Journal of Assisted Reproduction and Genetics, Vol. 21, No. 7, July 2004 (© 2004)

Assisted Reproduction

Does Seasonality Alter Intrauterine Insemination Outcomes: A 5-Year Study

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Submitted March 30, 2004; accepted May 4, 2004

***Purpose*:** To determine if seasonal changes alter pregnancy rate in intrauterine insemination (IUI) patients.
***Methods*:** One-thousand and eighty semen analyses prepared for IUI were evaluated in this retrospective cohort study of 496 patients.
***Results*:** Volume, pH, sperm concentration, and pregnancy rates were not altered by season. However, the percent motility, the total motile spermatozoa in the ejaculate, the straight-line velocity (VSL) of spermatozoa, as well as the morphology of sperm were altered by season. In a subset of these patients that were defined as normal, only the VSL and the morphology of the spermatozoa were altered by seasonal changes.
***Conclusions*:** Seasonality alters sperm motility parameters as well as morphology, but these changes are not significant enough to alter pregnancy rates.

KEY WORDS: Intrauterine insemination; seasonality; semen parameters.

INTRODUCTION

Intrauterine insemination (IUI) with the husband's washed spermatozoa has been used routinely in the treatment of infertility since the early 1980s (1). In contrast to intravaginal insemination, IUI involves the deposition of washed sperm directly into the uterine cavity, thereby increasing the number of motile sperm arriving at the ampullary region of the oviduct and thus improving the chance of fertilization.

Studies on the effectiveness of the washed IUI technique report a wide range of results, with pregnancy rates varying between 5 and 66% (2–4). Differences among pregnancy rates could be caused by small study populations, variations in stimulation protocols and/or insemination techniques.

Few studies exist that evaluate seasonal effects over numerous years in a large number of patients, even though there is extensive research in mammals that indicates that seasonal variations do exist. Seasonality alters reproductive performance in most economically important animals (cattle and sheep), as well as tame and feral mammals (5).

The purpose of our study was to evaluate seasonal effects on sperm parameters and pregnancy rates in a large population of patients undergoing IUI.

MATERIALS AND METHODS

Patients

This retrospective cohort study (6) was based on data collected from couples treated in the IUI program at Reproductive Endocrinology and Infertility of the Greenville Hospital System. Our institutional review committee approved the study, and patients signed consent forms indicating awareness that their data might be used to monitor and possibly improve methodologies used for IUI.

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Those patients undergoing ovarian stimulation received daily injections of gonadotropin (human menopausal gonadotropin, urofollitropin or recombinant follicle stimulating hormone). Ovulation was triggered by the use of human chorionic gonadotropin (hCG). Patients were determined to be pregnant if they had a quantitative beta-hCG value of>5 mIU/mL and a viable sac visible during ultrasound monitoring.

Patients were inseminated the morning after a reported LH surge (Ovu Quick® One-Step, San Diego, CA) or 36-h post hCG injection. For those patients receiving a second insemination, the procedure was repeated the next morning.

Protocol

Males were instructed to abstain from ejaculation for a minimum of 48–72 h before specimen collection. More than 95% of the specimens were collected into a sterile plastic specimen cup via masturbation; the remaining specimens were collected through the use of a nonspermicidal condom. Semen specimens were brought to the laboratory and placed into a 37°C incubator for approximately 30 min to allow liquefaction to take place.

Specimens were vortexed gently and, with a sterile pipette, evaluated for volume. The majority of the specimens (Fraction A) were placed into a Falcon 2095 tube (Becton Dickinson, Franklin Lakes, NJ) and diluted 2:1 with sperm-washing medium (SWM; Irvine Scientific, Santa Ana, CA). A small amount of each specimen (Fraction B) was reserved in the container for the semen analysis. Fraction A was centrifuged at room temperature for 5 min at $300 \times g$. The supernatant was removed and placed into a second Falcon 2095 tube and centrifuged for 3 min at $800 \times g$ at room temperature. The supernatant from the second centrifugation was removed and discarded. Pellets from both centrifugations were combined and resuspended, usually in 0.2 mL of SWM. The 0.2 mL specimen was inseminated into the woman's uterus via a Tom Cat catheter (Sherwood Medical, St. Louis, MO).

Fraction B was evaluated for pH, sperm concentration (M/mL), sperm agglutination, sperm morphology and concentration of white blood cells. In addition, the following sperm motility values were obtained: percent motile sperm, forward progression of sperm, total motile sperm, straight-line velocity (VSL), and sperm motility index. Measurement of total motile sperm was based on a mathematical value derived from multiplying the concentration by the percent motility. Sperm motility index was obtained by multiplying the percent of rapidly moving sperm by 4, the percent of moderately moving sperm by 3, the percent of slow-moving sperm by 2, and then adding all values together.

Semen Analysis

Semen specimens (diluted when sperm concentrations were $>50 \times 10^6$/mL) were loaded into 20-μm MicroCell chambers (Conception Technologies, San Diego, CA), allowed to settle and analyzed at 37°C, either via an Internal Visual Optical System (IVOS; Hamilton Thorne, Beverly, MA) or by a manual method. Both methods produced similar results in our laboratory (7). The IVOS (Version 10.8×) was used for semen specimens with more than 20×10^6 sperm/mL or greater and little debris. Manual methods of semen analysis were used for semen specimens with $<20 \times 10^6$ sperm/mL and/or substantial debris.

The importance of reporting CASA parameter settings has been mentioned elsewhere (8). The CASA parameters used in this study for semen analysis were reported in a previous article (7). Approximately seven fields and a minimum of 200 sperm cells were analyzed per specimen (9).

Statistical Analysis

Continuously distributed data were first examined for statistical normality. Many of the semen parameters were not normally distributed; therefore, median values, along with minimums and maximums, were used to describe these data. The Kruskal–Wallis test was used to test for a difference in medians between the four season groups. If this test was statistically significant then winter vs. summer groups were compared using the Wilcoxon-Rank Sum test. These two groups were selected because they provide the widest diversity in environmental temperatures and photoperiods. Proportions were compared using the chi-square test for homogeneity (e.g., comparison of pregnancy rates).

RESULTS

Patient data and their cycle characteristics are described in Table I. The majority of the 496 women in this study were between the ages of 26–35 with ovulatory dysfunction as their primary diagnosis of infertility. Eighty-three percent of all IUIs in this study occured within the first three cycles. Approximately

Table I. Frequency Distributions of Patient and IUI Characteristics ($n = 1080$ IUI Cycles)

Variable	No. IUIs	%
Age distributions ($n = 496$ women)		
≤25	31	6.3
26–30	168	33.9
31–35	191	38.5
36–40	90	18.1
>40	16	0.3
Primary diagnosis ($n = 496$ women)		
Ovulatory dysfunction	328	66.1
Endometriosis	61	12.3
Male factor	44	8.9
All other	63	12.7
No. of IUI attempts		
1	496	45.9
2	288	26.7
3	156	14.4
4	69	12.3
5	40	3.7
6	17	1.6
7	9	0.8
8	4	0.4
9	1	0.1
Stimulation		
No	862	79.8
Yes	218	20.2
No. of days inseminated		
1	815	75.5
2	265	24.5
Pregnancy outcome		
Not pregnant	957	88.6
Pregnant	123	11.4

Note. n = Number; IUI = intrauterine insemination; No. = number.

80% of the cycles were nonstimulated and 75% of the cycles were treated with a single insemination. The average pregnancy rate for the 1080 cycles was 11.4%.

When investigating all cycles collectively, volume, pH, and sperm concentration were not altered by seasonal conditions ($P > 0.05$; Table II). Other than the Motility Index (a mathematical computation) all motility parameters measured (percent motility, total motile sperm in an ejaculate, and straight line velocity [VSL]) were altered by season ($P < 0.05$). Likewise, morphology was significantly affected by seasonal conditions ($P < 0.001$). Pregnancy rate was not affected by season ($P > 0.05$).

In addition to analyzing the total dataset, we investigated a subset of patients defined as infertile (sperm concentrations $<30 \times 10^6$ per mL, sperm motility <40%, or normal sperm morphology <10%). There were 264 women and 488 cycles within this subset (Table III); results were similar to the collective set of data reviewed previously. The only exception was that the average motility only approached a statistically significant difference in this subset compared to reaching significance in the complete set of data.

The remaining subset of patients was defined as fertile (sperm concentrations $\geq 30 \times 10^6$ per mL, sperm motility ≥40%, and sperm morphology ≥10%). The individuals within this subset had little seasonal variation in their semen specimens except for the VSL ($P = 0.013$) and sperm morphology ($P < 0.001$).

When we investigated only those women whose age ranged from 26 through 35, 359 women with 780 cycles were evaluated (Table IV and Table V). In this group of women, volume, pH, and concentration were not altered by seasonal conditions ($P > 0.05$). No motility values (motility index, total motile, and percent motility) except VSL ($P < 0.001$) were altered by seasonal changes ($P > 0.05$). Morphology continued to be affected by season ($P < 0.001$). Pregancy rate was not altered by seasonality in this group of individuals ($P > 0.05$).

DISCUSSION

In this retrospective cohort study, we attempt to find seasonal, semen, and IUI characteristics that are associated statistically and independently with a patient's achieving a pregnancy after IUI. Our study includes the evaluation of 1080 cycles from 496 women over 57-months. Whether the analysis compared cycles from all couples, the infertile couples, or the fertile couples, sperm concentration and pregancy rates are not altered by seasonal events. However, sperm motility measured as VSL and sperm morphology are altered by season for the infertile couples as well as the fertile couples.

It has long been known that there is a seasonal effect on reproduction in mammals. Many studies indicate that hot weather decreases fertility rates and semen quality (10–13). While we see a trend toward a decline in pregancy rate in humans with an increase in seasonal temperatures, we are unable to confirm this seasonal effect on pregnancy rates. This seasonal effect is not observed when we compare the 12 individual months or when we group the months into four seasons on the basis of our hottest and coldest months. However, Centola and Eberly (13) demonstrate that there is a seasonal variation only in fertile patients and not in infertile patients. Their data indicate that infertile patients maintain certain semen parameters all year long, information that in turn suggests that subfertility is caused by a medical condition not affected by seasons or temperature.

Like the research of Centola and Eberly, some of the semen parameters of our normal patients

Table II. Semen Characteristics for All Intrauterine Insemination Patients

Sperm parameter	Spring ($n = 246$)	Summer ($n = 287$)	Fall ($n = 278$)	Winter ($n = 268$)	Winter vs. summer (P value)
Volume					
Median	3.0	2.8	3.0	2.9	
Min, max	0.4, 9.1	0.3, 8.9	0.2, 8.9	0.5, 7.5	0.85
pH					
Median	7.6	7.6	7.4	7.6	
Min, max	6.8, 9.0	6.4, 9.0	6.8, 9.0	5.3, 9.0	0.80
Concentration					
Median	48.4	54.7	54.2	48.9	
Min, max	3, 331	1, 541	2, 450	2, 358	0.17
Motility					
Median	48	54	53	51	
Min, max	10, 92	4, 96	5, 92	5, 92	0.027[a]
Total motile					
Median	59	77	75	64	
Min, max	2, 554	1, 740	1, 876	1, 977	0.029[a]
VSL					
Median	42.1	40.4	42.8	42.6	
Min, max	13.7, 72.6	18.7, 69.5	22.4, 65.8	20.9, 70.2	0.001[a]
Motility index					
Median	189	196	205	194	
Min, max	35, 348	14, 367	18, 356	23, 356	0.43
Morphology					
Median	20	20	23	27	
Min, max	2, 54	1, 53	3, 56	4, 64	<0.001[a]

[a] Significant differences observed when comparing the median values of winter and summer quarterlies.

Table III. Semen Characteristics for Infertile Intrauterine Insemination Cycles (Defined as Concentration <30 M/mL Motility <40% or Normal Morphology <10%)

Sperm parameter	Spring ($n = 120$)	Summer ($n = 121$)	Fall ($n = 119$)	Winter ($n = 128$)	Winter vs. summer (P value)
Volume					
Median	3.2	3.2	3.2	3.3	
Min, max	0.4, 8.2	0.3, 8.9	0.2, 8.9	0.5, 7.5	0.90
pH					
Median	7.8	7.6	7.6	7.6	
Min, max	6.8, 9.0	6.4, 9.0	7.0, 9.0	7.0, 9.0	0.11
Concentraion					
Median	25.5	26.5	25.0	23.2	
Min, max	3, 209	1, 541	2, 450	2, 175	0.13
Motility					
Median	36	37	36	35	
Min, max	10, 74	4, 89	5, 84	5, 92	0.07
Total Motile					
Median	23	36	29	27	
Min, max	2, 198	1, 740	1, 325	1, 155	0.038[a]
VSL					
Median	38.6	36.2	39.2	40.2	
Min, max	13.7, 65.6	18.7, 58.7	24.7, 58.8	20.9, 60.0	0.013[a]
Motility Index					
Median	136	136	146	136	
Min, max	35, 276	14, 340	18, 303	23, 334	0.58
Morphology					
Median	14	15	18	24	
Min, max	2, 40	1, 44	3, 56	4, 55	<0.001[a]
Pregnancy rates	5 (4.2)	8 (6.6)	13 (10.9)	10 (7.8)	0.25

[a] Significant differences observed when comparing the median values of winter and summer quarterlies.
Note. Methods: P values from Wilcoxon two-samples test for difference in medians and chi-square test for difference in proportions (pregnancy).

Table IV. Frequency Distributions of Patient and Intrauterine Insemination Characteristics for Women Aged 26–35

Variable	*n*	%
No. of IUI attempts ($n = 780$ IUIs)		
1	359	46.0
2	211	27.0
3	112	14.4
>4	98	12.6
Pregnancy outcome ($n = 780$ IUIs)		
Not pregnant	690	88.5
Pregnant	90	11.5
Stimulation ($n = 780$ IUIs)		
No	636	81.5
Yes	144	18.5
Age distributions ($n = 359$ women)		
26–28	90	25.1
29–31	117	32.6
32–34	118	32.9
35	34	9.5
Primary diagnosis ($n = 359$ women)		
Ovulatory dysfunction	249	69.4
Endometriosis	44	12.3
Male factor	27	7.5
All other	39	10.9

Note. n = number; IUI = intrauterine insemination.

(≥30 M/mL, motility of ≥40%, and ≥10% normal forms) are altered by season (Table VI). The VSL of the spermatozoa as well as the morphology of the individual sperm is changed in favor of more normal values during the winter months. However, unlike the Centola and Eberly data, our data indicate that season does not change concentration of spermatozoa, or overall sperm motility. Perhaps the difference between the data of Centola and Eberly and our data reflect the differences in temperature and photoperiod within the two different latitudes (Rochester, New York [43.154° north] vs. Greenville, South Carolina [34.9° north]).

Our data show significant seasonal differences among sperm motility and morphology values for all IUI patients. The median value for motility was significantly higher in the summer months compared with the winter ($P = 0.027$). These data agree with the data from Baker *et al.* (14), who analyzed 177 infertile men that had participated in three or more semen analysis in their laboratory and indicate that there is a 7.6% decrease in motility in the winter. Although our data for infertile men is not statistically significant ($P = 0.07$), it was consistent with Baker's findings.

Baker *et al.* (14) and Chen *et al.* (15) found that the percent normal morphology as assessed by strict criteria was higher in the winter than in the summer. Baker noted that oval heads, tapered heads, and abnormal tails were more common in summer, whereas amorphous heads were more numerous in winter. Our data

Table V. Semen Characteristics Used for Intrauterine Insemination in 26–35-Year-Old Women

Sperm parameter	Spring ($n = 177$)	Summer ($n = 199$)	Fall ($n = 198$)	Winter ($n = 206$)	Winter vs. summer (P value)
Volume					
Median	3.0	2.8	3.2	2.8	
Min, max	0.5, 8.0	0.3, 8.9	0.2, 8.9	0.5, 7.5	0.99
pH					
Median	7.6	7.6	7.4	7.6	
Min, max	6.8, 9.0	6.5, 9.0	7.0, 9.0	5.3, 9.0	0.97
Concentration					
Median	49.9	53.6	56.0	51.2	
Min, max	3, 331	2, 541	9, 450	2, 358	0.57
Motility					
Median	51	55	53.5	52	
Min, max	12, 92	4, 96	22, 90	5, 92	0.10
Total motile					
Median	63.5	73	84.5	65.5	
Min, max	2, 554	1, 740	10, 876	1, 977	0.23
VSL					
Median	41.9	40.3	43.8	42.5	
Min, max	18.7, 72.6	19.8, 63.6	28.4, 65.8	20.9, 70.2	<0.001[a]
Motility index					
Median	194	198	205	198	
Min, max	44, 345	14, 367	18, 340	26, 356	0.72
Morphology					
Median	20.5	20	24	26	
Min, max	2, 54	2, 53	3, 56	4, 64	<0.001[a]
No. (%) pregnant	21 (11.9)	21 (10.6)	25 (12.6)	23 (11.2)	0.93

[a] Significant differences observed when comparing the median values of winter and summer quarterlies.
Note. Methods: P values from Wilcoxon two-samples test for difference in medians and chi-square test for difference in proportions (pregnancy).

Table VI. Semen Characteristics for Fertile Intrauterine Insemination Cycles (Defined as Concentration ≥30 M/mL, Motility ≥40%, and Normal Morphology ≥10%)

Sperm parameter	Spring ($n = 126$)	Summer ($n = 166$)	Fall ($n = 159$)	Winter ($n = 140$)	Winter vs. summer (P value)
Volume					
Median	2.6	2.6	2.7	2.5	
Min, max	0.5, 9.1	0.5, 7.8	0.6, 6.8	0.5, 7.4	0.87
pH					
Median	7.5	7.6	7.4	7.8	
Min, max	6.8, 8.8	6.5, 9.0	6.8, 9.0	5.3, 9.0	0.07
Concentration					
Median	84.1	69.1	78.9	72.4	
Min, max	30, 331	30.1, 419	31, 407	31, 358	0.50
Motility					
Median	64	63	64	63	
Min, max	40, 92	40, 96	40, 92	40, 92	0.56
Total motile					
Median	126	119	135	114	
Min, max	27, 554	16, 596	14, 876	14, 977	0.86
VSL					
Median	43.2	43.0	45.3	44.0	
Min, max	27.1, 72.6	22.0, 69.5	22.4, 65.8	26.0, 70.2	0.013[a]
Motility index					
Median	234	229	242	233	
Min, max	128, 348	135, 367	140, 356	149, 356	0.58
Morphology					
Median	24.8	22	29	32	
Min, max	10, 54	10, 53	10, 54	13, 64	<0.001[a]
No. (%) pregnant	21(16.7)	20(12.1)	25(15.7)	21(15.0)	0.69

[a] Significant differences observed when comparing the median values of winter and summer quarterlies.
Note. Methods: P values from Wilcoxon two-samples test for difference in medians and chi-square test for difference in proportions (pregnancy).

also indicate a trend for more normal morphology forms in the winter compared to those in the summer ($P = 0.001$); however, unlike Baker *et al.* (14), our data are based on percent normal forms alone. Abnormal forms are not recorded by type of defect on a "by season" basis.

Because changes in motility and morphology do not alter pregnancy rates, perhaps these parameters are of less importance than once thought. Perhaps total concentration of available sperm is the primary factor with motility and morphology playing a much less important role. If this is true, the once intricate semen analysis may only require a sperm concentration and a cursory look at sperm motility and morphology.

Many reports indicate that sperm concentration and quality are reduced in the summer months when compared to others. Peaks of high sperm concentration were found in the spring (usually March), with a tendency for overall normal semen parameters in the winter (14–17). Are these declines in sperm parameters because of elevated seasonal heat? The data suggest that elevated summer heat may have an adverse affect on spermatogenesis. The overall effect of elevated summer temperatures, mixed with people's "summer time" lifestyles, may be responsible for degenerating effects on spermatogenesis, due to the inability of the testicles to thermoregulate properly.

Levine *et al.* (11) performed a study in New Orleans during July–August 1989 and January–February 1990 on indoor workers ($n = 64$) and outdoor workers ($n = 76$). If increased temperature is the problem, then the outdoor workers should exhibit decreased sperm parameters compared to indoor workers. The results show that a decrease in summer semen parameters are evident in both indoor and outdoor workers regardless of exposure to elevated temperature. The effect of summer heat on semen quality on outdoor workers was small and not significantly different from the indoor workers. Levine *et al.* (11) gives evidence to suggest that elevated summer heat may not be the only variable for decreased summer sperm quality.

Besides elevated temperature, photoperiod may play a key role in seasonal changes in semen quality. Like temperature, photoperiod (length of day) also changes with season with longer days in the summer and shorter days in the winter. This change in photoperiod has a biological effect on reproductive hormones in mammals, and may play a key

role in seasonal variation of semen parameters in humans.

The pineal gland appears to exert an important role in the neuroendocrine regulation of human reproduction (18). The pineal gland produces the hormone melatonin, which is secreted in response to dark or light, depending on if the species is a long day or short day breeder. The secretion of melatonin may then activate or inactivate the hypothalamic gonadotrophin-releasing hormone (GnRH) pulse generator, which in turn may then activate or inactivate the pituitary gonadal axis (19). In the mammalian reproductive system, the GnRH pulse generator is the key to seasonal breeding; requiring one pulse of GnRH secreted every hour or so (19). Although Humans are neither short day nor long day breeders, it is hypothesized that melatonin may still interact with the process of human reproduction. If humans secrete melatonin at night, then maybe melatonin plays a role in the regulation of seasonal variation in gonadal activity in humans, particularly in the infertile adult (19,20). Also, if there is seasonal variance in melatonin and gonadotrophins, this suggests that melatonin may affect seasonal sperm quality and production.

Seasonal changes in semen analysis may directly be a result of lifestyle and environment changes, along with seasonal temperature and photoperiods. Prolonged sitting and activities that require little or no physical activity interfere with the ability of the scrotum to thermoregulate, which causes damage to sperm production. Because sperm production requires the testes to be 3–4°C cooler than core body temperature, tight clothing, hot baths, steam rooms, etc. also have adverse effects on spermatogenesis (21).

Increased rates of smoking, consumption of alcohol, and exposure to endocrine disrupters have been associated with some men experiencing infertility problems (21,22). Smokers, for example, have been diagnosed with poor seminal quality, higher percentage of sperm head defects, and lower sperm density when compared to nonsmokers (22). Excessive alcohol consumption has been associated with decreased testosterone levels, which could have adverse effects on fertility. Endocrine disrupters can be constituents of plastics, such as bisphenolic and alkylphenolic compounds, phthalates, etc. Although there is no concrete evidence that these compounds are responsible for increased infertility in males, they appear to be "estrogen like," and may have negative effects on fertility (22).

In conclusion, our study indicates that season alters the motility and morphology sperm parameters of couples undergoing IUI cycles at our institution. Our data agree with others that sperm motility parameters are significantly higher in the summer and that percent normal morphology is significantly higher in the winter. This was basically true in all study groups (overall fertile and infertile). When the data are limited to women aged 26–35 years undergoing IUI cycles ($n = 780$), seasonal variations still held true for higher percent normal morphology in the winter and higher motility parameters approaching significance in the summer. The underlying cause for these results, whether it is temperature, photoperiod, environmental factors, or as yet unknown factors, is yet to be determined. While each of the previous factors could contribute to seasonal differences in sperm parameters, more research is needed to investigate other areas such as effects of geographical location, social status, etc.

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