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Effects of Ejaculation-to-Analysis Delay on Levels of Markers of Epididymal and Accessory Sex Gland Functions and Sperm Motility

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ABSTRACT: This study aimed to examine the association between the interval from ejaculation to analysis and epididymal and accessory sex gland function in relation to sperm motility. Ejaculates from 1079 men assessed for infertility were analyzed according to World Health Organization guidelines. Biochemical markers were measured in semen to assess the function of the epididymis (neutral α-glucosidase [NAG]), prostate (prostate-specific antigen [PSA] and zinc), and seminal vesicles (fructose). Three groups were defined according to time from ejaculation to analysis: $G_{\leq 30}$ (24–30 minutes), G_{31-60} (31–60 minutes), and $G_{>60}$ (63–180 minutes). The proportion of progressively motile sperm was significantly lower in $G_{>60}$ than in $G_{\leq 30}$ (mean difference, 8.0%; 95% confidence interval [CI], 2.0%–13%) or G_{31-60} (mean difference, 6.0%; 95% CI, 1.0%–12%). The proportion of rapid progressive sperm motility was significantly higher in $G_{\leq 30}$ compared with G_{31-60} (mean difference, 3.0%; 95% CI, 1.0%–5.0%) and $G_{>60}$ (mean difference, 6.0%; 95% CI, 1.0%–10%). Sperm morphology and viability did not vary significantly between the groups. However, PSA levels in $G_{>60}$ were 29% and 31% significantly lower than in $G_{\leq 30}$ (95% CI, 3.0%–54%) and G_{31-60} (95% CI, 7.0%–58%), respectively. Moreover, men in $G_{>60}$ had 29% and 17% significantly lower zinc compared with those in $G_{\leq 30}$ (95% CI, 4.0%–69%) and G_{31-60} (95% CI, 4.0%–64%), respectively. Levels of NAG and fructose did not differ significantly between the groups. There were negative associations between the ejaculation-to-analysis interval and sperm motility and levels of PSA and zinc. In male infertility assessments, semen analysis should be performed within 60 minutes of ejaculation.

Key words: Biochemical markers, morphology, semen analysis, viability.

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Sperm motility is believed to be one of the most important parameters for evaluating the fertilizing ability of ejaculated spermatozoa both in vivo and in vitro (Bongso et al, 1989; Eimers et al, 1994; Donnelly et al, 1998; Larsen et al, 2000; Hirano et al, 2001). The ability of the spermatozoa to move is determined by a multitude of factors, including contributions from properly functioning epididymal and accessory sex glands (Malm et al, 2000; Elzanaty et al, 2002). The qualities of semen are also influenced by several factors, such as the time elapsed from ejaculation/collection to analysis. However, only 1 study has addressed this issue (Mortimer et al, 1982), and the results suggested that sperm motility and viability decline when the interval from ejaculation to analysis exceeds 2 hours.

The analysis of semen quality plays an important role in clinical decisions regarding the strategy for infertility treatment. Therefore, it is essential to minimize the impact of variation in sample delivery and analytic conditions on the outcome of this testing. In the World Health Organization (WHO) manual (1999), which is the most accepted guideline for semen analysis, it is recommended that assessment of semen in infertility investigations be performed within 60 minutes of ejaculation. However, both the European Society of Human Reproduction and Embryology (ESHRE) and the Nordic Association for Andrology (Kvist and Bjorndahl, 2002) strongly advise that semen analysis be done within 30 minutes of collection. In contrast to both those levels, Mortimer et al (1982) have postulated that analysis can done up to 3 hours after ejaculation, although it is preferable that it be achieved within 2 hours.

In addition to the very limited scientific knowledge about the effects of the ejaculation-to-analysis interval on sperm motility, information is also lacking about the mechanisms underlying that association. Therefore, the aim of the present study was to investigate sperm motility in relation to the impact of the time from

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ejaculation to analysis on markers of the functions of the epididymis and accessory sex glands.

Materials and Methods

Subjects

The study was based on semen samples obtained from 1079 consecutive nonazoospermic men undergoing infertility assessment at the Fertility Center, Malmö University Hospital, Malmö, Sweden, between October 2000 and September 2006.

Semen Samples

The ejaculates were obtained by masturbation after 1–30 days (median, 4 days) of sexual abstinence. Only completely collected semen samples were included. For men delivering more than 1 sample during the study period, only the first ejaculate was included in the analysis. For each semen sample, the time of delivery to the laboratory and the time of semen analysis were recorded on the semen analysis form.

Semen Analysis

The semen samples were allowed to liquefy at 37°C. After liquefaction, within 24–180 minutes of ejaculation, aliquots of the samples were subsequently analyzed for semen volume, sperm concentration, motility (graded as follows: a, rapid progressive motility; b, slow progressive motility; c, local motility; or d, immotility), viability, and morphology. All these tests were performed according to the WHO recommendations (1999). Semen volumes were measured by weighing the containers with and without semen using Sartorius balances (Tillquist Analysis AB, Stockholm, Sweden). Sperm concentration was assessed using positive displacement pipettes and improved Neubauer hemocytometer. Sperm morphology was assessed after Papanicolaou staning, and viability was assessed using eosin-nigrosin–stained smears using WHO criteria. The analyses of ejacuiates were performed by 3 laboratory assistants, and the interobserver coefficient of variation for motility assessment was 8.5%. This laboratory participates in an external quality control program organized by the Nordic Association of Andrology and ESHRE.

For each sample, 450 μL of the remaining ejaculate was collected using a common air displacement pipette and then mixed with 50 μL of benzamidine (0.1 M) to stop the biochemical processes involved in liquefaction. The mixture was centrifuged for 20 minutes at 4500 × *g*, and the seminal plasma was decanted and stored at −20°C until analyzed for neutral α-glucosidase (NAG) activity and concentrations of prostate-specific antigen (PSA), zinc, and fructose.

Biochemical Markers

Biochemical markers of function were assessed for the epididymis (NAG), prostate (PSA and zinc), and seminal vesicles (fructose) as previously described (Elzanaty et al, 2002). NAG was analyzed by first measuring total α-glucosidase activity using an Episcreen kit (Fertipro, Beernem, Belgium) according to the instructions of the manufacturer and thereafter estimating the NAG activity by use of the table included in the kit. The concentrations of PSA, zinc, and fructose in seminal plasma were determined using a PROSTATUS kit (Wallac Oy, Turku, Finland), a colorimetric method (Makino et al, 1982), and a spectrophotographic technique (essentially as described by Wetterauer and Heite, 1976), respectively.

Background Characteristics

The subjects included in the present study were 20–64 years of age (median, 34 years). Ninety-five percent of the samples were analyzed within 24–60 minutes of ejaculation, and 5% were analyzed within 63–180 minutes. After subtracting the volume of semen required for routine analysis, only 915 of the neat samples contained a sufficient amount of semen for analysis of biochemical markers. Moreover, the biomarkers PSA, zinc, and fructose were analyzed first, and thereafter only 504 of the 915 samples contained enough semen for analysis of NAG. The proportions of semen samples delivered in different seasons were as follows: 24.4% in spring (March–May), 19.1% in summer (June–August), 29.3% in autumn (September–November), and 27.2% in winter (December–February). Samples found to have high viscoelasticity (n = 60) were excluded from the analyses.

Statistical Methods

Statistical analysis was performed using SPSS 11.0 software (SPSS Inc, Chicago, Ill). The normal distribution of residuals was determined using normal probability plots, after which logarithmic transformations of total activity of NAG and total amounts of PSA, zinc, and fructose were done to ascertain the normal distribution of residuals. The remaining data were not transformed. The subjects were divided into 3 groups according to the interval from ejaculation to analysis: $G_{\leq 30}$ (24–30 minutes), $G_{31–60}$ (31–60 minutes), and $G_{>60}$ (63–180 minutes). Linear regression analysis models were applied to investigate the effects of the ejaculation-to-analysis interval on sperm motility, viability, and morphology and on amounts of NAG, PSA, zinc, and fructose. As potential confounding factors, we considered the age of the donors (years), the length of sexual abstinence (number of consecutive days), and the season of semen collection (spring [March–May], summer [June–August], autumn [September–November], and winter [December–February]). *P* values below .05 were considered statistically significant.

Results

Table 1 summarizes the descriptive statistics for the following characteristics: age of the donor; abstinence time; semen volume; sperm concentration, total count, motility, morphology, and viability; and biochemical markers. Among the samples with an interval from ejaculation to analysis exceeding 60 minutes, 8 samples were produced at home and delivered to the laboratory after 60 minutes of collection, whereas the remaining 53

Table 1. *Distribution of data on samples from the 3 study groups**

Variables	$G_{\leq 30}$ n = 337	G_{31-60} n = 681	$G_{>60}$ n = 61
Age of donors (y)	33 (20–58)	34 (20–64)	35 (23–57)
Abstinence time (d)	4.0 (1.0–21)	4.0 (1.0–30)	4.0 (1.0–30)
Time from ejaculation to analysis (min)	30 (24–30)	45 (31–60)	70 (63–180)
Semen volume (mL)	4.0 (1.0–14)	4.0 (1.0–13)	4.0 (0.4–13)
Sperm concentration (10^6/mL)	45 (0.3–370)	48 (0–568)	51 (0.1–360)
Total sperm count (10^6/ejaculate)	170 (1.0–2400)	179 (0–2730)	159 (0.4–1730)
Rapid progressive motility (%)	17 (0–72)	14 (0–78)	10 (0–63)
Slow progressive motility (%)	28 (2.0–67)	30 (0–78)	24 (0–60)
Progressive motility (%)	51 (17–79)	50 (0–92)	46 (0–85)
Local motility (%)	16 (2.0–43)	17 (0–53)	16 (0–47)
Immotility (%)	32 (2.0–92)	32 (1.0–100)	41 (1.0–100)
Normal forms (%)	5.0 (0–18)	5.0 (0–20)	5.0 (0–16)
Viability (%)†	60 (8.0–79)	55 (0.4–85)	56 (8.0–73)
Biochemical markers	n = 285	n = 583	n = 47
NAG (mU/ejaculate)‡	29 (6.0–106)	29 (4.0–107)	29 (10–56)
PSA (μg/ejaculate)	3600 (320–1650)	3460 (160–19 140)	2683 (240–22 810)
Zinc (μmol/ejaculate)	7.0 (0–32)	7.0 (0.2–57)	5.0 (0.03–48)
Fructose (μmol/ejaculate)	59 (0.3–470)	55 (0–277)	46 (2.0–140)

* Values are presented as median (range). NAG indicates neutral α-glucosidase; PSA, prostate-specific antigen.
† A total of 118 samples were analyzed for viability: 35 in $G_{\leq 30}$, 74 in G_{31-60}, and 9 in $G_{>60}$.
‡ A total of 504 samples were analyzed for NAG: 180 in $G_{\leq 30}$, 312 in G_{31-60}, and 12 in $G_{>60}$.

samples were collected at the clinic but kept in the incubator for more than 60 minutes because of technical reasons.

Sperm Motility, Morphology, and Viability

The proportion of rapid progressive sperm motility (grade a) was significantly higher in the $G_{\leq 30}$ compared with G_{31-60} (mean difference, 3.0%; 95% confidence interval [CI], 1.0%–5.0%; $P = .01$) and $G_{>60}$ (mean difference, 6.0%; 95% CI, 1.0%–10%; $P = .02$), but there was no significant difference between the G_{31-60} and $G_{>60}$ samples. The proportion of progressive motility (grades a + b) was significantly lower in $G_{>60}$ compared with $G_{\leq 30}$ (mean difference, 8.0%; 95% CI, 2.0%–13%; $P = .01$) and G_{31-60} (mean difference, 6.0%; 95% CI, 1.0%–12%; $P = .02$), whereas there was no significant difference between $G_{\leq 30}$ and G_{31-60}. The proportion of immotile spermatozoa (grade d) was significantly higher in $G_{>60}$ compared with $G_{\leq 30}$ (mean difference, 8.0%; 95% CI, 3.0%–13%; $P = .004$) and G_{31-60} (mean difference, 8.0%; 95% CI, 3.0%–13%; $P = .003$), although there was no significant difference between $G_{\leq 30}$ and G_{31-60}. Furthermore, the proportions of spermatozoa exhibiting slow progressive motility (grade b) and local motility (grade c) did not differ significantly between groups (Table 2); nor did the proportions of morphologically normal and viable cells (Table 2).

Markers of Epididymal and Accessory Sex Gland Function

PSA levels in the $G_{>60}$ were 29% and 31% significantly lower than those found in the $G_{\leq 30}$ (95% CI, 3.0%–54%) and G_{31-60} (95% CI, 7.0%–58%) samples, respectively. Also, the zinc levels in $G_{>60}$ were 29% and 17% significantly lower than those in $G_{\leq 30}$ (95% CI, 4.0%–69%) and G_{31-60} (95% CI, 4.0%–64%), respectively. There were no significant differences between $G_{\leq 30}$ and G_{31-60} with regard to levels of PSA and zinc. Markers of epididymal function (NAG) and seminal vesicle performance (fructose) did not differ significantly among groups (Table 3).

Discussion

Semen samples from 1079 men assessed for infertility were analyzed in the present study, and the results show that the motility of spermatozoa and the levels of markers of prostate function (PSA and zinc) were significantly lower 1 hour after collection of the samples as compared with 30 or fewer or 31–60 minutes after collection. On the other hand, the ejaculation-to-analysis interval had no apparent impact on sperm morphology or viability or on the markers of epididymal and seminal vesicle function (NAG and fructose).

Table 2. *Association between ejaculation-to-analysis interval and sperm motility, morphology, and viability analyzed in semen from 1079 men assessed for infertility**

Variables	$G_{\leq 30}$ n = 337	G_{31-60} n = 681	$G_{>60}$ n = 61
Rapid progressive motility (%)	21 (17)‡§	18 (16)‡	15 (15)§
Slow progressive motility (%)	29 (14)‡	30 (15)‡	26 (16)
Progressive motility (%)	49 (20)§	48 (20)‖	41 (21)§‖
Local motility (%)	17 (8.0)	18 (8.0)	16 (9.0)
Immotility (%)	34 (19)§	34 (20)‖	43 (24)§‖
Normal forms (%)	6.0 (4.0)	5.0 (4.0)	5.0 (4.0)
Viability (%)†	55 (17)	54 (18)	53 (24)

* Values are presented as mean (SD); linear regression models were used for analysis, and adjustment was done for age of the semen donor, length of sexual abstinence, and season of collection (spring [March–May], summer [June–August], autumn [September–November], and winter [December–February]).

† A total of 118 samples were analyzed for viability: 35 in $G_{\leq 30}$, 74 in G_{31-60}, and 9 in $G_{>60}$.

‡ Significant difference between $G_{\leq 30}$ and G_{31-60}.

§ Significant difference between $G_{\leq 30}$ and $G_{>60}$.

‖ Significant difference between G_{31-60} and $G_{>60}$.

Sperm motility is considered to be one of the most important factors predicting the fertilizing ability of ejaculated spermatozoa both in vivo and in vitro (Bongso et al, 1989; Eimers et al, 1994; Donnelly et al, 1998). Notably, an earlier study indicated that a delay of up to 3 hours after ejaculation to the time to analysis had no significant adverse effect on the mobility of spermatozoa (Mortimer et al, 1982), whereas we observed significantly lower sperm movement when analysis was performed more than 1 hour after collection of semen samples.

Furthermore, during spermatogenesis, the locomotor apparatus of the spermatozoa is formed and becomes functional (Mohri and Ishjima, 1989), and considerable amounts of zinc are incorporated into the spermatids (Parizek et al, 1966). It has also been observed that the spermatozoa liberated from the rete testis and caput epididymis show only sluggish, nonprogressive movement (Cooper, 1986). The capacity for progressive motility is gained solely during maturation of the spermatozoa as they are transported through the epididymis (Haidl et al, 1994). In the course of that journey, the zinc content of the sperm is reduced by approximately 60% (Kaminska et al, 1987), which leads to the increased stabilization of the outer dense fiber (ODF) proteins that is induced when sulfhydryl groups are oxidized to form disulfide bridges (Calvin et al, 1973). In our study, prolonging the time from ejaculation to analysis to more than 1 hour was associated with significantly lower levels of zinc in the semen samples. A plausible explanation for that observation is that the binding of zinc to spermatozoa was augmented with increasing time from ejaculation to analysis, resulting in greater flexibility of the ODF proteins and consequently diminished motility of the spermatozoa. Perhaps future studies will confirm this assumption and, if so, they might also explain why the incorporation of zinc increases with time from ejaculation.

PSA is considered to be the primary proteolytic enzyme in seminal plasma, and it has been shown that

Table 3. *Association between ejaculation-to-analysis interval and markers of function of the epididymis (neutral α-glucosidase [NAG]), prostate (prostate-specific antigen [PSA] and zinc), and seminal vesicles (fructose) measured in semen from 915 men assessed for infertility**

Variables	$G_{\leq 30}$ n = 285	G_{31-60} n = 583	$G_{>60}$ n = 47
NAG (mU/ejaculate)†	29 (2.0)	28 (2.0)	27 (2.0)
PSA (μg/ejaculate)	3350 (2.0)‡	3400 (2.0)§	2600 (2.0)‡§
Zinc (μmol/ejaculate)	7.0 (2.0)‡	6.0 (2.0)§	5.0 (3.0)‡§
Fructose (μmol/ejaculate)	54 (2.0)	52 (2.0)	43 (2.0)

* Values represent geometric means (SD); linear regression models were used for analysis, and the data were adjusted for age of the donor, length of sexual abstinence, and season of collection (spring [March–May], summer [June–August], autumn [September–November], and winter [December–February]).

† A total of 504 samples analyzed for NAG: 180 in $G_{\leq 30}$, 312 in G_{31-60}, and 12 in $G_{>60}$.

‡ Significant difference between $G_{\leq 30}$ and $G_{>60}$.

§ Significant difference between G_{31-60} and $G_{>60}$.

this protein degrades the 2 major components of the semen coagulum (semenogelins I and II [SgI and II]; Lilja et al, 1989) into lower molecular weight fragments (Lilja, 1985; Robert and Gagnon, 1996) and thereby facilitates free movement of the spermatozoa (Malm et al, 2000). In the current study, we found that the levels of PSA decreased as the ejaculation-to-analysis time increased to more than 1 hour, which might be at least partially attributable to a modification in the antigenic epitopes. The biochemical mechanism behind such an alteration is not known, although it does seem to be of practical importance considering the time-related drop in PSA.

The fructose present in seminal plasma is believed to be the main source of energy for sperm metabolism and motility in vitro (Mann, 1964). Therefore, it is reasonable to assume that an increased interval between ejaculation and analysis will be associated with a decline in the levels of fructose. However, that notion is not supported by our results, possibly because there are other sources of energy present in seminal plasma, including glucose, which has been reported to constitute almost half of the sugar consumed by spermatozoa (Martikainen et al, 1980).

The morphologic development of spermatozoa is decisive for the motility of these gametes (Bedford, 1979). We found no significant difference in sperm morphology between the groups of semen samples investigated in our study, which agrees with earlier results reported by Mortimer and colleagues (1982). However, in contrast to the findings of Mortimer et al, we did not observe any effect of the time from ejaculation to analysis on the proportion of viable spermatozoa.

Our observations have obvious practical implications. Semen analysis is the cornerstone in male infertility assessment, and the information provided by such evaluation serves as a basis for the diagnosis and treatment of infertile men. Therefore, it is highly important to standardize semen investigation procedures to include shortening of the interval from collection to analysis of ejaculates, because that will improve the possibility of comparing the results of repeated analyses. Our study strongly suggests that investigation of semen be done within 60 minutes of ejaculation.

Our findings may also have therapeutic value. That conclusion is made in light of a study showing that intrauterine insemination performed with spermatozoa from semen samples processed more than 60 minutes after ejaculation resulted in no pregnancy, whereas the use of spermatozoa from semen processed within 30 and 60 minutes of ejaculation led to pregnancy rates of 29% and 13%, respectively (Yavas et al, 2004).

In conclusion, we found lower sperm motility with ejaculation-to-analysis time of more than 1 hour, and that finding was supported by measurements of PSA and zinc as markers of prostatic function. Further studies are needed to ascertain whether there is a connection between those 2 observations. In contrast, the interval from ejaculation to analysis had no apparent effect on sperm morphology or viability or on the markers of epididymal and seminal vesicle function (NAG and fructose). Our results also clearly indicate that semen analysis as a part of male infertility assessment should be done no longer than 1 hour after ejaculation.

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References

Bedford JM. Evaluation of the sperm maturation and sperm storage functions of the epididymis. In: Fawcett DW & Bedford JM, eds. *The Spermatozoon*. Baltimore, Md: Urban and Schwarzenberg; 1979:7–21.

Bongso TA, Ng SC, Mok H, Lim MN, Teo HL, Wong PC, Ratnam SS. Effect of sperm motility on human in vitro fertilization. *Arch Androl*. 1989;22:185–190.

Calvin HI, Yu CC, Bedford JM. Effects of epididymal maturation, zinc (II) and copper (II) on the reactive sulfhydryl content of structural elements in rat spermatozoa. *Exp Cell Res*. 1973;81: 333–341.

Cooper TG. *The Epididymis, Sperm Maturation and Fertilization*. Berlin, Germany: Spinger-Verlag; 1986.

Donnelly ET, Lewis SEM, McNally JA, Thompson W. In vitro fertilization and pregnancy rates: the influence of sperm motility and morphology on IVF outcome. *Fertil Steril*. 1998;70:305–314.

Eimers JM, Te Velde ER, Gerritse R, Vogelzang ET, Looman CW, Habbema JD. The prediction of the chance to conceive in subfertile couples. *Fertil Steril*. 1994;61:44–52.

Elzanaty S, Richthoff J, Malm J, Giwercman A. The impact of epididymal and accessory sex gland function on sperm motility. *Hum Reprod*. 2002;17:2904–2911.

Haidl G, Badura B, Schill WB. Function of human epididymal spermatozoa. *J Androl*. 1994;15:23S–27S.

Hirano Y, Shibahara H, Obara H, Suzuki T, Takamizawa S, Yamaguchi C, Tsunoda H, Sato I. Relationships between sperm motility characteristics assessed by the computer-aided sperm analysis (CASA) and fertilization rates in Vitro. *J Assist Reprod Gene*. 2001;18:213–218.

Kaminska B, Rozewicka L, Dominiak B, Mielnicka M, Mikulska D. Zinc content in epididymal spermatozoa of metoclopramide-treated rats. *Andrologia*. 1987;19:677–683.

Kvist U, & Bjorndahl L, eds. *ESHRE Monographs: Manual on Basic Semen Analysis*. Oxford, United Kingdom: Oxford University Press; 2002:1–24.

Larsen L, Scheike T, Jensen TK, Bonde JP, Ernst E, Hjollund NH, Zhou Y, Skakkebæk NE, Giwercman A. Computer-assisted semen analysis parameters as predictors for fertility of men from the general population. *Hum Reprod*. 2000;15:1562–1567.

Lilja H. A kallikrein-like serine protease in prostatic fluid cleaves the predominant seminal vesicle protein. *J Clin Invest*. 1985;76:1899–1903.

Lilja H, Abrahamsson PA, Lundwall A. Semenogelin, the predominant protein in human semen. Primary structure and identification of closely related proteins in the male accessory sex glands and on the spermatozoa. *J Biol Chem*. 1989;25:1894–1900.

Makino T, Saito M, Horiguchi D, Kina K. A highly sensitive colorimetric determination of serum zinc using water-soluble pyridylazo dye. *Clin Chim Acta*. 1982;120:127–135.

Malm J, Hellman J, Hogg P, Lilja H. Enzymatic action of prostate-specific antigen (PSA or hK3): substrate specificity and regulation by Zn(2+), a tight-binding inhibitor. *Prostate*. 2000;45:132–139.

Mann T. Fructose, polyols, and organic acids. In: Nam T ed. *The Biochemistry of Semen and of the Male Reproductive Tract*. London, United Kingdom: Methuen & Co Ltd; 1964:237–264.

Martikainen P, Sannikka E, Suominen J, Santti R. Glucose content as a parameter of semen quality. *Arch Androl*. 1980;5:337–343.

Mohri H, Ishjima S. Epididymal maturation and motility of mammalian spermatozoa. In: Serio M, ed. *Perspectives in Andrology*. New York, NY: Raven Press; 1989:291–298.

Mortimer D, Templeton AA, Lenton EA, Coleman RA. Influence of abstinence and ejaculation-to-analysis delay on semen analysis parameters of suspected infertile men. *Arch Androl*. 1982;8:251–256.

Parizek J, Boursnell JC, Hay MF, Babicky A, Taylor DM. Zinc in the maturing rat testis. *J Reprod Fertil*. 1966;12:501–507.

Robert M, Gagnon C. Purification and characterization of the active precursor of a human sperm motility inhibitor secreted by the seminal vesicles: identity with semenogelin. *Biol Reprod*. 1996;55:813–821.

Wetterauer U, Heite H-J. Eine empfehlenswerte Methode zur gleichzeitigenenzymatischen Bestimmung von Citrat und Fruktose im Seminalplasma. *Akt Dermatol*. 1976;2:239–248.

World Health Organization. *WHO Laboratory Manual for the Examination of Human Semen and Sperm-Cervical Mucus Interaction*. 4th ed. Cambridge, United Kingdom: Cambridge University Press; 1999.

Yavas Y, Selub MR. Intrauterine insemination (IUI) pregnancy outcome is enhanced by shorter intervals from semen collection to sperm wash, from sperm wash to IUI time, and from semen collection to IUI time. *Fertil Steril*. 2004;82:1638–1647.