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Seasonal Variations in Testicular Volume and Seminal Parameters in Human

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ABSTRACT

The present descriptive study was designed to analyze the seasonal variation in the semen parameters and testicular volume in Azad Jammu & Kashmir. This study was included twenty men age ranged from 20 to 30 years. The data was statistically analyzed by Student's T test by using SPSS. It was found that left and right testes have significant variation in summer vs winter and spring vs winter, while no significant difference was found between summer and spring season. Significance difference was found in semen volume in summer vs winter (*p= 0.002*) and spring vs winter (*P= 0.002*). There was no significant difference in semen volume between spring and summer *(p= 0.071*). No significant difference was found in total sperm count percentage, dead sperm percentage and sluggish sperm percentage in three studied seasons of year. Abnormal sperm percentage has shown significant variation in summer vs spring (*p= 0.001)* but no significance difference was found in winter vs summer (*p= 0.42)* and spring vs summer (*p= 0.012).*

Key words: Testicular size, Semen volume, Sperm count, Abnormal sperm, Active sperm, Dead sperm and Sluggish sperm.

INTRODUCTION

Semen is an organic fluid that subsume spermatozoa, which is primitively exposed by Antonie van Leeuwenhoek in 1677, by adopting microscope procuring magnification power of 200 x. Fluctuations in the semen quality is antecedent by some factors such as; season of year, scrotal temperature, geographical region, marijuana use and smoking status (Kidd, Eskenazi, & Wyrobek, 2001). Animals as well as mammals have demonstrated seasonal rhythm at reproductive phase. No seasonal pattern of reproduction is known in human. Testes act as essential and fundamental part of male reproductive system, endocrine system, sperm and androgen (Zorgniotti & Macleod, 1973). Sperms conceive one to five percent of volume of

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semen ejaculation. The quantitative and qualitative range of semen components varies different locations. One to five percent of total semen is covered by testes, twenty five to thirty by prostate gland, sixty five to seventy five by seminal vesicle and less than one percent of semen by bulbourethral glands. Impact of seasonal variations on condensation of chromatin of human sperm was investigated in Germany in 2001 (Badia, Pinart, Briz, Pastor, & Sancho, 2005)

From statistical point of view variation in results of semen are strongly associated with seasons of year. Seasonal variations have significant impact on total count of sperm which were at peak in number in April and also maximum value of chromatin condensation was found in January. There was no significant difference of seasonal variation on motility of sperm in semen fluid (Henkel, Menkveld, Kleinhappl, & Schill, 2001). Pathogenesis of medical condition effects on production of sperm, maturation of sperm and longevity of sperm (Omu, 2013).

To analyze the impact of geographical variations on quality of semen, study was performed in Japan in 2013. 1792 fertile men were comprehended in this study and all these males were belonging to four different areas of Japan having median age of thirty one year. Four groups were organized on their ecological base. Statistical significant divergence was not found in total sperm count but slight difference was found in volume of semen, sperm motility percentage and normal morphology of sperm (Iwamoto et al., 2013).

In 2009, World Health Organization has announced some standard reference values for normal semen characteristics. According to which, volume is about 1.5 ml, pH is about 7.2, sperm concentration is 15 million, total sperm count 39 million per ejaculate, progressive motility 32 percent or more, total (progressive and non-progressive) motility is 40 percent, morphology is 4.0 percent and normal forms vitality is 58 percent. These reference values were not fixed and may deviate from their mean reference value (Cooper et al., 2010). In China, data has been analyzed from 2318 healthy subjects during 1987-1997 which demonstrated that there was no evidence of decline on sperm concentration during the studied period (Zhu, Walker, Oakey, Setchell, & Maddocks, 2004). Another work was also performed by scientist in China from 1981-1986 which shows decline in semen quality during this duration due to the exposure of subjects to environmental pollutants (Li, Lin, Li, & Cao, 2011). It has been reported that there is a large difference in mean sperm concentration among different cities and countries (Swan et al., 2003).

Testes function is regulated by quality and amount of light. Temperature fluctuations of environment disturbs testis function which leads to variations in feedback of hormone secreted by anterior lobe of pituitary gland (Owen & Katz, 2005). Testicular hyperthermia was also moisturized due to destitution of thermoregulation of scrotal temperature and it leads to genital heat stress. Epididymal sperm and testicular germ cells are sensitive to damage by heat stress (Zhu et al., 2004). Exposure of germ cells to heat stress release molecular factors such as anti-apoptotic Bcl-2, pro-apoptotic Bax, cytochrome C, caspases (Kim, 2013).

Quality of semen of healthy men was found to be decreasing with the passage of time. This diminution in semen may be reached to critical level which has impact on reproduction. Enormous reduction in sperm concentration in the semen was reported in European men while limited reduction in sperm concentration was reported in United State men (Rao et al., 2015). Threshold value of semen parameters guides us to define fertility and this concept is fundamentally flawed. Twenty nine studies from United States on semen quality of fertile men

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by meta-analysis suggested that normal mean sperm concentration is 98 million/ml. The normal sperm motility which is considered to be best predictor of fertility is from fifty three to sixty two percent. Inter ejaculate coefficients of variation for sperm concentration and motility are estimated to be 44.7 percent and 15 percent (Natali & Turek, 2011).

Testes perform normal spermatogenic function when temperature of scrotum is constant at 2°C to 3°C which is lower than body internal temperature. Pampniform plexus assists to maintain thermoregulatory mechanism by heat dissipation of scrotum and by counter current heat exchange. Factors that can distract thermoregulatory function of scrotum may result to increase in scrotal temperature which decreases in number of sperm in semen (Thonneau, Bujan, Multigner, & Mieusset, 1998) Expose of testes to very high or very low level of estrogen provokes spermatogenesis process (Sierens, Sneddon, Collins, Millar, & Saunders, 2005). A survey was carried out to get information about methodology of semen analysis. For this purpose, questionnaire was filled from 410 centers related to fertility. According to this study, different methods used to analyze semen. Out of 410 fertility centers only eighty two centers have same methodology to analyze the semen parameters (Ombelet, Pollet, Bosmans, & Vereecken, 1997). It means that there are different methods used to analyze the semen in different areas. Technique called hemizona assay and this assay to determine the potential of sperm to fertilize the egg. Mannual hand cutting and micromanipulation are two methods which are used to cut zona pellucida of human egg. This study was performed to check the results of both techniques and it was concluded that the result of both techniques were comparable. It was concluded that the manual of hand cutting is more useful as compared to expensive micromanipulation (Janssen et al., 1997).

There was lack of data on seasonal variation of semen parameters as well as testicular volume from Pakistan specially Azad Jammu Kashmir. So, the present study was designed to analyze and find out the seasonal variation in testicular size and other seminal parameters in healthy volunteers from AJ&K.

The present study was designed to investigate the relationship of human semen parameters and testicular volume to seasonal variation and also to determine the influence of seasonal variation upon semen quality and testicular volume in human.

MATERIAL AND METHODS

Sampling population

The present study was longitudinal study, including a cohort of about 20 young men enrolled after a written consent.

Inclusion and exclusion criteria

All the men included were normal, healthy and between the age of twenty to thirty years. The vigorous physical workers, smokers, occupationally exposed to heat and radiations and drug addicts were excluded from the study. Moreover, men with known psychological, physical or reproductive factors were excluded from the study.

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Data collection

The ethnicity, age, abstinence period, smoking status, occupational exposure or hazards, presence or absence of any psychological, physical or reproductive factor were recorded. Ejaculation time, fever and abstinence period were also recorded.

Testicular volume

Vernier caliper

The volume (V) of the testes was calculated by using vernier caliper. The length (L), breadth (B) and thickness (T) of the testes were calculated with the help of vernier caliper and recorded. Testicualr size was then estimated by using the following formula (Setchell and Waites, 1964).

$$V = 4/3 \times \pi \times L/2 \times W/2 \times T/2.$$

Semen collection and analysis

Collection

Semen samples from men were collected in private room by masturbation after three days of abstinence. Sterile plastic bottles having wide mouth were used to collect semen samples. Samples were brought into laboratory within 30 minutes of collection.

Analysis

The seminal fluid analysis included; the macroscopic properties of the semen such as volume and liquefaction time. The microscopic tests included concentration of sperm, motility of sperm and morphology of sperm. According to World Health Organization guidelines volume of semen, total sperm count, motile sperm percentage, normal and abnormal sperm percentage were count to analyze the semen. After liquefaction of semen in incubator, all assays were performed within one hour of semen analysis. The semen samples which remained viscous were liquefied by mechanical method by using disposable pipette.

Liquefaction time

Liquefaction time was calculated by gentle aspiration in pipette having size of 5ml. Then the semen was allowed to drop by gravity and the length was observed. Small discrete drops were formed by the normal semen sample. In case of abnormal consistency, thread formed by drop was greater than 2cm. Another method used to estimate consistency was performed by introducing a glass rod into the sample and was observed the thread on withdrawal of the rod.

Semen volume

The volume of semen was measured by aspiratory the semen in graduated micropipette of 5 ml along with disposable tips.

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Sperm concentration

Neubauer haemocytometer was used to measure the concentration of sperm. Dilution of 1:20 was made by using 50 µl of semen, 950µl of sperm diluent solution was prepared by sodium carbonate (50g) and formalin (10 ml of 35 percent v/v). Optionally trypan blue (0.25 mg) or saturated aqueous gentian violet (5 ml) were added to water which was distilled to make the final volume of solution upto 1000 ml. 10 µl of solution was used from well shake mixture and introduced to slide chamber and then covered by glass cover slip. Another chamber was used for another sample. Spermatozoa were counted by using binocular microscope. Spermatozoa with tail were counted in both chambers and if the difference between two chambers was greater than 10 percent than another haemocytometer was set to repeat it.

Sperm motility

The motility of sperms was determined by using the slide method. The sperm which

covered the distance of 20µm in one second was supposed to be actively motile.

Classification of 100 different spermatozoa was done by this method.

Sperm viability

One drop of eosin was added at room temperature to one drop of semen to distinguish between live and dead sperms. Membrane of cell through which stained passed was considered as dead cell while cell which was unable to stain was considered as living cell. At least hundred spermatozoa were classified as either dead or alive.

Sperm defects

Technique of traditional feathering was used to make the smear of spermatozoa. In this technique, a drop of semen on surface of one slide was dragged by edge of another slide. Meyer's haematoxiline, Harris haematoxiline and Giemsa staining were used to determine head defects, shape defects and size defects.

Statistical analysis

All the quantitative semen parameters and testicular volume were compared among different seasons of the year by student's T test for paired sample using SPSS.

RESULTS AND DISCUSSION

Semen parameters and testicular size varies in different seasons of year human. The current study was a longitudinal study, designed to analyze the seasonal variations in contrasting semen parameters and testicular volume. In this study the mean size of right and left testes was asset to be significantly larger (*p=0.000*) in summer as compared to the size of testes in winter. The size of right and left testes was also significantly larger in spring having *p value* of *0.000* and *0.001* as compared to winter. While there was no significance difference in the size of testes between summer and spring, *p value* for variations in right testicular volume was *0.782* and for left it was *0.74*. In all these comparisons, the testicular size has shown strong positive correlation (Table I). Different factors are responsible for variation in testicular size such as temperature,

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area and altitude. In the multivariate model, high temperature was found to be associated with a decrease of size of testes, but moderate and low temperature did not effect on testicular size (Ku, Kim, Jeon, Lee, & Park, 2002). Strong positive relationship lies between size of testes and testosterone circulating level. Stipulation of testosterone has significant effect on production of androgen (Preston et al., 2012).

Semen volume is crucial parameter in male reproductive health. The typical volume of semen is 2.5 ml (Iwamoto, Nozawa, & Yoshiike, 2007). Another study has delineated that typical volume of semen is about 1.5 ml (Franken & Oehninger, 2012). The mean semen volume was computed to be 3.20 ± 0.89 ml in winter, 4.28 ± 0.73 in spring and 3.85 ± 0.73 in summer. Mean semen volume in the studied samples was significantly larger in summer (*p=0.002*) and spring (*p=0.001*) as compared to winter while there was no significant difference between spring and summer having p value *0.07*. Accession activity of accessory sex gland results in increase of semen volume (Pérez-Pé, Cebrián-Pérez, & Muino-Blanco, 2001). In spring season, glands work more actively as compared to summer and winter so, volume of semen in spring was found to be greater. The normal sperm count ranges from 40 million to 300 million per ml of semen. 20 million counts per ml may be fine if their physiology and morphology is normal. One of another manual of 2012 has shown that normal sperm count ranges from 33-36 million (Franken & Oehninger, 2012). Mean value of total sperm count was found to be 82.6 ± 18.94 million in winter, 80.25 ± 16.67 in spring and 80.60 ± 18.39 in summer. No significant difference (*p= 0.94*) was found in total sperm count of the studied subjects among different seasons of the year (Table II). In human, the production of sperm was remarked to be reduced when it exposed to increase in the temperature of testis. Concentration of the sperm in the semen was at peak in spring season as compared to other season (Chennaiaha, Rasheedb, & Patilb). An association was also detected between high level of phthalate and reduced sperm count. It has been noticed that mobile phones have adverse effect on sperm count as well as other parameters of semen (Jurewicz, Hanke, Radwan, & Bonde, 2009).

According to manual of WHO, about 40 percent of sperm having accurate morphology in semen sample is revolved as normal range(Cooper et al., 2010). The average percentage of abnormal sperm was found 26.25 ± 12.71 in winter, 34.3 ± 14.44 in spring and 24.40 ± 11.59 percent in summer. This abnormal sperm percentage in semen was significantly higher in spring (*p=0.012*) as compared to winter and summer (*p=0.001*). The mean percentage of abnormal sperms was statistically equal between winter and summer. A protein is synthesized by liver called sex hormone binding globulin (SHBG) and function of this protein is to help in the transport of sex hormone testosterone and estradiol. Testosterone circulates in blood is mostly attached to SHBG and albumin protein and decrease in the level of SHBG and circulating testosterone in the body may reduce the quality of sperm. Fifty percent of sperms should be active to fertilize the egg (Cohen-Dayag, Tur-Kaspa, Dor, Mashiach, & Eisenbach, 1995). Percentage of active sperms was calculated to be 61.65 ± 22.17 in winter, 56.70 ± 15.49 in spring and 62.25 ± 23.06 in summer. There was no statistically difference among the percentage of active sperm in different studied seasons (Table III). Previous studies have reported that in spring, pesticides such as DDT (Dicholoro diphenyldichloroethylene) and DDE (Dichloro Diphenyl ethylene) are used to kill the pests (Kidd et al., 2001). These pesticides can also affect the sperm motility if human are exposed to them.

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According to WHO about 58 percent of live sperm in given sample of semen are considered as reference value for achievement of pregnancy in ≤ 12 months. In winter, the mean percentage of dead sperm was found to be 27.85 ± 20.29, 32.25 ± 12.69 in spring and 25 ± 18.96 in summer. The highest percentage of dead sperm was computed in spring which is significantly higher as compared to winter. In young and healthy men of United State, impact of seasonal variations on amino acid was measured in summer, winter and fall season of year. The amino acid found in men were valine, threonine , serine, methyl histidine, histidine, lysine, glycine, glumatic acid, cysteine, agrnine and alnine. Significant difference was shown by some of amino acid were alanine, serine, lysine, glysine, glutamic acid, cysteine and argentine (Cooper et al., 2010). This seasonal change in amino acid level may lead to change in dead sperm percentage in different seasons of year. Sluggish sperm is diminished of sperm motility and normal forward sperm progression of other terms of sperm which we analyze under microscope. In man, testis require steadily kept scrotal temperature of 2-2.5 ^{0}C lower than normal core body temperature (37 ^{0}C) and is maintained by pampiniform plexus and by scrotal dissipation of heat (Capitalists & Nilekani). The sluggish sperm percentage was also measured in all three seasons. Mean percentage of sluggish sperm was found to be 10.15 ± 3.84 in winter, 12.7 ± 7.12 in spring and 13.25 ± 6.75 in summer. There was no statistically significant difference in sluggish sperm percentage in different studied seasons (Table IV). Sulphyldryl are important antioxidants which secure the spermatozoa to react it with reactive oxygen species. Decrease of these antioxidants is accounted for cell death due to oxidation occurs on spermatozoa membrane. Decrease in level of these antioxidants in summer may also leads to Greater percentage of sluggish sperm in summer (Lampiao, 2009). The graphical representation of all tabulated data has also shown in fig 1, 2 and 3.

CONCLUSION

From present study it was concluded that seasonal variations have significant as well as non significant affect on testicular volume and seminal parameters.

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Statement of conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this article.

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Figures

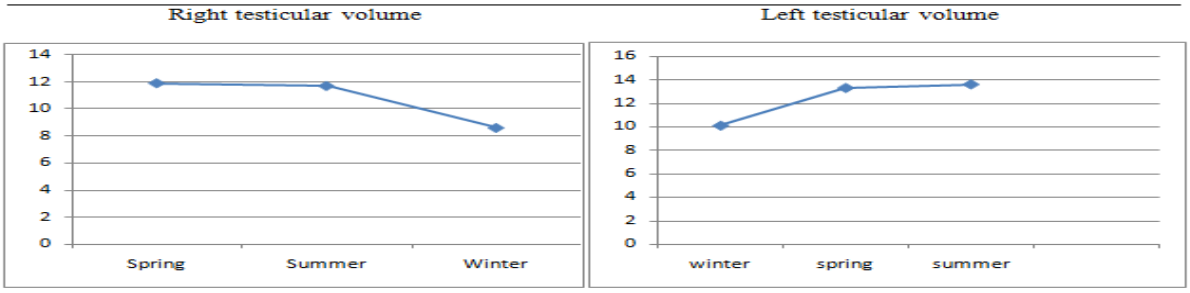

Figure 1. Graphical representation of right and left testicular volume in cm³ on Y axis along with month variations on X axis

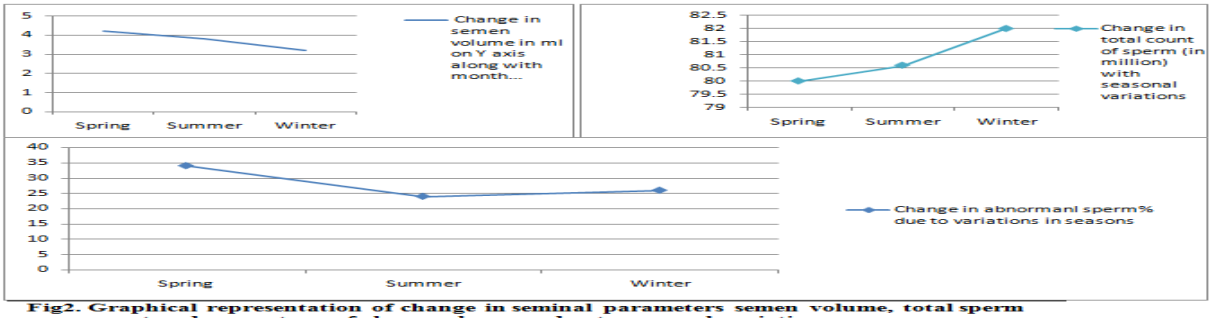

Fig2. Graphical representation of change in seminal parameters semen volume, total sperm count and percentage of abnormal sperm due to seasonal variations.

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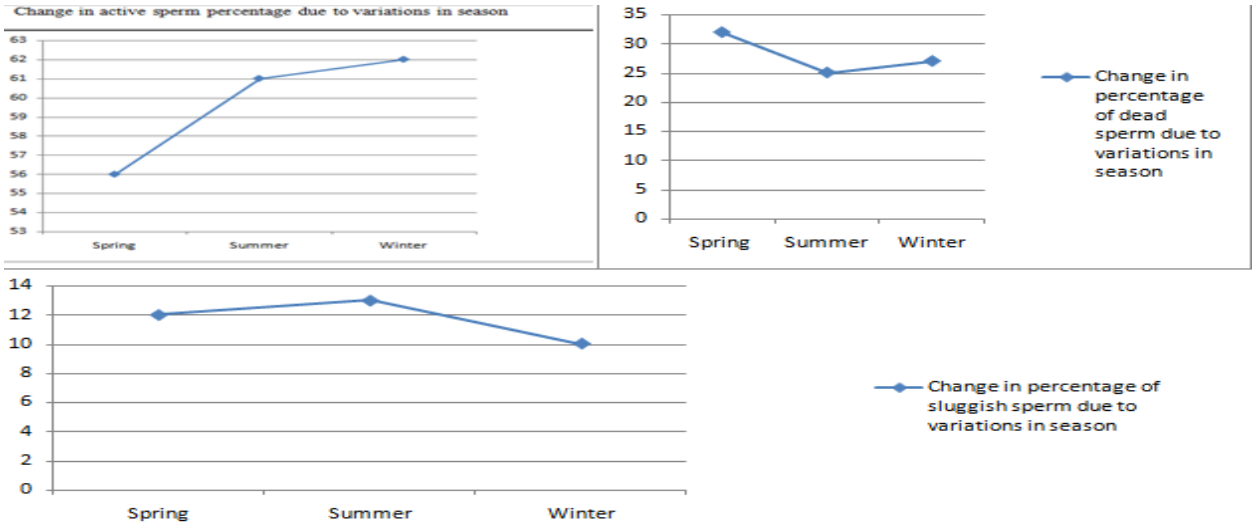

Fig3. Graphical representation of change in seminal parameters percentage of active sperm, dead sperm and sluggish sperm due to seasonal variations.

Tables

Table I. Comparison of left and right testicular size among different seasons.

Right testicular volume						Left testicular volume					
Comparison	Mean Difference	*P Value*	95% CI for Difference	R	*P Value*	Comparison	Mean Difference	*P Value*	95% CI for Difference	R	*P Value*
Summer vs Winter	3.12	*0.000*	1.82 - 4.40	0.85	*0.000*	Summer vs Winter	3.55	*0.000*	2.20-4.88	0.84	*0.000*
Spring vs Winter	3.36	*0.000*	4.78-1.94	0.92	*0.000*	Spring vs Winter	3.24	*0.001*	1.53-4.95	0.79	*0.000*
Spring vs Summer	0.25	*0.782*	-1.66 -2.16	0.78	*0.000*	Summer vs Spring	0.31	*0.74*	1.56 -2.16	0.76	*0.000*

Table II. Comparison of semen volume and total sperm count among different seasons of year.

Comparison of Semen volume among different seasons						Comparison of Sperm count among different seasons					
Comparison	Mean Difference	*P Value*	95% CI for Difference	R	*P Value*	Comparison	Mean Difference	*P Value*	95% CI for Difference	R	*P Value*
Summer vs Winter	0.66	*0.002*	-1.04-.27	.490	*.028*	Winter vs Summer	2	*.705*	-8.90-12.90	.22	*.346*
Spring vs Winter	1.08	*0.001*	-1.64-.55	.412	*.071*	Winter vs Spring	2.35	*.615*	-7.27-11.97	.34	*.615*
Spring vs Summer	0.43	*0.071*	-.04-.90	.57	*0.009*	Summer vs Spring	0.35	*.94*	-9.45-8.75	.39	*.937*

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Table III. Comparison of abnormal sperm and total active sperm among different seasons of year.

Comparison of abnormal sperm among different seasons						Comparison active sperm among different seasons					
Comparison	Mean Difference	*P Value*	95% CI for Difference	R	*P Value*	Comparison	Mean Difference	*P Value*	95% CI for Difference	R	*P Value*
Winter vs summer	1.85	*0.422*	-6.04-3.34	.659	*0.002*	Summer vs Winter	0.6	*.803*	-5.57-4.34	.891	*0.000*
Spring vs Winter	8.05	*0.012*	3.06-14.54	.556	*.001*	Spring vs Winter	4.95	*.057*	-.158-10.6	.892	*0.000*
Spring vs Summer	9.9	*0.001*	-15.15-5.15	.672	*.000*	Summer vs Spring	5.55	*0.90*	-12.06-.96	.809	*0.000*

Table IV. Comparison of dead sperm and total sluggish sperm among different seasons of year.

Comparison dead sperm among different seasons						Comparison sluggish sperm among different seasons					
Comparison	Mean Difference	*P Value*	95% CI for Difference	R	*P Value*	Comparison	Mean Difference	*P Value*	95% CI for Difference	R	*P Value*
Winter vs Summer	2.85	*3.28*	-3.09-8.79	.793	*0.000*	Summer vs Winter	3.1	*.098*	6.31-6.831	-.062	*.794*
Spring vs Winter	4.4	*.172*	-10.88-2.08	.740	*0.000*	Spring vs Winter	2.55	*1.78*	-6.37-1.27	-0.17	*.942*
Spring vs Summer	7.25	*.018*	1.41-13.09	.758	*0.000*	Summer vs Spring	0.55	*7.37*	-2.83-3.93	.459	*.042*