

Studies on human sperm motility and viability when treatment with rock salt (Saindhav)

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Abstract - Each sperm structure is composed of a head which contains the genetic material of father in its nucleus part, a tail which lashes back and forth to propel sperm along, and a midpiece which has mitochondria (power house of sperm) which provide energy for sperm motion. The presences of sugar fructose produced by seminal vesicles provide energy for sperm motility. An alkaline pH of sperm is an important characteristic. Camphor, from the above study is regarded as poisonous if ingested in large doses, it acts as a contraceptive agent, an abortifacient, as suppressor of sperm motility reducing its capacity to fertilize in rats. On other hand, it has several health benefits. In this study of effect of camphor on human sperm motility and its viability, a decrease in sperm motility and sperm viability investigated with increased concentration of camphor solution. It may be due decrease in fructose levels, or denaturation of proteins, cholesterol which are indirectly connected with energy source for sperm motility. Sperm motility and sperm viability decreased because increased concentration of Rock salt solution. Hence, it can be said that there is a positive relation between Rock salt and sperm parameters. And hence it can be concluded that Rock salt can be used as contraceptive.

Key words- Sperm motility, sperm viability and rock Salt

Introduction

Human fertilization is the union of a human egg and sperm, usually occurring in the ampulla (the second portion) of the uterine tube. Spermatozoa, after passage through the epididymis (coiled segment of the spermatid ducts that serves to store, mature and transport spermatozoa between the testis and the vas deferens), are motile cells. Sperm motility becomes critical at the time of fertilization because it allows or at least facilitates passage of the sperm through the zona pellucid (1). Without technologic application, a non-motile or abnormally-motile sperm is not going to fertilize [1].

Sperm motility is one of the biological characteristics of the spermatozoa. The quality of the sperm is more significant than the count. Motility is graded by the criteria according to the World Health Organization (WHO) Manual as “a” to “d”. This is detailed as below: Grade a (fast progressive) sperms are those which swim forward fast in a straight line - like guided missiles;

1. Grade b (slow progressive) sperms swim forward, but either in a curved or crooked line, or slowly (slow linear or non linear motility);
2. Grade c (nonprogressive) sperms move their tails, but do not move forward (local motility only);
3. Grade d (immotile) sperms do not move at all.

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Sperms of grade c and d are considered poor. Poor motility is called asthenozoospermia which is associated with reduced viability of spermatozoa. It is seen that high percentage of spermatozoa in the sample have less than normal motility. Frequent causes of asthenozoospermia are abnormal spermatogenesis (sperm manufacture), epididymal sperm maturation problems, transport abnormalities, varicocele. Non-motile spermatozoa are called necrozoospermia. It is characterized by total absence of moving sperm. The factors causing necrozoospermia are varied from diet, disease, injury, medications, alcohol or illicit drug use etc. It is has been reported that sperm with normal reproductive genetics are deficient in one or several of the factors necessary to produce motility [2, 3, 5].

There are number of studies has been carried out for estimation of the viability of the sperms by staining with Eosin stain and percent viability is calculated by using standard formula. Non motile sperms are tested for its viability by the mixing fresh semen with a supra-vital dye such as eosin or trypan blue, or by the use of the hypo osmotic swelling test (HOS), based on the semi-permeability of the intact cell membrane, which causes spermatozoa to "swell" under hypo-osmotic conditions, when an influx of water results in an expansion of cell volume (Drevius & Eriksson, 1966). Jeyendran et al. (1984) introduced above mentioned test for Non-motile viable sperm and suggested that Non-motile viable sperm for Intra cytoplasmic sperm injection (ICSI) may be used for *in vitro* fertilization procedure in which a single sperm is injected directly into an egg [7].

Rock salt is the common name for the mineral "halite". Its chemical formula is NaCl. It is typically formed by the evaporation of salty water (such as sea water) which contains dissolved Na^+ and Cl^- ions. Rock salt deposits are found in dry lake beds, inland marginal seas, and enclosed bays and estuaries in arid regions of the world. A large amount of the commercially mined rock salt is prepared for human consumption [10]. Rock salt has been used in numerous ways in health and diseases. Some of the common usages of rock salts are mentioned herewith. Natrum muriaticum commonly known as table salt or sodium chloride is used for colds, sneezing, eczema, thrush, urticaria (hives), menstrual and premenstrual problems, and migraine. In Ayurvedic medicine, it is given to promote digestion, improve appetite, and relieve constipation. In orthodox medicine, sodium chloride solution is used for dehydration, to irrigate the eye, to relieve nasal congestion, and as a mouthwash. Rock salt helps in exfoliating dead skin cells, cleansing skin pores and protecting the natural skin layer to produce healthy and energized skin type. It also helps in strengthening skin tissue to rejuvenate the skin to make it look younger and firm. Rock salt helps in getting rid of toxic minerals and refined salt deposits by stimulating blood circulation and mineral balance. The use of rock salt helps in stabilizing blood pressure by maintaining a balance of high and low blood pressures. It also helps in controlling weight by equalizing minerals which inhibit cravings and eliminate fat dead cells. Rock salt is used to treat many kinds of skin disorders and other ailments such as rheumatic pains and herpes. It can also be consumed along with lemon juice to eliminate stomach worms and control vomiting. The common cold and cough and other nasal and chest ailments can be treated with recipes that involve rock salt as the main ingredient. It is even used to reduce pain, inflammation and irritation from insect bites. In spite of wide application of rock salts in health and diseases, no experimental studies elaborating its physico-kinetic pathways are reported [4, 7].

Though the rock salts is essential for nourishment of minerals, water absorption, enhancing communication between cells and overall functioning of the body, the tome Haremekhala mentions one of the ancient Indian classics local contraceptive method, is used frequently in the some of the healthy couple, in which sesame oil is smeared on a small piece of rock salt and

place it at the cervix (mouth of the uterus; garbhashaya vadana). This procedure is still used in many parts of the world.

It has been reported that this process does not have any adverse effects on the woman's ovulation process. Nor does it affect the menstrual cycle but prevent conception. However there are no underlying mechanisms have been reported [8].

The present study is aimed to study the effect of rock salt and common salts on sperm motility and its viability *In vitro*. Attempt will also be made to analyze the effects of rock salt vis-à-vis use as one of the local contraceptive method for prevention of concept. Francis y. Kim et al have evaluated a total of 14 paired semen for infertility and reported significant reduction in enteric contaminants, which would otherwise be erroneously treated, can be achieved with an effective antibacterial skin preparation [13]. Their experimental study Assessment of VEGF action on sperm motility concluded that VEGF had a positive effect on some parameters of sperm motility in a concentration-dependent manner. Maximal effect was observed at a concentration of 15 ng/ml; motility, progression, straight-line velocity and curvilinear velocity of VEGF-exposed spermatozoa were significantly increased ($P < 0.05$) at this concentration [14]. The effects of alprostadil and prazosin on motility, viability and membrane integrity of human sperm and suggested that Alprostadil and prazosin hydrochloride at doses used in transurethral therapy for erectile dysfunction have no effect on the motility, viability and membrane integrity of human sperm. A study on Bacterial effect on sperm motility by George B. et al reported significant decrease in motility was observed at 106 colonies per cubic centimeter. It is suggested that immediate immobilization of sperm is not a major mechanism of action of the copper IUD in the human. The effect of metallic copper and copper salts on the motility of human spermatozoa was studied as part of an investigation of the mechanism of action of the copper-containing intrauterine device (IUD). Copper released from IUD inhibited sperm motility *in vitro* and *in vivo* within three to four hours. Comparable concentrations of copper sulfate were less effective [15]. *In vitro* inhibition of sperm motility by some local mineral water drinks. They suggested that local mineral water drinks have inhibitory effect on sperm motility. They carried out the *In vitro* study to analyze the effect of eight common local mineral water drinks on sperm motility and found no significant difference in the pH values of all the drinks but after one minute, Pepsi-Cola had the least inhibitory effect on sperm motility, Bitter-lemon had the strongest effect, while the other brands had varying effects between the two extremes. The percentage motility inhibition by the mineral water drinks is also a function of the initial total sperm count in the semen [16]. It is observed a severe, immediate reduction in sperm motility after exposure to undiluted standard solutions of methylene blue and Renografin. Yamaguchi K et al studied the motility of ejaculated human sperm which was washed thoroughly by Ficoll gradient centrifugation to remove seminal plasma was investigated in the presence of various concentrations of sugars It is suggested that sperm motility was higher than that with an individual sugar and the effect of glucose on human sperm motility is due to a different mechanism from that of fructose. It is further reported that Ciprofloxacin affected hyperactivation by altering membrane properties, whereas doxycycline inhibited the capacitation process. Cessation of motility in cefuroxime axetil was linked to disrupted sperm head membranes. Sperm motility and fertilizing capacity were decreased in nitrofurantoin because of decreased metabolism. The positive effect of ioxacin on fertilizing capacity did not involve changes in acrosome [17]. It is observed a significant decrease in sperm motility after exposure to concentrations of *Escherichia coli* varying from 500 to 108 colonies per cubic centimeter. A significant decrease in motility was observed at 106 colonies per cubic centimeter [18]. It is studied the influence of copper suggests that immediate immobilization of sperm is not a major mechanism of action of the copper IUD in the human. They carried out investigation to study the mechanism of action of the copper-containing

intrauterine device (IUD) on the motility of human spermatozoa. Copper released from an IUD inhibited sperm motility in vitro and in vitro within three to four hours. Comparable concentrations of copper sulfate were less effective. Preliminary experiments in which sperm were incubated up to four hours within a Millipore chamber in the uterine cavity of a patient using a copper device indicate that copper has little effect on sperm motility in utero. The three to four hours-long latent period required for the inhibition of sperm motility by copper suggests that immediate immobilization of sperm is not a major mechanism of action of the copper IUD in the human [19]. It is seen in the review of literature that the studies on sperm motility & viability have been carried out but the effect of rock salt per se has not been done. Hence the present study protocol is designed to study the effects of various concentrations on motility and viability of mature sperms invitro to evaluate its potential as local contraceptive.

MATERIALS AND METHOD

Preparation of salt solution: - Take 29.22 gm of rock salt curst it in fine powder add to sterile conical flask and add 89.2 ml of sterile distilled water to each conical flask. Then mix well with the help of stirrer.

Procedure for Sperm motility: - Fresh semen samples were collected from a healthy single donor to avoid inter-donor variability. Seminal fluid was collected in a clean container and kept warm (body temperature) until immediately prior to examination. The semen was tested for liquid consistency before proceeding with the test. The sample that not liquefied was checked at 10 minute intervals until it liquefied. The efficacy of 10 µl of sperm suspension and 10 µl of salt solution (1:1) was tested at different concentrations (1, 2.5, 5 and 10%) and placed on a glass slide and was mixed uniformly. As a control, 10 µl of Ham's-F10 solution was used. Immediately the specimen was examined under the high power objective (40x). The motility of sperm was observed at various time intervals starting from 0 sec and once in every 15 seconds up to 150 seconds. Sperm motility was determined by counting all motile and immotile spermatozoa in several randomly chosen fields using a 40x objective. Only free spermatozoa were assessed.

Samples with more than 25% of sperm clumps were not assessed. Spermatozoa with abnormalities such as pin-heads and "free tails" are not counted. Approximately 200 sperms were observed and classified according to their motility (defined as a percentage). The percentage motility per sample was calculated based on the following formula:

$$\text{Motility} = \frac{\text{Motile sperms}}{\text{Motile + non motile sperms}} \times 100$$

Procedure for Sperm Viability:- One drop of Eosin y stain was added in vial one drop of sperm sample with different salt concentration was added to it at room temperature and was kept undisturbed for one to two minutes and smeared the mixture on a microscopic slide. 100 spermatozoa were classified as either colored, if the stain has passed through the membrane and therefore the cell was considered dead, or non-stained, the cell than being considered alive.

The staining technique helps to differentiate spermatozoa that are immotile but alive from those that are dead. The percentage viability per sample was calculated based on the following formula:

$$\text{Viability} = \frac{\text{Viable sperms} \times 100}{\text{Viable} + \text{non viable sperms}}$$

RESULT AND DISCUSSION

The effects of Rock salt on the motility of sperm are presented in Table 1 and Figure 1. The result is the proportion of motile spermatozoa, expressed as an integer percentage. It was observed that an increase in concentration of the extracts decreased the motility of sperms. The effects of Rock salt on the viability of sperm are presented in Table 2 and Figure 2. The result is the proportion of viable spermatozoa, expressed as an integer percentage. It was observed that an increase in concentration of the extracts decreased the viability of sperms. The results of this study demonstrated that Rock salt *have* decreased the motility and viability of human sperms with increase in the salt concentration. The use of different types of contraceptives has various disadvantages depending on the contraceptive. The different contraception methods have many side effects on its use on both male and female. The oral contraceptive has many disadvantages like fluid retention, irregular bleeding, amenorrhea, nausea and vomiting, headache, multiple drug interactions, several contraindications and many more; barrier contraceptive also has many disadvantages like allergic reactions, vaginal dryness or irritation; and other methods also has some disadvantages like irregular bleeding, pelvic pain, spontaneous expulsion. The easily available substances should be used rather than using hormones or complex chemicals which have very adverse effects on the user. Rather than use of naturally available substances should used as contraceptive and it should be promoted. The findings of the present study clearly indicate that Rock salt also have an effect on the motility and viability of the human sperms and points to the prospective of the use of Rock salt as local contraceptive, which deserves further investigation.

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Table 1- Effect of Rock salt on Sperm motility

Treatment	Percent motility of spermatozoa at different time intervals (sec)						
	0	15	30	60	90	120	150
Control	82 ± 1.5	81 ± 2.2	81 ± 1.5	80 ± 2.3	80 ± 1.5	79 ± 2.3	79 ± 2.2
1%	82 ± 1.5a	66 ± 1.2a	57 ± 1.0a	45 ± 1.8a	34 ± 2.0a	27 ± 1.6b	9 ± 1.2a
2.5%	82 ± 1.5a	63 ± 1.7a	55 ± 1.1b	41 ± 1.2a	28 ± 1.7a	20 ± 1.1 b	5 ± 1.8a
5%	82 ± 1.5 b	53 ± 2.0b	47 ± 2.0a	36 ± 1.5a	20 ± 1.1a	10 ± 1.0a	2 ± 1.7a
10%	82 ± 1.5 b	31 ± 1.2b	23 ± 1.8a	14 ± 1.7a	9 ± 18a.	2 ± 1.4a	NIL

Values are mean + SE

^ap < 0.05 by comparison with control.

^bp < 0.01 by comparison with control.

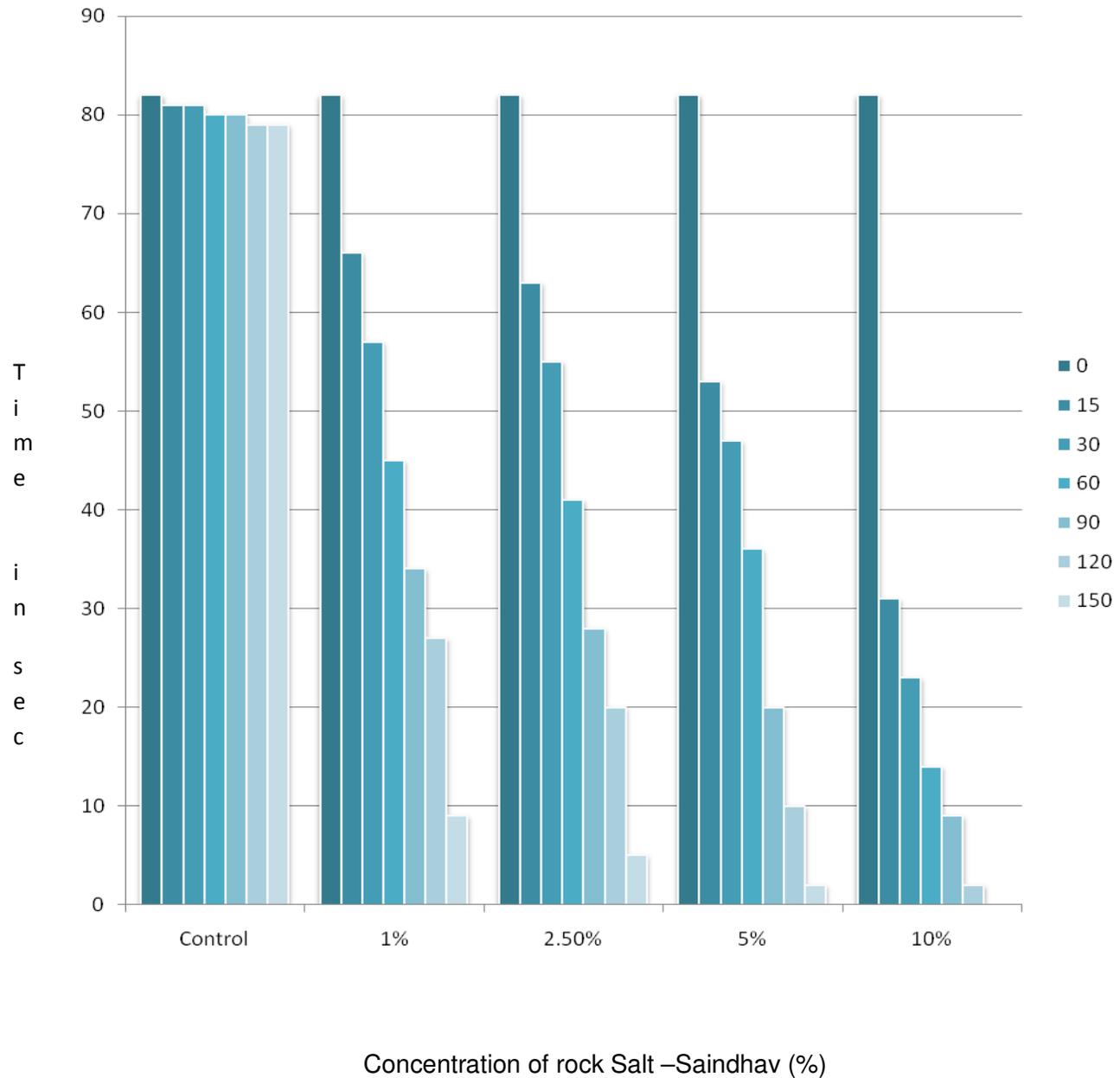


Fig. 1-Graphical representation of Effect of Rock salt on Sperm motility

Table 2- Effect of Rock salt on Sperm viability

Treatment	Percent motility of spermatozoa at different time intervals (sec)						
	0	15	30	60	90	120	150
Control	84 ± 1.7	83 ± 2.4	83 ± 1.7	82 ± 2.3	80 ± 1.7	81 ± 2.5	81 ± 2.4
1%	84 ± 1.7a	68 ± 1.4a	59 ± 1.2a	47 ± 1.9a	36 ± 2.2a	29 ± 1.9b	11 ± 1.2a
2.5%	84 ± 1.7a	65 ± 1.9a	57 ± 1.3 b	43 ± 1.4a	29 ± 1.8a	21 ± 1.3 b	6 ± 1.9a
5%	84 ± 1.7 b	55 ± 2.2b	49 ± 2.2a	38 ± 1.7a	21 ± 1.3a	11 ± 1.2a	3 ± 1.9a
10%	82 ± 1.7 b	33 ± 1.4 b	24 ± 1.9 a	16 ± 1.9a	9 ± 1.9a.	3 ± 1.6a	NIL

Values are mean + SE

^ap < 0.05 by comparison with control.

^b p < 0.01 by comparison with control.

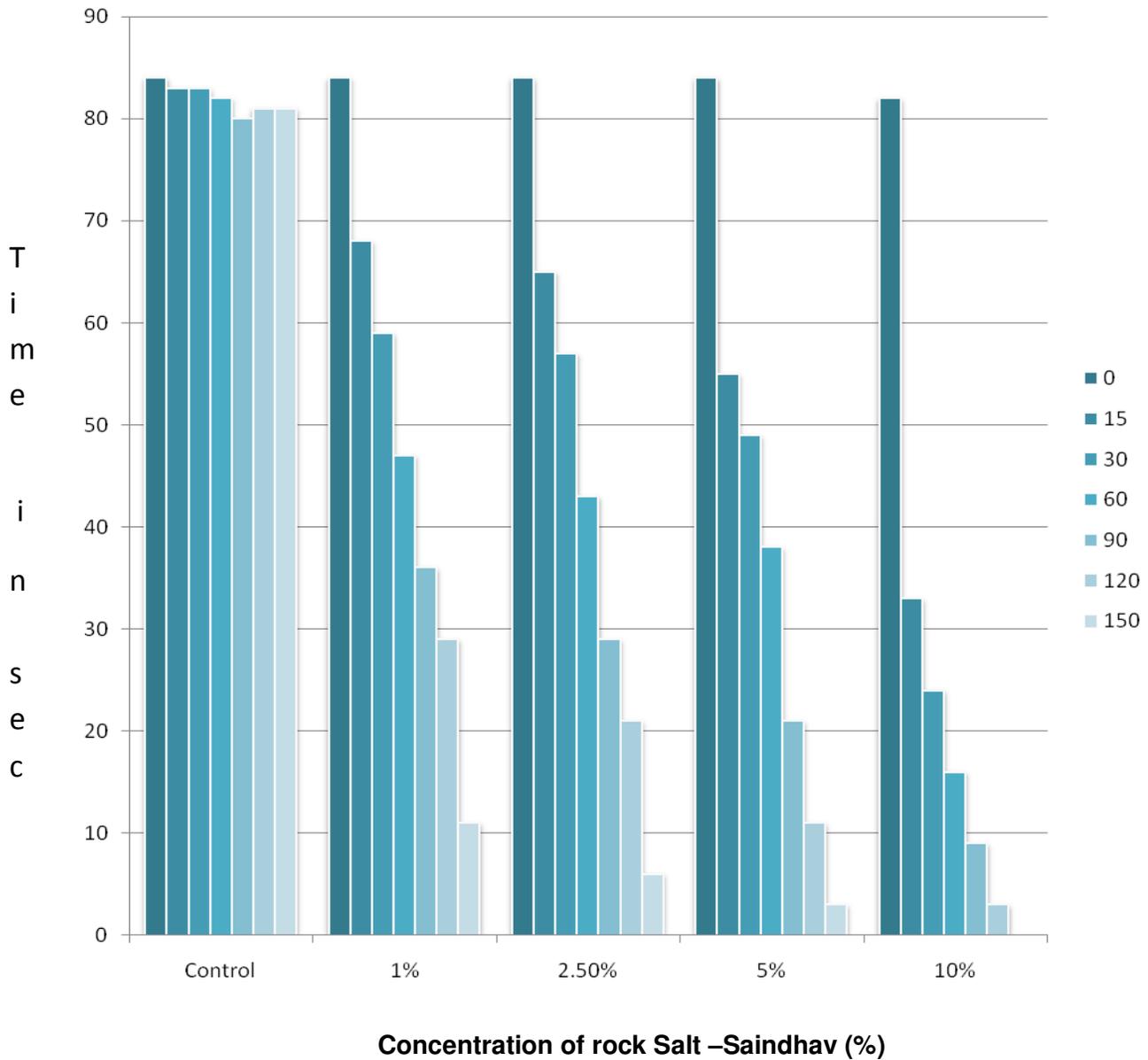


Fig. 2- Graphical representation of Effect of Rock salt on Sperm Viability