Comparative study of the metabolic markers of the seminal fluid between normospermia and azoospermia

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Abstract:
Objective: The present study is to compare the principal metabolites in the seminal liquids obtained with patients in age to procreate presenting azoospermia and normospermia.
Méthodology: With the glance as standards of the World Health Organization, 39 samples (22 azoospermia and 17 normospermia) were collected by masturbation. The seminal plasma of each sample was recovered after centrifugation thus allowing to the various parameters such as glucose, urea, creatinine, uric acid, total cholesterol, triglycerides and total proteins.
Results: According to the statistical analysis, a precision of P < 0.05 was considered significant. Average concentrations of urea showed a significant difference (P = 0.0319) in the two samples. This parameter can be used as discriminant in the diagnosis of the azoospermia profiles with a median value of 0.495 ± 0.059 g/L compared to normospermia which value averages is of 0.923± 0.204 g/L. Whereas the age of patients, as well as the others cannot be discriminating in the diagnosis of the azoospermia profiles compared to the normospermia. Nevertheless, the average concentrations of glucose, total cholesterol and triglycerides in the azoospermia samples were lower than those obtained in the samples normospermia what in the other hand is the reverse for creatinin, the uric acid and total proteins.
Conclusion: the evaluation of urea in plasma seminal could be one of the biochemical markers differentials between the normospermia and the azoospermia.

Key Words: Metabolites, seminal fluid, azoospermia, normospermia

Introduction
The perpetuation of the human species and its increasing global population is affected by many plagues and phenomena including the couple’s infertility prominently. According to WHO, infertility is defined as the absence of conception after two years of exposure to the risk of pregnancy [1]. Although estimates are hardly accurate and the figures vary from one region to another in Africa, 12-21% of couples experience a fertility problem during their reproductive years [2].
Generally, the woman is the first to be questioned, but according to some studies, in about half of cases, the man is wholly or partly responsible because the degradation of sperm quality is increasingly observed [3, 4].
Azoospermia is one of the first causes of infertility. It is defined as the absence of spermatozoon in the ejaculate during at least two or three semen analyses performed under optimal conditions [4]. However, according to WHO (2010) normospermia is a sperm that quality parameters are normal after a spermogram and a spermocytogram [5]. The seminal fluid is a favorable biochemical environment for the sperm survival. Thus, it contributes to ensure a good quality of the sperm. Depending on the species, its biochemical composition is very complex and variable [6].
However, the biochemical study of that liquid has permitted to bring out variations in the secretion and to establish a functional mapping of the male reproductive tract, especially during the exploration of azoospermia, oligozoospermia and asthenozoospermia highlighted by spermogram [7]. At this point, each segment in the genital tract produces a specific molecule which is found in the seminal fluid. On one hand, the epididymis, located at the testicular exit, produces alpha-glucosidase and carnitine while the seminal vesicles, connected to the vasa deferentia, produce fructose. On the other hand, the prostate, located at the intersection of the genital and urinary tracts produces citric acid and uses zinc. The biochemistry of sperm is therefore part of an exploration of the different fertility for dosing those elements called markers [8].
In the biochemical exploration of infertility, mainly in the case of azoospermia, in addition to the determination of markers, the one of the FSH that regulates the exocrine testes, is more important because it can differentiate a secretory azoospermia (increased or decreased rate depending on the site of involvement) of an excretory azoospermia (normal rate) [9].

In Côte d'Ivoire there are few statistics in the field of infertility. The unity of the reproductive biology of Institut Pasteur de Côte d'Ivoire (IPCI) has initiated this study in order to determine the metabolic markers that can be discriminative in the diagnosis between the azoospermia and the normospermia profiles. In this context, the specific objectives are firstly to determine azoospermia and normospermia after spermogram and spermocytogram, and then to evaluate metabolites in the seminal fluid of the two identified profiles, and finally to assess the significant differences in the metabolites concentration values measured between the two groups.

Materials and methods
This study was carried out in the laboratory of the reproductive biology unit of Institut Pasteur de Côte d'Ivoire (IPCI) from November 2010 to November 2012. The study was approved by the National Council of Ethics and Research of the country (Reference n° 036/MSLS/CNER/TB), and was conducted in accordance to the legal and regulatory provisions of Helsinki Declaration.

The biological material used was human sperm collected from patients who came at the IPCI for a fertility test. The number of samples for the study was 39 with 22 azoospermia and 17 normospermia. In addition to a Cobas Integra 400 controller spectrophotometer, an optical microscope, a cell Mackler, a centrifuge and an oven of which the technical material is made up, it also comprises a set of elements essential to the spectrophotometer and the routine microscopy. The reagents were composed of strips of pH, 0.6 % of eosin, to 5 % of nigrosine, and RAL kit 555. Kits for the assays of glucose, urea, creatinine, uric acid, total protein, total cholesterol and triglycerides are added to these elements.

The sampling of the biological material was made by masturbation. It was obtained from the patients who came for spermogram and spermocytogram after three (3) days abstinence [5]. The tests were required in an assessment of male fertility. We use the optical microscope to achieve the spermogram and the spermocytogram involve various important informations. Thus, the data considered for the fresh spermogram are the volume, the color, viscosity, pH, the sperm count and round cells with the percentage of polynuclear, vitality, and the different mobilities [5]. After the staining with RAL kit, the assessed parameters are the percentage of the normal form, the different shape abnormalities according to the classification of DAVID, and the multiple anomalies index (IAM) for the spermocytogram [5]. After these operations, the samples with normospermia and azoospermia selected for the assays of the biochemical metabolites.

At the end of the tests, the sperm were centrifuged and the seminal fluid is collected for the various dosages with an automatic spectrophotometer.

Thus, at a wavelength $\lambda$ 500 nm the determination of creatinine is achieved in alkaline area and in the presence of picric acid; glucose in the presence of the glucose oxidase and peroxidase; cholesterol in the presence of cholesterol esterase, oxidase and peroxidase; and finally the triglyceride in the presence of lipase, glycerol kinase, oxidase and peroxidase. At the wavelength $\lambda$ 510 nm, uric acid is determined in the presence of uricase and peroxidase. As to the wavelength $\lambda$ 550 nm, the determination of the protein is achieved in the presence of cupric ions and in alkaline area. For urea, the determination is done in the presence of urease at the wavelength $\lambda$ 600 nm. After dosage, the values of the concentrations of the studied parameters were recorded using Excel spreadsheet software. To these values, we applied the student T-test (and Nonparametric tests) using Graph Pad prism 5 software with the use of the Mann-Whitney U-test and a precision of $P < 0.05$ was considered significant.

Results
The current study allowed obtaining data on the distribution of samples and assaying results in order to educated and represent the significant parameter. The results showed a substantially homogeneous distribution of samples as described in figure 1. For the dosage of metabolites and the statistical analysis, table shows the means of concentrations of different metabolites in both samples; namely azoospermia and normospermia and the significativity indexes. Statistical analysis, of urea showed a significant difference in the two samples.
Figure 1: The distribution of the proportions between the number of normospermia and azoospermia patients

Table 1: Comparison of mean value of metabolic markers in both samples by Mann Whitney test

<table>
<thead>
<tr>
<th>Identified Groups</th>
<th>P &lt; 0.05</th>
<th>Significativity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azoospermia (N=22)</td>
<td>Normospermia (N=17)</td>
<td></td>
</tr>
<tr>
<td>Average age (year)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (year)</td>
<td>39.23 ± 0.87</td>
<td>37.65 ± 0.97</td>
</tr>
<tr>
<td>Average parameters metabolites</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose (g/L)</td>
<td>1.022 ± 0.076</td>
<td>1.194 ± 0.130</td>
</tr>
<tr>
<td>Urea (g/L)</td>
<td>0.496±0.059</td>
<td>0.923±0.204</td>
</tr>
<tr>
<td>Creatinine (mg/L)</td>
<td>67.23 ± 4.42</td>
<td>66.36 ± 10.40</td>
</tr>
<tr>
<td>Uric acid (mg/L)</td>
<td>66.11 ± 3.81</td>
<td>65.58 ± 7.83</td>
</tr>
<tr>
<td>Cholesterol (g/L)</td>
<td>0.204±0.033</td>
<td>0.209±0.037</td>
</tr>
<tr>
<td>Triglycerides (g/L)</td>
<td>0.247±0.079</td>
<td>0.258±0.072</td>
</tr>
<tr>
<td>Protein (mg/L)</td>
<td>45.65 ± 2.765</td>
<td>40.68 ± 3.660</td>
</tr>
</tbody>
</table>
Discussion

The comparative study of the metabolic markers in the seminal fluid of azoospermia and normospermia samples has permitted to assess several aspects. The patients’age was not a decisive factor in the diagnosis of azoospermia and normospermia profiles. The effective dosage of the metabolites of all samples were statistically analysed to identify significant values.

Generally, the ages of patients coming for spermogram and spermocytogram at the Institut Pasteur of Côte d’Ivoire varied. During the period of this study, the ages of the selected persons varied from 29 to 48 years. This showed that the population submitted to the study is adult, and according to figure 1, the distribution showed that normospermia represent 44% while azoospermia represent 56%. The average age of patients with azoospermia profile (39.23 ± 0.87 years), is higher than that of patients with normospermia one’s (37.65 ± 0.97 years). That corroborates with previous studies showing that the sperm quality deteriorates with age [10]. However, the statistical analysis reported in table 1 showed that there is no significant difference between the age as a discriminatory means in the diagnosis of azoospermia in relation to normospermia.

For the metabolic parameters, this study showed the presence of metabolites in different samples confirmed by other studies [9]. Indeed, these studies have shown that all the blood components are present in the seminal fluid with concentrations more or less close to the blood concentration.

With regards to the non significant parameters, this study has shown that in assays of metabolites in the seminal fluids, initially, the average concentrations of creatinine (67.23 ± 4.420g / L), uric acid (66.11 ± 3.81 mg / L), and total protein (45.65 ± 2.765 mg / L), in the azoospermia samples are higher than those obtained in normospermia samples which are respectively 66.36 ± 10.40 mg / L, 65.58 ± 7.834 mg / L and 40.68 ± 3.660 mg / L as indicated in table 1.

These data could be explained by the fact that in the presence of spermatozoa, total protein are more used since their predominant functions in the seminal fluid in the formation of coagulum and liquefaction, the protection and the metabolic support of spermatozoa [11]. Moreover, the average value of the concentration of total protein in normospermia, obtained in this study, does not differ from that obtained in other studies [12].

The formation of creatinine requires amino acids such as glycine and arginine which come from the degradation of proteins. In these conditions, the formation of creatinine is also explained by the assessment of the average concentration of total proteins in azoospermia [13, 14]. The catabolism of nucleic acids into uric acid comes from the cell destruction, but if the averages of uric acid concentrations are almost similar in azoospermia and normospermia semen samples, this could be explained by the fact that the metabolism of uric acid could be very small in the presence of spermatozoa. In the azoospermia, a complete absence of spermatozoa was detected in the
sperm. Therefore, how can we understand that there is a nucleic acid catabolism in azoospermia semen samples in the absence of cells? This question could be answered with the fact that azoospermia occurs in two forms: a secretory or non-obstructive form characterized by a complete absence of spermatozoa in the testes and a so-called excretory or obstructive form, in which the ejaculate does not contain spermatozoa [15].

However, the study showed that the average concentrations of glucose (1.019 ± 0.079 g/L), total cholesterol (0.204 ± 0.033 g/L) and triglycerides (0.246 ± 0.078 g/L) in azoospermia semen samples are lower than those obtained in normospermia semen samples which are respectively 1.194 ± 0.130 g/L, 0.209 ± 0.037 g/L and 0.072 ± 0.258 g/L, as shown in the table, the statistical analysis of the comparative metabolic parameters of normospermia and azoospermia.

Indeed, the human spermatozoa produces the great part of its own energy in the ATP form of anaerobic glycolysis [16], and these metabolites are involved in many catabolic reactions coupled to the process of oxidative phosphorylation, leading to the production of energy in the ATP form in the mitochondria. Many previous studies have shown that the absence of glucose from the seminal fluid significantly decreases the progressive motility and it consequently causes the decrease of fertility [17]. In addition, triglycerides are essential for the proper functioning of the body because they fall in the energy processes and are carriers of vitamins A, D, E, K in the blood [18]. A contrario, cholesterol is involved in the molecular changes of the spermatozoa during the phenomenon of capacitation. Indeed, the cell membrane of the spermatozoa is modified by the elimination of the membrane cholesterol by proteins of the albumin present in the female genital tract [9].

The statistical analysis showed that these differences are not significant as shown in the table 1. This allows to state that glucose, creatinine, uric acid, total cholesterol, triglycerides and total protein are not discriminant in metabolites during the diagnosis of azoospermia profiles in relation to normospermia profiles. These data support for glucose, with the previous works indicated, that there is almost no difference between the values of the glucospermia of the infertile subjects and those of the fertile subjects [19, 20, 21].

The study of urea is the only significant parameter highlighted in the azoospermia and normospermia semen samples. The values reported in table 1 represent the statistical analysis and indicate that the average concentration with the standard deviation of urea in the seminal fluids of normospermia (0.923 ± 0.204 g/L) is greater than the one obtained in the seminal fluids of the azoospermia semen samples (0.496 ± 0.059 g/L). This could be explained by the metabolic activity by the spermatozoa in the normospermia semen samples compared to the azoospermia semen samples where they are absent. Indeed, urea is a degradation product of the protein [22]. The role of the latter is to protect and support the metabolic spermatozoa.

Moreover, the difference in values of urea in the two samples could be explained by the role of mitochondria in the formation of urea. Indeed the urogenesis requires a mitochondrial stage with the formation of citrulline and a cytosolic stage with urea formation [23]. This makes clear that in addition to urea, these precursors could exist in the seminal fluid. Thus, the presence of the precursors of urea and the presence of spermatozoa promote an increase of the concentration of urea in the seminal fluid of the normospermia semen samples compared to the azoospermia semen samples.

The statistical analysis showed that there is a significant difference between the concentrations of urea in normospermia and azoospermia semen samples as shown in the table 1. That could have a great interest in the exploration of male infertility, namely in the diagnosis of azoospermia. Indeed, as the plasma measurements of FSH and LH allow the diagnosis of the azoospermia secretory compared to the azoospermia excretory, urea may be a parameter regarded metabolite discriminant in diagnosing azoospermia profiles relatively to the normospermia profiles [15].

**Conclusion**

The study on the seminal fluids from data obtained with assays of metabolites such as glucose, urea, creatinine, uric acid, total cholesterol, triglycerides and total protein, permitted to show that discriminative factors between azoospermia and normospermia semen samples contain all these metabolites to more or less different concentrations.

The average concentrations of glucose and urea in normospermia semen samples are higher than those obtained in azoospermia semen samples whereas those of creatinine and total protein in azoospermia semen samples are superior to those obtained in normospermia semen samples, and those of triglycerides, uric acid and total cholesterol remain substantially identical in both samples.

Statistically, the study enabled to have two types of parameters. The first type, which is not significant, is composed of glucose, creatinine, uric acid, total cholesterol, triglycerides and total protein. These metabolites can’t be regarded as discriminant for the azoospermia profiles. However, for the second type, only the differences in concentration of urea in both samples are considered as a significant parameter in the exploration of male infertility. The last
meaningful difference of the average concentrations of urea in both samples with regard to the results of this study could be decisive in the diagnosis of azoospermia and normospermia profiles as well as FSH and LH hormones that distinguish the secretory excretory azoospermia. However, a study with a larger sample could contribute to the validation of this metabolite as discriminant between normospermia and azoospermia.

Ethical approval
This study presented by the authors was approved by the National Council of Ethics and Research in Côte d'Ivoire (Reference N° 036/MSLS/CNER/RB).

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References


