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Semen hyperviscosity: causes, consequences, and cures

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1. ABSTRACT

The prevalence of semen hyperviscosity (SHV) is estimated to be between 12-29% and can lead to male factor infertility both *in vivo* and *in vitro*. Semen is composed of fluids secreted by the male accessory glands, which contain proteins essential to the coagulation and liquefaction of semen. Hypofunction of the prostate or seminal vesicles causes abnormal viscosity of seminal fluid. Infection and high levels of seminal leukocytes may also result in the development of SHV. Oxidative stress and biochemical and genetic factors can furthermore contribute to this condition. Hyperviscosity can impair normal sperm movement in the female reproductive tract, and can lead to decreased sperm count. SHV is treated with a hypodermic needle, mucolytic enzymes, antibiotics and anti-inflammatory agents in certain cases. Further research is needed to better understand the contributors to SHV and the treatments that can be used for infertile males with hyperviscous semen.

. INTRODUCTION

Male factor infertility contributes to the fertility problems of roughly 30-40% of subfertile couples, and is the sole contributor in 20% of infertile couples (1). Known causes of male factor infertility include oxidative stress (OS) and reactive oxygen species (ROS), as well as clinical diseases such as leukocytospermia, varicocele, and unexplained infertility, which can lead to a decrease in semen parameters and DNA damage of spermatozoa. These diseases potentially result in abnormal semen characteristics, which affect semen parameters. Other contributing factors include infection, inflammation, and dysfunction of the male sex glands, which gives rise to decreased sperm motility and increased levels of leukocytes. Semen hyperviscosity (SHV) is another condition that can contribute to male subfertility as it is associated with changes in the chemical and physical characteristics of semen (2).

Directly after ejaculation semen coagulates before gradual liquefaction occurs. Semen usually loses its viscosity during the liquefaction process, both *in vitro* and *in vivo* (in the female reproductive tract) (3). However, in the event that semen retains some of the viscous characteristics, it can be identified and is regarded as hyperviscous (2). Seminal hyperviscosity occurs in 12%–29% of ejaculates (4, 5) and may be a cause of male infertility. Hyperviscous seminal fluid has been shown to have a negative impact of sperm motility and semen quality (2, 6), and contributes to a poor outcome with *in vitro* fertilization (7). SHV is likely caused by infection, inflammation, dysfunction of the male sex glands, and diseases that directly impact male fertility. While these conditions are related to SHV, the exact mechanisms through which the coagulated characteristics are retained are still not clear (8). Hyperviscous seminal fluid results in a negative impact on fertility through its role in the female genital tract. The various causes of hyperviscosity contribute to male subfertility outside the scope of SHV. Assessment and treatment of hyperviscous semen has improved in recent years, but the mechanisms through which hyperviscosity affect fertility are still being debated.

The purpose of this review is to evaluate and appraise current research and literature concerning SHV. The focus is on the causes of SHV and the conditions associated with the diagnosis, the role and consequences of SHV in male fertility, as well as the treatments available both *in vivo* and *in vitro* to improve fertility for those with hyperviscous semen.

3. SEMEN AND ITS CONSTITUENTS

Semen consists of spermatozoa suspended in a fluid medium referred to as seminal plasma. The seminal plasma component of semen provides nutrients and protection to spermatozoa in the female reproductive tract as it protects spermtozoa from the acidic environment and possible DNA damage. Seminal plasma is composed of fluids secreted by the testes, epididymis, seminal vesicles, prostate, and bulbourethral glands (9). The fluid secreted by the seminal vesicles makes up the majority of the seminal plasma and is high in fructose (10), which provides the necessary energy for normal sperm function. Secretions from the prostate contain lipids, citric acid, proteolytic enzymes, zinc, and acid phosphatase, while the fluid secreted by the bulbourethral glands functions as a lubricant for the urethra. The testes contribute to the composition of semen by producing millions of spermatozoa, while the Sertoli cells secrete a quantity of fluid to act as a suspension medium and assist in the transport of sperm from the seminiferous tubules, after spermiation, through the different genital ducts.

Subsequently to ejaculation, semen coagulates as a result of vesicular and epididymal proteins present in the seminal plasma (11, 12). Sperm gain motility, after being freed from the seminal clot by the action of proteolytic enzymes secreted by the seminal vesicles following liquefaction of the coagulum, approximately 20-30 minutes after ejaculation (13).

4. DEFINING AND ASESSING SHV

Once liquefied, seminal fluid exhibits varying degrees of tensile strength and thickness, commonly referred to as viscosity. Normally, liquefied semen has low viscosity. Semen that retains some of its viscous properties post-ejaculation, which does not change over time, can be regarded as hyperviscous. SHV has varying grades of disease. According to the World Health Organization (WHO), diagnosis is based on the thread length formed by liquefied semen (14). The viscosity of the semen sample can be estimated by gently aspirating it into a wide-bore plastic disposable pipette (±1.5 mm diameter) and subsequently allowing it to drop by gravity. A normal sample leaves the pipette in small discrete drops, but if viscosity is abnormal (increased), the drop will form a thread more than 2 cm long. Alternatively, the viscosity can be evaluated by placing a glass rod into the sample and observing the length of the thread that forms upon withdrawal of the rod. The viscosity should also be recorded as abnormal when the thread exceeds 2 cm in length (14). These evaluations measure the visco-elasticity of the semen.

In contrast to a partially liquefied sample, a viscous semen specimen exhibits homogeneous stickiness and its consistency will not change with time. SHV can be recognized by the elastic properties of the sample, which adheres strongly to itself when attempts are made to pipette it (14).

The thread lengths of the semen drops can be measured on a centimeter scale in order to determine the grade of viscosity. Men whose semen has a thread length between 2cm and 4cm are diagnosed with mild SHV; a thread length between 4cm and 6cm is labeled as moderate SHV; and a thread length greater than 6cm is diagnosed as severe SHV (15). In a study performed by Elia and co-workers it was shown that the prevalence of SHV was as high as 26.6% in males from infertile couples (15). It was found that 13.1% of the men had mild SHV, while 6.6% of men had moderate SHV, with 6.4% of men diagnosed with severe SHV (15).

A more quantitative method of assessing viscosity levels in semen samples is through the use of capillary-loaded semen analysis chambers. Despite this being a time consuming and expensive exercise, results are believed to be more objective and reliable (16). Semen viscosity is measured by the filling time of the capillary; theoretically, there should be a relationship between the degree of viscosity and the time taken to fill a capillary of a certain diameter and at a certain angle (17). A study by Rijnders *et al.* found that this method of measuring semen viscosity was accurate 89.1% of the time (16). They concluded that both diameter of the capillary and angle of contact between semen and capillary were important in assessment, as well as whether or not seminal plasma was filtered. This method can be used in conjunction with viscosity meters; however, the use of a viscosity meter requires a large semen sample and is time consuming in itself

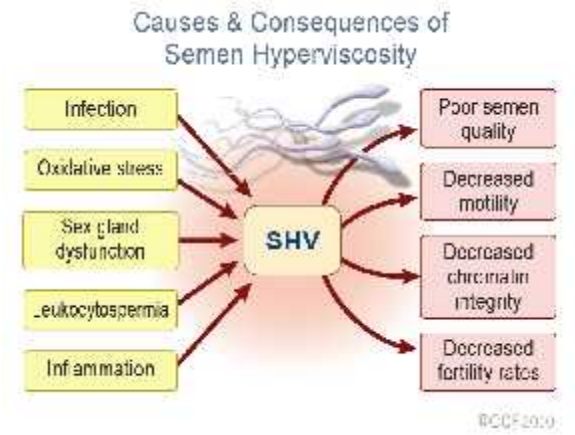

Figure 1. The various causes and consequences of semen hyperviscosity. Several contributors may ultimately lead to infertility or subfertility.

Normal semen rarely prevents sperm movement; however, hyperviscous semen produces impaired sperm motility and asthenozoospermia. SHV may create a trapping effect due to the visco-elasticity of the seminal plasma that inhibits normal sperm motility (6, 18). In addition, the tensile properties of semen have been shown to have an impact on the amount of energy spermatozoa need to expend in order to achieve a certain velocity. Emami *et al.* recently found kallikrein-related peptidases (KLKs), a type of human protease, to be differentially expressed when compared between semen samples grouped by sperm quality, including motility, speed, concentration, volume, and pH. They found that the expression level of seminal plasma KLKs correlates with liquefaction and viscosity, and may be used in diagnosis of SHV (19).

SHV can also potentially be used as a biomarker for the level of secretory activity of the seminal vesicles and prostate (5). Hyperviscosity of semen has been correlated with decreased fructose and phosphorous levels. A similar correlation was observed in semen that took longer than two hours to liquefy. Zinc concentrations have been found to be lower in hyperviscous samples. Hyperviscosity has been shown to correlate with prostate and seminal vesicle dysfunction, although hyperviscosity is commonly found in men with functionally normal accessory sex glands (5).

5. CONTRIBUTORS TO SHV

There are many known causes of SHV as well as several hypothesized contributors and associated factors (see Figure 1), although the exact cause of abnormal semen viscosity after coagulation and liquefaction is unclear (8). SHV is most attributed to male accessory gland infection, increased levels of leukocytes, and inflammation, as well as dysfunction of the sex glands or even the immune system (20). While these hypotheses are generally accepted by clinicians, conflicting scientific evidence have been reported in the literature. Munuce *et al.* found no association between hyperviscosity of semen samples and the number of positive bacterial cultures or the number of species in each sample, including *Mycoplasma hominis*, *Ureaplasma urealyticum*, and *Chlamydia trachomatis* (21). In addition, they reported no association between the presence of leukocytospermia or sperm antibodies and SHV. They concluded that SHV does not necessarily reveal a genito-urinary tract infection (21). In another study, researchers again did not find an association between antisperm antibodies and leukocytospermia (22), although leukocytospermia is sometimes associated with conditions other than infection, such as varicocele (23). Leukocytes still play an important role in the development of SHV, as they are present in cases of infection, are major sources of ROS, and are involved in the transmission of retroviruses (24).

It has been reported that bacteria culture-positive semen samples were more likely to be hyperviscous than non-positive samples (25). This is explained by inflammation and the presence of leukocytes or antibodies, or possibly the metabolic by-products of bacterial growth (26, 27). The WHO determined that increased semen viscosity or impairment of liquefaction is often established in patients with a genito-urinary tract infection and other abnormal sperm characteristics as opposed to in men with infection and normal sperm characteristics (28). In a study by Moulik *et al.* it was determined that a correlation existed between the presence of sperm antibodies and hyperviscous semen (29).

Elevated amounts of seminal leukocytes contribute to OS. When excess levels of ROS and reactive nitrogen species (RNS) are imbalanced in respect to levels of antioxidants, which are agents that are able to scavenge free radicals, OS can occur. ROS and oxidative damage may play a role in SHV; OS is associated with conditions that affect male fertility such as leukocytospermia, varicocele, idiopathic infertility, and genito-urinary tract infection, along with other factors including smoking and exposure to environmental pollution. It has already been reported that higher levels of ROS are found in the seminal plasma and sperm of infertile men (30). Similarly OS and OS-related conditions have been associated with hyperviscous blood (31). Malondialdehyde (MDA) and carbonyl detection are indicative of ROS levels and oxidative damage. High levels of MDA and carbonyl in blood have been associated with higher blood viscosity (31). Correlations have been found between hyperviscous semen and levels of MDA and carbonyl in males with subfertility (32). This suggests that OS may be a contributor to SHV. Increasing levels of MDA and protein carbonyls were shown to correlate with increasing degrees of viscosity of semen in infertile men (32). Low levels of antioxidants have been correlated with SHV (33); decreased antioxidant capacities in seminal fluid likely lead to an imbalance between antioxidants and ROS, resulting in OS.

Several biochemical factors contribute to SHV. Mendeluk *et al.* reported that semen samples with high structural viscosity were often less consistent throughout the sample due to highly organized protein networks (34),

possibly due to the presence of structurally complex molecules (35). The liquefaction of semen is activated by ethylenediaminetetraacetic acid (EDTA) (36). What is more is that Menduluk *et al.* was able to show that the presence of EDTA did not have a significant effect on SHV (34). This supports the theory by Amelar which suggests that normal semen coagulation and SHV are controlled by different factors (37). Furthermore other biochemical factors have been associated with SHV, including zinc, calcium, and prostate-specific antigen concentrations (6, 34). The liquefaction of coagulated semen is inhibited by the presence of zinc; lower zinc concentrations have been found in hyperviscous semen samples when compared to samples with normal viscosity (38).

Semen coagulation and liquefaction depends on the proteins in the secretions of the male accessory sex glands, including the seminal vesicles and prostate (39, 40, 41). Dysfunction of either of these glands results in hyperviscosity. A study by Gonzales *et al.* showed that SHV is associated with low sperm concentration, low sperm motility, low sperm vitality, and hypofunction of the seminal vesicles (2). They also reported that with hypofunction of the prostate or dysfunction of both the prostate and seminal vesicles, no significant correlation exists between hypofunction and SHV. This suggests that proper functioning of the seminal vesicles is required for normal viscosity. When there is less secretion from the seminal vesicles, the prostatic fluid secretion increases; the excess prostatic fluid may account for increased semen viscosity (2). However, in another study it was found that significantly lower levels of prostate-specific antigen (PSA) in hyperviscous semen when compared to normal controls (6). PSA is a proteolytic enzyme secreted by the prostate that is involved in the liquefaction process. Zinc is secreted by the prostate, and both the decreased levels of PSA and zinc in semen with SHV suggest that hypofunction of the prostate contributes to the development of SHV.

Genetic factors can furthermore play a role in the development of SHV. SHV is considered a clinical consequence of cystic fibrosis; Rossi *et al.* found an association between hyperviscous consistency of semen and gene mutations and variant tracts of the cystic fibrosis transmembrane conductance regulator (CFTR) (42). Rossi *et al*. subsequently theorized that cystic fibrosis can possibly be a genetic contributor to SHV.

6. SPERM AND SEMEN PARAMETERS IN SHV

SHV, and the factors that contribute to SHV, negatively affect sperm and semen parameters. Hyperviscous semen physically affects the movement of sperm. SHV has been shown to affect kinetics parameters such as motility, velocity, and the linear direction of sperm (43). SHV produces a trapping effect, inhibiting normal sperm movement through the female reproductive tract (2).

SHV gives rise to asthenozoospermia through biochemical factors. Past studies have shown that zinc concentrations have a positive correlation with sperm motility (44, 45). However, Mankad *et al.* found no significant correlation (38), and it has been shown that high seminal zinc concentrations negatively affect progressive sperm motility (46). Decreased total antioxidant capacity has also been associated with asthenozoospermia (47) and may play a role in SHV. OS and high seminal ROS levels in SHV are related to SHV decreased sperm parameters such as motility and increased sperm DNA damage (7). Studies have shown that the viscosity of seminal fluid affects the energy required by sperm to reach translational velocity (19). In the study by Gonzales *et al.*, sperm concentration, motility, vitality, and fructose concentrations were decreased in samples with high viscosity (2). Elzanaty *et al*. reported that seminal fructose levels were the only parameter to significantly predict hyperviscosity in semen samples (6). As increased ATP content per sperm has been found in semen samples with higher viscosity when compared to samples with normal viscosity (43, 48), it can point to the fact that normal sperm movement is impaired and ATP is not being consumed in hyperviscous semen.

SHV has been associated with poor semen quality, changes in chromatin stability, and decreased sperm count (2, 49). Gopalkrishnan *et al.* found that in semen samples with abnormal viscosity, sperm chromatin integrity was significantly lower when compared to controls with normal semen viscosity (49). The results from another study showed that semen with hyperviscosity exhibited high sperm chromatin stability, which is attributed to the presence of zinc-chelating agents (50). These characteristics in SHV may be due to the fact that sperm chromatin stability depends on secretions of the prostate and seminal vesicles (51), including zinc chelators that reduce the zinc content in sperm chromatin (52). A significant decrease in sperm count in men with SHV has been found (49), while Mankad *et al.* reported a statistically significant positive correlation between zinc concentrations and sperm count (38).

7. SHV AND FERTILITY

SHV can lead to infertility in males through impairment of normal sperm movement. SHV and the factors that contribute to the condition can also lead to *in vivo* complications as well as have consequences *in vitro* and in an assisted reproductive technology (ART) setting.

As seminal plasma plays a role in events leading to fertilization, hyperviscosity of the seminal plasma contributes to a decrease in sperm function. Seminal plasma has a significant effect on human sperm-zona pellucida binding both *in vivo* and *in vitro* (53). In one study, researchers determined that seminal plasma improved the entry of sperm into the cervical mucus, and played a role in maintaining sperm swimming speed after mucus penetration (54).

Soluble proteins secreted into the seminal fluid play a role in the maturation of sperm by affecting the distribution of surface charges across the sperm membrane (55). In addition, seminal plasma has a protective mechanism in preventing peroxidative breakdown of phospholipids by fatty acid peroxides, which could lead to

membrane damage and loss of motility (56). Nuclear decondensation of sperm and sperm chromatin instability has also been associated with seminal plasma (51).

The hyperviscosity of semen may impair any of these functions. Studies have shown that hyperviscous semen samples have infrequent consistency, possibly due to the presence of structurally complex molecules such as glycoproteins (7). These glycoproteins are incorporated into the sperm membrane and are involved in zona pellucida recognition by sperm and the acrosome reaction (57). Because of this, SHV contributes to lower fertilization rates after *in vitro* fetilization (IVF) and impairment of embryo development (7). Similarly, SHV has been associated with poor outcome of controlled ovarian hyperstimulation (COH) and intrauterine insemination (IUI) (58), although SHV does not have a negative impact on inctracytoplasmic sperm injection (ICSI) (7). OS present in SHV causes potential infertility through sperm membrane and DNA damage, which not only has consequences in sperm function but also embryo development and pregnancy rates (59). These factors indicate that SHV can more than likely be used as an indicator of poor outcome from certain ART procedures, as well as disrupted embryo development and failed pregnancy (7).

8. TREATMENT

Semen hyperviscosity can be treated successfully *in vitro* by traditional methods as well as by recently developed methods. Hyperviscous semen is commonly diluted or drawn into a hypodermic needle and forced through in order to overcome the elevated viscosity, although these methods are unlikely to be completely effective because SHV is not completely a mechanical phenomenon (7). More direct treatments of patients presenting with SHV include over hydration, prostatic massage, and the use of parenteral hyaluronidase. These methods showed limited success and have proven not to be too effective (60).

Less dated methods used in sperm preparation for ART procedures have been shown to improve semen and sperm parameters of infertile males. Honea *et al.* found that the use of limited proteolysis by using α-chymotrypsin in the treatment of SHV was effective for an *in vitro* setting such as IVF or IUI (61). Zavos *et al.* more recently reported that limited proteolysis by α-chymotrypsin was effective in improving the use and handling of hyperviscous semen samples, and that treatment with α-chymotrypsin assisted in the recovery of high quality sperm in greater numbers than in hyperviscous samples not treated with α-chymotrypsin (62). Other mucolytic agents that have been used in the treatment of SHV include α-amylase, dithiothreitol, and pancreatic dornase (63, 64). Anti-inflammatory agents can be used *in vivo* to treat patients with lesser degrees of SHV, while treatment in patients with severe SHV showed some improvement (15). Antibiotics can also be implemented *in vivo* for the management of SHV in cases of infection and leukocytospermia (21).

9. CONCLUSION

SHV is caused by multiple contributing factors, such as infection, inflammation, leukocytospermia, hypofunction of the male sex accessory glands, OS, and genetic factors. SHV leads to subfertility through a trapping effect, asthenozoospermia, decreased sperm count, and through disruption of events leading to fertilization including sperm-oocyte fusion, entry into the cervical mucus, distribution of surface charges on the sperm membrane, prevention of the peroxidation reaction, and the maintenance of sperm chromatin stability. SHV often leads to consequences in fertilization rate, pregnancy rate, and embryo development. SHV can be treated by conventional methods such as treatments with a hypodermic needle, mucolytic enzymes, and antibiotics and anti-inflammatory agents in certain cases. While the causes and consequences of SHV have been studied in the past, further research is still necessary in order to better understand the contributors to the development of hyperviscous semen and the mechanisms through which hyperviscosity impairs male fertility both *in vivo* and *in vitro*.

10. PERSPECTIVE

SHV affects a significant number of the male population and can lead to decreased fertility. While there are several known causes and consequences of SHV, some are simply hypothesized or not entirely confirmed through scientific research. Methods do exist for measuring degrees of viscosity, as well as for treatment of the conditions and infections that lead to subfertility associated with SHV. However, further research into possible treatments for and causes of SHV is necessary in order to improve fertility and the success of current ART procedures.

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