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# Study the efficiency of nano beet extract (Beta Vulgaris) on some productive and reproductive parameters in lambs and rams

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## Abstract

Beetroots (*Beta vulgaris*), commonly known as beets, it is a flowering plant (Amaranthaceae). The particular lab techniques are used for synthesizing and preparing silver nanoparticles of Beetroot (S-NPs-BR) after making an aqueous extract of the Beetroot. Our study aims to evaluate the effects of (S-NPs-BR) on some production and reproduction markers such as body weight, testosterone level, total semen account, dead and defect sperms rate, and active sperms rate in Iraqi Awassi rams. The Beetroot was provided from the local markets, extracted by the Soxhlet apparatus, and then transformed into (S-NPs-BR). Ten Iraqi Awassi rams are housed together under identical conditions (atmosphere and feeding); they are divided into two groups, five for each. The first group was administrated (S-NPs-BR) orally (3 mg/kg/Bw) daily for (8) weeks, while the control group was administered normal saline. The parameters were evaluated biweekly at (2, 4, 6, and 8) weeks after the administration of (S-NPs-BR). The results showed at the end of the study body weight were increased significantly ($p \leq 0.05$) compared with the second and fourth periods (week). However, gradually increased significantly in testosterone levels by time in G1 at ($P<0.05$). There are no statistical differences between G1 and G2 at the second period and fourth periods (weeks), while it reveals significant differences in 6 and 8 weeks at ($P<0.05$). However, total sperm count in G1 demonstrated an increase gradually in the total sperm count and showed great significant differences in 8 weeks at ($P<0.05$). There are significant differences ($P<0.05$) in active sperm rate between G1 and G2 groups along the different periods of the experiment. The dead and defect sperms rate decreased significantly with time in G1 at ($P<0.05$). As well as, the results showed differences significantly ($P<0.05$) between G1 and G2 in 2, 4, 6, and 8 weeks at ($P<0.05$). The S-NPs-BR increases testosterone level, total sperms account, active sperms rate, and decreases dead and defected sperms rate in Awassi rams.

**Keywords:** Awassi rams, Nano-Beet, Sperms, Testosterone.

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## 1. Introduction

The Beetroot is a root of a plant, Beet, called in America as beets, in British as table beet, golden and dinner beet [1].

Beets are rich in vit B9, which helps cells function and grow. Vit B9 have role in control the blood vessels damage, reducing the stroke and heart disease. Beets are contains nitrates which have role in the body [2].

Raw Beetroot consists of fat, water, protein, and carbohydrates. The beetroot (100) gram have (43 kilocalories), the beetroot is a rich vit B9, manganese [3]. The Beetroot can stimuli testosterone production. According to studies, regularly consuming beet can metabolize estrogen and testosterone levels [4].

Beetroot can remove built-up hormones in the bowel and liver by metabolizing estrogen. There is also evidence that Beetroot can lower stress hormone levels and boost libido by increasing testosterone in males [5]. The Beetroot can help lower the stress hormones, cortisol; when the cortisol increase, the testosterone is decreased, and vitamin C decreases the blood circulation of cortisol and another well-known stress hormone, adrenaline, in athletes. High hormone levels can harm the body by elevating blood pressure [6].

Anotechnology has an important role in veterinary medicine, animal health and diseases treatment [7].

Infertility has been well known since ancient times, and Beetroot used for treatment of infertility [8].

Root and leaves of Beta vulgaris is have been used for different ailments [9]. Beetroot provides micronutrients such as Na, K, Mg, P, Fe, Zn, folic acid, carotene, ascorbic acid, pyridoxine, thiamine, and riboflavin [10].

Our work aims to determine the effects of Silver NPs-Beetroot on the testosterone level, the total and active sperm count and the dead and defected sperms rate in Iraqi Awassi rams.

## 2. Material and Methods

### *2.1. Study Design*

Ten Iraqi Awassi rams are housed together under identical conditions (atmosphere and feeding); they are divided into two groups, five for each. The first group was administrated Nano-Beetroot extract orally (3 mg/kg/Bw) daily for (8) weeks, while the control group was administered normal saline. The parameters were determined biweekly at (2, 4, 6, and 8) weeks after the administration.

### *2.2. The Beetroot Extraction*

The Beet was provided from the local markets, extracted by the Soxhlet apparatus, and then transformed into silver Nano-beetroot [11]. Washing of the beetroot, and peeling of the shells. The beetroots were chopped inside small cubes. Mixing of the beetroot with water (contain citric acid 0.5%) at a ratio of 1:3 (beetroot:water). By using shaking water bath, the extraction was carried out (WiseBath, South Korea) for (60) minutes at 80 °C with shaking. Then cooling of the beetroot extract into the (25) $C^0$ after that it filtered with paper of Whatman. Concentering of the beetroot extract are done by using rotary evaporator (Heidolph Instruments, Germany) by 200 mm Hg vacuum and tempreture of 50 Ċ. Some of the extracts were dried by (Operon fdu, South Korea).

### *2.3. Preparation of Ag-Nano-beetroot*

(AgNO3) 10 mM (1.7 g) 100 mL was prepared as a stock solution by used diluted levels (1, 1.5, 2, 2.5, 3 and 5 mM). Adding of the Beetroot, the solution color are change from ruby red to brown, that occur due to interaction of silver and production Ag NPs [12].

### *2.4. Testosterone Estimation*

Testosterone level was determined by using indirections of ELISA Kit (MyBioSource, USA) (MBS701270). Which is microtitter plate and pre-coated with goat anti-rabbit antibody. Incubate standards or samples with HRP-conjugated androgen and a testosterone-specific antibody. Each well receives substrate solutions. Add sulphuric acid to stop the enzyme-substrate reaction, and measure the colour change at 450 nm 2 nm. Comparing the samples' O.D. to the calibration curves determines the testosterone levels.

### *2.5. Procedure*

At room temperature, utilize all reagents and samples and should be duplicated. Add all reagents to the well's liquid level. Pipette shouldn't touch well wall. Create a blank well. 50 µl Standard/Sample per well. Double-check standards. Add 50 µl HRP-conjugate and 50l Antibody for all the wells (not Blank). Incubate at 37°C for 1 hour. Fill the wells with 200 µl Wash Buffer, wait 10 seconds, then spin. Three times, for good performance requires complete liquid elimination at each phase. After the final wash, aspirate or decant the Wash Buffer. Invert and blot the plate with paper towels. Mix 50 µl of Substrates A and B in each well. 15 min at 37°C. In the dark, avoiding draughts and temperature swings. Fill each well with 50 µl Stop Solution. If color change isn't consistent, tap plate to mix. Measure each well's optical density in 10 minutes using a 450-nm microplate reader.

### *2.6. Calculation of Results*

For a standard curve, use professional soft. Determine optical density by averaging duplicate values for each standard, sample, and subtraction. Data reduction creates a standard curve. Construct a standard curve by graphing the absorbance for every standard on the y against the level on the x-axis. Log testosterone level vs O.D. log may linearize the data. Regression analysis determines the best fit line Figure 1.

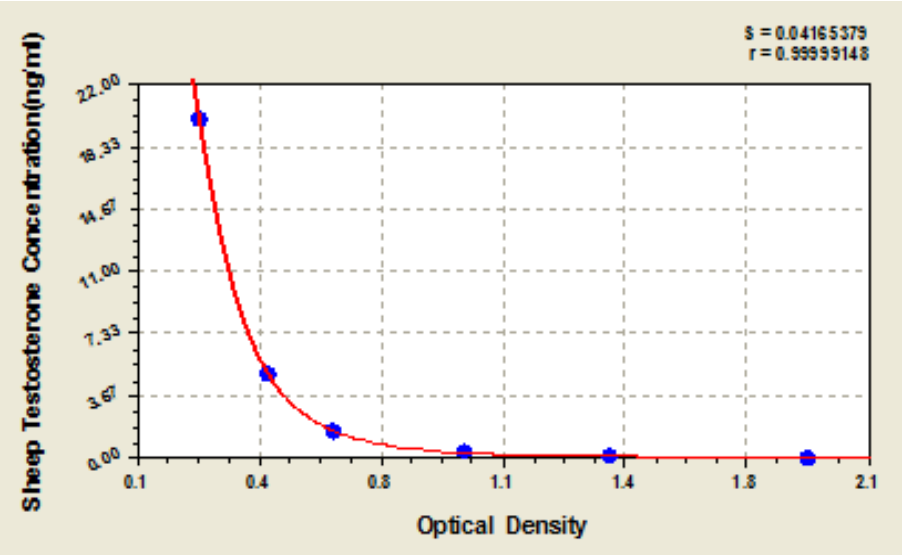


**Figure 1.**
The cure showed the optic density for sheep testosterone concentration antibodies (ng/ml).

*2.7. Semen Examination*

A semen analysis is a lab test that evaluates sperm count, active sperm rate, dead and defected sperms rate. Upon collection, semen can be evaluated at (1-2) ml in volume.

Gross motility is determined by examining non-diluted semen under low magnification. A drop of semen mixed with sodium citrate is put on a preheated glass slide and inspected under a microscope to assess sperm cell motility (40X). (100) sperm should be enumerated to identify motile and non-motile sperm. 100 sperm cells are counted and categorised as normal or abnormal [13].

*2.8. Statistical Analysis*

Data are represented as (mean ± SD). The test used for results analysis is Two way ANOVA with LSD at (0.05) level of probabilities. SPSS software (27) are used for performed the comparisons [14].

## 3. Results

The findings showed gradually increased significant differences in testosterone levels through the eighth week in the first group and significant differences between the periods in testosterone level. They showed an increase with the time at ($P<0.05$). A higher testosterone level occurred in 8$^{th}$ period compared with other periods.

On the other hand, second group (G2) showed no significant differences between the different periods of the study. The results also revealed that there were no significant differences between the treated (G1) and control (G2) groups at 2$^{nd}$ and 4$^{th}$ periods. At the same time, results revealed that significant differences ($p<0.05$) (G1) and (G2) in the 6$^{th}$ and 8$^{th}$ period of the study as showed in Table 1 and Figure 2.

**Table 1.**
Testosterone levels in experimental group and control group during the weeks.

| **Groups** | **Periods** | | | |
|---|---|---|---|---|
| | **2$^{nd}$ period** | **4$^{th}$ period** | **6$^{th}$ period** | **8$^{th}$ period** |
| G1 | 5.39±1.78Ad | 6.51±0.39Ac | 7.76±0.04Ab | 8.6±0.05Aa |
| G2 | 5.2±0.06Aa | 5.83±0.09Aa | 5.86±0.007Ba | 5.90±0.05Ba |
| LSD($P<0.05$) | 0.836 | | | |

The different capital letters are used for columnar comparison while the small letters used for horizontal comparison.

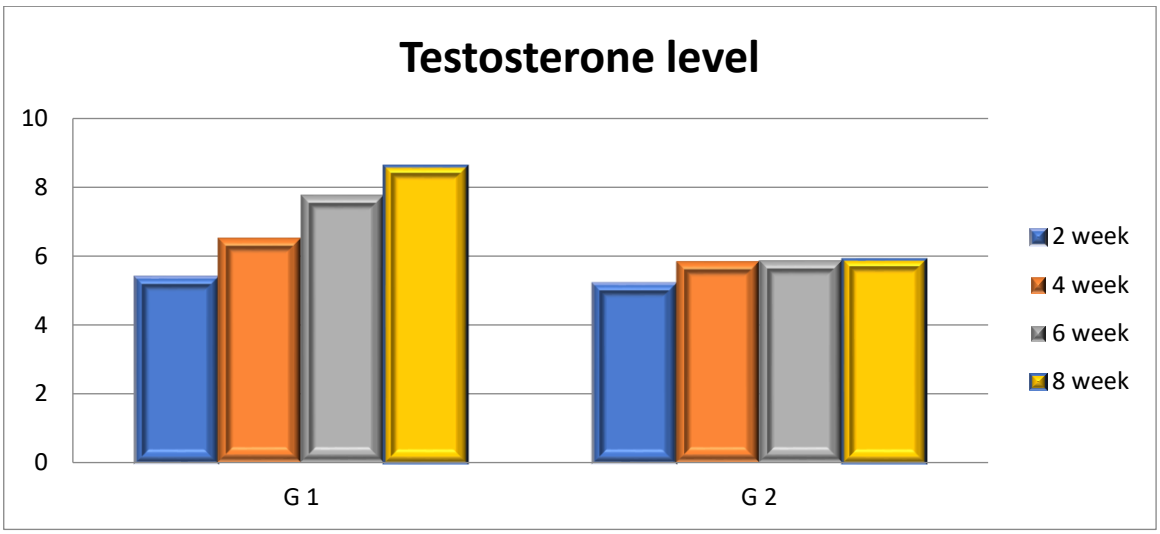


**Figure 2.**
Chart represented Testosterone levels in G1 and G2 at different periods.

On the other hand, the total sperm count increased significantly ($p<0.05$) in the eighth period when compared with other periods in the treated group (G1), but is demonstrated to increase gradually in the total sperm count, between the 2nd and 6th week as compared with the 8th weeks.

The second group (control group) showed non-significant differences between the periods at ($P<0.05$) in total sperm count. Furthermore, there were no significant differences in total sperm count between G1 and G2 in the second week. However, it reveals significant differences between both groups in the fourth, sixth, and eighth group in total sperm count at ($P<0.05$), as shown in Table 2 and Figure 3.

**Table 2.**
Total sperm count ($10^9$ Sperm／ml) in the experimental group and control group during the weeks.

| Groups | Periods | | | |
|---|---|---|---|---|
| | 2nd period | 4th period | 6th period | 8th period |
| G1 | 2.71±0.05Ac | 2.88±0Abc | 2.92±0.08Ab | 3.34±0Aa |
| G2 | 2.56±0.19Aa | 2.54±0.04Ba | 2.61±0.36Ba | 2.65±0Ba |
| LSD($P<0.05$) | 0.193 | | | |

The different capital letters are used for columnar comparison while the small letters used for horizontal comparison.

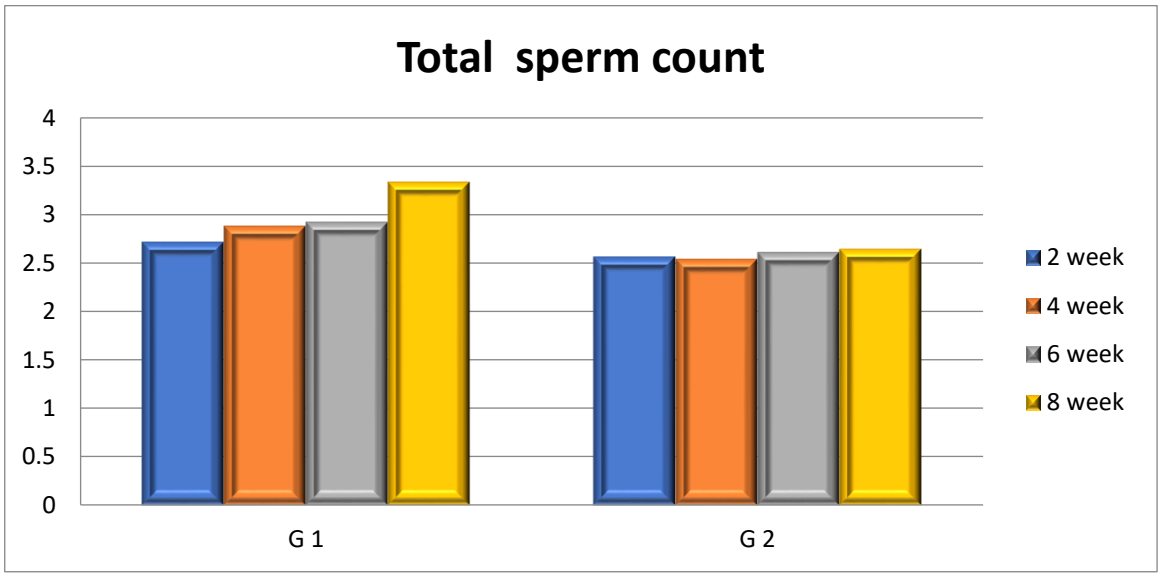


**Figure 3.**
Chart represented total sperm count in G1 and G2 at different periods.

There are no significant differences in active sperm rate between the second week and fourth week in the first group, but there is an increase significantly in active sperm rate in the G1 at 6th period and 8th period at ($P<0.05$). Generally, there is significant increase gradually in active sperm rate with time progress in the first group. Table 3 and Figure 4 showed that there are non-significant differences in the individual motility percentages in the control group (G2). However, Active sperm rate showed apparent significant differences ($p<0.05$) in all the periods between both groups (G1 and G2).

**Table 3.**
Active sperm rate (individual motility%) in the experimental group and control group during the weeks.

| Groups | Periods | | | |
|---|---|---|---|---|
| | 2nd period | 4th period | 6th period | 8th period |
| G1 | 95.67±0.82Ab | 96.06±0.72Ab | 97.51±0.39Aa | 98.1±0.07Aa |
| G2 | 94.69±0.06Ba | 95.26±0.81Ba | 94.7±0Ba | 94.7±0.35Ba |
| LSD (P<0.05) | 0.671 | | | |

**Source:** The different capital letters are used for columnar comparison while the small letters used for horizontal comparison.

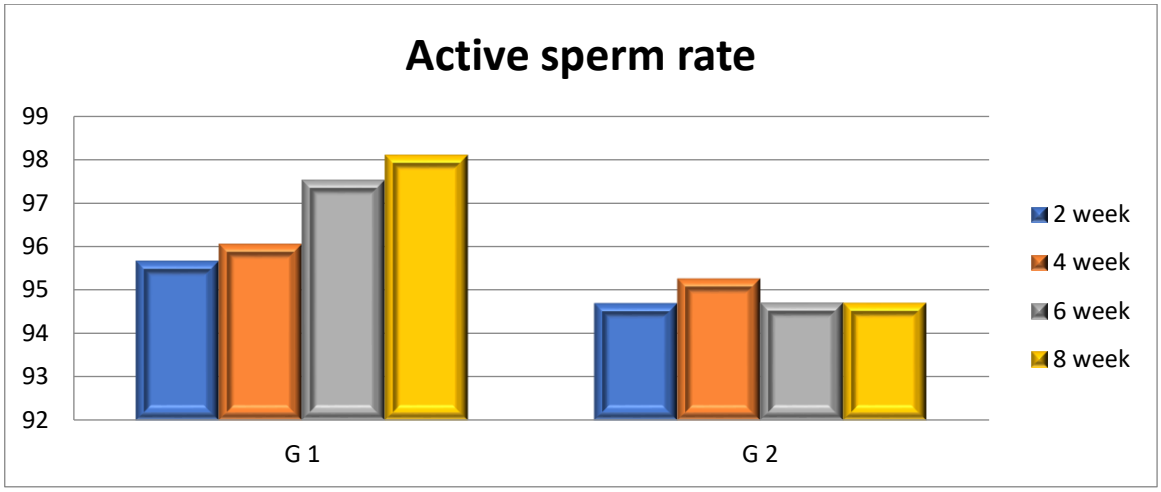


**Figure 4.**
Chart represented the active sperm rate in G1 and G2 at different periods.

The dead and defect sperms rate showed a decreased significant differences with time (during the weeks) in G1 at (P<0.05). As well as, the dead and defect sperms rates showed significant differences between the first group and second group in 2, 4, 6, and 8 weeks at (P<0.05).

Generally, there is a significant decrease (p<0.05) gradually (positive change) in dead and defect sperms rate in the experimental group (G1) (Table 4 and Figure 5).

**Table 4.**
Dead and defect sperms rate in the experimental group and control group during the weeks.

| Groups | Periods | | | |
|---|---|---|---|---|
| | 2nd period | 4th period | 6th period | 8th period |
| G1 | 4.32±0Ba | 3.94±0.74Ba | 2.50±0.36Bb | 1.90±0.1Bc |
| G2 | 5.31±0.07Aa | 5.32±0.26Aa | 5.3±0Aa | 5.28±0.72Aa |
| LSD (P<0.05) | 0.517 | | | |

**Source:** The different capital letters are used for columnar comparison while the small letters used for horizontal comparison.

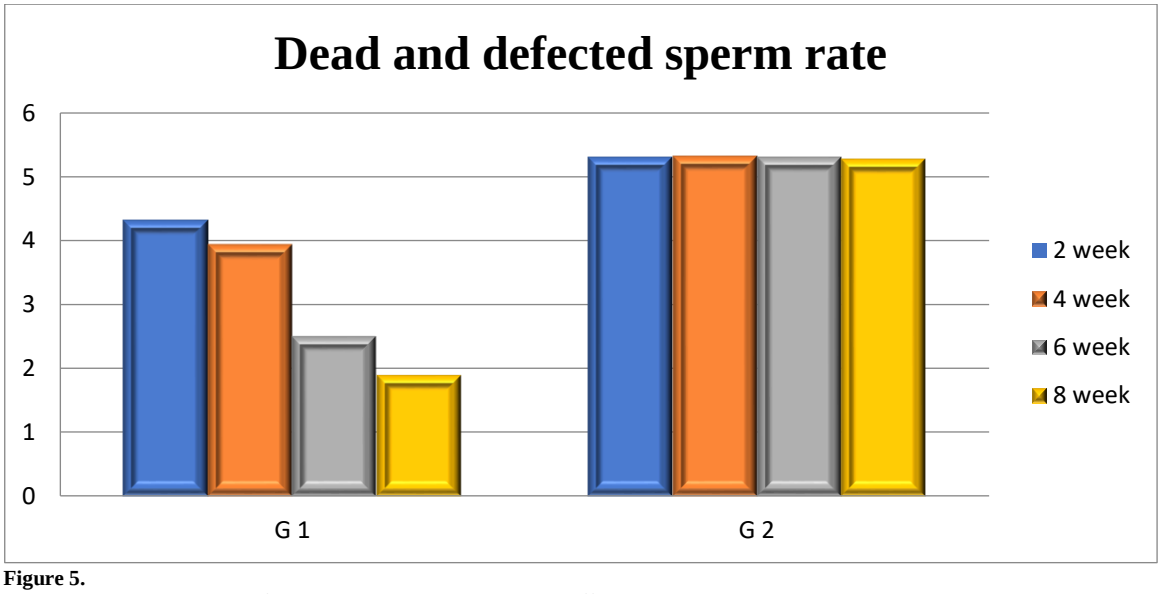


**Figure 5.**
Chart represented dead and defected sperm rates in G1 and G2 at different periods.

Table 5 showed significant differences in the body weight of the rams in the treated group (G1) in the 8th period of the study when compared with the second and fourth weeks. While the control group (G2) recorded significant differences in the last week compared with second and fourth weeks. However, there were no significant differences between G1 and G2 groups.

**Table 5.**
Nano Beet extract (*Beta vulgaris)* effect on body weight during the different weeks.

| Groups | Periods | | | |
|---|---|---|---|---|
| | **2nd period** | **4th period** | **6th period** | **8th period** |
| G1 | 28.55±0.72Ab | 30.25±0.68 A b | 31.55±0.82 A ab | 34.33±0.61 A a |
| G2 | 28.30±1.92 A c | 30.10±0.71 A bc | 31.54±1.31 A ab | 33.22±0.61 A a |
| LSD(P<0.05) | 3.0956 | | | |

## 4. Discussions

Our work is included for the first-time effect of silver nanoparticles Beetroot on Iraqi Awassi rams. Our results showed a gradual increase in testosterone levels through the eighth week in the first group that administrated silver NPs beetroot, wherever also showed significant differences between the periods in testosterone levels. The eighth week reveals higher testosterone concentration when compared with other periods and control group at (P<0.05).

Testosterone is a hormone that is secreted in the testicles. Testosterone is first responsible for the primary signs and secondary signs of males. Also, it have helps maintain the bones, muscle, and energy. Furthermore, it have role in behavior such as concentration and mood [15]. However feed additives in the rams feed cause significant effect on their health through their effectiveness on the rams digestibility efficiency [16].

Beetroot is provide ascorbic acid, phenolic acids, carotenoids, betaline, flavonoids, and antioxidant. Beetroot provides polyphenols, therefore has health benefits. Phytochemicals which extracted from of Beetroot inhibit and reduce the inflammation [17].

Nitric Oxide (NO) were one of beetroot content which effect on the performance of the athletics Beetroot has several effects on athletic performance because it contains nitric oxide (NO). (NO) is a regulating the cellular functions and leading dilation of the blood vessels and increasing bloods flow. (NO) improving athletic performance by increasing glucose, and oxygen for the muscles, and have role in hormones production and neurotransmitters [18].

Some the reports found that NO contribute on testosterone formation. Testosterone induces vasodilation. Different NO level stimuli different hormonal levels, although, some studies showed that the testosterone have little change after beetroot intake [19].

In study on mice, the testosterone were increased at (p<0.001) as compared to control group, and found that Beetroot improved testosterone concentration after taking of beetroot [19].

In another study are carry out for (9 weeks) of Trained Male Triathletes by administration 2.1 gram/day of Beet root (300 mg/day of nitrates ($NO_3^-$). However, beetroot prevent cortisol and testosterone hormones to increase [20]. NO increasing blood flow through the testis [21]. Beetroot reduce free radicals, aging, and testosterone secretion as compared with the control group and placebo group [22].

The level of serum testosterone proved that animals treated beetroot have improved testosterone level. Administration of BM-MSCs and Beetroot enhanced the improvement of the testosterone concentration [23].

According to the current study, the total and active sperm count in G1 (Beetroot administrated) demonstrated to increase gradually with the time, the first group showed significant differences as compared with the control group in the total sperm count at (P<0.05).

The poor levels of folate (vitamin B9) are associated with low sperm count and decreased sperm mobility. Beetroot contains a several vitamins; it helps boost male fertility by increasing the sperm number. Beetroot contains C and E vitamins which have important for activity and healthy sperms [24]. A study published in 2020, found that Beetroot improving fertility, hormonal level and sperms count [25]. Treatment with Beetroot (3 mg/kg body weight) revealed that there were slight increase in the count of the sperms when compared with the control group [26].

Based on our results, generally, there is decrease gradually (positive change) in the dead and defect sperms rate in G1 as compared with G2 at (P<0.05). Beetroots was contains antioxidant that have role in in combat the infertility. Furthermore, Beetroots supply nitrates that improve blood flow to all the organs body [19]. The study included twenty male rats, the study found that treatment by beetroot juice have great role for positive improvement for the shape and nature of the sperms become normal like the control [27]. Beetroot may increase the spermatogenesis of the sperms in the testis [27]. After administration of beetroot, the testosterone levels were comeback to the normal levels in rabbit with hormones distributed. The beetroot in improving the fertility, and reduce the anomalies sperms [19] Our results were agreed with several researcher who found that Beets are rich in vit B9, which helps cells function and grow [2] and because it Raw Beetroot consists of fat, water, protein, and carbohydrates. The beetroot (100) gram have (43 kilocalories), the beetroot is a rich vit B9, manganese [3]

## 5. Conclusion

*5.1. Theoretical Contributions*

This study contributes significantly to the theoretical understanding of how nano-extracts derived from *Beta vulgaris* (beetroot) influence reproductive physiology in ruminants. First, it reconceptualizes the role of phytochemicals and

nanotechnology in reproductive health by demonstrating that silver nanoparticles of beetroot extract (S-NPs-BR) produce measurable enhancements in testosterone levels, total sperm count, and motility, while reducing defective sperm rates. This positions nano-beetroot not merely as a dietary supplement but as a potential functional reproductive enhancer in veterinary medicine.

Second, the study enriches veterinary reproductive science by empirically validating the relationship between antioxidant-rich nanomaterials and hormonal regulation. By showing that nitric oxide pathways and vitamin B9 content can positively modulate spermatogenesis, the research extends the theoretical models linking nutrition, oxidative stress reduction, and reproductive success.

Third, it highlights the importance of contextual physiological responses in livestock, emphasizing that local breeds such as Iraqi Awassi rams demonstrate significant sensitivity to nutraceutical nanomaterials. This provides a foundation for integrating nanotechnology into theoretical frameworks of animal fertility management.

### *5.2. Practical Implications*

The findings provide several practical implications for animal production, veterinary practitioners, and livestock breeders. For breeders, the study offers evidence that supplementation with silver nano-beetroot can enhance reproductive outcomes, leading to higher fertility rates and improved semen quality in rams. This could translate into increased productivity in sheep breeding programs. For veterinarians, the results suggest that integrating nano-extracts in dietary regimens may provide a cost-effective and natural alternative to synthetic fertility enhancers, reducing dependency on pharmaceuticals. Moreover, the demonstrated reduction in defective sperm rates implies long-term genetic and herd quality improvement. The positive impact on body weight further indicates dual benefits, enhancing both growth and reproductive capacity.

### *5.3. Limitations and Future Research Directions*

Despite its contributions, the study faces certain limitations. The sample size of ten rams, though sufficient for pilot evaluation, restricts broader generalization. Future studies should adopt larger cohorts to validate and expand upon these results. Additionally, the research design was limited to an eight-week experimental period; longitudinal studies would be necessary to determine the long-term effects of nano-beetroot supplementation on reproductive performance across multiple breeding seasons. The study was also conducted under controlled experimental conditions, which may differ from variable field environments. Comparative investigations across different breeds and ecological settings would illuminate potential breed-specific and environmental interactions. Furthermore, mechanistic exploration at the molecular level—including gene expression analysis of spermatogenesis pathways—would enrich the theoretical grounding of the observed outcomes. Finally, as nanotechnology rapidly advances, examining the integration of other nanoparticle carriers or combined phytochemical formulations could reveal more efficient modalities for improving livestock reproductive health.

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