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Free Androgen Index and Steroid Hormones Profile during Reproductive Season of Two Breeds of Camels with Ovarian Abnormalities

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ABSTRACT

Background: This study investigated the free androgen index (FAI) and steroid hormone profiles in two breeds of dromedary camels (Wadhha and Magaheem) with ovarian abnormalities during the reproductive season to assess hormonal disruptions linked to reproductive disorders.

Methods: A total of 129 female camels with ovarian abnormalities were categorized by breed (Wadhha and Magaheem) and reproductive season (early: Nov-Dec 2021; late: Jan-May 2022). Each group was further divided into nine subgroups based on ovarian pathology. Each group was divided into nine subgroups according to the types of ovarian abnormalities. The total testosterone (TT), sex hormone binding globulin (SHBG), estrone (E1) and 17β-estradiol (E2) measurements were done using ELISA kits and FAI was calculated.

Result: Inactive ovaries exhibited the highest TT levels in both breeds, with significant elevations also observed in camels with organized follicles (P<0.001). Hydrobursitis was associated with markedly increased E1, while inactive ovaries showed the lowest levels (P<0.001). Inactive ovaries had severely suppressed E2 in both breeds (P<0.001), with reductions also noted in hemorrhagic and organized follicles. Magaheem camels with multiple small follicles displayed the highest SHBG levels (P<0.001). Elevated free androgen index (FAI) was observed in camels with inactive ovaries and hydrobursitis (P<0.001), indicating hyperandrogenism. Ovarian abnormalities, particularly inactivity and organized follicles, disrupt steroidogenesis, elevating TT and FAI while reducing E2. Breed-specific and seasonal variations highlight the need for tailored reproductive management. The FAI emerges as a potential biomarker for ovarian dysfunction in camels.

Key words: Dromedary camel, Free androgen index, Physiology, Ultrasound.

INTRODUCTION

The one-humped camel (*Camelus dromedaries*) is a unique animal that survives and reproduces under severe climatic conditions that do not suit the survival of other species of domestic mammals (Burger *et al.,* 2019). Camel production in the study area faced constraints from feed shortages, water scarcity and diseases (Demlie Mulu *et al.,* 2023). Saudi Arabia has been classified as one of the countries with a high proportion of camel livestock (Kandeel *et al.,* 2023). Several breeds of Saudi camels are specifically bred for meat production, others are used in transport, work and races (El-Hanafy *et al.,* 2023). The camel is of significant socio-economic importance in the Saudi Arabia and its milk constitutes an important component of human diets in this country (Alghizzi and Shami, 2021; El-Hanafy *et al*., 2023). Camel milk, a superfood, benefits health with antihyperglycemic, antihyperlipidemic, anticancer, liver and kidney support (Mohammed *et al.,* 2024). The camel has been serving various purposes with a high economic value by providing meat, milk and wool as well as transportation especially in Saudi Arabia, contributes significantly to the economy (Bekele *et al.,* 2022).

Ultrasonic technologies have been employed in dromedary camels to observe the reproductive tract and investigate follicular dynamics. Camels develop both follicular and luteal cysts, however follicular cysts are more prevalent (El-Badry *et al.,* 2020) in addition to ovarian inactivity and hydrobursitis (Mansour and karen, 2021 and Ghallab *et al.,* 2022). Hormones and enzymes are very

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important in affecting the reproductive health of animals. Research studies have focused on many hormonal causes in cases of abnormal ovaries. However, some important hormonal and enzymatic indicators that reflect reproductive activity have not yet been studied (El-Badry *et al.,* 2020). Disruptions in hormonal and metabolic balances can cause the creation and persistence of ovarian follicles, leading to infertility issues (El-Badry *et al*., 2020). The development of ovarian follicles and the subsequent oocytes can be positively or negatively impacted by nutritional and nonnutritive feed additives and supplements as well as hormone injections (Mohammed *et al.,* 2024).

Traditional practices among desert camel raisers significantly influence female dromedary reproductive outcomes (Alharbi 2022). Hormonal patterns, including 17β-estradiol (E2) and testosterone (TT), are linked to follicular dynamics (Perez-Guerra *et al*., 2022). Sex hormone binding globulin (SHBG), primarily produced by the liver, binds and transports TT and E2, regulating their bioavailability. Higher SHBG levels may reduce free hormone availability, affecting reproduction, while factors like age, nutrition and disease influence SHBG (Pezzaioli *et al*., 2021). Only 2-4% of serum TT is biologically active, with the majority tightly bound to SHBG or loosely to albumin (Pezzaioli *et al*., 2021). Elevated hepatic lipogenesis may suppress SHBG production, leading to hyperandrogenism and ovarian dysfunction (Ong *et al*., 2019).

The free androgen index (FAI) is a unique ratio used to assay abnormal TT status in primates. The ratio is the TT level divided by the SHBG level and then multiplying by100 without unit (Zhao *et al.,* 2024). Their results proved that the elevation of FAI was correlated with the lowest clinical pregnancy outcomes. Ashraf *et al.* (2019) said that increased FAI recorded in women with ovarian abnormality. Chen *et al.* (2021) reported that FAI is a significant measure related to fertility outcomes in infertile PCOS women. Escobar-Morreale (2010) demonstrated that FAI, but not TT, effectively identified women with hirsutism or acanthosis nigricans. The aromatase activity index (AAI) quantifies aromatase's conversion of androgens (*e.g*., TT) to estrogens (*e.g.*, E2). García-Sánchez *et al*. (2022) found associations between E1 and AAI in breast cancer patients, highlighting AAI as the most reliable frailty biomarker, surpassing hormonal levels in predictive accuracy for frail individuals. Cheewasopit *et al*. (2018) demonstrated aromatase expression and estradiol (E2) production in bovine granulosa cells. Subsequent studies by Che *et al*. (2020) and Panghiyangani *et al*. (2020) revealed that reduced aromatase activity contributes to elevated androgen levels. Liu *et al*. (2021) further highlighted that impaired aromatase function may trigger estrogen-dependent disorders, including ovarian cancer and polycystic ovary syndrome (PCOS), underscoring its therapeutic relevance. Synthesis of estradiol by cytochrome P450 Family 19 subfamily mRNA expression the ovarian granulosa cells' aromatase enzyme, encoded by CYP19A1, transforms androgens into estrogen (Tej *et al.,* 2024).

However, measurements of these indices in camels with ovarian abnormalities remain unexplored. Therefore, the present study was used sera from 129 of two breeds of dromedary camels admitted to Salam Veterinary Hospital and Qassim University Teaching Veterinary Hospital with abnormal ovaries. The animals were divided into two main groups according to the date of sampling. Each group was divided into 9 subgroups according to the types of ovarian abnormalities. The TT, SHBG, E1 and E2 measurements were done using ELISA kits and FAI and AAI were calculated.

MATERIALS AND METHODS

Animal selection

129 female camels that admitted to Salam Veterinary Hospital and Qassim University Teaching Veterinary Hospital with abnormal ovaries where initially divided into white colored Wadhhaa and black colored Magaheem breeds based on the phenotypic classification of Saudi Arabian camels (Abdallah and Faye, 2013). Medical condition data such as age, color, disease problem, medical history of the condition and general health status were recorded. Each animal's reproductive system was inspected by a licensed veterinarian. Female camels that suffer from infertility or camels that prepared for the breeding season are initially diagnosed with routine transrectal palpation. Some females were examined while standing in closed cages or the females were restrained in sternal recumbence while the inspections were conducted on specially outfitted tractors.

Ultrasound examination

Ovaries were examined by ultrasonography. Ultrasound settings included a scanning depth of 6-8 cm, gain at 65-75%, dynamic range of 60-80 dB and the focal zone aligned at the ovary. Frame rate was maintained at 25-30 fps to ensure clear, real-time imaging. To obtain complete information about the investigated organ, the transducer was positioned over the proper organ and slightly shifted from one side to the other. The ovaries were examined for the presence of different kinds of follicles, corpora lutea and any aberrant structures after transmission gel had been applied to the transducer. Ovaries were examined utilizing real-time, B mode ultrasonography connected to a 5 MHz probe and linear transducers, along with routine transrectal palpation. Two ultrasound devices were used during the examination, SonoScape ultrasound, Model Number: SonoScape E2 (Guangdong, China, Mainland) and Esaote ultrasound**,** Model Number: MyLab™Omega**,** (Italy).

Animal grouping and sampling

Animals were divided according to the date of taking the samples into early season (Nov-Dec. 2021) and late season (Jan to May 2022). The animals with abnormal ovaries were divided into 9 groups (Fig 1): G1: Control, with normal ovary contain follicles with a diameter of 8 mm to 9 mm G2: Ovarian hydrobursitis, G3: Hemorrhagic follicle less than 35 mm, G4: Hemorrhagic follicle over 35 mm,

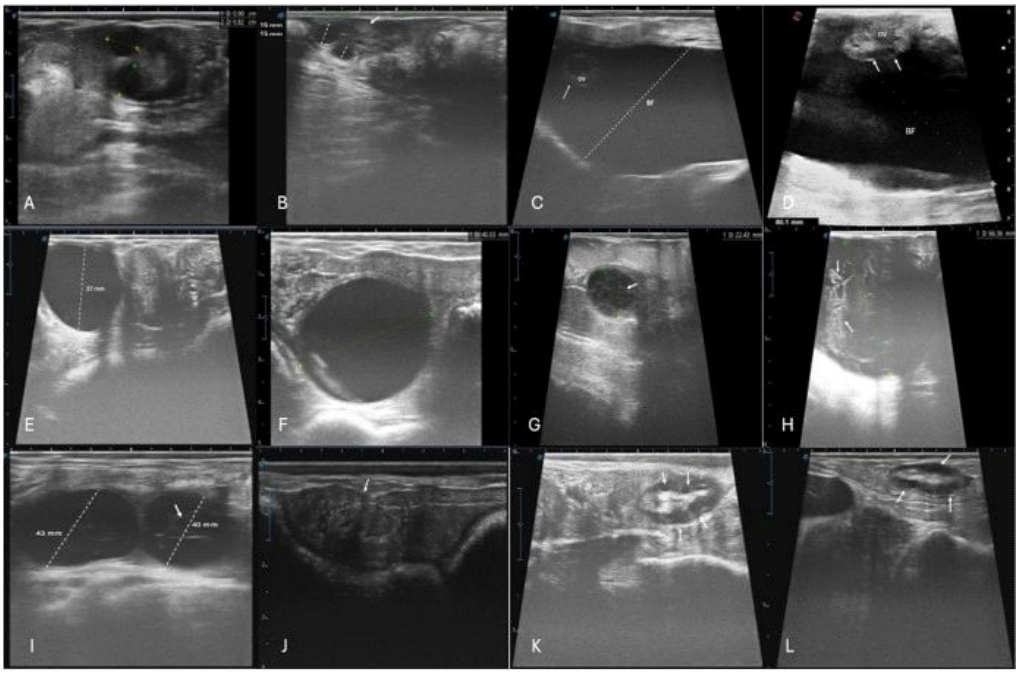
G5: organized folliculitis less than 25 mm, G6: Organized folliculitis over 25 mm, G7: Organized and hemorrhagic follicles, G8: Ovarian inactivity, G9: multiple small follicles under 8 mm. Blood samples were taken straight from the jugular vein in clean tubes and separating the serum in a centrifuge at 6000 r.p.m for 6 minutes, while preserving the ultrasound image of the abnormal ovaries. The serum kept under freezing -20°C until the time of analysis.

Hormonal analysis and indices calculation

The TT, SHBG, E1 and E2 measurements were done using camel ELISA kits from SunLong Biotech following the manufacturer's instructions. The catalogue numbers were SL 0011Cm, SL0068Cm, SL 0066Cm and SL 0027Cm respectively. The intra-assay variability is less than 10%, while inter-assay variability is less than 12% for all hormones. Sensitivity varies from 0.6 pg/ml for E2 to 8 pg/ml for TT. The regression equations for each hormone describe the relationship between optical density (OD) and concentration in pg/ml or ng/ml. TT follows OD = 0.2203 + 0.005318 pg/ml, SHBG has OD = -0.0758+0.1342 ng/ml, Estrone (E1) follows OD = 0.3226+0.006055 pg/ml and E2 follows OD = 0.1167+0.01039 pg/ml, indicating their respective detection and quantification parameters. All the analysis were done in Department of Medical Biosciences, College of Veterinary Medicine, Qassim University, Buraydah, Saudi Arabia.

Statistical analysis

Data values were represented as a means of standard errors. A straightforward one-way analysis of variances (ANOVA) test was conducted with the SAS program 20 (SAS, USA) for each measured parameter. The Tukey's HSD test was used in a post hoc analysis to compare the control normal group to other abnormal groups within the breed and season. A straightforward two-way ANOVA test was



A: Control group, normal ovaries contain follicles with a diameter of 9 mm and 8mm. B: Normal ovaries contain follicles with a diameter of 15 mm and 16 mm. C and D: Ovarian hydrobursitis group appeared as a large, well-demarcated, hypoechogenic sac encapsulating the ovary, the bursal fluid (BF) was clear and hypoechogenic. E: Hemorrhagic follicle less than 35 mm group, one hemorrhagic follicle cysts (diameter in 27 mm) on the left ovary. It had thin walls with clear hypoechogenic fluid. F: Hemorrhagic follicle over 35 mm group, one hemorrhagic follicle cysts (diameter in 40 mm) on the right ovary of she-camel. It had thin walls with clear hypoechogenic fluid. G: Organized follicle less than 25 mm group, one organized hemorrhagic follicle cysts (diameter in 23 mm) on the right ovary and many echogenic, transecting fibrinous strands (arrows). H: Organized folliculitis over 25 mm group, one organized hemorrhagic follicle cysts (diameter in 66 mm) on the right ovary and many echogenic, transecting fibrinous strands (arrows). I: Organized and hemorrhagic follicles group, one organized hemorrhagic follicle cysts (diameter in 40 mm) and many echogenic, transecting fibrinous strands (arrows). and one hemorrhagic follicle cysts (diameter in 43 mm). It had thin walls with clear hypoechogenic fluid on the right ovary. J: Ovarian inactivity group, inactive ovary (arrow) in both the right and left ovaries. K and L: Ovary with normal multiple small follicles (the follicles under 8 mm in diameter) on the right and left ovaries.

Fig 1: Ultrasonography images of different camel ovaries.

conducted to obtain the complex interactions of breed, season and ovarian conditions.

RESULTS AND DISCUSSION

TT levels of Wadhha and Magaheem camels with ovarian abnormalities

TT levels in Wadhha breed with normal ovaries varied significantly between seasons, reflecting the natural hormonal rhythm of the reproductive cycle (Table 1). Early-season levels were recorded at 35.79±7.84 pg/ml, decreasing substantially to 19.377±0.654 pg/ml in the late season. Small hemorrhagic F. caused significant elevations in TT levels, particularly in the early season, where levels spiked to 45.90±6.60 pg/ml. In the late season, levels decreased to 64.28±3.59 pg/ml ($p<0.001$), though they remained elevated relative to the control. Large hemorrhagic F. exhibited slightly lower TT levels compared to their small counterparts but remained significantly elevated. Early-season levels were recorded at 69.75±4.58 pg/ml ($p<0.001$), decreasing marginally to 60.93±9.86 pg/ml in the late season. Small, organized F. had a profound effect on TT levels. Early-season levels were significantly elevated at 80.89±9.26 pg/ml ($p<0.001$), while late-season

Table 1: SHBG, TT and FAI levels of dromedary camels with ovarian abnormalities.

Breeds and season	Ovarian problem	SHBG (ng/ml)	TT (pg/ml)	Free androgen index (FAI)
Wadhha	Normal ovary (N=4)	8.817±0.460	35.79±7.84	0.116±0.029
(Early -Nov-Dec)	Hydrobursitis (N=3)	9.957±0.709	20.13±1.01	1.524±0.198b
	Hemorrhagic F. (<35mm) (N=3)	9.250±1.020	45.90±6.60	1.13±0.353
	Hemorrhagic F. (>35mm) (N=4)	9.461±0.785	69.75±4.58c	0.741±0.013
	Organized F. (<25mm) (N=3)	10.054±0.693	80.89±9.26c	0.797±0.037
	Organized F. (>25mm) (N=4)	9.331±0.189	53.53±8.50	0.58±0.103
	Hemorrhagic F. + Organized F. (N=4)	12.021±0.241	84.28±5.88c	0.705±0.063
	In active ovary (N=3)	8.096±0.308	170.5±1.96c	2.127±0.109c
	Multiple small F. (N=3)	18.06±2.960b	84.30±21.10	0.495±0.146
Wadhha	Normal ovary (N=3)	9.10±1.01	19.377±0.654	0.224±0.032
(Late -Jan - May)	Hydrobursitis (N=3)	13.58±2.77	28.811±0.218	1.657±0.211c
	Hemorrhagic F. (<35mm) (N=4)	9.44±1.20	64.28±3.59c	0.701±0.051
	Hemorrhagic F. (>35mm) (N=4)	10.23±1.53	60.93±9.86	0.652±0.164
	Organized F. (N=4) (<25mm)	9.140±0.559	166.48±4.28c	1.866±0.153b
	Organized F. (>25mm) (N=4)	9.748±0.882	79.30±13.50a	0.878±0.208
	Hemorrhagic F. + Organized F. (N=3)	10.996±0.832	107.10±19.10c	1.031±0.251
	In active ovary (N=4)	8.171±0.250	177.20±11.50c	1.483±0.484b
	Multiple small F. (N=3)	15.84±4.88	84.60±26.70	0.532±0.122
Magaheem	Normal ovary (N=3)	8.507±0.665	20.23±1.140	0.239±0.005
(Early- Nov-Dec)	Hydrobursitis (N=4)	11.732±0.896a	25.73±1.26	1.473±0.395b
	Hemorrhagic F. (<35mm) (N=3)	8.742±0.678	67.68±9.92a	0.756±0.065
	Hemorrhagic F. (>35mm) (N=3)	8.113±0.867	75.32±9.10c	0.999±0.219
	Organized F. (<25mm) (N=4)	7.267±0.065	158.53±2.56c	2.183±0.055c
	Organized F. (>25mm) (N=4)	11.447±0.688	135.70±9.20c	1.213±0.153
	Hemorrhagic F. + Organized F. (N=4)	9.491±0.037	109.0±18.0c	1.146±0.185
	In active ovary(N=4)	9.378±0.184	176.61±9.18c	1.884±0.092c
	Multiple small F. (N=4)	24.815±0.770c	22.96±1.59	0.092±0.004
Magaheem	Normal ovary (N=4)	27.174±0.041	89.30±41.40	0.328±0.152
(Late -Jan - May)	Hydrobursitis (N=4)	13.161±0.951c	16.830±0.926	1.431±0.297a
	Hemorrhagic F. (<35mm) (N=3)	7.688±0.191c	54.00±7.68	0.696±0.083
	Hemorrhagic F. (>35mm) (N=4)	10.598±0.112c	96.70±23.00	0.906±0.207
	Organized F. (<25mm) (N=4)	8.314±0.316c	169.28±6.15c	2.054±0.152b
	Organized F. (>25mm) (N=4)	9.073±0.493c	93.47±8.65c	1.057±0.139
	Hemorrhagic F. + Organized F. (N=3)	8.295±0.654c	77.774±0.054c	0.955±0.075
	In active ovary (N=3)	8.663±0.555c	175.73±7.33c	2.066±0.147c
	Multiple small F. (N=4)	18.05±3.28c	72.50±28.40	0.541±0.255

Mean±standard error. Values with letter a, b and c are measuring the difference than control group within the same season at $P<0.05$. $P<0.01$ and $P<0.001$ respectively. TT: Total testosterone; SHBG: Sex hormone binding globulin; F: Follicle.

levels surged dramatically to 166.48±4.28 pg/ml (p<0.001). Larg organized F., TT levels were recorded 53.53±8.50 pg/ml, while late-season levels rose to 79.30±13.50 pg/ml (p<0.05). The coexistence of hemorrhagic and organized F. caused compounded disruptions in TT regulation. Early- season levels reached 84.28±5.88 pg/ml (p<0.001), while late-season levels increased further to 107.10±19.10 pg/ml (p<0.001). Inactive ovaries exhibited the highest TT levels among all conditions, with early-season levels at 170.51±1.96 pg/ml (p<0.001) and late-season levels slightly increasing to 177.20±11.50 pg/ml (p<0.001).

TT levels in Magaheem breed with normal ovaries exhibited significant seasonal variation, with early-season levels recorded at 20.23±1.140 pg/ml, increasing sharply to 89.30±41.40 pg/ml in the late season. Small hemorrhagic F. caused significant elevations in TT levels, particularly during the early season, where levels spiked to 67.68±9.92 pg/ml (p<0.05). Late-season levels, though reduced, remained elevated at 54.00±7.68 pg/ml. Larger hemorrhagic F. demonstrated even greater TT elevations, with early-season levels at 75.32±9.10 pg/ml (p<0.001) and late-season levels increasing further to 96.70±23.00 pg/ml. Small organized F. resulted in the most significant elevations in TT levels. Early-season levels were recorded at 158.53±2.56 pg/ml (p<0.001), with late-season levels increasing further to 169.28±6.15 pg/ml (p<0.001). Larger organized F. also caused significant TT elevations, early-season levels were 135.70±9.20 pg/ml (p<0.001), while late-season levels decreased to 93.47±8.65 pg/ml (p<0.001). The coexistence of hemorrhagic and organized F. compounded the effects on TT levels. Early-season levels reached 109.0±18.0 pg/ml (p<0.001), while late-season levels decreased to 77.774±0.054 pg/ml (p<0.001). Inactive ovaries exhibited the highest TT levels among all conditions. Early-season levels were 176.61±9.18 pg/ml (p<0.001) and late-season levels remained consistent at 175.73±7.33 pg/ml (p<0.001).

E1 levels of Wadhha and Magaheem camels with ovarian abnormalities

E1 levels were significantly elevated in Wadhha breed with hydrobursitis (Table 2). During the early season, levels reached 21.21±1.89 pg/ml and during the late season, they remained similarly high at 21.13±2.32 pg/ml (p<0.01). For other abnormalities, E1 levels were slightly lower or higher but remained close to normal. Among all conditions in Magaheem breed, hydrobursitis exhibited the highest E1 levels, with significantly elevated values in both the early (29.519±0.928 pg/ml) and late (28.51±1.47 pg/ml) seasons (p<0.001). Small hemorrhagic F. significantly reduced E1 levels compared to the control group, particularly in the early season (3.464±0.715 pg/ml, p<0.01). Small organized F. significantly suppressed E1 levels, particularly in the late season, where levels dropped to 2.770±0.710 pg/ml compared to 4.82±1.04 pg/ml in the early season (p<0.05). Inactive ovaries exhibited persistently low E1 levels across seasons, with 4.437±0.138 pg/ml in the early season (p<0.01) and 4.383±0.830 pg/ml in the late season. Camels with multiple small F. showed significantly elevated E1 levels, with 28.26±3.17 pg/ml in the early season and 20.64±7.52 pg/ml in the late season (p<0.001).

E2 levels of Wadhha and Magaheem camels with ovarian abnormalities

Large hemorrhagic F. of Wadhha breed displayed a significant E2 reduction in the late season. Early-season levels were 490.6±23.0 pg/ml, decreasing to 384.1±13.3 pg/ml (p<0.05) in the late season. Larger organized F. exhibited significantly reduced E2 levels. Early-season levels were 385.34±4.78 pg/ml (p<0.05), increasing to 423.8±35.1 pg/ml in the late season. Inactive ovaries exhibited the lowest E2 levels among all conditions. Early-season levels were 138.8±11.5 pg/ml (p<0.001), increasing slightly to 158.5± 24.2 pg/ml (p<0.001) in the late season. Camels with multiple small follicles demonstrated mild E2 reductions. Early-season levels were 391.2±12.3 pg/ml (p<0.05), further decreasing to 353.9±34.1 pg/ml in the late season. Magaheem breed with hydrobursitis displayed a substantial reduction in E2 levels. Early-season levels were 360.4± 34.0 pg/ml, decreasing further to 331.1±18.1 pg/ml (p<0.05) in the late season. Large hemorrhagic F. demon-strated a significant E2 reduction in the late season. Early-season levels were 568.6±35.2 pg/ml, dropping to 334.1± 14.0 pg/ml (p<0.05). Larger organized F. showed significantly reduced E2 levels. Early-season levels were 378.3±16.6 pg/ml (p<0.05), decreasing to 354.2±7.0 pg/ml (p<0.05) in the late season. Inactive ovaries exhibited the most significant E2 suppression. Early-season levels were 202.5±33.4 pg/ml (p<0.001), with a sharp late-season decrease to 146.6±19.6 pg/ml (p<0.001). Camels with multiple small follicles showed mild E2 reductions. Early-season levels were 378.2±21.4 pg/ml (p<0.05), remaining stable at 373.9±11.0 pg/ml (p<0.05) in the late season.

SHBG of wadhha and magaheem camels with ovarian abnormalities

Different abnormalities of Wadhha breed were associated with elevated SHBG levels, particularly in the late season, without significant difference. Multiple small follicles led to the most significant elevation in SHBG levels, with early-season levels reaching 18.06±2.960 ng/ml (p<0.01) and late-season levels at 15.84±4.88 ng/ml. SHBG levels in control Magaheem breed with normal ovaries showed a stark seasonal variation, with early-season levels at 8.507±0.665 ng/ml and late-season levels surging to 27.174±0.041 ng/ml. Hydrobursitis was associated with elevated SHBG levels, especially in the late season, where levels reached 13.161±0.951 ng/ml (p<0.001). Early-season levels, though slightly lower at 11.732±0.896 ng/ml (p<0.05), still exceeded the control group. Small hemorrhagic F. displayed a unique pattern, with SHBG levels at 8.742±0.678 ng/ml in the early season and a significant reduction to 7.688±0.191 ng/ml (p<0.001) in the late season. Larger hemorrhagic F. had a more pronounced impact on

SHBG dynamics, with levels increasing from 8.113± 0.867 ng/ml in the early season to 10.598±0.112 ng/ml (p<0.001) in the late season. Small organized F. demonstrated a complex interaction with SHBG levels. Early-season levels were significantly reduced at 7.267±0.065 ng/ml, while late-season levels increased to 8.314±0.316 ng/ml (p<0.001). Larger organized F. maintained consistently elevated SHBG levels across seasons, with early-season levels at 11.447±0.688 ng/ml and late-season levels at 9.073±0.493 ng/ml (p<0.001). The coexistence of hemorrhagic and organized F. caused significant SHBG elevation in both seasons. Early-season levels reached 9.491±0.037 ng/ml, while late-season levels were slightly lower at 8.295±0.654 ng/ml (p<0.001). Inactive ovaries exhibited near-normal SHBG levels during the early season (9.378±0.184 ng/ml) but showed a significant reduction in the late season (8.663±0.555 ng/ml, p<0.001). Small multiple F. caused the most dramatic elevation in SHBG levels. Early-season levels were significantly elevated at 24.815±0.770 ng/ml (p<0.001) and late-season levels, though reduced, remained high at 18.05±3.28 ng/ml (p<0.001).

FAI of Wadhha and Magaheem camel with ovarian abnormalities

Wadhha breed with hydrobursitis exhibited significantly elevated FAI levels. Early-season values reached

Table 2: E1 and E2 levels of dromedary camels with ovarian abnormalities.

Breeds and season	Ovarian problem	E1 (pg/ml)	E2 (pg/ml)
Wadhha (Early-Nov-Dec)	Normal ovary (N=4)	9.000±0.805	520.9±40.8
	Hydrobursitis (N=3)	21.21±1.89b	380.1±29.4
	Hemorrhagic F. (<35 mm) (N=3)	7.93±1.05	519.0±21.8
	Hemorrhagic F. (>35 mm) (N=4)	10.393±0.189	490.6±23.0
	Organized F. (<25 mm) (N=3)	7.20±1.47	480.03±5.77
	Organized F. (>25 mm) (N=4)	8.836±0.521	385.34±4.78a
	Hemorrhagic F. + Organized F. (N=4)	7.607±0.757	430.7±76.8
	In active ovary (N=3)	4.820±0.393	138.8±11.5c
	Multiple small F. (N=3)	16.62±5.86	391.2±12.3a
Wadhha (Late-Jan-May)	Normal ovary (N=3)	9.328±0.237	527.7±40.6
	Hydrobursitis (N=3)	21.13±2.32b	454.6±48.9
	Hemorrhagic F. (<35 mm) (N=4)	8.84±1.37	533.2±30.3
	Hemorrhagic F. (>35 mm) (N=4)	8.71±1.43	384.1±13.3a
	Organized F. (N=4) (<25 mm)	4.109±0.958	463.0±31.7
	Organized F. (>25 mm) (N=4)	8.836±0.142	423.8±35.1
	Hemorrhagic F. + Organized F. (N=3)	8.016±0.994	453.8±41.0
	In active ovary (N=4)	4.93±1.11	158.5±24.2c
	Multiple small F. (N=3)	16.84±3.60	353.9±34.1
Magaheem (Early-Nov-Dec)	Normal ovary (N=3)	13.51±1.33	575.7±26.3
	Hydrobursitis (N=4)	29.519±0.928c	360.4±34.0
	Hemorrhagic F. (<35 mm) (N=3)	3.464±0.715b	597.4±30.2
	Hemorrhagic F. (>35 mm) (N=3)	11.049±0.379	568.6±35.2
	Organized F. (<25 mm) (N=4)	4.82±1.04a	473.5±47.6
	Organized F. (>25 mm) (N=4)	7.443±0.379	378.3±16.6a
	Hemorrhagic F. + Organized F. (N=4)	7.85±1.09	417.9±30.3
	In active ovary(N=4)	4.437±0.138b	202.5±33.4c
	Multiple small F. (N=4)	28.26±3.17c	378.2±21.4a
Magaheem (Late-Jan-May)	Normal ovary (N=4)	9.246 ±0.663	543.2±24.0
	Hydrobursitis (N=4)	28.51±1.47c	331.1±18.1a
	Hemorrhagic F. (<35 mm) (N=3)	4.492±0.473	404.7±86.6
	Hemorrhagic F. (>35 mm) (N=4)	9.656±0.047	334.1±14.0a
	Organized F. (<25 mm) (N=4)	2.770±0.710	449.5±39.8
	Organized F. (>25 mm) (N=4)	7.28±1.22	354.2±7.0a
	Hemorrhagic F. + Organized F. (N=3)	5.148±0.473	346.6±41.0
	In active ovary (N=3)	4.383±0.830	146.6±19.6c
	Multiple small F. (N=4)	20.64±7.52	373.9±11.0a

Mean±Standard Error. Values with letter a, b and c are measuring the difference than control group within the same season at P<0.05. P<0.01 and P<0.001 respectively.; F: follicle E2: estradiol; E1: estrone.

1.524±0.198 ng/ml (p<0.01), increasing slightly to 1.657±0.211 ng/ml (p<0.001) in the late season. Smaller organized F. displayed variable FAI values. Early-season levels were 0.797±0.037 ng/ml, while late-season values increased significantly to 1.866±0.153 ng/ml (p<0.01). Inactive ovaries displayed significantly elevated FAI values. Early-season levels were 2.127±0.109 ng/ml (p<0.001), while late-season values decreased to 1.483±0.484 ng/ml (p<0.01). Magaheem breed with hydrobursitis displayed significantly elevated FAI values. Early-season levels reached 1.473± 0.395 ng/ml (p<0.01), with a slight decrease to 1.431±0.297 ng/ml (p<0.05) in the late season. Smaller organized F. displayed significantly elevated FAI values. Early-season values were 2.183±0.055 ng/ml (p<0.001), with late-season values remaining high at 2.054±0.152 ng/ml (p<0.01). The coexistence of hemorrhagic and organized F. resulted in moderate FAI levels. Early-season values were 1.146±0.185 ng/ml, decreasing slightly to 0.955± 0.075 ng/ml in the late season. Inactive ovaries showed significantly elevated FAI values. Early-season levels were 1.884±0.092 ng/ml (p<0.001), which increased to 2.066± 0.147 ng/ml (p<0.001) in the late season.

TT, E1, E2 and SHBG levels of of wadhha and magaheem camels with ovarian abnormalities

The present study provides critical insights into the relationship between serum hormone levels and ovarian abnormalities in Wadhha and Magaheem camels during early and late breeding seasons. Testosterone (TT) levels exhibited significant seasonal variations in camels with normal ovaries, reflecting natural reproductive cyclicity. Notably, hemorrhagic follicles caused substantial TT elevation, particularly during the early season (November-December), likely due to increased theca cell activity mediated by CYP17 enzyme dysregulation (Secchi *et al*., 2021). This finding aligns with established knowledge about theca cell function in androgen production. The highest TT levels occurred in inactive ovaries, suggesting a severe endocrine disruption in this condition. Estrone (E1) levels showed an inverse pattern, with significant reductions in hemorrhagic follicles and inactive ovaries during the early season, consistent with Madhi *et al*. (2022) findings on altered steroidogenesis in inactive ovaries. The study revealed intriguing breed-specific responses, with Wadhha camels showing elevated E1 levels in hydrobursitis cases, potentially due to fluid accumulation disrupting follicular function (Benaissa *et al*., 2014). Estradiol (E2) dynamics demonstrated clear seasonal patterns, with marked late-season reductions across both breeds. The positive correlation between E2 levels and follicular dimensions (El-Badry *et al*., 2020) was evident, as multiple small follicles showed the highest E2 levels indicating active folliculogenesis, while inactive ovaries exhibited the lowest levels. These hormonal patterns were significantly influenced by seasonal factors, with winter months showing greater ovarian activity compared to summer (Ashour *et al*., 2017; Senthilkumar *et al*., 2025), likely due to favorable environmental conditions during the traditional breeding season (November-March) as described by Ainani *et al*. (2018).

SHBG dynamics in ovarian pathologies

The investigation of sex hormone-binding globulin (SHBG) revealed important patterns in camels with ovarian abnormalities. Complex follicular conditions, particularly hemorrhagic follicles combined with organized follicles, were associated with elevated SHBG levels in both seasons. This elevation may stem from increased estradiol production stimulating hepatic SHBG synthesis (Brianso-Llort *et al*., 2024), consistent with SHBG's known role in regulating steroid bioavailability (Simó *et al*., 2015). The most dramatic SHBG elevation occurred in camels with multiple small follicles, strongly supporting the link between follicular activity and SHBG production. Interestingly, inactive ovaries showed near-normal SHBG levels early in the season but significant late-season reductions, suggesting progressive endocrine dysfunction. These findings align with murine models showing altered SHBG synthesis in ovarian abnormalities (Qu and Donnelly, 2020), though the stable SHBG levels in organized follicles present an intriguing discrepancy requiring further investigation. The study also highlighted nutritional influences on SHBG levels, as demonstrated by Brianso-Llort *et al*. (2024), emphasizing the complex interplay between metabolic factors and reproductive endocrinology in camels.

Free androgen index as a diagnostic marker

The free androgen Index (FAI), calculated as (TT/SHBG)× 100, emerged as a valuable indicator of hyperandro-genemia in camel ovarian disorders. The study found that hydrobursitis cases showed significantly elevated FAI levels, suggesting that inflammatory conditions disrupt hormonal equilibrium and promote androgen synthesis. This aligns with Mohammadi *et al*. (2017) and Ong *et al*. (2019) work on proinflammatory cytokines upregulating CYP17 and androgen production. Notably, inactive ovaries demonstrated the highest FAI levels, indicating a potential camel analogue to polycystic ovary syndrome (PCOS) in other species. Conversely, camels with multiple small follicles exhibited the lowest FAI values, suggesting minimal androgenic influence despite active folliculogenesis. The late season brought further FAI elevation in hydrobursitis cases, reinforcing the connection between ovarian inflammation and androgen excess. These findings support FAI's utility as a diagnostic tool for ovarian dysfunction in camels, though its reliability may be compromised when SHBG concentrations are low (Keevil *et al*., 2018). The consistent elevation of FAI in inactive ovaries across both seasons strongly suggests this condition represents a distinct endocrine disorder in dromedaries.

Comparative breed analysis of hormonal profiles

The comparative analysis between Wadhha and Magaheem breeds revealed significant strain-specific hormonal variations. Magaheem camels showed more

pronounced late-season TT increases in hemorrhagic follicles, while Wadhha camels exhibited greater elevations in severe conditions like inactive ovaries. In hydrobursitis cases, Magaheem consistently displayed higher E1 levels than Wadhha, whereas Wadhha showed stronger E2 compensatory responses in this condition. The breeds also differed in their SHBG dynamics, with Magaheem showing more dramatic seasonal variations and severe disruptions compared to Wadhha. These differences likely reflect genetic distinctions between the breeds, as noted by AlAskar *et al*. (2020) and Mahmoud *et al*. (2020), though both populations show considerable genetic admixture. The FAI patterns further highlighted breed differences, with Magaheem exhibiting higher values in organized and hemorrhagic follicles, while Wadhha maintained elevated values in hydrobursitis and multiple small follicles. These findings underscore the importance of breed-specific considerations in camel reproductive management.

Clinical implications and future directions

The study's findings have significant implications for camel breeding and veterinary practice. The identification of hormonal patterns associated with specific ovarian abnormalities provides valuable diagnostic markers for reproductive disorders. The PCOS-like profile observed in inactive ovaries suggests potential avenues for therapeutic intervention, possibly including anti-inflammatory approaches as suggested by Mohammadi *et al*. (2017). The breed-specific differences highlight the need for tailored management strategies, particularly given the economic importance of both Wadhha (valued for milk, meat and beauty contests) and Magaheem breeds in Arabian Peninsula (Burger *et al*., 2019). Future research should focus on elucidating the molecular mechanisms underlying these hormonal disruptions, particularly the role of CYP17 in TT elevation and the factors influencing SHBG production in different follicular conditions. Longitudinal studies tracking individual camels across multiple seasons could further clarify the progression of these endocrine abnormalities. Additionally, investigation of nutritional interventions to modulate SHBG levels, as suggested by Brianso-Llort *et al*. (2024), may offer practical management strategies for improving reproductive outcomes in camel herds.

CONCLUSION

This comprehensive investigation of hormonal dynamics in Wadhha and Magaheem camels with ovarian abnormalities has revealed complex interactions between reproductive endocrinology, seasonal factors and breed characteristics. The study establishes clear patterns of TT, E1, E2 and SHBG variation associated with different pathological conditions, providing a foundation for improved reproductive management in dromedary camels. The identification of FAI as a potential diagnostic marker for ovarian dysfunction offers practical clinical applications, while the breed-specific findings emphasize the need for customized approaches to camel reproduction. These results significantly advance our understanding of camel reproductive endocrinology and highlight important areas for future research to enhance breeding efficiency and animal health in these economically and culturally important species.

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Disclaimers

The authors' views and conclusions in this study are solely their own and do not necessarily reflect the views of their affiliated institutions. They are responsible for the accuracy and completeness of the information provided, but they accept no liability for any direct or indirect harm resulting from the use of this content.

Informed consent

On October 29, 2023, the Bioethics Certificate was obtained from King Abdulaziz City for Science and Technology's National Committee for Bioethics. The animal experiment used in the current study was approved by the Standing Committee for Scientific Research Ethics at Qassim University, No. 24-06-05. There were no operations conducted that may cause pain, suffering, or discomfort to the animals. Blood samples were collected via jugular vein.

Conflict of interest

The authors of this work state that they have no conflicts of interest related to its publication. There was no impact from funding or support on the study's design, data collection, analysis, manuscript preparation, or publication decision.

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