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# A COMPARATIVE STUDY BETWEEN EMBRYO QUALITY FERTILIZED WITH NORMAL EJACULATE SPERMS VS. TESA/PESA SPERMS

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## Abstract

Our study aims to compare the quality of embryos fertilized using sperm obtained from testicular sperm aspiration (TESA) or percutaneous epididymal sperm aspiration (PESA) against those sourced from normal ejaculate in the intracytoplasmic sperm injection (ICSI). Male infertility is a significant issue. A particularly challenging form is azoospermia, a condition in which there are no sperm in the ejaculate. Azoospermia can be obstructive (OA) or non-obstructive (NOA). The causes for azoospermia vary; they can include genetic disorders, such as cystic fibrosis, or physical blockages. For patients with azoospermia, surgical methods like TESA & PESA offer hope for biological parenthood. This study's goal is to assess how effective these methods are in embryo fertilization by observing embryos till day 5 -blastocyst stage. These blastocysts serve as an important indicator of embryo viability.

**Keywords**: TESA/PESA, male infertility, azoospermia

## INTRODUCTION

Male infertility is a critical issue within reproductive health. Azoospermia, a complete absence of sperm in the ejaculate, represents one of the most serious forms of male infertility. Nearly 15% of infertility in men caused by azoospermia (Cocuzza, 2013). It is categorized into two types, (a) Obstructive azoospermia (OA), where sperm production is normal but cannot reach the ejaculate due to a blockage, and (b) Non-obstructive azoospermia (NOA), which is caused by compromised sperm production. The causes of azoospermia can vary from genetic disorders (cystic fibrosis) and hormonal issues to infections, and injuries.

For patients affected by azoospermia, advanced assisted reproductive technologies (ART) have offered new possibilities. Surgical techniques like testicular sperm aspiration (TESA) and percutaneous epididymal sperm aspiration (PESA) have enabled the retrieval of sperms directly from the testicles or epididymis, even in the absence of sperm in the ejaculate. These methods can then be combined with intracytoplasmic sperm injection (ICSI) to achieve fertilization. The significance of TESA/PESA is that it allows azoospermic patients to achieveparenthood (Lacey, 2021). However, they also has challenges such as restricted quantity of sperm obtained, the invasive characteristics of the operations, elevated expenses, and the possibility of diminished sperm quality, which could influence embryo development.

Embryos fertilized with different sources of sperms are later analyzed using Gardner grade system. The purposeof this study is to assess how well this sperm sources produce healthy embryos. This objective will help in to clarify the reproductive potential of surgically obtained sperm and enhance treatment outcomes for azoospermicpatients, as the available data is inconsistent.

## METHODOLOGY

**Inclusion Criteria**

In this study, the participants were classified into two groups through certain clear inclusion criteria.

***Group A***: patients with a diagnosis of azoospermia (no sperm in the ejaculate). The existence of this category is important for the assessment of sperm retrieval techniques like TESA and PESA when natural sperm productiondoes not exist.
***Group B***: the semen of normal males with normal parameters, without any known disease was used.

Group A: The men of the group were required to meet certain health criteria, in order for the sperm collected tohave optimal quality and reliability. All subjects in two groups were of the same age, i.e., 25–40 years. This age range was selected to increase measurement precision by minimizing miscorrelation resulting from age- dependent decreases in sperm quality and reproductive health.

In addition, participants had to be free from any known present or past medical condition that could affect reproductive health. This meant, review of any medications they had been on; for all those on sperm toxic medications were excluded from entering the trial. An external health conditions criterion, to ensure that theactual results are not confounded by other factors.

**Exclusion criteria**
Participants outside the specified age range (younger than 25 or older than 40 years) were excluded to focus ona demographic where reproductive health is typically at its peak. Males with any significant medical history, whether ongoing or in the past, were also excluded, as such conditions might affect sperm quality and compromise reproductive outcomes.

Furthermore, individuals who smoke or drink alcohol were not included, because these habits are known to negatively impact sperm production and quality. This strict implementation of both inclusion and exclusion criteria ensured homogeneity to study population, enhancing the reliability and accuracy of findings regarding sperm retrieval techniques and their effectiveness in assisted reproductive technologies.

**Sample Collection**
In this study, a total of 20 samples were collected to assess the efficiency of sperm retrieval techniques and successive fertilization outcomes. Specifically, 10 testicular sperm aspiration (TESA) or percutaneous epididymal sperm aspiration (PESA) samples were obtained surgically by a qualified gynecologist. These procedures were performed under general anesthesia to ensure patient comfort and minimize any potential discomfort associated with the extraction process.

In parallel, 10 normal semen samples were collected from patients via masturbation. This method is standard forobtaining ejaculated sperm and allows for a non-invasive collection technique typically yielding higher volumesof sperm.

**Sperm Retrieval Procedures**
The TESA/PESA samples were carefully collected by urologist using precise surgical methods to ensure bestpossible sperm yield. After collection samples were processed to isolate healthy viable sperms. This involvesgentle extraction of sperms from tissues using a teasing technique followed by lab techniques to check their viability. The viable sperms were then enriched using swim-up technique.
Oocyte Retrieval and Record Keeping

Throughout the procedures, careful documentation was maintained, particularly concerning the numberof oocytes retrieved for each patient. This information is essential for correlating the fertilization rates and subsequent embryo development with the sperm source.
ICSI Procedure
After processing, ICSI was performed using oocytes and sperm from both the TESA/PESA and normal ejaculate groups. The embryos were assessed on day 2 to check any sign of successful fertilization. This gave usinsights into assessing how effective each method was to fertilize embryos.

By day 3 embryos were graded using GARDNER grading system, which assesses important factors like cell numbers, fragmentations and overall morphology. This grading system is crucial for identifying embryos withbest chance of fertilization

At last on day 5 which is blastocyst stage of embryo, embryos were assessed using same system for properblastocyst formation. These thorough investigations not only help us identifying embryos with high implantation potential but also provide data about viability of sperms through different retrieval methods.

## STATISTICAL ANALYSES

**CHI-SQUARE test of independence** was used to analyze relation between our two variables, embryo fertilizedwith normal sperms and tesa/pesa extracted sperms.

Null hypothesis (Ho): If there is no significant difference between embryo fertilized with normal sperms andtesa/pesa extracted sperms.

Alternate hypothesis (H1): If there is significant difference between embryo fertilized with normal sperms andtesa/pesa extracted sperms.

Total samples= 20 (10 for each group)Normal:

| NO. | AGE(25 - 40) | NO. OF MATURE OOCYTE | FERTILISED(2PN) | DAY 3 | DAY 5 |
|---|---|---|---|---|---|
| | | | | | |
| **1** | 30 | 9 | 8 | 7 | 6 |
| **2** | 32 | 11 | 10 | 10 | 4 |
| **3** | 25 | 13 | 11 | 11 | 6 |
| **4** | 39 | 8 | 7 | 6 | 4 |
| **5** | 28 | 13 | 12 | 12 | 4 |
| **6** | 34 | 6 | 6 | 6 | 1 |
| **7** | 27 | 5 | 5 | 4 | 2 |
| **8** | 26 | 4 | 4 | 3 | 1 |
| **9** | 37 | 8 | 7 | 6 | 4 |
| **10** | 30 | 7 | 6 | 6 | 5 |

***Mean:***

***Age: =30.8***
***M2 =8.42pn=7.6D-3=7.1D-5=3.7***

**Surgical:**

| NO. | AGE | NO. OF MATURE OOCYTE | FERTILISED(2PN) | DAY 3 | DAY 5 |
|---|---|---|---|---|---|
| | | | | | |
| **1** | 33 | 2 | 2 | 2 | 2 |
| **2** | 25 | 2 | 1 | 1 | 0 |
| **3** | 25 | 10 | 10 | 9 | 8 |
| **4** | 27 | 19 | 17 | 17 | 7 |
| **5** | 39 | 2 | 2 | 2 | 0 |
| **6** | 32 | 16 | 16 | 15 | 2 |
| **7** | 28 | 15 | 15 | 15 | 4 |
| **8** | 30 | 4 | 4 | 3 | 1 |
| **9** | 29 | 3 | 7 | 6 | 4 |
| **10** | 36 | 5 | 6 | 6 | 5 |

**Mean:**
**Age: =30.4**
**M2 =7.8**
**2pn=8 D-3=7.6D-5=3.3**

**Observed frequencies:**

| | NORMAL | SURGICAL | TOTAL |
|---|---|---|---|
| AGE | 30.8 | 30.4 | **61.2** |
| M2 | 8.4 | 7.8 | **16.2** |
| 2PN | 7.6 | 8 | **15.6** |
| DAY 3 | 7.1 | 7.6 | **14.7** |
| DAY 5 | 3.7 | 3.3 | **7** |
| TOTAL | **57.6** | **57.1** | **114.7** |

Expected frequencies:

| | NORMAL | SURGICAL | TOTAL |
|---|---|---|---|
| AGE | 30.7333 | 30.4666 | 61.2 |
| M2 | 8.13531 | 8.06469 | 16.2 |
| 2PN | 7.83400 | 7.76599 | 15.6 |
| DAY 3 | 7.38204 | 7.31796 | 14.7 |
| DAY 5 | 3.51525 | 3.48474 | 7 |
| TOTAL | 57.6 | 57.1 | **114.7** |

**Test statistics:**

**Formula used for chi square statistics:** $x^2 = \sum \frac{(O-E)^2}{E}$

where:

- $\chi 2$ = Chi-square statistic
- O = Observed frequency (actual count from the data)
- E = Expected frequency (theoretical count if there were no relationship between the variables)
- The sum ($\sum$) is taken over all the cells in the contingency table.

| | NORMAL | SURGICAL | TOTAL |
|---|---|---|---|
| AGE | 0.00014 | 0.00014 | 0.00029 |
| M2 | 0.00861 | 0.00868 | 0.01729 |
| 2PN | 0.00699 | 0.00705 | 0.01404 |
| DAY 3 | 0.01077 | 0.01087 | 0.02164 |
| DAY 5 | 0.00970 | 0.00979 | 0.01950 |
| TOTAL | 0.03623 | 0.03654 | **0.07277** |

| | |
|---|---|
| **SIGNIFICANCE LEVEL** | **0.05** |
| **DEGREE OF FREEDOM** | **4** |
| **TEST STATISTICS** | **0.07277** |
| **P VALUE** | **0.99935** |
| **CRITICAL VALUE** | **0.71072** |

## RESULTS

The Chi-square test analysis generated a p-value of 0.999, which is greater than the significance level of 0.05. Hence it states that we fail to reject the null hypothesis, indicating no statistically significant difference in embryo quality between those fertilized with normal sperm and those fertilized with sperm extracted via TESA/PESA.

## DISCUSSION

The findings of our above Statistical studies shows that there is absolutely no significant difference in embryoquality fertilized with TESA/ PESA sample versus normal ejaculated sample. The above statement is supported by our Statistical analysis performed for this topic. The p value that we obtained from chi square test was 0.999 which is significantly higher than our set significance level which was 0.05 for this research.

Therefore based on these Statistical results, we can say that the method of sperm retrieval which is whether surgical or via ejaculation does not have a statistically significant influence on the embryo quality. These findings are critical particularly for patients undergoing surgical retrieval methods like tesa pesa due to conditions like azoospermia as it is proved that surgical methods doesn't compromise the quality of the resultingembryos.

However it is important to note that the conducted study is solely assessed for embryo quality and does not anticipate its findings to other crucial factors such as incidence of pregnancy or live birth outcomes. Where embryo quality is an important factor in the success of assisted reproductive technologies (ART), pregnancies or live birth rates may depend on other factors like uterine environment, embryo implantation potential, and other variables too which were not a part of this conducted research. Also there is a greater possibility of physical andphysiological stress related with surgical sperm retrieval methods like Tesa Pesa which is also a crucial factor totake into account.

Even though the embryo quality stays same, male patients may experience extra stress, discomfort or other difficulties due to the invasiveness of these procedures compared to semen collected through normal ejaculation. It is counselor's responsibility to discuss these questions to the couple during treatment planning so that the couple can have idea about possible pros and cons of each sperm retrieval techniques.

Along with this discussion we can point out that while there isn't significant difference between embryo fertilized with normal ejaculate sperms and TESA/PESA extracted sperms but the invasiveness of surgical techniques and multiple operations may cause damage to the blood supply of the seminiferous tubules, can produce hematoma, and bring about fibrosis of the testicular tissue and even testicular atrophy, which may lead to long-term complications such as male autoimmune response, osteoporosis, insulin resistance and depression. (Wu1).

## CONCLUSION

The results of this study found no statistical significant difference in embryo quality fertilized with normal sperms to those fertilized with TESA/PESA extracted sperms. The chi-square analysis yielded p value above significance level of 0.05 which means we fail to reject null hypothesis. Hence we can state that whether normally or surgically retrieved healthy sperm does not affect the overall embryo quality however further studies with larger sample sizes is necessary to validate this findings.

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