Influence of the Period of Abstinence on Semen Quality in a Patient with Systemic Lupus Erythematosus: A Case Report and Review of the Literature

Tarah H. B. Waters, Carol Pui Shan Chan, Tin Chiu Li, and David Yiu Leung Chan

Assisted Reproductive Technology Unit, Department of Obstetrics and Gynecology, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong, China

Correspondence should be addressed to David Yiu Leung Chan; drdcyl16@cuhk.edu.hk

Received 20 March 2019; Revised 6 January 2020; Accepted 23 January 2020; Published 9 March 2020

Academic Editor: Gregory J. Tsay

Copyright © 2020 Tarah H. B. Waters et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Systemic lupus erythematosus (SLE) is a chronic inflammatory disease that can affect fertility. There is currently little information regarding the semen profile of males with SLE. Moreover, there is no consensus on an appropriate period of sexual abstinence for semen analysis and on the use of DNA fragmentation assay, together with multiple semen analyses to document the semen profile in this clinical population. In this case report, multiple semen analyses, with DNA fragmentation assays, were performed for a male with SLE undergoing fertility treatment at our andrology unit. A 4-day period of abstinence improved the semen concentration, total sperm count, total progressive motile sperm, and sperm morphology, with minimal DNA fragmentation. In conclusion, multiple semen analyses obtained after different periods of sexual abstinence, together with DNA fragmentation assays, may be useful to develop a semen profile for patients with SLE, providing information on the optimal abstinence period to yield the best semen quality for subsequent fertility treatment. For patients with fluctuating semen results, concomitant semen cryopreservation should be considered to preserve the better quality semen before starting assisted reproductive technologies if pregnancy is planned in the future.

1. Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune disorder that causes systemic inflammation, with a female-to-male incidence ratio of 9:1 [1]. The exact etiology of SLE remains unknown; however, common pathologies associated with SLE include inflammation, vasculitis, immune complex complement deposition, and vasculopathy [2]. The impact of SLE on male fertility has received little attention, with no study to date having used consecutive multiple semen analyses together with DNA fragmentation assays to assess sperm quality specifically for patients with SLE. This is an important issue as current therapies for SLE have been shown to reduce fertility in both males and females, particularly the use of cyclophosphamide (CY) which is associated with gonadal toxicity [3, 4]. CY suppresses spermatogenesis, which often results in abnormal semen parameters and testicular function that may or may not recover depending on the dose and duration of treatment [5]. Therefore, there is a risk for permanent azoospermia from exposure to CY. A meta-analysis of 30 studies indicated a dose-related effect of CY on the rate of gonadal failure in adult males, with a rate of 20% for a CY dose of 100–200 mg/kg (equivalent to 7.5–15 g in a 75 kg male) compared with an almost 100% failure rate with a dose > 400 mg/kg (equivalent to > 30 g in a 75 kg male) [6]. Of note, effects of CY exposure on gonadal failure were sustained for several years after treatment [6].

A study of 35 postpubertal males with SLE revealed abnormal semen analyses and reduced testicular volume in all patients, compared with healthy controls [7]. It was suggested that SLE disease activity causes damage to the
seminiferous tubules, resulting in a significant reduction in testicular volume, made worse by treatment using intravenous CY. Another study also reported abnormal semen analyses among 4 men with SLE undergoing prednisone and/or immunosuppressive therapy who had normal erectile function, ultrasound examination of the testicles, and libido [8]. There is also evidence of an association between SLE, and its treatments, and hypothalamic-pituitary-axis dysfunction and increased gonadotrophin levels [9, 10].

Diagnosis of male infertility relies on semen analysis, based on the reference limits proposed by the World Health Organization (WHO, 5th edition, 2010). According to current guidelines, semen samples for analysis should be collected after a minimum of 2 days and a maximum of 7 days of sexual abstinence. Moreover, the quality of semen cannot be reliably evaluated from a single semen sample, with 2 to 3 samples being required to obtain accurate baseline data. However, to date, there is no clear guideline regarding the effect of the period of sexual abstinence on the quality of semen and DNA fragmentation in males with SLE.

One available study investigating the effect of abstinence duration on semen quality among normozoospermic men undergoing infertility testing (n = 16; 11 of whom strictly complied with abstinence requirements) found that abstinence did not influence the pH, viability, morphology, or motility of the sperm [11]. However, a longer period of abstinence did improve sperm concentration and semen volume. Specifically, a 24-hour period of abstinence significantly increased the proportion of the normospermic sperm having immature chromatid, but with no effect on DNA fragmentation. No such information for males with SLE was identified.

DNA damage in sperm has been shown to reduce fertilization rates, embryo development, and pregnancy outcomes in a general population [12, 13]. Although diagnostic testing of sperm DNA fragmentation is not yet routine in most fertility clinics, there is increasing evidence that it is a valuable diagnostic tool [14]. Various optimal cutoff points for the DNA fragmentation index (DFI) have been proposed. Two studies in particular used the Halosperm G2® Sperm Chromatin Dispersion (SCD) testing kit (Halotech®, Madrid, Spain), which we used in this case report [15, 16]. Optimal DFI cutoff values of 26.1% [15] and 25.5% [16] were suggested to differentiate fertile and infertile males or for testing only males seeking fertility treatment, respectively. The impact of DFI on pregnancy outcomes was also evaluated in these studies.

Cytotoxic treatments, such as CY, are known to cause DNA damage due to their alkylating effects on the germinial epithelium [17, 18]. A recent study in mice found that CY-exposed sperm (exposure dose, 300 mg/kg for 7 days) used for in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) (22 cycles producing a total of 1212 embryos) negatively affected in vitro embryo development and the subsequent live birth rate, compared with the nonexposed sperm [19]. Although the mouse model is not completely translatable to humans, we are lacking direct data from human studies. For those patients who require semen cryopreservation after CY treatment, it may be necessary to utilize sperms that have potentially been damaged by CY, and, therefore, patients must be counselled appropriately regarding this risk. The WHO 2010 guideline recommends an abstinence period of 2–7 days before semen collection [11, 20] unless the epididymal function is abnormal [21]. However, there are studies that have suggested that a shorter abstinence time may improve sperm quality. One study [22], based on retrospective analysis of 9,489 semen samples, recommended an abstinence period for oligozoospermic males (non-SLE) of just 1 day, and not exceeding 10 days, due to an observed decrease in sperm motility and normal morphology. Abstinence beyond 4 days was associated with deterioration in sperm morphology. It also appears that males with reduced semen parameters may benefit from a slightly shorter abstinence period compared with normozoospermic males [22]. In fact, another study suggested that an abstinence period of 1-2 days, which is shorter than the WHO recommendation, is optimal for sperm quality and reduced DNA fragmentation [23]; however, only normozoospermic males were included in this study.

Bahadur et al. [24] reported markedly improved semen parameters (such as progressive motility, morphology, and sperm concentration) in oligozoospermic males (n = 73) after a short abstinence time (consecutive sample produced up to 40 minutes after the first). Effects on DNA fragmentation from the short abstinence times were not recorded, and there are few studies that have investigated the impact of a shorter period of abstinence on DNA fragmentation to benefit subfertile males. One study, however, did find that a short abstinence period (1 day) could reduce DNA fragmentation in a select group of subfertile patients. Specifically, in 35 males with increased sperm DNA fragmentation (≥30%), a 1-day abstinence protocol, with up to three ejaculation attempts, improved DNA fragmentation to normal levels in 90% of patients [25].

Current evidence regarding the optimal abstinence period is mixed, with no clear consensus to inform practice for the collection of sperm samples with higher quality in special groups of patient [26], such as males with SLE. While obtaining a semen profile with multiple semen samples may not be essential for normozoospermic males, it may be valuable for patients with SLE in whom cytotoxic treatment may lead to poor semen quality and the potential for DNA damage.

The potential impact of SLE and its associated treatment on male fertility is of major concern to young males who develop the condition, as their future fertility options may become limited. At the point of fertility assessment, a semen profile may improve the quality of sperm available for subsequent assisted reproductive technology (ART) treatment. This could be especially important as SLE treatment is likely to be ongoing, and, as such, the reduction in semen quality may persist. In this case report, we describe our approach to developing a patient-specific semen profile for a male with SLE and evaluate the effect of different periods of abstinence, over a 1-month period, on the semen quality. Based on our experience, we propose that a 4-day period of abstinence, combined multiple sample analysis, and DNA fragmentation may be clinically relevant for these patients.
2. Case Presentation

This male patient first received a probable diagnosis of SLE at a private hospital in 2006, at the age of 22 years, with the diagnosis confirmed 1-year later at Prince of Wales Hospital, Shatin, Hong Kong. The diagnosis of SLE was based on the presence of the following: alopecia, face rash, polyarthritis, lymphopenia, and an antinuclear antibody test titer of 1:640. He had a positive family history for SLE, with two paternal cousins (female) having a diagnosis of SLE. The patient had previously used ketamine, until 2010, and gave up smoking and drinking in 2014 and 2013, respectively. He had previously fathered one child, a daughter who was 8 years old, prior to starting CY treatment to manage his SLE-related symptoms.

The patient was referred to us by the IVF unit for fertility investigation in February, 2017. The patient was 33-years-old at the time of semen analysis. Data regarding the size of testes were not available as this is not a component of the routine examination in our fertility unit. Of note, the patient was fertile in spite of his early SLE diagnosis at the age of 22 years, having naturally conceived a child at the age of 25 years. He had since become infertile. In more recent years, as his SLE condition worsened, treatment with monthly intravenous (IV) CY was initiated, as follows: 6 monthly doses of 1 g in 2014 and 3 monthly doses of 1 g in 2016. Since that time, treatment with CY had been intermittent, with the last dose received in April 2016. At the time of semen analysis for this case report, the patient was being treated with corticosteroid (prednisolone) and immunosuppressant drugs, mycophenolate mofetil (MMF), and tacrolimus (TAC).

For semen analysis, the patient submitted three samples over a 1-month period, with analyses performed by an andrologist who had fulfilled the external quality assessment criteria set by the United Kingdom National External Quality Assessment Service (UK NEQAS). The samples were collected after 2, 4, and 7 days of sexual abstinence, determined as per the patient’s convenience over a 1-month period. We do note that the patient had no change in his health status in the index month. Semen analysis was performed manually according to the WHO 5th edition recommendations, 2010 [27], and following the advised checklist on “How to count sperm properly” published by Björndahl et al. [28]. In brief, semen collection was obtained at the clinic by masturbation, and, after liquefaction at 37°C, samples were assessed within 1 h of ejaculation. Sperm concentration and motility were assessed manually under a phase contrast microscope (Olympus BX43, Japan) at a total magnification of either ×200 or ×400. Hemocytometers, with improved Neubauer ruling, were used for the measurement of sperm concentration. To assess sperm morphology (strict Tygerber criteria), sample slides were stained with a Diff-Quik staining kit (Dade Behring AG, Switzerland) and observed under an oil immersion microscope (×100 total magnification). At least 200 sperms were counted in duplicate for each assessment.

DNA fragmentation was measured using a Halosperm G2® Sperm Chromatin Dispersion (SCD) testing kit (Halotech®, Madrid, Spain), according to the manufacturer’s instructions. The detection method is based on the sperm chromatin dispersion (SCD). In brief, sperm with intact DNA will appear as a halo ring around the sperm head; different degrees of DNA fragmentation will decrease the size of the halo ring. Hydrogen peroxide- (\(H_2O_2\)) induced DNA fragmentation is used as a positive control resulted in the negative halo ring, while a negative control, by omitting denaturing solution, resulted in sperm cells with the halo ring. A minimum of 300 sperms were scored per sample across two replicates, and the number of sperms with fragmented DNA (i.e., sperm without a halo or with a small or degraded halo) was represented as a percentage of the whole sample. The halo assays were performed within 1 h after the samples were submitted.

All three samples included in the analysis had normal physical properties, with the exception that the volume was below the WHO reference value of 1.5 ml. The volume did increase from 0.5 ml, 0.7 ml, and 1.0 ml with an abstinence period of 2, 4, and 7 days, respectively. The percentage of viable sperm, however, decreased as a function of increased period of abstinence from 84%, 63%, and 33% at 2, 4, and 7 days of abstinence. All samples were below the WHO reference values for motility, morphology, and total sperm count (Table 1). With a 2-day period of abstinence, the proportion of motile sperm was 28%, with the proportion decreasing to 18% and 15% for an abstinence period of 4 and 7 days, respectively. Only the sample obtained after an abstinence of 4 days had a satisfactory sperm concentration (37.3 M/ml), with the concentration after a 2- and 7-day period of abstinence being below the WHO reference value of 15 M/ml. Interestingly, the concentration gradually increased from 9.8 M/ml after a 2-day abstinence to a maximum of 37.3 M/ml after a 4-day abstinence, subsequently decreasing 18.8 M/ml after a 7-day abstinence. Normal sperm morphology also improved after a 4-day abstinence, but decreased after a 7-day abstinence, along with decreases in semen volume and concentration and total sperm count. In addition, the level of sperm DNA fragmentation was elevated after a 2-day abstinence (55.3%), reaching its lowest level after 4 days (34.7%) and then began to rise again after 7 days (38.3%). Considering our results, overall, a 4-day period of abstinence yielded the best semen quality, compared with an abstinence period of 2- and 7-days.

3. Discussion

It is generally accepted that an increase of abstinence time can improve total motile sperm count and viability and increase the level in DNA fragmentation [23, 29, 30]. However, the situation may vary in special patient groups, such as those with SLE, due to previous gonadotoxic treatment. Since DNA fragmentation assay is not routinely performed in fertility clinics, this is the first report to use multiple semen analyses, with different lengths of abstinence, together with DNA fragmentation assay to demonstrate the relationship between the period of abstinence and the level of DNA fragmentation in the semen of a patient with SLE. For this patient, a 7-day period of abstinence was associated with decreased sperm motility and slightly
increased DNA fragmentation, compared with a 2- and 4-day period of abstinence. To our surprise, the shortest (2 days) abstinence time produced a sample of increased sperm motility, but with a marked increase in the level of DNA fragmentation. The current recommendation by the WHO (2010) is for semen samples to be collected after an abstinence period of between 2 and 7 days. However, in the literature, there appears to be no guideline to suggest the optimum period of abstinence in relation to semen parameters and DNA fragmentation level for patients with SLE or in those who have had prior exposure to gonadotoxic treatment. A very recent study by Tiseo et al. [31] revealed the DNA fragmentation level to be significantly higher in nonazoospermic patients with SLE when compared with a non-SLE control group. However, the period of abstinence in this study was not mentioned. In our case report, we identify that a 4-day period of abstinence, in our patient with SLE, yielded more balanced semen parameters, in terms of the level of DNA fragmentation, sperm motility, sperm concentration, and sperm morphology, with these parameters having the most influence on fertility potential. However, we do note that our case is not representative of the whole SLE population. Accordingly, we propose that a cohort study of male SLE patients would be warranted to confirm our findings. As well, semen profiles in patients with SLE would be more accurately developed from a series of semen analyses performed after different periods of abstinence and should include DNA fragmentation assay to obtain a better quality of available semen for patients with SLE undergoing subfertility treatment. Semen preservation may be applied in parallel with semen analyses in order to save the best quality sample among the multiple samples for future ART intervention.

### Table 1: Summary of three semen analysis reports of an SLE male from semen samples submitted after 2, 4, and 7 days of sexual abstinence.

| Sex parameter | 2 days | 4 days | 7 days | WHO (2010) lower reference limits |
|---------------|--------|--------|--------|----------------------------------|
| Concentration (×10⁶/ml) | 9.8 | 37.3 | 18.8 | 15 |
| Total sperm number in ejaculate (×10⁶) | 4.9 | 26.1 | 18.8 | 39 |
| Total progressive motile sperm in ejaculate (×10⁶) | 1.0 | 2.6 | 1.7 | — |
| Normal morphology (%) | 1.0 | 2.7 | 2.0 | 4 |

### Motility assessment

| Parameter | 2 days | 4 days | 7 days | WHO (2010) lower reference limits |
|-----------|--------|--------|--------|----------------------------------|
| Total motility (%) | 28 | 18 | 15 | 40 |
| Progressive motility (%) | 21 | 10 | 9 | 32 |
| Nonprogressive motility (%) | 7 | 8 | 6 | — |
| Immotility (%) | 72 | 82 | 85 | — |

### Macroscopic examination

| Parameter | 2 days | 4 days | 7 days |
|-----------|--------|--------|--------|
| Volume (ml) | 0.5 | 0.7 | 1.0 |
| Liquefaction (within 60 min) | Complete | Complete | Complete |
| pH | 8.0 | 8.0 | 8.0 |
| Colour | Normal | Normal | Normal |
| Viscosity | Slightly viscous | Slightly viscous | Slightly viscous |
| Gel clumps | None | None | None |

### Microscopic investigation

| Parameter | 2 days | 4 days | 7 days |
|-----------|--------|--------|--------|
| Debris | None | None | None |
| Round cell | None | None | None |
| Red cell | None | None | None |
| Nonspecific aggregation | None | None | None |
| Agglutination of sperm | None | None | None |
| Sperm vitality | Viable sperm (%) | 84 | 63 | 33 |
| DNA fragmentation | Average DNA fragmentation (%) | 55.3 | 34.7 | 38.3 |

*Total motility = progressive + nonprogressive motility.

### 4. Conclusions

A more rigorous study of the semen of patients with SLE is needed to confirm the best abstinence time. For male patients with SLE who are seeking ART treatment, it may be beneficial to develop a semen profile by obtaining multiple semen samples combining DNA fragmentation assay from the patient in order to increase the availability and quality of sperm. Cryopreservation of semen prior to SLE treatment is recommended as there is a risk of fertility impairment from current therapies.

### Abbreviations

| Acronym | Description |
|---------|-------------|
| SLE | Systemic lupus erythematosus |
| CY | Cyclophosphamide |
| ART | Assisted reproductive technology |
| WHO | World Health Organization |
**DFI:** DNA fragmentation index
**SCD:** Sperm chromatin dispersion
**IVMP:** Intravenous methylprednisolone
**IVCY:** Intravenous cyclophosphamide
**UKNEQAS:** UK National External Assessment Service.

**Ethical Approval**

This study was approved by the Joint Chinese University of Hong Kong-New Territories East Cluster Clinical Research Ethics Committee (CREC no. 2016499).

**Consent**

Written informed consent was obtained from the patient for publication of this case report and any accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal.

**Conflicts of Interest**

The authors declare that they have no conflicts of interest.

**Authors’ Contributions**

TW analyzed the data obtained from semen analyses and wrote the manuscript. CC performed all semen analyses and testing in the andrology laboratory. TC and DC supervised the study and contributed to the writing of the manuscript. All authors read and approved the final manuscript.

**Acknowledgments**

The authors specially thank for Ms Cosy Cheung Wing Ching in recruiting the SLE patient in this report and the patient for the donation of semen samples.

**References**

[1] R. W. McMurray and W. May, “Sex hormones and systemic lupus erythematosus: review and meta-analysis,” *Arthritis & Rheumatism*, vol. 48, no. 8, pp. 2100–2110, 2003.

[2] C. C. Mok and C. S. Lau, “Pathogenesis of systemic lupus erythematosus,” *Journal of Clinical Pathology*, vol. 56, no. 7, pp. 481–490, 2003.

[3] R. A. Hickman and C. Gordon, “Causes and management of infertility in systemic lupus erythematosus,” *Rheumatology*, vol. 50, no. 9, pp. 1551–1558, 2011.

[4] J. F. Wetzelz, “Cyclophosphamide-induced gonadal toxicity: a treatment dilemma in patients with lupus nephritis?” *The Netherlands Journal of Medicine*, vol. 62, no. 10, pp. 347–352, 2004.

[5] J. Mersereau and M. A. Dooley, “Gonadal failure with cyclophosphamide therapy for lupus nephritis: advances in fertility preservation,” *Rheumatic Disease Clinics of North America*, vol. 36, no. 1, pp. 99–108, 2010.

[6] S. A. Rivkees and J. D. Crawford, “The relationship of gonadal activity and chemotherapy-induced gonadal damage,” *JAMA: The Journal of the American Medical Association*, vol. 259, no. 14, pp. 2123–2125, 1988.

[7] P. M. F. Soares, E. F. Borba, E. Bonfa, J. Hallak, A. L. Corrêa, and C. A. A. Silva, “Gonad evaluation in male systemic lupus erythematosus,” *Arthritis & Rheumatism*, vol. 56, no. 7, pp. 2352–2361, 2007.

[8] C. A. Silva, J. Hallak, F. F. Pasqualotto, M. F. Barba, M. I. Saito, and M. H. Kiss, “Gonadal function in male adolescents and young males with juvenile onset systemic lupus erythematosus,” *The Journal of Rheumatology*, vol. 29, no. 9, pp. 2000–2005, 2002.

[9] C. A. A. Silva and H. I. Brunner, “Review: gonadal functioning and preservation of reproductive fitness with juvenile systemic lupus erythematosus,” *Lupus*, vol. 16, no. 8, pp. 593–599, 2007.

[10] R. M. Suehiro, E. F. Borba, E. Bonfa et al., “Testicular sertoli cell function in male systemic lupus erythematosus,” *Rheumatology*, vol. 47, no. 11, pp. 1692–1697, 2008.

[11] C. De Jonge, M. LaFromboise, E. Bosmans, W. Omolet, A. Cox, and M. Nijs, “Influence of the abstinence period on human sperm quality,” *Fertility and Sterility*, vol. 82, no. 1, pp. 57–65, 2004.

[12] G. López, R. Lafuente, M. A. Checa, R. Carreras, and M. Brassesco, “Diagnostic value of sperm DNA fragmentation and sperm high-magnification for predicting outcome of assisted reproduction treatment,” *Asian Journal of Andrology*, vol. 15, no. 6, pp. 790–794, 2013.

[13] B. Wiweko and P. Utami, “Predictive value of sperm deoxyribonucleic acid (DNA) fragmentation index in male infertility,” *Basic and Clinical Andrology*, vol. 27, no. 1, p. 1, 2017.

[14] S. E. M. Lewis, “Should sperm DNA fragmentation testing be included in the male infertility work-up?” *Reproductive BioMedicine Online*, vol. 31, no. 2, pp. 134–137, 2015.

[15] S. E. M. Lewis, R. John Atikken, S. J. Conner et al., “The impact of sperm DNA damage in assisted conception and beyond: recent advances in diagnosis and treatment,” *Reproductive BioMedicine Online*, vol. 27, no. 4, pp. 325–337, 2013.

[16] L. Simon, K. Murphy, M. B. Shamsi et al., “Paternal influence of sperm DNA integrity on early embryonic development,” *Human Reproduction*, vol. 29, no. 11, pp. 2402–2412, 2014.

[17] I. D. Morris, “Sperm DNA damage and cancer treatment,” *International Journal of Andrology*, vol. 25, no. 5, pp. 255–261, 2002.

[18] D. Sakkas and J. G. Alvarez, “Sperm DNA fragmentation: mechanisms of origin, impact on reproductive outcome, and analysis,” *Fertility and Sterility*, vol. 93, no. 4, pp. 1027–1036, 2010.

[19] M. D. Johnson, C.-C. Lin, M. Sukhwani, K. Peters, S. Malik, and K. E. Orwig, “In Vitro fertilization (IVF) intracytoplasmic sperm injection (ICSI) using sperm exposed to cyclophosphamide reduces preimplantation embryo development and live birth after embryo transfer (ET),” *Fertility and Sterility*, vol. 103, no. 2, pp. e4–e5, 2015.

[20] J. P. Tyler, N. G. Crockett, and G. L. Driscoll, “Studies of human seminal parameters with frequent ejaculation II. Semen analysis,” *Human Reproduction*, vol. 599, 2007.

[21] J. M. Johnson, C.-C. Lin, M. Sukhwani, K. Peters, S. Malik, and K. E. Orwig, “In Vitro fertilization (IVF) intracytoplasmic sperm injection (ICSI) using sperm exposed to cyclophosphamide reduces preimplantation embryo development and live birth after embryo transfer (ET),” *Fertility and Sterility*, vol. 81, no. 4, pp. 1148–1150, 2004.

[22] E. Levitas, E. Lunenfeld, N. Weiss et al., “Relationship between the duration of sexual abstinence and semen quality: analysis of 9,489 semen samples,” *Fertility and Sterility*, vol. 83, no. 6, pp. 1680–1686, 2005.
[23] A. Agarwal, S. Gupta, S. Du Plessis et al., “Abstinence time and its impact on basic and advanced semen parameters,” *Urology*, vol. 94, pp. 102–110, 2016.

[24] G. Bahadur, O. Almossawi, R. Zeirideen Zaid et al., “Semen characteristics in consecutive ejaculates with short abstinence in subfertile males,” *Reproductive BioMedicine Online*, vol. 32, no. 3, pp. 323–328, 2016.

[25] I. Pons, R. Cercas, C. Villas, C. Brana, and S. Fernandez-Shaw, “One abstinence day decreases sperm DNA fragmentation in 90% of selected patients,” *Journal of Assisted Reproduction and Genetics*, vol. 30, no. 9, pp. 1211–1218, 2013.

[26] B. M. Hanson, K. I. Aston, T. G. Jenkins, D. T. Carrell, and J. M. Hotaling, "The impact of ejaculatory abstinence on semen analysis parameters: a systematic review,” *Journal of Assisted Reproduction and Genetics*, vol. 35, no. 2, pp. 213–220, 2017.

[27] World Health Organization, *World Health Organization Laboratory Manual for the Examination and Processing of Human Semen*, World Health Organization, Geneva, Switzerland, 2010.

[28] L. Björndahl, C. L. R. Barratt, D. Mortimer, and P. Jouannet, “How to count sperm properly: checklist for acceptability of studies based on human semen analysis,” *Human Reproduction*, vol. 31, no. 2, pp. 227–232, 2016.

[29] J. Gosálvez, M. González-Martínez, C. López-Fernández, J. L. Fernández, and P. Sánchez-Martín, “Shorter abstinence decreases sperm deoxyribonucleic acid fragmentation in ejaculate,” *Fertility and Sterility*, vol. 96, no. 5, pp. 1083–1086, 2011.

[30] P. Sunanda, B. Panda, C. Dash, P. K. Ray, R. N. Padhy, and P. Routray, “Prevalence of abnormal spermatozoa in tobacco chewing sub-fertile males,” *Journal of Human Reproductive Sciences*, vol. 7, no. 2, pp. 136–142, 2014.

[31] B. Tiseo, E. Bonfá, E. Borba et al., “Complete urological evaluation including sperm DNA fragmentation in male systemic lupus erythematosus patients,” *Lupus*, vol. 28, no. 1, pp. 59–65, 2019.