Research Article

Evaluation of Blood and Seminal Plasma Testosterone and Aldosterone Levels and their Consequences on Semen Parameters and Fertility in Dromedary Camels

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Abstract | Background; Numerous biomarkers had utilized by researchers for predicting fertility in camels or selecting fertile camels. Infertile dromedary camels could be identified with conventional semen quality assessments, but relative in vivo fertility varies significantly for camels considered fertile. **Aim;** Study the blood and seminal plasma aldosterone and testosterone levels of male dromedary camels and their outcome on semen parameters and fertility. **Methods;** From breeding history records, depending on fertility status, sixty camels had divided into two groups; control "fertile" (n = 20) and infertile (n = 40). Blood and seminal plasma have obtained from all camels for hormone analysis. **Results;** Blood testosterone level was lower ($p < 0.05$) in control "fertile" than that in infertile camels (3.05 ± 0.12 vs. 5.17 ± 0.24 ng/mL). Similarly, lower ($p < 0.05$) testosterone levels were found in the seminal plasma of control (1.29 ± 0.22 ng/mL) compared to infertile (2.78 ± 0.29 ng/mL) dromedaries. However, there were no differences ($p > 0.05$) in aldosterone levels between the two groups in the blood or seminal plasma. Blood and seminal plasma aldosterone showed a positive correlation ($r = 0.36$) in the control group. In infertile camels, there was a positive correlation ($r = 0.45$) between blood testosterone and aldosterone. Further, there was a negative correlation ($r = - 0.26$) between blood aldosterone and seminal plasma testosterone. In fertile camels, there was a negative correlation ($r = - 0.42$) between blood aldosterone and sperm motility percentage. Besides, plasma aldosterone levels in infertile animals were positively correlated ($r = 0.26$) with sperm abnormality ratios. **Conclusion;** The blood and seminal plasma aldosterone and testosterone levels could be used as a biomarker to predict fertility in male dromedary camels. Further, it could recommend measuring blood aldosterone relative to seminal plasma testosterone for early diagnosis of sub-fertility in male camels.

Keywords | Dromedaries, Semen quality, Aldosterone, Testosterone, Hormonal analysis, Fertility.

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INTRODUCTION

Dromedary camels reproduce less efficiently than other livestock (Kaufmann, 2005; Skidmore, 2005). Camels are known as seasonal breeders, but sexual behavior is very variable due to the wide geographical distribution of this species (Farh et al., 2018). A lack of assisted reproductive techniques, such as embryo transfer and artificial insemination, could also contribute to less efficient reproduction (Skidmore, 2005). Evaluating the semen quality and fertil-

ity of a male dromedary camels is crucial (Skidmore et al., 2013; El-Bahrawy et al., 2015). Even if infertile dromedary camels can be identified through conventional semen quality assessments, there is substantial variance in relative in vivo fertility in camels considered fertile. (Waheed et al., 2015). Biomarkers have been utilized by researchers to predict fertility in camels or select fertile camels. Dromedary camels and their seminal plasma and serum are analyzed for testosterone as a fertility-associated biomarker (Waheed et al., 2015; Ali et al., 2018). It is also possible to determine whether infertility is the result of a gonadotropin deficiency, primary testicular failure, spermatogenic failure, or androgen resistance through hormonal screening (Ali et al., 2018). Moreover, in male dromedaries, common affections of the reproductive tract include scrotal trauma, testicular degeneration, and hypoplasia has been reported (Skidmore et al., 2013). The most prevalent pathologies included testicular hypoplasia, cryptorchidism, and ectopic testes (Skidmore et al., 2013). Thus, for diagnosis of unexplained infertility or subfertility, it may be necessary to obtain testicular tissue by either biopsy or needle aspiration (Hoflack et al., 2008). Likewise, biomarkers are an important tool for selecting fertile camels or predicting fertility. Besides, selecting a camel with a high fertility ratio offers a tremendous economic benefit to dromedaries' breeders (Waheed et al., 2015).

During normal blood pressure, electrolytes, and fluid levels, the Renin-Angiotensin System (RAS) maintains blood pressure, electrolytes, and fluid levels. In the RAS system, aldosterone and angiotensin II are the circulating hormones that exert their functions (Paul et al., 2006). During dehydration, the RAS may maintain circulation and kidney function (Ali et al., 2012). Moreover, multiple members of the RAS family had been detected in the testis, sperm, and semen of males (Paul et al., 2006). In addition to regulating male fertility, they work in synergistic and/or independent ways with systemic RAS (Paul et al., 2006). As well as maintaining seminal plasma electrolytes, this local RAS is involved in steroidogenesis, spermatogenesis, and sperm function (Gianzo and Subirán, 2020). However, it is unclear how they are located at these locations. Accordingly, the purpose of this study was to investigate the levels of aldosterone and testosterone in male dromedary camels' blood and seminal plasma, and how these levels correlate with semen parameters and fertility.

MATERIAL AND METHODS

Following the guidelines of the Animal Welfare Committee, the experimental procedures had approved by the University Ethics Monitoring Committee, Qassim University, Saudi Arabia. Approval # 332/187/2021.

Animals and experimental design

The present study examined sixty male dromedary camels (*Camelus dromedarius*) in private camel farms in the Qassim region, of central Saudi Arabia. Animals aged 6 to 13 years with bodies weighed between 460 and 618 kg and a body condition score of 3 - 4.5 on a scale of 5 (Sghiri and Driancourt, 1999). Animals had kept under nomadic conditions in an open yard during the breeding season "November to February" (Farh et al., 2018). All studied camels received a commercial diet with 13% crude protein (CP) and 2.9 Mcal metabolizable energy (ME) in addition to mineral-vitamin premix (Table 1). Additionally, Rhodes grass, and water were provided ad libitum. Using the breeding history records, camels were divided into two groups: control (served as sires active for breeding females, n = 20) and infertile (unable to achieve conception, even after several attempts during the breeding seasons, n = 40). The duration of the infertility problems ranged from one to five rutting seasons. Camels' fertility was evaluated using the camel fertility index (the number of females mated with this camel divided by the number of conceived).

Table 1: The ingredients and chemical composition of the diet used in the experiment

Item	Content
Ingredients, % of dietary dry matter	
Barley	60.22
Wheat bran	9.63
Soybean meal	4.25
Alfalfa hay	19.03
Salt	0.47
Limestone	2.10
Molasses	2.00
Mineral and vitamin premix*	1.00
Nutrient composition, dry matter basis	
Dry matter, %	90.20
Crude protein, %	13.50
Ether extract, %	1.72
Neutral detergent fiber, %	25.34
Acid detergent fiber, %	15.55
Ash, %	4.92
Metabolizable energy, Mcal/kg	2.9

* Contained per kg, 10,000 IU vitamin A, 1000 IU vitamin D, 20 IU vitamin E, 300 mg Mg, 24 mg Cu, 0.6 mg Co, 1.2 mg I, 60 mg Mn, 0.3 mg Se, 60 mg Zn.

Blood sampling, semen collection, and evaluation

Blood samples were collected from the jugular vein in a heparinized tube from all camels, centrifuged at 4000 ×*g* for 10 min, and plasma was harvested aliquot and stored at

-80 °C until analyzed.

Semen was collected from all animals by an electro-ejaculator (ElectroJac® 6; Ideal Instruments, Neogen Co., Lansing, MI) described previously (Ali et al., 2014). The ejaculate was evaluated by conventional methods using Sperm Vision® 3.5 (Minitube of America, Inc.) with software validated for camel semen. Semen parameters were volume (ml), viscosity, pH, motility (%), sperm cell concentration (× 10^6/ml), and sperm cell abnormalities (%) using nigrosine–eosin stain. The seminal plasma was separated by centrifugation at 4000 ×*g* for 10 min, aliquot, and stored at -80 °C until analyzed.

Hormone determinations

The blood and seminal plasma testosterone levels had measured by a commercial ELISA kit (Absorbance Microplate Reader ELx 800 BioTek, USA; Microplate Strip Washer ELx 50 BioTek, USA). Samples were diluted (1:10) and run in duplicate. This kit has a lower detection limit of 5.38 pg/mL, an inter-assay coefficient of variation (CV) of 10.22%, and an intra-assay CV of 10.0%. The cross-reaction was testosterone 100%, 19 hydroxytestosterone 12.48%, androstenedione 8.24%, dehydroepiandrosterone 0.62%, estradiol 0.37%, and <0.001% for dihydrotestosterone, estriol, aldosterone, corticosterone, cortisol, cortisone, estrone, progesterone. The results were expressed in ng/ml. The blood and seminal plasma aldosterone levels had measured using a commercial ELISA kit (Diagnostics Systems Laboratories Inc., TX, USA). All samples were analyzed in duplicate. Absorbance was measured using a Multiscan reader (primary robotic immunoassay operator, BRIO; Radim, Pomezia, Rome, Italy). The inter and intra-assay CV was 5% and 7%, respectively. The results were expressed in pg/ml.

Statistical analysis

All data are shown as means ± SEM. The data were analyzed by Student's t-test (t) for comparisons between the two tested groups, and correlations coefficients (r) were tested using the SPSS program, version 24.0 (SPSS, 2016). The normal distribution of data was analyzed with the test of Kolmogorov-Smirnov. The differences were considered to be statistically significant at $p < 0.05$.

RESULTS

The pregnancy rate for the control and infertile camels was 52.45 % and 0.00 %, respectively. Blood testosterone level was lower ($p < 0.05$) in control "fertile" than that in infertile camels (3.05 ± 0.12 vs. 5.17 ± 0.24 ng/mL, Fig.1A). Similarly, lower ($p < 0.05$) testosterone levels were found in the seminal plasma of control (1.29 ± 0.22 ng/mL) compared to infertile (2.78 ± 0.29 ng/mL, Fig.1B) dromedaries. However, there were no differences ($p > 0.05$) in aldosterone levels in the blood or seminal plasma between the two groups (Fig.2A, B). Moreover, there was a positive correlation between blood aldosterone and seminal plasma aldosterone levels in fertile camels ($p < 0.05$, $r = 0.36$). Additionally, in the infertile camels, there was a positive correlation ($p < 0.01$, $r = 0.45$) between blood testosterone and blood aldosterone levels. Furthermore, a negative correlation ($p < 0.05$, $r = -0.26$) was demonstrated between blood aldosterone and seminal plasma testosterone levels in infertile dromedaries.

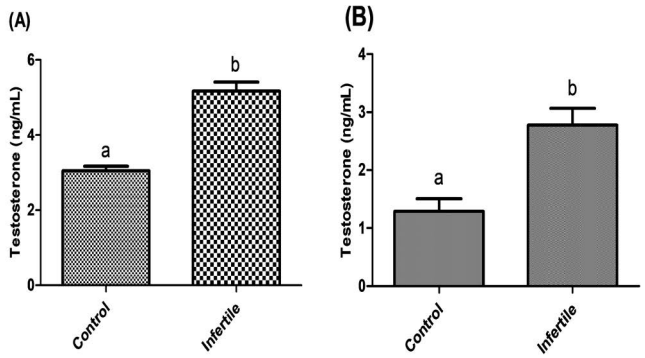

Figure 1: Testosterone concentration (ng/mL) in (**A**) the blood plasma and (**B**) the seminal plasma of control (served as sires for breeding females, n = 20) and infertile (inability to achieve conception in fertile females after persistent attempts over one [7 months] or more breeding seasons, n = 40) dromedary camels. Different letters denote significant differences at $p < 0.05$.

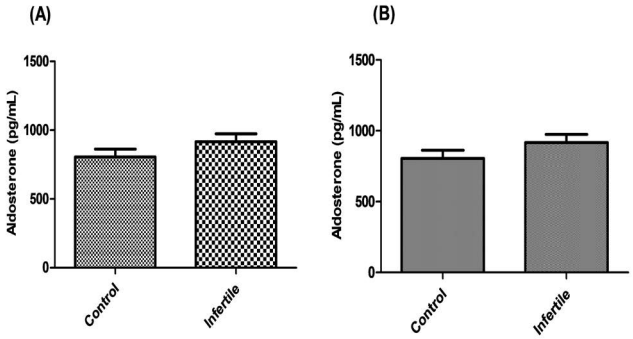

Figure 2: Aldosterone concentration (pg/mL) in (**A**) the blood plasma and (**B**) the seminal plasma of control (served as sires for breeding females, n = 20) and infertile (inability to achieve conception in fertile females after persistent attempts over one [7 months] or more breeding seasons, n = 40) dromedary camels.

The percentages of sperm motility, live sperm, and sperm cell concentration were lower ($p < 0.05$) in the infertile camels compared to control camels (Table 2). Moreover, abnormal sperm value was higher ($p < 0.05$) in the infertile than fertile dromedaries, respectively (48.26 ± 3.84 vs.

Table 2: Semen characteristics (mean ± S.E.) of control and infertile dromedary male camels.

Item	Control n = 20	Infertile n = 40
Volume (ml)	5.63 ± 0.27	4.90 ± 0.85
Viscosity (0–5)	4.36 ± 0.26	3.63 ± 0.25
pH	7.56 ± 0.16	8.07 ± 0.17
Sperm motility (%)	64.60 ± 1.41[a]	17.64 ± 2.91[b]
Live sperm (%)	71.60 ± 2.62[a]	34.54 ± 3.98[b]
Sperm abnormality (%)	14.20 ± 1.20[a]	48.26 ± 3.84[b]
Sperm concentration ($x10^6$/ml)	234.60 ± 12.22[a]	54.50 ± 12.67[b]

Means within the same row with different superscripts are significantly different at $p < 0.05$.

14.20 ± 1.20 %). Furthermore, in fertile camels, a negative correlation ($p < 0.05$, $r = -0.42$) was shown between blood aldosterone and sperm motility percentage. However, infertile dromedaries positively correlated with seminal plasma aldosterone and sperm abnormalities ratio ($p < 0.05$, $r = 0.26$).

DISCUSSION

As observed in the present study, infertile camels had higher testosterone levels in their blood and seminal plasma than control fertile camels, suggesting spermatogenesis defects (Babu et al., 2004; Ali et al., 2018). This may be caused by an increase in interstitial cell density at the expense of seminiferous tubule density (Ali et al., 2018). Additionally, infertile camels' blood plasma testosterone levels may rise as a result of changes in the structure and function of leydig cells (Tibary, 2004; Ali et al., 2014). Infertile dromedary male camels may also have high blood testosterone levels due to increased LH levels (Fraietta et al., 2013). In contrast to previous findings, Waheed et al. (2015) observed a higher testosterone concentration in the serum of fertile dromedary males than infertile males. Several factors have been studied to determine what contributes to male infertility, including gonadotropin deficiency (Babu et al., 2004), spermatogenic failure (Bhasin, 2007), and androgen resistance (Fraietta et al., 2013). Moreover, infertile camels with elevated seminal testosterone levels in the present study suggest that they may be experiencing spermatogenic failure or androgen resistance. Consequently, testosterone can be used as a fertility-associated biomarker in the seminal plasma and blood of dromedary camels (Waheed et al., 2015).

The current study found no difference between the two groups in terms of aldosterone levels in the blood or seminal plasma. Dehydration, however, slightly altered aldosterone levels in dromedary camels (Ali et al., 2012). Water and electrolyte balances of the body are affected by aldosterone because it regulates sodium, potassium, and chloride metabolism (Yang and Ma, 2009). Thus, the detected aldosterone level in the present study indicates that both fertile and infertile camels were experiencing similar dehydration and water balance conditions. Both groups may be equally responsible for circulating aldosterone levels in the blood and in the presumed osmolality of plasma due to sodium levels. The current study confirmed that infertile camels' blood plasma testosterone and aldosterone were positively related. In contrast, experiments in male rats (Kau et al., 1999) suggest that testosterone inhibits aldosterone release at basal and in response to Ang II and corticotropin (ACTH). This mechanism occurs via inhibition of aldosterone synthase activity, cytochrome P-450 side-chain cleavage (P450scc) activity, and ACTH-stimulated c-AMP accumulation in the zona glomerulosa cells. Also, endogenous androgens (testosterone) exert anti-hypertensive effects that appear to involve non-genomic and possibly genomic mechanism(s), resulting in reductions in RAS expression in the kidney and enhanced systemic vasodilation in male rats (Hanson et al., 2020). However, the present study showed a negative association between blood aldosterone and seminal plasma testosterone in infertile camels.

According to the current findings, there was a significant difference in the motility, live sperm, concentration, abnormalities of sperm, and fertility status between the control and infertile male camels. Similar data were reported previously in dromedary camels (Mostafa et al., 2014, Waheed et al., 2015; Waheed et al., 2018). Poor semen quality can be caused by various factors, including impaired spermatogenesis, endocrine malfunctions, altered seminiferous tubular microenvironment, and accessory gland infections (Robaire and Hamzeh, 2011). There is also evidence that dromedary camels are genetically predisposed to testicular hypoplasia, which results in absent or atretic seminiferous tubules of the testicular parenchyma (Tibary, 2004; Ali et al., 2014). Likewise, the present study showed a negative association between blood plasma aldosterone and sperm motility in control camels. Furthermore, a positive relationship was indicated between seminal plasma aldosterone and sperm abnormality in the infertile camels. A possible link between aldosterone and semen quality is the local RAS regulator of seminal plasma electrolytes, steroidogenesis, spermatogenesis, and sperm function (Gianzo and Subirán, 2020). Also, the RAS acts locally through different paracrine and autocrine mechanisms (Gianzo and Subirán, 2020). However, the definitive explanation for aldosterone's influence on the semen quality of the dromedary camel still needs to be resolved.

CONCLUSIONS

Dromedary camels with infertility cases had elevated testosterone levels in their blood and seminal plasma. Consequently, low fertilization rates and poor-quality semen were experienced by infertile camels, possibly due to spermatogenic failure, electrolyte disruption, or androgen resistance. Furthermore, sub-fertility in male camels could be diagnosed early by measuring blood aldosterone relative to seminal plasma testosterone.

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ETHICS APPROVAL

Following the guidelines of the Animal Welfare Committee, the experimental procedures were approved by the Qassim University Ethics Committee, Saudi Arabia Kingdom.

FUNDING

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CONFLICT OF INTEREST

The authors declare no competing interests.

AVAILABILITY OF DATA AND MATERIALS

The authors ensure that all data and materials support the findings and comply with field standards. The farmer was informed of the study, and he gave his consent to data collection from his farm and animals.

NOVELTY STATEMENT

Assessing the semen quality and fertility of a male dromedary camels is fundamental. Even if infertile dromedary camels can be recognized through usual semen evaluations, the biomarkers are an important tool for choosing fertile camels or predicting fertility. Thus, the current study showed that evaluating blood aldosterone comparative to seminal plasma testosterone could be a promising alternative tool for early diagnosed of sub-fertility in male camels.

AUTHOR CONTRIBUTION

Zeitoun M.M. and Mansour M.M. conceived and designed research. Zeitoun M.M. conducted experiments. Mansour M.M analyzed data. Zeitoun M.M. and Mansour M.M. wrote the manuscript. All authors read and approved the manuscript.

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