selected from the group of 63 individuals described above.

The pelleted samples were fixed in 2.5% glutaraldehyde that was buffered with 0.05 M sodium cacodylate (pH 7.4) with 5% added sucrose. After fixation, the pelleted tissue was washed in three changes of buffer (5% sucrose added) and post-fixed in an osmium:ferrocyanide mixture (Russell and Burguet, 1977) for 1½ hours. Standard dehydration, infiltration, and embedding in Araldite (CY-212) followed. The embedded tissue blocks were thin-sectioned perpendicular to the contour of the tube in which the samples were pelleted to obtain a sampling of all regions of the pellet. Sections showing silver-grey interference colors were placed on slot grids that were Formvar-coated such that all regions of the sections were easily visualized. The tissues were subsequently examined on a Phillips 201 electron microscope. Electron micrographs, taken at an original magnification of $\times 3,000$, were enlarged to $\times 7,600$ original magnification. Micrographs were taken as composites from three regions within the tissue block; the area first pelleted, that centrifuged next and the area last pelleted.

Sectioned profiles or "hits" of tissue or amorphous elements, as seen on electron micrographs, were scored as being either normal or abnormal sperm elements (heads or tails), cellular elements other than spermatozoa (exfoliated cells), amorphous material or cellular fragments. Two hundred consecutive hits were scored on the micrographs from the three regions of the pellet of the rise and non-rise samples. To obtain volume density, the cross-sectional area of the rise and non-rise samples was calculated using a digitizer to determine the area of the aforementioned 200 hits in each individual rise and non-rise sample. The digitized area of normal and abnormal sperm profiles, as well as amorphous debris, cellular debris and exfoliated genital tract cells, was recorded. Head or acrosomal shapes that deviated from recognized literature descriptions of normal human spermatozoa (Holstein and Roosen-Runge, 1981) were considered abnormal. Other examples of abnormal heads included double heads and heads containing excess cytoplasm. Flagella in the head region were scored as abnormal. Asymmetry of head shape alone, as seen in a section, was scored as abnormal only if the plane of section could not have reasonably produced such asymmetry. Spermatozoa whose flagella had missing or disrupted axonemes, fibrous rings or fibrous sheathes were among those considered abnormal. Mean values from morphometric measurements were compared statistically (paired t-test) in rise and non-rise samples.

Penetration of Rise and Non-Rise Samples Using the Sperm Penetration Assay

Twenty-one samples from nineteen individuals participating in an *in vitro* fertilization program were utilized to assess fertilizing potential.* The rise and non-rise samples from the same individual were each diluted to a concentration of 10×10^6 spermatozoa per ml. Frequently, one ml of

*The term "fertilizing potential" is used to distinguish the ability of human spermatozoa to penetrate hamster eggs (see discussion).

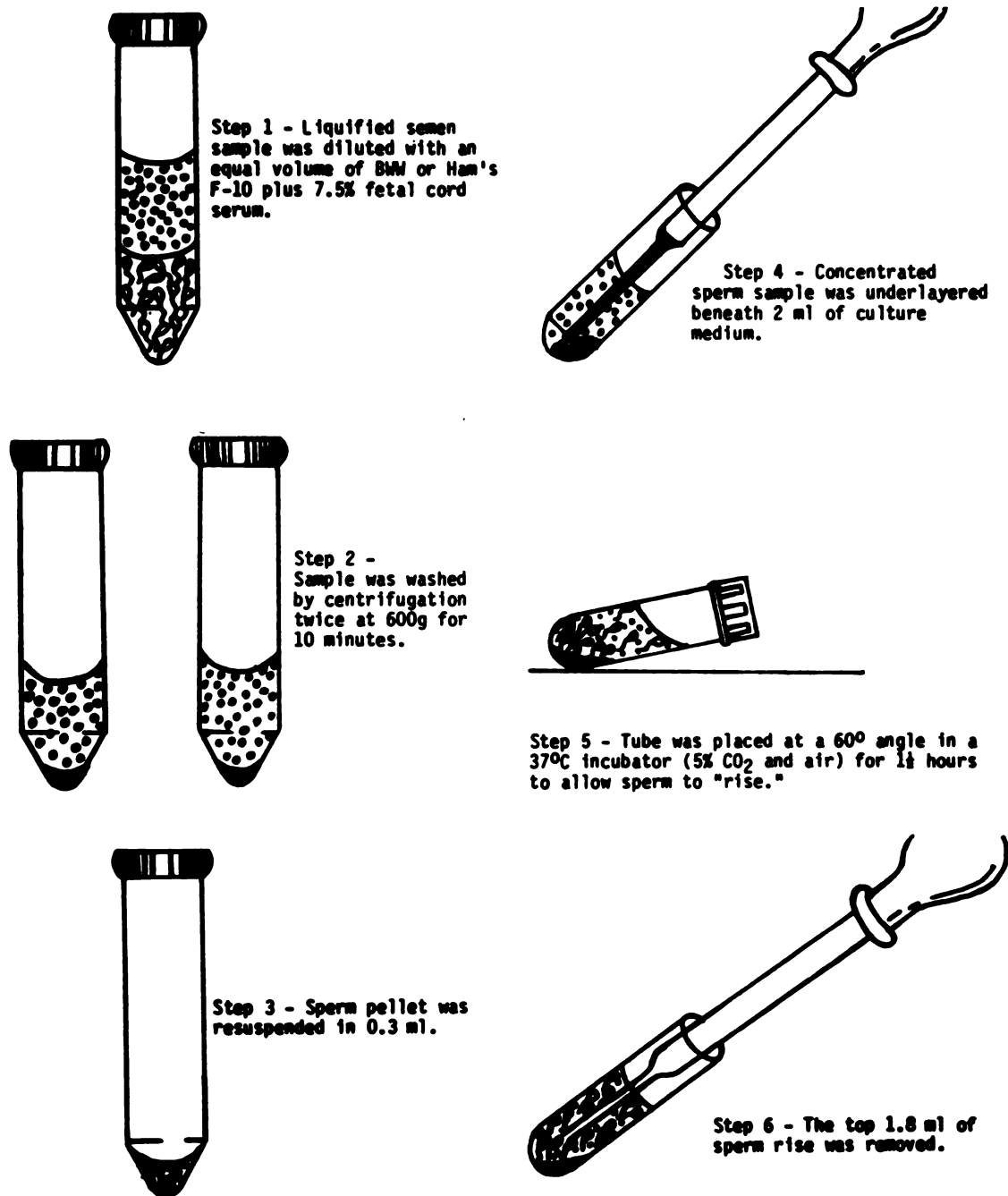


Fig. 1. Diagrammatic representation of the sperm rise procedure.

the rise sample contained less than 10×10^6 spermatozoa. In these cases, the rise was used at the highest concentration obtained and the unprocessed and the non-rise samples were limited to 10×10^6 spermatozoa. (We have shown that rise concentrations over the range used had no effect on penetration rate.) Details of the sperm penetration assay have been published (Rogers et al, 1979; Rogers, 1985) and are summarized briefly below. Superovulated hamsters were sacrificed and their eggs were recovered.

Cumulus cells were removed by hyaluronidase treatment ($0.1 \mu\text{g}/\mu\text{l}$), the eggs were washed three times and the zona pellucida removed by trypsin ($0.1 \mu\text{g}/\mu\text{l}$). The eggs were washed again three times and placed in 100- μl drops of capacitated spermatozoa (preincubated 18 to 20 hours) in a Falcon dish under mineral oil. A total of 30 to 50 eggs per sample were evaluated. After three hours of coincubation, eggs were washed once, mounted under coverslips, and examined using phase contrast microscopy. The presence

TABLE 1. Comparison of Rise and Unprocessed Semen Samples*

Sample	Concentration ($\times 10^6$ spermatozoa/ml)		% Motile Spermatozoa	% Normal Morphology
Unprocessed Sample	92.8 (± 1.00)	51.8 (± 0.44)	57.8 (± 0.39)	
Rise Sample	10.2 (± 0.36)	89.1 (± 0.44)	79.2 (± 0.44)	

*Mean values are represented with standard error in parentheses. N = 63 for each sample. $P < 0.001$ for each unprocessed and rise comparison.

of swollen heads with attached tails was regarded as evidence of penetration. The percentage of eggs penetrated was recorded as well as the number of swollen heads per egg. The data were expressed as either the percentage of hamster eggs penetrated by human spermatozoa or as the penetration index (number of swollen heads per egg). The data were analyzed using the paired t-test.

Results

For the 63 individuals whose rise and unprocessed samples were analyzed by light microscopy, there was a significant ($P < 0.001$) enhancement in the percentage of motile forms, which increased from 51.8% to 89.1%, respectively. Morphologically normal spermatozoa significantly ($P < 0.001$) increased from 57.8% to 79.2% (Table 1). In the subset of 25 individuals, the non-rise portion of the sample was also analyzed and the data are presented separately (Table 2). The non-rise portion showed significantly ($P < 0.001$) fewer motile (38.5%) and normal forms (53.0%) compared with the unprocessed sample, which was apparently the result of the selective accumulation of normal appearing and motile forms in the rise. The total number of rise spermatozoa represented, on the average, a 5.9% recovery of the number of spermatozoa in the unprocessed sample (Table 2). On the average, 14.6% of the spermatozoa

TABLE 3. Distribution of Various Abnormal Sperm Forms

Abnormality	Sample*		
	Unprocessed %	Rise %	Non-rise %
Tapered Head†	4.1	1.8	5.0
Microcephalic‡	7.7	6.9	6.6
Macrocephalic†	2.8	1.0	3.4
Amorphous†	3.6	1.4	3.9
Tail Abnormality†	9.1	3.1	13.6
Bent Midpiece†	7.8	2.7	9.1
Cytoplasmic Droplet†	6.8	1.7	6.0

*N = 25 for each sample.

† $P < 0.001$ for rise vs. either unprocessed or non-rise sample.

‡ $P > 0.05$ for rise vs. either unprocessed or non-rise sample.

were lost during washing or pipetting and 79.5% remained in the non-rise. The breakdown of morphologic abnormalities in the smaller group of 25 individuals showed a significant ($P < 0.001$) decrease in all categories (except microcephalic where $P > 0.05$) in the rise as compared with the unprocessed sample (Table 3).

The unprocessed sample (Fig. 2), non-rise (Fig. 3) and rise (Fig. 4) were examined by electron microscopy. Figure 2 shows a typical unprocessed sample from one individual. For comparison, Figures 3 and 4 show the non-rise and rise from another individual. The unprocessed sample is characterized by normal-appearing profiles of sperm heads and flagella, abnormal-appearing sperm heads and flagella, cytoplasmic fragments of unknown origin, exfoliated genital tract cells, and amorphous debris. The sample shown is typical of numerous samples one investigator (L.R.) has examined with the electron microscope over several years.

A morphometric analysis of the rise and non-rise portion of the samples was undertaken to quantitate aspects of the rise and non-rise samples from nine individuals (Table 4). The non-rise and rise differed

TABLE 2. Semen Parameters in Unprocessed, Rise and Non-rise Samples*

	Concentration ($\times 10^6$ spermatozoa/ml)	Total Spermatozoa ($\times 10^6$)	% Motile Spermatozoa	% Normal Morphology
Unprocessed	98.7 (± 1.3) [13-215]	293.8	52.4 (± 0.7) [25-72]	58.0 (± 0.6) [36-71]
Rise	11.5 (± 0.6) [0.5-28]	17.5†	90.3 (± 0.7) [52-99]	80.8 (± 0.7) [36-95]
Non-rise	409.2 (± 2.9) [26-809]	233.4	38.5 (± 0.8) [13-66]	53.0 (0.6) [37-69]

*N = 25 for each sample. Mean values are presented with standard error in parentheses and ranges in brackets.

†17.5 is 5.9% of the unprocessed initial ejaculate and 7.4% of the non-rise sample. $P < 0.001$ for rise vs. non-rise and rise vs. unprocessed sample.

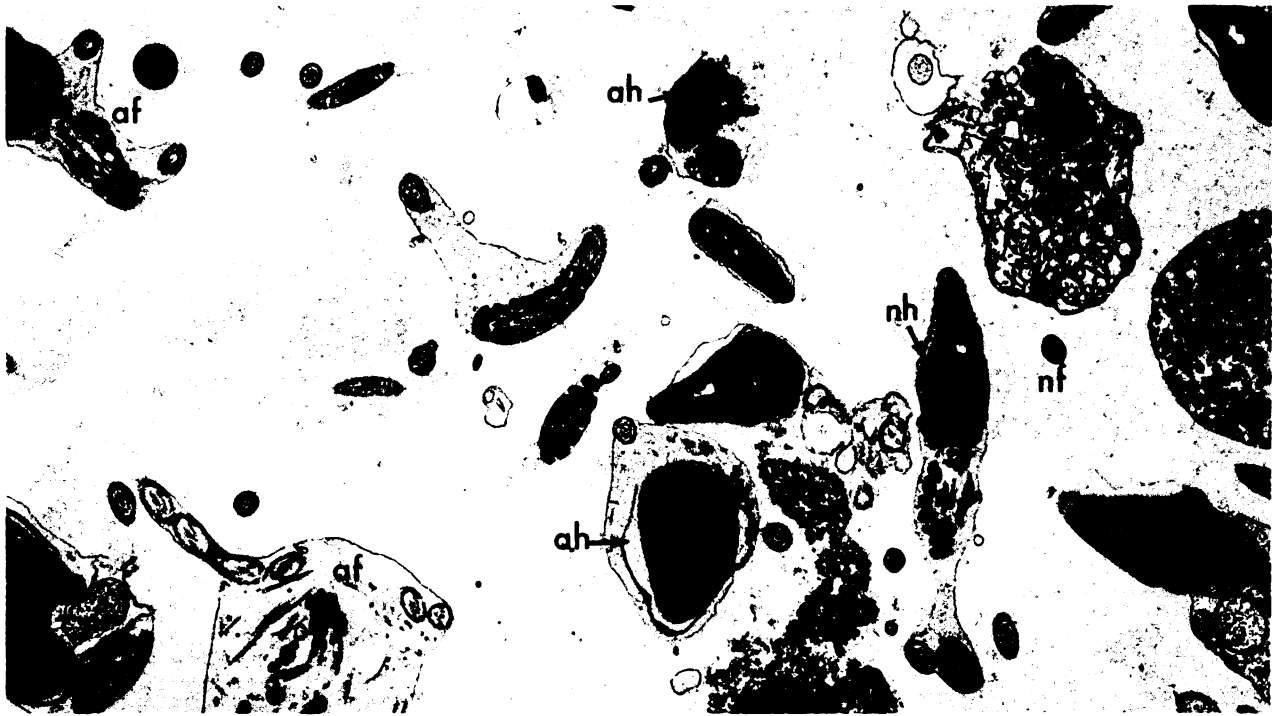


Fig. 2. Electron micrograph depicting an unprocessed, but washed sample. A variety of normal and abnormal profiles are depicted ($\times 8200$).

Figs. 2-4. Various semen fractions obtained during processing of spermatozoa using the rise technique. Selected elements are designated as being either normal sperm heads (nh) or flagella (nf), abnormal sperm heads (ah) or flagella (af), cytoplasmic fragments (cf), cellular elements (ce) or amorphous material (am).

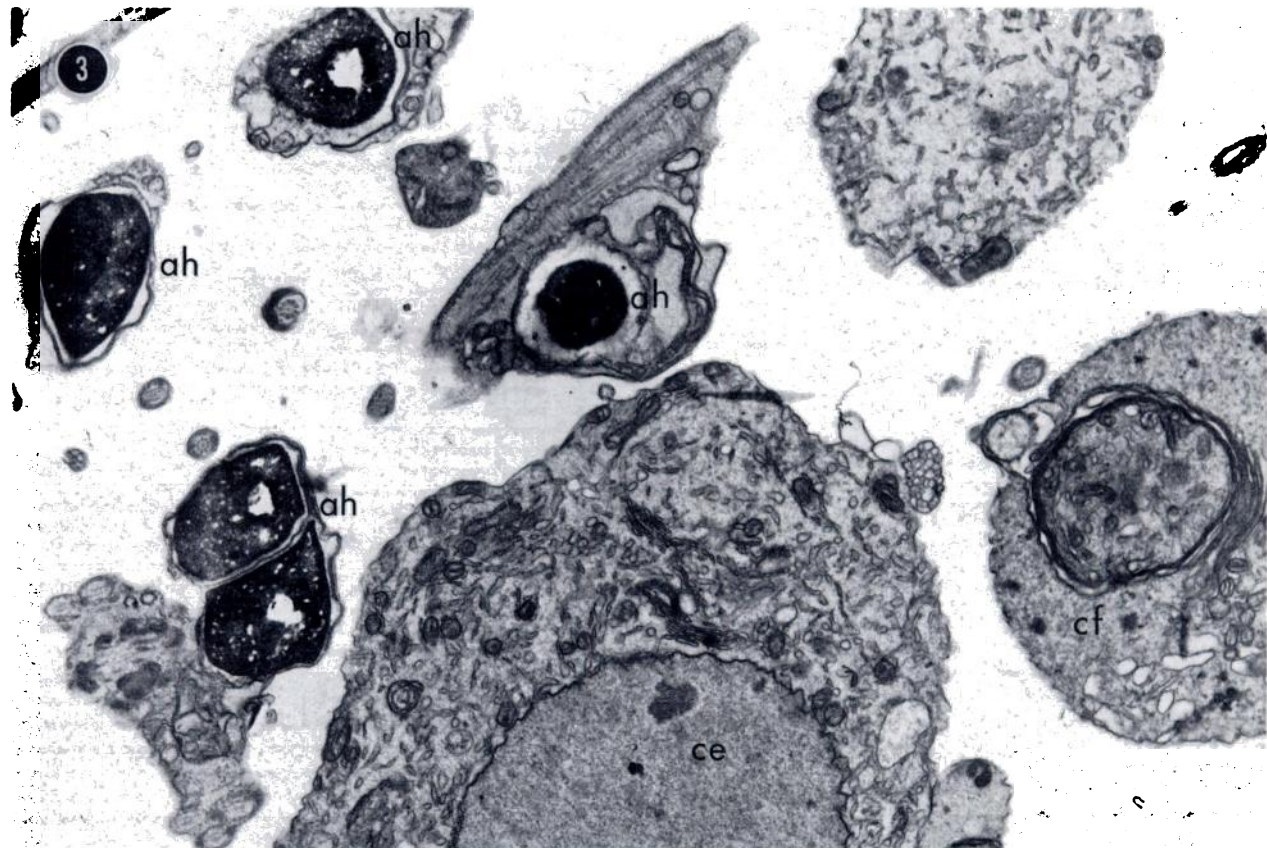


Fig. 3. Electron micrograph of the non-rise portion of the sample show many of the same types of elements seen in the unprocessed sample. In this field, only abnormal forms or extraneous cellular material are observed ($\times 9500$).

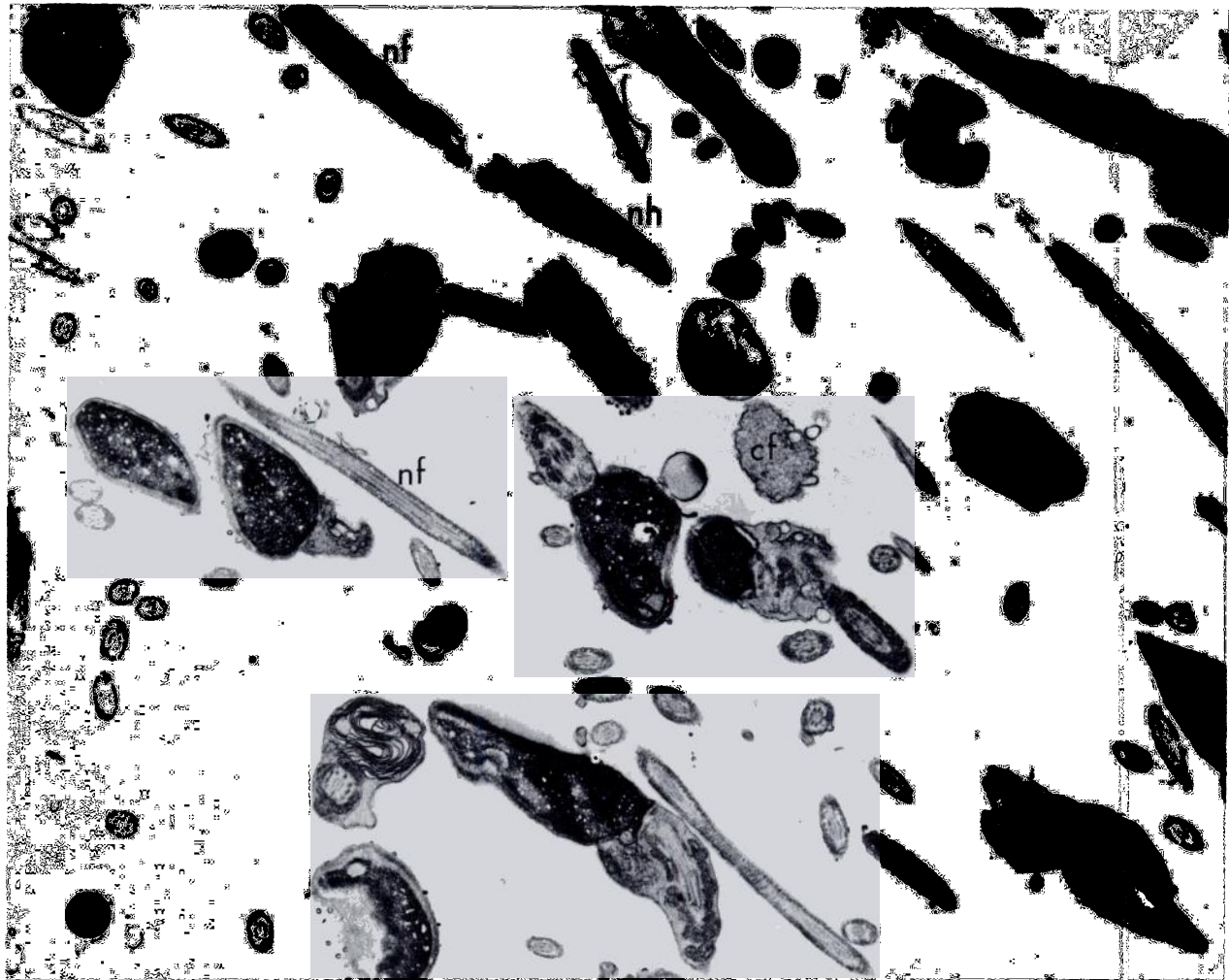


Fig. 4. Electron micrograph of the rise portion of the sample. For the most part, profiles depicted are classified as normal spermatozoa ($\times 8400$).

TABLE 4. Morphometric Analysis of Rise and Non-rise Semen Samples*

		Non-rise†	Rise
Normal "Hits" of Spermatozoa	$\times 100$	56.4	92.1
Total "Hits" of Particulate and Cellular Material		(± 6.0)	(± 2.6)
Normal Sperm Head "Hits"	$\times 100$	36.2	86.8
Total Sperm Head "Hits"		(± 8.0)	(± 6.4)
Cross-sectional Area of Normal Spermatozoa	$\times 100$	18.1	83.4
Total Cross-sectional Area of Particulate and Cellular Material		(± 3.9)	(± 5.5)

*Mean values are presented with standard error in parentheses. N = 9 for each sample.

† $P < 0.001$ for each rise and non-rise comparison.

significantly in the percentage of normal forms, 56.4% and 92.1%, respectively ($P < 0.01$), and there was a significant increase ($P < 0.001$) in sperm heads in the rise (86.8%) as opposed to the non-rise (36.2%). Volume density measurements of normal spermatozoa expressed as a percentage of all elements in the sample showed that 83.4% of the rise was occupied by normal spermatozoa whereas normal spermatozoa in the non-rise occupied only 18.1% of the volume of the sample ($P < 0.001$).

Fertilization potential was evaluated using the sperm penetration assay for three types of spermatozoa: rise, non-rise and washed. Fertilization potential in the heterologous sperm penetration assay was expressed as a penetration percentage and penetration index (Table 5). In 21 samples, the percentage of motile spermatozoa (59.8, 49.6 and 89.3) was similar to that previously described for unprocessed (fresh),

non-rise and rise samples, respectively, as was the percentage of spermatozoa with normal morphology (62.4, 57.1 and 80.2). The percentage of hamster eggs penetrated in the normally processed (washed) sample was 37.9. The rise spermatozoa demonstrated a significant enhancement to 67.2%. The non-rise was only slightly less than the washed sample in percentage penetration. The trend of the penetration index paralleled the data on penetration of hamster eggs, being lowest in the non-rise sample (1.0), intermediate in the washed sample (1.5) and highest in the rise sample (3.0). The rise samples differed significantly ($P < 0.01$ or $P < 0.001$) from the non-rise or the unprocessed sample with regard to both percentage of eggs penetrated or penetration index.

Discussion

The human male is frequently described as being on the verge of infertility by virtue of the number and quality of spermatozoa in the ejaculate. The testis is, in part, at fault for low efficiency in sperm production and the many abnormal forms seen in the ejaculate. Also, the ejaculate has a large amount of exfoliated germ cells, genital tract cells, cellular debris and amorphous material, some of which are not readily visible by phase contrast microscopy. Electron microscopy more clearly reveals the poor quality of the human ejaculate, since the increased resolution affords a more detailed view of the individual constituents.

With the advent of *in vitro* fertilization and other clinical techniques, it is considered important to use the best spermatozoa available from a semen sample. The procedure investigated here takes advantage of sperm progressive motility which is, in itself, an important characteristic for achieving fertility. Those spermatozoa that "rise," because of their motility, to the overlying medium are captured and utilized.

The rise procedure as employed here (Fig. 1) is slightly modified from the rise procedures previously described (Drevius, 1971; Overstreet et al, 1980; Cohen et al, 1981, 1985; Makler et al, 1984; McDowell et al, 1985). First, the procedure allows spermatozoa to rise from a uniform suspension of a population of spermatozoa rather than a pellet; second, it provides for a prewashing of spermatozoa that minimizes the damaging effects of seminal plasma; third, the entire ejaculate is used, which provides more spermatozoa from which the rise population can be isolated. Some other procedures call for spermatozoa to rise directly from seminal plasma (Makler et al, 1984; Cohen et al, 1985) or from a

TABLE 5. Comparison of Fertilization Potential and Related Semen Parameters for Rise, Non-rise and Washed Spermatozoa*

Sample	% Motile	% Normal Morphology	% Penetration	P.I.†
Rise	89.3 (± 1.7)	80.2 (± 2.7)	67.2 (± 8.0)	3.0 (± 1.0)
Non-rise	49.6 (± 3.2)	57.6 (± 4.3)	31.3 (± 6.6)	1.0 (± 0.6)
Washed‡	59.80 (± 2.4)	62.4 (± 2.3)	37.9 (± 7.0)	1.5 (± 0.7)

*Mean values are presented with standard errors in parentheses. N = 21 for each sample.

†P.I. = penetration index = $\frac{\text{number of swollen heads}}{\text{total number of eggs}}$

‡Fresh semen was used to determine initial % motile and % normal while a washed sample was used to determine penetration parameters. $P < 0.001$ for rise sample vs. non-rise or unprocessed samples except $P < 0.01$ for rise vs. unprocessed in the penetration index.

sperm pellet (McDowell et al, 1985). We believe, however, that our rise technique has potential for additional modifications that would further improve the sample. For example, it might be possible to increase the surface area in which underlain spermatozoa could swim into the media above, which in turn would allow more spermatozoa to be captured in the rise.

The data obtained from light and electron microscopy clearly demonstrate enhancement in the morphology and motility of spermatozoa. Especially impressive are the volume density differences in non-rise and rise samples, indicating that the volumetric components of the non-rise comprise only 18.1% normal spermatozoa in contrast to 83.4% in the rise. The approximate volume occupied by normal spermatozoa in the unprocessed sample, although not determined morphometrically, can be calculated. Since 5.9% of the spermatozoa in the initial ejaculate were, on the average, able to rise, and over 80% of these spermatozoa displayed normal forms, then approximately 23% [18.1% (normal sperm volume in non-rise) + 4.9% (calculated % volume lost from non-rise)] of the volume of the unprocessed sample contained normal forms! This figure (23%) reflects the contribution of cellular debris, exfoliated cells and amorphous material to the volume of the sample.

The data also emphasize the need to enrich human sperm preparations in spermatozoa with normal forms prior to their use in research studies either by using the rise or other techniques (Steen et al, 1975; Lopata et al, 1976; Harris, 1976; Ericsson, 1977; Paul-

son and Polakoski, 1977; Hellema and Rumke, 1978; Harris et al, 1981). Research using human ejaculates that is largely grounded in biochemistry or enzymology may need to be reexamined on the basis of the purity of the sample. Even with the rise procedure, the normal spermatozoa in the sample occupy only 83% of its volume density.

The rise technique or a variation thereof has been used for years with the assumption that it is an improvement over existing techniques such as simple washing. In a small number of samples, Cohen et al (1981) found an enhancement in the sperm penetration assay with rise samples; however, Wolf and Sokoloski (1982) reported inconsistent results with four donors. The functional benefit of using the rise population compared with other portions of the sample for fertilizing human eggs has never been demonstrated due to ethical considerations. We have approached the question also by using the sperm penetration assay as the measure of improvement. To relate the penetration data using hamster eggs to the homologous human system requires the assumption that the *in vitro* fertilization capability of the washed, non-rise and rise samples in the human correlates with the sperm penetration assay results. If true, then a significant improvement in human fertilizing ability can be attributed to the rise sample as compared with the unprocessed sample.

The enhancement of penetration by the rise sample impacts on both *in vitro* fertilization methodology as well as the technique for performing the sperm penetration assay. The rise sample appears to be preferable for fertilizing human eggs *in vitro*. In an instance where the rise sample was quantitatively inadequate, use of the non-rise sample to fertilize the human egg was unsuccessful (Rogers, unpublished data). Such an anecdotal report does not prove our contention, but lends support to the use of rise samples where possible. In the sperm penetration assay, the rise sample will provide higher values than the unprocessed or washed sample and thus help eliminate false negatives. If a patient gets a negative result using the standard washing technique, it would seem prudent to repeat the assay using a rise sample.

What is the basis for such enhanced fertilizing capacity in the sperm penetration assay? Two explanations are suggested. The fertilization success of the rise in the sperm penetration assay might be attributed to an increase in the concentration of motile spermatozoa. The simply washed sample is capable of providing a higher absolute number of motile spermatozoa, but the percentage penetration

and penetration index are relatively low in comparison with that of the rise. Nonetheless, sperm concentration improvement by itself has never been shown to potentiate the fertilization ability of a single spermatozoon. A more appealing explanation is that the rise procedure eliminates immotile and dead spermatozoa along with the exfoliated cells, cellular debris and amorphous material. In the unprocessed sample, we calculate that only approximately 23% (see above) of the sample volume is normal sperm elements in contrast to the rise, which demonstrates about 83% normal spermatozoa. If the assumption is made that collisions of spermatozoa with the egg are important in fertilization, then the rise sample would be nearly ideal since the volume purity approaches 100%. Certainly, collisions of motile spermatozoa with elements other than the egg are much more commonplace in the unprocessed or simply washed sample. Physical barriers in a semen sample whose volume density of abnormal components is over 80% of the sample would interfere more than a sample in which these represent about 23% of the sample. White cells, which are eliminated in the rise, interfere with penetration of hamster eggs by human spermatozoa (Maruyama et al, 1985) and dead spermatozoa that may release detrimental materials (enzymes, etc.) also are virtually eliminated. Such elements are eliminated *in vivo* by barriers such as cervical mucus, the uterotubal junction and the sheer distance of the egg from where the spermatozoa are deposited. In this respect, the rise technique may provide a more natural setting for fertilization since the human female reproductive system sets up its own "rise" conditions.

Acknowledgments

The use of the electron microscope facility at the Springfield campus is acknowledged. The technical assistance of Tina Krivacka, Wendy Holmgren, Tom Thompson, Alan Buck, and Cristina Bastias was greatly appreciated.

References

- Cohen J, Edwards R, Fehilly C, Fishel S, Hewitt J, Purdy J, Rowland G, Steptoe P, Webster J. *In vitro* fertilization: a treatment for male infertility. *Fertil Steril* 1985; 43:422-432.
- Cohen J, Felton P, Zeilmaker GH. *In vitro* fertilizing capacity of fresh and cryopreserved human spermatozoa: a comparative analysis of freezing and thawing procedures. *Fertil Steril* 1981; 36:356-362.
- Drevius L-O. The "sperm-rise" test. *J Reprod Fertil* 1971; 24:427-429.
- Ericsson RJ. Isolation and storage of progressively motile human sperm. *Andrologia* 1977; 9:111-114.
- Harris RAP. A highly efficient method for washing mammalian spermatozoa. *J Reprod Fertil* 1976; 48:347-353.

- Harris SJ, Milligan MP, Masson GM, Dennis KJ. Improved separation of motile sperm in asthenospermia and its application to artificial insemination homologous (AIH). *Fertil Steril* 1981; 36:219-221.
- Hellema HWJ, Rumke P. The micro-sperm immobilization test: the use of only motile spermatozoa and studies of complement. *Clin Exp Immunol* 1978; 31:1-11.
- Holstein AF, Roosen-Runge EC, eds. *Atlas of human spermatogenesis*; Berlin: Grosse, 1981.
- Kanwar KC, Yanagimachi R, Lopata A. Effects of human seminal plasma on fertilizing capacity of human spermatozoa. *Fertil Steril* 1979; 31:321-327.
- Lopata A, Patuillo MJ, Change A, James B. A method for collecting motile spermatozoa from human semen. *Fertil Steril* 1976; 27:677-684.
- Makler A, Murillo O, Huszar G, Tarlatzis B, DeCherney A, Naftolin F. Improved techniques for collecting motile spermatozoa from human semen. I. A self migratory method. *Int J Androl* 1984; 7:61-70.
- Maruyama DK, Hale RW, Rogers BJ. Effects of white blood cells on the *in vitro* penetration of zona-free hamster eggs by human spermatozoa. *J Androl* 1985; 6:127-135.
- McDowell JS, Veeck LL, Jones HW. Analysis of human spermatozoa before and after processing for *in vitro* fertilization. *J In Vitro Fertil Embryo Transf* 1985; 2:23-26.
- Overstreet JW, Yanagimachi K, Katz DF, Hayashi K, Hanson FW. Penetration of human spermatozoa into the human zona pellucida and the zona-free hamster egg: A study of fertile donors and infertile patients. *Fertil Steril* 1980; 33:534-542.
- Paulson JD, Polakoski KL. A glass wool column procedure for removing extraneous material from the human ejaculate. *Fertil Steril* 1977; 28:178-181.
- Rogers BJ. The sperm penetration assay: Its usefulness reevaluated. *Fertil Steril* 1985; 43:821-840.
- Rogers BJ, Perrault SD, Bentwood BJ, McCarville C, Hale RW, Soderdahl DW. Variability in the human-hamster *in vitro* assay for fertility evaluation. *Fertil Steril* 1983; 39:204-211.
- Rogers BJ, Russell LD. Comparison of the rise and non-rise samples of human sperm as employed in fertility programs. *J Androl* 1985; 6[Abstr]:P-31.
- Rogers BJ, Van Campen H, Ueno M, Lambert H, Bronson R, Hale R. Analysis of human spermatozoal fertilizing ability using zona-free ova. *Fertil Steril* 1979; 32:644-670.
- Russell LD, Burguet S. Ultrastructure of Leydig cells as revealed by secondary tissue treatment with a ferrocyanide-osmium mixture. *Tissue Cell* 1977; 9:751-766.
- Steen O, Adimoelja A, Steeno J. Separation of X- and Y-bearing human spermatozoa with the Sephadex gel-filtration method. *Andrologia* 1975; 7:95-97.
- Wolf DP, Sokoloski JE. Characterization of the sperm penetration bioassay. *J Androl* 1982; 3:445-451.
- Yovich JL, Stanger JD. The limitations of *in vitro* fertilization from males with severe oligospermia and abnormal sperm morphology. *J In Vitro Fertil Embryo Transf* 1984; 1:172-179.

Sustaining Members of the Society

The following companies are sustaining members of the American Society of Andrology. The Society is grateful for their support.

Buckeye Urological Associates	Syntex Company
Knoll Pharmaceutical Company	Syva Company
National Medical Enterprises, Inc.	TAP Pharmaceuticals
Ortho Pharmaceutical Corporation	The Upjohn Company
Schering Corporation	West Michigan Reproductive Institute
Serono Laboratories, Inc.	Wyeth Laboratories, Inc.

Serono Symposium on Inhibin, Tokyo, 1987

The Serono Symposium on Inhibin will be held in Tokyo, Japan, May 21 and 22, 1987. Subjects to be covered will include: FSH Regulation, Inhibin—Purification and Characterization, Molecular Biology, Radioimmunoassay, Physiology, Mechanisms, Standards, Contraceptive Applications, and Local Actions. The Scientific Committee for the meeting consists of: H. Burger (Australia) and M. Igarashi (Japan), Chairmen; D. T. Baird (UK), F. de Jong (Netherlands), D. M. de Kretser (Australia), P. Franchimont (Belgium), R. Guillemin (USA), R. Iizuka (Japan), W. Vale (USA), and G. Waites (WHO).

Enquiries should be directed to: Prof. Henry G. Burger, Medical Research Centre, Prince Henry's Hospital, St. Kilda Road, Melbourne, Australia 3004.