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### **Correlation of Immature Germ Cells in Semen in Severe Oligospermic and Azoospermic Males**

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#### **ABSTRACT**

Recent studies suggest suggests that male factor contributing to infertility is up to 30% of the total cases. Semen analysis report “round cells” as without further differentiating them into leucocytes or immature germ cells. The aim of this work was to study a method for differentiating the round cells in semen into immature germ cells and leucocytes and to correlate shedding of immature germ cells in severe Oligospermic and Azoospermic males. Semen samples from 100 males, who had come for investigation for infertility, were collected, semen parameters recorded, and stained with Leishman smears studied for different round cells. In this study, out of 100 cases 32 were Azoospermic whereas 68 were severe Oligospermic males. The round cells were counted as percentage of the total count and it was seen that their percentage of immature germ cells were more in Oligospermic as compared to Azoospermic males. The differential counts of round cells showed 70% were immature germ cells in Azoospermic males and 80% in Severe Oligospermic males. In semen analysis round cells should also be given due importance, as they can be differentiated and counted into immature germ cells. Presence of immature germ cells in Azoospermic males rules out Obstructive type. The differential counts mentioned in a semen report give valuable and clinically relevant information.

**KEYWORDS:** Immature germ cells; round cells; semen; total sperm count.

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## **INTRODUCTION**

In Medical career treating Infertility is a challenge, also the cases of infertility are on rise. Recent studies report the incidence of infertility as almost one in every seven couples. Rate of infertility in India is 9% of the reproductive population. Considering etiology of infertility cases, male causes amount to 50%. Due to all these reasons, lots of studies related to advanced diagnostic test and treatment for male infertility are reported. In developing country like India, investigations and treatment are huge financial burden on the couple. So, semen analysis which is basic investigation for diagnosis for male infertility. In semen analysis report, cells mentioned as “round cells” are leucocytes or immature germ cells. These cells cannot be differentiated in an unstained wet preparation.<sup>1</sup>

The manual for semen analysis mentioned by WHO suggest there is need for differentiation of round cells into immature cells and Leucocytes, if count of the round cells are more than  $1 \times 10^6/\text{ml}$ .<sup>2</sup> Many studies clearly conclude that in semen report mention of percentage of spermatogenic and non-spermatogenic origins is important.<sup>3</sup> Only mentioning total number of “round cells” will be incomplete information to the treating Andrologists.<sup>4</sup> Also review of literature suggest that differentiating round cells can help further treatment and will guide regarding pregnancy outcome, sperm micro-manipulation and in-vitro fertilization techniques.<sup>5</sup> The absence of germ cells in semen report can easily lead to diagnosis of obstructive and rule out non-obstructive azoospermia.<sup>6</sup> Recent advances in procedure like TESA (testicular sperm extraction) which retrieve the immature germ cells (spermatids) in semen samples from azoospermic males can be used for producing viable embryos by Intra-cytoplasmic sperm-injection technique. So this information is really important especially in azoospermic.<sup>7</sup> For all mentioned reasons, there is a need for semen report to mention differentiation of round cells into immature germ cells and leucocytes.

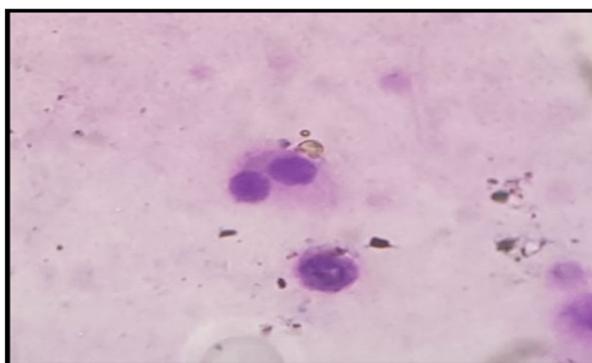
The aim of the this study was to differentiate the round cells by a Leishman’s stain into leucocytes and immature germ cells and also to compare percentage of immature germ cells in severe Oligospermic and Azoospermic males.

## **METHODOLOGY**

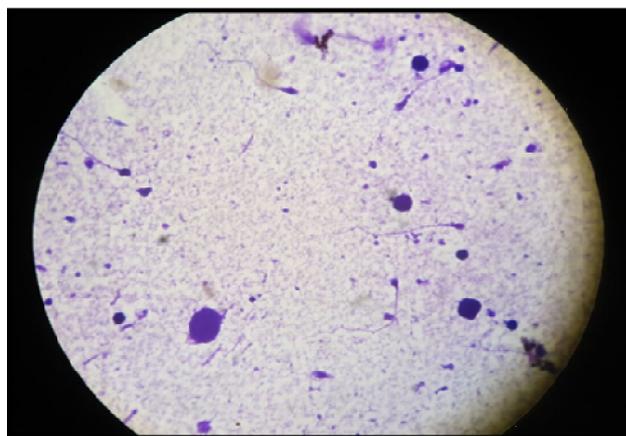
Data was collected after clearance from Institutional Ethical committee. Data of 100 semen samples collected in the laboratory from males coming for semen analysis for routine infertility work-up during the period of 1 and half year from March 2017- July 2018. After obtaining written informed consent, samples of semen were collected by masturbation. Samples were collected into a clean wide-mouthed plastic semen container by masturbation. Initially using a drop of semen, Semen

parameters were recorded. Later smears were prepared by the feathering method described in the 5th edition of the WHO manual for semen analysis.<sup>8</sup> Smears were stained by using Leishman stain.<sup>9</sup>

The round cells were counted and differentiated into immature germ cells and leucocytes. Smears in duplicate were seen under oil immersion lens of microscope for differential counts of Round cells into immature germ cells and leucocytes. Cells were identified according to their size, shape, and morphology.<sup>3,4</sup> Immature germ cells seen were Primary spermatocyte, identified by their large size, large spherical nucleus with woolly appearance and evenly distributed chromatin granules [Figure 1]. Spermatids were smaller, round to oval cells with a dark nucleus [Figure 2]. Leucocytes were differentiated by their smaller size and multilobed nuclei.



**Figure 1: Leucocyte and Primary Spermatocyte (Leishman Stain, x100)**



**Figure 2: Sperm, Spermatid and Primary Spermatocyte (Leishman Stain, x100)**

## **OBSERVATIONS**

In our study out of 100, 32 were Azoospermic while 68 were severe Oligospermic. Routine semen parameters like the total count and motility were recorded. Out of 32 Azoospermic, 31 semen were Fructose positive one was Fructose negative. The round cells were counted as percentage of the total count and it was seen that their percentage were more in Oligospermic as compared to Azoospermic males as shown in Table 1.

Table 1: "Round Cell Count and Percentage of cells"

| Group            | Semen Leishman Stain   |                       |              |
|------------------|------------------------|-----------------------|--------------|
|                  | Total cells million/ml | Immature germ cells % | Leucocytes % |
| Azoospermic(32)  | 2                      | 70.06                 | 30.25        |
| Oligospermic(68) | 2.53                   | 82.63                 | 20.3         |
| P value          | P < 0.0001**           | P < 0001**            | P < 0.0001** |

## DISCUSSION

The differential counts of round cells showed that out of the total round cells, 70% to 80% were immature germ cells and 10% to 20% were leucocytes. Our study results are similar to studies done by Fedder et al.,<sup>10</sup> Gandini et al.,<sup>1</sup> and Ariagnoet al.<sup>11</sup>

Semen report mentioning percentage of shedding immature germ cell is definitely a useful tool for knowing the etiology of infertility. Also this is could be a good indicator of a dysfunction at the testicular level, by giving information about germ cell maturation arrest.

If this staining method reports no immature germ cells, it suggest obstructive cause so avoiding invasive procedure of Testicular biopsies.<sup>1</sup> In this study, there was one azoospermic Sample having absence of fructose and immature germ cells which confirmed obstructive azoospermia. Thus, the presence of germ cells can easily and definitely differentiate non-obstructive from obstructive azoospermia.<sup>8</sup>

These immature germ cells in semen can be used for diagnostic and cytogenetic studies. These isolated germ cells can be studied by cytogenetics for chromosomal aberrations – numerical or structural.

Recent advances in procedure like TESA (testicular sperm extraction) which retrieve the immature germ cells (spermatids) in semen samples from azoospermic males can be used for producing viable embryos by Intra-cytoplasmic sperm-injection technique. So this information is really important especially in azoospermic.<sup>7</sup>

## CONCLUSION

In semen analysis round cells should also be given due importance, as they can be differentiated and counted into immature germ cells. The count of immature germ cells could be obtained by simple staining procedures and will add clinically relevant information to the semen report.

Further, the immature germ cells in semen can be separated and isolated for diagnostic, cytogenetic studies and can be used for in-vitro culture.

Absence of fructose and immature germ cells in semen confirms Obstructive azoospermia.

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