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Research Article

Semen and Serum Biochemical Parameters are Influenced By Age of Iraqi Local Black Bucks

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Abstract | The objective of this study was to evaluate the influence of age on semen characteristics and serum metabolites in Iraqi local black goat bucks. A total of 12 local bucks were categorized into three age groups: Group 1 (1–3 years), Group 2 (3–5 years), and Group 3 (over 5 years). Blood samples were collected, and serum was separated for biochemical and hormonal analysis. The results showed that zinc ion concentration was significantly higher ($P \leq 0.01$) in bucks older than 4 years compared to the other groups. Testosterone and calcium levels were also significantly higher ($P \leq 0.01$) in bucks aged 3–4 years. In contrast, potassium and iron ion concentrations were significantly higher ($P \leq 0.01$) in bucks aged 1–2 years. Furthermore, a significant negative correlation was observed between iron and zinc concentrations with testosterone levels. Conversely, a significant positive correlation was found between testosterone and calcium levels. These results exhibited that goats aged 3–5 years showed the best results across most parameters, with a strong positive correlation between zinc and testosterone. Blood serum minerals influenced semen quality, with the highest mineral levels found in the 3–5-year age group.

Keywords | Local black bucks, Semen, Iron, Zinc, Testosterone

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INTRODUCTION

Goats are economically important livestock and represent an important part of the economy of many countries of the world because they are inexpensive for rearing (Hafizuddin *et al.*, 2023). The introduction of reproductive techniques to assist in the reproduction and genetic improvement of goats and to overcome fertility problems is one of the important factors in improving the reproductive performance of goats (Luo *et al.*, 2019; Husnurrizal *et al.*, 2023). In Iraq, the number of goats is about 1.360 million (FAO, 2022), and they are considered an important livestock wealth, there are three breeds local to the region, namely black goats, mountainous black goats, which are raised mainly for meat, and milk, and Meriz goat which is used primarily for hair in addition to meat and dairy (Al-Kass and Jumaa, 2005). Black goats are found in the central and southern regions and are smaller than those found in other breeds, their productivity is low, but they can withstand harsh environmental conditions (Alkass and Mustafa, 2023).

Chemical metabolites play a critical role in goat reproduction, as imbalances in their concentrations can disrupt spermatogenesis, reduce libido, and ultimately impair male fertility (Tvrda *et al.*, 2015). Semen quality and quantity are influenced by age, health status, and sexual activity. Several studies have shown that biochemical factors, environmental conditions, and animal activity levels indirectly but significantly affect sperm vitality (Krzastek *et al*., 2020; Chao *et al*., 2023). Sperm production is hormonally regulated, with follicle-stimulating hormone (FSH) playing a key role. When FSH levels decrease, the body compensates by increasing testosterone production to sustain normal spermatogenesis (Oduwole *et al*., 2018; Hafizuddin *et al*., 2021).

Recent studies emphasize the essential roles of calcium (Ca), zinc (Zn), and iron (Fe) in male fertility, mainly in relation to function of sperm and hormonal balance. Calcium is crucial for sperm motility and the acrosome reaction, both vital for fertilization (Yang *et al*., 2024). Zinc supports sperm membrane integrity, antioxidant defense, and testosterone regulation, with higher seminal Zn levels linked to improved motility and morphology (Ferrer *et al*., 2024; Fallah *et al*., 2018). Iron, while necessary for cellular respiration, must be tightly regulated, as excess can lead to oxidative damage, whereas deficiency impairs sperm quality (Nawito *et al*., 2015; Tsao *et al*., 2022). Age-related changes in the levels of these minerals may significantly influence semen quality and reproductive performance in bucks. This study aimed to evaluate the effect of age on selected serum biochemical minerals and reproductive hormones in Iraqi local goat bucks.

MATERIALS AND METHODS

Animal sample

Twelve Iraqi local black goat bucks, weighing between 36.23 and 50.55 kg, were used in this study. The animals were sourced from local private farms and divided into three age-based groups, with four bucks in each group:

- Group 1: 1–3 years old
- Group 2: 3–5 years old
- Group 3: Over 5 years old

Blood sample collection

All laboratory analyses were conducted at the Al-Musayyab Technical College laboratory. Blood samples were collected from the jugular vein of each buck twice per month over a three-month period during the spring season. Approximately 4–6 mL of blood was drawn using a 10 mL disposable syringe into non-heparinized tubes, following hair removal and sterilization of the collection site with ethyl alcohol. Samples were centrifuged at 3,000 rpm for 10 minutes to separate the serum. The resulting supernatant was carefully transferred using a digital pipette into 5 mL plastic tubes and stored at −20°C until biochemical and hormonal analysis.

Semen quality and blood serum analysis procedures

Blood serum ion concentrations were determined using commercial diagnostic kits (Human, Germany), following the method described by Henry (1974). Serum testosterone levels were measured using ELISA kits (Monobind, USA).

To assess sperm abnormalities, 200 sperm cells were examined under a microscope at 40× magnification. Counts were performed in multiple fields on each slide to ensure accuracy, as described by Chemineau *et al*. (1991). For mass motility evaluation, a drop of fresh semen was placed on a glass slide and examined at 10× magnification at 37°C, following the method of Salisbury *et al*. (1978). Individual motility was assessed by mixing a drop of semen with a drop of 2.9% sodium citrate solution on a slide, then observing at 40× magnification under the same temperature conditions (Chemineau *et al*., 1991).

Statistical analysis

Data were analyzed using the Statistical Analysis System (SAS, 2012) to evaluate the effect of age groups on the studied parameters, following a completely randomized design (CRD). Significant differences between group means were determined using Duncan's multiple range test, with significance levels set at $P < 0.05$ and $P < 0.01$ (Duncan, 1955). The mathematical model was as follows:

$$Yij = \mu + Ai + eij$$

Where; Yij: View value j of treatment I, μ: the general average of the trait studied, Ai: the effect of age group i (1-3, 3-5, older than 5 years), eij: the normally distributed random error with a mean of zero and a variance of σ^2e.

RESULTS AND DISCUSSION

As shown in Table 1, the progressive motility of black Iraqi goat bucks was not significantly affected by age group, with values recorded as 71.71 ± 0.77%, 72.87 ± 0.27%, and 72.91 ± 0.29% for young, middle-aged, and older bucks, respectively. These values were lower than those reported by Aguiar *et al*. (2013), who found a progressive motility rate of 91.92 ± 1.21%. The discrepancy may be attributed to differences in nutrition, as high-energy diets can lead to fat accumulation in the scrotal area, impairing thermoregulation and, consequently, spermatogenesis thus reducing sperm motility (Karasahin *et al*., 2022).

In contrast, mass motility showed a significant difference among age groups ($P \le 0.01$), with older bucks recording the highest value (70.54 ± 0.11%), followed by middle-aged (68.95 ± 0.98%) and young bucks (65.33 ± 0.19%). This finding aligns with Ahmad *et al*. (2014), who reported lower motility in younger bucks and higher values in older ones. However, Sundararaman and Edwin (2008) observed the opposite trend, with younger animals exhibiting better motility. Such inconsistencies may be due to differences in breed or experimental conditions.

Regarding sperm abnormalities, young bucks showed a significantly higher rate of deformities (2.30 ± 0.16%) compared to middle-aged and older groups ($P \leq 0.01$), which is consistent with findings by Syafruddin *et al.* (2020) and Abah *et al.* (2023).

Sperm motility is critical for successful fertilization, as it enables sperm to reach the oocyte. The most effective type of motility is linear, forward movement, which ensures timely arrival at the egg (Hackerova *et al.*, 2025). Additionally, mass motility should not fall below 70%, as optimal movement appears under the microscope as wave-like motion patterns (Armansyah *et al.*, 2018).

Table 1: Influence of age on progressive motility, mass motility, and sperm abnormalities in black Iraqi goat bucks.

Age groups (years)	Average ± Standard Error (SE)		
	Progressive movement (%)	Mass movement (%)	Deformities (%)
1-3 years (Young)	71.71 ±0.77	65.33± 0.19c	2.30 ±0.16a
3-5 years (Middle-aged)	72.87 ±0.27	68.95 ± 0.98b	1.75 ± 0.19c
> 5 years (Older)	72.91 ±0.29	70.54 ± 0.11a	2.05 ± 0.24b
Significance level	NS	**	**

The averages with different letters within the same column are different significantly. ** ($P \leq 0.01$), NS: Non significant.

Statistical analysis in Table 2 showed that calcium levels were significantly higher ($P \leq 0.01$) in the 3–5-year-old group (11.37 ± 0.14 μg/dL) compared to the younger (7.78 ± 0.12 μg/dL) and older groups (10.64 ± 0.17 μg/dL). Calcium influences mass motility, particularly in older animals, consistent with Towaje *et al.* (2018), who reported that calcium regulates sperm motility through its association with adenosine triphosphate (ATP), controlling motility and energy use. Additionally, calcium's role in reducing primary sperm malformations aligns with findings by Al-Zubaedi *et al.* (2019), who noted that rams with low serum calcium are more prone to sperm abnormalities.

Additionally, the results showed that zinc was significantly superior ($P \leq 0.01$) in the old age group (Greater than 5 years), which reached 18.01 ±0.09 μmol/L compared with the young and middle ages, which reached 14.45 ±0.13 μmol/L and 12.48 ±0.11 μmol/L respectively. The superiority of zinc in the old age group led to a decrease in the percentage of malformations, and increase in the vitality and concentration of the sperm and these results are in agreement with what was mentioned by (Souza *et al.*, 2011; Liu *et al.*, 2016).

Iron ions were significantly higher ($P \leq 0.01$) in the young age group (1–3 years), measuring 94.03 ± 7.34 μg/dL, compared to the middle (75.76 ± 4.22 μg/dL) and older groups (85.09 ± 4.03 μg/dL). This higher iron level contributed to a reduction in initial sperm deformities. These findings align with Lerias *et al.* (2015) and Syarifuddin *et al.* (2022), who reported that iron in seminal plasma is crucial for maintaining sperm motility and vitality, supporting proper sperm function.

Table 2: Influence of age on mineral levels in blood serum of black Iraqi goat bucks.

Age groups (years)	Average ± Standard Error (SE)		
	Calcium (μg/dL)	Zinc (μmol/L)	Iron (μg/dL)
1-3 years (Young)	7.78 ±0.12c	14.45±0.13b	94.03±7.34a
3-5 years (Middle-aged)	11.37±0.14a	12.48±0.11c	75.76±4.22c
> 5 years (Older)	10.64±0.17b	18.01±0.09a	85.09±4.03b
Significance level	**	**	**

The averages with different letters within the same column are different significantly between them. ** ($P \leq 0.01$), NS: Non significant.

The results in Table 3 show that testosterone levels were significantly higher ($P \leq 0.01$) in the middle age group (3-5 years), at 1.86 ± 0.23 pg/L, compared to the young (1.15 ± 0.12 pg/L) and older groups (1.11 ± 0.14 pg/L). These findings align with previous studies (Tesema *et al.*, 2020; Pareek *et al.*, 2023) and may be due to fewer Leydig cells in younger and older bucks (Ngcobo *et al.*, 2023). Leydig cells, the primary source of testosterone and androgens, are crucial for sperm production, sexual development, and secondary sexual characteristics (Zirkin and Papadopoulos, 2018). Testosterone levels in male goats also vary based on breed, age, nutrition, season, and female estrus behavior (Kandemir *et al.*, 2023).

Table 3: Effect of the ages on testosterone level in black Iraqi goat bucks .

Age groups (years)	Average ± Standard Error
	Testosterone hormone (pg/L)
1-3 years (Young)	1.15 ± 0.12 b
3-5 years (Middle-aged)	1.86 ± 0.23 a
> 5 years (Older)	1.11 ± 0.14 c
Significance level	**

Averages carrying different letters within the same column differ significantly between each other ** ($P \leq 0.01$).

The results in Table 4 show a significant positive correlation ($P \leq 0.01$) between testosterone and both calcium ($r = 0.34$) and zinc ($r = 0.42$). These findings align with Farshad *et al.* (2012) and Yatoo *et al.* (2013), who reported a positive relationship between zinc and testosterone levels. Zinc plays a crucial role in maintaining germ cells, promoting testicular growth, aiding sperm maturation, and supporting testosterone synthesis (Sultan *et al*., 2023). Zinc deficiency negatively affects the development of seminiferous tubules. Additionally, Zarazaga *et al*. (2022) found that rams with normal testosterone levels had higher zinc concentrations than those with low testosterone. Conversely, iron showed a significant negative correlation with testosterone ($r = -0.37$, $P \leq 0.01$), suggesting that elevated iron levels may reduce testosterone production, potentially impacting the reproductive health of goat bucks (Amrane *et al*., 2013).

Table 4: Correlation coefficient between minerals and testosterone hormone in black Iraqi goat bucks **.**

Minerals	Correlation coefficient(r)
	Testosterone hormone
Calcium	0.34 **
Zinc	0.42 **
Iron	- 0.37 **
** ($P \leq 0.01$)	

CONCLUSIONS

The 3–5-years old goat group showed higher results in most studied parameters, with a highly significant positive correlation between zinc and testosterone. Blood serum minerals notably influence sperm characteristics, with the middle age group (3–5 years) having the highest levels of key minerals. We recommend correlating these traits with pregnancy rates to determine the optimal breeding age.

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NOVELTY STATEMENT

This study showed the relationship and an effect between minerals, age, and sperm characteristics in Iraqi local black bucks, which is important for artificial insemination programs as well as for semen freezing.)

AUTHOR'S CONTRIBUTION

The laboratory work, and statistical analysis of data by M. K. H. AL-dulaimi, while M. K. A Al-juhaishi sample collection and writing. The article was reviewed by all authors.

Ethical approval

The laboratory work was conducted at the College of Technology – Al-Musayyab, Al-Furat Al-Awsat Technical University, with approval from the Animal Welfare Committee (Approval No. 7/18/7765, dated 24/11/2024).

Generative AI and AI-assisted technology statement

During the writing process, generative AI technology was used to assist in improving the language and enhancing the readability of the text. The grammarly tool was used for this purpose [link: https://app.grammarly.com/?source=doc-title-bar]. All content generated by the AI was carefully reviewed, edited, and proofread by human writers to ensure accuracy and quality.

Conflict of interest

The authors report no financial or any other conflicts of interest in this research.

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