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# Effect of exogenous supplementation of coenzyme Q10 on sperm quality of bull processed by swim up technique

**M Elanchezhian, K Brindha, Sathaiah Gunaseelan and U Dhanalakshmi**

## Abstract
The quality of sperms in any semen sample plays an important role in ART irrespective of any procedure that is carried out. Various parameters are considered in evaluating a semen sample to assess its fertility potential before any ART procedure is performed. Basic criteria like the concentration of the sperms, motility, and morphology are usually assessed when semen is used in artificial insemination to increase the success percentage in animals. The same protocol is followed in humans to evaluate the male fertility. Despite this analysis, there are other semen factors involved in determining the male fertility potential. Factors such as viability, acrosomal integrity, DNA fragmentation in sperms, etc., also affect the fertility potential of sperms which in turn affects the embryo quality and ART outcomes. Hence it is mandatory to study those factors to get a clear picture on the male fertility. It has also become a necessity to treat those cases to improve the quality of the sperms to avoid various challenges such as poor embryonic development, implantation failures, miscarriages, etc., The aim of this study was to find out whether exogenous supplementation of the anti- oxidant Coenzyme Q10 to sperm wash medium which is used to process the raw semen has any positive effects on the quality of sperms post preparation. Bull semen samples were processed using sperm wash medium and sperm wash medium fortified with CoQ10. The semen parameters of the post wash samples were observed and studied. There were observable significant differences found between post wash samples processed using sperm wash medium and sperm wash medium fortifed with CoQ10. Exogenous supplementation of CoQ10 to sperm wash medium had positive effects on semen parameters.

**Keywords:** Bull fertility, semen analysis, viability, antioxidant, coenzyme Q10, DNA fragmentation

## Introduction
The prediction of bull fertility has a major economic importance in the dairy breeding industry. The fertilizing potential of sperm is determined by their ability of reaching the oocyte, fertilize it, and sustain embryogenesis, which is partly determined by the quality of sperm DNA. The sustainability and economics of bovine husbandry depends on obtaining high conception rates through artificial insemination. The success of artificial insemination and optimal use of genetically superior bulls is determined by the fertility of the bull, which in turn depends on sperm quality in frozen-semen doses. [1] A routine semen analysis is considered inconsistent predictor of reproductive efficiency because it helps only by eliminating samples with very poor quality [2] Hence, it has become a necessity to perform various other tests to check the quality of the sperms more accurately and also measures have to be taken to improve the quality in order to increase the fertility potential of the sperms. There are several reports available that review and emphasize on various semen parameters like sperm motility [3], velocity parameters [4], viability and membrane integrity [5], morphology [6], capacitation status [7], acrosome reaction status [8], chromatin integrity [4], and lipid peroxidation status [9] related to bull fertility.

It is evident that many studies have reported an adverse effect of sperm DNA damage on fertility. Sperm DNA fragmentation is associated with failure to conceive [10] longer times to pregnancy [11] poor outcome following stimulated intrauterine insemination [12, 13] impaired embryo development [14] higher miscarriage rates [15] and increased risk of pregnancy loss after both in -vitro fertilization [IVF] and intracytoplasmic sperm injection [16] Hence, one of the parameters to be investigated to get a clear picture on sperm quality or fertility potential is its DNA fragmentation. There are many methods employed in ART to process the semen samples to separate the motile sperms from them. Though these methods are helpful in separating motile sperms from the raw semen sample, the quality of the sperms remain undiscovered after

processing. It has been reported that the preparation of semen involving centrifugation affects the DNA of sperms causing DNA fragmentation in them [17]. On the other hand, oxidative stress [OS] has been recognized as a significant cause of suboptimal assisted reproductive outcome. Many of the sperm preparation and manipulation procedures that are necessary in the *in vitro* environment can result in excessive production of reactive oxygen species [ROS] thereby exposing the gametes and growing embryos to significant oxidative damage. Antioxidants have long been utilized in the management of male subfertility as they can counterbalance the elevated levels of ROS inducing a high state of OS [18]. In sperm cells, antioxidant coenzyme Q10, is concentrated in the mitochondria of the midpiece, so that the energy for movement and all other energy-dependent processes in the sperm cell also depend on the availability of CoQ10. The reduced form of CoQ10-ubiquinol also acts as an antioxidant, preventing lipid peroxidation in sperm membranes. It has been reported that the sperms treated with CoQ10 *in vitro* has showed some positive benefits such as increased fertilization rates in humans [19]. In this study few other parameters are been taken into consideration to study if there are any other positive benefis of Coenzyme Q10 on sperm factors.
Parameters such as viability, acrosomal integrity and DNA fragmentation index are taken into consideration in this study. Though there are many other parameters that together determine the male fertility potential, the above three factors are considered to be more important in ART procedures such as Conventional IVF, ICSI. Hence the effect of CoqQ10 on these factors is studied here in this experiment.

## 2. Materials and Methods
### 2.1 Materials
#### 2.1.1 Samples
Frozen bull semen straws available at Centralized Embryo Biotechnology Unit, Department of Animal Biotechnology were used for this study.

#### 2.1.2 Chemicals and Reagents
#### 2.1.2.1 Sperm wash medium
Commercially available sperm wash medium (SAGE, Cooper surgical, USA) was used.

#### 2.1.2.2 Diff-quick stain Kit
Diff-Quick Stain (Microptic Diagnostic Systems, Barcelona, Spain) was used to stain the spermatozoa to study their morphology.

#### 2.1.2.3 Eosin-Nigrosin Stain
Eosin-Nigrosin stain (Microptic Diagnostic Systems, Barcelona, Spain) was used to stain the spermatazoa to study their viability.

#### 2.1.2.4 Halo Sperm Kit
Halo sperm kit (Halotech DNA, Madrid, Spain) was used to study the DNA fragmentation Index of Spermatozoa.

#### 2.1.2.5 Coenzyme Q10
Sperm wash medium was fortified with Coenzyme Q10, at 50 µM concentration and used.
Both sperm wash medium and sperm wash medium fortified with Coenzyme Q10 were equilibrated at 37 $^{0}$C for 1 hr prior to usage.

#### 2.1.3 Disposables
All disposables were of 'embryo tested' quality (Nunc, Roskilde, Denmark).

### 2.2 Methods
#### 2.2.1 Thawing of frozen semen samples
Straws having frozen bull semen were thawed at 37 $^{0}$C in water bath for 15 seconds in a water bath and were collected in 15 ml centrifuge tubes. Ten different samples were collected in ten labelled centrifuge tubes and each tube had 0.25 ml of semen which was used as sample for the study. Semen analysis was carried out for each sample and the values were recorded.

#### 2.2.2 Preparation of sperms by swim up method
One ml of raw semen sample was underlayed in 15 ml centrifuge tube containing 1ml of equilibrated sperm wash medium and was incubated at 37 $^{o}$C for 45 minutes. The tube was incubated at 45$^{o}$ slanting position to increase the surface area for maximum sperm retrieval. After the incubation period, 0.7 ml of the supernatant was transferred to a new centrifuge tube and was centrifuged at 1200 rpm for 10 minutes. The supernatant was discarded post centrifugation and the pellet was re-suspended in 2 ml of sperm wash medium and this mixture was again centrifuged at 1200 rpm for 5 minutes. The supernatant was discarded and the pellet was resuspended in 0.5 ml of fresh sperm wash medium. This product was analysed and used for further studies such as viability, acrosomal integrity and DNA fragmentation.

#### 2.2.3 Preparation of sperms by swim up method using sperm wash medium fortified with Coenzyme Q10
Sperms were retrieved from frozen semen straws as described in 3.2.2 except that swim up was carried in CoQ10 fortified sperm wash medium. The sperm pellet was also resuspended in 0.5 ml of fresh sperm wash medium fortified with CoQ10. The treated sperms were used for further studies such as viability, acrosomal integrity and DNA fragmentation.

#### 2.2.4 Analysis of semen
The analysis of semen was carried out for the raw sample, post wash sample prepared by swim-up technique and post wash sample prepared by fortification with CoQ10. The semen analysis was performed according to the standard procedures using a compound microscope.

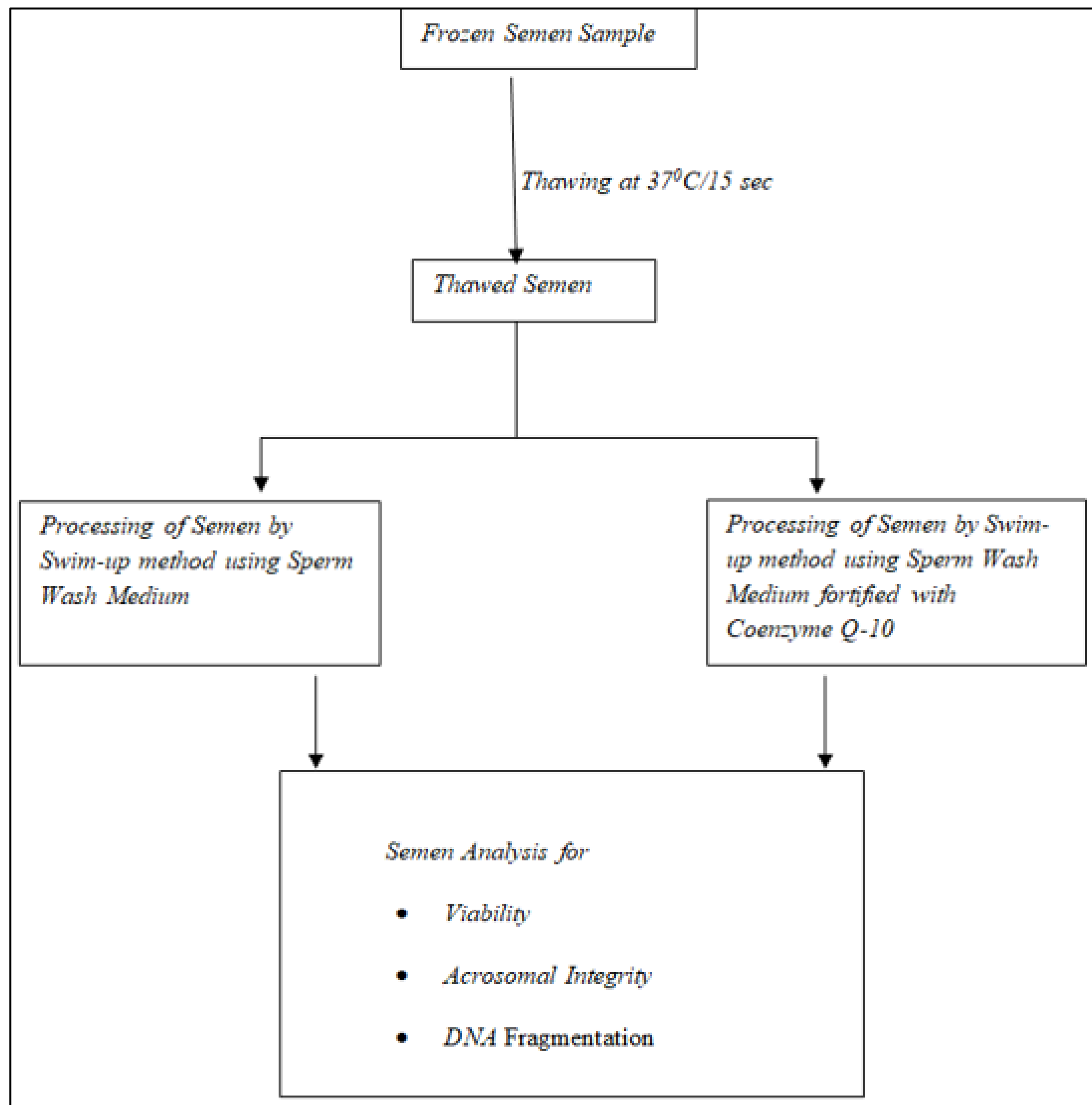



**Fig 1:** Experimental design

### 2.2.5 Evaluation of concentration of sperms
The sperm concentration was studied using a Makler chamber (Microptic Diagnostic Systems, Barcelona, Spain). The sample was mixed well and a drop of it was placed in the centre of the disc area of makler chamber. Cover glass was gently placed and the drop was allowed to spread on the entire area of the disc into a thickness of 10 µ. The sperm heads within the squares of the grid were counted. A strip of 10 squares were counted and was repeated in another strip or two to determine the average. This number was considered as the concentration in millions per milliliter.

### 2.2.6 Evaluation of motility of sperms
Motility of the sperms was assessed using differential count method. The sample was mixed well and a drop of it was placed on a glass slide. A cover slip was gently placed on the drop ensuring there is no air bubble formation and observed under phase contrast microscope using 20X objective lens. Two hundred sperms were counted from at least five different fields randomly where motile and immotile sperms were included. The motility according to the progression was categorized as:

a) Rapid progression
b) Slow progression
c) Non Progression
d) Immotile

Sum of rapid and slow progressive sperms (a+b) was together reported as progressive motile sperms whereas sum of all the three progressions (a+b+c) was reported as total motility of the semen sample.

### 2.2.7 Morphological evaluation of sperms
A thin smear of semen was made on clean grease free slide and air dried. The slide was dipped for one minute in solution RAL Diff-Quik Fixative. Surplus solution was drained onto a filter paper. The smear was stained for 25 seconds in RAL Diff-Quik Solution. The excess solution was drained onto a filter paper. The slide was dipped for 25 seconds in RAL Diff-Quik Solution II. The slide was then rinsed with distilled water, air dried and visualized under high power field of compound microscope.

### 2.2.8 Evaluation of viability of sperms
One part of staining solution (0.1 ml) was mixed with an equal volume (0.1 ml) of semen. After one minute, a smear was made on a clean grease-free slide and air dried. The slide was then examined for viability under oil immersion objective of the microscope. A total of 200 sperms were counted. All viable sperms were not stained whereas dead sperms were stained. The viability percentage was determined by, Viability % = (Number of unstained sperms/Total number of sperms counted) x 100

### 2.2.9 DNA Fragmentation test

The DNA fragmentation test was carried out using Halosperm Kit that is based upon the sperm chromatin dispersion technique. All the procedures were followed according to the protocol provided in the kit. The agarose screw tube was placed into the float and melted using a water bath at 95-100ºC for 5 minutes until it was completely melted. Ten microfuge tubes were aliquoted with 100 microlitres of the melted agarose. Immediately after that, the tube to be used was kept at 37ºC for 5 minutes to prevent the gelification.

Solutions 1 (DA) and 2 (LS) provided in the kit were set at room temperature (22 °C) during the whole process. The Super-Coated Slides provided in the kit were used. The sperm sample was diluted with Phosphate buffered saline to a maximun of 20 million sperm per milliliter. Immediately, 50 µl of the sperm sample was transferred to the tube and mixed gently with a micropipette. The formation of bubbles was prevented.

Following that, a drop of 8 µl of the cell suspension was placed onto the centre of sample well ("S") and covered with a coverslip. It was pressed gently, avoiding air bubbles formation. Slides were held in a horizontal position throughout the entire process. The control "C" well was used to process a control sample. The slide was placed on a cold surface and transferred into the fridge at 4 °C for 5 minutes to solidify the agarose. The slide was taken out of the fridge and coverslip was removed by sliding it off gently. All the processing were performed at room temperature (22 ºC). The slide was placed horizontally in an elevated position in a tray.

Solution 1 (DA) was applied on the well making sure it was fully covered by the reactant during the whole process and was incubated for 7 minutes. Then, reactant was removed by tilting until complete drying and the slide was placed horizontally in an elevated position. Solution 2 (LS) was applied on the well making sure it was fully immersed and incubated for 20 minutes. Then, the reactant was removed by tilting until complete drying and the slide was placed horizontally in an elevated position. The slide was then washed for 5 minutes covering with abundant distilled water and using a disposable pipette. Then, the reactant was removed by tilting until complete drying and slide was placed horizontally in an elevated position.

It was then dehydrated by flooding with 70% ethanol, using a disposable pipette and incubated for 2 minutes. It was then drained and 100% ethanol was applied for 2 minutes. It was then drained and allowed to dry horizontally on filter paper. After drying, the slide was horizontally placed in an elevated position into a Petri dish or similar tray and solution 3 (SSA) was applied on the wells making sure that those were fully immersed. It was then incubated for 7 minutes and then the stain was removed by tilting until complete drying. Solution 4 (SSB) was applied on the wells making sure these are fully immersed and incubated for 7 minutes. Then, stain was removed by tilting. Excess of stain was removed and allowed to dry at room temperature.

Slides were then visualized under bright field microscopy. A minimum of 300 sperm per sample were counted using the following criteria:

a) Spermatozoa without DNA fragmentation- Spermatozoa with big halo (halo width is similar or higher than the diameter of the core) and Spermatozoa with medium-sized halo- (halo size is between those with large and with very small halo).
b) Spermatozoa with fragmented DNA- Spermatozoa with small halo (halo width is similar or smaller than 1/3 of the diameter of the core) and Spermatozoa without halo or degraded (those that show no halo and present a core irregularly or weakly stained core).

DNA fragmentation index was calculated as follows:
DNA fragmentation index = (Small Halo + No Halo)/3

### 2.2.11 Statistical Analysis

The statistical analysis was performed in Origin Pro 2021 tool. One-way ANOVA and Tukey-post hoc test were used for analyzing the seminal parameters – sperm concentration, motility, morphology, viability, acrosomal integrity and DNA fragmentation index.

## 3. Results

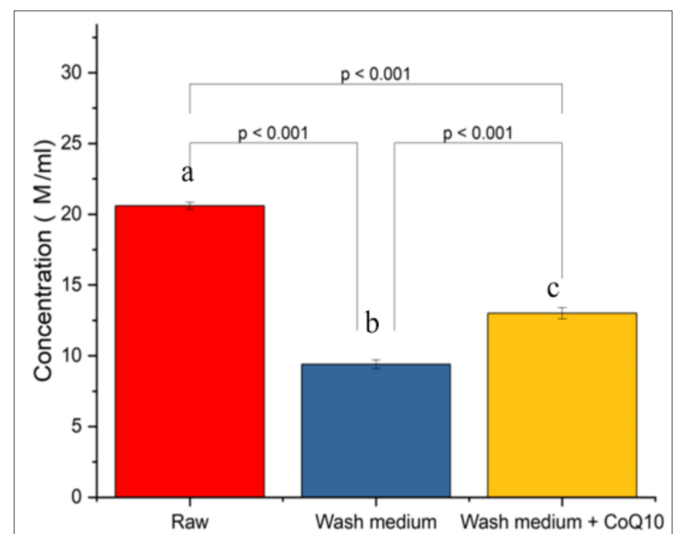
### 3.1 Semen Analysis

The semen parameters of the raw sample, post-wash sample prepared by swim-up method using sperm wash medium with and without CoQ10 were analyzed for their motility, viability, acrosomal integrity and DNA fragmentation index.

### 3.2 Effect of different treatments on sperm concentration

The concentration of the bull sperms as observed after different treatments are presented in Table 1. It was found that raw sample had a sperm concentration of 20.6±0.8[a] million / ml, while post wash samples had a sperm concentration of 13 ± 1.18[b] million/ml and 9.4 ± 0.92[c] million / ml for the swimup samples with and without CoQ10 fortification respectively.

Post wash sample processed using sperm wash medium fortified with CoQ10 had a significantly higher sperm concentration statistically ($p<0.001$) than post wash sample prepared using sperm wash medium alone. However, the concentration of sperms in raw sample was significantly higher than post wash samples (Figure 2).




Values are expressed as Mean ± SD (n=10).
Values superscripted with a, b and c differ significantly

**Fig 2:** Comparison of sperm concentration between raw frozen semen sample and post swim up samples with and without CoQ10 supplementation.

### 3.3 Effect of different treatments on sperm motility

The motility and progressive motility of the bull sperms as observed after different treatments are compared in Table 1. It was found that raw sample had motility and progressive motility percentage of 53.6 ± 2.24[a] % and 62.8 ± 3.06[a] %

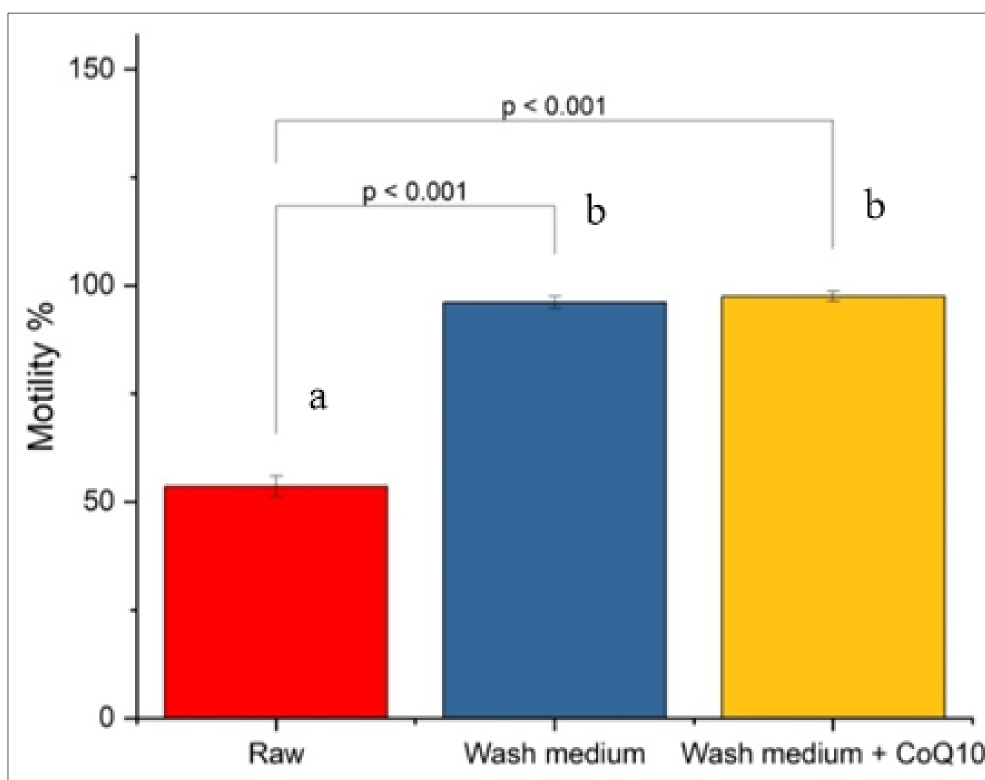
respectively, while post wash samples had motility and progressive motility percentage of 97.5 ± 1.12[b]%, 91.1 ± 2.84[b] % and 96.1 ± 1.3[b] %, 87 ± 3.79[b] % for the swim up samples with and without CoQ10 fortification respectively.

As represented in figure 3a and 3b, among the treatments the post wash sample processed by sperm wash medium supplemented with CoQ10 showed higher level of sperm motility and progressive motility percentage when compared to raw sample. There was significant difference ($p$<0.001) in motility between raw sample and post wash sample after swim up.

**Table 1:** Comparison of sperm parameters between raw frozen semen sample and post swimup samples with and without CoQ10 supplementation

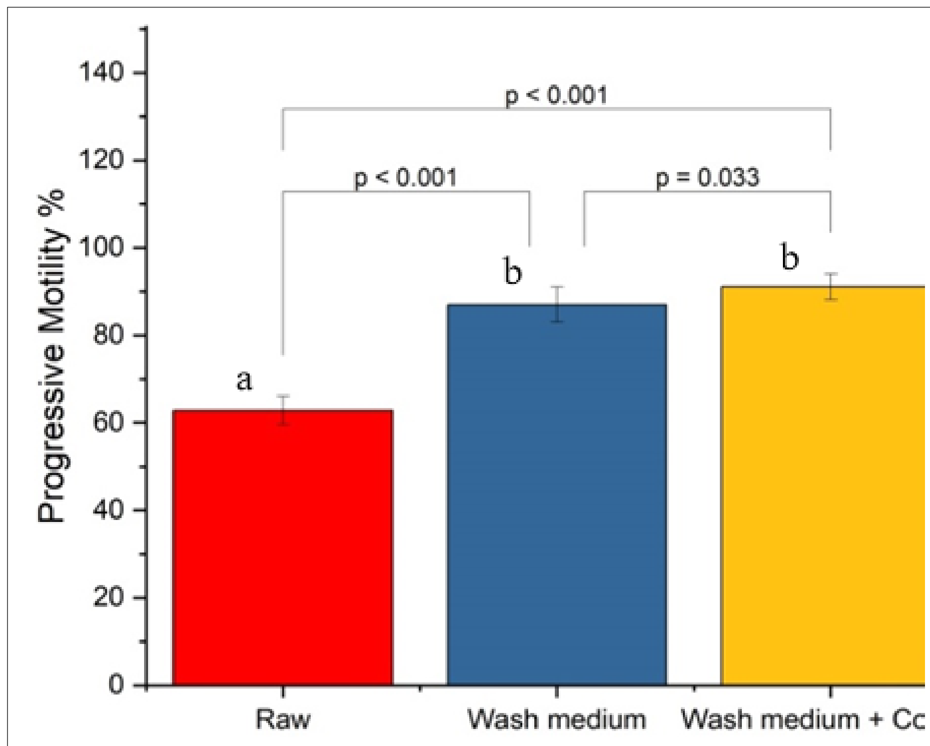
| Treatment | Concentration | Motility | Progressive Motility | Morphology | Viability | DNA fragmentation index |
|---|---|---|---|---|---|---|
| Raw (Frozen Semen} | 20.6±0.8[a] | 53.6±2.24[a] | 62.8±3.06[a] | 79.7±2.05[a] | 79.2±2.18 [a] | 9.4±1.28 [a] |
| Post Wash Sperms with CoQ10 | 13±1.18[b] | 97.5±1..12[b] | 91..1±2.84[b] | 93.7±1,49[a] | 91..9±1.22[b] | 154±1.2[b] |
| Pest Wash Sperms without CoQ10 | 9.4..+0.92[C] | 96.1± 1.3[b] | 87±3.79[b] | 93.6± 1.56[b] | 89±1.84[b] | 7,3±0.9[c] |

Values are expressed as Mean ± SD (n=10).
Values superscripted with a, b and c differ significantly




Values are expressed as Mean ± SD (n=10).
Values superscripted with a and b differ significantly

**Fig 3a:** Comparison of sperm motility between raw frozen sample and post wash sperms with and without CoQ10 supplementation




Values are expressed as Mean ± SD (n=10).
Values superscripted with a and b differ significantly

**Fig 3b:** Comparison of sperm progressive motility between raw frozen sample and post wash sperms with and without CoQ10 supplementation.

### 3.4 Effect of different treatments on Sperm Morphology

The morphological assessment of the bull sperms as observed after different treatments is represented in figure 4. It was found that raw sample had 79.7 ± 2.05 $^{a}$ % of sperms with normal morphology, while post wash samples had 93.7 ± 1.49 $^{a}$ % and 93.6 ± 1.56$^{b}$ % of sperms with normal morphology for the swim up samples with and without CoQ10 fortification (Table 1).

As represented in figure 5, among the treatments the post wash sample prepared using sperm wash medium supplemented with CoQ10 showed higher percentage of sperm with normal morphology when compared to raw sample and post wash sample without CoQ10.

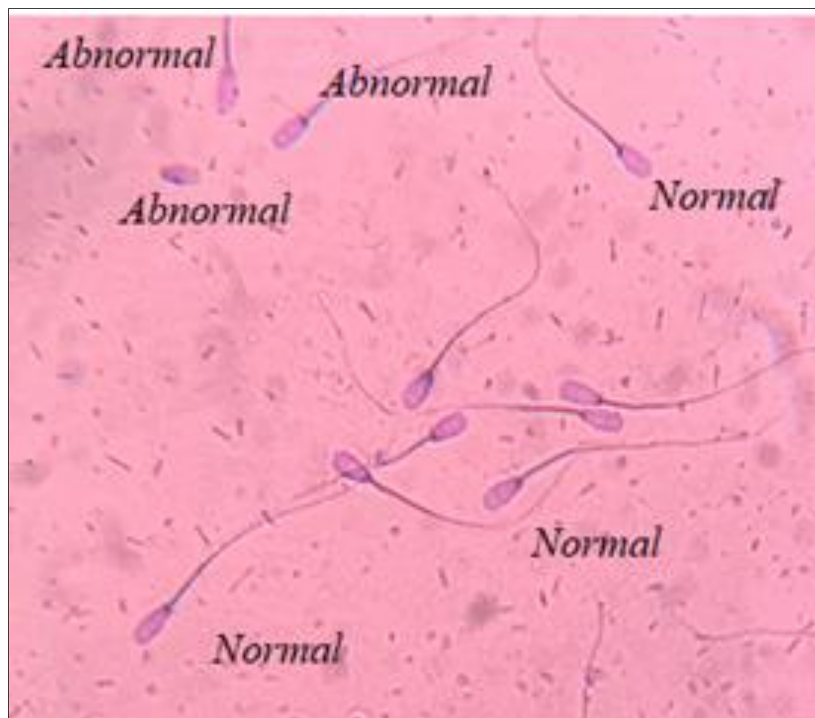
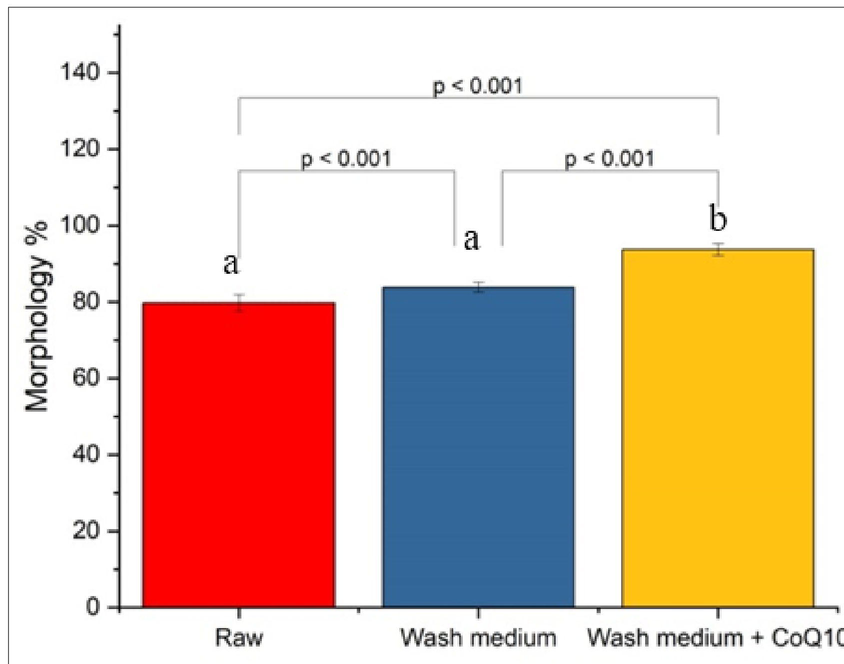



**Fig 4:** Diff-Quick stain for assessing sperm morphology




Values are expressed as Mean ± SD (n=10).
Values superscripted with a and b differ significantly

**Fig 5:** Comparison of sperm morphology between raw frozen sample and post wash sperms with and without CoQ10 supplementation.

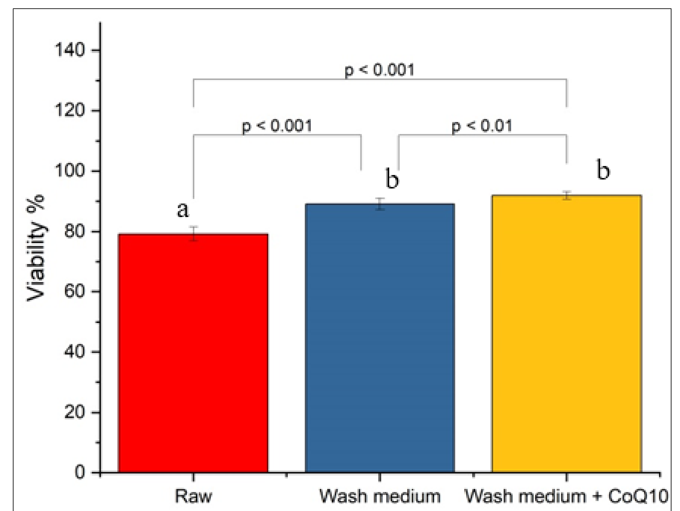
**3.5 Effect of different treatments on sperm viability**

The assessment of sperms for their viability is illustrated in figure 6. It was found that raw sample had a sperm viability percentage 79.2 ± 2.18 [a] %, while post wash samples had 91.9 ± 1.22 [b] % and 89 ± 1.84 [b] % of viable sperms after swim up samples with and without CoQ10 fortification respectively (figure 7).

**Fig 6:** Eosin-Nigrosin stain for sperm viability

**3.6 Effect of different treatments on sperm DNA fragmentation**

Sperm DNA fragmentation assessed using Halosperm kit is illustrated in figure 8. As shown in figure 9, post wash sperms processed with supplementation of CoQ10 had a significantly lesser ($p<0.001$) DNA fragmentation index (3.54±1.2 [a]) than raw sample (9.4±1.28 [b]) and post wash sample prepared without CoQ10 (7.3±0.9 [c]).




Values are expressed as Mean ± SD (n=10).
Values superscripted with a and b differ significantly

**Fig 7:** Comparison of sperm viability between raw frozen sample and post wash sperms with and without CoQ10 supplementation.

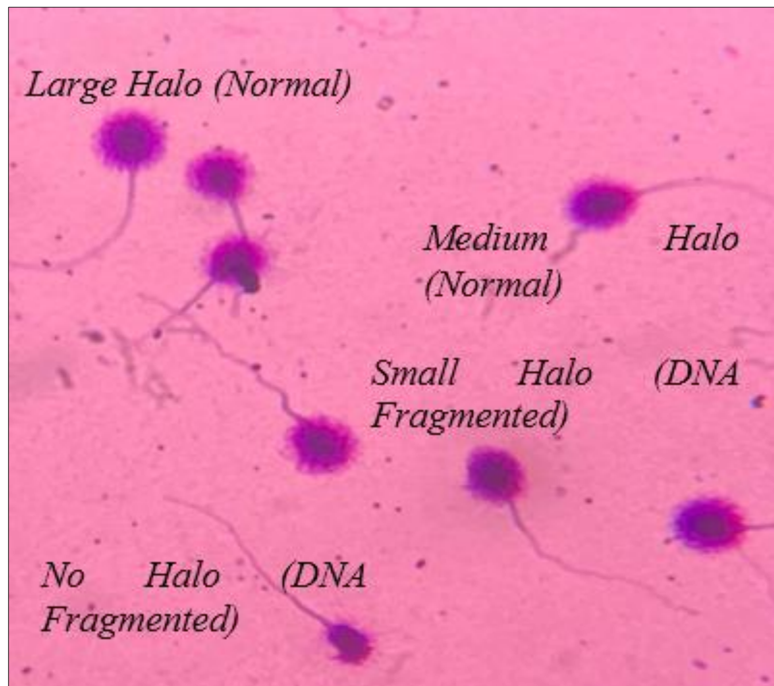
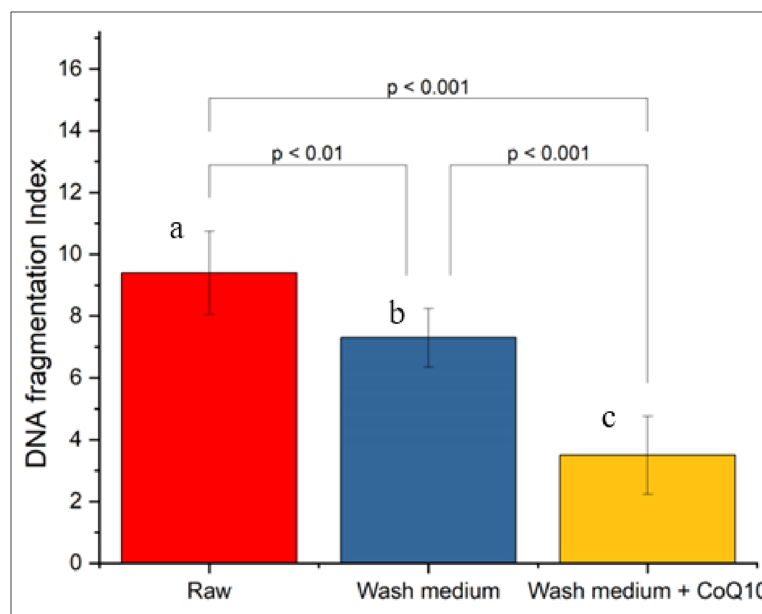



**Fig 8:** Halosperm analysis for sperm DNA fragmentation




Values are expressed as Mean ± SD (n=10).
Values superscripted with a, b and c differ significantly

**Fig 9:** Comparison of sperm DNA fragmentation index between raw frozen sample and post wash sperms with and without CoQ10 supplementation

## 4. Discussion

### 4.1 Coenzyme Q10

CoQ10, a vital antioxidant that is omnipresent in almost all body tissues is particularly present at high concentrations in sperm mitochondria and plays an integral role in energy production [20]. CoQ10 biosynthesis is very active in the testes and a high level of ubiquinol is present in sperm [21, 22]. It is found that there is direct correlation between seminal plasma CoQ10 concentration and sperm motility [23]. An increase in spontaneous pregnancy rates along with improvement in sperm concentration and motility was reported by studies evaluating the oral use of CoQ10 on male infertility [24, 25] which also influenced the outcome of ICSI [20].

One of the studies that used CoQ10 during freezing of buffalo semen illustrates that exposure to CoQ10 *in vitro* enhances most sperm characteristics such as motility, viability, plasma membrane integrity decreasing sperm abnormalities and emphasizes that it is a strong power against acrosomal damage of buffalo and cattle spermatozoa [26]. It was reported that exogenous CoQ10 supplementation at more than 100 µM concentration in *in vitro* maturation medium failed to improve the outcomes of *in vitro* embryo production system whereas lower concentration did not have any effect [27]. However, another study reported that supplementation of 40µM CoQ10 improved mitochondrial function in IVM where unwanted stress, higher AMPK activity, and Oct4 potency loss were induced [28]. Similarly, it is suggested that supplementation of sperm wash media with zinc, D-aspartate and co-enzyme Q10

could protect sperm from oxidative stress damage during *in vitro* handling in assisted reproductive technologies [29]. Hence, the present study was undertaken to assess whether supplementation of CoQ10 would improve the quality of frozen thawed semen.

### 4.2 Evaluation of *in vitro* semen parameters

Semen analysis includes examining the physical characteristics of semen like colour, odor, pH, viscosity, and liquefaction time. It also includes the microscopic examination like volume, concentration, morphology, sperm motility and progression

It is suggested that after thawing, the bull's semen previously frozen in a straw should contain not less than 50% of spermatozoa of correct progression and 80% of spermatozoa without any morphological changes [30]. These values were similar to those of the frozen semen samples in this study in which the mean concentration of sperms was 20.9 million per ml whereas the mean motility and morphology were 53.8% and 79.7% respectively.

It should be noted that sperm viability, acrosomal integrity, and DNA fragmentation index differs among the bulls with different fertility status and these parameters play an important role in assisted reproductive technology or artificial insemination [31].

#### 4.2.1 Evaluation of concentration of sperms

Various methods are employed to assess concentration of sperms like using hemacytometer, flow cytometer and nucleoCounter SP-100, improved Neubauer chamber, Makler chamber etc., [32, 33].

In this study the concentration of sperms was evaluated using a Makler chamber and the concentration of frozen raw sample when thawed was found to be (20.6 ± 0.8 million/ml). As shown in figure 2, the concentration of post wash sample treated with CoQ10 (13±1.18) was significantly better ($p< 0.001$) than post wash sample without CoQ10 supplementation (9.4±0.92). This is in line with the findings of Saha *et al.,* [34] wherein CoQ10 supplementation was found to bring about a moderate improvement in sperm concentration.

#### 4.2.2 Evaluation of motility of sperms

The evaluation of sperm motility provides important information on the energy status of mammalian sperm [35]. It is emphasized that progressive motility is a vital functional characteristic of ejaculated human spermatozoa which governs their ability to penetrate into and migrate through cervical mucus and the oocyte vestments, and ultimately fertilize the oocyte [36]. They also illustrated several methods to study concentration and motility of sperms like wet preparation, assessment by Makler chamber, capillary loading method using fixed depth chambers, drop loading method using fixed depth chambers and assessment using haemocytometer. Thus, spermatozoa were either classified as having forward progressive motility or non-motile [37].

In this study, as shown in figure 3a and figure 3b, there was significant difference ($p< 0.001$) in motility between raw sample and post wash sample after swim up. There was also a significant difference ($p< 0.001$) in motility between raw sample (53.6±2.24) and CoQ10 treated post wash sample (97.5±1.12) whereas the progressive motility tended to be significantly higher in CoQ10 treated post wash sample (91.1±2.84) when compared post wash sample without CoQ10 supplementation (87±3.79 )( p=0.033). Our findings are in agreement with those of Balercia *et al.* [38] who reported that significant increase was found in sperm motility after treating the sperms with COQ 10 by computer-assisted analysis. Similarly, Boonsimma *et al.* [39] reported that the total motility of the CoQ10 supplemented human spermatozoa was significantly higher than in the control ($p$ =0.009) and progressive motility tended to be higher ($p$ =0.053).

#### 4.2.3 Evaluation of morphology of sperms

It is reported that accurate morphological screening of the ejaculates allowed elimination of bulls with low fertility, prior to the entrance of bulls to progeny testing program and the preservation of semen, thus contributing to a major saving for AI enterprises [40, 41].

In this study, as illustrated in figure 5, the post wash sample supplemented with CoQ10 showed higher percentage (93.7±1.49) of morphologically normal sperms when compared to raw sample ($p< 0.001$).

Similar finding has been reported by Masoudi *et al.* [42] wherein no significance difference was observed in rooster semen morphology after treating with CoQ10. However, Saha *et al.* [34] reported there was a moderate improvement in sperm morphology when rat semen was treated with CoQ10.

#### 4.2.4 Evaluation of viability of sperms

Although a conventional method, the usage of eosin-nigrosin stain showed similar results to flow cytometry in terms of assessing the viability of spermatozoa [43] which is also more economical.

Masoudi *et al.* [42] reported a higher viability rate after treating rooster sperms with CoQ10. Pîndaru *et al.,* [43] documented there was a significant increase in boar semen viability from 79.89 ± 3.76% to 82.24 ± 3.56% when treated with CoQ10 during semen freezing. Saeed *et al.,* [26] also reported a significant elevation in viability of spermatozoa in cattle and buffalo when treated with CoQ10 at 30 µM concentration ($p< 0.05$).

Similarly, as shown in figure 7, the post wash sample supplemented with CoQ10 had a significantly better viability percentage (91.9±1.22) statistically than raw sample (79.2±2.18) and post wash sample without CoQ10 (89±1.84) ($p< 0.001$).

#### 4.2.5 Evaluation of sperm DNA fragmention

The acridine orange staining is a simple microscopic procedure based on the same principle as the SCSA [44]. Another simple procedure to study the DNA fragmentation is sperm chromatin dispersion (SCD) test. This test is based on the principle that sperm with fragmented DNA fail to produce the characteristic halo of dispersed DNA loops that is observed in sperm with non-fragmented DNA, following acid denaturation and removal of nuclear proteins and it is considered to be a simple, accurate, highly reproducible, and inexpensive method for the analysis of sperm DNA fragmentation in semen and processed sperm [45].

Giacone *et al.* [46] reported that supplementation of Coenzyme Q 10 along with zinc and D-aspartic acid did not have any statistically significant effect on the percentage of spermatozoa with fragmented DNA in both normozoospermic and asthenozoospermic patients. However, Talevi *et al.* [47] reported that a significant decrease in DNA fragmentation of sperms using the same supplementation.

As shown in figure 9, the post wash sample processed with

medium supplemented with CoQ10 had a significantly lesser DNA fragmentation index (3.54±1.2) raw sample (9.4±1.28) ($p< 0.001$) and post wash sample without CoQ10 (7.3±0.9) ($p< 0.001$).

## 5. Conclusion

Exogenous supplementation of CoQ10 is found to exhibit positive effects on semen parameters such as morphology, viability, motility, acrosomal integrity and DNA fragmentation index in bull sperms. Further research in formulating culture media with exogenous supplementation of antioxidants such as Coenzyme Q10 could improve the artificial insemination outcomes and better success rates in assisted reproductive technology procedures.

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## Conflicts of interest

There are no conflicts of interest

## 7. References

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