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Research Article

Within Subject Variation Of Seminal Parameters After 5 Days Of Abstinence

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Abstract: World over, male infertility is on rise. Several causes for infertility are known. Semen examination report is an important document, of which total sperm count and percentage of sperm motility are very well considered for understanding the status of fertility. Several factors affecting these parameters are discussed by different groups of workers. Abstinence maintained prior to semen collection differed among different studies. Abstinence may have an influence on semen parameters. Several reports show submission of sample is done after random collection. If collection is done after number of days of abstinence the semen will be poor in quality. It is known that sperms are damaged if they remain more number of days in male system prior to its release. As spermatogenesis is a continuous process, number of sperms will be more in testes if not released out. Percentage of sperm motility in such samples will be poor. Similarly the quality of semen of daily collected is likely to be poor where sperms are less in number. Considering the said facts we have opted to find out if any variation in semen takes place if abstinence period is strictly maintained. In the present study, a total number of thirty five young healthy men supplied semen samples on three different occasions at a fixed time after maintaining abstinence for a fixed period of five days. The time of collection of semen is also an important factor as semen collected at different timings of the day after maintaining same fixed number of days of abstinence showed significant difference in values. Knowing seasonal changes lead to difference in parameters of semen, we completed the present study in one season. Our results on all parameters showed change in values in all three samples, collected on three different days but they were statistically insignificant. Our study favors collection of sample after fixed number of five days of abstinence.

Keywords: Semen Parameters, Abstinence, Biological Variations, Sperm Count, Sperm Motility, Fertility

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1. INTRODUCTION

Childless couples live in despair. Psychologically they are much disturbed. They approach one or more clinicians for consultation where they undergo detailed history taking, body examination and essential haematological investigation. Husband is advised semen evaluation[1,2]. On the basis of semen report, clinician reaches on conclusion whether patient is normal or infertile. When semen report shows his sample as below normal and he is responsible for childlessness. Depressed husband would submit samples to different laboratories out of eagerness to find himself as eligible to become a father. Male infertility is on rise world over[3-11]. Reasons for this condition are different. The two important parameters in semen study responsible for fertility are total sperm count and percentage of sperm motility[12]. Semen studies concentrated working on these two important parameters. It is reported an interdependence of these two parameters exists[12]. Normal sperm count is widely accepted as 40 millions/ml. Macomber and Sanders have introduced the sperm counting technique in 1929[13]. They considered a man as normal where spermatozoa at or above 60 million/ml was present. Since then several workers contributed in similar form and their conclusions are shown below.

Investigators	Lower limit for normozoospermia count In million/ml
Macomber, Sanders (1929)[13]	60
Mac Leod, Hotchkiss (1946)[14]	60
Amelar, Dubin, Schoenfeld (1973)[2]	40
Eliasson (1973)[15]	40
Skandhan,Mazumdar (1983)[12]	40
Mac Leod, Gold (1951)[16]	20
Baeyertz (1967)[17]	10
Santamauro et al. (1972)[18]	10

Abstinence prior to semen collection is an important factor which function as responsible for showing the values of count and motility. Daily ejaculation increases pH, decreases the volume of semen, sperm count and sperm motility showing the importance of maintaining abstinence prior to semen collection[19]. In bulls, the increased pH in repeated ejaculation seen is related to increased secretion of bulbourethral gland[20]. We have identified pseudo causes for male infertility[21]; 1.Wearing tight undergarments leading to oligozoospermia or azoospermia [22] ; 2. Directly hot water falling on scrotum while taking bath in sitting posture; 3. Drivers of three wheeler vehicle whose engine is fitted below driver's seat and 4.Traders as well as clerical staff daily sitting long time, in all these cases spermatogenesis is disturbed due to increased temperature in testis causing damage to spermatogenesis. Harrison [23] reported increase in temperature in testis disturbs spermatogenesis. Spermatozoa production was calculated as 1.4 to 6.3 times 10^6/gram of testis[24] which may reduce due to increase in temperature. Pollutants in environment like DDT (Dichlorophenyl trichloro ethane, $C_6H_4Cl_2$) is reported as toxic to male reproductive system[25]. One of the causes of infertility even in normal semen is shown as antigens present in it or on spermatozoa causing agglutination or immobilization [26,27]. The discrepancy in semen report of person from different laboratories was experienced by many. One of the probable causes is difference in abstinence maintained prior to submission of sample. Daily collected semen specimen showed difference in sperm count and motility[19]. Aim of the present study is to understand if any difference in semen picture of persons who submitted sample on three occasions after maintaining an abstinence for fixed five days.

2. MATERIALS AND METHODS

The present study was conducted in the Institute for Post Graduates Studies and Research in Ayurveda, Gujarat Ayurved University. The study was initiated after getting approval from Institutional Ethical Committee GAU/PGIR/EC/7-17. Prior to the study, participants were informed about the study and their consent was taken. They were assured not publishing their identity. The study was conducted in two months period of summer season. All 35 men who participated in this study gave their consent. They belonged to the age group of 21-23 years. They had no previous history of andrological pathology. Their habits were uniform; no medications, unusual meals, physical exercise or sexual habits. They submitted semen samples on three different occasions after maintaining an abstinence of fixed number of 5 days[19]. All precautions were taken while collecting sample; which was done by masturbation close to the laboratory at 8.00 am (± 30 minutes) and the samples were collected without missing even a drop onto well cleaned container provided from laboratory [28] Throughout the study in laboratory, same brightness of light and on microscope the intensity of light was maintained [29]. Each sample was carefully evaluated [1, 2, 15, 30]. The percentage of sperm motility was rated from 100 (all sperm motile) to 0 (no motile sperm) [29]. The final assessment was percentage of sperm with rapid linear progressive (RLP) motility, slow linear progressive (SLP) motility, non-progressive (NP) motility and non-motile (NM) [31, 32]. Standard decimal system for percentage was followed by majority of laboratories [33].

3. STATISTICAL ANALYSIS

We have done statistical study to find out significant difference if any, was present among three groups. The simple method we have employed was Graphpad Instat (https://www.graphpad.com/scientific-software/instat/).

4. RESULTS

The study was completed in two months period of one season. The result of the three samples after five days abstinence is presented in Table 1. Viscosity, liquefaction time and pH remained within normal range. Fructose was present in all samples. Semen volume showed a very minimal fluctuation. Fluctuation was present in total sperm count, RLP, SLP, NP, NM and IM but statistically no difference was seen (Table1). Morphology of spermatozoa was within normal level.

Table 1 : Variations observed in 3 semen samples of 35 participants after maintaining 5 days of abstinence

Parameter	Mean of 35 samples				Range of fluctuations
	1	2	3	Mean	
Volume (ml)	1.6	1.7	1.6	1.6	0-0.1*
Total sperm Count (mill/ml)	42.6	42.1	45.3	43.33	0.5-3.2*
Motility%					
RLP	12..6	15.6	11.1	12.1	1-3.5*
SLP	22.6	28.8	28.2	26.53	0.6-6.2*
NP	22.7	22.7	19.4	21.6	2.2-3.3*
IM	42.1	32.9	41.3	39.76	5.4 - 13.8*

* ***Statistically insignificant***

5. DISCUSSION

Results of painstaking efforts of several research workers on human semen gave us the details of the present knowledge. Semen study is the most suitable diagnostic tool to understand the fertility potential of a male [1,2]. Biological variation in semen parameters is present[37]. Within subject biological variation is known [29, 35, 36, 38]. The aim of the study is to understand within subject variation in semen parameters, after maintaining a fixed number of five days of abstinence. Change of place, season, time and mode of collection of sample shall influence the results. Care was taken to exclude them. Selection of subjects is important. Age is an important factor as the quality of semen may not be same at all ages. Advanced age deteriorated the semen quality affecting especially progressive motility [37, 38] and morphology [38]. Participants of this study were young, physically and mentally healthy. Their difference in age was 1-2 years. Our participants maintained an abstinence for a fixed number of 5 days considering it as a responsible factor for determining the quality of semen [29]. Reports showed semen parameters reduced significantly after an abstinence of 4[30], 18 [39], 24 [19, 28, 40] or 36 hours of collection [39]. Abstinence advised by different authors differed from 2 [39], 3[41], 3-5 [19, 41], 2-7 [1], 3-7 [42] to 3-8 days [43]. Abstinence of long period is associated with an increase in dead and abnormal shaped cells [44] and fall in semen quality [45]. It is not advisable to characterize a man's semen quality by evaluating single semen sample [1]. Base line data of semen is made by examining 2 or 3 samples [38, 46, 47]. Before characterizing a man as normal or infertile, multiple ejaculates have to be studied [46]. WHO [48] recommends repeated two semen samples for analysis. Others suggested three [49] or four [50] samples for full account of sperm motility. Three[51] or four samples[49] were preferred to ascertain sperm count. Experimental studies differed preferring hours or days according to the plan of the study[19, 30, 39]. However, most intra individual variation in semen parameters are not explained with duration of abstinence[45]. Quality of semen specimen depends on the mode of collection[34, 53-55]. Masturbation is the accepted method for a thorough semen analysis[34]. Our subjects collected samples by masturbation onto specially designed simple glass device which excluded error in volume as no transferring of sample from one to other[31]. Wrong collection may lead to loss of a fraction of the ejaculate and show low in semen volume and possibly other parameters. Ejaculates produced by masturbation and collected into containers in a room near the laboratory can be of lower quality than those recovered from non – spermicidal condoms used during intercourse at home[55], probably due to the difference in types of sexual arousal[56]. Sperms rapidly lose motility after collection done by electro ejaculation or penile vibratory stimulation[57]. In animals, electrical stimulation made difference in semen parameters[54]. The intensity of light is an important factor for motility. It was shown that sperm motility was higher in percentage and quality and survived for longer time in dark[32]. A change in light intensity lead to change in sperm motility. Throughout the study, brightness in the laboratory as well as on microscope was carefully maintained. The present study was completed in two months period of one season to exclude any change in semen parameters due to seasonal changes. De Giorgi et al.[58] in a retrospective study of 11 years observed a positive correlation between semen parameters, total sperm count and percentage of sperm motility and seasons. Sperm count was seen more in winter[58] and sperm motility was seen in summer. Levine et al.[59] observed semen quality deteriorated during summer. They explained this factor as responsible for the reduction in birth rate during spring season in regions with warm climates. Ombelet et al.[60] observed a chronobiological fluctuation in semen parameters. The test for viscosity was done and the grade was given from 0 (normal) to 4+[2]; the thickness of sample may also be caused by large number of sperms[2]. Ray et al.[61] did not find any significant difference in the viscosity of normal and azoospermic semen. High viscosity may be associated with poor liquefaction[34]. Highly viscous semen causes poor sperm transport[34]. In the present study viscosity remained normal in all samples. Human semen coagulum liquefies within 5-20 minutes; which is earlier in vivo than in vitro[62]. Non liquefied coagulum leads to infertility as spermatozoa gets entrapped. When liquefaction starts, an amorphous material consisting of small globules appear on the surface of fibers until which disappear and globules appear[63]. Liquefaction time observed in this study was within normal range. Semen pH was studied immediately after the liquefaction of sample. Average value observed in this study was 7.2. Semen pH increases with time. when natural buffering decreases , pH falls below 7 [64]. Change in pH is possible due to ejaculatory duct obstruction or congenital bilateral absence of the vas deferens or change in season[58]. In the present study semen was collected into a specially designed container which directly measures the volume thus exclude the error otherwise would have caused by transferring sample from one to other. The mean semen volume observed in this study was 1.6 ml. There was no statistical difference among three groups of samples included in this study (Table 1). Similar result was seen when abstinence period was 3-5 days[19]. Other reports show semen volume as 2-6 ml[15,42,54]. Semen volume is reported as low as 1.1 ml [12, 53, 65]. Normal sample with 11[12] or 20 ml[66] is also reported. Total volume decreases with daily [19, 34] or frequent ejaculation[35]. After prolonged abstinence, semen volume increases with increase

in the death and abnormal shape of cell [2, 44] and fall in semen quality[45]. In retrograde ejaculation in androgen deficiency reduction in volume is reported [1]. Other known cause is obstruction of the ejaculatory duct or congenital bilateral absence of the vas deferens[64]. We observed fructose in all samples; which is secreted by seminal vesicle and ampulla[54,59]. Fructose in normal semen is from 5-800 mg/ ml[60]. We observed it from 36-139 mg/ 100 ml[67]. Inter individual variation of fructose in semen exists [60]. It is the main glycolysable sugar and fructolysis represents the major metabolic process. It induces motility and fertilizing ability of spermatozoa[54].Which is influenced by age [68] and inflammatory conditions of SV[69]. The true glucose content of seminal plasma reported by Eliasson[70] was 5 to 10mg/100ml. We observed it from 0-83 mg/100 ml[67]. Though the glucose level is very low, the main energy source of spermatozoa is glucose[70.] Which alone may be sufficient to maintain the energy for sperm cells to travel some distance. The two important interdependent parameters of semen which deserved more attention are sperm count and motility[12]. Sperm concentrations in semen from young and old men may be the same, but total sperm numbers may differ due to decrease output in both the volume and total sperm in old age[71]. Present study on three different days showed total sperm count, from 42.1-45.3 mill/ml (Table1). No significant difference among three samples was seen (Table 1).The difference in three groups was anticipated due to biological variation. The study of 20 normal subjects submitted samples for 10 times, once a week with an abstinence of 3-4 days[72] observed wide biological variations within subjects on sperm concentration though the production of sperms on daily basis remains same[36]. The abstinence for number of days leads to increased number of spermatozoa[73]. Daily spermatozoa production is not influenced by period of abstinence [33]. A hypothesis predicts increase in sperm production after repeated ejaculation after keeping short duration of abstinence[74]. A significant association between long abstinence and increased total sperm count is shown[34, 36, 39, 75, 76]. With frequent ejaculation, the number of sperm in cauda epididymis[77] and in semen is reduced [19, 47, 73]. Normal lower limit of the total sperm count is considered as 60[13], 40 [2, 15] or 20 million per ml of semen[18]. The size of the testis influences the total number of spermatozoa per ejaculate[78]. We have observed variation in percentage of RLP, SLP, NP and IM (Table 1). Variation in all types of motility as well as immotile sperms was seen. We consider this variation as biological and a natural phenomenon. Statistically the difference seen in each sperm motility pattern was insignificant. All these factors were taken care in our study. The percentage system for sperm motility is the best method for assessment [30]. Some follow grading system[31]. Different factors like length of abstinence, temperature, time gap between sample collection and evaluation, infections, diseases or other pathological conditions affect sperm motility. After maintaining an abstinence of 18-30 hours, no significant difference in motile sperm count was seen[39]. Sperm motility provides vital information on the functional competence of spermatozoa. In men, different patterns of motility seen are vibratory, circular, darting, rotating and asymmetrical[32]. Types of movement influence fertilizing capacity. Straight swimmer succeed in fertilizing an ovum[79]. Total percentage and quality of motility of spermatozoa deteriorated in light; in dark the quality of motility remained superior and they survived for long number of hours[36]. In the present study, we did not find any significant difference in sperm motility of any nature (Table 1). Alvarez et. al[72] observed insignificant difference in total percentage of motility among their subjects. We observed an increase in percentage of progressive sperm motility in daily ejaculates[34]. The reason is likely to be the minimum exposure time for sperm in epididymal micro environment. Epididymis may not be a suitable place for sperm to initiate and maintain motility[80]. The different morphologic types of mature spermatozoa were counted by the examination of minimum 200 spermatozoa[30]. Our results on morphology of sperms were within normal range. We included abnormal shaped and immature cells. Increased numbers of coiled tails of spermatozoa reduce the fertilizing capacity of semen[53, 81]. Long abstinence leads to increase in abnormal shape of sperm cell[44]. After an abstinence of 18-30 hours a significant change in DNA and the percentage of normal morphological sperm was seen[39]. Low level DNA content of spermatozoa considered as a cause for abortion[50].

6. CONCLUSION

In conclusion, this semen study conducted on three different occasions in one season, subjects submitted samples after maintaining five days of abstinence. The difference observed in parameters studied was non-significant. The present study suggests that while conducting semen study, following points like the season, number of days of abstinence prior to semen collection, time and mode of collection are to be considered.

7. AUTHORS CONTRIBUTION STATEMENT

Godatwar P, Skandhan KP, Prasad BS, Mehra BL and Sing G have contributed equally in conducting experimental work, analysing results and preparing the manuscript.

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9. CONFLICT OF INTEREST

Authors have no conflict of interest

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