Comparison between semen parameters in specimens collected early in the morning and in the evening.

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Key words
male factor, infertility, intrauterine insemination (IUI), circadian rhythms, semen analysis

List of abbreviations
IUI: intrauterine insemination
OAT: oligoasthenoteratozoospermia
TSC: total sperm count
TMSC: total motile sperm count

Abstract

The majority of facilities in Japan that offer artificial insemination as part of assisted reproduction programs currently perform semen collection in the early morning. The total motile sperm count of the semen used in intrauterine insemination is an important factor in achieving successful fertilization and subsequent childbirth. The present study was initiated to determine whether semen parameters varied with the time of day at which the semen sample was collected. The study subjects were 20 fertile males and 20 infertile males with abnormal seminograms who attended our Reproduction Center. Semen was collected early in the morning (morning collection group) and in the evening (evening collection group) from the same subjects, and total motile sperm count was assessed as the primary outcome measure. As secondary outcome measures, semen volume, sperm concentration, sperm motility and total sperm count were assessed. A sexual abstinence period of 3 days was set for all participants. The semen samples were analyzed using CASA CEROS, a sperm motility analysis system, and the data from the

morning and evening collection groups were compared using a Wilcoxon signed rank test. We found that the fertile males had a significantly higher total motile sperm count and total sperm count in the evening collection group than in the morning collection group. In contrast, the male infertility patients showed no significant difference in total sperm count between the two collection times; however, the total motile sperm count was significantly higher in the evening collection group than the morning collection group. Our analyses indicate that total motile sperm count in ejaculated semen is significantly higher after evening collection than after morning collection. From a male side perspective, we suggest that successful intrauterine insemination might be easier to achieve using semen collected in the evening than in the early morning.

Key words

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Introduction

Approximately half of the cases of infertility in couples are due to sperm deficiencies, such as low sperm counts or low numbers of motile sperm (Miyamoto et al. 2017; Rumbold et al. 2019). As a consequence, intrauterine insemination (IUI) has been developed as a treatment option for infertility (Goverde et al. 2000) and is indicated for oligoasthenoteratozoospermia (OAT) syndrome, ejaculation disorder, sexual dysfunction, cervical factor infertility, and infertility with unknown cause that does not lead to pregnancy even after timing therapy for a certain period (Luco et al. 2014). Several factors have been reported to affect the achievement of pregnancy by IUI, and various characteristics of semen can provide positive predictors (Ombelet et al. 2014). At present, most infertility treatment facilities in Japan collect semen for intrauterine insemination early in the morning. However, as sexual intercourse most frequently occurs in the evening or at night, this collection time may not be appropriate. In this study, we carried out semen collections early in the morning and in the evening.

Various semen characteristics were compered to determine whether the two collections showed significant differences.

Results and Discussion

Table 1 shows the luteinizing hormone values, follicle stimulating hormone values, total testosterone values, and prevalence of varicocele in the male infertility group. We assessed the hormonal assays using Electrochemiluminescence immunoassay. Varicocele was assessed by physical examination and color doppler ultrasonography. The semen characteristics of the fertile volunteers are shown in Table 2. In these men, the total sperm count (TSC)s of the morning and evening groups were 140.2 (65.8-252.5) × 10^6 and 266.1 (148.1-408.6) × 10^6, respectively (p = 0.035); the total motile sperm count (TMSC)s of the two groups were 140.2 (65.8-252.5) × 10^6 and 266.1 (148.1-408.6) × 10^6, respectively (p = 0.024). Thus, both TSC and TMSC were significantly higher in the evening group than the morning group. The semen characteristics of the infertile male patients are shown in Table 3. No significant difference was found for TSC between the morning and evening groups. The TMSCs of the morning and evening groups in infertile men were 4.9 (2.2-21.9) × 10^6 and 44.3

(6.5-66.5) × 10^6, respectively ($p = 0.047$). Thus, TMSC was significantly higher in the evening group than the morning group.

IUI is widely used as a fertility treatment (Ombelet and Van Robays 2015). As described earlier, IUI is the first-line treatment for a range of male-derived infertility conditions; however, IUI is an invasive and expensive form of assisted reproduction (Buckett and Sierra 2019). In general, infertility treatment facilities in Japan tend to use semen collected early in the morning for IUI. This is simply because the male partner may not be able to attend the medical examination due to work commitments; he produces an ejaculate before going to work and his partner visits hospital alone in many cases. By contrast, sexual intercourse generally occurs in the evening or night (Nagao 2007). This raises the possibility that IUI might provide better results if semen collected in the evening was used.

The results of the present study showed that TMSC was higher in semen collected in the evening than the morning in both fertile volunteers and male infertile patients. The

purpose of IUI is to increase gamete density at the fertilization site, and there are some reports that increased rates of pregnancy are achieved with higher TMSCs (Ombelet et al. 2014; Dong et al. 2011; Hajder et al.2016; Gubert et al. 2019) . This suggests that using semen collected in the evening for IUI might be more advantageous for achieving pregnancy than semen collected in the early morning.

In 1999, Cagnacci *et al*. investigated diurnal changes in semen characteristics by collecting semen at 7 a.m. and 5 p.m. from 54 male patients visiting a fertility clinic. They reported that both sperm concentration and sperm motility were significantly higher in semen collected in the evening than in the morning (Cagnacci et al. 1999) . In the present study, there was no significant difference in sperm concentration and sperm motility between morning and evening collections from infertile men, but TMSC was significantly higher in the evening collection group. Although they are not completely consistent, both studies agree that evening-collected semen is better than morning-collected semen. Cagnacci *et al*. did not evaluate a normal male group with proven

fertility; here, we showed that TMSC was significantly higher in the evening collection group than the morning collection group in fertile males.

Spermatogenesis involves a complicated process of cell differentiation from spermatogonia to sperm in the seminiferous tubules (Neto et al. 2016) ; the sperm then mature in the epididymis and are ejaculated (Sullivan and Mieusset 2016; Zhou et al. 2019). It is difficult to suggest potential causes of the diurnal variation between morning and evening ejaculates given the lengthy process of differentiation and development undergone during spermatogenesis. Possible factors may be the environment during ejaculation and variation in the neuromuscular mechanism that triggers ejaculation (Setchell 2006). Furthermore, it is possible that the characteristics of the semen might improve if ejaculation takes a longer time (Elzanaty 2008). In the case of early morning collections, time pressures may encourage rapid ejaculation. The causes of the differences between morning and evening ejaculates require further investigation (Elzanaty and Malm 2008).

There are several limitations in this study. First, the number of men involved in the study was comparatively low; therefore, the conclusions will need to be confirmed by larger sample sizes. Next is the problem of semen collection time. We presume that sexual intercourse most frequently occurs at night rather than in the early evening. Therefore, it would be more appropriate to compare semen collected at night with that collected early in the morning, rather than semen collected in the early evening. However, in the present study, semen collection in the early evening was selected for reasons of convenience in the facility where the study was conducted. It is possible, that if semen were collected at night rather than the early evening, different results would have been obtained. In addition, this study only considered sperm characteristics. As the purpose of assisted reproduction is achieving pregnancy, then our study needs to be extended to determine whether fertilization rates vary between morning and evening sperm collections; a different study design would be required to examine this aspect. Although there are limitations to our study, our findings nevertheless add to the limited

data on diurnal variations in semen quality in the same person. Our analyses indicate that the TMSC of semen collected in the evening is higher than that of semen collected early in the morning. Therefore, we suggest that for IUI, pregnancy might be more easily achieved using semen collected in the evening compared to in the early morning.

Materials and Method

This study was approved by the Institutional Review Board at Dokkyo Medical University Saitama Medical Center. All subjects provided written informed consent. Twenty healthy adult males, who had fathered a child by natural pregnancy (average age 35.2 ± 4.16 years), and 20 new male infertile patients with abnormal seminograms at the Reproduction Center of the Dokkyo Medical University Saitama Medical Center between April 2017 and June 2017 (average age 35.5 ± 5.05 years), were included in the study. All partners of 20 infertile male patients had undergone gynecologic check-ups and had no major problems affecting fertility. Azoospermic patients were excluded from this study. A sexual abstinence period of 3 days before semen collection was set for the men who participated in the study. Semen was collected in the early morning (7 a.m., morning collection group) at the hospital. In another day, semen was collected in the evening (5 p.m., evening collection group) after three days of abstinence period. As the primary outcome measure, TMSC was assessed, and the following semen

characteristics were assessed as secondary outcome measures: semen volume, sperm concentration, sperm motility rate, and TSC. Semen analyses were performed according to the WHO2010 manual (WHO 2010). After ejaculation, the semen was liquefied for 30 minutes and semen motility was then measured using CASA CEROS, a sperm motility analysis system (Lammers et al. 2014). The results from the semen analyses were compared between the morning and evening collection groups using a Wilcoxon signed rank test. *P* values <0.05 were considered statistically significant. Statistical analysis was performed using the software package SPSS, version 18.0 (IBM, Armonk, NY, USA).

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Disclosure of interest

The authors report no conflict of interest.

Author contributions

Conceived and designed the experiments: Y. S., H.O.

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Contributed reagents/materials/analysis tools: Y. S., T. I.,

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