# EFFECT OF WHITE RADISH JUICE ON SPERM VIABILITY AND SEMINAL OXIDATIVE STRESS OF BUCK RABBIT SEMEN DURING CRYOPRESERVATION.

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## ABSTRACT
The study was conducted to evaluate the effect of white radish juice on sperm viability and seminal oxidative stress of buck rabbit semen during cryopreservation. 12 rabbits of 24 weeks weighing 4-6kg were randomly allocated into 3 treatments replicate of 4 containing 0ml (control), 5ml, and 10ml of white radish juice respectively. After 8 weeks of feeding, semen was collected with the use of an artificial vagina, using a doe as a teaser. Parameters such as motility, livability, acrosome integrity, membrane integrity, seminal leukocytes counts and Malondialdehyde concentrations were evaluated. The results showed that motility, acrosome integrity were significant ($p<0.05$) with higher percentage at 10ml inclusion level, membrane integrity was significant ($p<0.05$) at 5ml inclusion level. Seminal leukocytes counts and Malondialdehyde concentrations were showed to be significant ($p<0.05$) as they were reduced across level of inclusion. In conclusion, white radish juice was observed to preserve the semen viability and maintained a low oxidative stress status thereby making the semen suitable for artificial insemination post thawed.

**Keywords**: Antioxidant, Oxidative Stress, Semen and cryopreservation, White radish juice

## INTRODUCTION
As breeders typically select genetically better animals for breeding, semen cryopreservation is vital for maintaining sperm cells, which when thawed can be used for artificial insemination to boost livestock development (Cseh *et al*., 2012). As an alternative or complement to conventional medicine, natural compounds derived from plants are employed (Elkordy *et al*., 2021). Antioxidant, antibacterial, anticancer, anti-proliferative, anti-inflammatory, DNA-protecting, hepatoprotective, and allergenic qualities are only a few of the advantages that plants by product (Nazir, *et al*., 2021).*White radish (Raphanus sativus) a* common cruciferous vegetable contain different classes of biologically active phytochemicals (Hanlon and Barns, 2011). In several ethnic cultures, radish has been used as a laxative, stimulant, digestive aid, appetizer, and in the preparation of (methylthio)-3-butenyl isothiocyanate. It contains flavonoids including antioxidants, peroxidases, and kaempherol glycosides (Kim *et al*., 2016; Salah-Abbès *et al*., 2008).
Oxidative stress is a condition when there is an imbalance between the production and accumulation of oxygen reactive species (ROS) in cells and tissues and the inability of the body to detoxify these reactive products, causes oxidative stress (Pizzino *et al*., 2017). One of the most important factors contributing to poor quality semen has been reported to be oxidative stress (Bucak *et al*., 2010) The buildup of reactive oxygen species (ROS), which includes free radicals and peroxides, is the most harmful component of oxidative stress. Reactive oxidative species (ROS) have a negative impact on the sperm plasma membrane, DNA, and physiological processes, which compromises the viability of sperm cells (Juan *et al*., 2021).
Animals' physiological and productive states can be improved by dietary antioxidant supplementation through altering metabolic processes as antioxidants are the primary protective factors against oxidative stress brought on by free radicals (Seifried *et al*., 2007). According to research, feeding white radish to rabbits buck has no negative effects on the animals because of the antioxidants in the vegetable. However, no considerable research has been done on white radish juice to determine its health benefits and appropriate graded level for rabbit. Therefore, this study was carried out to determine the effect of white radish juice on sperm viability and seminal oxidative stress of buck rabbit semen during cryopreservation.

## MATERIALS AND METHOD
### Experimental site

The experiment was conducted at the rabbitry unit, College of Animal Science and Livestock Production (COLANIM) Farm, Federal University of Agriculture, Abeokuta, Nigeria. The University is situated on latitude 7˚10´ N, longitude 3˚2´ E and altitude of 76m above sea level (Google Earth, 2019).

**Experimental animals**

12 rabbit buck of 24 weeks old were used for the experiment, weighing between 4-6kg. The animals were reared for 8 weeks in an intensive battery cage management system and housed individually. Fresh clean water with grower mash concentrate feed and forage were made available to them.

**Experimental design**

After the rabbits have been acclimatized, the rabbits were randomly assigned to 3 treatment replicate of 4 bucks in each replicate. $T_0$ serve as control and receive 0ml of white radish juice, $T_1$ and $T_2$ received 5 and 10mls of white radish juice respectively. Treatment (white radish juice) was administered through gavage twice per week for a period of 8 weeks.

**Preparation of test ingredient**

White radish was sourced from an organic farm in Ogun State, Nigeria, it was washed thoroughly, sliced, blended and poured into a muslin to extract the juice which was used for the experiment.

**Semen collection**

The lubrication of the inner sleeve was done using a white Vaseline. The semen was mixed with an already prepared extender and the sample kept in a thermo flask at 37˚C for transportation to the laboratory for evaluation for evaluation.

**Conventional slow freezing**

Ejaculate from buck rabbits were pooled together and diluted with Tris-based extender composed of two fractions at 32˚C. The semen was diluted in Fraction 1 solution (contain 250.25mM Tris-Based extender) then the Fraction 2 solution containing 7% glycerol which was subsequently added to Fraction 1 at a ratio of 1:1. The pH of the extenders was measured using a digital pH meter. The diluted semen was then loaded into 2ml Eppendorf tubes, sealed with paraffin sealant and cooled gradually in a refrigerator from 32˚C to 4˚C at a rate of 1˚C/4mins (0.25˚C/mins.), thereafter, the tubes were equilibrated by placing them 4cm above liquid nitrogen in the vaporous phase for 10 minutes before gradually plunging them into liquid nitrogen. Semen samples were then extracted after 24 hours, thawed, and tested for viability and oxidative stress characteristics.

**Thawing and semen evaluation**

The frozen semen was thawed in Clifton water bath (Model: 74178 by Nickel Electro Ltd, Weston-S-Mare Somerset, England) at 37°C for 30mins following 24 hours of storage. For semen evaluation, sperm motility and livability were evaluated as described by Bearden and Fuquay (1997), sperm membrane integrity by Correa *et al.* (1997) and acrosome integrity by Ahmad *et al.* (2014)

**3.7.7 Statistical Analysis and model**

Data collected was analyzed using one-way analysis of variance (ANOVA) for a completely randomized design using a statistical package SPSS 2010. Differences between treatments means were separated using Duncan multiple Range Test (Duncan, 1955).

## RESULTS AND DISCUSSION

The result of semen characteristic of buck rabbit administered different levels of white radish juice is shown in Table1. The result from the table indicates that semen characteristics of rabbit bucks were significantly ($p<0.05$) affected by white radish juice especially progressive motility, acrosome and membrane integrity across the different levels of inclusion except for Livability which was not significantly ($p>0.05$) affected by white radish juice at all level of inclusion. This result is supported by Abu *et al*. (2013) who fed graded level of Moringa oleifera leaf meal and Ansa *et al*., (2004) who reported sperm motility of 70% and above for good quality fresh sperm.

The result of seminal oxidative stress parameters of buck rabbit administered different levels of white radish juice is shown in Table 2. The result from the table indicates that seminal leukocytes counts and malondialdehyde concentrations of rabbit bucks were significantly ($p<0.05$) affected by white radish juice across the different levels of inclusion. Although, there was no significant ($P>0.05$) difference across the treatment groups, rabbit bucks administered 5mL white radish juice recorded the lowest value in seminal leukocytes counts and malondialdehyde concentrations.

This result is supported by Abed-Alazeez *et al*., 2016 who administer white radish seed extract and obtained a significant decrease in level of MDA in rabbit. Sperm cells of Mammals have been observed to have substantial polyunsaturated fatty acids contained in their plasma membrane, which makes them prone to lipid peroxidation (Niki *et al*., 2005; Mandal *et al*., 2014). The measures of oxidative stress are essential and valuable indicators of sperm viability (Das *et al*., 2009). Therefore the inclusion of white radish juice in this study, has sustained the quality of cryopreserved rabbit sperm and demonstrated its capacity to effectively preserve sperm cells and reduce oxidative stress. This protective effects could be linked to constituents of white radish that are important to promote sperm optimal survival during freezing such includes; vitamin C, flavonoids such as kaempherol glycosides, peroxidases and antioxidants ( Baskar *et al*., 2018; Shen, 2015; Charles and Charles, 2013).

**Table 1: Results showing the effect of white radish on sperm viability of buck rabbits**

| PARAMETERS | CONTROL | 5ML (WRJ) | 10ML (WRJ) | SBM | P-VALUE |
|---|---|---|---|---|---|
| MOTILITY (%) | $63.00^{b}$ | $66.08^{ab}$ | $69.75^{a}$ | 1.963 | 0.014 |
| LIVABILITY (%) | 80.00 | 84.00 | 75.50 | 1.920 | 0.524 |
| ACROSOME INTEGRITY (%) | $42.00^{b}$ | $55.25^{a}$ | $59.00^{a}$ | 2.960 | 0.001 |
| MEMBRANE INTEGRITY (%) | $35.33^{b}$ | $42.29^{a}$ | $33.75^{b}$ | 1.683 | 0.001 |

$^{a,b,ab}$ *means within same role having different superscript differs significantly (p<0.05).WRJ(White radish juice)*

**Table 2: Result showing the effect of white radish on oxidative stress parameters of buck rabbits**

| PARAMETERS | CONTROL | 5ML (WRJ) | 10ML (WRJ) | SBM | P-VALUE |
|---|---|---|---|---|---|
| LEUKOCYTES (%) | $55.00^{a}$ | $42.25^{b}$ | $50.25^{a}$ | 2.250 | 0.001 |
| MDA (n/mol/$10^{-5}$) | $4.81^{a}$ | $2.43^{b}$ | $3.26^{b}$ | 0.311 | 0.001 |

$^{a,b}$ *means within same role having different superscript differs significantly (p<0.05).WRJ(White radish juice)*

## CONCLUSION

The results obtained in this study suggests that rabbits fed white radish juice appeared generally healthy as most semen characteristics were within the normal range for rabbits suggesting that inclusion of white radish juice does not have any negative effect on semen viability and oxidative stress parameters of rabbit bucks. Inclusion of 10ml improves the semen quality and 5ml inclusion level reduce the oxidative stress of rabbit buck

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