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ANCILLARY TREATMENT FOR PREVIOUSLY FAILED IVF CYCLES BY THEOPHYLLINE IN MALE FACTOR PARAMETERS

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ABSTRACT: Theophylline are used to identify viable sperm for ICSI in patients with all immotile spermatozoa. Theophylline is another similar compound that is efficient in stimulating immotile spermatozoa. The objective of this study was to compare outcome of ICSI cycles after use of these theophylline / sperm mobil . **Key Words:** Theophylline, ICSI, fertilization, pregnancy.

INTRODUCTION:

Sperm motility is an important parameter for success in intracytoplasmic sperm injection (ICSI) treatment. The sperm motility is an important pre requisite's for fertilizing an egg during an ICSI procedure . Mainly targeting male infertility sub group . If the sperm is not moving, it could be a non viable . Normal live births have been obtained after ICSI with immotile sperm. Various procedures have been applied to pick viable sperm for ICSI. In addition theophylline is activation factor. Regardless of which method is employed, it results in higher fertilization rates and higher pregnancy rates in the different patient groups with asthenozoospermia / necrozoospermia / azoospermia cases where immotile spermatozoa are obtained for ICSI .

Materials and Instruments:

Theophylline, PVP, Culture media, oil.

Instruments:

Microscope, Incubators , Laminar Air Flow, Micromanipulator, Heating blocks, Stopwatches, Culture dishes, Centre well dishes, Glass pipette, Collection containers.

METHODOLOGY:

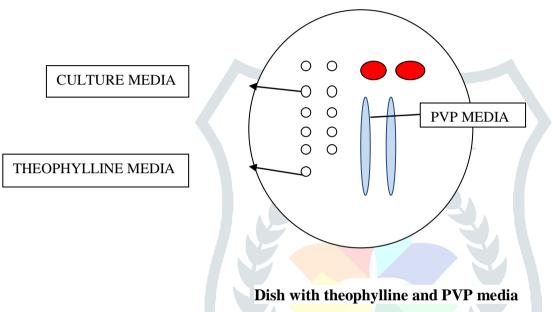
- Artificial sperm activation was used when sperm motility after preparation was low or nil motility in our standard procedure.
- The source of sperm were : 09 samples from ejaculated sperm with nil motility or necrozoopsermia samples , 10 samples from Testicular Extracted Sperms (TESE), 20 samples of Testicular EPIDYDIMAL ASPIRATION (TESA), 5 samples from MICRO-TESE and remaining 6 samples from MESA was studied.
- Each sample was diluted with equal amount of sperm wash medium (V SPERM WASH, VitroMed, Germany), mixed gently and centrifuged at 1200 rpm for 12 minutes. The supernatant was removed; pellet re-suspended to fresh culture medium and kept until the preparation of intracytoplasmic sperm injection dish (ICSI) dish preparation.
- All procedures were performed in the sterile room attached to the Andrology laboratory.
- This study was conducted from period September 2022 to January 2023.
- Couples divided into two groups
- Group A control group (n=30) where no theophylline was used.
- Group B (n=20) processed semen samples from patients having all immotile sperm were treated with theophylline (Sperm Mobil) was used to activate the sperm/ to identify viable sperm for ICSI.
- The sources of sperm were normal ejaculates and the Mean ± SD sperm processing, embryo culture and embryo transfer were performed by standard protocols.

Preparation of ICSI dish with Theophylline

- In this study, commercially available solution of theophylline (GM501 SpermMobil, GYNEMED, Germany) was used. The ICSI dish was prepared as shown in Figure.
- The ICSI drops were 5 μ L, the media drop containing immotile sperm to which theophylline solution was added and the media drop where sperm was rinsed after exposure to theophylline was 10 μ L.
- The theophylline solution was warmed little.
- To facilitate sperm activation, 2 μ L solution of the ophylline solution was added to the sperm containing drop.

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- The ICSI dish was then placed on a warm plate at 37°C for 10 min before search for viable sperm for ICSI.
- The viable sperm showing visible movement were picked, rinsed in media drop, immobilized in PVP drop • and injected into the egg.
- In case of non-availability of motile sperm, oocytes were injected with immotile sperm having twitching • motility of tail.
- The remaining procedures like oocyte denudation, ICSI, embryo culture, fertilization check, embryo • development check and embryo transfer were performed as per our standard laboratory protocols.
- Parameters were calculated as Mean± SD and percentages. •



RESULTS:

Comparison of outcome theophylline in male factor parameters

| Parameters | Control group | Test group |
|--------------------------------|----------------------------|----------------------------|
| No of patients | 30 | 20 |
| Sperm source | Fresh ejaculate | Fresh ejaculate |
| Sperm count | <1mil/ml | <1mil/ml |
| | (immotile/occasionally | (immotile) |
| | motile) | |
| Age of men | $31.7 \pm 6.2 \text{ yrs}$ | $31.7 \pm 6.2 \text{ yrs}$ |
| Motility enhancer | No theophylline used | Theophylline used |
| Oocytes retrieved in each case | 11 ± 6 | 11 ± 6 |
| Mature oocytes | 8 ± 5 | 8 ± 5 |
| Total no of oocytes injected | 348 | 234 |
| (ICSI done) | | |
| Fertilization rate % | (326/348) 93.6% | (221/234) 94.4% |
| Clinical pregnancy rate% | 53.2% | 54.8% |

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In our study, there was no much significant difference was observed in cases treated with theophylline however, this difference was statistically non-significant (P<0.05). Slightly more percent fertilization rate and clinical pregnancy rate was seen in test group. Further large group study is required to prove the efficiency of sperm enhancers.

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