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Seasonal Variation and Age-Related Changes in Human Semen Parameters

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ABSTRACT: Although semen quality has been discussed extensively with regard to age and season in the andrology literature, the results vary and firm conclusions are still outstanding. To investigate seasonal and age-related variations in human semen parameters, we analyzed data that were collected from an andrology clinic population. We performed a retrospective review of 551 semen analysis records collected from 1989 to 2000 from the Vincent Memorial Andrology Laboratory at Massachusetts General Hospital. Semen volume, sperm concentration, total sperm count, motility, total motile sperm, and morphology significantly decreased as age increased. In addition, as age increased, the percentage of sperm with tail defects increased. Sperm concentration was significantly higher in winter (mean 157.9 million /mL) than in fall (mean 119.1 million /mL) ($P < .05$). The mean percentage of sperm with normal morphology was significantly higher in winter (9.2%) than in summer and spring (7.0% and 7.5%, respectively; $P < .05$). The mean percentage of sperm with head defects was significantly higher in fall and summer (74.0% and 72.3%, respectively) than in winter (68.6%; $P < .05$). Seasonal variations were found in sperm concentration and morphology, with higher sperm concentrations in winter than in fall, and a greater percentage of sperm with normal morphology in winter than in spring and summer. Sperm concentration was lowest in the fall, whereas the percentage of sperm with normal morphology was lowest in summer. Semen volume, sperm concentration, total sperm count, motility, total motile sperm, and morphology decreased as age increased.

Key words: Epidemiology, sperm concentration, motility, morphology.

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During the last several decades, the number of annual office visits for infertility in the United States has risen from 600 000 in 1968 to 2 million in the 1990s (Seibel, 1997). Several factors may have contributed to this rise. They include an increase in public awareness of infertility as a treatable condition, as well as an increase in couples with infertility. Approximately 15% of couples are unable to conceive after 1 year of unprotected intercourse. A male factor is solely responsible in about 20% of infertile couples and is contributory in another 30%–40% (Thonneau et al, 1991).

Semen analysis is frequently used to evaluate male infertility. Assessment of semen quality is based on an evaluation of several parameters, including semen volume, pH, sperm concentration, sperm motility, and sperm morphology.

Seasonal variations in semen parameters have been reported in both fertile and infertile men (Levine et al, 1988; Saint Pol et al, 1989; Centola and Eberly, 1999). Saint Pol and coworkers (1989) found a significant seasonal variation in sperm count, with the highest sperm counts observed in late winter and early spring and the lowest in late summer. In age-adjusted analyses, Centola and Eberly (1999) found significant seasonal variation in the percentage of rapid motile sperm and progressive straight-line velocity, as well as in the percentage of tail defects, immature sperm, and tapered sperm.

Several studies have suggested that an increase in age is associated with a decline in semen parameters (Schwartz et al, 1983; Haidl et al, 1996; Centola and Eberly, 1999; Kidd et al, 2001). However, Paulson and coworkers (2001) identified an inverse association between age and total sperm count, but no age-related decrease in fertilization rate or a decrease in live birth rate in the oocyte donation model was found.

The present study was designed to evaluate seasonal variation and age-related changes in human semen parameters. In this retrospective study we reviewed data that were collected from men who attended the Vincent Memorial Andrology Laboratory of Massachusetts General Hospital (MGH) for semen analysis from 1989 to 2000.

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Materials and Methods

Participants and Data Source

The majority of patients who presented to the MGH Andrology Laboratory for semen evaluation were part of couples undergoing medical evaluation for an inability to conceive. An individual may or may not be infertile or subfertile, because the couple's fertility depends on the fertility of both partners.

Because this was a retrospective review of an existing laboratory database, the subjects were not contacted for informed consent. The MGH Human Subject Committee Institutional Review Board approved this study.

Information was collected retrospectively on 551 semen analyses that were performed from July 1989 to December 2000. The first 4 semen analysis records were retrieved for each month of the study period and comprise the study population. Each record contained the patient's date of birth, date of semen analysis, and semen analysis results (volume, pH, sperm concentration, motility, progressive motility, and morphology). These data were used to derive the patient's age at the time of the semen analysis, as well as the season in which each semen analysis was performed. Winter was defined as December, January, and February; spring as March, April, and May; summer as June, July, and August; and fall as September, October, and November.

All duplicates were eliminated before selecting laboratory records for inclusion in the study database. No demographic information was available from the Vincent Memorial Andrology Laboratory records for this sample population.

Semen volume and pH data were available for 551 records. However, only 408 records had semen analyses performed using a computer-assisted semen analysis (CASA; Hamilton Thorn Research IVOS, Beverly, Mass); 143 records that were manually counted were excluded from the analyses for sperm concentration and motility. The CASA analyzer was used on semen analyses performed from July 1992 to December 2000. In December of 1992, the laboratory implemented the Tygerberg-Kruger strict morphology assessment, thereby changing from the World Health Organization (WHO) criteria for morphology assessment. Therefore, the morphology data were not included before December 1992 because the assessment criteria had been changed. A total of 388 records from December 1992 to December 2000 were included for evaluation of morphology variables. During the study period, 4 technologists performed the semen analyses.

Collection of Semen Samples

Semen was collected by masturbation into a sterile, wide-mouthed polystyrene container in a private collection room in the hospital near the laboratory. The recommended period of abstinence was a minimum of 48 hours but not longer than 7 days. Semen specimens were allowed to liquefy for at least 20 minutes in an incubator at 37°C and were analyzed within 60 minutes after the samples were collected. A routine semen analysis was performed and included several parameters: semen volume, pH, sperm concentration, sperm motility, progressive motility, and sperm morphology.

Laboratory Evaluation

Semen Volume and pH—The samples were well mixed in the original container and were not vigorously shaken. The volume was determined using a disposable polycarbonate serologic pipette. The sample color and viscosity were recorded. Semen pH was measured within 1 hour of ejaculation. A drop of semen was spread evenly onto pH strips (color pHast indicator strips pH 6.5–10.0; EM Science, Gibbstown, NJ, made in Germany). This brand of pH strips was the only one used during the period of data collection. After 30 seconds, the color of the stained zone of the strip should have been uniform and was compared with the calibration strip to read the pH. The pH strips were compared with known pH standards of 7.0, 8.0, 9.0, and 10.0 (Buffer Solution, Fisher Scientific, Pittsburgh, Pa).

Concentration and Motility—All fresh samples were analyzed for sperm concentration and motion parameters by CASA. Sperm concentration, percentage motility, and percentage of progressive motility were determined. Setting parameters and the definition of measured sperm motion parameters for CASA were established by Hamilton-Thorn (frames acquired, 30; frame rate, 60 Hz; straightness threshold, 80.0%; medium average path velocity cutoff, 25.0 μm/s; and duration of tracking time, 0.38 seconds). Aliquots of semen samples (5 μL) were placed into a prewarmed (37°C) Makler counting chamber (Sefi Medical Instruments, Haifa, Israel). A minimum of 200 spermatozoa from at least 4 different fields was analyzed from each specimen. The percentage of motile sperm was defined as WHO grades ''a'' (rapidly progressive ≥25 μm/s at 37°C) plus ''b'' (slow/sluggish progressive with a velocity ≥5 μm/s but <25 μm/s).

Morphology—Using the ''feathering'' method described in the WHO manual (1999), at least 2 slides were made for each fresh semen sample. The resulting thin smear was allowed to air dry for 1 hour before staining, which was carried out using a Diff-Quik staining kit (Dade Behring AG, Düdingen, Switzerland). Morphological assessment was performed with a Nikon microscope using an oil immersion 100× objective (Nikon Company, Tokyo, Japan). As the slide was examined from one microscopic field to another, all spermatozoa were assessed and scored as normal or abnormal. Head defects, midpiece defects, and tail defects were scored. Sperm morphology was determined using the strict criteria described by Kruger et al (1988). A minimum of 200 spermatozoa were counted from 2 slides for each specimen. Results were expressed as the percentage of normal spermatozoa, head defects, midpiece defects, and tail defects.

Statistical Analysis

To investigate whether there were differences in semen parameters across season and associated with age, we performed regression analyses (SAS version 8.2, SAS Institute, Cary, NC). Winter was used as the reference season. We also investigated month-to-month variations in semen parameters. For each semen parameter, a separate multiple regression was performed. Semen analysis parameters were entered into the models both untransformed and after square root transformation because of their skewed distribution. Because the square root–transformed results were similar to the untransformed results and are simpler to interpret, only the untransformed results are presented. To explore whether the semen parameters and age relationships were linear,

Table 1. *Distribution of age and semen parameters for the study population*

Semen Parameter	Number	Mean	(SD)	Median	Minimum	Maximum
Age (years)	551	36.3	(6.5)	35.3	20.3	65.6
Volume (mL)	551	3.0	(1.5)	2.8	0.1	8.0
pH (unit)	551	8.2	(0.4)	8.3	6.8	9.5
Concentration (sperm*/mL)	408	136.1	(142.0)	90.9	2.2	847.0
Total sperm count (sperm)*	408	399.5	(463.9)	252.3	1.2	3364.0
Motility (%)	392	62.3	(28.8)	69.0	0.0	100.0
Total motile sperm (sperm)*	392	194.7	(143.7)	159.0	0.0	697.5
Normal morphology† (%)	388	8.1	(5.2)	7.0	0.0	27.0

* Number of sperm $\times 10^6$.
† Tygerberg Kruger strict morphology.

age was used as both a continuous and categorical variable (less than 30 years, 30 to 40 years, and greater than 40 years of age). $P < .05$ was considered statistically significant.

Results

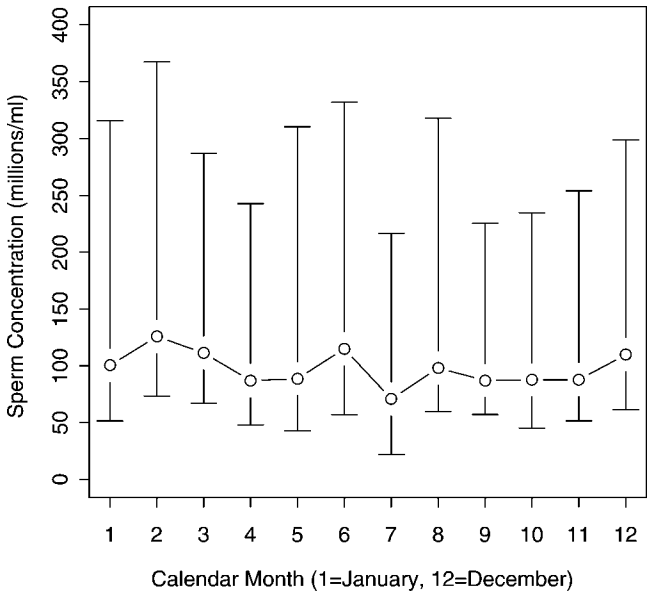
During the 11-year period of data collection, the age of all patients included in this sample ranged from 20 to 66 years (mean, 36.3 years; SD, 6.5). The majority of the subjects (n = 340, 62%) were between 30 and 40 years old, 13% (n = 71) were younger than 30 years, and 25% (n = 140) were older than 40. Although the mean sperm concentration and motility were on average above the WHO reference values, and the mean percentage of normal morphology was above 4% normal (strict criteria), a substantial portion of the study population was below these reference values. The average sperm concentration was 136.1 million/mL (SD 142.0) with a range from 2.2 to 847 million/mL. Sperm concentration had a skewed distribution, with the mean larger than the median. Forty-three (10.5%) subjects had <20 million sperm/mL. Six subjects had a sperm concentration >750 million/mL. The mean total sperm count was 399.5 million/mL (SD 463.9). The mean motility and normal morphology percentages were 62.3% (SD 28.8) and 8.1% (SD 5.2), respectively. One-hundred thirty-two subjects (33.7%) had <50% motility and 79 (20.4%) had <4% normal morphology. The mean semen pH was 8.2 (SD 0.4), ranging from 6.8 to 9.5, and 21% of samples were between pH 7.2 and 8.0. Distributions of semen parameters for the study population are presented in Table 1.

Seasonal variations in semen quality are shown in Table 2. The mean sperm concentration in autumn (119.1 million/mL) was significantly lower than in winter (157.9 million/mL; $P < .05$). The mean sperm concentrations in summer (132.9 million/mL) and spring (135.9 million/mL) were also lower than in winter, although this was not statistically significant. The seasonal differences remained after adjusting for age as both a continuous and categorical variable and after square root transformation of semen parameters. The figure shows the month-to-month median sperm concentration across all 11 years of the study. The interquartile ranges (25th and 75th percentiles) are used to describe the variability about the median. The spring, summer (except for June), and fall months had lower median sperm concentrations than winter months. Total sperm counts were significantly lower in fall than in winter (Table 2). In addition, total sperm counts in

Table 2. *Seasonal variations in semen quality*

	Spring			Summer			Fall			Winter		
Semen Parameter	Mean	(SD)	Median	Mean	(SD)	Median	Mean	(SD)	Median	Mean	(SD)	Median
Volume (mL)†	2.8	(1.5)	2.8	3.1	(1.4)	3.0	3.1	(1.7)	2.9	3.0	(1.5)	2.8
pH (unit)†	8.2	(0.4)	8.3	8.3	(0.4)	8.3	8.3	(0.4)	8.3	8.2	(0.4)	8.3
Concentration (sperm*/mL)‡	135.9	(145.2)	90.0	132.9	(131.7)	83.5	119.1¶	(125.7)	87.7	157.9	(163.5)	111.6
Total sperm count (sperm)*‡	375.8	(426.5)	238.0	418.6	(455.3)	292.9	331.2¶	(359.5)	208.6	476.3	(585.4)	288.2
Motility (%)§	62.6	(28.5)	70.5	62.6	(30.6)	73.5	63.1	(27.8)	69.0	60.9	(28.4)	65.5
Total motile sperm (sperm)*§	177.0	(130.5)	150.9	211.4	(153.7)	190.0	193.4	(143.5)	145.5	196.1	(145.7)	173.1
Progressive motility (%)‖	35.2	(18.6)	37.0	37.0	(20.6)	40.5	34.7	(19.9)	37.0	33.6	(18.2)	31.0

* Number of sperm $\times 10^6$.
† Number of subjects for volume and pH (spring, n = 132; summer, n = 139; fall, n = 144; winter, n = 136).
‡ Number of subjects for concentration and total sperm count (spring, n = 96; summer, n = 104; fall, n = 108; winter, n = 100).
§ Number of subjects for motility and total motile sperm (spring, n = 96; summer, n = 100; fall, n = 96, winter, n = 100).
‖ Number of subjects for progressive motility (spring, n = 96; summer, n = 104; fall, n = 108; winter, n = 100).
¶ $P < .05$ for the regression coefficient comparing the indicated season to winter as the reference season.



Median and interquarterile range (25th and 75th percentiles) for sperm concentration by month for the 11-year period 1989–2000. O, median; I, interquarerile range.

spring and summer were lower than in winter but this was not statistically significant.

There were also seasonal variations in sperm morphology parameters (Table 3). The mean percentage of normal morphology in winter (9.2%) was significantly greater than in spring (7.5%) and summer (7.0%), ($P < .05$) and nonsignificantly higher than in fall (8.7%). The mean percentage of head defects in summer (72.3%) and fall (74.0%) were significantly higher than in winter (68.6%; $P < .05$). The mean percentage of midpiece defects was significantly lower in fall (10.4) than in winter (14.0; $P < .05$. These seasonal differences remained after adjusting for age as both a continuous and categorical variable and after square root transformation of the semen parameters.

Mean semen volume and pH were similar across seasons. Although the month-to-month median sperm motility was lowest in July and August and in November and December, there was no consistent seasonal pattern.

The relationships between age, which was used as a continuous variable in the regression models, and semen parameters are shown in Table 4. There was a significant age-related decline in volume (−0.38 mL/decade; $P = .0002$), sperm concentration (−25.4 million/decade; $P = .02$), total sperm count (−101.1 million/decade; $P = .004$), motility percentage (−5.12%/decade; $P = .02$), progressive motility percentage (−4.27%/decade; $P = .004$), total motile sperm (−36.6 million/decade; $P = .001$), normal morphology percentage (−1.06%/decade; $P = .009$), and an increase in percentage of tail defects (+2.46%/decade; $P < .0001$). Analyses in which age was used as a categorical variable did not show differences between categories for sperm concentration, motility, or morphology. This was not unexpected because the range of ages was narrow and few subjects were over 40 years old.

Table 4. *Age-related change in human semen parameters*

Semen Parameter	Regression Coefficient (for 10-y Interval)	*P* Value
Volume (mL)	−0.38	.0002
pH (unit)	0.03	>.1
Concentration (sperm*/mL)	−25.40	.02
Total sperm count (sperm)*	−101.1	.004
Motility (%)	−5.12	.02
Total motile sperm (sperm)*	−36.6	.001
Progressive motility (%)	−4.27	.004
Normal morphology (%)	−1.06	.009
Head defects (%)	−0.18	>.1
Midpiece defects (%)	−0.84	>.1
Tail defects (%)	2.46	<.0001

* Number of sperm × 10^6.

Discussion

Although semen quality has been discussed extensively with respect to age and season in the andrology literature, the results vary considerably and firm conclusions are still outstanding. To further our understanding of seasonal and age-related affects on semen quality, we undertook the present retrospective study of patients evaluated in the

Table 3. *Seasonal variations in sperm morphology*

Semen Parameter Morphology*	Spring‡			Summer‡			Fall‡			Winter‡		
	Mean	(SD)	Median	Mean	(SD)	Median	Mean	(SD)	Median	Mean	(SD)	Median
Normal (%)	7.5†	(5.2)	6.5	7.0†	(4.7)	6.0	8.7	(5.4)	8.0	9.2	(5.2)	9.0
Head defects (%)	69.1	(13.6)	70.0	72.3†	(10.6)	72.0	74.0†	(10.9)	75.0	68.6	(12.7)	70.0
Midpiece defects (%)	16.2	(10.5)	14.5	13.2	(7.1)	12.0	10.4†	(7.9)	9.0	14.0	(9.3)	12.0
Tail defects (%)	6.1	(7.6)	4.0	6.9	(6.0)	5.0	5.8	(5.2)	4.5	7.0	(7.3)	5.0

* Tygerberg Kruger strict criteria

† $P < .05$ for the regression coefficient comparing the indicated season to winter as the reference season.

‡ Number of subjects for spring, summer, and fall was n = 96; winter, n = 100.

andrology laboratory of MGH. The majority of men were partners in a couple undergoing medical evaluations for an inability to conceive. Seasonal variations were found in total sperm count, sperm concentration, and morphology, with higher total sperm counts and sperm concentrations found in winter than in fall and a greater percentage of sperm with normal morphology found in winter than in spring and summer. Total sperm counts and sperm concentrations were lowest in the fall, whereas the percentages of sperm with normal morphology were lowest in summer. This may suggest that percent of normal sperm morphology recovers more rapidly than total sperm count and sperm concentration. In addition, age-related decreases were found in semen volume, total sperm count, sperm concentration, percent motility, total motile sperm, and percent progressive motility.

The present study shows a higher sperm concentration and total sperm count in winter than in other seasons and is lowest in fall. A similar finding was reported by Gyllenborg and coworkers (1999), who found lower sperm counts during summer and autumn than in late winter and spring among young Danish men. Two other retrospective studies (Politoff et al, 1989; Saint Pol et al, 1989) found peak sperm concentrations in the winter and spring, whereas the lowest sperm concentrations occurred in summer. A prospective study (Levine et al, 1992) reported reductions in semen quality during summer compared with winter. Levine (1994) conducted a semiquantitative meta-analysis of seasonality in human reproduction. In all 8 studies that reported sperm concentration, the values were lowest during summer. The highest values were noted during winter or spring. This is partially consistent with our findings of highest sperm concentration values in winter, although our study found the lowest values in fall followed by summer. Overall, our data are in agreement with previous reports of seasonal variation in sperm concentration, with winter having the highest concentration.

In the present study, the percentage of sperm with normal morphology was significantly higher in winter than in spring and summer. In addition, the percentage of sperm with abnormal head defects was significantly higher in fall and summer than winter. Centola and Eberly (1999) found similar variations, with a higher percentage of tapered forms in fall than in spring.

The present study is consistent with several other studies (Mortimer et al, 1983; Saint Pol et al, 1989; Centola et al, 1999) in that it did not find seasonal variations in semen volume or motility. In contrast, Reinberg and coworkers (1988) found a peak semen volume during April and May in prevasectomy patients.

The effects of temperature and hours of daylight may partially explain these statistically significant seasonal variations in sperm concentration (Levine 1994). Sperm production in humans is known to decrease when testicular temperature is raised by experimental techniques (Mieusset et al, 1987). Normal spermatogenesis requires a temperature 2–3°C lower than the rectal temperature (Snyder et al, 1990). The effect of higher temperature is manifested at a later time (about 90 days after exposure). This may partially explain why mean sperm concentrations were lowest in the fall but not in summer, and highest in winter. Chia and coworkers (2001) reported no significant month-to-month fluctuations in semen volume and sperm density among men who resided in the tropics, where there are minimal changes in temperature.

In the present study, semen volume, sperm concentration, total sperm count, sperm motility, progressive motility, total motile sperm, and normal morphology decreased as age increased. In addition, tail defects increased significantly as age increased. Significant decreases in semen parameters linked to aging were recognized by Centola and Eberly (1999), Schwartz et al (1983), and Haidl et al (1996). A review of the literature by Kidd et al (2001) on the association between age and semen quality and fertility status suggested that increased age was associated with a decline in semen volume, sperm motility, and sperm morphology, but not with sperm concentration.

The present study has several limitations. Although patients were told to abstain from ejaculation for at least 48 hours and no longer than 7 days before their clinic visit, we were not able to confirm this. Abstinence time data were not retrospectively available. The length of the period of abstinence may confound the relationship between season and semen parameters if, for instance, there was a seasonal variation in the frequency of sexual intercourse. Furthermore, if there was a relationship between age and length of sexual abstinence, the relationship between age and semen parameters may also be biased. However, because older men generally have less frequent intercourse, and therefore longer abstinence times, the relationship between age and semen parameters may by biased toward the null by not adjusting for abstinence time. We also lacked data on smoking history and other lifestyle factors, which may alter semen parameters. For bias to occur, these would need to be related to age or season, and be predictive of the semen parameters.

Although several technologists analyzed the semen samples over the study period, this is unlikely to bias our results. Because season and age-related associations are averaged temporally, intertechnologist variability is unlikely to account for our results. However, for temporal trend analysis of this data, intertechnologist variability may introduce bias. Although the Makler counting chamber was replaced several times during the study period, for the same reasons as noted above, this is also unlikely

to account for the seasonal and age-related associations with semen parameters.

Because the study subjects were men who were partners in infertile couples, it may not be possible to generalize the results of the present study to the general population, which includes fertile men. However, if there are seasonal trends among men who attend infertility clinics, this would be important to determine because these men generally represent the subset of the population that is most vulnerable to reproductive insults. The inability to generalize does not alter the internal validity of the study.

In conclusion, the present study found both seasonal and age-related associations with several semen parameters in a sample of subjects from an infertility clinic population. These results are consistent with previously reported results. However, because this was a retrospective review of semen analysis data, we were unable to collect information on potential confounders, including abstinence time and lifestyle factors. Future analysis of a prospectively collected dataset will be used to evaluate seasonal and age related associations with semen parameters.

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