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Serum trace elements associated with different forms of infertility in rams

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Abstract

The main objective of the present study was to investigate the association between the concentration of trace elements and different forms of infertility, genital affections and testosterone concentration of rams in central Saudi Arabia. A total of 54 infertile and 18 fertile rams were used in this study. Infertile rams were admitted to the veterinary hospital at Qassim University, Saudi Arabia for breeding soundness examination. Animals averaged 27.21 months of age, body condition score of 3, and weight of 57.92 kg. Data including owner's complaint, breeding history, and signalment were recorded. Blood samples were taken immediately upon admission and the animals were examined clinically. Sera of the studied animals were used for the estimation of serum trace elements and testosterone concentrations. The animals were categorized according to their breed (Awassi, n = 27; Najdi, n = 13; Harry, n = 8; Blackhead Persian, n = 6), age (less than 12 months, n = 14; ≥ 12–24 months, n = 16; ≥ 24–36 months, n = 14; more than 36 months, n = 10), form of infertility (impotentia generandi, n = 40; impotentia coeundi, n = 8; lack of sexual desire, n = 6) and affected organ (testicular, n = 22; epididymal, n = 8; spermatic cord, n = 3; penile/preputial, n = 4; idiopathic, n = 17). Results revealed that rams older than 36 months had lower Zn ($P = 0.04$) than younger ones. Serum Mn, Se, and Zn were lower in infertile rams with different forms of infertility ($P = 0.000$, $P = 0.000$, $P = 0.02$, respectively) and various genital affections ($P = 0.0001$, $P = 0.001$, $P = 0.02$, respectively). It can be concluded that trace elements may be implicated in the pathogenesis of genital affections in infertile rams.

Sheep, reproductive issues, testosterone, minerals, fertility

It is important to note that rams' fertility is affected by a variety of factors, such as their ability to breed, sexual desire, sperm production, age and nutritional status (Petrovic et al. 2017). The infertility of male animals takes three main forms; decrease or lack of libido, copulatory impotence (impotentia coeundi) and failure to fertilize (impotentia generandi) (Ucar and Ulsu 2021). The susceptibility of rams to nutritional infertility is affected by breed. Mutton breeds are more susceptible to reproductive disorders than wool breeds and even within the same breed there is an individual variation regarding the ability to accommodate to harsh nutritional conditions and the resulting disrupted fertility (Ali et al. 2021). Age of the ram has an impact on the ram's resilience to infertility attributed to nutritional deficiency but the soundness of the toes and teeth should be considered especially with older rams (Petrovic et al. 2017). Impotentia generandi is the most common form of ram's infertility and represents more than 60% of the reproductive failure in rams and the other two forms represent less than 40% (Ali et al. 2019). Testicular affections are the main causes of impotentia generandi in rams followed by epididymal ones and characterized by deteriorated semenogram (Naoman et al. 2022).

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Trace elements play a major role in all the biochemical processes in the animal's body though they are required in small quantities to achieve their tasks (Derar et al. 2023). The inadequacy of these elements affects the general health and reproductive function of the animal. Reproductive system and its physiological functions are rapidly affected by any unconventionality in the concentration of these elements in blood and tissues caused by either inadequate or excess intake in the animal's feed (Derar et al. 2022). Trace elements are essential for normal libido, copulation, spermiogram, capacitation and fertilization (Maciejewski et al. 2022).

Zinc (Zn) is known for its role in maintaining the integrity of the reproductive system defense mechanism, providing indispensable input in sustaining the sex drive of rams (Page et al. 2020). Zinc could be an indicator for sperm anomalies and male infertility. It plays a major role in semen ejaculation as well as being a cofactor for the DNA-binding proteins with Zn fingers (Ghorbani et al. 2018). Zinc in the seminal plasma stabilizes the cell membrane and nuclear chromatin of the sperm. It controls the effects by modulating the activity of the $Ca^{2}+$ ATPase enzyme and plays an important role in the development of testes and secondary sexual characteristics, and in a few sperm physiologic functions (Esfiokhi et al. 2023). Decrease in the Zn concentration in the seminal plasma causes hypogonadism, decrease in the testical size, inadequate development of secondary sexual characteristics, and atrophy in seminiferous tubules (Ucar and Ulsu 2021). It has been shown to stimulate sperm capacitation and acrosomal response by activating the epidermal growth factor receptor and the G protein-coupled receptor (Esfiokhi et al. 2023).

Selenium (Se) has long been used together with vitamin E to promote reproductive performance of rams especially to improve the quantity and quality of semen in small ruminants through its direct effect on the spermatogenesis or indirectly via its role on the biological and metabolic pathways in the reproductive organs and its associated endocrine organs (Maquivar et al. 2021). Both Zn and Se are involved in maintaining the oxidant/antioxidant balance which is critical for the reproductive system health and soundness (Derar et al. 2022).

Manganese (Mn), another important trace element, is irreplaceable for its involvement in the wear and tear of the male reproductive system (Ghorbani et al. 2018). Long standing decrease in Mn level in the ram's blood results in atrophy and impaired functions of the affected genital organ (Wdowiak et al. 2015).

Iron (Fe) is believed to be necessary for the male reproductive function due its implication in most metabolic aspects of the genital system. Excess or insufficient Fe intake has drastic effects on both the soundness and function of the ram's fertility (Ucar and Ulsan 2021).

The main effect of unbalanced intake of trace elements is usually associated with their inadequate supply. The resultant effect depends on the age-related reproductive stage of the animal (before or after puberty), ranging from disrupted to completely impaired reproductive functions (Boitani and Puglisi 2008). There is a general agreement that supplementation of these trace elements in the feed of rams in considerable amounts promotes their fertility particularly prior to breeding season (Carrillo-Nieto et al. 2018). Due to their role in the synthesis of reproductive hormones, trace elements were used to improve the fertility outcomes in humans and animals (Shan et al. 2017; Alves et al. 2020; Te et al. 2023). Zinc, Se, and vitamin E were prescribed for patients with decreased libido, erectile dysfunction, and deteriorated semenogram through their direct and indirect effect on the hormonal biosynthesis (Ali et al. 2021).

The association between trace elements and infertility has been intensively investigated in ewes and does (Derar et al. 2022) and their impact on promoting fertility in other species has also been well documented (Ali et al. 2021). However, the relation between these elements and the incidence of reproductive disorders in rams in central Saudi Arabia has not yet been investigated. This study was, therefore, aimed to investigate the status of five selected trace elements in serum of infertile rams and their associations with the clinical findings and testosterone concentrations.

Materials and Methods

Animals

A total of 54 infertile and 18 fertile rams were used in this study. Infertile rams were admitted to the university veterinary hospital, Qassim University, Saudi Arabia for breeding soundness examination during the breeding season autumn-winter. Investigated rams averaged 27 months age (9–42 months), a body condition score of 3 (Kenyon et al. 2014), and a weight of 57.92 kg. Data including owner's complaint, breeding history and signalment was recorded. The infertility problems lasted between one and three seasons. Eighteen fertile rams (control group) aged between 17–30 months with body condition scores of 3 and average weight 59.23 kgs were used as a control group. Studied rams were fed adequate rations for breeding according to National Research Council (2007) and water *ad libitum*.

Breeding soundness examination

Infertile rams were examined andrologically as previously described (Ali et al. 2019). Briefly, the testes and epididymis were evaluated for size and consistency using visual inspection, palpation, calipers, and ultrasound. The internal genitalia were examined by transrectal palpation and ultrasonography using a B-mode scanner equipped with a 5-MHz linear-array transducer (Aloka SSD-500; Aloka Co., Ltd, Tokyo, Japan).

Blood sampling

To estimate the serum trace elements in the investigated and control rams, the animals were bled immediately after admission and before clinical examination. Blood was collected from the jugular vein using plain serum tubes (no anticoagulant). Samples were kept at 4 °C for ≤ 2 h before centrifugation. The collected blood was centrifuged at 1,700 × *g* for 15 min, and serum was collected and kept at −20 °C until the assessment of trace elements and testosterone.

Assessment of serum trace elements and testosterone

According to the method detailed by Pompilio et al. (2021), atomic absorption spectrometry was implemented to determine the contents of Mn, Se, Fe and Zn using a PerkinElmer PinAAcle 900T atomic absorption spectrophotometer (PerkinElmer Inc., Waltham, MA, USA). Graphite furnace atomization was used for Zn, Fe, and Mn, while hydride generation was used for Se determination. Certified reference materials (Seronorm™ Trace Elements, Sero AS, Billingstad, Norway) were used for quality control. Serum concentrations of testosterone were determined by ELISA using commercial kits (Prechek Bio., Inc., Lot No: 119011502, Yongin, South Korea). The coefficients of variation of the inter- and intra-assays were 3.7 and 5.1%. The sensitivity of the assay was measured at 0.3 ng/ml.

Statistical analysis

The data were presented as means ± SE, and statistical analysis was carried out using the SPSS program, version 25 (2017). Data were tested for normality using Shapiro-Wilk test and homogeneity of variance using Levene's test. Independent samples *t*-test (two-tailed) was used for the comparison between infertile and control groups. For comparisons among multiple groups (forms of infertility, genital affections, age categories, breeds), one-way ANOVA was used followed by Tukey's *post hoc* test. For groups with less than six animals, non-parametric Kruskal-Wallis test with Dunn's *post hoc* test was applied. Correlation coefficient was used to determine the effect of different factors and concentrations of trace elements. Significance was set at $P < 0.05$.

Results

The inability to fertilize (impotentia generandi) was the most prevalent form of infertility among the investigated rams (Table 1). Testicular affections represented the most frequently diagnosed genital disorder, followed by epididymal lesions, whereas idiopathic infertility accounted for 31.48% of cases (Table 2). Breed (Table 3) had no effect on any of the studied trace element serum concentrations whereas age (Table 4) had a significant effect on serum Zn concentration. Results revealed that rams older than 36 months had lower Zn ($P = 0.04$) than younger ones (Table 4). Age had a positive correlation with serum Zn concentration (r = 0.264, $P = 0.052$), with rams ≥ 36 months showing significantly lower Zn compared to younger groups (Table 4). Infertile rams associated with different forms of infertility had lower serum Mn ($P = 0.000$), Se ($P = 0.000$), and Zn ($P = 0.02$) compared with fertile ones. Genital affections had a significant effect on serum Mn ($P = 0.0001$), Se ($P = 0.001$)

and Zn ($P = 0.02$) of infertile rams compared to fertile ones. Correlation analysis revealed significant relationships among serum trace elements; testosterone concentration, age, and body condition score (Table 5). Age demonstrated a positive correlation with serum zinc concentration ($r = 0.264$, $P = 0.052$), with rams aged ≥ 36 months exhibiting significantly lower Zn concentrations compared to younger age groups. Testosterone had a significantly negative association with all trace elements [Mn ($r = -0.36$, $P = 0.007$); Se ($r = -0.42$, $P = 0.001$); Fe ($r = -0.26$, $P = 0.05$); Zn ($r = -0.41$, $P = 0.002$)]. Interaction between age and breed, and between age and complaint had a significant effect on Se ($P = 0.04$) and Zn ($P = 0.04$) concentrations, respectively.

Table 1. Serum manganese (Mn), selenium (Se), iron (Fe), zinc (Zn) and testosterone (T) concentrations in rams with different forms of infertility (mean ± SEM).

Form of infertility	n	Mn (mg/l)	Se (mg/l)	Fe (mg/l)	Zn (mg/l)	T (ng/ml)
Control	18	2.88 ± 2.28^a	0.76 ± 0.37^a	18.12 ± 2.26^a	6.20 ± 2.70^a	11.41 ± 1.16^a
IC	8	0.35 ± 0.09^b	0.07 ± 0.01^b	16.84 ± 8.46^a	3.20 ± 0.46^b	4.11 ± 4.11^a
IG	40	0.35 ± 0.09^b	0.09 ± 0.02^b	15.98 ± 3.42^a	3.24 ± 0.43^b	9.33 ± 1.31^a
LSD	6	0.10 ± 0.02^b	0.06 ± 0.03^b	17.07 ± 5.77^a	3.09 ± 0.94^b	9.54 ± 1.16^a

SEM: Standard error of the mean; IC: impotentia coeundi, inability to copulate; IG: impotentia generandi, inability to fertilize; LSD: lack/decrease of sexual desire. Values with the same superscript in the same column are not significantly different, with significance set at $P < 0.05$.

Table 2. Serum manganese (Mn), selenium (Se), iron (Fe), zinc (Zn) and testosterone (T) concentrations in infertile rams associated with different genital affections (mean ± SEM).

Genital affections	n	Mn (mg/l)	Se (mg/l)	Fe (mg/l)	Zn (mg/l)	T (ng/ml)
Control	18	3.70 ± 3.68^a	0.65 ± 0.61^a	21.59 ± 12.13^a	8.04 ± 3.42^a	11.41 ± 1.16^a
Testicular affections	22	0.35 ± 0.08^b	0.11 ± 0.02^b	20.15 ± 3.46^a	4.36 ± 1.03^b	8.27 ± 1.98^a
Epididymal affections	8	0.17 ± 0.04^b	0.04 ± 0.01^b	15.97 ± 4.89^a	1.97 ± 0.18^b	12.64 ± 2.71^a
Other genital affections*	7	0.39 ± 0.20^b	0.23 ± 0.15^a	12.16 ± 2.30^a	2.66 ± 0.52^b	7.83 ± 3.97^a
Idiopathic	17	0.09 ± 0.03^b	0.03 ± 0.01^b	15.26 ± 7.75^a	2.16 ± 0.09^b	9.12 ± 1.54^a

SEM: Standard error of the mean. Values with the same superscript in the same column are not significantly different, with significance set at $P < 0.05$.

*Other genital affections: combined spermatic cord (n = 3) and penile/preputial (n = 4) affections

Table 3. Serum manganese (Mn), selenium (Se), iron (Fe), zinc (Zn) and testosterone (T) concentrations in rams of different breeds in the Qassim region (mean ± SEM).

Breed	n	Mn (mg/l)	Se (mg/l)	Fe (mg/l)	Zn (mg/l)	T (ng/ml)
Awassi	27	0.65 ± 0.22^a	0.16 ± 0.05^a	18.28 ± 2.53^a	3.58 ± 0.39^a	9.55 ± 1.12^a
Najdi	13	0.13 ± 0.02^a	0.04 ± 0.01^a	14.96 ± 2.62^a	3.13 ± 0.83^a	11.34 ± 1.56^a
Harry	8	0.08 ± 0.02^a	0.04 ± 0.01^a	18.72 ± 5.24^a	2.79 ± 0.61^a	8.72 ± 3.59^a
Blackhead	6	0.51 ± 0.12^a	0.09 ± 0.03^a	14.72 ± 1.33^a	3.02 ± 0.52^a	5.85 ± 3.84^a

SEM: Standard error of the mean. Values with the same superscript in the same column are not significantly different, with significance set at $P < 0.05$.

Table 4. Serum manganese (Mn), selenium (Se), iron (Fe), zinc (Zn) and testosterone (T) concentrations in rams of different ages in the Qassim region (mean ± SEM).

Age	n	Mn (mg/l)	Se (mg/l)	Fe (mg/l)	Zn (mg/l)	T (ng/ml)
> 12 months	14	0.08 ± 0.03[a]	0.06 ± 0.03[a]	24.92 ± 6.60[a]	3.03 ± 0.97[a]	10.67 ± 1.92[a]
≥ 12–24 months	16	0.36 ± 0.12[a]	0.09 ± 0.02[a]	17.40 ± 2.72[a]	2.77 ± 0.28[a]	9.17 ± 1.49[a]
≥ 24–36 months	14	0.67 ± 0.34[a]	0.14 ± 0.06[a]	15.18 ± 2.74[a]	3.42 ± 0.55[a]	10.45 ± 1.36[a]
≥ 36 months	10	0.44 ± 0.14[a]	0.19 ± 0.10[a]	18.46 ± 4.94[a]	4.84 ± 1.21[b]	8.99 ± 2.35[a]

SEM: Standard error of the mean. Values with the same superscript in the same column are not significantly different, with significance set at $P < 0.05$.

Table 5. Pearson correlation coefficients between Serum manganese (Mn), selenium (Se), iron (Fe), zinc (Zn) and testosterone (T) concentrations, age, and body condition score (BCS) in rams.

Variable	Mn	Se	Fe	Zn	T	Age	BCS
Mn	1.00	0.45*	0.22	0.31*	−0.36*	0.12	0.08
Se		1.00	0.18	0.40*	−0.42*	0.10	0.05
Fe			1.00	0.15	−0.26*	0.07	0.11
Zn				1.00	−0.41*	0.26	0.14
T					1.00	−0.20	−0.09
Age						1.00	0.33*
BCS							1.00

*$P < 0.05$

Discussion

In accordance with previous reports (Ali et al. 2019; 2021), the findings of the present study demonstrated that inability to fertilize (impotentia generandi) is the most common form of infertility and that testicular affections are the primary pathological lesion encountered in subfertile rams. Our findings are consistent with the literature, where orchitis, peri-orchitis, and epididymitis are frequently detected in infertile rams (Ali et al. 2021). Brucellosis is often diagnosed in such cases, with the tunica vaginalis of the scrotum showing strong granulomatous inflammation and focal necrosis that may extend to the epididymis and spermatic cord (Alshahed 2018). *Chlamydiae* have also been identified as infectious agents in the male genital organs of rams and bucks, and their presence in semen suggests the possibility of venereal transmission (Ali et al. 2019). Epididymitis is principally associated with *Brucella ovis*, a contagious pathogen that impairs ram fertility by inducing alterations in the epididymis, testicle, and accessory sexual glands (Picard-Hagen et al. 2015). Chronic epididymitis is commonly linked to the development of spermatocele, sperm granuloma, or abscess (Alshahed 2018).

The present study revealed that low fertility in rams is associated with decreased serum concentrations of Mn, Se, and Zn, but not Fe. Trace elements, particularly Se and Zn, are widely supplemented in feed to promote fertility in animals (Contri et al. 2011) and humans (Hawkes et al. 2009). Zinc has been shown to exert a direct effect on sperm motility, morphology, concentration, viability, and androgen secretion in rams (Page et al. 2020). Serum Zn reflects its dietary availability, and a deficiency directly impairs the biosynthesis and function of Sertoli and Leydig cells. It has been proposed that Zn mediates the action of IGF-I on reproductive processes in males, likely through its abundance in the mitochondria of reproductive organs (Jafarpour et al. 2015). Dietary Zn also plays a critical role in the bioavailability of Mn in ram seminal plasma, although the precise mechanism by which Zn enriches accessory glands with Mn remains under debate (Colagar et al. 2009). Testicular tissue is highly sensitive to even minor deviations in Zn intake, responding rapidly with impaired spermiogram and reduced androgen levels (Ali et al. 2021). The latter study demonstrated that Zn supplementation for approximately three weeks improved fertility and semen indices in infertile camels, including those with azoospermia. Our observation of reduced serum Zn in infertile rams is in accordance with Zhao and Xiong (2005), who documented a strong association between low serum Zn and subfertility in males. Conversely, our results differ from Osadchuk et al. (2021), who found no correlation between serum Zn and idiopathic infertility in men, suggesting potential species-specific differences in trace-element metabolism.

In human studies, men with idiopathic infertility often exhibit lower Zn concentrations in seminal plasma than in serum, challenging the assumption that serum concentrations reliably indicate fertility status (Osadchuk et al. 2021). However, other reports argue for a strong consistency between Zn concentrations in seminal plasma and serum, with both decreasing in idiopathic infertility (Page et al. 2020). In men, seminal-plasma Zn concentrations correlate positively with semen indices such as sperm concentration, morphology, progressive motility, and viability (Sundaram et al. 2013). Zinc is a key component of over 350 enzymes that regulate diverse biological processes in the reproductive system, especially its defense mechanisms (Ucar and Ulsan 2021). To further clarify these relationships, future studies should measure trace elements in seminal plasma and correlate them with serum concentrations, clinical findings, and testosterone concentration.

Selenium was significantly lower in infertile rams compared to fertile controls in this study. Selenium and vitamin E are often regarded as biological partners due to their synergistic action (Ali et al. 2021). Both are commonly used to enhance the reproductive performance of rams before the breeding season (Osadchuk et al. 2021). Their beneficial effect on semen quality is attributed to antioxidant activity and involvement in numerous metabolic pathways of the reproductive system (Jafarpour et al. 2015). Supplemented males typically show higher numbers of progressively motile, morphologically normal, and viable spermatozoa, along with improved libido, compared to non-supplemented counterparts (Ali et al. 2021). The addition of organic Se to cryopreserved ram semen has been shown to improve its post-thaw quality and motility (Rodrigues et al. 2024). Selenium also helps protect sperm genetic material, particularly DNA integrity (Moya et al. 2021). Because Se is involved in testosterone secretion from Leydig cells and in spermatogenesis within the seminiferous tubules, its deficiency is implicated in testicular and epididymal disorders (Behne et al. 1996). The impact of Se deficiency on the integrity of the male genital tract—especially the testes and epididymis—and on semen quality can be severe and often irreversible (Maquivar et al. 2021).

In the present study, testosterone showed a significant negative association with all trace elements measured. This finding is consistent with reports in human males; for example, Cheng et al. (2005) observed an inverse correlation between androgen levels and basal concentrations of Se, Zn, and Mn in endurance runners. The primary effect of trace-element imbalance appears to be on Leydig cells, impairing their ability to produce testosterone. Luteinizing hormone, a pituitary hormone that regulates androgen synthesis, is also influenced by trace-element status, particularly Se and Zn (Alves et al. 2020). Moreover, inadequate concentrations of these elements adversely affect other hormones involved in reproduction, such as growth hormone, IGF-I, and cortisol (Comitato et al. 2015). As a component of manganese superoxide dismutase, Mn was negatively correlated with testosterone, supporting the notion that Mn is closely involved in testosterone synthesis by Leydig cells (Alves et al. 2020).

This study was conducted under routine clinical conditions; therefore, the distribution of cases reflects naturally occurring reproductive disorders rather than experimentally balanced groups. Genital affections with limited individual representation were combined into anatomically and clinically related categories to ensure robust statistical interpretation. The investigation focused on serum trace element profiles; assessment of vitamin E and seminal-plasma trace element concentrations was beyond the scope of the present work, though their potential relevance is acknowledged for future research.

In conclusion, the present findings demonstrate that disturbances in serum Mn, Se, and Zn concentrations are closely associated with different forms of infertility and genital affections in rams, with testosterone showing a consistent inverse relationship to trace element concentrations. Age-related variation, particularly affecting Zn status in older rams, further emphasizes the importance of targeted nutritional management before and during the breeding season. These results highlight the clinical relevance of serum trace element profiling as a practical tool in the evaluation of ram infertility under field conditions. Future studies integrating seminal plasma assessment and dietary interventions may further optimize fertility management strategies in breeding rams.

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