Original Article

Study Of Variation In Incubation Time Of Semen Processing Media On Progressive Motility Of Human Spermatozoa

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Abstract

AIM: The aim of this study, was to check the effect of variation in media incubation time on progressive motility of spermatozoa of men attending Acharya Vinoba Bhave Rural Hospital, Wardha Testube Baby Center. METHOD: A total 55 semen samples of patients were used in this study. Semen Analysis was performed according to WHO guidelines 2010 and observations for Sperm Count and motility was recorded as Pre-wash data. Sperm Processing was performed using density gradient method in which samples randomly divided into 3 group with media of variant incubation time at 37ºC-36ºC (i.e. 18 samples in ±15 min; 19 samples in ±30 min; 18 samples in ±60 min), and observations for Sperm Count and motility was recorded as Post-wash data. Retrieval rate of progressive motility of sperm in all 3 groups were evaluated and compared via Pre and Post wash data. RESULT: A timeline of individual sample in all three groups was recorded to trace the lower limit, upper limit and average retrieval rate of the group. For group with media incubation time ±15Min, upper limit value was 24%; lower limit value was 0.56% and average of progressively motile sperm retrieval rate was 8.26%. For group with media incubation time ±30Min, upper limit value was 23.3%; lower limit value was 2.0% and average of progressively motile sperm retrieval rate was 8.22%. For group with media incubation time ±60Min, upper limit value was 17.6%; lower limit value was 2.1% and average of progressively motile sperm retrieval rate was 8.14%. Variation in incubation time of sperm preparation media affects the retrieval rate of progressive motility sperms from the native semen sample which subsequently may influence the assisted reproductive technology (ART) outcomes. Sperm Processing media should be incubated at 37ºC-36ºC for ±15min for better retrieval outcome.

KEYWORDS Progressive motility, Retrieval rate, Variation in incubation time.

INTRODUCTION

The World Health Organization (WHO) states that currently 60 to 80 million couples worldwide suffer from infertility. [1] According to WHO Infertility is "a disease of the reproductive system defined by the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse." [2] A steady increased rate for assisted reproduction technology (ART) procedures is observed to establish pregnancies worldwide. Annually, about 7% of

births are thought to be established by ART procedures, which is approximately around 1 million treatments. [3] Therefore, it becomes necessarily important to optimize the efficiency of sperm preparation technique used for retrieval of high-quality spermatozoa which eventually contributes to the formation of high quality embryos. [4] One of the major aim of sperm preparation process is to yield highly motile spermatozoa for ART treatments. The quality of sperm preparation media used in an ART lab affects the motility, vitality and DNA integrity of spermatozoa. [5]

Previous studies have shown that the recovery of a good high-quality spermatozoa depends on the quality of the semen sample, the sperm preparation method, time and storage temperature of the prepared sperm suspension. [6][7][8] It is also seen that in humans, variation in temperature affects sperm to lose its motility if stored at 37ºC for longer time before Semen analysis. [9] One might suppose that, like other living cells, sperms would be kept at a lower temperature (i.e.20ºC) than normal (37ºC) before examining or processing it, but many observers have found decrease in 4°C than normal temperature has an adverse effect on their motility. This so-called thermal shock may be related to decreased sperm adenosine triphosphate or 3‘: 5’-cyclic monophosphate, which may be necessary to the propagation of the flagellar wave. [10][11] The sperm processing media generally contains; BWW, Earle’s, Ham’s F-10/human tubal fluid (HTF), human serum albumin (HSA) highly purified and free from viral, bacterial and prion contamination and endotoxins; sodium pyruvate, sodium lactate, sodium bicarbonate buffer system and Isotonic density-gradient medium. [12] Variation in incubation of this media could be one of the idiopathic step towards unsuccessful ART outcome as some of these contains of sperm processing media are sensitive to temperature and exposure to the atmospheric air and light radiations. This may result to oxidation/reduction of buffer system, denaturation of protein contains and degradation of gradient medium, eventually this can affect metabolism rate in sperm during processing step. Over expose of media to environmental air and temperature at 37 º/36ºC may increase chances of bacterial contamination to the media (if antibiotics are not present in media). These bacterial contaminations can cause Sperm-immobilization which may then affect the quality of spermatozoa (Progressive Motility).

In India, from year 2012-2019 the average annual temperature was between 25.8 – 25.9ºC [13]. Thus it becomes necessary that, sperm processing media should be at its ideal condition before introducing semen sample to it as semen sample is in contact with it for approximately 30 minutes during complete processing.

The aim of this study, was to check the effect of variation in media incubation time on progressive motility of spermatozoa of men attending Acharya Vinoba Bhave Rural Hospital, Wardha Testube Baby Center.

METHOD

SAMPLE

This study involved 55 semen samples of men attending Wardha Test Tube Baby Center for infertility treatment from September 2019 to February 2020. Sever Oligozoospermic, Asthenozoospermic, Oligoasthenozoospermic and semen sample of men primarily contributing towards infertility were excluded from the study.

SEMEN ANALYSIS

Semen Analysis was performed according to WHO guidelines 2010. [12] Makler’s chamber (1mm 2 grid**,** subdivided into 100 squares, each one of 0.1 x 0.1 mm) was used to count and screen the sperms for motility and morphology using compound microscope at 20x. Sperm count was estimated into million/mL as in Makler’s chamber the space bounded in a row of 10 squares is exactly one millionth of mL. Thus, the number of sperm in 10 squares indicates its concentration in million/mL. Similarly, Sperm motility was estimated into percentage as motile or non-motile. Further the motile sperms where graded into Grade IV (Fast Progressive motile); Grade II & III (Slow Progressive/Swirl/Woggle); and Grade 1 (Sluggishly motile/Vibrating). Here, Sperm count, Total Motility and progressive motility of samples were recorded as pre wash data.

SPERM PROCESSING

After Semen Analysis, Sperm samples were processed via Density gradient method [12] using PURESPERM-II kit (Puresperm-A: 80% Density gradient; Puresperm-B: 40% Density gradient; Puresperm-C: 80% Wash solution). After washing, the sample was observed for Sperm count and motility. Sperm count was estimated in million/ml and Total and Progressive motility was estimated in percentage. Here, Sperm count, Total Motility and progressive motility of samples were recorded as post wash data.

SPERM RETRIEVAL RATE (Estimation & Statistical Analysis)

Samples were randomly divided into 3 groups varying in media incubation time (i.e. 18 samples in ±15 min; 19 samples in ±30 min; 18 samples in ±60 min). Progressively motile Sperm Retrieval Rate was evaluated by comparing Post wash observation against Prewash as following:

Pre-Wash	**Post-Wash**
$\mathbf{T_m} = \frac{S_c \times T_m\%}{100}$	$\mathbf{T_m} = \frac{S_c \times T_m\%}{100}$
$\mathbf{P_r} = \frac{T_m \times P_r\%}{100}$	$\mathbf{P_r} = \frac{T_m \times P_r\%}{100}$

T_m = No. of Totally Motile Sperms (Million/ml)

P_r = No. of Progressively Motile Sperms (Million/ml)

S_c = Sperm Count (Million/ml)

T_m% = Total Motility observed (%)

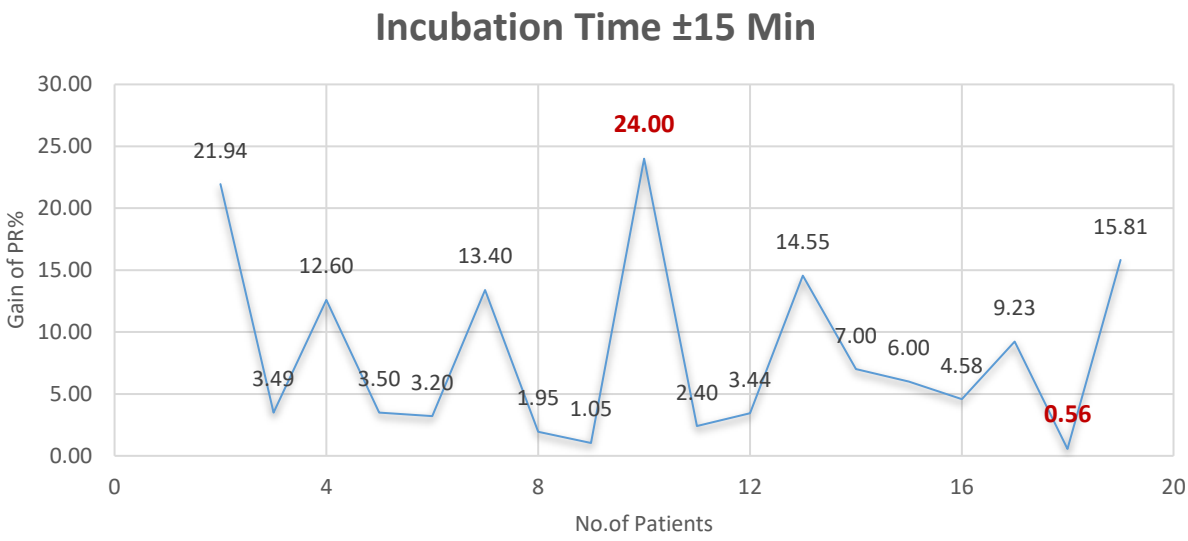
P_r% = Progressive Motility Observed (%)

Retrieval Rate or Gain was evaluated as = $\mathbf{P_r}$ (Post-Wash) - $\mathbf{P_r}$ (Pre-Wash).

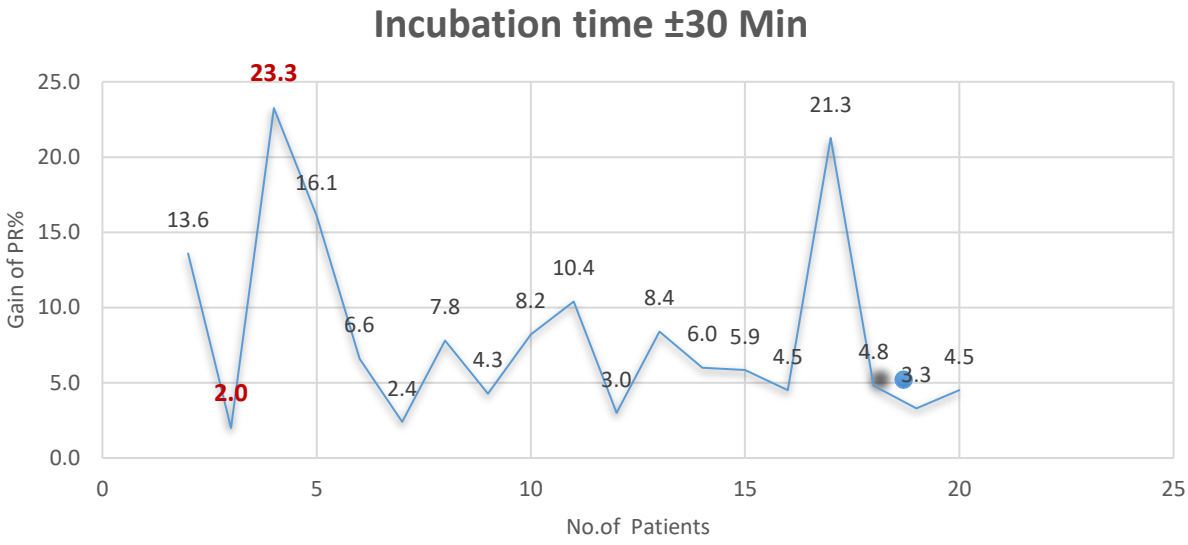
Clustered dot plot chart of Retrieval Rate of each group was shown. Mean of Progressively Motile Sperm Retrieval Rate of samples was evaluated in all 3 groups and was statistically compared and represented as clustered bar graph with error bars using Microsoft Excel 2016.

RESULT

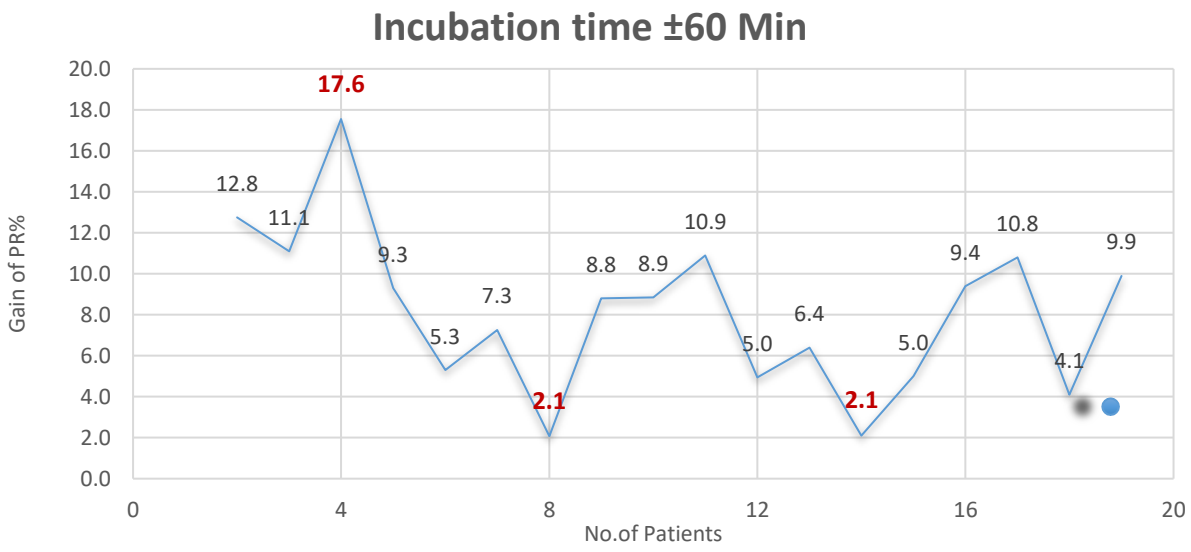
All 55 samples were randomly divided into 3 group of varyingly incubated media (i.e. 18 samples in ±15Min; 19 samples in ±30Min; 18 samples in ±60Min) and density gradient method was used to wash the sample. A timeline of Sperm Retrieval rate of individual sample in all three groups was recorded to trace the lower and upper limit of the group. Graph.1 shows timeline of group with media incubation time ±15Min, the upper limit value was found 24% and lower limit value was found 0.56%. Similarly, Graph.2 shows timeline of group with media incubation time ±30Min, the upper limit value was found 23.3% and lower limit value was found 2.0% and; Graph.3 shows timeline of group with media incubation time ±60Min, the upper limit value was found 17.6% and lower limit value was found 2.1%. Average of progressively motile sperm retrieval rate for Group with media incubation time ±15Min was found to be 8.26%. Similarly, average of progressively motile sperm retrieval rate for group with media incubation time ±30Min was found to be 8.22% and average of progressively motile sperm retrieval rate for Group with media incubation time ±60Min was found to be 8.14%. Graph.4 shows comparison of average of rate of progressively motile sperm between all three groups, linear forecast of the data shows simultaneous decrease in rate of progressively motile sperm with increase in media incubation time before using it.



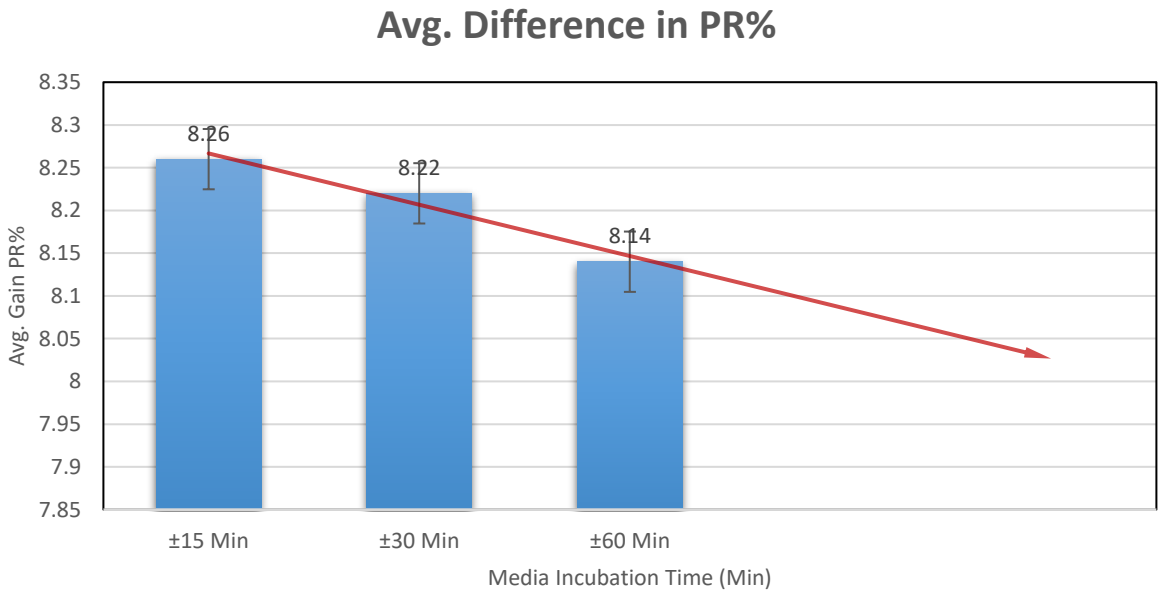
Graph.1. Timeline of Sperm Retrieval Rate of 18 samples in group of media incubated for ±15Min before sperm processing. Upper limit of the group is 24% and lower limit is 0.56%.



Graph.2. Timeline of Sperm Retrieval Rate of 19 samples in group of media incubated for ±30 Min before sperm processing. Upper limit of the group is 23.3% and lower limit is 2.0%



Graph.3. Timeline of Sperm Retrieval Rate of 18 samples in group of media incubated for ±60 Min before sperm processing. Upper limit of the group is 17.6 % and lower limit is 2.1%.



Graph.4. Comparison of average of rate of progressively motile sperm between all three groups. Linear forecast of the data shows simultaneous decrease in rate of progressively motile sperm with increase in media incubation time.

DISCUSSION

In ART laboratories, where multiple cycles of treatment are performed including both IUI and IVF/ICSI may face problem of maintaining the consistency of incubation time of media at 37 º /36ºC before using it for sperm processing. This can affect the quality of sperm if the components of the media react with the spermatozoa by getting oxidized/reduced, denature or degrade by over exposure to temperature and environmental air. This problem is generally not considered as a serious issue in many ART laboratories and no similar study was reported previously

for comparison, but as in aspect of media un doubtfully, many research work has shown Impact of quality of media on motility, vitality and DFI of sperm [5-15]; Relation between incubation of sperms in the culture media with respect to DNA damage/fragmentation index[14]; Effect of temperature on sperm motility and viability [15]; Effects of temperature and storage time on the motility, viability, DNA integrity and apoptosis of processed human spermatozoa[16-21]. This study shows that maintaining consistency of incubation time of sperm processing media at 37 º/36ºC before using it plays an important role in obtaining good retrieval rate of progressively motile sperm. Graph.4 shows that, with increase in incubation time of media from ±15 Min to ±60 Min the retrieval rate of progressively motile sperm is simultaneously decreasing. The difference in the retrieval rate of progressive motility was seen may be due to variation in metabolism rate of sperm in different incubation of media for same temperature. Most probably it seems that, in media which was incubated for ±60Min at 37 º/36ºC sperm metabolized at a greater rate than in media incubated ±15Min 37 º/36ºC, and therefore use up more of the nutrients than normal rate. In order to metabolize, unlike other cells sperms too requires energy in form of ATP, in sperms most of its energy source is stored in mitochondria in middlepiece, which generates ATP. Higher rate of metabolism in sperm at processing step can lead in depletion of ATP in sperm, which is generally, in normal conditions invested to gain motility in sperms when introduced in female reproductive tract. In any ART laboratory, there is always effort to retrieve best quality of sperms from the native semen sample of the patient for the treatment, by maintaining the consistency or reducing variation in media incubation time before using it may lead one step further to achieve this goal.[22-31]

Due to time concern and COVID-19 scenario, this work was only limited to checking sperm progressive motility as influencing factor with respect to variation in media incubation time at 37ºC to 36ºC. In further aspects of study, effects of variation in incubation time of sperm preparation media on other physiological and structural integrity of spermatozoa will be evaluated.

CONCLUSION

Variation in incubation time of sperm preparation media affects the retrieval rate of progressive motility sperms from the native semen sample which subsequently may influence the assisted reproductive technology (ART) outcomes. Sperm Processing media should be incubated at 37ºC-36ºC for ±15min for better retrieval outcome.

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