Optimal timing for repeat semen analysis during male infertility evaluation

Nahid Punjani, M.D., M.P.H.,[a] Gal Wald, M.D.,[b] Omar Al-Hussein Alwamlh, M.D.,[a] Miriam Feliciano,[a] Vanessa Dudley, M.S.H.S.,[a] and Marc Goldstein, M.D., D.Sc. (Hon)[a]

[a] Center for Reproductive Medicine and Surgery, Institute for Reproductive Medicine and Department of Urology, Weill Cornell Medicine, New York, New York; and [b] Weill Cornell Medical College, New York, New York

Objective: To assess whether the 4-week time period between semen analyses during the workup of male infertility is optimal and whether two samples are needed.
Design: Retrospective study.
Setting: Tertiary hospital.
Patient(s): Men whose semen samples were obtained within 90 days of each other, without known fertility intervention, treatment, and/or azoospermia.
Intervention(s): Semen analysis.
Main Outcome Measure(s): Correlation between semen parameters and agreement among consecutive semen analyses.
Result(s): A total of 2,150 semen samples from 1,075 men were included in the analysis. The optimal correlation for volume occurred at weeks 2, 8, and 12 ($r = 0.803$, $r = 0.802$, and $r = 0.821$, respectively). For concentration, the correlation was maximized at weeks 1, 4, and 5 ($r = 0.950$, $r = 0.841$, and $r = 0.795$, respectively). Total sperm count correlated at weeks 1, 2, and 4 ($r = 0.929$, $r = 0.727$, and $r = 0.808$, respectively). Motility was maximally correlated at weeks 1, 10, and 13 ($r = 0.711$, $r = 0.760$, and $r = 0.708$, respectively). Morphology was optimally correlated at weeks 1, 2, and 9 ($r = 0.935$, $r = 0.815$, and $r = 0.839$, respectively). Semen volume was correlated in 55% of men, sperm concentration in 64% of men, sperm motility in 52% of men and sperm morphology 64% of men.
Conclusion(s): Our data suggest that four weeks may not be the optimal time for repeat semen analysis and that one sample is insufficient to assess any abnormalities in the result of semen analysis. The optimal time between repeat semen analyses should be individualized depending on the results of the initial analysis and additional factors, suggesting the need for future large-scale studies to investigate this trend. (Fertil Steril Rep® 2021;2:172–5. ©2021 by American Society for Reproductive Medicine.)
Key Words: Semen analysis, repeat testing, optimal timing

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Male factor infertility occurs in up to 50% of infertility cases and is a growing problem worldwide (1). Semen analysis is the gold standard assessment of male factor infertility (2). The standard semen analysis values have been determined by the World Health Organization (WHO) and consist of the lowest 5% of the values of healthy fertile men (3). The reported parameters in a standard semen analysis include count, concentration, volume, pH motility, and morphology, although additional features may also be reported such as the presence of leukocytes.

The standard protocol for semen analysis collection consists of an abstinence period of 2–7 days before clean collection via masturbation as mandated by the American Urologic Association (AUA) and the American Society of Reproductive Medicine (ASRM) (3, 4). The AUA and ASRM also suggested that the optimal time for repeat collection is at least one month (5). However, there are limited data to support this period, given that the time required for the sperm to develop is approximately 74 days (6). Additionally, there has been recent controversy surrounding the need for a repeat semen analysis, or whether a single sample is sufficient. Some studies have suggested that because of significant within-patient variability, two samples provide a more robust result (7). To assess male infertility, a

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Reprint requests: Marc Goldstein, M.D., D.Sc. (Hons), Matthew P. Hardy Distinguished Professor of Reproductive Medicine, and Urology Surgeon-in-Chief, Male Reproductive Medicine and Surgery, Weill Cornell Medicine, New York Presbyterian Hospital, 525 East 68th Street, Starr Pavilion, Suite 900, New York, New York 10065 (E-mail: mgoldst@med.cornell.edu).

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single sample may be sufficient; however, to characterize infertility, a second sample is needed (8, 9).

With limited evidence supporting the optimal repeat semen analysis timing, we assessed a large sample of semen analyses to evaluate various possible time periods. As an alternative, to eliminate the need to determine an optimal time, we also assessed if it was possible to obviate the need for a repeat semen sample by assessing the agreement between consecutive patient samples.

MATERIALS AND METHODS

Study Population

Data were retrospectively reviewed for men between 2007 and 2018 at Weill Cornell in New York City. Patient data were retrieved from an electronic database, which contained prospectively collected semen analysis data. Patients were included if they underwent two semen analyses at our institution within 90 days of each other. If multiple semen analyses were completed during this period, only the first two analyses were considered. Individuals who received fertility interventions, or were referred from other reproductive urologists, were excluded. Additionally, men with azoospermia were also excluded. The study was approved by the Weill Cornell Institutional Review Board.

Sample Collection and Preparation

All patients received the same counseling instructions for semen collection. All samples were obtained via masturbation into a sterile container without the use of sperm damaging lubricants. The samples were provided immediately to the laboratory for processing and analysis by a certified andrologist.

The samples were processed and prepared as per the WHO manual (10). The semen volume was measured in milliliters, and the semen pH was determined. All samples were assessed after preparing the smear on a glass slide and analyzed at 400× magnification by a certified andrologist. Sperm count was measured in millions and sperm concentration was measured in millions/ml. Sperm morphology was recorded as per the WHO version 4, that is, a percentage of morphologically normal sperm based on adequate substructures including sperm heads, midpieces, and tails. Sperm motility was reported as a percentage of motile sperm visualized under the microscope.

Semen Analysis Timing and Correlations

The patient's age was calculated using the date of collection and the patient's date of birth. The time between samples was documented in days based on the collection dates between the two consecutive patient samples. The period of abstinence was recorded in days.

Agreement between the samples was classified using the WHO standard cut-off values for normal semen parameters. Specimens that showed abnormal results on the first analysis were compared with those that showed abnormal findings on the second analysis regardless of the time period.

Statistical Analysis

The semen analysis data were grouped in weeks based on the time between specimen collection. Age in years and abstinence period in days were reported as medians based on weekly time periods between sample collection with associated interquartile ranges (IQRs). The Pearson correlation coefficients were calculated for each respective week for each parameter. Values of >0.7 were indicated a strong correlation; 0.4–0.7, moderate; and <0.4, weak correlation. Each semen parameter was recorded as normal or abnormal for both the first and second samples, and the agreement between these was assessed and reported as a percentage. The overall agreement for all samples was reported as a Cohen's kappa statistic (κ), with 1.00 indicating perfect agreement. Analysis was performed using Stata v16.

RESULTS

A total of 1,075 men with 2,150 semen analyses were included. The overall median age was 36 years (IQR, 33–41 years), and the median age by weeks between consecutive

TABLE 1

Age and abstinence period by numbers of weeks between consecutive samples.

Weeks between samples	No. of samples	Median abstinence period, days (IQR)	Median age, years (IQR)
1	19	3.9 (3.0–4.0)	42.0 (38.0–52.0)
2	47	3.5 (2.5–4.0)	38.0 (34.0–45.0)
3	57	4.0 (3.5–5.0)	36.0 (33.5–41.0)
4	203	3.5 (3.0–4.5)	36.0 (32.0–41.0)
5	270	3.5 (3.0–4.0)	37.0 (33.0–41.0)
6	133	3.0 (2.5–3.5)	36.0 (33.0–39.5)
7	90	3.0 (2.5–3.0)	39.0 (34.0–45.0)
8	77	3.0 (2.5–3.5)	36.0 (33.0–40.0)
9	48	2.9 (2.5–3.0)	35.0 (32.0–39.0)
10	41	3.0 (2.5–3.3)	38.0 (35.0–43.0)
11	43	2.8 (2.3–3.0)	37.0 (34.0–40.0)
12	23	3.0 (2.5–3.8)	37.0 (33.0–41.0)
13	24	3.0 (2.5–3.5)	35.0 (32.0–39.0)

Note: IQR = interquartile range.

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TABLE 2

Pearson correlation coefficients of semen samples based on period of time between specimens.

Semen parameters					
Weeks between samples	**Volume (Pearson, r)**	**Concentration (Pearson, r)**	**Count (Pearson, r)**	**Motility (Pearson, r)**	**Morphology (Pearson, r)**
1	0.615	0.950	0.929	0.711	0.935
2	0.803	0.709	0.727	0.623	0.815
3	0.720	0.671	0.601	0.565	0.797
4	0.700	0.841	0.808	0.487	0.709
5	0.774	0.795	0.713	0.630	0.651
6	0.767	0.590	0.701	0.468	0.735
7	0.674	0.777	0.704	0.500	0.656
8	0.802	0.741	0.727	0.605	0.728
9	0.726	0.649	0.649	0.619	0.839
10	0.286	0.751	0.743	0.760	0.444
11	0.730	0.670	0.441	0.519	0.701
12	0.821	0.632	0.476	0.582	0.704
13	0.259	0.757	0.552	0.708	0.646

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samples ranged from 35 to 42 years (Table 1). The overall median age was 36 years (IQR, 33–41 years) (Table 1). The overall median time between repeat semen analyses was 35 days (IQR, 28–49 days). The majority were completed between 4 and 6 weeks (n = 609, 56.4%).

The correlation by week for each parameter is presented in Table 2. For semen volume, the most significant correlations were observed at weeks 12, 2, and 8 (r = 0.821, r = 0.803, and r = 0.802, respectively). For sperm concentration, the greatest correlations were observed at weeks 1, 4, and 5 (r = 0.950, r = 0.841, and r = 0.795, respectively). Sperm count had the greatest correlation at weeks 1, 4, and 2 (r = 0.929, r = 0.808, and r = 0.727, respectively). Sperm motility was most significantly correlated at weeks 10, 1, and 13 (r = 0.760, r = 0.711, and r = 0.708, respectively). Sperm morphology was most correlated at weeks 1, 9, and 2 (r = 0.935, r = 0.839, and r = 0.815, respectively).

Table 3 illustrates the correlative predictive ability of the patient's first semen analysis to their second semen analysis. The Cohen's kappa statistic for agreement of semen parameters for all included samples was lowest for motility (0.4403) and greatest for morphology (0.6131). When focusing only on the abnormal values, the semen volume was below 1.5 ml in 212 men (19.6%) in the first semen analysis and in 208 men (19.3%) in the second analysis. However, only 116 (54.7%) of those who showed abnormal results on analysis 1 were found abnormal on analysis 2. Disagreement occurred in 45.3% of the cases. Sperm concentration was abnormal ($<15 \times 10^6$/ml) in 155 men (14.4%) in the first analysis and 139 men (12.9%) in the second analysis, with an agreement of 63.9% and a disagreement of 36.1%. Sperm motility was classified as abnormal (<40%) in 195 men (18.1%) and 186 men (17.2%) in analysis 1 and 2, respectively, with an agreement of 52.3% and disagreement of 47.7%. Finally, sperm morphology was abnormal (<4%) in 201 men (18.6%) in their first analysis and in 176 men (16.3%) in the second analysis, with an agreement of 63.7% and disagreement of 36.3%. The

TABLE 3

Predictive ability of first and second semen analyses for detecting an abnormality.

	Semen analysis 1	Semen analysis 2	Cohen's kappa (κ)	Agree (n/total)	% Agree	% Disagree
Concentration						
</= 15 × 10^6/ml	155 (14.4%)	139 (12.3%)	0.5946	99/155	63.9%	36.1%
>15 × 10^6/ml	925 (85.6%)	941 (87.7%)				
Total motility						
</= 40%	195 (18.1%)	186 (17.2%)	0.4403	102/195	52.3%	47.7%
>40%	885 (81.9%)	894 (82.8%)				
Volume						
</= 1.5 ml	212 (19.6%)	208 (19.3%)	0.4469	116/212	54.7%	45.3%
>1.5 ml	868 (80.4%)	872 (80.7%)				
Morphology						
</= 4%	201 (18.6%)	176 (16.3%)	0.6131	128/201	63.7%	36.3%
>4%	879 (81.4%)	904 (83.7%)				

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average absolute differences for each parameter were 0.0 ± 1.1 ml for volume, 0.1 ± 59.8 × 10^6/ml for concentration, 1.1 ± 16.5 % for motility, and 1.2 ± 9.9% for morphology.

DISCUSSION

Semen analysis remains the gold standard for the assessment of male factor infertility with clear guidelines provided by national societies such as the AUA and ASRM (5)Although it is suggested that a repeat semen analysis should be performed at least one month after the original sample, our series suggests that the optimal timing for assessing some parameters may be different. Furthermore, our data suggest that one semen analysis may not be sufficient to assess male infertility.

In our series, for most parameters, significant correlation was observed within the first two weeks of repeat sample collection. There were also multiple semen parameters that displayed increased correlation at a time greater than eight weeks apart. These findings were regardless of a fairly consistent median abstinence period and age across time periods, both of which are known to impact semen quality (11, 12). Generally, a longer time between samples correlates with the sperm life cycle and its development in 74 days (6). Conversely, a shorter time between samples likely reflects a similar quality to the sperm recently compared in the previous sample given the time period for complete spermatogenesis. That said, since semen analysis remains a mainstay in the evaluation of male infertility, consideration of the interval between repeat semen analyses should be left at the discretion of the treating physician especially if there is suspicion of an acute or transient source for impaired semen parameters.

The guidelines suggested by national organizations do not cite any evidence for their recommendation on consecutive sample collection (5). To our knowledge, no report has specifically addressed the optimal time for repeat semen analysis testing. This is likely because of the presence of significant confounding factors and the shortcomings of semen analysis testing (13). Alternatively, perhaps more emphasis should be placed on discovering more predictive laboratory assessments for male factor infertility that correlate directly with pregnancy and fertilization outcomes.

When assessing the necessity for using a single or repeat semen analysis, our series is in agreement with other studies, which reported that two specimens should be recommended (8). Certain reasons to support this have been suggested including significant intrapatient variability as well as the subjective quality of the technician assessing the semen analysis. In our series, we reported an agreement of the second specimen with the first specimen; however, in the context of an abnormal semen parameter, it remains unclear which sample better represents the true value. In these situations, practicing urologists should consider using results from reliable laboratories and technicians that they feel most comfortable with.

The limitations of our study include the retrospective study design. Our overall sample size is large; however, when stratified by weekly periods of time, the individual sample size for some parameters is much smaller, especially for the shortest and longest time periods.

CONCLUSION

Our data suggest that a repeat semen analysis four weeks after the initial analysis may not be the optimal time. Timing should be individualized depending on the results of the initial analysis, the quality of the semen analysis laboratory, and other relevant patient factors. Future large-scale studies are required to investigate this trend.

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