Semen Parameters Are Seriously Affected in Acephalic Spermatozoa Syndrome

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Research Article

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Abstract

Objective: To evaluate the effect of acephalic spermatozoa syndrome on sperm quality in semen with different proportions of headless sperm.

Design: Case control study.

Setting: Andrology Laboratory.

Patient(s): A total of 391 patients with headless sperm and 400 prenatal examination patients with no headless sperm who underwent semen analysis at the andrology laboratory.

Intervention(s): None.

Main Outcome Measure(s): The correlation of the proportion of headless sperm in semen with semen parameters.

Result(s): All semen parameters except the semen volume were negatively ($P<0.05$) correlated with the proportion of headless sperm in the semen. The semen samples were divided into three groups based on the proportion of headless sperm (PHS) as follows: $0<PHS\leq10\%$ (n=249, group A), $10<PHS\leq20\%$ (n=71, group B) and $PHS>20\%$ (n=71, group C). Nearly all semen parameters were significantly lower in group B and group C than in the control group ($P<0.05$). However, in group A, only the vitality and motility parameters were lower than those of the control group.

Conclusion(s): Semen samples containing headless sperm tend to have lower semen parameters than samples without headless sperm. Increases in the proportion of headless sperm in semen samples are associated with decreases in semen quality.

Introduction

Reproductive health is essential for ensuring the continuity of human populations. However, recent reports indicate that approximately 15% of couples suffer from fertility problems, and it is known that up to half of cases of infertility may be due to male factors [1, 2]. Teratozoospermia is an important cause of male infertility. Headless sperm are those severely deformed sperm that have only flagella. This abnormal morphology was first described as “minute-head sperm”, but in 1981, Perotti et al. demonstrated that minute-head sperm actually have no head at all and that the “minute heads” were actually small cytoplasmic droplets [3]. Headless sperm are produced when there are abnormalities in the formation of the sperm head-tail coupling apparatus (HCTA), an important structure that anchors the sperm flagellum to the sperm head [4, 5].

Acephalic spermatozoa syndrome has been confirmed to cause male infertility, as the semen contains many headless sperm [4, 6–9]. Kamal reported few headless sperm in semen, suggesting that the sperm are easily decapitated, which may also be the cause of male infertility or failure of assisted reproduction [10].

Moreover, both the incidence of headless sperm and the proportion of headless sperm are higher in the infertile population than in the fertile population; the percentage of headless sperm infertile men is 2.7±3.1%, while in infertile men, it is increased to 9.0±8.8%[11, 12]. Therefore, the proportion of headless sperm might be a cause of infertility or impaired fertility in males.

Previous studies reported that some patients with headless sperm in ejaculates have poor semen parameters [13, 14], but until now, there has been no published study of a systematic analysis of semen quality in patients with different proportions of headless sperm in the semen. In this study, we aimed to explore the effect of acephalic spermatozoa syndrome on semen quality by evaluating the parameters of semen containing distinct proportions of headless sperm.

Materials And Method

Study population and participants

In West China Second University Hospital, Sichuan University, between January 2018 and July 2019, headless sperm were found in ejaculates of 391 patients (age 30.3±4.8 years). As matched controls, we selected 400 prenatal examination patients (age 32.33±5.0 years) who did not produce headless sperm (excluding azoospermia) but otherwise showed roughly the same characteristics as the headless sperm group. All patients were subjected to routine semen analysis after an abstinence period of 2–7 days. Monthly mean monitoring, intertechnician comparison and daily semen concentration and semen motility quality control were used to ensure the reliability of semen analysis. Ethics approval for this study was obtained from the ethics board of West China Second University Hospital of Sichuan University, and all experimental protocols for human subjects were performed in accordance with guidelines approved by the Institutional Review Board of West China Second University Hospital Sichuan University (WCSUH-SCU IRB 2020-(102)).

Semen analysis

Semen samples were obtained by masturbation and after liquefaction in a 37 ℃ incubator all samples were analyzed according to WHO guidelines with some modifications. The volume of the samples was measured by weighing of the collection container. The sperm concentration, sperm motility (percentage of progressive motility), and round cells were evaluated in a Makler counting chamber with the help of computer-assisted sperm analysis (CASA), which also provides the following objective sperm motility parameters: straight-line velocity (VSL), curvilinear velocity (VCL), average path velocity (VAP), beat cross frequency (BCF), and amplitude of lateral head displacement (ALH). In addition, after staining with the cell eosin Y technique, we calculated the sperm vitality (percentage of alive sperm) under the microscope. Moreover, we analyzed sperm morphology (percentage of normal sperm morphology) after Papanicolaou staining according to the standards in the WHO manual (fifth edition).

Semen parameter cutoff values (lower reference limits; LRL), as established by the WHO manual (fifth edition), were as follows: semen volume, 1.5 mL; sperm concentration, $15*10^6$ spermatozoa/mL; sperm motility, 32% progressive motile; sperm vitality, 58% live; and morphology, 4% normal forms. In addition, the percentage of headless sperm was calculated as the headless sperm concentration/(headless sperm concentration+normal sperm concentration) × 100%.

It is worth noting that all the semen parameters described in the WHO manual (fifth edition) standards do not include headless sperm but only intact spermatozoa (defined as having both head and tail); headless sperm need to be counted separately as a specific type of structural defect.

Statistical analysis

The results from the different groups were compared by the nonparametric Mann-Whitney test. Each group of statistics is indicated with medians and interquartile ranges. Spearman correlation coefficients were determined for different proportions of headless sperm and semen parameters. We also constructed receiver operating characteristic (ROC) curves to assess the specific proportion of headless sperm, which may lead to abnormal semen parameters. All evaluations were performed using PRISM software. Differences between groups were considered statistically significant at $P<0.05$.

Results

Characteristics of the headless sperm group and control groups

Compared with the control group, the median semen concentration, total sperm count, sperm vitality, progressive motility, normal sperm morphology and some sperm motility parameters (VCL, ALH, VAP, BCF) were lower in the headless sperm group ($p<0.05$). However, there were no significant differences between the headless sperm group and the control group in semen volume, number of round cells, or VSL ($p>0.05$) (Table 1). Moreover, a notable morphological difference was observed between the two groups; small acrosome deficiency was observed in 27.6% (108/391) of patients in the headless sperm group but just 2.7% (11/400) of patients in the control group.

Table 1
The comparison of semen parameters in test group and control group

Semen parameters	Headless sperm group (n=391)	Control group (n=400)	p
Volume(ml)	3.3(2.5-4.1)	3.5(2.6-4.4)	0.1351
Round cells	0.2(0.2-0.5)	0.3(0.2-0.5)	0.0753
Concentration(million /ml)	73.8(36.9-131.6)	89.4(54.7-139.9)	0.0008
Count(million)	236.5(108.7-419.4)	299.3(187.5-446.7)	<0.0001
Vitality (%)	77(70-81)	79(72-84)	0.0001
Progressive motility(%)	49(37-61)	59(46-69)	<0.0001
Normal sperm morphology(%)	3.9(2-6)	4.9(3-6.9)	<0.0001
VCL (um/sec)	35.1(25.0-45.2)	38.2(28.6-47.1)	0.0119
VSL (um/sec)	19.6(13.6-26.5)	20.5(15.6-26.2)	0.1178
VAP (um/sec)	25.3(17.6-32.7)	27.6(20.5-33.8)	0.0246
ALH (um/sec)	3.14(2.3-3.9)	3.3(2.5-4.2)	0.0076
BCF(Hz)	9.9(7.6-12.1)	10.7(8.7-12.5)	0.0010

Note: Abbreviations: VSL (straight-line velocity), VCL (curvilinear velocity), VAP (average path velocity), BCF (beat cross frequency), ALH (amplitude of lateral head displacement). Data are presented as Median (interquartile range), and comparison between headless sperm group and control group was determined by nonparametric Mann-Whitney test. P<0.05 indicated a significant difference.

Semen parameters and proportion of headless sperm in semen

To explore the relationship between the proportion of headless sperm and semen parameters, we conducted a correlation analysis of the proportion of headless sperm and their sperm parameters in the headless sperm group. Interestingly, all the semen parameters except semen volume were negatively (P<0.05) correlated with the proportion of headless sperm in the semen (Table 2).

Table 2
The correlation of the percentage of headless sperm and semen parameters.

Semen parameters	Median (interquartile range)	Correlation coefficient	P
Volume(ml)	3.3(2.5-4.1)	0.08849	0.0809
Concentration(millions /ml)	73.8(36.9-131.6)	-0.7391	<0.0001
Count(millions)	236.5(108.7-419.4)	-0.6835	<0.0001
Vitality (%)	77(70-81)	-0.1177	0.0214
Progressive motility(%)	49(37-61)	-0.2661	<0.0001
Normal sperm morphology(%)	8(2-6)	-0.2074	<0.0001
VCL (um/sec)	35.1(25.0-45.2)	-0.3030	<0.0001
VSL (um/sec)	19.6(13.6-26.5)	-0.2899	<0.0001
VAP (um/sec)	25.3(17.6-32.7)	-0.3075	<0.0001
ALH (um/sec)	3.14(2.3-3.9)	-0.3370	<0.0001
BCF(Hz)	9.9(7.6-12.1)	-0.3697	<0.0001

Note: Abbreviations: VSL (straight-line velocity), VCL (curvilinear velocity), VAP (average path velocity), BCF (beat cross frequency), ALH (amplitude of lateral head displacement). P<0.05 indicated a significant difference.

To identify the appropriate cutoff for the specific proportion of headless sperm in the semen that may predict abnormal semen parameters, we selected the sperm concentration (15*10^6/ml), sperm motility (32%), and sperm morphology (4%) reference values (lower reference limits) as the cutoff values to draw ROC curves. The headless sperm proportion threshold with the best balance of sensitivity and specificity was calculated for each parameter (sperm concentration, sperm motility, sperm morphology) separately for a better distinction between normal and abnormal values. The cutoff values for proportion of headless sperm were found to be between 7.15% and 17.96% (Table 3, Fig. 1).

Table 3

The comparison of semen parameters in group A，group B, group C and control group.

Groups	Control group	Group A	Group B	Group C
n	400	249	71	71
Volume（ml）	3.5（2.6-4.4）	3.3(2.5-4.0)	3.4(2.6-4.3)	3.4(2.5-4.3)
Concentration（million /ml）	89.4（54.7-139.9）	108.9(68.3-168.6) [a]	45.1(31.8-61.1) [ab]	13.9(7.1-30.8) [abc]
Count（million）	299.3（187.5-446.7）	368.7(200-525.6) [a]	159.5(106.7-220.7) [ab]	49.7(20.3-90.5) [abc]
Vitality（%）	79（72.0-84.0）	77(71.0-82.0) [a]	77(68.0-81.0) [a]	73(67.0-79.0) [ab]
Progressive motility（%）	59（46.0-69.0）	53(40.8-62.3) [a]	45(36.5-55.0) [ab]	38.5(27.0-52.5) [ab]
normal sperm morphology（%）	4.9（3.0-6.9）	4(2.5-6.4)	3.5(2.5-5.0) [a]	2.5(1.0-4.0) [abc]
VCL (um/sec)	38.2(28.6-47.1)	37.5(28.3-47.1)	32.2(23.2-40.4) [ab]	24.8(15.9-38.8) [abc]
VSL (um/sec)	20.5(15.6-26.2)	21.3(15.4-27.6)	19.0(12.6-24.3) [ab]	13.7(8.7-21.6) [abc]
ALH (um/sec)	3.3（2.5-4.2）	3.4(2.6-4.2)	2.9(2.1-3.5) [ab]	2.2(1.49-3.1) [abc]
VAP (um/sec)	27.6（20.5-33.8）	27.2(20.4-34.7)	22.8(16.5-30.2) [ab]	17.5(11.5-26.8) [abc]
BCF（Hz）	10.7(8.7-12.5)	10.6(8.5-12.5)	8.7(7.3-11.2) [ab]	7.0(5.3-10.4) [abc]

Note: n=the number of samples. Data are presented as Median (interquartile range). group A= <PHS≤10%，group B=10<PHS≤20%, group C=PHS>20%.Comparison among group A, group B, group C and control group was determined by nonparametric Mann-Whitney test. a indicated to comparison with Control group has a significant difference. b indicated to comparison with Group A has a significant difference. c indicated to comparison with Group B has a significant difference.

Based on these findings, the headless sperm group was divided into three subgroups based on the percentage of headless sperm (PHS) as follows: 0<PHS≤10% (n=249, group A), 10<PHS≤20% (n=71, group B) and PHS>20% (n=71, group C). As expected, nearly all semen parameters of group B and group C were significantly lower than those of the control group (P<0.05). However, in group A, only the vitality

and motility were lower than those in the control group (Table 4, Fig. 2). We conclude that sperm parameters decrease with increasing proportion of headless sperm.

Table 4
Receiver operating curves.

Semen parameters	Cut-off	Area	Sensitivity(%)	Specificity(%)
Sperm Concentration	17.96	0.9382	93.02	88.47
Sperm Motility	7.15	0.6432	63.64	59.11
Sperm Vitality	13.63	0.5891	44.83	76.2
Normal sperm morphology(%)	12.06	0.6109	38.22	80.85

Discussion

Headless sperm is a specific type of structural defect. Previous research highlighted that patients with high proportion of headless sperm may face infertility and that their semen parameters are usually relatively lower [13, 15, 16]. In addition, the incidence and proportion of headless sperm in the infertile population were higher than those in the fertile population. For fertile men, the headless sperm proportion is typically below 13% [12]. Our data shows that semen parameters are negatively correlated with the proportion of headless sperm in the semen. There was no noticeable deterioration in semen parameters when the proportion of headless sperm in the semen was under 10%, and the semen parameters declined significantly when the proportion of headless sperm in semen was more than 20%. Therefore, we suggest that a proportion of headless sperm >10% may be accompanied by a decline in semen parameters and should be considered in clinical diagnosis.

A large amount of literature has confirmed that headless sperm can be caused by mutations in *SUN5*, *PMFBP1*, *TSGA10*, or *BRDT* [9, 14, 17–21]. Male exposure to khat or methyl chloride and ligation of the vas deferens can also lead to the production of headless sperm [22–24]. Investigations in male mice have found that loss-of-function mutations in genes involved in the production of headless sperm, such as *Spata6*, *Hook1*, *Prss21*, *Oaz3*, and *Odf1*, can cause fertility reduction or infertility[25–28]. Interestingly, no mutations of these genes have been identified in humans, which might be explained by either genetic heterogeneity underlying this syndrome or differences in the functions of these genes or in the molecular pathogenesis between mice and humans. Research on the causes of headless sperm has focused on patients with a high proportion of headless sperm in the semen. However, it is very important to also study the causes of lower proportions of headless sperm, which may explain some cases of idiopathic male infertility.

Alterations in any of the above factors could lead to abnormalities in HCTA structure, in turn causing the detachment of the sperm tail from the sperm head during spermatid elongation. Because the sperm neck is unstable, sperm heads are usually phagocytosed by Sertoli cells [4, 8, 14, 18, 29, 30] or present a higher risk of sperm fracture when subjected to mechanical stress (mix, centrifugation or micromanipulation) [26, 31].

Sperm concentration as evaluated according to the WHO manual (fifth edition) standards considers only whole sperm (i.e., sperm with both a head and a tail), while free tails and heads are not counted [32]. This may explain why a higher proportion of headless sperm could be associated with a lower sperm concentration.

Sperm mitochondria play a major role in sperm motility, as they are the powerhouse of the sperm [33, 34]. The sperm tail is an important structure for sperm motility, and human sperm swim forward by moving their tail symmetrically from side to side [35]. Under transmission electron microscopy, the intact sperm of patients whose semen also contains headless sperm often have abnormal structures, such as disassembled mitochondria and sperm tail malformations[15, 36–38]. These morphological abnormalities may be responsible for the decrease in sperm motility. In addition, our data also show that the motility parameters (VCL, ALH, VAP, BCF) of intact sperm are lower in semen samples containing headless sperm, confirming that among intact sperm, not only the percentage of progressive motility but also the movement type is altered. These motility parameters were reported to predict the success of IUI and IVF in couples receiving infertility treatment[39–42]. The results indicate that even with the same semen concentration, semen motility and normal semen morphology, males with headless sperm in semen samples may demonstrate lower fertility.

Our data show that semen samples containing headless sperm present other defects in sperm morphology and a higher incidence of abnormally small acrosomes. Previous studies also reported acrosome abnormalities in the intact sperm of semen samples containing headless sperm [7, 31, 43]. The acrosome is formed by the trans-Golgi, and it is the unique structure of mature sperm and plays an important role in the binding of sperm and zona pellucida during fertilization [44]. In 1984, Bacetti et al. reported that headless sperm can occur due to overproduction of vesicles by the Golgi complex in the region between the centrioles and nucleus [45]. In a patient with headless sperm, the expression of Golgi-related genes (GOPC and VPS54) was changed; GOPC and VPS54 are important constituent proteins in the Golgi apparatus in tissue culture cells, and loss of GOPC and VPS54 affected sperm acrosome formation [19, 46–49]. Our data further confirm that headless sperm and small acrosomes may be a possible result of the same pathologic morphogenic mechanism. Sperm acrosomes play an important role in sperm binding to the zona pellucida during fertilization, and abnormal acrosomes may be a reason for male infertility or fertility reduction in semen containing headless sperm.

Conclusion

Our findings have strengthened the understanding of the parameters of semen samples containing headless sperm. Semen samples containing headless sperm tend to have lower semen parameters than samples without headless sperm. Higher proportions of headless sperm are associated with lower-parameters semen. This may be because different proportions of headless sperm are produced by different pathogenic mechanisms; this possibility requires further study. Previous research has reported that patients whose semen contains headless sperm may experience infertility or decreased fertility [4, 12,

[13, 25–28, 31, 50–52]. Our research supported some of these statements regarding sperm quality. However, the decrease in semen parameters is only a symptom, and the specific reasons for the decrease need to be further explored in combination with the factors that lead to the production of headless sperm. At the same time, sperm functional tests, such as nuclear maturity, mitochondrial membrane potential, and acrosomal function tests, are also important indicators for evaluating male fertility and also need greater attention. More importantly, headless sperm should be assessed seriously and counted accurately in semen analysis.

Declarations

Ethics approval and consent to participate

Written informed consent for sample donation for research purposes was obtained from all patients prior to sample collection. Ethics approval for this study was obtained from the ethics board of West China Second University Hospital of Sichuan University, and all experimental protocols for human subjects were performed in accordance with guidelines approved by the Institutional Review Board of West China Second University Hospital Sichuan University (WCSUH-SCU IRB 2020-(102)).

Consent for publication

Not applicable

Availability of data and materials

The datasets generated and/or analysed during the current study are not publicly available due local institutional patient data is considered confidential but are available from the corresponding author on reasonable request.

Competing interests

The authors declare no competing interests

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Authors' contributions

Li-juan Ying and Qing-ting Liu designed the project, reviewed and analyzed the data, and wrote the paper. Ying-bi Wu, Jing-yan Xu, Ye-lin Jia, Yan Zheng conducted semen analysis, Lin Yu and Tingting Yang conducted the statistical analysis. Dong-Deng and Fu-ping Li conceived this study, performed data analysis, and prepared the manuscript. All authors have read and approved the final manuscript.

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Figures

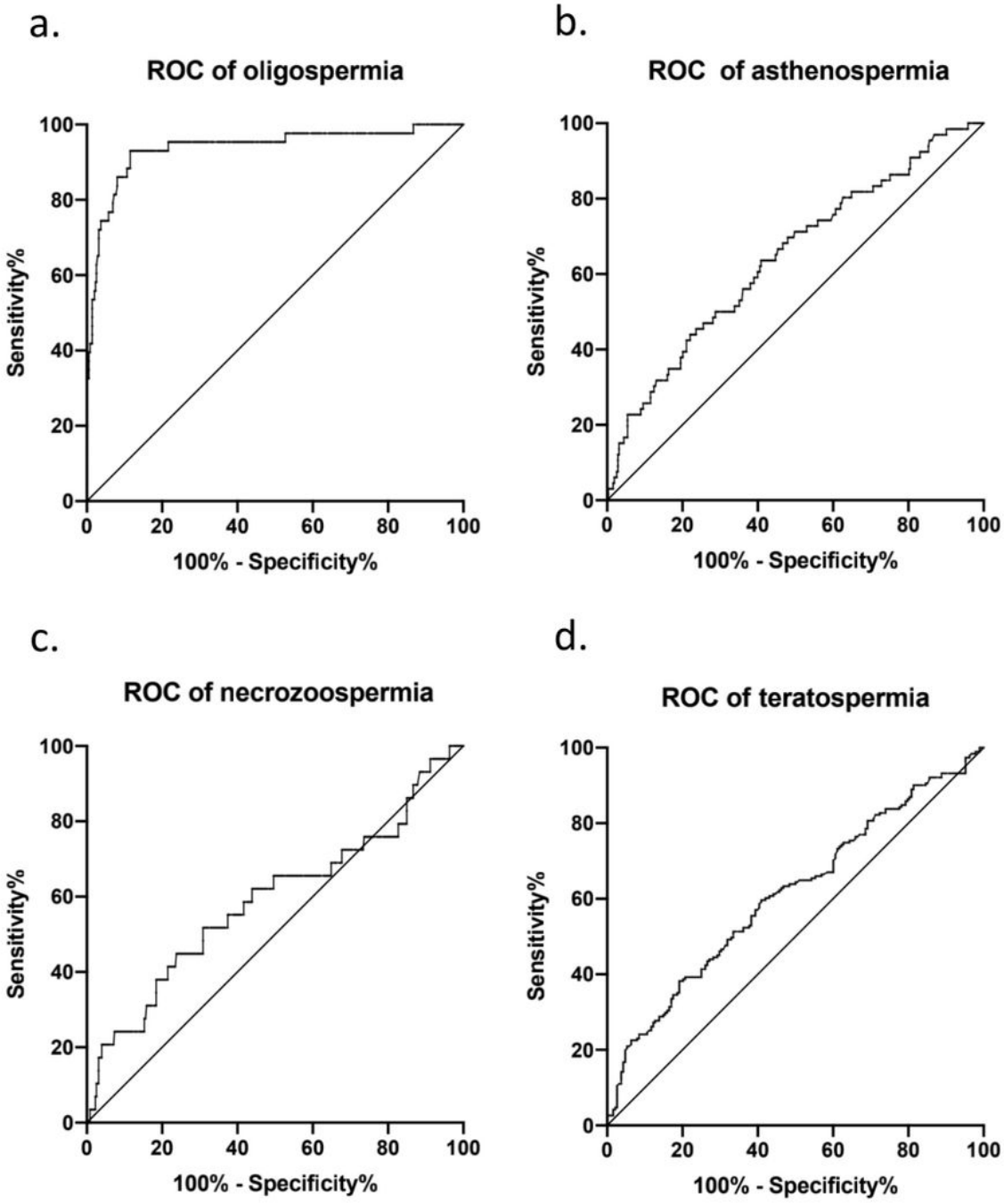

Figure 1

Receiver operating curve. Analysis of the proportion of headless sperm in diagnosis (a) oligospermia(sperm concentration<15*106 spermatozoa/mL), (b) asthenospermia(the percentage of progressive motile sperm < 32%), (c) necrozoospermia(the percentage of alive sperm < 58%)%), (d) teratospermia(the percentage of normal morphologysperm<4%).

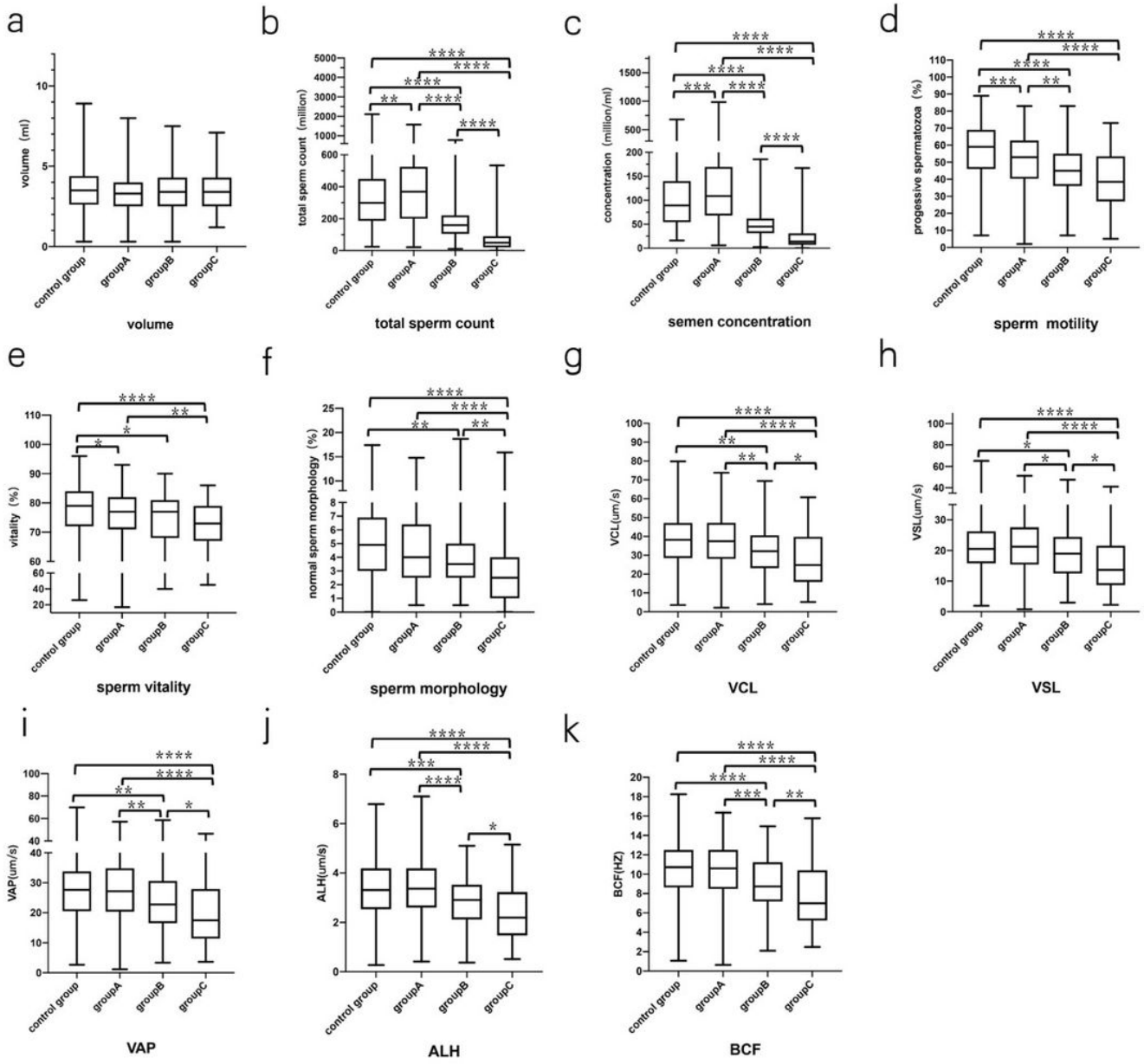

Figure 2

The proportion of headless sperm and semen parameters. group A=20%. * P< .05; ** P< .005; *** P< .0005; **** P< .0001.