J. Multidiscip. Res. Healthcare. Vol. 9, No. 2 (2023), pp.51–58

CHITKARA

Journal of Multidisciplinary Research in Healthcare

Journal homepage: https://jmrh.chitkara.edu.in/

Oligoasthenozoospermic and Normozoospermic Indian males yielded similar Fertilization capability by Intracytoplasmic Sperm Injection

Ram Dayal

Neelam Hospital, NH-07, Rajpura–Chandigarh Highway, Opposite Chitkara University, Punjab –140401, India

ramdayal2509@gmail.com

ARTICLE INFORMATION

Received: November 11, 2022
Revised: January 02, 2023
Accepted: March 07, 2023
Published Online: April 12, 2023

Keywords:
Capacitati, Intra-cytoplasmic sperm injection, sperm concentration, Sperm motility, Normozoospermia, Igoasthenozo ospermia

DOI: 10.15415/jmrh.2023.92005

ABSTRACT

Background: The present study evaluated the fertilization outcomes by intra-cytoplasmic sperm injection (ICSI) technique with oligoasthenozoospermic (OAZ) compared to normozoospermic (NZ) semen.

Purpose: To find the fertilizing capability of human sperm in oligoasthenozoospermic and normozoospermic semen samples.

Methods: Fresh semen ejaculates belonging to NZ and OAZ groups (n=50 in each group) patients attending the infertility clinics were evaluated for semen volume, sperm motility, and sperm concentration. Fertilization rates with ICSI along with post-capacitation motility and concentration were studied in both groups.

Results: The motility and concentration in fresh semen samples patients of the NZ group (32.44±1.50%, 71.96±6.45 M/ml, respectively) were significantly ($P<0.05$) higher compared to the OAZ group (26.76±1.38, 10.31±0.48 M/ml, respectively). However, the results of our study revealed similar fertilization rates in-vitro by ICSI in the NZ (98%) and OAZ (98%) groups.

Conclusion: The in-vitro processing by the ICSI technique is an effective approach to achieving good fertilization rates in sub-fertile males.

1. Introduction

Infertility is a condition in which a female is unable to conceive despite having unprotected timed sexual intercourse with her male partner for one year. Infertility has been recognized as a public health issue worldwide by the World Health Organization (Zegers-Hochschild *et al.*, 2009). One in seven couples is affected by infertility in urban India and approximately 13-19 million couples were expected to be infertile at a given point in time (Unisa, 2010). The causative factors related to male (male factor) are one of the major reasons for infertility in India and accounts for approximately 40-50% of total infertility problems (Kumar *et al.*, 2015). Although, the male factor in infertility cases is common, but yet it is poorly understood. Despite many defined causes, 50% of male infertility cases remain idiopathic, and low sperm concentration and or poor/abnormal sperm motility (oligoasthenozoospermia) remains the single most important etiology of male infertility (Jequier, 2004).

Infertility treatment of oligoasthenozoospermic patients mostly relies on the utilization of assisted reproductive technologies viz *in-vitro* fertilization (IVF) or intra-cytoplasmic sperm injection (ICSI) techniques. The application of ICSI in assisted reproduction has opened new avenues for the treatment of previously untreatable cases of female and male infertility. Poor sperm concentration and sperm motility in fresh semen ejaculates are the most common reasons for the rejection of potential semen donors (Sidhu *et al.*, 1997). However, the evaluation of sperm motility is the most important microscopic criterion to predict the fertility outcome with ICSI for *in-vitro* processed semen. The *in-vitro* processes involved during ICSI enable oligoasthenozoospermic semen to fertilize the oocyte. Hence, improvement in sperm motility ensures a better fertilizing capacity of semen. Keeping the above facts in view, the present study evaluated the effects of differential seminal variables in capacitated and uncapacitated semen of oligoasthenozoospermic males on the fertilization outcomes using ICSI.

2. Methodology

The study was conducted on patients enrolled for infertility treatment at various hospitals/clinics in North India during the period of June 2017 to April 2020.

ISSN No.: 2393-8536 (Print) ISSN No.: 2393-8544 (Online) Registration No. : CHAENG/2014/57978

2.1. Study Groups

The fresh semen ejaculates from male patients were collected and examined (as per the standard protocol of WHO, 2010) for seminal parameters viz. volume, motility, and concentration. On basis of the results, males with sperm concentration of >15 million/ml as well as sperm motility >32% (type A+B) of forward progressive (type A) and slow progressive (type B) were categorized as normozoospermic (NZ group, n=50) whereas those with sperm concentration of <15 million/ml and sperm motility of <32% of (type A+B) as oligoasthenozoospermic (OAZ group, n=50). Then the samples were processed by density gradient and swim-up method (Mortimer, 2000) to analyze the sperm motility and concentration after capacitation. Later, the post-capacitated semen was used for ICSI and fertilization rates were evaluated in the two groups.

2.2. Protocol for Down-regulation

The first and foremost procedure was the down-regulation of oocyte donors i.e. ovarian stimulation by exogenous hormones to increase the number of eggs (follicles) and control the time of ovulation (Macklon *et al.*, 2006). Briefly, the treatment plan included oral intake of birth control pills (Ovral L, ethinylestradiol (0.03mg) + levonorgestrel (0.15mg)) for two weeks to prevent the release of hormones that could stimulate natural ovulation. Two weeks after the start of the pills, GnRH agonist (leuprolide acetate or Lupron 0.3 ml/day OD subcutaneously) was given one day prior to ovum pick-up (OPU) to stimulate the growth of multiple follicles. During the stimulatory treatment, ultrasonography was done on alternate days to assess the follicular growth till the follicles became 'ready' (at least two follicles reach 17 mm in size) for trigger (10,000 IU of hCG intramuscularly) to final maturation. This allowed the final maturation of follicles (18 mm size) and was then aspirated within 35-38 hours of the post-hCG administration.

2.3. Ovum pick-up and Grading of Oocytes

Patients were allowed to lie down in a supine position under general anesthesia. Both ovaries were scanned to determine the number and size of follicles. Further, the cleaning of the vagina and the perineal area was done with normal saline. Then, the trans-vaginal probe assembly was prepared and the probe was inserted into the vagina, scanning was focused over the ovary and then the OPU needle was advanced into the follicle along with the application of the negative pressure (120 mm to 140 mm of Hg), and rotation of the needle to aspirate all the content. The collapsing of each follicle was observed to confirm the complete aspiration. Thereafter, the collection line was rinsed with the human tubal fluid medium (HTF) to prevent any clogging of the assembly by oocyte cumulus complex (OCC) /oocyte (s). The collection tube was maintained at 37^0C and immediately passed through the pass box/window for searching the oocyte from the follicular fluid. Oocytes of good quality were equilibrated for about 2h in a continuous single culture (CSC) medium in a CO_2 incubator (at 37^0C, 6% CO_2, and 95% relative humidity). Meanwhile, during the oocyte equilibration, the semen samples were prepared for ICSI procedures.

2.4. Semen Processing

Freshly collected semen ejaculates were evaluated for volume and sperm concentration and sperm motility after complete liquefaction (using Counting Chamber Makler, WHO, 2010). Semen samples were prepared for ICSI procedures with density gradient and swim-up as described (Agarwal *et al.*, 2016). In brief, liquefied semen samples were gently laid over density gradient media (in a 15.0 ml conical tube sterile) and centrifuged for 12 minutes at 1500 rpm. After aspirating the supernatant, the pellet was transferred into a new centrifuge tube with 4.0 ml washing media (HTF) and then centrifuged again at 1500 rpm for five minutes. The supernatant was then discarded to obtain pellets. The pellets were overlaid with 0.5 ml of CSC medium and the tubes were kept in a CO_2 incubator (6%) for 5-10 minutes in a slanting position (at 45^0 angle) for swim-up of motile spermatozoa. Then 5.0 µl of each swim-up sample was evaluated for sperm concentration and sperm motility by using Counting Chamber Makler (Sefi Medical Instruments, Israel) and phase contrast microscopy (20x objective) (Makler *et al.*, 1980; Sukcharoen *et al.*,1994).

2.5. Denudation of Oocyte for ICSI and Fertilization Rates

Denudation of oocytes was undertaken in hyaluronidase enzyme (Mahadevan, 2021) using 200 µl pipette tip, followed by 170 µm, 140 µm denuding pipettes with concentrated hyaluronidase (80 IU/ml) within a time span of 40 seconds or with 1:1 diluted hyaluronidase (40 IU/ml) within a time span of 80 seconds. Denuded oocytes were washed three times in HTF media followed by three items of washing with CSC media and subsequently transferred into the micro-culture dish to maintain 6% CO_2 at 37^0C for equilibration and reducing stress. After that ICSI dish was made and <1.0 µl of swim-up sperm suspension was added in one drop of 7% polyvinylpyrrolidone (PVP) and sperm crushing was performed by micro-injection needle and selection of

sperm was done (to check sperm defects). Thereafter the ICSI was performed as per the standard protocol (Palermo *et al.,* 1995). After 16 to 18 hours of ICSI procedure, fertilization was checked under an inverted microscope (20 x or 40 x objective), and two polar bodies (in the perivitelline space) and twopro-nuclei (in the center of cytoplasm and equal distribution of nucleoli, one from sperm cell and one from oocyte) as an indicator of the fertilization. The chemicals used in this study were from Irvine Scientific (FUJIFILM, USA) and plastic wares from FALCON® unless otherwise indicated.

2.6. Statistical Analysis

The statistical analyses of the experimental data were carried out using IBM SPSS Statistics 24.0 (SPSS Inc., Chicago, IL, USA). The semen characteristics (semen volume, sperm concentration sperm motility, fertilization rate, etc.) were compared between the groups using an independent t-test. The semen parameters within each group were compared using the paired sample t-test. P-values <0.05 were considered to be statistically significant.

3. Results

The study was conducted on pre- and post-capacitated semen characteristics and fertilization outcomes of oligoasthenozoospermic *vs* normozoospermic patients (n=50 each) treated for infertility by ICSI. The present study evaluated different semen characteristics *viz.* volume, motility, concentration in fresh ejaculates, and motility and fertilization rates in post-capacitation semen samples of two groups i.e. normozoospermic (NZ) and oligoasthenozoospermic (OAZ) males attending the infertility clinics. The results (Mean±SEM) and their comparative analysis in the two experimental groups are presented below.

3.1. Fresh Semen Characteristics

Different seminal parameters in fresh semen ejaculates of normozoospermic (NZ group) and oligoasthenozoospermic (OAZ group) infertile patients are depicted in Table 1. The sperm concentration, as well as sperm motility, were significantly (P<0.05) higher in the NZ group compared to OAZ patients. However, the semen volume was similar (P=0.079) among the two groups.

Table 1: Seminal characteristics of fresh semen ejaculates in normozoospermic and oligoasthenozoospermic infertile patients

Semen characteristics (Mean±SEM)	**Normozoospermic (NZ) Group** (n=50)	**Oligoasthenozoosprmic (OAZ) Group** (n=50)	**P-value**
Semen volume (ml)	2.14±0.16	2.64±0.23	0.079
Sperm concentration (millions/ml)	71.96±6.45^{b}	10.31±0.48^{a}	0.001
Sperm motility (%)	32.44±1.50^{b}	26.76±1.38^{a}	0.01

Values with different superscripts (a, b) within a row differ significantly (P<0.05).

3.2. Post-capacitation Semen Characteristics

Evaluation of the *in-vitro* post-capacitated semen parameters of normozoospermic and oligoasthenozoospermic groups are presented in Table 2. The sperm motility after capacitation was statistically similar among the two groups. However, the post-capacitated sperm concentration was significantly (P<0.01) greater in patients belonging to the NZ group compared to the OAZ group.

Table 2: Seminal characteristics of post-capacitation semen samples of normozoospermic and oligoasthenozoospermic patients

Semen characteristics (Mean±SEM)	**Normozoospermic (NZ) Group** (n=50)	**Oligoasthenozoospermic (OAZ) Group** (n=50)	**P-value**
Sperm motility (%)	93.00±0.86	90.10±1.15	0.05
Sperm concentration (millions/ml)	66.66±5.32^{b}	21.20±1.38^{a}	0.001

Values with different superscripts (a, b) within a row differ significantly (P<0.05).

3.3. Comparison of Semen Parameters in the Fresh and post-capacitation Semen Samples

The comparison of different seminal parameters (sperm concentration and motility) in fresh versus post-capacitation semen samples of normozoospermic and oligoasthenozoospermic patients is shown in Table 3. In the NZ group, the sperm concentration was similar between the fresh and post-capacitation semen samples. However, the OAZ group showed that sperm concentration increased

significantly (P<0.01) after post-capacitation as compared to respective fresh semen samples. In inpatients of both the groups i.e. NZ and OAZ; the sperm motility significantly increased after capacitation in comparison to the non-capacitated fresh semen.

Table 3: Seminal parameters in fresh and post-capacitation semen of normozoospermic and oligoasthenozoospermic patients

Semen characteristics (Mean±SEM)	**Group** (n=50)	**Fresh semen**	**Post-capacitation semen**	**P-value**
Sperm concentration (millions/ml)	Normozoospermic (NZ) group	71.96±6.45	66.66±5.32	0.287
	Oligoasthenozoosprmic (OAZ) group	10.31±0.48[a]	21.20±1.38[b]	<0.001
Sperm Motility(%)	Normozoospermic (NZ) group	32.44±1.50[a]	93.00±0.86[b]	<0.001
	Oligoasthenozoosprmic (OAZ) group	26.76±1.38[a]	90.10±1.15[b]	<0.001

Values with different superscripts (a, b) within a row differ significantly (P<0.05).

3.4. Fertilization Rates with Post-capacitation Semen Samples of Normozoospermic and Oligoasthenozoospermic Patients

The fertilization with ICSI using post-capacitated semen belonging to the patients of NZ (98±0.009%) and OAZ (98±0.005%) groups was similar (P>0.05).

4. Discussion

The present study evaluated variations in ejaculated volume, sperm concentration, and sperm motility in fresh semen which were within the expected range as per set standards values of WHO (WHO, 2010). The ejaculatory semen volume of patients belonging to the OAZ and NZ groups were similar whereas the sperm concentration and sperm motility were significantly lower in OAZ compared to NZ patients. However, the sperm fertilizing capacity of both groups during ICSI was similar in terms of fertilization rates. Similar variations in ejaculated semen volume, sperm concentration, and sperm motility have been reported earlier in OAZ (Micic *et al.*, 2019) and NZ (Shekha *et al.*, 2019) males who underwent infertility treatment. In the present study, semen volume in sub-fertile Indian patients with oligoasthenozoospermic and normozoospermic semen quality constituted 2.64±0.23 and 2.14±0.16 ml, respectively. This was close to the volume in sub-fertile patients reported in Saudi Arabia (Nadia, 2013) and in Iraq (Mamdooh *et al.*, 2020); but lower than previously reported in France (Geoffroy-Siraudin *et al.*, 2012) and Brazil (Camila *et al.*, 2018). However, the values are greater than only a few recently reported studies (Shah *et al.*, 2020). Similarly, in India, variations in seminal volume have been reported earlier (Mishra *et al.*, 2018), and possible reasons for low seminal volume were reviewed (Roberts *et al.*, 2019). The ejaculated semen volume in the OAZ group was higher than in NZ semen donors, which might be attributed to the more watery content in the semen of OAZ patients. Contrarily, oligozoospermic patients showed lesser ejaculatory volume on different days after abstinence than normozoospermic infertile men (Shah *et al.*, 2020). In concurrence with the present study (Mishra *et al.*, 2018) observed greater seminal volume in infertile males than in fertile Indian males.

In the present study, the observed significantly lower sperm concentration in the OAZ group compared to the NZ group patients stands in concurrence with an earlier report (Nadia, 2013), which might be due to more watery content in oligoasthenozoospermic patients. The values of the current study fall within the expected range of WHO (WHO, 2010). Normozoospermic patients of the current study showed sperm concentration equivalent to the baseline density (68.22 M/ml) of Indian men (Adiga *et al.*, 2008). Contrarily, lower sperm concentrations in males treated at infertility clinics in India have also been reported (Marimuthu *et al.*, 2003). Such variability might be due to the dissimilar composition of the studied populations, mostly to the severity of the male factor.The observed sperm motility in the present study is comparable with those reported in other studies of infertile men with oligozoospermia (26%) (Shah *et al.*, 2020) or Ghanaian sub-fertile men (23%) (Blay *et al.*, 2020). In another study, about 25% of men from infertile couples had motility of about 32% (Shekha *et al.*, 2019). Although there is no known critical age limit for gamete production in men, evidence suggests that there are declines in semen quality (e.g., volume, motility, and morphology) and male fertility associated with increasing male age (Andolz *et al.*, 1999; Girsh et al, 2008; Dain *et al.*, 2011; Zhu *et al.*, 2011). Furthermore, a reduction in sperm motility was observed from about 26% to 8.5% over a period of time (Geoffroy-Siraudin *et al.*, 2012; Oliveira, *et al.*, 2014). Sperm motility was lower than that reported in men from different countries like Brazilian sub-fertile men

(Nogueira *et al.*, 2018), and ejaculates of Italian (Manna *et al.*, 2020) and Indian males (Mukhopadhyay *et al.*, 2010), but was similar to the results reported in India (Camila *et al*, 2018). Such differences could be attributed to racial, geographical, cultural, environmental, genetic, hormonal, nutritional, or lifestyle variations. The high temperature and humidity during most of the year in any region is the most important cause that affects sperm motility (Nadia, 2013).

Fertilization rates in the present study were above 95% in both types of sub-fertile males, without significant differences in fertilization rates of the two groups during ICSI. The study observed that the positive outcomes of ICSI are not dependent on the three basic sperm parameters viz. semen volume, sperm motility, and sperm concentration of fresh semen ejaculates of sub-fertile males in which these characteristics are impaired. For ICSI, sperm with the ability to activate the oocyte and form a pronucleus is necessary, and rather the other semen parameters are not important (Nagy et al., 1998). Similarly, the probability of conception of intrauterine insemination is not correlated with the total sperm count, concentration, and motility (Findekleet et al., 2020). Furthermore, oocyte quality is the only other most important factor that has an impact on fertilization rate during ICSI. However, variation due to oocyte quality could be minimized by the selection of good-quality oocytes after denudation before ICSI. In agreement with the present study, fertilization rates of oligoasthenozoospermic and normozoospermic patients were similar when semen samples were processed by density gradient and a swim-up aliquot was used for ICSI (Borges et al, 2007). The motility of the sub-fertile semen sample was likely increased during the process of gradient centrifugation and swim-up (Malvezzi *et al.*, 2014), and fertilization rates during assisted reproduction (Borges *et al.*, 2013). The observed fertilization rates of above 95% in the present study have not been reported earlier, rather a little lower rate of about 70% was reported during IVF/ICSI (Xie et al, 2013. A fertilization rate of 70-80% was usually attained by the ICSI technique (Palermo *et al.*, 2009). Our observations are in concurrence with earlier reports (Borges *et al.*, 2007) of similar fertilization and conception rates in OAZ and NS patients undergoing ICSI. Contrarily, oligoasthenozoospermic patients showed reduced conception rates during IVF and ICSI (Xie *et al.*, 2013), and testicular sperm retrieval for ICSI (Testi-ICSI) was recommended for improved fertilization rate (Mehta *et al.*, 2015). This might be due to higher sperm DNA fragmentation (SDF) in oligoasthenozoospermic patients. Seminal parameters viz. semen volume, sperm concentration and sperm motility varies with age (Maya *et al.*, 2009), climatic conditions (Miyamoto, 2012), regions (Adiga *et al.*, 2008), dietary habits (Sanlier *et al.,* 2018), environment, lifestyle (Blay *et al.*, 2020), nature of occupation (Tayawade & More, 2018), mobile usage (Agarwal *et al.*, 2011), type of clothes wearing (Parazzini *et al.*, 1995; Mínguez-Alarcón *et al.*, 2018), sleep pattern (Jhuang *et al.,* 2021). In conclusion, in spite of some variations in the seminal parameters of oligoasthenozoospermic and normozoospermic males, the fertilization capacity of sperm in terms of fertilization rates of the semen of these males was similar during ICSI.

5. Conclusion

The results of this study revealed that the fertilization rate for Oligoasthenozoospermic and Normozoospermic semen samples was similar. Further, the ICSI is an effective method of achieving good fertilization rates in sub fertile males.

6. Ethical Statement

The study was conducted after obtaining a letter of approval (014/IRCCPKL/2017) dated 2nd June 2017 from the Institute of Reproduction and Child Cares (IRCC) Institutional Ethical Committee.

7. Competing Interest

The author declares that there is no conflict of interest.

8. Funding Source

This project was supported by the Institute of Reproduction and Child Cares, Panchkula, Haryana, India. Grant No.-IRCCPKL/2017

9. Acknowledgement

I would like to acknowledge the technical assistance of Dr. Kamla Singh, Chief Medical Director, Institute of Reproduction and Child Cares, Panchkula, Haryana, India

References

Adiga, S.K., Jayaraman, V., Kalthur, G., Upadhya, D., & Kumar, P. (2008). Declining semen quality among south Indian subfertile men: A retrospective study. *J. Hum. Reprod. Sci. 1*, 15–18.

Agarwal, A., Singh, A., Hamada, A., & Kesari, K. (2011). Cell phones and male infertility: a review of recent innovations in technology and consequences. *International braz j urol*, *37*, 432-454. https://doi.org/10.1590/S1677-55382011000400002

Agarwal, A., Gupta, S., & Sharma, R. (2016). *Andrological evaluation of male infertility*. Cham: Springer, 113-33. https://doi.org/10.1007/978-3-319-26797-5.

Aleisa, N. A. (2013), Semen characteristics of fertile and subfertile men in a fertility clinic and correlation with age. *Journal of King Saud University-Science*. *25*, 63-71. https://doi.org/10.1016/j.jksus.2012.06.002

Blay, R. M., Pinamang, A. D., Sagoe, A. E., Owusu, E. D. A., Koney, N. K. K., & Arko-Boham, B. (2020). Influence of lifestyle and environmental factors on semen quality in Ghanaian men. *International journal of reproductive medicine*, 2020. https://doi.org/10.1155/2020/6908458.

Andolz, P., Bielsa, M. A., & Vila, J. (1999). Evolution of semen quality in North-eastern Spain: a study in 22 759 infertile men over a 36 year period. *Human Reproduction*, *14*(3), 731-735. https://doi.org/10.1093/humrep/14.3.731

Borges Jr, E., Rossi, L. M., de Freitas, C. V. L., Guilherme, P., Bonetti, T. C. S., Iaconelli, A., & Pasqualotto, F. F. (2007). Fertilization and pregnancy outcome after intracytoplasmic injection with fresh or cryopreserved ejaculated spermatozoa. *Fertility and Sterility*, *87*(2) 316–320. https://doi.org/10.1016/j.fertnstert.2006.06.032

Borges, E. Jr., Setti, A.S., Vingris, L., Figueira Rde, C., Braga, D.P., & Iaconelli, A. Jr. (2013). Intracytoplasmic morphologically selected sperm injection outcomes: the role of sperm preparation techniques. *J Assist Reprod Genet, 30*, 849-54. https://doi.org/10.1007/s10815-013-9989-x

Camila N, Ivan H. Y., Fabia L. V., Waldemar P. C., Emerson B. C., & Caio P. B., (2018). Seminal profile of 23,504 patients over the years: 7 years of experience. *JBRA Assisted Reproduction 22*(4), 286-288. https://doi: 10.5935/1518-0557.20180055.

Dain, L., Auslander, R., & Dirnfeld, M. (2011). The effect of paternal age on assisted reproduction outcome. *Fertil Steril*, *95*, 1–8. https://doi.org/10.1016/j.fertnstert.2010.08.029

Dupesh, S., Pandiyan, N., Pandiyan, R., Kartheeswaran, J., & Prakash, B. (2020). Ejaculatory abstinence in semen analysis: does it make any sense?. *Therapeutic advances in reproductive health*, *14*, 2633494120906882. https://doi.org/10.1177/2633494120906882

Findekleet, S., Radosa, J. C., Radosa, M. P., & Mohamad Eid Hammadeh (2020). Correlation between total sperm count and sperm motility and pregnancy rate in couples undergoing intrauterine insemination. *Sci Rep, 10*, 7555. https://doi.org/10.1038/s41598-020-64578-0.

Geoffroy-Siraudin, C., Loundou, A. D., Romain, F., Achard, V., Courbière, B., Perrard, M. H., ... & Guichaoua, M. R. (2012). Decline of semen quality among 10 932 males consulting for couple infertility over a 20-year period in Marseille, France. *Asian journal of andrology*, *14*(4), 584. https://doi:10.1038/aja.2011.173.

Girsh, E., Katz, N., Genkin, L., Girtler, O., Bocker, J., Bezdin, S., & Barr, I. (2008). Male age influences oocyte-donor program results. *J Assist Reprod Genet*, *25*, 137–43. https://doi.org/10.1007/s10815-008-9215-4

Jequier, A. M. (2004). Clinical andrology—still a major problem in the treatment of infertility. *Human Reproduction*, *19*(6), 1245-1249. https://doi.org/10.1093/humrep/deh269

Jhuang, Y. H., Chung, C. H., Wang, I. D., Peng, C. K., Meng, E., Chien, W. C., & Chang, P. Y. (2021). Association of obstructive sleep apnea with the risk of male infertility in Taiwan. *JAMA network open*, *4*(1), e2031846-e2031846. https://doi.org/10.1001/jamanetworkopen.2020.31846

Kumar, N., & Singh, A. K. (2015). Trends of male factor infertility, an important cause of infertility: A review of literature. *Journal of human reproductive sciences*, *8*(4), 191-196. 10.4103/0974-1208.170370

Macklon, N. S., Stouffer, R. L., Giudice, L. C., & Fauser, B. C. (2006). The science behind 25 years of ovarian stimulation for in vitro fertilization. *Endocrine reviews*, *27*(2), 170-207. https://doi.org/10.1210/er.2005-0015

Mahadevan, M. M., & Trounson, A. O. (1985). Removal of the cumulus oophorus from the human oocyte for in vitro fertilization. *Fertility and sterility*, *43*(2), 263-267. https://dx.doi.org/10.1016/Soo15-0282(16)48383-4

Makler, A. (1980). The improved ten-micrometer chamber for rapid sperm count and motility evaluation. *FertilSteril*, *33*, 337-8.

Malvezzi, H., Sharma, R., Agarwal, A., Abuzenadah, A. M., & Abu-Elmagd, M. (2014). Sperm quality after density gradient centrifugation with three commercially available media: a controlled trial. *Reproductive biology and endocrinology, 12*(121). https://doi.org/10.1186/1477-7827-12-121.

Manna, C., Barbagallo, F., Manzo, R., Rahman, A., Francomano, D., & Calogero, A. E. (2020). Sperm Parameters before and after Swim-Up of a Second Ejaculate after a Short Period of Abstinence. *Journal of Clinical Medicine*, *9*(4), 1029. https://doi.org/10.3390/jcm9041029.

Marimuthu, P., Kapilashrami, M. C., Misro, M. M., & Singh, G. (2003). Evaluation of trend in semen analysis for 11 years in subjects attending a fertility clinic in India. *Asian J Androl*, *5*, 221–5.

Maya, W. C., Berdugo, J., & Jaramillo, A. C. (2009). The effects of maleage on semen parameters: analysis of 1364 men attending an Andrologycenter. *Aging Male, 12*, 100–103. https://doi.org/10.3109/13685530903322841

Mehta, A., Bolyakov, A., Schlegel, P. N., & Paduch, D. A. (2015). Higher pregnancy rates using testicular sperm in men with severe oligospermia. *FertilSteril, 104*, 1382–1387.

Micic S., Lalic N., Djordjevic D., Bojanic N., Bogavac-Stanojevic N., Busetto G. M., Virmani A., & Agarwal A. (2019). Double-blind, randomised, placebo-controlled trial on the effect of L-carnitine and L-acetylcarnitine on sperm parameters in men with idiopathic oligoasthenozoospermia. *Andrologia*, *51*, e13267. https://doi.org/10.1111/and.13267

Mínguez-Alarcón, L., Gaskins, A. J., Chiu, Y. H., Messerlian, C., Williams, P. L., Ford, J. B., ... & Chavarro, J. E. (2018). Type of underwear worn and markers of testicular function among men attending a fertility center. *Human Reproduction*, *33*(9), 1749-1756. doi: 10.1093/humrep/dey259.

Mishra, P., Negi M, P. S., Srivastava, M., Singh, K., &Singh, R. (2018). Decline in seminal quality in Indian men over the last 37 years. *Reproductive Biology and Endocrinology, 16*, 103. https://doi.org/10.1186/s12958-018-0425-z

Miyamoto, T., Tsujimura, A., Miyagawa, Y., Koh, E., Namiki, M., & Sengoku, K. (2011). Male infertility and its causes in human. *Advances in urology*, 2012. https://doi.org/10.1155/2012/384520

Mortimer, D. (2000). Sperm preparation methods. Journal of andrology, 21(3), 357-366. https://doi.org/10.1002/j.1939-4640.2000.tb03390.x

Mukhopadhyay, D., Varghese, A. C., Pal, M., Banerjee, S. K., Bhattacharyya, A. K., Sharma, R. K., & Agarwal, A. (2010). Semen quality and age-specific changes: a study between two decades on 3,729 male partners of couples with normal sperm count and attending an andrology laboratory for infertility-related problems in an Indian city. *Fertility and sterility*, *93*(7), 2247-2254. https://doi.org/10.1016/j.fertnstert.2009.01.135.

Nagy, Z. P., Verheyen, G., Tournaye, H., & Van Steirteghem, A.C. (1998). Special applications of intracytoplasmic sperm injection: the influence of sperm count, motility, morphology, source and sperm antibody on the outcome of ICSI. *Hum Reprod 13*(Suppl 1). 143-154. https://doi.org/10.1093/humrep/13.suppl_1.143

Nogueira, C., Yoshida, I. H., Vilarino, F. L., Carvalho, W. P., Cordts, E. B., & Barbosa, C. P. (2018). Seminal profile of 23,504 patients over the years: 7 years of experience. *JBRA assisted reproduction*, *22*(4), 286–288. https://doi.org/10.5935/1518-0557.20180055.

Oliveira, J. B. A., Petersen, C. G., Mauri, A. L., Vagnini, L. D., Baruffi, R. L., & Franco Jr, J. G. (2014). The effects of age on sperm quality: an evaluation of 1,500 semen samples. *JBRA Assisted Reproduction*, *18*(2), 34. https://doi.org/10.5935/1518-0557.20140002.

Palermo, G. D., Neri, Q. V., Takeuchi, T., & Rosenwaks, Z. (2009). ICSI: where we have been and where we are going, *Seminars in Reproductive Medicine, 27*, 191–201. https://doi.org/10.1055/s-0029-1202309

Parazzini, F., Marchini, M., Luchini, L., Tozzi, L., Mezzopane, R., & Fedele, L. (1995). Tight underpants and trousers and risk of dyspermia. *International journal of andrology*, *18*(3), 137-140. https://doi.org/10.1111/j.1365-2605.1995.tb00400.x

Roberts, M., & Jarvi, K. (2009). Steps in the investigation and management of low semen volume in the infertile man. *Canadian Urological Association Journal*, *3*(6), 479. 10.5489/cuaj.1180

Sanlier, N., & Yorusun, T. O. (2018). The relationship between infertility and nutrition. *J Nutr Health Sci*, *5*(2), 207.

Shekha, S. A., Narkhede, P., Fatima, T., & Bhople, K. S. (2019). Patterns of semen parameters in male partners of infertile couples attending rural medical college. *Int. J. Clin. Diagn. Pathol, 2* (2), 341-348. https://doi.org/10.33545/pathol.2019.v2.i2f.125

Sidhu, R. S., Sharma, R. K., Kachoria, S., Curtis, C., & Agarwal, A. (1997). Reasons for rejecting potential donors from a sperm bank program. *Journal of assisted reproduction and genetics*, *14*, 354-360. https://doi.org/10.1007/BF02765841

Sukcharoen, N., Ngeamjirawat, J., Chanprasit, Y. E. N. C. H. I. T., & Aribarg, A. (1994). A comparison of Makler counting chamber and improved Neubauer hemocytometer in sperm concentration measurement. *J Med Assoc Thai*, *77*(9), 471-476.

Tayawade, A. V., More, A., Salve, M., & Shrivastava, D. (2020). Effect of Occupational Environment on Male Infertility. *Int J Cur Res Rev*, *12*, S117-21.

Unisa, S. (2010). Infertility and treatment seeking in India: findings from district level household survey. *Social Aspects of Accessible Infertility Care in Developing Countries*, *59*, 65.

Xie, B. G., Zhu, W. J., & Huang, Y. H. (2013). Outcome of conventional IVF and ICSI on sibling oocytes in moderate ligoasthenozoospermia. *Pakistan Journal of Medical Sciences*, *29*(5), 1221.

Zegers-Hochschild, F., Adamson, G. D., De Mouzon, J., Ishihara, O., Mansour, R., Nygren, K., ... & Van der Poel, S. (2009). The international committee for monitoring assisted reproductive technology (ICMART) and the world health organization (WHO) revised glossary on ART terminology, 2009. *Human reproduction*, *24*(11), 2683-2687. https://doi.org/10.1093/humrep/dep343

Zhu, Q. X., Meads, C., Lu, M. L., Wu, J. Q., Zhou, W. J., & Gao, E. S. (2011). Turning point of age for semen quality: a population-based study in Chinese men. *Fertility and sterility*, *96*(3), 572-576. https://doi.org/10.1016/j.fertnstert.2011.06.058

CHITKARA

Journal of Multidisciplinary Research in Healthcare

Chitkara University, Saraswati Kendra, SCO 160-161, Sector 9-C, Chandigarh, 160009, India

Volume 9, Issue 2 **April 2023** **ISSN 2393-8536**