“Semen Analysis and effect of fruit juice on human sperm motility”

Dissertation submitted in partial fulfillment for the degree of

Master of Science in Biotechnology

Submitted By

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CERTIFICATE

This is to certify the dissertation entitled **"Semen Analysis and effect of fruit juice on human sperm motility"** Submitted by ***Anwesa Pratihar*** in partial fulfilment of the requirement for the degree of Master of Science in Biotechnology, KIIT School of Biotechnology, KIIT University, Bhubaneswar bearing ***Roll No. - 1661007*** & ***Registration No. - 16645851455*** is a *bona fide* research work carried out by her under my guidance and supervision from ***'01/12/2017'*** to ***'12/05/2018'***.

Date -
Place - Bhubaneswar

Dr.Kaninika Panda
Sr Scientist and learning officer
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CERTIFICATE

This is to certify that the dissertation entitled **"Semen Analysis and effect of fruit juice on human sperm motility"** submitted by ***Anwesa Pratihar, "Roll No. - 1661007"*** & ***"Registration No. - 16645851455".*** to the School of Biotechnology, KIIT University, Bhubaneswar-751024, for the degree of Master of Science in Biotechnology is her original work, based on the results of the experiments and investigations carried out independently by her during the period from ***'01/12/2017'*** to ***'12/05/2018'***of study under my guidance. Further, it is also to certify that the above said work has not been previously submitted for the award of any degree, diploma, or fellowship in any Indian or foreign University

Date -
Place - Bhubaneswar

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DECLARATION

I hereby declare that the dissertation entitled **"Semen Analysis and effect of fruit juice on human sperm motility"** submitted by me, for the degree of Master of Biotechnology to KIIT University is a record of *bona fide* work carried by me under the supervision and guidance of '***Dr.Kaninika Panda,*** Sr Scientist and learning officer of Santaan Fertility and research Institute, Bhubaneswar, Odisha, India.

Date:
Place: Bhubaneswar *Anwesa Pratihar*

Abstract

The present study was conducted to determine that 30 human semen sample analysis (Macroscopic and Microscopic analysis) and beside also semen sample diluted with different concentration pineapple juices like 5%, 10%, 15%, 20% and this solution stored at 37℃ up to 6 hours. Fruits are rich in sugars (Hulme, 1970), and sperm readily utilizes sugars for respiration. And also pineapple juice have many antioxidants such as vitamins and phenolic compounds known to function as antioxidants. These causes pineapple juice containing sperm more motile after 6 hours of analysis, than the control one (means where pineapple juice was not added, 0%.) But when the juice was added it have a optimum concentration. The optimum concentration of juice is 10%. after that, means in 15% and 20% pineapple juice containing semen sample shown that decrease the motility. That may be occurred for very low pH. So can concluded that after the experiment, human sperm motility will be increase at 37℃ after a longer time (6 hours) with the supplement of 10% pineapple juice.

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Last but not the least I express **my regards and respect to my parents** for their support without which the work could not be possible.

Anwesa Pratihar

Date -

Place - Bhubaneswar

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Abbreviations

AI - Artificial Insemination

ART - Assisted Reproductive Technology

HOS - Hypo-osmotic swelling

ICSI - Intracytoplasmic sperm injection

IM - Immotility

IUI - Intrauterine insemination

IVF - In-vitro fertilization

ml - millilitre

NPM - Non progressive motility

PM - Progressive Motility

WHO - World Health Organization

µl - Micro-litre

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Introduction

1. Introduction:

The modern day animal reproduction is based on better reproductive techniques, one of which is artificial insemination (AI). AI is an effective and globally accepted method of breeding animals. But In AI most Important and first this is preserve Semen of breed. For this need to preserve semen from the animal such that it could be used for subsequent AI over a period. Artificial insemination with preserved semen is a viable option for genetic upgrading of animal The survival of sperm after collection for longer periods during preservation in a low temperatures requires dilution with a specific extender in order to maintain viability of spermatozoa. Here the experiment was designed to develop simple extenders using available natural products that may have the capacity to preserve spermatozoa under room temperature condition.

Sperm cells are subject to oxidative stress resulting from lipid peroxidation, which can lead to reduced sperm viability and fertility (Donghue and Donoghue, 1997). Although semen contains antioxidants that counteract the damaging effects of lipid peroxidation and prevent excessive peroxide formation (Lewis et al., 1997), the endogenous antioxidative capacity of semen may be insufficient during storage (Maxwell and Salamon, 1993). In vitro studies suggested that the addition of some antioxidants to semen extenders could improve the motility and survival of spermatozoa (Sanchez-Partida et al., 1997; Krzyosiak et al., 2000; Bilodeau et al., 2002). Fruits are good sources of natural antioxidants, containing many different antioxidant components (Cao et al., 1996;Wang et al., 1996; Velioglu et al., 1998). These antioxidants include carotenoids, vitamins, phenolic compounds and flavonoids and have proved to function as singlet and triplet oxygen quenchers, free radical scavengers and peroxide decomposers (Larson, 1988). In addition, natural foods and food-derived antioxidants such as vitamin C, E and phenolic phytochemicals have been reported to act as chemo-preventive agents against oxidative damage (Kiwon et al., 2003; Ondei et al., 2009).

In this experiment pineapple juice used for preservation for sperm longer period under room temperature. Pineapple juice is rich natural antioxidants renowned for their high concentration of vitamins and other antioxidants (Cutler et al., 2008). Different Concentration Pineapple juice added with Sperm and after that check their motility after. This solution kept at 37℃ up to 6 hours after semen collection from different age group people (21-45 years).

1.1 Scope And Objectives:

Sperm Motility is decrease in room temperature after some period. After some time of collection sperm motility may be the decrease due to the oxidative stress or may be not enough energy not supply for their motile.

Pineapple juice is rich natural antioxidants and have different vitamins and also contain with carbohydrates. When sperm mix with different concentration juices (5% , 10 % , 15 % and 20 %) then give more motile and also more progressive sperm than control (where not added pineapple juice) in 37℃. This experiment also learn about which concentration of pineapple juice may the optimum motile sperm concentration.

1.2 Achievement:

This experiment was designed to develop simple extenders using available natural products like pineapple juice that may have the capacity to preserve semen under room temperature condition or 37℃. This is also loser cost. But adding pineapple juice may be vary the normal pH range of semen (pH 6.8 - pH 8.2) which is may be effect on morphology of sperm. But adding juice motility is increased.

1.3 Over view of Dissertation:

In my dissertation programme learn about whole andrology part. Andrology is the medical specialtythat deals with male health, particularly relating to

the problems of the male reproductive system that are unique men to men. Basic thing present in my andrology part is following:

1.3.1. Semen Collection:

- The sample should be collected in a private room near the laboratory, in order to limit the exposure of the semen to fluctuations in temperature and to control the time between collection and analysis.
- The sample should be collected after a minimum of 2 days and a maximum of 7 days of sexual abstinence.
- The following information should be recorded on the report form: the man's name, birth date and personal code number, the period of abstinence, the date and time of collection, the completeness of the sample, any difficulties in producing the sample, and the interval between collection and the start of the semen analysis.
- The sample should be obtained by masturbation and ejaculated into a clean, wide-mouthed container made of glass or plastic, from a batch that has been confirmed to be non-toxic for spermatozoa.
- The specimen container is placed on the bench or in an incubator (37 °C) while the semen liquefies.
- The specimen container should be kept at ambient temperature, between 20 °C and 37 °C, to avoid large changes in temperature that may affect the spermatozoa after they are ejaculated into it. It must be labelled with the man's name and identification number, and the date and time of collection.
- Semen samples may contain dangerous infectious agents (e.g. human immunodefi ciency virus (HIV), hepatitis viruses or herpes simplex virus) and should therefore be handled as a biohazard. If the sample is to be processed for bioassay, intra-uterine insemination (IUI), in-vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI), or if semen culture is to be performed, sterile materials and techniques must.

1.3.2. Macroscopic analysis:

- **Liquefaction :**

Immediately after ejaculation into the collection vessel, semen is typically a semisolid coagulated mass. Within a few minutes at room temperature, the semen usually begins to liquefy (become thinner), at which time a heterogeneous mixture of lumps will be seen in the fluid. As liquefaction continues, the semen becomes more homogeneous and quite watery, and in the final stages only small areas of coagulation remain. The complete sample usually liquefies within 15 minutes at room temperature, although rarely it may take up to 60 minutes or more.

Occasionally samples may not liquefy, making semen evaluation difficult. In these cases, additional treatment, mechanical mixing or enzymatic digestion may be necessary.Some samples can be induced to liquefy by the addition of an equal volume of physiological medium. Inhomogeneity can be reduced by repeated (6–10 times) gentle passage through a blunt gauge 18 (internal diameter 0.84 mm) or gauge 19 (internal diameter 0.69 mm) needle attached to a syringe.

- **Semen Viscosity:**

After liquefaction, the viscosity of the sample can be estimated by gently aspirating it into a wide-bore (approximately 1.5 mm diameter) plastic disposable pipette, allowing the semen to drop by gravity and observing the length of any thread. A normal sample leaves the pipette in small discrete drops. If viscosity is abnormal, the drop will form a thread more than 2 cm long.

- **Appearance of the ejaculate:**

A normal liquefied semen sample has a homogeneous, grey-opalescent appearance. It may appear less opaque if the sperm concentration is very low; the colour may also be different, i.e. red-brown when red blood cells are present (haemospermia), or yellow in a man with jaundice or taking certain vitamins or drugs.

- **Semen Volume:**

The lower reference limit for semen volume is 1.5 ml.

- **Semen pH:**

Normal semen pH range is 6.2 - 8.2.

1.3.3. Microscopic analysis:

The preparation should then be observed at ×200 or ×400 total magnification (i.e. a combination of a ×20 or a ×40 objective with a ×10 ocular). This permits assessment of sperm motility and determination of the dilution required for accurate assessment of sperm number. This also his provides an overview of the sample, to reveal: mucus strand formation; sperm aggregation or agglutination; the presence of cells other than spermatozoa, e.g. epithelial cells, “round cells” (leukocytes and immature germ cells) and isolated sperm heads or tails.

A simple system for grading motility is recommended that distinguishes spermatozoa with progressive or non-progressive motility from those that are immotile. The motility of each spermatozoon is graded as follows:

- Progressive motility (PR): Spermatozoa moving actively, either linearly or in a large circle, regardless of speed.
- Non-progressive motility (NP): All other patterns of motility with an absence of progression, e.g. swimming in small circles, the flagellar force hardly displacing the head, or when only a flagellar beat can be observed.
- Immotility (IM): no movement

The lower reference limit for total motility (PR + NP) is 40% and the lower reference limit for progressive motility (PR) is 32%.

1.3.4. Sperm vitality:

Sperm vitality is estimated by assessing the membrane integrity of the spermatozo and determined routinely on mainly all samples, but mainly for semen samples which one have less than about 40% progressively motile spermatozoa. This test can provide a data on the motility evaluation, since the percentage of dead cells should not exceed (within sampling error) the percentage of immotile spermatozoa. The percentage of viable cells normally exceeds that of motile cells.

Eosin - Nigrosin Stain mainly help to for sperm vitality test. Spermatozoa with red or dark pink heads are considered dead (membrane-damaged), whereas spermatozoa with

white heads or light pink heads are considered alive (membraneintact). And also Vitality sstest is done by using hypo-osmotic swelling. Swollen spermatozoa are identified by changes in the shape of the cell, as indicated by coiling of the tail. Live cells are distinguished by evidence of swelling of the sperm tail; score all forms of swollen tails as live spermatozoa.

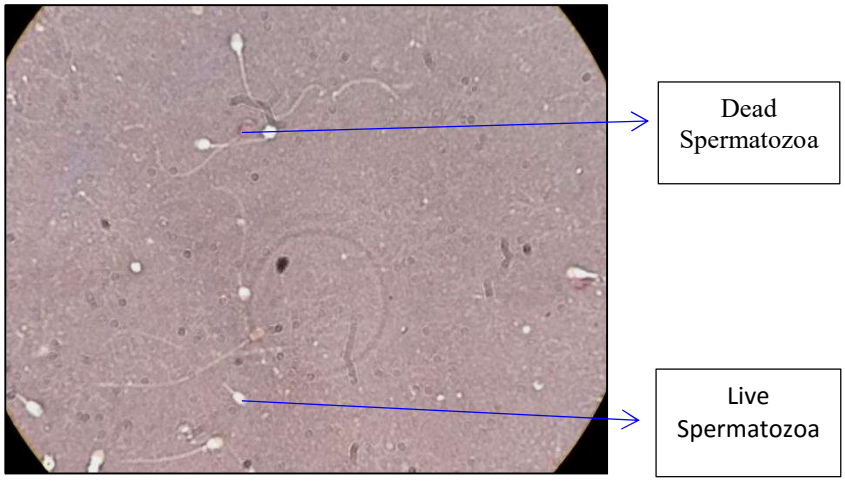

Fig 1: Spermatozoa stain with eosin nigrosin stain (Dead spermatozoa are pink coloured and alive spermatozoa are white coloured)

1.3.5. Sperm morphology:

For morphology assessment Diff Quick method is followed. By this method can difference between normal sperm, head defect, neck defect, cytoplasmic droplet, tail defect. Average normal sperm present in semen is 4%.

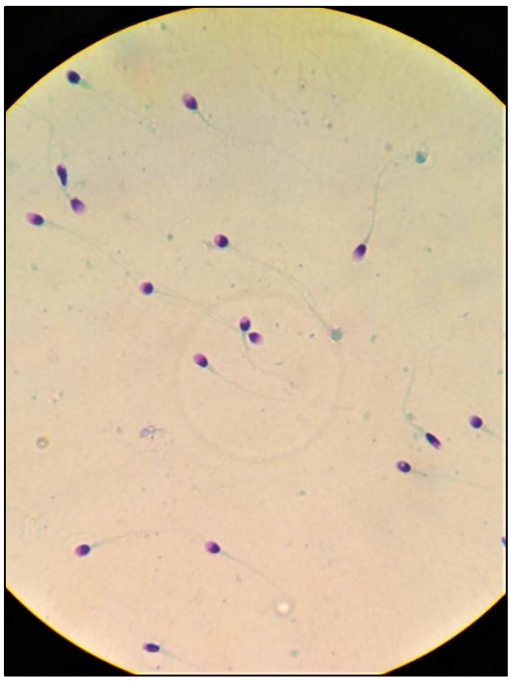

Fig 2: Morphological slide of spermatozoa by diff quick method

1.3.6 Sperm Preparation Techniques:

Three straightforward spermatozoa preparation techniques area unit delineated within the following sections. For all of them, the substance instructed could be a balanced salt answer supplemented with macromolecule and containing a buffer applicable for the environmental conditions within which the spermatozoa are going to be processed. For aided copy procedures, like intracytoplasmic spermatozoan injection (ICSI), in-vitro fertilization (IVF), artificial insemination (AI) or germ cell intrafallopian transfer (GIFT), it's imperative that the human albumin is very purified and free from microorganism, microorganism and particle contamination.

➢ Simple wash:

This simple laundry procedure provides the best yield of spermatozoa and is adequate if cum samples area unit of fine quality. it's usually used for getting ready spermatozoa for Intra female internal reproductive organ insemination (IUI).

➢ Direct swim-up:

Spermatozoa could also be elect by their ability to swim out of seminal plasma and into substance. this can be called the "swim-up" technique. The cum ought to ideally not be

diluted and centrifuged before swim-up, as a result of this could end in peroxidative injury to the spermatozoa membranes (Aitken & Clarkson, 1988). Thus, a right away swim-up of spermatozoa from cum is that the most well-liked methodology for separating out motile spermatozoa (see e.g. Mortimer, 1994a, b). The direct swim-up technique are often performed either by layering substance over the liquefied cum or by layering liquefied cum beneath the substance. Motile spermatozoa then swim into the substance. This procedure provides a lower yield of spermatozoa than laundry, however selects them for his or her motility and is helpful wherever the share of motile spermatozoa in cum is low, e.g. for IVF and ICSI.

➢ Discontinuous density gradients:

Discontinuous density gradients will provide the most effective choice of additionally an improved quality spermatozoa, giving sensible separation from alternative cell trash. it's easier to standardize than the swim-up technique, and results area unit additional constant. this system is employed to recover and additionally prepare spermatozoa to be used in IVF and ICSI and additionally IUI.

This methodology uses natural action of seminal plasma over density gradients consisting of mixture oxide coated with silane, that separates cells and cell trash by their density. additionally, motile spermatozoa swim actively through the gradient material to make a soft pellet at all-time low of the tube. an easy ballroom dance discontinuous density-gradient preparation methodology is most generally applied, generally with a forty five (v/v) density prime layer associated an ninetieth (v/v) density lower layer. spermatozoa preparation exploitation density gradient natural action typically ends up in a fraction of extremely motile spermatozoa, free from trash, contaminating leukocytes, non-germ cells and degenerating germ cells.

A number of business product area unit offered for creating density gradients appropriate for cum process. These product ought to be used in line with the manufacturers' recommendations. Any departure from procedural recommendations ought to be evidence-based. Most density-gradient media contain high relative molecular mass elements that have inherently low osmolality, so that they area unit typically ready in medium that's isoosmotic with feminine fruitful tract fluids.

1.3.7. Semen Cryopreservation:

Cryopreservation of spermatozoa is an important part of the work of many semen analysis laboratories, particularly those associated with infertility clinics. Liquid nitrogen was used and semen cryopreservation developed rapidly in many countries with the establishment of commercial sperm banks or coordinated national services (Perloff et al., 1964; David et al., 1980; Clarke et al., 1997; Leibo et al., 2002). A variety of cryopreservation protocols are now used with different cryoprotectants and freezing procedures. Cell survival after freezing and thawing depends largely on minimization of intracellular ice crystal formation. This is done by using appropriate cryoprotectants and applying rates of cooling and warming that minimize the amount of intracellular water subject to ice formation (Sherman, 1990; Keel &

Webster, 1993; Watson, 1995). If the spermatozoa spend significant periods of time above –130 °C (the glassy transition temperature), particularly during the thawing process, re crystallization can occur, with growth of potentially damaging intracellular ice crystals.

Beside this thing I also added a topic to my dissertation work. I added pineapple juice with semen sample then check its motility after 6 hours which is kept in 37℃. Pineapple juice give energy and antioxidant activity to the sperm and for this reason sperm motility can increase for some hours even 37℃. This topic is also discussion in my result and discussion portion.

Review And literature

2. Literature and Review

Semen extenders are used to create multiple insemination doses from a single ejaculate and contain buffers and nutrients suitable for storage of spermatozoa. The purpose of this study was to evaluate the survivability of sperm stored under room temperature in different extenders containing some natural products (Alafuro Akand et al). The modern day farm animal reproduction is hinged on better reproductive techniques, one of which is artificial insemination (AI). The development of the AI technique in pig production has been mostly prompted by the dissemination of genes from improved boar and the fact that results are equivalent or even better than those related to natural service (Gadea review). Semen collected from boars is ultimately diluted in any one of a variety of commercially available boar semen extenders. Among the commercially available boar semen extenders are Beltsville TS, Androhep+, Mulberry III, Acromax, X-CELL, Seminark, Vitasem LD, etc, (Pursel VG, et al) and most of them are either short- or long - term preservation media. These extenders are used to create multiple insemination doses from a single ejaculate and contain buffers and nutrients, which provide spermatozoa a congenial environment that maintains their preservation capabilities and viability for 3 or more days post collection (Kuster CE et al). Some of the protective compounds added to extenders include antibiotics, albumin, bovine serum, (Johnson LA et al) and recently, whey protein (van den Berg BM et al). The addition of antibiotics to semen extenders is a common feature in commercial extenders, usually aimed at avoiding bacteria growth in extended semen (Vyt P, Maes D et al). The addition of antibiotics as component of semen extenders may be justified because bacteria in semen are harmful to the quality of semen. Endotoxins produced by these bacteria interfere with spermatozoa survival time in semen and cause sperm agglutination and reduced motility (Althouse GC et al).

The West African Dwarf (WAD) goats (Capra hircus) possess certain valuable traits that confer adaptation to endemic trypanosomiasis challenge and hot humid tropics (Daramola and Adeloye, 2009). Some breeds that do

not have adaptive traits, however, are gradually replacing them. There is need to preserve semen from the breed such that it could be used for subsequent artificial insemination over an extended period of time. Artificial insemination with preserved semen is a viable option for genetic upgrading of this breed. The survival of sperm after collection in seminal plasma for longer periods during preservation at low temperatures requires dilution with appropriate extender in order to maintain viability of spermatozoa. Regardless of the extender constituents, however, viability of spermatozoa deteriorates at low temperatures during storage. Fruits are good sources of natural antioxidants, containing many different antioxidant components (Cao et al., 1996 ;Wang et al., 1996; Velioglu et al., 1998). These antioxidants include carotenoids, vitamins, phenolic compounds and flavonoids and have proved to function as singlet and triplet oxygen quenchers, free radical scavengers and peroxide decomposers (Larson, 1988). In addition, natural foods and food-derived antioxidants such as vitamin C, E and phenolic phytochemicals have been reported to act as chemo-preventive agents against oxidative damage (Kiwon et al., 2003; Ondei et al., 2009). Daramola and Adekunle (2015) recently observed improved progressive motility, acrosome and membrane integrities, reduced abnormalities and MDA following supplementation of extenders with pineapple and cucumber juices during refrigeration of semen obtained from WAD goat bucks.

Aims & Objectives

3. Aims And Objectives:

- At first collected total 30 human semen sample and analysis is done of the sample (Macroscopic analysis and microscopic analysis).
- Effects of different concentration pineapple juices on human sperm motility.

Materials & Methods

4. Materials And Methods:

The study was conducted at Santaan Fertility Center, Origio Academy in Bhubaneswar, Odisha. Semen Sample collected from clinic from different age group (mainly 21 - 43 years). Study evaluated by 30 different sample collecting from different age group people. Every Samples were collected in same environment.

4.1 Semen collection:

Semen were collected from different age group person (21 - 43 years). Semen were mainly collected in clinic or home if sample were collected in home then sample was must come between 30 minuets after the collection. After the collection it is kept in incubator or hot plate where temperature is already maintain 37℃.

4.2 Juice preparation:

The fruit-juice was prepared according to the procedure by Adeyemo et al. (2007) with some modifications as follows: pineapple was washed thoroughly using distilled water. The Pineapples were first peeled and thereafter cut into pieces and and then the fruit were mashed by the mortar pestle, placed in a sieve and squeeze the juice out from the mashed fruits by filter paper. The juices collected from pineapples were put in plastic test tubes and centrifuged at 3000 xg for 20 min. The supernatant fluid obtained was decanted into a clean beaker and used immediately for the experiment.

4.3 Semen Macroscopic Analysis:

Liquefaction :

- After the ejaculation, the seminal secretions from a coagulum, which gradually liquefies.
- In most cases, the semen sample should become fully liquefied in 15 - 30 minutes
- Semen sample should be mixed well in the original container once liquefaction is complete.

- If liquefaction does not occur then this abnormality be noted. Some time pipetting or syringing or media will be added for liquefaction.

Appearance :

- A normal liquefied semen sample has a homogeneous, grey-opalescent appearance.
- If creamy white - high sperm concentration.
- Clear - low sperm concentration.
- Brown / smoky red - presence of erythrocytes.
- Pale yellow - presence of leukocytes.

Volume :

- The ejaculated volume were accurately measured using a graduate plastic pasteur pipette or plastic syringes.

Viscosity :

- A normal sample leaves the pipette in small discrete drops.
- If viscosity is abnormal the drop will from a thread more than 2 cm long.

pH :

- A drop of semen evenly onto pH paper and comparing the colour change with calibration range provided.
- The pH of the semen is measured using the pH dipsticks range 6.0 - 10.0.

4.4 Semen Microscopic Analysis:

Concentration and motility Assessment:

- The thickness of Makler Chamber is 10µm and the grid on the lid has 10×10 Squares.
- A drop of about 10µl of sample is put on the pre - warm Makler chamber and covered with the lid which creates a chamber with sperm in only one layer.

- If sperm sample is too dense to analyse then it can be diluted with pre warmed media and the results should be noted as per the dilution.
- For concentration and motility, count the sperm in 10 squares which represents the sperm concentration of sperms in millions/ml and classify motility as per WHO 2010.

4.5 Experimental layout :

Semen samples were diluted with the freshly made pineapple juices. 4 different types concentration taking of pineapple juice. 5%, 10%, 15%, 20% pineapple juice taking and mix with semen sample and semen washing media. Discuss as a table 1.

Juice added semen sample then observed under microscope to check the motility of the sperm. The sperm motility check in makler chamber. The process were follow which is discuss in *4.4 Semen macroscopic analysis portion.* Same process follow to count the motile sperm in juice containing solution.

Pineapple juice Concentration	**0% / Control**	**5%**	**10%**	**15%**	**20 %**
Semen Sample (µl)	50	50	50	50	50
Pineapple juice (µl)	0	5	10	15	20
Washing Media ((µl)	50	45	40	35	30

Table-1: composition of different concentration pineapple juice containing semen sample per 100 µl

Result

5. Results:

Analysis done total 30 semen clinic collected samples. In table: 1 first 16 samples data ere evaluated and table: 2 other 14 samples were evaluated. In this section semen macroscopic analysis and microscopic analysis were done. In macroscopic analysis observed the apperence of the sample, then check the total volume of the sample, check the pH, check the viscosity whether it normal or highly viscous / low viscosity and observed also whether the sample is liquefied or not. These all data collected and also mention in the table: 1 and table: 2 Macroscopic analysis portion.

After doing the Macroscopic analysis then shift to the Microscopic analysis. Mainly in this project only discussion about the motility, immotility. These macroscopic analysis portion were done by two one in makler chamber and also slide base method. Observed progressive motile sperm, non progressive motile sperm and immotile sperm. All data mention in the table: 1 and table: 2 portion.

Id No.	Macroscopic Analysis					Microscopic Analysis			
	Appe-rence	*Vol-ume*	*Viscosi-ty*	*Liquefa-ction*	*pH*	*PM*	*NPM*	*TM*	*IM*
2	Grey Opalescent	2.5ml	Normal	Liquefied	8.5	90	60	150	78
3	Grey Opalescent	2.5ml	Normal	Liquefied	7.5-8	96	57	153	77
4	Grey Opalescent	1.5ml	High	Not Liquefied	8.8	29	12	41	23
5	Grey Opalescent	3ml	Normal	Liquefied	7-7.5	63	51	114	71
6	Grey Opalescent	2.4ml	Normal	Liquefied	7.3	133	45	178	42
7	Grey Opalescent	1ml	Normal	Liquefied	7.5	41	31	72	44
8	Whitish yellow	0.5ml	Normal	Liquefied	7.5	10	6	16	15
9	Grey Opalescent	3ml	High	Not Liquefied	8.5	53	19	72	28
10	Grey Opalescent	2ml	Normal	Liquefied	7.5	42	30	72	28
11	Grey Opalescent	2ml	Normal	Liquefied	8.5	40	37	77	31
12	Creamy white	1.8ml	High	Not Liquefied	8	48	27	75	28
13	Grey Opalescent	1ml	Normal	Liquefied	8	46	29	75	38
14	Grey Opalescent	3.2ml	High	Not Liquefied	8.5	50	26	76	42
15	Grey Opalescent	2.5ml	Normal	Liquefied	8	34	21	55	24
16	Grey Opalescent	2.5ml	Normal	Liquefied	7.5	6	4	10	19

Table-2: Sample Analysis (Macroscopic & Microscopic) of 16 Samples
[PM - progressive motility, NPM - non progressive motility, TM - progressive motility+non progressive motility, IM - Immotile}

Id No.	Macroscopic Analysis					Microscopic Analysis			
	Appe-rence	*Vol-ume*	*Viscosi-ty*	*Liquefa-ction*	*pH*	*PM*	*NPM*	*TM*	*IM*
17	Grey Opalescent	3.1ml	High	Not Liquefied	8.8	53	33	86	27
18	Grey Opalescent	2.3ml	Normal	Liquefied	7.5	58	37	95	56
19	Grey Opalescent	2.8ml	Normal	Liquefied	8	67	42	109	35
20	Grey Opalescent	3.2ml	High	Not Liquefied	9	47	25	72	27
21	Grey Opalescent	1.5ml	Normal	Liquefied	8.5	26	12	38	18
22	Grey Opalescent	2.5ml	Normal	Liquefied	8	3	-	3	-
23	Grey Opalescent	0.5ml	High	Not Liquefied	9.5	12	15	27	50
24	Grey Opalescent	1.5ml	Normal	Liquefied	8.5	43	42	85	24
25	Grey Opalescent	3.3ml	Normal	Liquefied	8.5	73	16	89	15
26	Grey Opalescent	3ml	High	Not Liquefied	8.5	51	21	72	18
27	Grey Opalescent	2ml	Normal	Liquefied	6.5	3	2	5	12
28	Grey Opalescent	2.2ml	Normal	Liquefied	8	27	8	35	8
29	Grey Opalescent	0.5ml	High	Not Liquefied	9.5	45	28	73	18
30	Grey Opalescent	3 ml	Normal	Liquefied	8	47	25	72	32

Table-3: Sample Analysis (Macroscopic & Microscopic) of other 14 Samples
[PM - progressive motility, NPM - non progressive motility, TM - progressive motility+non progressive motility, IM - Immotile}

In another side semen sample was diluted with different pineapple juice concentration, 5%, 10%, 15%, 20%. After adding the different pineapple juice concentration check the total motility (progressive motility + non progressive motility). This data denoted as “0 hour”. Progressive motility and Non progressive motility and total motility were observed
of 30 semen samples with there different juice concentration (5%, 10%, 15%, 20%) along with control one means 0% juice containing solution. Because these data were collected as soon as possible after adding the different concentration pineapple juices. In table 4 mention all data. There were evaluated all 30 semen samples with their different pineapple juice concentration 5%, 10%, 15%, 20%. 0% or control means there was no pineapple juice added in this solution. In figure 3 showing the graph about total motility of 30 semen samples Vs pineapple juice concentration at “0 hour” The graph was almost stable and in 15% and 20% slightly decrease.

After taking “0 hour” data 30 semen samples which is mixed with different concentration juice and also control or 0% pineapple juice were kept at 37℃ for 6 hours. After 6 hours again data were collected. data denoted as “6 hour”. Progressive motility and Non progressive motility and total motility were observed of 30 semen samples with there different juice concentration (5%, 10%, 15%, 20%) along with control one means 0% juice containing solution. In table 5 mention all data. There were evaluated all 30 semen samples with their different pineapple juice concentration 5%, 10%, 15%, 20%. 0% or control means there was no pineapple juice added in this solution. In figure 4 showing the graph about total motility of 30 semen samples Vs pineapple juice concentration at “0 hour” The graph was shown that in 0% pineapple juice solution, sperm have less total motility than “0 hour”. Because as the time pass on sperm have not that much motile than the time of first time analysis means “0 hour”. In 5 % juice concentration little increase than the 0%. In 10% juice containing solution, it was the optimized motile sperm get in the “6 hour” analysis. Because 15% & 20% juice concentration motilty of sperm further decease shown as a figure 4.

In figure 5 shown the combine of “0 hour” and “6 hour” and the graph was shown that total motility Vs 0%, 5%, 10%, 15%, 20% different pineapple juice concentration. In figure 5 “0 hour” motility is shown in green colour where graph was throughout stable bt in 15% & 20% slightly decrease. In figure 5 “6 hour” motility is shown in red colour where in 0 % was decrease with the compare of “0 hour”, 0% concentration. In 5 % juice concentration little increase than the 0%. In 10% juice containing solution, it was the optimized motile sperm get in the “6 hour” analysis. Because 15% & 20% juice concentration motility of sperm further decease shown as a figure 5.

Sample No.	Total motility in Different Pineapple Juice Concentration				
	0%	5%	10%	15%	20%
1	94	93	96	80	75
2	150	144	140	134	130
3	153	152	151	149	144
4	41	45	44	44	41
5	114	115	117	110	108
6	178	175	177	170	163
7	72	70	73	68	63
8	16	18	20	15	12
9	72	71	72	70	64
10	72	72	70	68	61
11	77	75	78	71	67
12	75	76	77	70	67
13	75	72	69	67	61
14	76	78	71	65	59
15	55	50	52	45	39
16	10	6	8	4	2
17	86	88	80	83	78
18	95	94	91	85	80
19	109	111	108	95	90
20	72	70	72	68	61
21	38	35	37	32	28
22	3	5	7	3	2
23	27	25	21	18	13
24	85	83	84	80	72
25	89	88	87	81	78
26	72	75	74	68	65
27	5	8	9	6	2
28	35	31	29	21	17
29	73	71	72	65	60
30	72	69	70	67	56

Table-4 : Total motility of different pineapple juice concentration in 0 hour

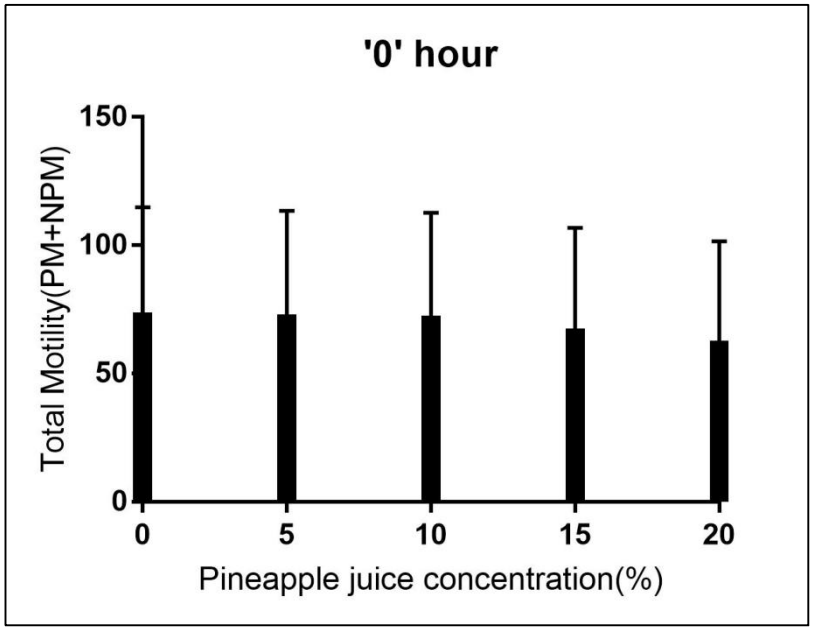

Fig 3- Graph showing pineapple juice concentration Vs total motility of 30 semen samples in 0 hour

Sample No.	Total motility in Diff Pineapple Juice Concentration				
	0%	5%	10%	15%	20%
1	51	55	66	59	51
2	121	133	143	134	121
3	125	120	131	112	128
4	23	29	41	27	23
5	88	93	110	98	92
6	109	117	134	121	128
7	54	74	74	68	70
8	6	8	17	12	3
9	48	54	60	51	33
10	51	55	68	57	43
11	61	67	78	64	55
12	54	59	77	57	45
13	48	52	71	62	56
14	61	65	75	61	44
15	32	39	52	39	37
16	5	6	8	2	0
17	75	77	87	77	61
18	78	80	95	71	56
19	89	90	110	88	72
20	54	60	78	52	38
21	23	27	39	28	16
22	0	0	3	1	0
23	12	17	25	16	8
24	52	57	72	65	43
25	39	43	77	72	61
26	55	60	72	62	52
27	0	2	7	2	0
28	21	29	34	18	6
29	67	70	78	64	51
30	44	51	67	54	42

Table-5: Total motility of different pineapple juice concentration in 6 hour

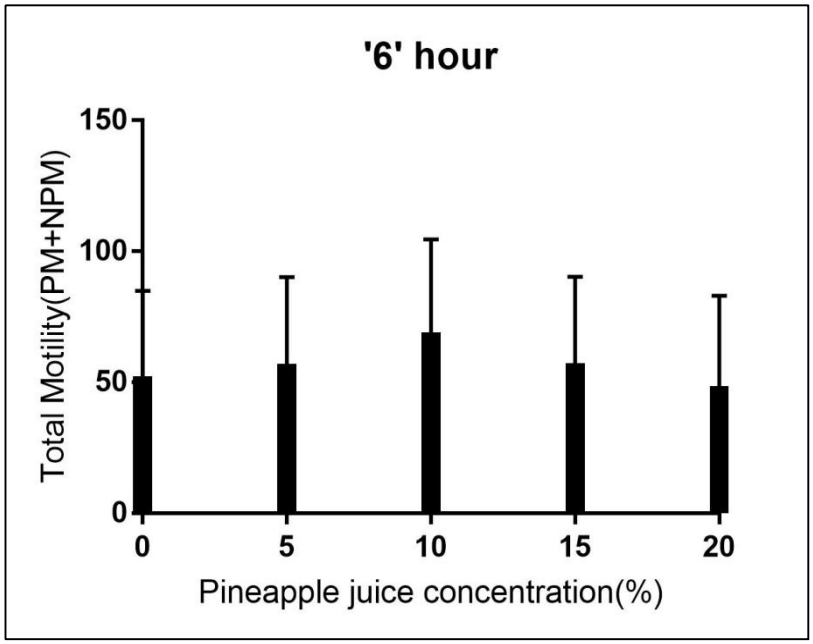

Fig 4 - Graph showing pineapple juice concentration VS total motility of 30 semen samples in 6 hour

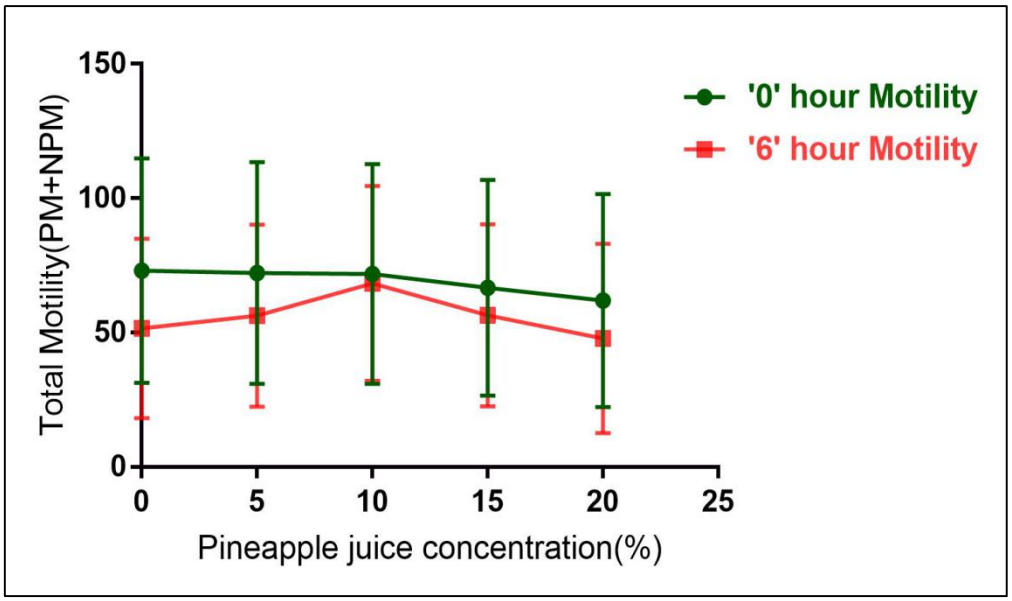

Fig 5 - Graph showing Comparing between o hour and 6 hour (Total motility Vs Pineapple juice Concentration)

Discussion

6. Discussion:

The present study clearly demonstrates that spermatozoa can be stored in pineapple juice up to 6 hours. But the survivability of sperm or increase the motility of the spermatozoa mainly observed in the 5% and 10% pineapple juice concentration solution. After increasing the concentration of juice like 15% and 20%, motility of the sperm was decrease.

Generally, sperm motility decreased with longer duration of storage in agreement with Lustyková et al. And also know that energy is very crucial for the maintenance of sperm motility and viability. The improvement in this parameter could be attributed to the presence of substances in pineapple juice such as vitamins and phenolic compounds known to function as antioxidants (Gebhardt and Thomas, 2002; Kiwon et al., 2003; Cutler et al., 2008). Gardner et al. (2000) had earlier reported that concentrations of vitamin and total phenolic contents in fruit-juices have a strong relationship with antioxidant capacity. Furthermore, fruits are rich in sugars (Hulme, 1970), and sperm readily utilizes sugars for respiration. Sugars also provide osmotic balance (Aboagla and Terada, 2004). The results further agreed with Fukuhara and Nishikawa (1973), who supported the role of sugar in semen diluents to provide energy and protection to sperm cells.

But in 15% and 20% juice concentration, motility of the sperm was decrease. It may be occurred for pH change. Previously known the maintenance of sperm at low pH prevents the onset of motility of sperm (Bencic et al., 2000a,b,2001). Pineapple juice pH is mainly ranges between 3.5- 5.2. That is the main reason of decrease the motility of the sperm.

Conclusion

7. Conclusion:

After doing all the experiment can concluded that spermatozoa can be stored in pineapple juice up to 6 hours at 37℃. But the optimized juice concentration is 10% pineapple juice containing solution. Because after that if increase the juice concentration motility will be gradually decrease. So that conclusion is human sperm motility will be increase at 37℃ after a longer time (6 hours) with the supplement of 10% pineapple juice.

And future study will be cryopreservation of the human semen sample supplement with these concentration pineapple juices along with the proper extender. After the cryopreservation of the human semen samples, motility of the sperm normally decrease comparing with before one. After adding the juice with the proper extender and after that cryopreserved the samples and then observed, it may be increase the motility of the sperm before that.

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