Spermocytogram: Comparison Between the Papanicolaou Staining and Modified May Grünwald Giemsa Staining

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Abstract

Introduction: The research and distribution of sperm abnormalities by the spermocytogram are an essential examination in the diagnosis of male infertility. The standard Papanicolaou staining method, recommended by the World Health Organization, for performing a spermocytogram, is too expensive in Congo. The work aimed to look for abnormalities in sperm forms by two comparative methods: Papanicolaou staining and modified May Grünwald Giemsa staining (less expensive), set up in our laboratories.

Materials and methods: A series of one hundred spermocytograms were performed by two staining methods: the Papanicolaou method and the modified May Grünwald Giemsa method. The results of two techniques were compared qualitatively and quantitatively.

Results: The mean detection of sperm form abnormalities was higher by the Papanicolaou method. However, the precision of the kind of abnormalities highlighted by the two techniques was superimposable.

Conclusion: Despite the superiority of the Papanicolaou staining method, which is a reference technique of the spermocytogram, the modified May Grünwald Giemsa staining method can be a first-line technique in the male diagnosis of infertility, especially in the deep Congo. It is efficient, reliable, inexpensive and easily achievable.

Introduction

Infertility is the absence of conception after at least 12 months of married life after regular sexual intercourse during ovulation [1-3]. It can be primary or secondary and affects about 1/6 of couples [4-6]. The male part is 20 to 30% [4, 7]. The diagnosis of infertility in men is based first of all on semen analysis, which makes it possible to detect abnormalities in the number (oligozoospermia, azoospermia) and form (teratozoospermia) of spermatozoa [8, 9]. In common practice, teratozoospermia is detectable from the spermocytogram, which gives an idea of male fertility, according to numerous studies [4, 8, 10, 11]. The study of sperm morphology is therefore an essential step in the management of male infertility. In 2010, the World Health Organization (WHO) indicated a reference method in the detection of these abnormalities: it includes the Papanicolaou staining technique and the...
Kruger classification [12]. However, in the Republic of Congo, the practice of the spermocytogram using the Papanicolaou staining technique is too expensive. To reduce the cost of the spermocytogram in Brazzaville, we propose to set up a less expensive diagnostic technique: May Grünwald Giemsa (MGG) staining modified in its steps. MGG staining is a staining technique usually used in hematology for the performance of peripheral blood smears. The objective of the present study was to look for abnormalities in sperm forms by two comparative methods: Papanicolaou staining and modified May Grünwald Giemsa staining, implemented in our laboratories.

Materials and Methods
This was a cross-sectional and analytical study spanning six-month period (July to November 2019). It was carried out at the National Public Health Laboratory (LNSP), at the Teaching Hospital of Brazzaville and at the National Institute of Research on Health Sciences (IRSSA) of Congo. Informed consent was obtained from all men involved in sperm collection.

Materials
The study was based on one hundred (100) sperm samples from men with known infertility in the couple. Patients previously consulted by their general practitioner were recruited from a pre-established questionnaire. Sperm collection was carried out according to usual procedures (WHO 2010) [13]. It was placed at room temperature for immediate analysis, and the remainder kept at -20°C for possible analysis. The different reagents: have been stored at room temperature.

Methods
The comparative analysis of sperm morphology was based on the techniques of modified MMG staining and Papanicolaou. Common preliminaries, including the preparation of air-dried smears for 24 hours, were performed in both methods.

Color with modified May Grunwald Giemsa (MMG).

- A bath of the spermatic smear slides successively in the undiluted May Grunwald for 2 minutes (min) then in the May Grunwald diluted to 1/2 for 5 min followed by a rinse with demineralized water and draining;
- A bath of the blades in Giemsa diluted to 1/20 for 10 min, followed by a rinse with demineralized water and air-dried for 24 hours. The reading was done under an optical microscope (o.m.), objective 100. A minimum of 200 sperm counts were required and results were expressed as a percentage (%).

Papanicolaou staining.
The principle is based on the use of several acidic and basic dyes that specifically stain the nucleus and cytoplasm of cells in purplish blue. The protocol incorporates:
- The spermatic laminae were immersed for 3 min each time, in three successive baths of alcohol decreasing at 80°, 70° and 50°; followed by a rinse with distilled water for 5 min.
- Bath in hematoxylin, 5 min, and rinse with distilled water 5 min.
- A 2nd series of stirred baths in alcohol rising to 50°, 70° and 80° then rinsing.
- A third series of stirred baths in 95° alcohol, then successively in a solution of orange G, and a solution of EA50, 5 min each time; interspersed with a bath of alcohol at 95° by stirring.
- Drying of the slides in the oven for 5 min, assembly of the slides with Eukitt and reading with the o.m. (objective 100).
- Morphological results of spermatozoa are reported according to the Kruger classification (WHO 2010) (Table 1).

Table 1: Kruger Classification

<table>
<thead>
<tr>
<th>Classes</th>
<th>Morphology</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Spermatozoa of normal morphologies</td>
<td>&gt; 4% (4 à 44%)</td>
</tr>
<tr>
<td>II</td>
<td>Spermatozoa with abnormal morphologies</td>
<td>&lt; 50%.</td>
</tr>
</tbody>
</table>

Results
Abnormal Forms Depending on the Methods Used
The staining techniques showed a slightly higher average teratozoospermia by the Papanicolaou method (Table 2). However, the kind of abnormalities observed were more or less the same in both techniques (Table 3).
Table 2: Spermocytogram Data According to the Kruger Classification

<table>
<thead>
<tr>
<th>Staining Method</th>
<th>Classes</th>
<th>Morphology</th>
<th>Mean (%)</th>
<th>Type of abnormalities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Papanicolaou</td>
<td>I</td>
<td>Normal</td>
<td>42.08</td>
<td>- Macrocephalic or microcephalic, double head</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>Abnormal forms</td>
<td>57.92±15.88</td>
<td>- Acrosome abnormalities</td>
</tr>
<tr>
<td>May Grunwald Giemsa modified</td>
<td>I</td>
<td>Normal</td>
<td>51.48</td>
<td>- Flagellum: short, double, or coiled</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>Abnormal forms</td>
<td>48.52±13.72</td>
<td></td>
</tr>
</tbody>
</table>

Comparison of the Two Methods
- Comparison of the means of these 2 methods showed a P-value of 0.3346, which was not significant (Table 3).
- For both techniques, there was no correlation between sperm count and abnormal forms of spermatozoa in all sperm of the cohort (P-value of 0.1339). On the other hand, acrosome and flagellum abnormalities show significant P-values for both techniques (Table 3).

![Comparative Staining of Teratozoospermia](image)

Table 3: Types of Sperm Abnormalities Observed by the Papanicolaou Staining Method and Modified MGG
<table>
<thead>
<tr>
<th>Types of abnormalities</th>
<th>Mean (%)</th>
<th>P. value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Papanicolaou</td>
<td>Modified MGG</td>
</tr>
<tr>
<td>Macrocephalic</td>
<td>7.167±10.43</td>
<td>3.444±1.649</td>
</tr>
<tr>
<td>Microcephalic</td>
<td>7.917±16.45</td>
<td>4.593±2.606</td>
</tr>
<tr>
<td>Elongated head</td>
<td>7.077±3.068</td>
<td>9.63±2.95</td>
</tr>
<tr>
<td>Thinned head</td>
<td>4.583±2.021</td>
<td>3.64±3.466</td>
</tr>
<tr>
<td>Multiple heads</td>
<td>5±1.581</td>
<td>1.5±2.301</td>
</tr>
<tr>
<td>Acrosomal abnormalities</td>
<td>4.909±2.256</td>
<td>1.71±1.863</td>
</tr>
<tr>
<td>Angulation</td>
<td>3.867±3.681</td>
<td>0.464±1.319</td>
</tr>
<tr>
<td>MP absent</td>
<td>5.33±3.447</td>
<td>2.5±2.769</td>
</tr>
<tr>
<td>MP irregular base</td>
<td>3±2.412</td>
<td>1.6±2.483</td>
</tr>
<tr>
<td>MP short</td>
<td>5.33±3.848</td>
<td>4.25±3.908</td>
</tr>
<tr>
<td>MP Coiled</td>
<td>7.786±2.293</td>
<td>8.75±5.654</td>
</tr>
<tr>
<td>MP Doubled</td>
<td>5.6±2.608</td>
<td>0.285±0.8545</td>
</tr>
<tr>
<td>Total mean</td>
<td>57.92±15.88</td>
<td>48.52±13.72</td>
</tr>
</tbody>
</table>

**Note:** MP: Midpiece; MGG: May Grünwald Giemsa.

### Discussion

The implementation of the modified MGG staining method showed that Papanicolaou's method is not the only staining used in the performance of the spermocytogram. Indeed, the literature reports several other sperm staining techniques, including: the staining of Bryan-Leishman, Giemsa, Shorr, Hemalun-Shorr, Wright, Hematoxylin-Eosin, Wright-Giemsa and the Spermo-Scan [12, 14, 15]. The Diff-Quik kit is a staining method also used [7, 16, 17]. All these methods aim to detect teratozoospermia.

The research for Teratozoospermia

The staining of Papanicolaou and the modified MGG in search of abnormal forms of spermatozoa had allowed us to observe the morphologies of the spermatozoa under an optical microscope (Table 3, Figure 1). Using both methods, the microscopic analysis, specifically identified the three major parts of the spermatozoa: the head, the midpiece and the main part. Macrocephalic heads, microcephalic heads, and elongated heads were well identified by both staining techniques (Figure 1). However, abnormalities of the acrosome, cytoplasmic remainder, and main piece were difficult to see with modified MGG staining.

In addition, the search for flagellum abnormalities by modified MGG staining required more attention in observation, therefore, leading to use a high magnification (100X, 200X). Note that the lack of data in the literature on MGG staining developed in our laboratories did not allow us to compare our results with other authors.

On the other hand, Papanicolaou staining had easily detected abnormalities in the form of spermatozoa. The number of sperm form abnormalities found by Papanicolaou staining was higher (Table 2). The latter staining method, which best identifies teratozoospermia, is widely used in other countries [3, 18]. The results found in this study were identical to those in the literature [3, 18].

### Types of Abnormal Forms of Spermatozoa

Regarding the kind of abnormalities detected by the two methods, there is a prevalence of macrocephalic and microcephalic heads, short and coiled flagella (Table 2, Figure 1). The present results are similar to those reported by some authors [19, 20]. However, Fall et al. [21] found in their study a predominance of cytoplasmic remainder abnormalities.

The types of abnormal forms of spermatozoa by the two staining methods did not show a big difference (Table 3). Indeed, the abnormalities found by Papanicolaou staining were also visualized by modified MGG staining (Figure 1).

In contrast, abnormalities in the main part (absence of the main part, p= 0.0077; double main part, p= 0.0086) and acrosome (p=0.0093) showed a statistically significant difference for the two methods (Table 3). These results show that the identification of abnormal forms of spermatozoa by these two methods can be superimposed.

### Conclusion

The Papanicolaou staining method proposed by the WHO, remains a method of choice in the analysis of the spermocytogram under light microscopy, for the diagnosis of teratozoospermia. However, its expensive cost prompted us to implement the modified May Grünwald Giemsa stain method. The results obtained show that the latter could be an alternative method to that of Papanicolaou. It could serve as a first-line treatment in the diagnosis of male infertility, especially in the deep Congo because it is effective, reliable, inexpensive and easily achievable in our laboratories.

### Acknowledgments

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Contributions to Authors
OAWS designed the study. YKP, BBR, LSBS and MC collected the cases and performed the stains. BBR, YKP, MC, LSBS and HP analyzed the data. MC, HP and OAWS wrote the paper.

Conflict of Interest
The authors do not declare any conflict of interest.

References