The present study aimed to clarify the phenomenon of presence of larger than normal follicles (OVGF) in female dromedary camels. Females with OVGF (n=125) were examined by manual palpation and ultrasonography. Accordingly, the OVGF were subdivided into those with thin walls and clear hypoechogenic content (OVGF-TH, n=18) and those with thick walls and fibrous trabeculae (OVGF-TK, n=107). Transvaginal follicle aspiration was performed in females with OVGF and from a control group with growing follicles (1-2 cm in diameter, GF group, n=5). Serum was collected at the same time of follicle aspiration and analyzed for Follicle-stimulating hormone (FSH), luteinizing hormone (LH), progesterone (P4) and estradiol-17β profiles (E2). The follicular fluid (FF) was analyzed for E2 and P4. The results showed that mean E2 concentration in FF and serum were lower in OVGF-TH and OVGF-TK groups than in the GF group (P<0.05). Difference between OVGF-TH and OVGF-TK groups was not significant. P4 in FF did not significantly differ among groups. Positive correlation was found between E2 in FF and E2 in serum (r = 0.495, r = 0.03). Mean FSH concentration in serum was higher in OVGF-TH and OVGF-TK groups than in the GF group (P = 0.03). Mean LH concentration was non-significantly (P=0.1) greater in OVGF-TH and OVGF-TK groups than in the GF group. In conclusion, female dromedary camels with OVGF had endocrine characteristics differed from camels with no OVGF. It seems that the high FSH and/or LH concentration(s) stimulated the continuing growth of the developing follicles to reach these large sizes, suggesting that the phenomenon of OVGF in camels is a pathological finding.

Key words:
Dromedary camels; oversized follicles; hormones; follicular fluid; ovary.

1. Introduction
The estrus cycle in camels has certain uniqueness, it did not have a luteal phase, the cycle is strictly follicular, and the ovulation is induced [1,2]. The follicular cycle has been subdivided into growth, mature and regression phases [3-5]. In non-mating females, the dominant follicle brings into being atretic, however, in certain females the dominant follicle continued to grow to Larger than typical follicles (OVGF, >2 cm in diameter) [3,4].

There is an argument regarding the pathogenicity of this phenomenon in camels. Certain studies assumed that it does not similar to the cystic ovarian disease (COD) of cattle. If the female camel is not pregnant, large numbers of follicles can develop some form of cystic ovaries, because ovulation is induced [2,6]. Others have observed the OVGF in combination with
some reproductive disorders including ovarian hydrobursitis and clinical endometritis [7]. Biochemical and hormonal contents of the follicular fluid in camels have been investigated by some researches, mostly from ovaries collected from the slaughterhouse with no attached breeding history [8-12]. None of these studies, however, tried to associate the presence of this OVGF with the peripheral gonadotropin concentrations to understand the occurrence of this phenomenon in camels. In cattle, a transient increase in FSH preceded detection of follicular cysts [13]. Moreover, an increase in FSH secretion following a decrease in inhibin initiated revenue of cystic follicles in dairy cows [14]. Besides, during the follicular phase, the concentration of LH was higher in cows with cysts than in cows without cysts [15].

The relativeness of endocrinological changes in camels with larger than normal follicles is still unclear. In the current study, the interrelationship between the occurrence of oversized follicles and the peripheral and intra-follicular concentrations of progesterone (P4), estradiol-17beta (E2), luteinizing hormone (LH) and follicle-stimulating hormone (FSH) were investigated in female dromedary camels.

2. Materials and Methods

This study was approved by the Animal Care and Welfare Committee, Deanship of Scientific Research, Qassim University, Kingdom of Saudi Arabia.

2.1. Animals

Female dromedary camels (n=1263) were examined for reasons of failure of conception at the Veterinary Teaching Hospital of Qassim University, during the breeding season from September 2017 to March 2018. Of them, 125 were found with oversized follicles (OVGF, > 2 cm in diameter, Skidmore, 2011) with no further detectable pathological findings. Most of the females with OVGF were multipara (101/125, 80.8%), while fewer were primi-(16/125, 12.8%) or nullipara (8/125, 6.4%). They were non-lactating and aged 7 to 12-year-old. Primi- and multipara were presented for examination 8-12 months after parturition. The animals were originated from herds using natural mating, where males were running with females all the time. The body condition scores of OVGF females ranged from 3 to 4 on a 5-point scale [16]. They were grazed on open pastures. Alfalfa (Medicago sativa) was provided. The herders seldom supplemented the diet. Five female camels (multipara, 7-12 years) with growing follicles (1-2 cm in diameter) were used as a control group (GF group).

2.2. OVDF Classification

The genital tract of each animal was examined through transrectal palpation and by ultrasonographic examination using Real-time, B-mode ultrasonic apparatus (Aloka SSD 500, Tokyo, Japan) attached to a 5 MHz transrectal transducer. The examinations were carried out on specially equipped tractors, whereas the females were secured in sternal recumbence. The transducer was placed over the appropriate organ and moved a little from one side to the other to get full information on the examined organ. The ultrasonic images were frozen on the monitor, and the dimensions were estimated at the maximum size by the electronic caliper. According to manual palpation and ultrasonographic examination, the OVGF were subdivided into two main groups, those with thin walls and clear hypoechogetic content (OVGF-TH, n=18, Figure 1A) and those with thick walls and fibrous trabeculae (OVGF-TK, n=107, Figure 1B, C).

Figure 1. Ultrasonographic images for the oversized ovarian follicles (OVGF) in female dromedary camels: (A) OVGF with thin wall, clear hypoechogetic content and of 3.5 cm maximum diameter; (B) OVGF with thick wall, moderate transecting trabeculae and of 5.7 cm maximum diameter; (C) OVGF with thick wall, heavy transecting trabeculae and of 7.3 cm maximum diameter.

2.3. Follicle aspiration

Follicle aspiration was carried out from females with OVGF and from the GF group. When more than one growing follicle was present in the GF group, the largest one was only aspirated. The OVGF and growing follicles were aspirated transvaginally using a specially designed 1.2 mm x 500 mm cannula with 45o angle tip [17]. Briefly, the ovaries were first examined ultrasonographically to identify the location of the OVGF. After eliminating the probe, the cannula was progressed in the anterior part of the vagina being covered by an insemination pipette. The OVGF carrying ovary was manipulated per
The cannula was then advanced to perforate the vaginal wall into the OVG. Follicular fluid (FF) was aspirated under transrectal manual control using a 20 mL syringe connected with the cannula. This technique was carried out without special precaution or sedation. The FFs were stored at -20 until analyzed for E2 and P4.

### 2.4. Hormonal analysis

Jugular blood was collected from all OVG and GF camels between 8.00 and 10.00 AM at the same time as follicle aspiration. The serum was harvested and stored until analyzed for LH, FSH, E2, and P4. The FSH and LH were determined by specific ELISA using kits designed for the quantitative determination of camel FSH and LH (Life Sciences Advanced Technologies Inc., 2900 72nd St, N Saint Petersburg, FL 33710, USA). Serum concentrations of E2 and P4 were determined by ELISA using commercial kits (Human Gesellschaft fur Biochemica und Diagnostica, Wiesbaden, Germany). The coefficients of variation of the intra- and inter-assays were 4.4, 5.6, 3.7 and 5.1 % and 4.5, 5.9, 6.4 and 6.8%, and the sensitivity of the assay was measured at 0.1 mIU/mL, 0.1 mIU/mL, 3 pg/mL and 0.12 ng/mL for the FSH, LH, E2 and P4, respectively.

### 2.5. Statistical analysis

The data were presented in mean ± SE, and statistical analysis was carried out using the SPSS program, version 24 (2016). Analysis of variance (ANOVA) was used for the comparison between groups. When a significant difference was recorded, the LSD test was used to determine how the means differed. Relationships between hormonal levels were estimated by the correlation coefficient. The level of significance set at P < 0.05.

### 3. Results

The diameter of the OVG ranged between 3 and 12 cm (average = 5.16 cm). Distribution of the OVG between right and left ovary did not significantly differ (59.2% vs. 48.8%). Most of the OVG were single (98/125, 78.4%), while few cases had double (19/125, 15.2%) or triplets (8/125, 6.4%) OVG. The OVG were detected in association with growing follicles in 69 cases (55.2%). The other cases had no further structures. No case with OVG had a corpus luteum. All OVG females had the history of repeat breeding with regular (n = 106) or irregular (n = 19) heat intervals.

Mean E2 concentration in FF was significantly lower in OVG-TH and OVGH-TK groups than in the GF group (P = 0.006). Difference between OVG-TH and OVGH-TK groups was not significant. P4 in FF did not significantly differ among groups (P = 0.5), (Figure 2).

Mean E2 concentration in serum was significantly lower in OVG-TH and OVGH-TK groups than in the GF group (P = 0.03). Difference between OVG-TH and OVGH-TK groups did not reach a significant level. Mean P4 in serum tend to be greater in camels with OVGH-TK than in other groups (P = 0.09), (Figure 3). Positive correlation was found between E2 in FF and E2 in serum (r = 0.495, r = 0.03). However, no correlation was detected between P4 in FF and P4 in the serum.

Mean FSH concentration in serum was significantly higher in OVG-TH and OVGH-TK groups than in the GF group (P=0.03). No significant difference was found between OVGH-TH and OVGH-TK groups (Figure 4).

Mean LH concentration in serum was non-significantly greater in OVG-TH and OVGH-TK groups than in the GF group (P=0.1), (Figure 4).
Figure 2. Estradiol 17β (E2) and progesterone (P4) concentrations (means ± SE) in follicular fluid (FF) of female dromedary camels having oversized follicles with thin wall and clear content (OVGF-TH, n=18) or thick walls and fibrous trabeculae (OVGF-TK, n=107) in compare to a group with growing follicles (1-2 cm in diameter, GF, n=5). a, b Significant at P = 0.006.
Figure 3. Estradiol 17\beta (E2) and progesterone (P4) concentrations (means ± SE) in serum of female dromedary camels having oversized follicles with thin wall and clear content (OVGF-TH, n=18) or thick walls and fibrous trabeculae (OVGF-TK, n=107) in compare to a group with growing follicles (1-2 cm in diameter, GF, n=5). a, b Significant at P = 0.03.
Figure 4. FSH and LH concentrations (means ± SE) in the serum of female dromedary camels having oversized follicles with thin wall and clear content (OVGF-TH, n=18) or thick walls and fibrous trabeculae (OVGF-TK, n=107) in compare to a group with growing follicles (1-2 cm in diameter, GF, n=5). a, b Significant at P = 0.03.

4. Discussion
In the present study, female dromedary camels with follicle larger than normal (OVGF) had endocrine characteristics differed from camels with no OVGF, especially concerning the serum FSH concentration. It seems that the higher than normal FSH concentration observed in OVGF groups stimulated the continuing growth of the developing follicles to these large sizes. Supporting this opinion, an increase in FSH secretion headed the detection of follicular cysts in dairy cows [13,14]. Endogenous or exogenous factors; e.g. day length, presence of the male within the herd, nutrition, and affection of the genital tract; have been suggested to contribute to this phenomenon in camels [2,6,7]. Unlike cattle, milk production and postpartum period do not play major roles in the cyst formation in camels, because all females here were non-lactation and were at distance from parturition.

High level of pulsatile secretion of LH endorses incessant growth of the dominant follicle in cattle [18,19]. Moreover, during the follicular phase mean
serum concentration of LH was higher in cows with ovarian cysts. Also, LH pulse frequency and amplitude were higher in cows with cysts than in non-cystic cows [15-20]. In the current study, the LH in serum tends to be higher in camels with OVGF than in those with no OVGF.

Intermediate level of circulating progesterone have been revealed to prevent ovulation and endorse the persistence of dominant follicles in cattle [21-22]. This subluteal progesterone levels may befall in camels from luteinization of some ovarian follicles. Follicle luteinization is frequently observed in camels [7,25]. In this study, follicle luteinization (OVGF-TK group) was associated with some increase in the peripheral progesterone levels. Treatment of cystic cows with exogenous progesterone resulted in a decrease in mean LH and LH pulse frequency and recruits ovulatory follicular growth [23,24].

Camels with the OVGF had lower E2 concentrations both in serum and follicular fluid than camels with GF. It is known that Intrafollicular concentrations of E2 and P4 alter according to follicular size, degree of atresia and stage of the cycle [11,12,17]. The ability of E2 production is the outcome of the increase in the capacity of the follicular theta to produce androgen as well as the aptitude of granulosa cells to aromatize androgen into E2 [25]. Aging and degeneration of follicles are associated with a decrease in their ability for E2 synthesis [17,26]. This low follicular and peripheral E2 concentration might explain, why any of these females did not show the symptoms of continuous estrus (nymphomania) as in cattle.

P4 concentration, in contrast, did not differ between camels with GF and those with OVGF. It is known that P4 concentration of dominant follicle and its developmental status [17]. Moreover, it has been reported that the P4 concentration of the dominant follicle increased only at the pre-ovulatory period and in the final stage of atresia [17,27,28].

No differences were found between OVGF-TH and OVGF-TK groups for any of the estimated hormones. Further investigations are needed to follow each follicle and to identify the hormonal status at the time when these follicles become atretic or continue to grow to larger sizes.

5. Conclusion

This study clarified that camels with follicles larger than normal (OVGF) have certain endocrine characteristics that may contribute to understanding the occurrence of this phenomenon in this species. Camels with OVGF had higher FSH concentration than those with no OVGF. Serum and FF of camels with OVGF had lower E2 than camels with GF. LH tends to be greater in the OVGF group than in the GF group. No differences were found between OVGF-TH and OVGF-TK groups for any of the estimated hormones. Further investigations are needed to follow each follicle and to identify the hormonal status at the time when these follicles become atretic or continue to grow to larger sizes.

Author contribution
Ahmed Ali: Supervision; Investigation; Methodology; Data curation; Formal analysis; Writing - review & editing.
Derar R Derar: Investigation; Methodology; Review.
Moustafa M. Zeitoun: Methodology.
Fahd Al-Sobayil: Funding acquisition; Review.

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