

# Could There Be An Association Of Blood Group O And Nutrition Level With The Seminal Parameters Of Individuals Who Suffer From Fertility Issues?

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**Abstract:** A cross sectional study (n = 105) was carried out on written consent in a fertility clinic of Sri Lanka during the period of August 2014 to August 2017 to find out whether there was a relationship of nutrition level (measured anthropometrically) and blood group O with seminal parameters such as seminal volume, count, mortality and morphology. Thus, the study was aimed to find out the biological effect on male infertility due to the fact that the male infertility has become increasing in the modern society irrespective of the economic status. The selected individuals were evaluated separately for seminal analysis (WHO method), anthropometric analysis (mid arm circumference) and blood group analysis (WHO method). It was found that neither nutrition level nor blood group O had a significant effect on seminal parameters so as the male infertility. Thus, the myth which is prevailing in the society that the blood group O bearers are more prone to be infertile is collapsing under the outcome of the study. The nutritional level of someone which was found via the mid arm circumference also has no significant effect on the seminal parameters of men with fertility issues. This could be due to the reason of satisfaction of the nutrition level of the selected study group as the infertile men are advised to maintain a good healthy diet. Anyway, this could be a better trend.

**Key words:** blood group, mid arm circumference, nutrition level, semen parameter, male infertility

## 1. Introduction

Male factor infertility, which contributes 30-40% for the infertility<sup>(1)</sup> has become a psychosocial issue in the modern society. The men's fertility, mainly depends on the quality of seminal parameters such as seminal volume, count, motility and morphology. The deviation of these parameters from their normal reference range could cause poor quality semen, which ultimately give rise to the male infertility. Various conditions and situations can cause for poor quality semen. Varicocele, infections (gonorrhea), hormonal imbalance (low level of testosterone), lifestyle factors (obesity, smoking), environmental factors (radiation, heat) are some of the examples for them<sup>(2), (3)</sup>. However, in most of the cases the male infertility is idiopathic. A myth is prevailing in the society that the blood group O bearers are more prone to be infertile. The blood group O bearers contains the anti A and B antibodies in the serum. Sometime this could have an effect on the semen production via a serological effect. Anyway the studies on the topic is a handful. The nutritional level of someone can also affect on seminal parameters. The formation of spermatozoa could depend on the nutrition of someone as it occurs in the other cells. The nutrition level can be assessed in various ways. One is anthropometric assessment which measures the mid arm circumference. The mid arm circumference, proportionate to the level of nutrition of a human being. Thus, the study was carried out to assess the effect of nutrition on seminal parameters via the measuring of mid arm circumference. The studies on the objective is less.

## 2. Methods and methodology

All procedures performed in the study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The consent of patients were obtained before the study and ethical approval was obtained from the ethic review committee of the institute where the study was carried out.

### 2.1. Design<sup>(4)</sup>

The cross sectional design was selected for the study due to the reasons such as;

- Ability to access many outcomes and risk factors
- Inexpensive feature
- Less time consumption
- Possibility of generating hypothesis to build up relevant studies.

### 2.2. Method

The male partner of infertile couples who visited the fertility clinic of an institute of Sri Lanka during the period of August 2014 – August 2017 was involved in the study. The individuals who wished to take part in the study were evaluated on exclusive and inclusive criteria on the consent.

### 2.3. The sample size<sup>(5)</sup>

$$\text{Sample size} = 4 \frac{Z_{\alpha}^2 P (1-P)}{D^2}$$

$Z_{\alpha}$  = standard normal deviate (at 95% confidence interval = 1.96)

P = prevalence of male infertility (8%)<sup>(6)</sup>

D = Total width of confidence (0.125)

Thus, the sample size at 95% of confidence interval =  $\frac{4}{(1.96)^2 \times 0.08 \times 0.92} = 72$

0.015625

N = 72

### **2.3.1. The control group**

The age matching, number equal (as much as possible) men who were compatible with each independent variable (blood group O, mid arm circumference) were selected from the same study population for the control groups<sup>(7)</sup>. These individuals were having normal semen profiles and their spouses were having the fertility issues. The control was considered from the same population to maintain the location equality<sup>(7)</sup>

### **2.4. Criteria to select the subjects**

The subjects were selected as follows according to the internal and external criteria.

#### **2.4.1. Inclusive criteria**

- All the male, who were over 18 years old.

#### **2.4.2. Exclusive criteria**<sup>(8), (9), (10)</sup>

- Individuals, who had been suffering from systemic diseases such as diabetes, hypertension, cancer, arthritis during the period (the conditions and the drugs used for could affect the reproductive hormones and sperm production in the testis).
- Individuals, who had been on drugs relevant to above disease conditions.
- Individuals, who had addicted to recreational drugs such as marijuana, abin and ganja (chemicals in the drug could cause negative effect on the sperms synthesis).
- Individuals, who had been on anti-gastric drugs such as cimetidine or any steroidal drugs (increase the hormone, prolactin which negatively affect on sperm production).
- Individuals, who were with pathological issues in reproductive system (varicocele, testicular problems, varicocele may raise the temperature in the area which could be unfavorable for the production of sperm).
- Individuals, who were unable to communicate (dumb, deaf and mentally handicapped).
- Individuals, who were on fertility treatment at the time (the semen quality could be changed on the ongoing treatment)
- Individuals, who were unwilling to participate in the study.

The subjects who were satisfactory according to the criteria, were selected for the study and interviewed orally to gather the essential demographic data such as age, residence, contact numbers. Then, the subject was asked to provide a semen sample (3 days abstinent from ejaculation). Finally, a blood sample was collected in to a plane tube from the subject under well aseptic condition for the purpose of dining the blood group of subject.

### **2.6. The laboratory investigations**

Laboratory investigation on semen and blood samples were made as follows.

#### **2.6.1. The blood grouping (WHO standard procedure)**

##### **Materials:**

1. Individual's anti-coagulated blood sample
2. Disposable plastic pipettes (1 per each blood sample)
3. Test tubes and racks
4. Microscopic glass slides
5. Group A, B and O blood
6. Antisera A reagent
7. Antisera B reagent
8. Anti-Rhesus reagent
9. Normal saline
10. Microscope
11. Refrigerator
12. Centrifuge

It is common in serological procedures, to prepare a 5% of red cell suspension to increase the sensitivity of outcome. Thus, in the procedure of identification of blood group a 5% of pooled cell samples were prepared for each group of A, B and O blood samples as follows. The three groups of each was found from the subjects who were aware themselves group and were confirmed from an accredited laboratory. Each anti-clotted blood samples were centrifuged at 1000 rpm for 1 minute to get separated the cells from serum. Thus, the cellular portion of each was added in to three test tubes separately and the tubes were filled up to  $\frac{3}{4}$  with normal saline. The mixture was mixed up gently and centrifuged at 1000 rpm for 1 minute to wash and clean the separated portion of cells. Eventually, a drop of washed cells from each centrifuged sample was added into a clean separate test tube and was mixed up with 19 drops of normal saline to prepare a 5% of red cell suspension. The suspensions were stored in 4 °C.

- Quality control of reagent

Each antisera vial was checked carefully for precipitate, gel formation, turbidity or color change and any change would cause to discard the reagent. Further, a drop of A, B and any Rh positive pooled blood cells were mixed up with 2 drops of anti-sera A, B and D (anti Rh reagent) in 3 separate tubes respectively and the outcome was checked for the agglutination. Non formation of agglutination would cause to discard the relevant antisera. For the negative control, all the cell types were replaced with O-cells and any formation of agglutination would cause to discard the relevant reagent.

- Blood group identification

Both serum as well as red cells of each individual were tested for an accurate result according to the WHO standard operating procedure (SOP) of identification of blood groups.

- Testing of red cells

Individual's anti-clotted blood sample was undergone for above-mentioned procedures to prepare a 20% cellular suspension (at the final stage one drop of washed red cells was diluted with 4 drops of normal saline). Then, the 4 test tubes were labeled as A, B, AB and Rh. Each test tube was added with 2 drops of antisera from reagent of A, B, AB and Rh respectively. This was followed by adding of 1 drop of individual's red cells from particular 20% suspension. The mixtures in all tubes were shaken gently and the formation of agglutination was observed after 3 minutes. The result was confirmed over the microscope. Pooled AB cells with anti AB reagent and pooled O+ cells with anti-rhesus reagent were reacted as positive control for the ABO and Rh system respectively. Further, O cells with anti AB reagent and O- cells with anti Rh reagent were reacted as negative control for the same system.

- Testing of serum

A drop of pooled red cells (5% suspension) from each group A, B and O were added in to 3 test tubes separately and 2 drops of serum of individual was also added to each. Tubes were mixed up gently and observed for the agglutination after 3 minutes. Every agglutination and non-agglutination was confirmed over the microscope. Pooled serum of AB group and AB red cells were considered as negative control and pooled serum of O group and AB cells were considered as positive control. The results were interpreted according to the following table.

**Table 2.1:** Interpretation of blood group according to the pattern of agglutination

Cell test/forward grouping				Serum test/reverse grouping			Blood group
Reagent Anti A + Test cells	Reagent Anti B + Test cells	Reagent Anti AB + Test cells	Reagent Anti Rh + Test cells	Pooled A cells + Test serum	Pooled B cells + Test serum	Pooled O cells + Test serum	
+	-	+	+	-	+	-	A+
-	+	+	+	+	-	-	B+
-	-	-	+	+	+	-	O+
+	+	+	+	-	-	-	AB+
+	-	+	-	-	+	-	A-
-	+	+	-	+	-	-	B-
-	-	-	-	+	+	-	O-
+	+	+	-	-	-	-	AB-

Positive - agglutination is positive

Negative - agglutination is negative

Contamination as well as inadequate mixing of antisera and blood could limit the test outcome. Thus, it was concerned to avoid the problem.

## 2.7. Semen analysis<sup>(11)</sup>

The semen samples obtained from the subject were analyzed as follows.

### Materials required

Semen sample

Semen diluting fluid

Measuring cylinder (10 ml)

Sahli pipette

Rotator

Improved Neubauer counting chamber

Microscope

Coverslips and slides

Leishman stain

### 2.7.1. Analysis of semen volume

After the liquefaction was taken place, the volume of semen was measured with 10 ml of measuring cylinder.

### 2.7.2. Analysis of sperm count

The liquefied semen mixture was gently shaken to mix the specimen and using a Sahli pipette semen was drawn up to 0.5 micro liter mark. Then the semen diluting fluid was placed up to 11 micro liter mark and placed the pipette on a rotator to mix the interior contents well.

Thereafter, the Improved Neubauer counting chamber was loaded with the mixture and allowed the sperm to settle in. Eventually, the number of sperms in four corner squares was counted.

$$\text{Number of sperm/ml} = \frac{n \times 10 \times 20 \times 1000}{4}$$

n = number of sperm counted in all four corner squares

### 2.7.3. Analysis of sperm motility

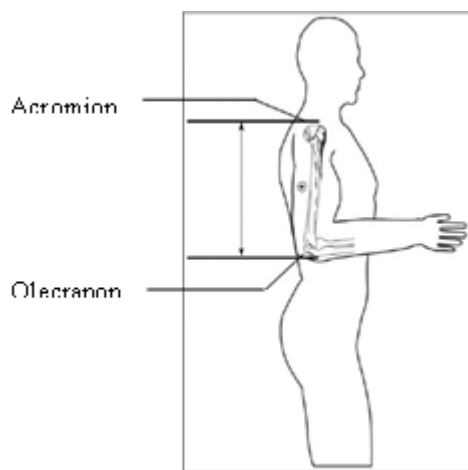
A drop of liquefied semen (10 µl) was placed on a clean slide and covered with a coverslip and rimmed the edge with petroleum jelly to prevent evaporation. It was observed the proportion of motile to non-motile sperms under high power field (× 40) in several microscopic field to obtain the average percentage of motile sperm.

### 2.7.4. Analysis of sperm morphology

A drop of liquefied semen (10 µl) was placed on a clean slide and made a thin smear and the smear was air dried. The dried smear was washed thoroughly with semen diluting fluid to remove the mucous. Then the smear was covered around 8 mints with the diluted Leishman stain which was prepared by mixing 10 ml of stain and 20 ml of distilled water. Thereafter the stain was washed off well with buffered distilled water. Finally, the slide with stained smear was made to dry. The slide was observed for morphology under high power field and the ration of normal to abnormal spermatozoa was observed in different microscopic fields to have the final average percentage of normal spermatozoa.

## 2.8 measuring of mid arm circumference of the individuals<sup>(12)</sup>

The person was asked to stand or sit with his arm hanging fully extended and relaxed by the side of the body Then, the midpoint of the upper arm was found at the midway between acromion process of the scapula and olecranon process of the ulna.



**Figure 2.1:** The midway between acromion process and the olecranon process

The midpoint was looped firmly but not so tightly with the tape. The measurement was recorded in centimeters to the nearest 0.1 cm. Due to the fact that the mid arm circumference depends on races, sex, ethnicity and others, it was difficult to find a firm normal reference value for Asians. Anyway in a review study of Tang et al.,<sup>(13)</sup> and a study of Suryanarayana et al.<sup>(14)</sup>, it has been mentioned that the figure of 22 cm or over for female and 23 cm or over for male as the normal reference value.

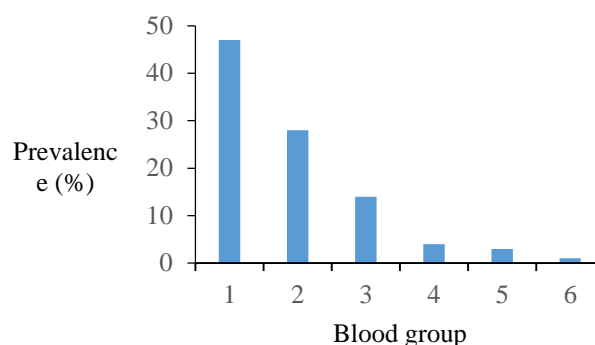
### 3. Data processing and statistical methods

All the results were subjected to normality test and non-normal distribution was indicated. Thus, non-parametric test was used in the analysis of results. The average seminal parameters (volume, count, motility and morphology) of the test group (group O bearers in the study group) were compared with that of the control group (other than group O bearers) with the Wilcoxon signed rank test. Further, the descriptive statistics were also obtained to find out the prevalence of each blood group in the target population. The linear regression analysis was used to find the relationship of mid arm circumference (nutrition level) with the seminal parameters of the individuals. All the statistical analysis were done with the IBM SPSS 20 versions.

## 4. Result and discussion

### 4.1. Effect of blood group (O) on semen parameters

A traditional myth is prevailing in the society that the male individuals with blood group O are more prone to be infertile than the individuals with other blood groups. Thus, in the study each seminal parameter of the male individuals who had possessed blood group O was compared with the same of the control group irrespective of the Rhesus factor. For the control group the age and number matching (nearly) healthy individuals who were bearing the blood groups other than O were selected (the individuals were selected from the study population itself and they were having the normal seminal parameters. Thus, the fertility issues were with their wives). Thus, in the analysis of overall result the prevalence of each defective parameter in the study group was as in fig 4.1.



**Figure 4.1:** The prevalence of each blood group in the study population

In the studied population (n = 105), individuals with blood group O were prominent and was around 50% (including both O+ and O-). The trend is usual even in the normal population. However, most of the O bearers (48%) were positive for the Rhesus factor (O+). Among the other groups, the group B (including both rhesus positive and negative) became the second most predominant (31%) and the group A was in the third rank (14%). According to the outcome of the study the blood group AB was in the fourth rank (4%). The average seminal parameters of the individuals of the test group were compared with those of the control group statistically and the result was as in the table 4.1.

**Table 4.1:** The average values of seminal parameters of the individuals with blood group O (test) and other than O (control).

Semen parameter	Control (n = 52)	Test (n = 53)	P value (Wilcoxon signed rank)
Volume (ml)	2.03 ± 0.40	2.37 ± 0.45	P > 0.05
Count (10 <sup>6</sup> cells/ml)	66.68 ± 13.33	59.32 ± 1.41	P > 0.05
Morphology (normal form %)	42.34 ± 8.40	41.15 ± 8.20	P > 0.05
Motility (%)	57.94 ± 11.28	54.97 ± 10.59	P > 0.05

Out of all, a number of 53 individuals were isolated for the test group as they were having the blood group O. Thus, for the control group a number of 52 individuals who had blood group other than O were selected. In the control group there were individuals who were carrying the blood groups such as A, B, and AB. The average semen volume of the group with blood group O (test) was 2.37 ± 0.45 ml and the same of the opposite group (control) was 2.03 ± 0.40 ml. According to the result the average semen volume in the test group had been higher than that of the other group which had left a result which was controversial to the myth. However, this increment was statically insignificant (P > 0.05, P = 0.65). Thus, it couldn't be considered as a causative differential. However, though the average seminal volume was slightly higher in the group with O blood, the other parameters were considerably low. This was obvious when it came to the average sperm count. It was found in 7.60 of reduction of the average sperm count in the test group and the



reduction was statistically insignificant ( $P > 0.05$ ). Thus, the reduction of average sperm count in the group with blood group O individuals (test), doesn't sound that the blood group O has a negative effect on sperm count. When it came to the motility of sperms, even though a slight decrease of average motility was seen in the test group, it was not significant statistically ( $P > 0.05$ ) as the sperm count described above. The average percentage of proper morphological spermatozoa in the O group were nearly similar to that of the control group and no significant difference was found ( $P > 0.05$ ). As the number of individuals in each group was nearly equal and the average age and the BMI values of both groups were also nearly equal and 34 years and 23 Kg/m<sup>2</sup> in figure, it is utmost clear that someone's semen parameter is independent of blood group factor (O). The same conclusion had been made in certain foreign studies (especially in the Middle East region) as well. In a Pakistan study, which was carried by Khan et al. (n = 1521), it had been mentioned that in the study population, they investigated (Pakistanians), the blood group O was the predominant and the AB was the less predominant<sup>(15)</sup>. Thus, the outcome regarding the prevalence of blood groups was similar to the finding of current Sri Lankan study. Moreover, the same scientists had concluded that due to the reason of finding of high prevalence of group O even in infertile men, the blood group had a relationship with male infertility. However, this interpretation of Pakistan result was not up to the scientific conclusion as the result was not analyzed statistically. Furthermore, as a concept it was accepted predominately that the group O was the most common blood group among human being including both fertile as well as in infertile. Thus, the finding of higher percentage value of blood group O, in infertile male couldn't be considered as a significant factor which affect the male infertility simply without any comparative statistical evaluation. Anyway, in the current study also it had been found that the blood group O was the predominated group in both groups with normal as well subnormal semen parameters and no relationship was found to have between the group O factor and seminal quality. Thus, the conclusion of Pakistan study becomes collapse. Another study (n = 250) of Omu et al.<sup>(16)</sup> in Kuwait, had explored that the blood group had not any effect on seminal parameters by making autoantibodies. The finding is an important additive to exclude the effect of group O via autoantibody pathway hence to confirm the conclusion of this study. The conclusion of the current study (this study) had further been proved by a group of Indian scientists (Ganitha et al study) with 100 test males in a fertility clinic<sup>(17)</sup>. However, in the study of Haas et al<sup>(18)</sup>, it had been found that the higher level of antigen H (antigen of the red cells of group O blood) in semen could reduce the motile ability of semen. Anyway, they have carried out the study with respect to the seminal plasma. Further, the finding of antigen H in semen at high concentration is rare<sup>(18)</sup>. Thus, the finding of that study has a low relative to the outcome of the current study. In addition to the human studies, certain animals (bulls) had also been involved in the studies to find out the effect of blood group factor (ABO group) on seminal parameters (infertility) and found out that there was a negative relationship between the two<sup>(19)</sup>, anyway, it's very

complicated to compare the outcome of animal studies with human studies, due to the species variances.

#### 4.2 The effect of mid arm circumference (anthropometric indicator of nutrition) on seminal parameters

The average mid arm circumference value of the whole population was 29.75 4.13 cm and the range was 22.5 - 52.5 cm. According to the result, almost all the subjects were within the normal reference range. Thus, the Spearman correlation test was used basically to analyze the relationship of nutrition level (anthropometric wise) with the seminal parameters. Further, the linear regression analysis was also used.

**Table 4.2:** The total result of the spearman correlation analysis between mid-arm circumference and each seminal parameter

Variables	Correlation coefficient	Significance
Mid arm circumference, semen volume	0.062	$P > 0.05$
Mid arm circumference, sperm count	-0.144	$P > 0.05$
Mid arm circumference, sperm motility	-0.113	$P > 0.05$
Mid arm circumference, sperm morphology	-0.36	$P > 0.05$

Though, an inverse correlation was seen between mid-arm circumferences and each seminal parameters such as sperm count, motility and morphology, no one was significant statistically (table 4.2). The correlation between mid-arm circumference and semen volume was also non-significant. Thus, no correlation was found between mid-arm circumference and any of the seminal parameters mentioned.

**Table 4.3:** The total result of the linear regression analysis between mid-arm circumference and each seminal parameter

Variables	R value	R square
Mid arm circumference, semen volume	0.284	0.080
Mid arm circumference, semen count	0.148	0.022
Mid arm circumference, sperm motility	0.198	0.039
Mid arm circumference, sperm morphology	0.002	0.000

The linear relationship was also analyzed further between the mid-arm circumference and seminal parameters (volume, count, motility and morphology) and found to have a weak connection between the mentioned variables which was statically non-significant (table 4.3). Further, it was clear that there was no considerable contribution from mid-arm circumference toward each seminal parameters according to the R square value of the test.

## 5. Conclusion

The blood group O has not any effect on the seminal parameters hence the semen quality and male infertility. Thus, the myth of the negative effect of blood group O on male infertility is collapsed with the study. Anyway, it is better to carry out more and more studies considering the present study as a platform. Further, the power of this study is limited by the relatively small overall sample size. This could be due to the strict adherence to exclusion criteria. The outcome is dependent on the exclusive criteria, hence the data gathered from the subjects. The mid arm circumference hence the nutrition level has no significant relationship with the seminal parameters. This could be due to the finding of well-nourished individuals in the study group (average mid arm circumference was within the normal reference range). That is because the prevailing concepts of the society to have a well-balanced diet by the male who are going to make their wives pregnant. Anyway this situation is a best trend in the male with fertility issues.

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