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*Review Article*

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# Characteristic of Flaviviridae and Diseases Caused by Viruses in this Family: A Briefing

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**Abstract**

Viruses are the sub-microscopic infectious agents and are the smallest and simplest of all life forms. The family Flaviviridae contains several important viruses that cause diseases in domestic and wild animals. Some of these viruses are emerging as very important not only for animal health but also for public health as they are highly zoonotic. The viruses of veterinary importance in this family belong either to the tick-borne virus group or the mosquito-borne virus group. So this article describes about the diseases caused by the family flaviviridae, its epidemiology, susceptible host, pathogenesis, clinical findings, diagnosis, prevention and treatment and its public health significance

## *General Features of the Family*

The family Flaviviriade is named after '***yellow fever***', 'flavus; is the Latin word for yellow. These are also called ***group B arbovirus***.The viruses under Genus flavivirus of veterinary importance are Japanese B Encephalitis virus; Louping Ill virus; West Nile virus.The viruses under Genus Pestivirus of veterinary importance are Bovine viral diarrhoea virus; Border disease of sheep virus and Classical Swine Fever Virus. Host for Pestivirus include even toed ungulates. The virus in the family Flaviviridae is all inactivated easily by heat and disinfectants as they are enveloped viruses.

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## *Classification*

| Realm | Ribovira |
|---|---|
| **Phylum** | *incertasedis* |
| **Family** | *Flaviviridae* |
| **Genera** | *Hepacivirus, Flavivirus Pegivirus Pestivirus* |

## *Virion Properties*

Virions of the family Flaviviridae, regardless of Genus are spherical and consist of tightly adherent lipid envelope that may display distinct protein spikes surrounding nucleocapsid with icosahedral symmetry.

Genome consist of a single molecule of positive sense single stranded RNA of approximately 11, 12.3, 9.6-10.4, kb and 11.2 kb for flavivirus, Pestivirus, Hepacivirus and Pegivirus respectively. Flavivirus has a 5' terminal cap whereas Pestivirus Hepacivirus and Pegivirus don't have cap, instead

they have an internal ribosomal entry site in 5′ end. Flavivirus don't have poly A tail at 3′ end of the genome.Genome of all members of family contains one ORF encoding 10 or more proteins that are created by co or post translational processing of single large polyprotein. Virions contains 3 structural proteins (genera: flavivirus, Hepacivirus and Pegivirus), or 4 structural proteins (Pestivirus) that are encoded at the 5′ end.

Structural proteins of flavivirus include C polypeptide, thenucleocapsid protein; envelope contain 2 virus encoded proteins ,the major and larger one is Envelope (E) protein, which is a major target site for neutralizing antibodies; smaller one is M protein, the transmembrane protein.M protein is generated from precursor polypeptide M(PrM). Pestivirus have 4 structural proteins including C (nucleocapsid protein) and Erns, E1 and E2 Envelope glycoprotein.

There are 7-8 virus encoded non- structural proteins including NS5, the RNA dependent RNA polymerase and NS3, which have several functions including helicase and protease activity in addition to contributing to RNA polymerase complex. NS2B and NS3 are largely responsible for cleavage of polyprotein and host cell proteases are responsible for rest of this processing. Flavivirus encodes only NS3 protease and Hepacivirus and Pestivirus encode a second protease in the NS2 region that cleaves between NS2 and NS3. Pestivirus also encode for a unique non-structural protein, Npro which auto catalytically releases itself from polyprotein.This protein is not responsible for viral replication in cell culture but modulates the interferon response in infected cells.

### *Diseases Caused by the Family Flaviviridae*

*Bovine Viral Diarrhoea*

It is a sub-acute, acute, in apparent contagious disease of bovines characterized by high fever, diarrhoea and erosive lesions on mouth, oesophagus, rumen, abomasum and intestine. It is caused by Bovine Viral Diarrhoea Virus.

### *Epidemiology*

It is one of themost significant infectious diseases in livestock industry with high prevalence, persistence and clinical consequences. It was first recognized in USA. BVDV-1 strain is the most predominant strain in the world. BVDV-2 was first isolated in UK in 2000. BVDV-3 has also been reported recently.It is responsible for significant economic losses in places like Africa, South and North America, Asia (India, Bangladesh, Pakistan, Burma, Sri Lanka)

### *Susceptible Host*

Principal host of BVD is cattle of age group 6-24 months. Buffalo, pig and sheep also affected whereas deer, wild ruminants are also susceptible and often affected. BVD occurs in all seasons but most common in rainy and winter season.

### *Mode of Transmission*

It can be spread by ddirect and indirect contact. Incidence of BVD is more in crowded herd. Virus is normally present in nasal secretionsoral dischargeurine. Other sources like transport vehicles, farm appliances, contaminated feed and water etc can also serve as a potential means to spread the virus.Ingestion of contaminated materials of diseased animals can also spread the virus. Sheep and swine no role in disease transmissionCalves harboring cytopathic strain act as principal source of infection

### *Clinical Features*

Clinical manifestation of the disease depends upon the age of animal infected and pregnancy status of the animal. Three types of situation arise that include post natal infection in non-pregnant cattle, infections in pregnant cows and persistent infections in calves and Mucosal disease (MD).

### *Post Natal Infection in Non-Pregnant Cattle*

All age groups susceptible (8-24 months common). In some animals there may be explosive diarrhea. Nasal and ocular discharge and erosive stomatitis, considerable drop in milk yield in dairy herds. Due to immunosuppression, there may be opportunistic respiratory and intestinal infections.

### *Infection in Pregnant Cows*

High frequency of transplacental spread depending on age of fetus and strain of virus. Infection very early in pregnancy results in death, resorption of fetus. Infection before immunological maturity (80-125 days) results in fetal death or low birth weight(weak calf syndrome), defective organogenesis like congenital defects in eye (retinal dysplasia) and C.N.S defects like cerebellar hypoplasia and cavitations of cerebrum. Surviving calves become persistently infected. (seronegative in all test); responsible for shedding virus throughout life. Their dams are called 'Trojan dams'. They have high probability of developing MD. Foetus infected after 125 days of gestation, usually survive.

***Persistentinfections in Calves and Mucosal Disease***

PI calves areillthrify and smaller than their peers. They continuously shed virus. Virus transmitted poorly from acutely infected cattle butefficiently from persistent cattle. PI dam always gives rise to PI calves. Only 20% PI survive up to 2 years of age. PI cattle that survive are susceptible to MD (fatal). If the PI animal is super infected with a cytopathic strain of BVDV due to mutation of non-cytopathic strain already circulating in the animal, develop MD.

***Diagnosis***

Diagnosis is made by observing the typical signs and syndromes, gross and microscopic lesions, leukopenia etc. Confirmatory diagnosis can be made by isolating and identifying the virus from suspected materials like blood, spleen and lymph node. Animal inoculation tests can also be performed. Cytopathogenic effects like degeneration of cytoplasm pyknosis ballooning elongation vaculation granulation of lymphoblastic cells can be looked for. Other tests like Viral Neutralization Test, CFT, and ELISA can be used. Serum conjugates against Hog Cholera can be used in FA test for BVDV. BVD must be differentially diagnosed from Malignant catarrhal fever FMD, Rinderpest, IBRJohne's disease, heavy parasitism etc.

***Prevention and Control***

Proper hygiene and sanitation must be followed. Chloroform, ether and trypsin kill them. Mild case recovered spontaneously with durable immunity. Modified live viral vaccine/Killed vaccine used to prevent the disease (first vaccination ~ 6 months, booster required for proper immune response).

Difference Between Cytopathic and Non-Cytopathic Bvdv Strains:

| CYTOPATHIC | NON-CYTOPATHIC |
|---|---|
| Produces cytopathic effects in MDBK cell line->vaculation being the earliest morphological change | No cytopathic change in MBBK cell line |
| Fail to establish chains of infections and unable to cause PI rather they are responsible for mucosal disease | Responsible for persistent infections and associated changes |
| Not common in occurrence as compared to cytopathic strains | More common in field condition |
| Emergence of cytopathic virus from non-cytopathic virus is attributed to mutations that are unique to each virus [Recombinant initiations within host-cell mRNA, gene translocations, duplications, point mutations] | NCP strains undergo mutation to cytopathic strain |
| It illustrates a case of viral emergence to extinction | Persistence of virus helps its survival inside host |
| Able to induce Type I interferon in infected cultured cells | Fails to induce INF type I in cultured cells |
| Show production of NS 3 proteins by mutation of NS2 gene | NS3 not expressed |

***Swine Fever***

Swine fever is also known as Hog cholera, pig typhoid, Schweine pest (German) Pestedupore (French) Pestesuina (Italian), Peste porcine. It is a highly fatal, highly infectious viral disease affecting pigs of all age groups. Pigs are the natural host and the only animal to be affected by the disease naturally. The disease is characterized by septicaemia, haemorrhages, leukopenia, ataxia, skin discolouration, reproductive failure, pneumonia, vomition, diarrhoean etc. It is caused by Swine Fever Virus which was previously considered as G- ve bacteria, ***Salmonella cholerasuis.*** Dorset et al identified Swine fever virus in 1904.It has anumber of strains with wide virulence and antigenicity which is a reason for vaccine outbreak. It has antigenic similarity with **BVDV**.

***Epidemiology***

Worldwide in distribution and occur in severe outbreak form Morbidity may be up to 100%. It is eradicated successful by primary policy of slaughter and quarantine in countries like Canada Australia New Zealand South Africa UK USA.

***Susceptible Host***

It is one of the most costly diseases affecting pig. Mortality is very high. Pigs of all breeds, sex and

ages are susceptible to it. Suckling white mice and rabbits are used as laboratory animals. Virus does not grow in chick embryo Cultivated in tissue cultureWild pigs remain as carrier.

### *Mode of Transmission*

Present in blood stream. All organs and all dischargesare infective Urine, dung, eye discharge and breathecan be the mode of transmission. It is excreted in urine for 2-3 weeks.The host gets infection, mostly byingestion of garbage or contaminated feed and water.Virus can enter respiratory tract through inhalation. It is a very contagious disease and can spread rapidly with direct contact. *Recovered pigs may act as carrier and carrier pigs and vaccinated pigs are potent source of infection.* In pregnant sows, it can pass or cross placental barrier leading to still birth, abnormal piglets. Transmission through breeding Insects flies. One peculiar method of transmission seen in case of this disease is that the virus is present in eggs of lung worm of affected pigs and when the earthworm ingests lung worm egg, it gets the virus and when in turn eaten by healthy pigs, the pigs get infected by the virus.

### *Pathogensis*

The virusmostly enters body through upper digestive tracts through ingestion or respiratory tract by breathe. It localises in tonsils which is the site of primary viral replication. It enters into blood through tonsilartissue. Secondary replication of the virus occurs in the endothelial cells, lymphoid organs, and bone marrow leading to haemorrhages, profound leukopenia and thrombocytopenia. Blood vessels undergo changes like hyaline degeneration, infiltration by lymphocytes, macrophages & plasma cellshaemorrhages necrosis, infarction in various organs. Inflammation in the lungs along with secondary bacterial infection (Salmonella choleraesuis Pasteurella suiseptica) can also occur.

### *Clinical Findings*

*The disease can occur in per acute, acute and chronic form.* Incubation period varies from three days to three weeks (commonly seven days).

### *Classical Form of Swine Fever*

In its Classical form, hog cholera is an acute infection. Common signs after IP of 2 to 4 days include high fever, depression, anorexia and conjunctivitis. Vomition, diarrhea, constipation is also seen.

***Nervous dysfunctions*** like paresis, paralysis, tremor, circling etc can be seen.Light skinned swine show hyperemia,purpuria on abdomen and ears. Severe leucopenia may occur. Mortality may reach 100%.

### *Chronic Form*

Chronic form is less severe with prolonged IP. Signs like Runting, chronic diarrhea, dermatitis, purpuria, secondary bacterial infections and death are generally seen. This form is associated with virus of moderate virulence. Infection in pregnant cows leads to foetal, embryonic death, abortion, mummification, still birth. New born piglets may die or survive with tremors, runting and progressive disease leading to death

### *Some Characteristic Clinical Signs of The Disease*

*Infarction of spleen is pathognomonic.* DIC, thrombosis of small vessels is commonly seen. Most prominent lesion in swine dying of hog cholera is general exhaustion of immune system. There is complete *atrophy of thymus***,** and germinal centers in spleen and lymph node. Live piglets (healthy\infected) remain *persistently infected, immunologically tolerant and are the lifelong shedders of the virus.*

### *Diagnosis*

Diagnosis is done byhistory and clinical signs, histopathologic findings isolation and identification from specimens like spleen, tonsils, lymph nodes and blood.

*FA Test is the fastest and most reliable* can be applied to cryostat sections of spleen and lymph node.Animal inoculation (susceptible and immune swine inoculated with the infected materials) can also be done. Lab tests like acute leucopenia, late stage leukocytosis can help in diagnosis.

### *Treatment*

As it is a viral disease, there is no specific treatment. Hyper immune serum,fluid therapy can be used for treating the animals.

### *Prevention and Control*

It is destroyed by heat at 60-70°C%, cresol, and 3% sodium hydroxide. It *survives in frozen pork for many years.* It can be preserved in 50% glycerin saline for 7 months. ***Garbage feeding prohibitions and garbage cooking regulationsare adopted by most of the developed countries where it is eradicated.*** Strict and rigorous sanitary prophylaxis as per ***OIE Terrestrial Animal Health Code is applied in order***

***to check the spread of the disease.*** Eradication by ***"Test and Slaughter" followed by safe disposal of carcass and thorough disinfection of the area.***

### *Control by Vaccination*

Attenuated lapinizedviral vaccine were used previously that provided immunity for 3- 6 months. ***Crystal violet vaccine*** which is a killed vaccine with efficacy 60% shows good results. Recently, Indian scientists at ICAR's IVRI developed Classical Swine Fever (CSF) cell culture based vaccine which provides immunity for about 2 years. Intervet swine fever vaccines, which is an attenuated, freeze dried, and tissue culture based vaccine is available in 5 ml vial. Blanket vaccination of pigs over 2 weeks of age can be done where the infection is endemic. Piglets born to vaccinated sows, can be vaccinated at 8 weeks age. New generation MLV (Modified Live Vaccine **'Marker Vaccine'** based ***on major envelope glycoprotein (E2-Subunit)*** is also available.

### *Border Disease of Sheep*

Border disease was first described on the border between England and Wales, hence the name used in Great Britain and North America. It is known as "hairy shaker disease:, in Australia and New Zealand due to its clinical signs

### *Clinical Features*

It appears as a congenital disease in lambs characterized by low birth weight, poor viability and poor conformation, tremors and excessively hairy birth coat in normally smooth-coated wood breeds.

### *Pathogenesis*

In adult sheep, infection is always subclinical, but infection in pregnant ewes results in foetal death or delivery of dead, deformed or mummified lambs. There is defective myelination of the nerve fibres in CNS which is manifested clinically by neurological signs. In some lambs, there is development of immune response whereas in others there is persistent infection, immunological; tolerance and seronegativity to the virus permanently. These organs become life time carriers of the virus and shed the virus in all body secretions and excretions whether they are showing clinical signs or not

### *Louping Ill*

It is an infectious ovine encephalomyelitis, a tick born disease in sheep and cattle caused by Louping ill virus (under genus Flavivirus). It occurs mostly in the British Isles and Iberian Peninsula.

### *Pathogenesis*

Louping ill is seen most commonly in sheepless than 2 years of age. Adult sheep are either immune or show a sub clinical infection. The disease is characterized by fever, CNS involvement, in coordination of movement, ataxia and paralysis. Gross lesions are not seen in infected sheep but microscopic lesions like meningoencephalitis is seen with damage to the Purkinje cells and Perivascular cuffing. The disease gains its name from peculiar leaping gail of ataxic sheep. Natural infection can occur in a variety domestic animals like horse, whereas rabbits and guinea pig are resistant.

### *Prevention and Control*

Formalin inactive vaccine provides effective immunity, antibody exist up to 3 years. Recently tissue culture vaccine is also developed. Acaricides can be used to control ticks which are the main vectors of the disease.

### *Public Health Significance*

Louping ill is zoonotic and it is transmitted to humans by ticks or occasionally by contact with infected sheep and sheep tissues. In humans, the disease is biphasic, the first phase is influenza type and the other phase is meningoencephalitis syndrome that usually resolves without sequel in 4- 10 days.

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*Review Article*

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# Semen Analysis as A Tool to Assess Infertility Among Males

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**Abstract**

Sperm analysis, also called sperm count, measures the amount and quality of sperm and semen. Sperm is a thick, white fluid that comes out of the vagina during orgasm. This release is called ejaculation. Sperm contains the sperm, the male cells carrying the genes. When a sperm unites with a woman's egg, it forms an embryo. Low sperm count or abnormal sperm formation or movement can make it difficult for a man to conceive a woman. Infertility is called infertility. Infertility can affect both men and women. About one-third of couples who are unable to have children, male fertility, an important factor needs to be considered. Sperm analysis can help determine the cause of male infertility.[3] The present study discussed about the sperm analysis and its use as per the standard guidelines to enable the researcher to carry out the research and get the quality outcome in terms of assessment of infertility among males.

**Keywords:** Azospermia, Sperm Analysis, Semen, Infertility.

## Introduction

Sperm, also known as semen, is a living fluid designed to produce sperm. It is released through the gonads (sex glands) and other genitals of the male or hermaphroditic organs and can fertilize the female ovum. In humans, sperm fluid contains several nutrients in addition to spermatozoa. These include proteolytic and other enzymes and fructose are the components of sperm fluid that promote spermatozoa survival, and provide a way to move or "swim". Sperm is also produced from the seminal vesicle, which is located in the pelvis. The process that leads to sperm is called ejaculation. Sperm is a type of gene. In animals, sperm are collected for cryoconservation. Cryoconservation of animal genetic resources is a practice that requires the collection of genes in conservation efforts of a particular species.

Sperm testing is essential for a variety of reasons:

Testing for male reproductive function and the ability of the genital tract to provide appropriate reproductive treatment and to monitor the response to treatment;

Evaluation of fertility and choosing the appropriate treatment for the infertile couple. Measuring the effectiveness of male contraception (e.g. vas occlusion and interventions that include male hormonal contraception and other possible

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alternatives). Semen analysis may direct the clinician to determine how to proceed with the investigation and management of the infertile couple.[2]

### *Basic tests-Methods*

Sperm count testing: Sperm cleansing has been simplified, but 200 spermatozoa should be counted. The laboratory must not stop testing the amount of sperm at low concentrations (2 million / ml). The total number of sperm per ejaculate (ejaculation output) has a diagnostic value beyond the concentration of sperm, but in this case, the sperm volume should be accurately measured. A test phase of azoospermia is maintained. Procedures for centrifugation and staining of live sperm are also included for detecting sperm from unprepared samples for post-vasectomy sperm testing or when semen is significantly suppressing contraceptives.

Sperm flow test. Separation of sperm motility reverts to a continuous rapid, slow-moving, continuous motion and motion (grade a, b, c or d) because the presence (or absence) of rapidly developing sperm is clinically significant

### *Sperm test*

The procedure for testing for sperm formation using a systematic method is described and should be followed. In this issue, several micrographs of the best quality spermatozoa from unprocessed sperm samples considered normal, borderline, or abnormal are included, accompanied by explanations as to why each sperm is classified in the way it has been classified. It should help train specialists to explain the subtle features of spermatozoal morphology further.

### *Extended tests-Methods*

Unlike many other bodily secretions that have been tested for diagnostic or therapeutic purposes, ejaculate is a combination of secretions that are not present within the body before being expelled. In males, the ejaculate is produced from a concentrated suspension of spermatozoa. It is then stored in the paired epididymides, mixed with, and diluted by, primarily the prostatic fluid in the urethra, followed by the removal of seminal vesicles. Therefore, consecutive ejaculate components are not equally integrated.

The ejaculate has two large measurable properties.

The number of spermatozoa indicates sperm production by the testicles, the strength of the post-testicular duct system, the efficiency of smooth muscle contraction in the epididymides and the vasa deferentia actively moving semen into the urethra, as well as the ability to ejaculate and ejaculate. Ejaculate filled with semen. The latter factors are influenced by sexual arousal - duration and quality - and are performed by emotional signals to smooth muscle cells (vasa deferentia, glands, urinary bladder sphincter) and smooth muscle cells that control blood flow and erectile dysfunction. The amount of fluid supplied by the various available glands reflects the secretory function of the glands and the smooth contraction of the muscles that secrete each gland. These activities are responsive to the emotional stimuli caused by arousal and as a preparation for arousal.[1]

The nature of spermatozoa (its strength, motility and morphology) and the formation of ejaculate fluid are also essential for sperm function. As a result of sexual intercourse, the first, affluent part of the prostatic sperm of the ejaculate may be connected to the cervical mucus that passes into the vagina without significant contact with the entire ejaculate. Conversely, in a laboratory setting, all ejaculate is collected in a single container, where sperm are trapped in a gel developed in proteins of seminal vesicular origin. In vitro, this gel is later converted to the action of prostatic protease, during which its osmolality increases. The total volume and content of spermatozoa for ejaculates varies depending on the conditions in which the ejaculate is produced. Ejaculates produced by masturbation and collected in containers in a room near the laboratory can result in lower yields than those found in non-sperm condoms used during sex at home. This difference may indicate a different level and duration of sexual arousal, as the time spent producing the sample by masturbation also affects ejaculate volume and content.3

Under the given conditions of collection, ejaculate factors depend on usually irreversible factors, such as sperm production, accessory discharge and recent infection (especially febrile), and other factors, such as urinary incontinence, should be recorded considered in interpreting results. The results of laboratory measurements of ejaculate symptoms will depend on the following. • That complete ejaculate is collected. During ejaculation, the first parts are excreted mainly by the prostatic fluid rich in semen, while the later parts are dominated by seminal vesicular fluid. Therefore, the loss of the first part (rich in sperm) of ejaculate has a more significant impact on the analysis results than the loss of the last part. Therefore, sperm concentration

is not a direct measure of testicular sperm release, as it is influenced by secretory function in other organs. The total amount of ejaculated spermatozoa (sperm concentration doubled by sperm volume) is, therefore, the best expression of sperm production capacity.

The time between ejaculated ejaculation and the most recent ejaculation (ejaculatory menopause "period of abstinence", sometimes called sexual abstinence). Spermatozoa collect in epididymides until they are complete, then excrete in the urethra and excrete in the urine; since epididymides are never wholly removed by a single discharge, some spermatozoa remain from the time of the previous discharge. This affects the age range and quality of spermatozoa in the ejaculate. Sperm strength and chromatin are not affected by the increased duration of sleep deprivation unless an epididymal function is impaired. In addition, extensive research to determine daily spermatozoa production has shown those 2-3 days of daily release is required to complete epididymal retention of spermatozoa. Therefore, a recommendation, based on clinical experience, asking men to collect ejaculate for testing after 2-7 days of abstinence can cause discrepancy and an unexplained boundary between expected and infertile outcomes. The extent of this influence is difficult to determine, and it is rarely considered.[4]

### *Sperm Collection*

*Patient information*

The man should be given clear written and verbal instructions regarding collecting sperm samples. The doctor should provide the same information to the patient. The main recommendation is to collect ejaculate by masturbation. Coitus interrupts is not recommended and should only be used in exceptional cases due to the risk of incomplete collection and contamination of vaginal fluid and cells. Contraceptive condoms cannot be used due to sperm killing agents. Ordinary latex condoms should not be used for sperm collection because they contain substances that interfere with the movement of spermatozoa. Lubricants should be avoided, as they may contaminate the ejaculate and alter its properties. If necessary, certified non-spermatozoic certified lubricants should be used. The ejaculate needs to be fully collected, and the male should report any loss of any part of the sample. Ejaculate should be collected after at least two days and more than seven days of abstinence. Avoid sperm exposure to temperature fluctuations and control the time between collection and analysis, it is recommended that the sample be collected in a private room near the laboratory. Ideally, the investigation should begin within 30 minutes after collection but at least within 60 minutes. Individual variations may be required, and each individual should be given appropriate advice about the possible risks and risks.[5]

Prior to ejaculating collection, the specimen container should be stored at a temperature of between 20 °C and 37 °C to avoid significant temperature changes that may affect sperm.

The sample container should be a clean, wide-brimmed plastic container from a collection that has been proven to be non-toxic to spermatozoa.[12]

The sample container, as well as the accompanying worksheets, should be labeled with identification consistent with the sampling acceptance procedures and continuous management, eliminating the risk of merging the samples with working papers. Legal requirements for container tag ownership may vary. It could be a man's name and ID number, date and time of collection, or unique identification numbers. The following information must be recorded on the acceptance sample and presented in the final report: the identity of the man (e.g. name, date of birth and personal code number) and his confirmation that the sample is his; pre-exit period; date and time of collection.13

The completeness of the sample and any difficulties in producing the sample (e.g. if the collection was not done in the laboratory); andturn off the volume.

### *Volume by weight*

Volume is best measured by measuring the sample in the container in which it is collected. It can best be done when a pre-heated container is received to incubate to drain the liquid.

Use a pre-measured container to collect the ejaculate, weight recorded on the container and lid. Empty sample containers usually have a different weight, so each container with a lid should be weighed in advance. The weight should be recorded on the container and its lid with a permanent marker pen before giving it to the patient. When labels are used - for example, tags - their weight should be included in the blank weight.[6]

Measure the vessel with ejaculate into it. 3. Remove the weight of the empty container. 4. Calculate the volume from the weighted sample, assuming the sperm concentration is 1 g / ml. (Sperm density reported to vary between 1.03 and 1.04 g / ml, 1.00 and 1.01 g / ml, and a dose of 1.01 g / ml).[1,13]

*Macroscopic examination*

Macroscopic tests include several vital observations that may not be possible to diagnose with the exact number of numbers - and thus control the most common methods - but are still very important clinically. The average liquid ejaculate is a macroscopically pale, cream/grey colour. It may appear slightly opaque if sperm concentration is too low; colour may also vary - i.e. slightly yellow after a long period of abstinence, reddish-brown when red blood cells are present (haemospermia), or clear yellow in a patient with jaundice or taking specific vitamins or drugs. If the ejaculate appears vicious, completely clear and colourless, then the pre-ejaculate may come out only from Cowper glands, which are produced by males in various quantities during arousal; in this case, this should be discussed with the patient to determine if orgasm associated ejaculation occurs.[6]

*Melting*

Immediately after ejaculation from the collection vessel, the ejaculate is usually a semi-solid coagulated mass or gel like clump. Usually, the ejaculate begins to melt (become thin) within a few minutes at a temperature equal to room, at which point a mixture of loose lumps will appear in the liquid. As liquefaction progresses, the ejaculate becomes homogeneous and watery but has a higher viscosity than water. In the final stages of melting, there are still small areas for thickening. A temperature of 37 °C will make it easier for the liquid. Also, a slow, rotating movement of the sample container will help the liquefaction dissolution complete. If a moving tray (orbital mixer) can be used during melting, the container must be rotated slightly for 15-30 seconds before starting a liquid macroscopic test.

Complete ejaculate liquefaction is usually achieved within 15-30 minutes at room temperature. • If immersion is not completed within 30 minutes, this should be recorded and recorded in the final report. The ejaculate may be left at 37 ° C for another 30 minutes. • If immersion is not completed within 60 minutes, this should be included in the final report. • Normal ejaculate can contain jelly-like granules (gelatinous bodies) that do not dissolve and do not appear to be clinically significant. The presence of mucous membranes, although it may interfere with ejaculate examination, should therefore be noted in the final report.

*Ejaculate viscosity*

After liquefaction, the viscosity of the ejaculate can be measured by gently squeezing it into a vast hole (approximately 1.5 mm wide) disposable plastic pipette (certified as non-toxic to the sperm and, if necessary, sterile), allowing sperm to drop. by gravity and to check the length of any string. Average fluid ejaculate falls like separate tiny droplets. If the viscosity is abnormal, the drop will form a thread more than 2 cm long.

*Smell out*

There is a significant difference in the ability of different people to detect the typical odour of human ejaculate. Knowledge of the strong odour of urine or decay may be necessary for the clinic; it is, therefore, essential to note this in the report.[7]

*Ejaculate pH*

The pH at the ejaculation rate depends on the relative contribution of acidic prostatic secretion and alkaline seminal vesicular secretion. In ejaculate, there is no effective control of the pH of the fluid. In vitro, there will be a continuous loss of CO 2, resulting in a gradual increase in pH. The clinical interest in ejaculate pH is low. If pH is to be tested, it should be done simultaneously, preferably 30 minutes after collection, but in any case, within 1 hour of discharge. For standard samples, pH test strips of 6.0–10.0 should be used. 1. Mix the sperm sample well. 2. Distribute the sperm evenly across the pH line. 3. Wait for the colour of the affected area to match (<30 seconds). 4. Compare colour with measuring line to read pH.8

Preparation for a microscopic investigation

For reliable results of very little research, the tested aliquots must represent the entire ejaculate. The nature of the liquid ejaculate, which is more visible than water, makes taking a representative sample of sperm for analysis more difficult. If the sample is not well mixed, analyzing the two different aliquots is less likely to represent the entire ejaculate. It may show significant differences in sperm concentration, mobility, strength and morphology. Even if the liquid ejaculate is macroscopically homogeneous, small aliquots can have very different textures. Ejaculate mixing before removing the sperm aliquot from any test; mix the sample well in the first container, but not so hard that air bubbles form.[9]

Mixing can be achieved by placing the sample container on a moving tray during the liquid in an incubator at 37 °C. In the absence of an orbital mixer, basic mixing can be achieved in about 15–30 seconds of manual rotation.

***Damaged sample***

Although liquefied macroscopically may look perfectly alike, there may be small but essential compounds with a different sperm structure and discharge. It is therefore essential: • To use at least 50 µl repeated aliquots to purify the sperm concentration • To use at least 10 µl repetitive aliquots to check sperm flow. Comparisons of those duplicate aliquots are required to reduce the risk of errors due to non-representative samples. Comparison methods are described under each testing strategy.[10]

To make a wet fix directly after the ejaculate is well mixed; remove the appropriate dose, not allowing time for the sperm to come out of the erection. Always re-mix the sperm sample before extracting duplicate aliquots. The movement test should be executed in two different ways, the newly prepared liquid (see the wet preparation instructions on page 65 for the background). 1. Place a 10 µl aliquot mixed well on a clean microscope slide that is best heated to 37 ° C (e.g. in a sample incubator).

2. Place the 22 mm × 22 mm coverslip by carefully tossing it horizontally over the drop. The weight of the coverslip disperses the sample (so use the weight slip # 1½). 3. Check for recent wet repairs as soon as the content is no longer flooded. • If the flow does not stop within 1 minute after installing the coverslip, a new wet fix should be made.

Testing under a microscope A clear-contrast optics microscope is required for all tests of fresh sperm setting (Section 8.3 on page 221 on how to set up a microscope). 2.4.4.1 Low magnification. The first small test of ejaculate aliquot involves scanning correction for a total magnification of × 100 (i.e. a combination of a target lens of × 10 with × 10 ocular). It provides an overview of the sample, indicating whether the sperm is evenly distributed throughout the preparation, any visible mucous membranes, and sperm fusion or fusion. In the case of uneven distribution, the cause may be: insufficient mixing, high viscosity, insufficient fluid , sperm accumulation.[11]

High magnification Adjustments should then be tested at × 200 or × 400 total magnifications (i.e. a combination of × 20 or × 40 purposes with × 10 oculars).

It allows: Sperm flow testing; determining the purification required for accurate sperm count testing. Determining the presence of circular cells that require further testing; • determining the presence of cells outside of spermatozoa (e.g. epithelial cells) or "circular cells" (leukocytes and immature virus cells).[12]

## Conclusion

The tests quality depends on the standard process which needs to be followed with minimizing errors. In case of sperm analysis the time management is crucial while performing a test for assessment of male infertility. The liquefaction after collection of the semen is important factor in addition to preservation of the sample. The process if followed meticulously then the battle is half win for the technologists performing the test.

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*Case Report*

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# Impact of willpower on Health in Humans

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**Abstract**

In whole world among other animal and plants, the human beings have capabilities of thinking and application of thoughts in the universe. There are several examples of thinking high and achieving high in the society. Whatever we see at present nearby us like airplanes, rockets, atomic bombs, electricity, gadgets like cars, bikes and much more, all were in our ancestors' thoughts then resulted in practical and visible one. Human brain is the key behind all these approaches. Now it is important to think that the brain of human which can make lots of technologies and gadgets more capabilities than making things. We can learn from various case studies where one person's got victim of accident or traumatic shocks or paralytic attack. The doctors also not confirmed for speedy recovery in such cases, but it was well seen that such persons through their strong willpower regain his/her organ's lost strength. The willpower is the power of our thoughts which impacted us in very positive ways.

The present study hypothesized that one should always pay gratitude to nature, do not release negative thoughts for self and for others, always adopt an approach for conservation of nature instead of destroying nature. These all efforts will ensure that nature, the Mother Earth, will conserve the adopting the above-mentioned practices. Healthy world and Vasudev Kutumbkam need to be the best practiced by Indians and need to be popularized on mass scale.

**Keywords:** Willpower, Positive Thoughts, Health, Wellness

## Introduction

The human beings three powers greater than other animals, these includes power of writing, power of thoughts, power of communication by languages. If a person centralizes his/her power including all these means to a particular goal, then it is possible he/she will achieve the possible goal in short span of time. We all born and live in nature, the Earth is mother for all of us and thus whatever we do, will impact the Earth and in return impacted us. There is law in science that indicated that "every action there is equal and opposite reaction" and this law is well applicable on our relationship with our mother Earth. If we throw garbage like polythene, canes, plastics it will return to us only in form of ailments like cancers and other problems. If conservational actions for Earth, then we in return get best living environment, good air, good water and good food quality. The time to think on this issue micro level.

Positive thoughts mean that you keep your head in the sand and ignore life's less pleasant situations. Positive thoughts just means that you approach unpleasantness in a more positive and productive way. You think the best is going to happen, not the worst.

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Positive thoughts often start with self-talk. Self-talk is the endless stream of unspoken thoughts that run through your head. These automatic thoughts can be positive or negative. Some of your self-talk comes from logic and reason. Other self-talk may arise from misconceptions that you create, because of lack of information.

If the thoughts that run through your head are mostly negative, your outlook on life is more likely to be pessimistic. If your thoughts are mostly positive, you're likely anoptimist, someone who practices positive thoughts.

### *The health benefits of positive thoughts*

Researchers continue to explore the effects of positive thoughts and optimism on health. Health benefits that positive thoughts may provide include:

1. Increased life span
2. Lower rates of depression
3. Lower levels of distress
4. Greater resistance to the common cold
5. Better psychological and physical well-being
6. Better cardiovascular health and reduced risk of death from cardiovascular disease
7. Better coping skills during hardships and times of stress

It's unclear why people who engage in positive thoughts experience these health benefits. One theory is that having a positive outlook enables you to cope better with stressful situations, which reduces the harmful health effects of stress on your body.

It's also thought that positive and optimistic people tend to live healthier lifestyles, they get more physical activity, follow a healthier diet, and don't smoke or drink alcohol in excess.

### *Identifying negative thoughts*

## Methodology

Not sure if your self-talk is positive or negative? Some common forms of negative self-talk include:

### *Filtering*

You magnify the negative aspects of a situation and filter out all the positive ones. For example, you had a great day at work. You completed your tasks ahead of time and were complimented for doing a speedy and thorough job. That evening, you focus only on your plan to do even more tasks and forget about the compliments you received.

### *Personalizing*

When something bad occurs, you automatically blame yourself. For example, you hear that an evening out with friends is canceled, and you assume that the change in plans is because no one wanted to be around you.

### *Catastrophizing*

You automatically anticipate the worst. The drive-through coffee shop gets your order wrong and you automatically think that the rest of your day will be a disaster.

### *Polarizing*

You see things only as either good or bad. There is no middle ground. You feel that you have to be perfect or you're a total failure.

### *Focusing on positive thoughts*

You can learn to turn negative thoughts into positive thoughts. The process is simple, but it does take time and practice — you're creating a new habit, after all. Here are some ways to think and behave in a more positive and optimistic way:

Identify areas to change: If you want to become more optimistic and engage in more positive thoughts, first identify areas of your life that you usually think negatively about, whether it's work, your daily commute or a relationship. You can start small by focusing on one area to approach in a more positive way.

### *Check yourself*

Periodically during the day, stop and evaluate what are your thoughts. If you find that your thoughts are mainly negative, try to find a way to put a positive spin on them.

Be open to humor. Give yourself permission to smile or laugh, especially during difficult times. Seek humor in everyday happenings. When you can laugh at life, you feel less stressed.

### *Follow a healthy lifestyle*

Aim to exercise for about 30 minutes on most days of the week. You can also break it up into 10-minute chunks of time during the day. Exercise can positively affect mood and reduce stress. Follow a healthy diet to fuel your mind and body, learn

techniques to manage stress.

***Surround yourself with positive people***

Make sure those in your life are positive, supportive people you can depend on to give helpful advice and feedback. Negative people may increase your stress level and make you doubt your ability to manage stress in healthy ways.

Practice positive self-talk: Start by following one simple rule: Don't say anything to yourself that you wouldn't say to anyone else. Be gentle and encouraging with yourself. If a negative thought enters your mind, evaluate it rationally and respond with affirmations of what is good about you. Think about things you're thankful for in your life.

One study suggested self-control is based on glucose as an energy source. The various laboratory tests of self-control like the Stroop task, thought suppression, emotion regulation, attention control along with the social behaviors like helping behavior, coping with thoughts of death, stifling prejudice during an interracial interaction proved the outcome of the study. (Gailliot, M. T.et.al 2007)

Another study provides critical new findings that glucose level depleted during self-control and cognitive performance. (Gregory M. Waltonet.al 2013)

The meta-analytical study of 83 studies and as per the study of the strength model, self-control was observed as a finite resource which establishes ability for effortful control over dominant responses and once expended, leads to impaired self-control task performance which is called as ego-depletion. The results revealed a significant effect of ego depletion on self-control task performance. (Haggeret.al 2013)

An outcome of a study for Breast cancer and the outcome of study revealed that the work situation after Breast Cancer is a critical issue. The strongest predictors factors of work disability in employed Breast Cancer survivors were observed in the study (MR Stradaet.al 2010)

One study revealed that decision making, impulse control and loss of willpower to resist drugs: a neurocognitive perspective revealed that the addiction is the outcome of an imbalance between two separates, but interacting, neural systems that control decision making an impulsive, amygdala system for signaling pain or pleasure of immediate prospects, and a reflective, prefrontal cortex system for signaling pain or pleasure of future prospects.

**Summary and Conclusion:**

The study is currently needed in willpower and its association with management of various diseases. The symptoms can be minimized by willpower strengths along with other factors like diet control, meditations. The meet to nature is best practice for the patients in addition to diet control, meditations to combat the disease as worrier. The disease and its treatment side effects are the two major enemies for a patient. A patient must fight with these two at a same time. The comorbidity also plays wide role in weakening of will power to live. When the person accepts his or her failure in front of disease then only slowly, he/she moved to death. The willpower manages the disease effects and helps the patients to stand again. The present review study is important for new researchers in the field for using spirituality as connecting tool in disease management through willpower.

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# Subject Index

# Author Index

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**Standard journal article**

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**Article in supplement or special issue**

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**Reference from electronic media**

[9] National Statistics Online—Trends in suicide by method in England and Wales, 1979-2001. www.statistics.gov.uk/downloads/theme_health/HSQ20.pdf (accessed Jan 24, 2005): 7-18. Only verified references against the original documents should be cited. Authors are responsible for the accuracy and completeness of their references and for correct text citation. The number of reference should be kept limited to 20 in case of major communications and 10 for short communications.

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