

# Effect of Oral Ambroxol HCl Treatment on Characteristics and Recovery of Spermatozoa from Abnormally High Viscous Semen in Infertile Men

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## ABSTRACT

**Objective:** The objective of this study was to assess the impact of treating abnormally high viscous semen in infertile men with oral Ambroxol HCl by changing the qualitative and quantitative characteristics of spermatozoa recovered by SWIM UP methods to be use in assisted reproductive techniques (ART).

**Study Design:** Controlled clinical study

**Material and Methods:** Fifty sub fertile men producing abnormally high viscous semen participated in this study. The use of oral Ambroxol HCL for improving the liquefaction of semen in these patients leading to impact on the semen specimen produced by the patient was assessed. The spermatozoa recovered with swim up methods were used in ART to treat the sub fertile couple. The method of semen liquefaction used in this study was helpful in the recovery of greater numbers and higher quality spermatozoa (PiO.05) as compared to the conventional method. All the characteristics of spermatozoa were assessed both from quantitative and qualitative point of view in both, the conventional and the Ambroxol liquefied specimens.

**Conclusions:** The liquefaction of abnormally high viscous semen with Oral Ambroxol was shown to be a treatment, which can help significantly in the handling and preparation of abnormally high viscous semen specimen. The oral use of Ambroxol HCL in sub fertile men with abnormally high viscous semen assisted in the recovery of qualitatively and quantitatively improved spermatozoa, which can be used, assisted reproductive techniques.

**Key words:** Semen viscosity, coagulation, liquefaction, Ambroxol HCL

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## INTRODUCTION

The Assisted Reproduction Techniques (ART) by using isolated motile spermatozoa came into clinical practice for the management of infertility in human about three decades ago. Over the last many years, the indications to use ART have become more common in the management of male factor infertility and this urged the researchers to find better and more suited techniques to separate functional spermatozoa from immotile with poor morphology. The conventional swim up procedure is the most simple and cheapest of all available presently<sup>1</sup>.

Fresh human semen is normally ejaculated in the viscous liquid state followed by immediate coagulation and it does not liquefy for at least 20-30 minutes<sup>2-6,8</sup>. So-called coagulation proteins which are derived from the seminal vesicle, promote the coagulation and liquefaction is caused by secretions from prostate gland<sup>8</sup>.

Some times semen samples are rather viscous even after 30 to 45 minutes<sup>4,5</sup>. However, if

liquefaction is not complete and the semen remains viscous, it is considered as an abnormality because of a lack of proteolytic enzyme present in the prostatic secretions<sup>4,5</sup>. The coagulation and subsequent liquefaction of semen and its physiological significance is not clear. Delayed or failure of liquefaction, and high viscosity (hyper viscosity) are conditions equated with infertility, since it is claimed that the spermatozoa are tangled in the fibrous or mucous mass in the semen and are prevented from migrating properly from the seminal plasma into the cervical track Fluids and ascending to the site of fertilization<sup>3,9-11</sup>.

The viscosity of the liquefied semen different from coagulation is gauged by aspirating into a wide-bore 5-ml pipette and then allowing the semen to drop by gravity and observing the length of the thread. A normal sample leaves the pipette as small discrete drops. In cases of abnormal viscosity the drop will form a thread more than 2cm long. Alternatively, introducing a glass rod into the sample and observing the length of the thread that forms on withdrawal of the rod may evaluate the viscosity. Again, the thread should not exceed 2cm.

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Viscous specimens are extremely difficult to manipulate in vitro and may not allow the proper separation and isolation of spermatozoa for assessment, sperm preparation or performance of ART procedures such as intrauterine insemination (IUI) and IVF<sup>7,14,15</sup>. Semen liquefaction can be induced in vitro by addition of mucolytic agents, diluting by mixing with culture media or mechanical disruption of the mucous materia<sup>2,3,7,9,14,16-21</sup>. Mucolytic agents such as  $\alpha$ -chymotrypsin,  $\alpha$ -amylase and dithiothreitol have been employed to improve semen liquefaction and viscosity difficulties<sup>7,14,20</sup>. In general, it has been shown the treatment of semen with  $\alpha$ -chymotrypsin improves the in vitro fertilizing ability of high viscosity semen specimens<sup>14,22</sup>.

Mixing the semen with media will not work. Forcing the viscous semen through a narrow-gauge needle may cause severe damage to the spermatozoa. Liquefying the ejaculate with an enzyme e.g.,  $\alpha$ -amylase,  $\alpha$ -chymotrypsin, lysozyme or hyaluronidase or a special trypsin-based dissolving solution, Sperm Solute, can also damage spermatozoa<sup>9</sup>. We therefore tried an oral use of mucokinetic agent Ambroxol HCl "Cyclohexanol, 4-(2-amino-3,5-dibromophenylmethyl amino, trans-" Mucosolvan tab is equivalent to 30 mg of Ambroxol-HCl

The objective of this study was to assess the impact of using oral use of Ambroxol HCl in high viscosity semen specimens, on the qualitative and quantitative characteristics of spermatozoa recovered for possible use in ART.

Ambroxol HCL is a metabolite of bromhexine that stimulates mucociliary action and clears the air passages in the respiratory tract. It is usually administered as expectorant in the form of hydrochloride.

The antioxidant effects and inhibition of hyaluronic acid degradation induced by hydroxy radicals is responsible for reducing the semen viscosity.

In 46 patients in whom the semen became liquefied within 30 minutes after, the therapy was successful making the ejaculate to carry out normal swim up process easy. Other than reduction in the semen viscosity, Ambroxol HCL showed other effects on different semen parameters as well.

## MATERIAL AND METHODS

**Collection of semen:** 50 sub fertile men between 25-45 years of age with abnormally high semen viscosity were selected. Semen sample after 2-3 days of abstinence was produced with masturbation/coitus interruptus and was collected in a glass container with no rubber seal of the lid. The

sample was allowed to stand on bench at room temperature (37 C) for 30 minutes and was gently shaken by rotating the glass container gently. After which the viscosity was noted.

The ejaculate was considered to be of abnormally high viscosity if the semen cannot be expelled from the pipette and mucus-like string in accordance with measuring the 'Spinbarkeit' of cervical mucus<sup>24,25</sup> is more than 2 cm long is formed when the pipette was removed after insertion into the semen sample.

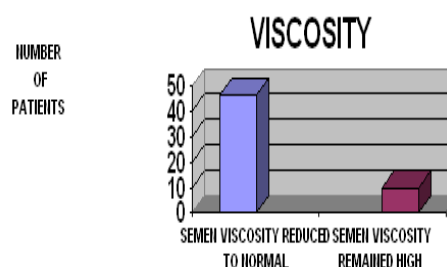
Semen specimens were assessed for viscosity, sperm concentration ( $\times 10^6$  sperm/ml) by using a Horwell Fertility Counting Chamber, grade of motility (0-4), morphology (% normal) of spermatozoa. Sperm agglutination and the presence of debris were also noted.

**Drug regimen:** All of these fifty men in this study were prescribed Ambroxol HCL (Bo ringer, Germany), 30 mg tab orally three times a day for 10 days. After 10 days of treatment, the ejaculates were collected from these men according to the protocol already mentioned. The specimens were then processed by Swim-up method similar as described by Lopata et al.(1976) using Earl's medium(GIBCO USA) PH was tested to 7.4 containing 10%v/v heat inactivated patients own serum. After swim up, the recovered sperms were counted and assessed without dilution.

## RESULTS

The method of semen liquefaction used in this study was helpful in the recovery of greater numbers and higher quality spermatozoa (PiO.05) as compared to the conventional method. All the characteristics of spermatozoa were assessed both from quantitative and qualitative point of view in both, the conventional and the Ambroxol liquefied spe

### EFFECTS OF AMBROXOL HCL ON SEMEN



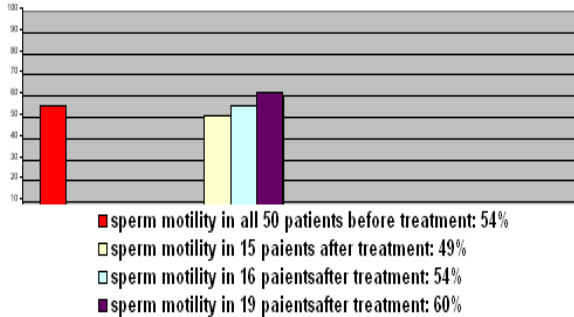
■ 46(92%) patients semen showed normal viscosity after treatment

■ 6(8%) patients semen showed no reduction in viscosity

The effect of Ambroxol of HCL in reducing the Viscosity of abnormally high Viscous semen was significant. In total no. 50 patients of abnormally high viscous semen, 46 patients semen sample showed reduced viscosity near to normal. (Fig-1)

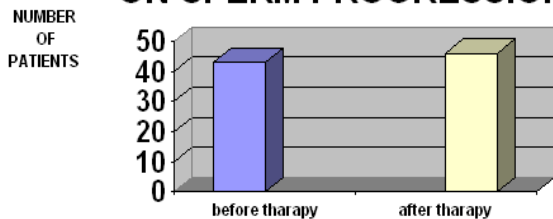
The sperm motility was improved revealed in the semen samples of 19 out of 50 patients.(Fig-2)

**EFFECTS OF AMBROXOL HCL ON SPERM MOTILITY**



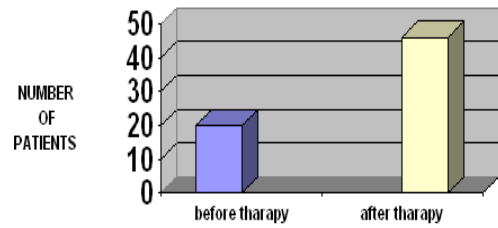
Oral Ambroxol HCL though did not have convincing effects on the Sperm progression how ever a good sperm progression was shown in the semen simple of 43 patients before treatment where as semen simple of 46 patients showed improved sperm progression after treatment (Fig-3)

**EFFECTS OF AMBROXOL HCL ON SPERM PROGRESSION**



There was significant improved sperm morphology after treatment (43 out of 50 patients comparing with before treatment (20 out of 50 patients). (Fig-4)

**EFFECTS OF AMBROXOL HCL ON SPERM MORPHOLOGY**



- > 60% abnormal sperms in 20 patients before treatment
- > 60% abnormal sperms in 7 patients after treatment

**DISCUSSION**

Assisted Reproduction Techniques (ART) by using isolated motile spermatozoa came into clinical practice for the management of infertility in human about three decades ago. The conventional swim up procedure is the most the most simple and cheapest of all available presently<sup>1</sup>. Many IVF centers, including ours, currently use the standard swim up method to obtain motile sperms for the purpose of insemination of oocytes

Normal semen is a mixture of spermatozoa suspended in secretions from the testis and epididymus, which, at the time of ejaculation, are combined with secretions from the prostate, seminal vesicles, and bulbourethral glands. In human and most mammals, semen coagulates shortly after ejaculation and liquefies again later on. Fresh human semen is normally ejaculated in the viscous liquid state followed by immediate coagulation and it does not liquefy for at least 20-30 minutes. [2-6,8]. Semenogelin ,a protein secreted from the seminal vesicle promotes the coagulation and Prostate Specific Antigen (PSA), a serine-proteinase secreted by prostate gland is responsible for semen liquefaction to occur later<sup>8</sup>.

The concept and significance of physiological process of coagulation and subsequent liquefaction of semen is not very well understood. Delayed liquefaction leading to a semen specimen of abnormally high viscosity may be responsible for a male factor infertility as spermatozoa, even of normal

qualitative characters find difficult to gain a required motility in this sticky, thick and fibrinous mucus vehicle and so are not able to ascend through the cervical track Fluids to the site of fertilization<sup>3,9-11</sup>.

Aspirating into a wide-bore 5-ml pipette and then allowing the semen to drop by gravity and observing the length of the thread gauges the viscosity of the liquefied semen. A normal sample leaves the pipette as small discrete drops. In cases of abnormal viscosity the drop will form a thread more than 2 cm long. Alternatively, introducing a glass rod into the sample and observing the length of the thread that forms on withdrawal of the rod may evaluate the viscosity. Again, the thread should not exceed 2 cm.

Viscous specimens are extremely difficult to manipulate in vitro and may not allow the proper separation and isolation of spermatozoa for assessment. Sperm preparation or performance of ART procedures such as intrauterine insemination (IUI) and IVF<sup>7,14,15</sup>.

The objective of this study was to assess the impact of using oral use of Ambroxol HCL in high viscosity semen specimens, on the qualitative and quantitative characteristics of spermatozoa recovered for possible use in ART.

As highly viscous semen can reduce sperm motility thus being a cause for male infertility, it is necessary in the clinical set-up of assisted reproduction either to reduce semen viscosity or to liquefy the ejaculate artificially.

## CONCLUSION

According to our results, the use of oral Ambroxol in the treatment of male infertility because of abnormally high viscous semen is beneficial and effective in improving the qualitative and quantitative sperm parameters both in vitro and in vivo and is helpful in making the handling of a highly viscous semen specimen easier for the recovery of sperm to be used in Assisted Reproduction Techniques.

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