EFFECTS OF SPERM PARAMETERS ON THE OUTCOMES OF INTARCYTOPLASMIC SPERM INJECTION: A RETROSPECTIVE STUDY FROM LAHORE PAKISTAN

Haroon Latif Khan,[1] Yousaf Latif Khan,[1] Kausar Siddiqui,[1] Ayesha Awais,[1] Abdul Rahman Khawaja[1]

ABSTRACT

Background: Semen analysis is the basic step when investigating sub fertility disorders related to male genital tract. Valuable clinical information can be obtained from Semen analysis report about the spermatogenesis and functional capabilities of spermatozoa**. Objective**: To determine the fertilization rate and pregnancy outcomes among intracytoplasmic sperm injection patients and its association with sperm parameters. **Methodology**: This retrospective analytical study was carried out at Lahore Institute of Fertility and Endocrinology, Lahore. Male partners of 272 couples were included in the study as they came for evaluation for assisted reproduction in between 1st July and 31st December 2014. Before samples were collected these males were asked to remain abstinent for 3 days. Specimens were collected by masturbation, and in cases of azoospermia it was retrieved by Percutaneous Epididymal Sperm Aspiration (PESA) or Testicular Sperm Apiration (TESA). Semen analysis was performed according to the methods and standards outlined by the World Health Organization (WHO). Oocytes were aspirated after 35-36 hours approximately, by ultrasound-guided transvaginal retrieval. About 4-5 hours after the aspiration of oocyte, in the IVF cycles, they were inseminated. After 18 to 20 hours all oocytes were examined for pronuclei followed by cleavage assessment within 24 hours. Embryos were examined and best embryos two or three were transferred into the uterine cavity on the 3rd day. **Results**: Fertilization did occurred in 182 (95.8 %) patients with normal sperm count, in 30 (90.9%) with subnormal sperm count and in 43 (87.8%) with Azoospermia. Fertilization did occur in 209 (95.4%) patients with normal sperm morphology, in 12 (92.3%) with subnormal sperm morphology and in 34 (85.0%) with Azoospermia. Fertilization did occur in 160 (98.2%) patients with normal sperm motility, in 59 (88.1%) with subnormal sperm motility and in 36 (85.7%) in the Azoospermia group. **Conclusion:** Sperm motility and morphology abnormality affected fertilization in intracytoplasimic sperm injection. Though value of semen analysis is limited but gives direction to the investigation. Mere semen examination is not at all sufficient to make definitive diagnosis. It is not at all predictive about the functional abilities of the sperms. There is a need for adding an andrology component to the training of the gynecologists.

Key Words: Sperm Parameters, ICSI, Outcome

JSZMC 2016;7(2):968-973

INTRODUCTION

Semen analysis is the basic step when investigating sub fertility disorders related to male genital tract. Valuable clinical information can be obtained from Semen analysis report about the spermatogenesis and functional capabilities of spermatozoa.[1] Results of semen analysis are used by clinicians to choose appropriate option of treatment for the sub fertile couple, the laboratory performing semen analysis should have trained technicians and use reliable procedures.[2]

Clinicians and assisted reproductive technology (ART) specialists have relied upon (WHO) criteria to decipher the results of basic semen analysis; though these criteria have been regarded controversial. But to comment about fertility potential of a male just on the basis of routine semen analysis may be impractical and not justified.[3,4] A properly performed and comprehensive semen analysis with a complete history and physical examination can become first step in right direction in investigating sub fertile couples especially in a primary care setting.[5] Clinical data in conjunction with robust semen analysis serves as a useful tool in diagnosis of male sub fertility and also guides to make a choice of best treatment modality.[6] Unexplained subfertility is the commonest type of male sub fertility which has one or more semen abnormalities with no visible cause.[7] One fourth of the sub fertile males may have either severe forms of sub fertility or sterility.[8] An insightful understanding of sperm characteristics is essential before initiating the process of sub fertility investigation and management.[9]

Sperm motility, count and morphology were found to have unequal potential in occurrence of pregnancy.

1. Lahore Institute of Fertility and Endocrinology, Hameed Latif Hospital, Lahore, Pakistan.

Correspondence: Dr. Abdul Rahman Khawaja, Biostatistician and Research Coordinator LIFE, Pakistan.

E-mail: arkhaja@gmail.com **Mobile:**+92 3218460270 **Received:**12-11-015 **Accepted:** 30-04-2016

Detailed analysis of sperm morphology including six different anomalies of head, tail and cytoplasmic droplet were more frequent in the couples who did not have pregnancy. Multiple Anomalies Index (MAI) was shown to have high predictability of occurrence of pregnancy.[10,11]
Sperm morphology, count and motility are examined in semen analysis along with other semen parameters and compared with reference values to make an assessment about the fertility potential of the male partner.[6] The measurements of sperm concentration, motility, and morphology all provide useful information for diagnosing male sub fertility. Sperm morphology, as measured according to strict criteria, appears to be the most informative semen measurement for discriminating between fertile and sub fertile men. However, none of the measures, alone or in combination, can be considered diagnostic of sub fertility.[12]
If semen analysis is performed correctly and according to high standards then sperm morphology report is of a highly predictive significance especially in deciding the assisted reproductive techniques to be employed.[13] The cause of these abnormalities in the male leading to sub fertility in a couple may not be evident even after complete evaluation of the couple presenting to a fertility center; but this may have huge impact on the life of the subfertile couple along with psychosocial implications.[14]
When a couple approaches a physician for evaluation of sub fertility first of all male is asked for semen analysis.[15-18] After viewing the semen analysis report further screening and evaluation of the female partner is carried out.[19] Standardization of functional characteristics of sperms and their count, morphology and motility led to development of WHO guidelines which helped to look at male fertility factors objectively. This standardization helped in communication among professionals and to compare information among various research centers but did not imply a cause-effect relationship between male fertility and sperm count.[20-23]
Repeated revisions of the WHO guidelines were carried out in the light of new scientific evidence and latest version was published in 2010.[24-29]
Semen analysis remains a cornerstone of male fertility evaluation despite doubts about its clinical value. New semen analysis manual of World Health Organization (WHO) uses evidence-based publications as a source to decide the cutoff normality values.[28] Sperm morphology assessment is a difficult task as compared to sperm motility assessment or sperm counting. It needs knowledge, training and expertise. Without stricter quality control interpreting sperm morphology assessment may become meaningless for the clinicians.[30]
The present study was done to look into sperm parameters in males presenting in the sub infertility clinic for semen evaluation according to WHO criteria and correlate it with fertilization and pregnancy outcomes.

METHODOLOGY

This retrospective analytical study was carried out at Lahore Institute of Fertility and Endocrinology, Hameed Latif Hospital Lahore. Sample was selected through non probability consecutive time based sampling from 1st July to 31st December 2014. Semen analysis was performed according to the methods and standards outlined by the World Health Organization (WHO).
Male partners, 272 couples were included in the study as they came for evaluation for assisted reproduction in this period. Before the samples were collected these males were asked to remain abstinent for 3 days. Specimens were collected by masturbation and sterile wide mouthed containers were used to store the semen for examination.
Semen was examined within 60 minutes of its collection. After looking at pH, appearance, liquefaction, volume, and pus cells, spermatozoa were examined for morphology, motility and count. Graduated disposable pipette was used to determine the volume and pH was checked with the pH paper. The semen specimen was thoroughly mixed with a pipette and thin drop of this specimen was placed on a glass slide. This drop was spread with the help of a cover slip. Meckler's counting chamber was used for counting of spermatozoa. Microscopic assessment for morphology and motility, of 200 spermatozoa, was done by examining different fields.
A gonadotropin-releasing hormone analogue (Decapeptyle) was used to down regulate the women undergoing assisted reproduction. Long protocol Daily intramuscular injections (Menogon, IVF-M, Mesone and Fostimone) or daily subcutaneous injections (G.F, Puregon) were used for stimulation. It was followed by HcG i.e. human chorionic gonadotrophin (Pregnyl, IVF-C, Choriomon) and

oocytes were aspirated after 35-36 hours approximately, by ultrasound-guided transvaginal retrieval.

About 4-5 hours after the aspiration of oocyte, in the IVF cycles, they were inseminated. In the Intracytopplasmic Sperm Injection (ICSI) cycles a spermatozoon was deposited in the cytoplasm of the oocyte after denuding them with hyaluronidase. After 18 to 20 hours all oocytes were examined for pronuclei followed by cleavage assessment within 24 hours. Embryos were examined and best embryos, two or three, were transferred into the uterine cavity on the 3rd day. Data was entered into SPSS version 15 and descriptive analysis was done. Mainly morphology, motility and counts were included. Fertilization was seen in these couples and association was checked between morphology, motility and counts and fertilization.

RESULTS

Fertilization did occur in 182 (95.8%) patients with normal sperm count, in 30(90.9%) with subnormal sperm count and in 43 (87.8 %) with Azoospermia. In these cases sperm retrieval was carried out either by PESA/ TESA. Fertilization did occur in 209 (95.4%) patients with normal sperm morphology, in 12 (92.3%) with subnormal sperm morphology and in 34 (85.0%) with Azoospermia. Fertilization did occur in 160(98.2%) patients with normal sperm motility, in 59 (88.1%) with subnormal sperm motility and in 36 (85.7%) in the Azoospermia group (Table I). Strong statistically significant association was found between sperm motility and fertilization (0.01).

Table I: Sperm parameters and fertilization

Variable	Categories	Fertilization		Total
		Occurred	No fertilization	
Sperm count	Normal	182 (95.8%)	8 (4.2%)	190 (100%)
	Subnormal	30 (90.9%)	3 (9.1%)	33 (100%)
	Azoospermia	43 (87.8%)	6 (12.2%)	49 (100%)
Sperm morphology	Normal	209 (95.4%)	10 (4.6%)	219 (100%)
	Subnormal	12 (92.3%)	1 (7.7%)	13 (100%)
	Azoospermia	34 (85.0%)	6 (15.0%)	40 (100%)
Sperm motility	Normal	160 (98.2%)	3 (1.8%)	163 (100%)
	Subnormal	59 (88.1%)	8 (11.9%)	67 (100%)
	Azoospermia	36 (85.7%)	6 (14.3%)	42 (100%)

Strong statistically significant association was also found between sperm morphology and fertilization (0.042). Statistically insignificant association was found between sperm count and fertilization (0.09). Out of 272 couples who had undergone ICSI fertilization occurred in 255(93.75%), p-value 0.02 (significant). Out of 272 couples undergone ICSI pregnancy occurred in 66 (24.3%), p-value 0.69 (not significant).

DISCUSSION

In our study fertilization was seen with respect to sperm count, morphology and motility. Fertilization occurred in above 95% of the couples with normal sperm count, morphology and motility. Fertilization also occurred in 88% in couples with subnormal count, morphology and motility. Fertilization was also seen in more than 85% couples with azoospermia. Association was found to be strong and statistically significant between sperm morphology, motility and fertilization.

ICSI is an assisted reproductive technique which was introduced in 1992 it involves injecting a single sperm directly into an egg in situations where sperm cannot penetrate an egg or in cases of azoospermia.[30] The chances of fertilization were enhanced in couples with unsuccessful IVF cycles with undetectable sperm abnormalities and couples with severe male factor subfertility.[31]

ICSI outcome was examined in 308 cases of azoospermia by Palermo in 1999. Sperms were extracted by MESA (Micro-epididymal Sperm Aspiration) or TESE (Testicular Sperm Extraction). On the basis of etiology, epididymal obstruction was found to be, acquired in 103 cases and congenital in 138 cases. Reproductive tract obstruction was present in 14 cases and absent vas deference in 53 cases. Sperms extracted by TESE fertilized 57.0% of 533 eggs. In non-obstructive cases 80.5% of 118 eggs were fertilized. (P value=0.0001) Twenty six out of fifty three (49.1%) non-obstructive cases showed to have pregnancy and eight out of fourteen cases (57.1%) of obstructive azoospermia seemed to have pregnancy. Fifty out of one hundred and three (48.5%) cases had acquired epididimal obstruction they showed to have pregnancy in eighty five out of one hundred and thirty eight cases (61.6%) of congenital epididimal obstruction seemed to have pregnancy.[32]

In a study published by Khan in 2011, which was done on 1521 sub fertile men and their semen parameters were compared with the parameters of fertile men. Results showed that teratospermic were 2.4%, azoospermic 13.3%, normospermic 14.5%, oligospermic 23.2%, polyspermic 0.9%, asthenospermic 35.2%, oligoasthenospermic 10.5.

Semen of proven fathers had greater sperm count and motility than that of infertile men and it was also statistically significant ($p<0.05$). Similar results were seen when sperm morphology was compared.[33] Fertilization in ICSI is dependent on the source of the sperm. Sperm may be from ejaculates of abnormal and normal semen or there may be a sperm that has been extracted from testes with azoospermia that is non-obstructive in nature. Sperm's fertilizing ability in ICSI is highest in with sperms from ejaculates of normal semen and is lowest with sperms from testicular biopsy. Clinical pregnancy rates are also lower with sperms from testicular biopsy.[34] Sperm from males having non obstructive azoospermia effects both fertilization as well as implantation.[35,36] ICSI with testicular sperms yielded higher fertilization and implantation rates.[37]

Hameed and Ozturk in 2010 conducted a retrospective evaluation of 111, ICSI treatment cycles which were performed at ACU (Assisted Conception Unit), UCH (University College Hospital) London, in five years. Surgical extraction of sperms was done from epididymis and testes and the outcome was studied in terms of fertilization and pregnancy. Epididymal spermatozoa showed a higher fertilization rate as well as clinical and ongoing pregnancy as compared to spermatozoa extracted from the testes. Clinical pregnancy rate was found to be statistically significant.[38]

In men with obstructive azoospermia rates of embryo transfer, pregnancy and delivery seemed to have no statistically significant difference between epididymal and testicular spermatozoa. Birth rates seemed to differ significantly between obstructive and non-obstructive azoospermia. Pregnancy rates were higher in obstructive azoospermia as compared to non-obstructive azoospermia.[39] Ninety five percent of males with severe infertility can become fathers of their own genetic children due to ICSI combined with TESE and PESA and donor spermatozoa are no more needed.Naru studied the outcome in couples undergoing Intracytoplasmic sperm injection in 2008. The outcome was compared between the ejaculated sperm and retrieved sperm with PESA and TESE in azoospermic men. They concluded that no difference was seen between outcomes of both groups of men.[40]

Clinical decisions about diagnosis, prognosis and management based solely on results of semen analysis may not be reliable as various studies have concluded.[41] Semen analysis is very useful investigation for identifying male infertility as a first step in the initial workup for sub fertility.[42] But its utility in making accurate prognosis and choice of treatment of sub fertility has been questioned. It has been suggested that it is the patient who needs real attention and not the semen analysis and its result.

It has been proposed that as male sub fertility is being evaluated and treated by gynecologists; there is an ample need for enhancing andrology skills of the gynecologists.[43,44] A multi-disciplinary team, consisting of gynecologist, urologist and embryologist, has become essential for making clinical decisions in an assisted reproductive setting for diagnosis, prognosis and treatment of subfertility. Suitably trained staff for fertility, psychology and genetic counseling may also be necessary.[45]

If a couple in a family suffers from sub fertility a general practitioner is the first person to be approached. A general practitioner is a family physician who not only explains the phenomenon of fertility to the couple and seniors of the family but also helps in initial workup and referral to the fertility physician.[46] Ashrafi et al, recommended after a study done in Iran in 2013 that the physicians must be properly educated and trained to help sub fertile couples to treat sub fertility. They should know what to ask from the couples approaching for evaluation of subfertility. If these physicians are having enough information about the causes of subfertility they will be able to help them effectively. Though ICSI is an effective procedure for severe male sub fertility it can be useful for subfertility from many other causes.[47]

CONCLUSION

Sperm motility and morphology abnormality affected fertilization in intracytoplasinic sperm injection. Semen analysis is an important basic test, which is needed by the physician starting workup evaluation for male sub fertility. Though its value is limited but gives direction to the investigation. Mere semen examination is not at all sufficient to make definitive diagnosis. It is not at all predictive about the functional abilities of the sperms. There is a need for adding an andrology component to the training of the gynecologists as male infertility most of the time is being treated by the gynecologists.

Acknowledgment:
Miss Saba Sardar and Mrs. Rameen Makhdoom Assistant Biostatistician in Lahore Institute of

Fertility and Endocrinology (LIFE) are acknowledged for their help in data extraction and analysis.

Conflict of interest:
The authors have declared no conflict of interest.

REFERENCES

1. Andrade-Rocha FT. Semen analysis in laboratory practice: an overview of routine tests. J Clin Lab Anal 2003; 17(6):247-58.
2. McLachlan RI, Baker HW, Clarke GN, Harrison KL, Matson PL, Holden CA, de Kretser DM. Andrology Australia Australian Centre of Excellence in Male Reproductive Health; Fertility Society of Australia Scientists in Reproductive Technology Subcommittee; Board of Education of the Royal College of Pathologists of Australia. Semen analysis: its place in modern reproductive medical practice. Pathology. 2003; 35(1):25-33.
3. Lewis SE. Is sperm evaluation useful in predicting human fertility? Reproduction. 2007; 134(1):31-40.
4. Agarwal A, T.M. Said. Interpretation of Basic Semen Analysis and Advanced Semen Testing. Current Clinical Urology: Male Infertility: Problems and Solutions. 2011; Springer Science+Business Media, LLC.
5. Agarwal A, Bragais FM, Sabanegh E. Assessing sperm function. UrolClin North Am. 2008;35(2):157-71.
6. Catanzariti F, Cantoro U, Lacetera V, Muzzonigro G, Polito M. Comparison between WHO (World Health Organization) 2010 and WHO 1999 parameters for semen analysis - interpretation of 529 consecutive samples. Arch ItalUrolAndrol. 2013;85(3):125-9.
7. Kantartzi PD, GoulisChD, Goulis GD, Papadimas I. Male infertility and varicocele: myths and reality. Hippokratia. 2007;11(3):99-104.
8. Baker HW. Male infertility. EndocrinolMetabClin North Am.1994;23(4):783-93.
9. Steger K, Cavalcanti MC, Schuppe HC. Prognostic markers for competent human spermatozoa: fertilizing capacity and contribution to the embryo. Int J Androl.2011;34(6 Pt 1):513-27.
10. Jouannet P, Ducot B, Feneux D, Spira A. Male factors and the likelihood of pregnancy in infertile couples. I. Study of sperm characteristics. Int J Androl. 1988;5:379-94.
11. Ducot B, Spira A, Feneux D, Jouannet P. Male factors and the likelihood of pregnancy in infertile couples. II. Study of clinical characteristics--practical consequences. Int J Androl. 1988;(5):395-404.
12. Guzick DS, Over street JW, Factor-Litvak P, Brazil CK, Nakajima ST, Coutifaris C, Carson SA, Cisneros P, Steinkampf MP, Hill JA, Xu D, Vogel DL. National Cooperative Reproductive Medicine Network. Sperm morphology, motility, and concentration in fertile and infertile men. N Engl J Med.2001;8;345(19):1388-93.
13. Menkveld R. Sperm morphology assessment using strict (tygerberg) criteria. Methods Mol Biol. 2013;927:39-50.
14. Greil AL, Slauson-Blevins K, McQuillan J. The experience of infertility: A review of recent literature. Sociology of Health & Illness, 32(1), 140–162. Sociol Health Illn. 2010;32(1):140-62.
15. Cobb M. An amazing 10 years: the discovery of egg and sperm in the 17th century. Reprod Domest Anim. 2012;47 Suppl 4:2-6.
16. Cooper TG, Noonan E, von Eckardstein S, Auger J, Baker HW, Behre HM, Haugen TB, Kruger T, Wang C, Mbizvo MT, Vogelsong KM. World Health Organization reference values for human semen characteristics. Hum Reprod Update. 2010;16(3):231-45.
17. Butt F, Akram N. Semen analysis parameters: experiences and insight into male infertility at a tertiary care hospital in Punjab. J Pak Med Assoc.2013;63(5):558-62.
18. Whitman-Elia GF, Baxley EG. A primary care approach to the infertile couple. J Am Board FamPract 2001;14(1):33- 45.
19. Cooper TG, Noonan E, von Eckardstein S, Auger J, Baker HW, Behre HM, Haugen TB, Kruger T, Wang C, Mbizvo MT, Vogelsong KM. World Health Organization reference values for human semen characteristics. Hum Reprod Update.2010;16(3):231-45.
20. Steger K, Cavalcanti MC, Schuppe HC- Prognostic markers for competent human spermatozoa: fertilizing capacity and contribution to the embryo. Int J Androl. 2011; 34(6 Pt 1):513-27.
21. World Health Organization. Geneva; World Health Organization;. WHO Laboratory Manual for the Examination and Processing of Human Semen.2010 5th ed.
22. Vieira M. New World Health Organization reference values for semen analysis: where do we stand? Einstein (Sao Paulo). 2013;11(2):263-4.
23. Franken DR, Oehninger S. Semen analysis and sperm function testing. Asian J Androl. 2012;14(1):6-13.
24. World Health Organization. Cambridge; Cambridge University Press; 1987. World Health Organization Laboratory Manual for the Examination of Human Semen and Semen–Cervical Mucus Interaction. 2nd ed.
25. World Health Organization. Cambridge; Cambridge University Press; 1992. World Health Organization Laboratory Manual for the Examination of Human Semen and Semen–Cervical Mucus Interaction. 3rd ed.
26. World Health Organization. Cambridge; Cambridge University Press; 1999. WHO Laboratory Manual for Examination of Human Semen and Semen–Cervical Mucus Interaction. 4th ed.
27. Menkveld R. Clinical significance of the low normal sperm morphology value as proposed in the fifth edition of the WHO Laboratory Manual for the Examination and Processing of Human Semen. Asian J Androl.2010;12(1):47-58.
28. Souter VL, Irvine DS, Templeton AA. Laboratory techniques for semen analysis: a Scottish survey. Health Bull (Edinb). 1997;55(3):140-9.
29. Yao KS, Zhang XZ, Wu Y. Assessment of sperm morphology without quality control may be meaningless for clinicians. Asian J Androl. 2010;12(4):607-8.
30. Palermo G, Joris H, Devroey P. Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. Lancet.1992; 340(8810):17-8.
31. Benadiva CA, Nulsen J, Siano L, Jennings J, Givargis

HB, Maier D. Intracytoplasmic sperm injection overcomes previous fertilization failure with conventional in vitro fertilization. Fertil Steril.1999;72(6):1041-4.
32. Palermo GD, Schlegel PN, Hariprashad JJ, Ergün B, Mielnik A, Zaninovic N, Veeck LL,Rosenwaks Z. Fertilization and pregnancy outcome with intracytoplasmic sperm injection for azoospermic men. Hum Reprod.1999;14(3):741-8.
33. Khan MS, Deepa F, Ahmed Z, Tahir F, Khan MA. Assessment of male reproductive health by conventional method of semen analysis. J Ayub Med Coll Abbottabad.2011;23(1):84-8.
34. Göker EN, Sendag F, Levi R, Sendag H, Tavmergen E. Comparison of the ICSI outcome of ejaculated sperm with normal, abnormal parameters and testicular sperm. Eur J ObstetGynecolReprod Biol.2002;104(2):129-36.
35. Nicopoullos JD, Gilling-Smith C, Almeida PA, Ramsay JW. The results of 154 ICSI cycles using surgically retrieved sperm from azoospermic men. Hum Reprod. 2004;19(3):579-85.
36. Verza S Jr, Esteves SC. Sperm defect severity rather than sperm Source is associated with lower fertilization rates after intracytoplasmic sperm injection. IntBraz J Urol.2008;34(1):49-56.
37. Devroey P, Nagy P, Tournaye H, Liu J, Silber S, Van Steirteghem A. Outcome of intracytoplasmic sperm injection with testicular spermatozoa in obstructive and non-obstructive azoospermia. Hum Reprod. 1996;11(5):1015-8.
38. Hameed N, Ozturk O. Testicular versus epididymal spermatozoa inintracytoplasmic sperm injection treatment cycles. J Ayub Med Coll Abbottabad.2010; 22(4):159-63.
39. Schwarzer JU, Fiedler K, Hertwig Iv, Krüsmann G, Würfel W, Mühlen B, Pickl U, Löchner-Ernst D, Schleyer M, Ovens-Räder A, Hennig M. Male factors determining the outcome of intracytoplasmic sperm injection with epididymal and testicular spermatozoa. Andrologia.2003;35(4):220-6.
40. Naru T, Sulaiman MN, Kidwai A, Ather MH, Waqar S, Virk S, Rizvi JH. Intracytoplasmic sperm injection outcome using ejaculated sperm and retrieved sperm in azoospermic men. Urol J.2008; 5(2):106-10.
41. Small DR, Collins JA, Wilson EH, Wrixon W. Interpretation of semen analysis among infertile couples. CMAJ.1987;136(8):829-33.
42. Lewis SE. Is sperm evaluation useful in predicting human fertility? Reproduction. 2007;134(1):31-40.
43. Jequier AM. Is quality assurance in semen analysis still really necessary? A clinician's viewpoint. Hum Reprod. 2005;20(8):2039-42.
44. Holt WV. Is quality assurance in semen analysis still really necessary? A spermatologist's viewpoint. Hum Reprod. 2005;20(11):2983-6.
45. Göker EN, Sendag F, Levi R, Sendag H, Tavmergen E. Comparison of the ICSI outcome of ejaculated sperm with normal, abnormal parameters and testicular sperm. Eur J ObstetGynecolReprod Biol.2002;104(2):129-36.
46. Case AM. Infertility evaluation and management. Strategies for family physicians. Can Fam Physician. 2003;49:1465-72.
47. Ashrafi M, Jahanian Sadatmahalleh S, Akhoond MR, Ghaffari F, Zolfaghari Z. ICSI Outcome in Infertile Couples with Different Causes of Infertility: A Cross-Sectional Study. Int J FertilSteril.2013;7(2):88-95.