Assessment of Exfoliated Prostate Cells in Semen

Relationship With the Secretory Function of the Prostate

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Key Words: Basic science; Cytology; Prostate function; Prostate cells; Semen

Abstract

The percentage of exfoliated prostate cells (EPCs) in 139 semen specimens was determined. A receiver operating characteristic curve showed a cutoff of 17% for EPCs, and specimens were distributed into 4 groups: EPCs 17% or fewer without (group 1; n = 44) and with (group 2; n = 31) leukocytospermia and EPCs more than 17% without (group 3; n = 39) and with (group 4; n = 25) leukocytospermia. Acid phosphatase, calcium, and zinc levels; volume and pH of ejaculate; and leukocyte count were assessed. Between groups 1 and 2, the only significant difference was in leukocyte count (P < .001). Between groups 3 and 4, differences were significant in zinc levels (P = .026) and leukocyte count (P < .001). Comparisons of groups 1 and 2 vs 3 and 4 showed lower levels of acid phosphatase activity (P = .017) and calcium (P = .019), and increased seminal volume (P = .019) between groups 1 and 3. Between groups 2 and 4, the difference was significant only in leukocyte count (P < .001). EPCs of 17% or fewer with and without leukocytospermia suggest normal prostate function; EPCs of more than 17% without leukocytospermia suggest abnormal function. EPCs of more than 17% with leukocytospermia were inconclusive.

Semen analysis is of great interest in clinical practice to investigate disorders affecting male genital organs. This analysis aimed to evaluate a wide number of semen parameters, such as physical properties of the ejaculate, seminal biochemical markers, sperm characteristics, sperm function tests, leukocyte count, antisperm antibodies, acrosome status, DNA integrity, and ultrastructural defects of spermatozoa. The majority of these procedures were standardized by World Health Organization guidelines.

Semen analysis helps in the investigation of several disorders of the male genital tract such as infertility, infections, diseases, and other clinical conditions. However, only a few parameters of semen analysis are used to study prostate diseases, despite their high prevalence, chiefly in elderly men. Assumptions have been made indicating that the analysis of biochemical markers of the prostate might be useful in this regard. Nevertheless, they have hitherto been poorly explored in clinical practice. One such parameter that could perhaps be related to the aforementioned, ie, the physical analysis of the ejaculate, has also been neglected. As far as can be ascertained, this is partly attributable to the extensive efforts to provide reliable, direct support for evaluating the capacity of fertilization of spermatozoa, to the detriment of the semen parameters used to study prostate diseases, because semen analysis is particularly prescribed for infertile men.

Such evidence encouraged me to study exhaustively the role of semen parameters when assessing the secretory function of the prostate and seminal vesicles through semen analysis. Focusing on this purpose, the clinical value of the physical analysis of ejaculate was studied and reported, and abnormal results were related with gland dysfunctions.
In the present study, I established a new focus of research based on the determination of the percentage of exfoliated prostate cells (EPCs) in semen. On several occasions, these cells were studied in prostate diseases.\textsuperscript{15-17} However, they are not assessed in routine semen analysis, partly owing to the lack of affordable and time-efficient diagnostic techniques. Overall they are recorded as “round cells.” Nevertheless, it is expected that the individual analysis of EPCs may be of clinical interest, taking into account certain aspects in particular, such as why and when the desquamation of the prostate epithelium increases and its relationship with normal and abnormal secretory functions of the prostate.

As a part of routine semen analysis, I studied EPCs in semen smears and differentiated them from other seminal cells, using a simple modified Leishman blood staining method.\textsuperscript{18} Based on this practice, the purposes of the present study were as follows: (1) report the method to determine the percentage of EPCs in semen smears; (2) determine a cutoff level for EPCs; (3) study the relationship between EPCs and biochemical markers of the prostate, acid phosphatase activity, calcium and zinc, and the physical properties, volume and pH, in semen with and without leukocytospermia; and (4) determine the clinical value of the percentage of EPCs in assessing prostate secretory function in routine semen analysis.

Materials and Methods

Specimens

Semen specimens were obtained from 139 consecutive untreated men aged 19 to 60 years (mean, 38.4 years) routinely referred for initial semen analysis for infertility, genital infections, varicocele, impotence, testicular pain, undescended testicles, or recurrent miscarriage in the partner before treatment. Specimens were collected at the laboratory, into sterile containers by masturbation, after 4 to 6 days of sexual abstinence.

Analysis of Physical Properties of the Ejaculates

Semen volume was measured using a 15-mL graduated cylinder with a conical base (accurate to 0.1 mL), and dipsticks ranging from 6.0 to 8.0 and 8.2 to 10.0 (Merck, Rio de Janeiro, Brazil) were used for estimation of pH.

Biochemical Analysis

Acid phosphatase and total calcium and zinc levels were measured in the seminal plasma obtained by centrifugation of liquefied semen for 10 minutes at 1,000g, according to procedures described previously.\textsuperscript{14} Semen with abnormal liquefaction was analyzed 1 hour after ejaculation.

Leukocyte Count

The count was performed according to the World Health Organization protocol,\textsuperscript{5} by histochemical study of peroxidase activity in polymorphonuclear granulocytes (PMNs). Leukocytospermia was defined as a leukocyte count of more than 1.0 × 10\(^6\) PMNs/mL of semen.

Assessment of EPCs

Semen smears were produced using pellets obtained by centrifugation of fresh semen for 5 minutes at 1,000g. After the smears were mounted and dried, they were stained by a modified Leishman method, as described previously,\textsuperscript{18} and examined under light microscopy at a magnification of ×1,000. The percentage of EPCs was determined after the count of 100 exfoliated nongerminal epithelial cells of the seminal tract, such as prostate cells, transitional cells, seminal vesicle cells, and urethral cells according to the following formula:

\[
\text{Total of EPCs} \times 100
\]

\[
\text{Total of Nongerminal Epithelial Cells}
\]

For each specimen, 2 semen smears were evaluated, and the mean value was registered.

Statistical Analysis

A receiver operating characteristic (ROC) curve analysis (MedCalc, version 8.1.0.0, MedCalc Software, Mariakerk, Belgium) was constructed to derive the most suitable statistical cutoff level for EPCs in semen that provided the best combination of sensitivity and specificity. The ROC curve depicts sensitivity (true-positive rate) and specificity (false-positive rate). The value that combines the highest true-positive rate with the lowest false-positive rate is usually considered the optimum cutoff level. The area under the curve and 95% confidence intervals were also calculated. Based on the cutoff obtained, semen specimens were distributed in 2 study groups (≤17% EPCs and >17% EPCs), and each of these also was distributed in 2 groups, without or with leukocytospermia. The data were statistically analyzed, using the unpaired t test and Mann-Whitney test, for normally and nonnormally distributed variables. A P value lower than .05 was regarded as significant.

Results

Three morphologic characteristics of EPCs were identified in the semen smears: small cells, large cells, and columnar cells Image1A. Overall, the cells presented round or oval morphologic features; pale cytoplasm, sometimes with small narrow strips; and a central or epicentric nucleus. The cells were well-differentiated from those originating in the seminal vesicles, which resemble EPCs but contain intracytoplasmic granules Image1B. Also, EPCs were well differentiated from the large polygonal or oval squamous epithelial cells.
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Image 1C and from the cuboidal or oval transitional epithelial cells from the bladder and urethra Image 1D.

Because the prevalence of EPCs in semen has not been described in previous reports, an ROC curve was constructed to determine the cutoff point providing the best combination of sensitivity and specificity. The ROC curve showed a cutoff at 17% for EPCs in semen Figure 1. The area under the curve was 0.563 (SE, 0.058; 95% confidence interval, 0.460-0.662) with 78.0% sensitivity and 35.6% specificity.

By taking into account the cutoff found, semen specimens were distributed in 2 study groups: 17% or fewer EPCs (n = 75) and more than 17% EPCs (n = 64). Both were also divided in 2 subgroups: without leukocytospermia for EPCs 17% or fewer (n = 44) and EPCs more than 17% (n = 39) and with leukocytospermia for EPCs 17% or fewer (n = 31) and for EPCs more than 17% (n = 25). The physical characteristics of the ejaculate, volume and pH, and the seminal biochemical markers, acid phosphatase and total calcium and zinc levels, were evaluated in the study groups, together with the leukocyte count.

Descriptive statistics are summarized in Table 1 as mean ± SD. No statistically significant differences were observed for any of the seminal parameters analyzed between the subgroups of EPCs 17% or fewer (P > .05), with the exception of the leukocyte count (P < .001). On the other hand, the levels of zinc were significantly lower in semen without leukocytospermia than semen with leukocytospermia with EPCs more than 17% (P = .026). The differences were not significant for
other semen parameters \((P > .05)\), with the exception of the leukocyte count \((P < .001)\).

The study also compared semen samples with EPCs 17% or fewer vs EPCs more than 17%. When comparisons were made between semen samples without leukocytospermia, acid phosphatase activity and the level of total calcium were significantly lower in samples with EPCs more than 17% \((P = .017\) and \(P = .019\), respectively), whereas the semen volume was significantly higher \((P = .019)\). The level of zinc, the pH, and the leukocyte count did not show significant differences \((P > .05)\). On the other hand, the differences were not significant in the biochemical markers or volume and pH between semen samples with leukocytospermia \((P > .05)\). The leukocyte count was significantly higher in EPCs more than 17% \((P < .001)\).

**Discussion**

Although human semen is a potential source of prostate epithelial cells, the assessment of EPCs in semen was hitherto the subject of few reports. Likewise, EPCs are not used in clinical practice to evaluate the secretory function of the prostate gland. The aim of the present study was to determine if the percentage of EPCs in semen is associated with abnormalities in the prostate function, as indicated by levels of acid phosphatase activity and total calcium and zinc and the physical properties of the ejaculate, volume and pH, and leukocyte count.

Direct comparison of the semen without and with leukocytospermia in samples with EPCs 17% or fewer demonstrated that the levels of the prostate biochemical markers and seminal volume and pH did not differ significantly. Accordingly, these findings seem to support that no abnormality could be ascertained with this seemingly “normal” desquamation of the prostate epithelium. They suggest a normal prostate secretory function, even in semen with leukocytospermia.

**Table 1**

<table>
<thead>
<tr>
<th>EPCs ≤17%</th>
<th>EPCs &gt;17%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without Leukocytospermia</td>
<td>With Leukocytospermia</td>
</tr>
<tr>
<td>(n = 44)</td>
<td>(n = 39)</td>
</tr>
<tr>
<td>Acid phosphatase (IU/mL)</td>
<td>728.8 ± 610.1</td>
</tr>
<tr>
<td>Calcium (µg/mL)</td>
<td>334.7 ± 187.8</td>
</tr>
<tr>
<td>Zinc (µg/mL)</td>
<td>116.0 ± 48.3</td>
</tr>
<tr>
<td>Volume (mL)</td>
<td>3.42 ± 1.57</td>
</tr>
<tr>
<td>pH</td>
<td>765.0 ± 0.44</td>
</tr>
<tr>
<td>Leukocytes ((×10^6))</td>
<td>109.6 ± 120.9</td>
</tr>
</tbody>
</table>

EPCs, exfoliated prostate cells.

† Values are expressed as mean ± SD.

‡ \(P = .017\), EPCs ≤17% vs EPCs >17% (without).

§ \(P = .019\), EPCs ≤17% vs EPCs >17% (without).

|| \(P = .026\), EPCs >17% (without vs with).

|| \(P < .001\), EPCs ≤17% and EPCs >17% (without vs with) and EPCs ≤17% vs EPCs >17% (with).
were also lower, but the differences were not significant. In contrast, the biochemical markers did not show significant differences between semen samples with leukocytospermia (EPCs ≤17% vs EPCs >17%). These data suggest that the increase of EPCs in semen without leukocytospermia is indicative of abnormal prostate secretory function. Moreover, they did not present convincing proof that any prostate secretory function was also affected in semen with leukocytospermia, because the levels of prostate biochemical markers in semen samples were not significantly altered.

The normal prostate epithelium is basically composed of a heterogeneous population of cells (stem cells, basal cells, intermediate cells, luminal secretory cells, and neuroendocrine cells), which are identified according to their androgen dependence, biochemical composition, capacity of proliferation, apoptotic index, and expression of high-molecular-mass cytokeratins. Usually, prostate cells exfoliate into the ejaculate, and they can be increased in semen from men with prostate diseases. Because the accumulation of biochemical compounds and their secretion into the prostatic fluid are essential functions of the luminal secretory cells, under androgen regulation, it is likely that the increased desquamation of EPCs in semen is associated with disturbances in their secretory patterns. Data from the current study suggest that functional changes occur and these can negatively affect substances such as acid phosphatase, calcium, and zinc. Nevertheless, functional changes were consistent and indicative of prostate dysfunction only in semen samples without leukocytospermia.

Indeed, previous investigators have found low levels of prostate biochemical markers in men with abnormal prostate secretory function, including in prostate cancer. However, such abnormalities have also been found in men with prostatitis, a feature that apparently contrasts with the current findings because no significant differences were detected in the biochemical markers in samples from men with leukocytospermia. In fact, comparisons between results obtained in the present study and those of other studies can hardly be drawn because, to the extent of my knowledge, this is the first study of its kind to evaluate EPCs.

Also, it is noteworthy that EPCs more than 17% were associated with a significant increase in the seminal volume in semen without leukocytospermia. This finding was somewhat surprising because one would expect subjects with abnormal prostate secretory function to have production of prostatic fluid negatively affected, thus impairing its dynamic interaction with the seminal vesicle fluids in the formation of the ejaculate. It is well known that prostate and seminal vesicle fluids contribute 30% to 35% and ±60% of the ejaculate, respectively. Therefore, it would be expected that low levels of the biochemical markers and a decrease in the production of the prostate fluid in samples with EPCs more than 17% would yield a prevalence of seminal vesicle fluids in the ejaculate. Nevertheless, to increase the semen volume in the presence of impaired production of prostate fluid, it would also be necessary for the amount of seminal vesicle fluids in the ejaculate to increase significantly, which seldom occurs because, although they interact with one other, these glands are functionally self-sufficient, even in gland function. Thus, the higher volume in samples with EPCs more than 17% and without leukocytospermia suggests that prostate dysfunctions increase the contribution of prostatic fluid and the desquamation of EPCs in the ejaculate, while concurrently affecting negatively the production of biochemical compounds. Despite this evidence, further study is necessary because it has been shown that secretion of prostate fluid into the ejaculate can seem reduced in men with prostate diseases, mainly in older men.

The ROC curve performed in this study demonstrated that the determination of the percentage of EPCs in semen presented poor specificity, suggesting its ineffectiveness as a single diagnostic screening test for prostate disease. However, the alterations observed in the biochemical markers and semen volume in samples with EPCs more than 17% without leukocytospermia indicate an abnormal prostate secretory function, which was mainly detected owing to the distribution of the study groups according to the percentage of EPCs in semen. Therefore, it can also be used as an adjunct test in semen analysis for monitoring prostate function. It is my hope that semen analysis may shed new light on the pathophysiology of the prostate.

**Concluding Remarks**

Data from the present study suggest that EPCs of 17% or fewer in semen are indicative of normal secretory function of the prostate, even in semen with leukocytospermia. On the other hand, EPCs of more than 17% indicate abnormal secretory function of the prostate in the lack of leukocytospermia, which affects negatively the levels of prostate biochemical markers and, seemingly, increases the volume of prostate fluid in the ejaculate. This study does not produce consistent evidence that prostate secretory function was also abnormal in semen samples with EPCs more than 17% and leukocytospermia because the biochemical markers and semen volume were not significantly affected.

Further studies are necessary to determine which diseases harm prostate function by increasing the exfoliation of prostate cells. Although the percentage of EPCs did not reveal a clear relationship with leukocytospermia, its role in prostatitis should also be further investigated. It is suspected that the diverse forms of prostatitis (acute and chronic bacterial, chronic pelvic pain syndrome/chronic prostatitis, and asymptomatic) could bring about degenerative changes in the prostatic...
epithelium, increasing the EPCs in semen, and, thus, simultaneously affecting (or not) the biochemical marker levels and semen volume.

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Acknowledgment: I thank Elciro José Duarte for helpful aid in reviewing the photomicrograph.

References
