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Study on Infertility Related to Persistent Corpus Luteum in Dromedary She-camels *Camelus dromedarius*)

دراسة حول ضعف الخصوبة المرتبط باستمرار الجسم الاصفر في إناث الإبل وحيدة السنام

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DEDICATION

I am dedicating this research to someone who started with me from the first step, and she is the one, who always raises my spirits, and because of difficult circumstances, she is not present now in my life, but I have all the respect for her.

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All the praises and thanks are to Almighty ALLAH, the cherisher, and the sustainer of the worlds, and the only, who makes every difficult thing easy for us.

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Table of Contents

List of figures

List of tables

List of abbreviations

Abbreviation	stand for
CL	Corpus luteum
GF	Graafian follicle
LH	Luteinizing hormone
$PGF_{2\alpha}$	Prostaglandin F_2 Alpha
µg	Microgram
FAO	Food and Agriculture Organization
Hz	Hertz
IU	International Unit
Mg	Milligram
GnRH	Gonadotropin Releasing Hormone
I/M	Intra-muscular
ADAFSA	Abu Dhabi Agricultural & Food Safety Authority
UAE	United Arab Emirate
AOAD	Arab Organization for Agricultural Development
MIC	Minimal Inhibitory Concentration
RBT	Rose Bengal test
PMN	Poly morphonuclear leukocytes

List of Appendices

ABSTRACT

The present study was conducted to evaluate the efficacy of different doses of prostaglandin F_2 alpha ($PGF_{2\alpha}$) for treatment of persistent corpus luteum (CL) and monitor genera of bacteria causing endometritis in dromedary she- camels. A total of 20 animals with history of infertility were randomly and equally divided into two groups (n= 10) during the breeding season. Group I: received 3ml (0.75 mg) and group II: received 5 ml (1.25 mg) of cloprostenol intramuscularly, 3 doses every 10 days. Trans-rectal ultrasonographic examinations and sexual receptivity assessment were performed for all camels at the day of admission and every 10 days (days 0, 10, 20, 30 and 40). Coinciding with ultrasonographic scanning, blood samples were collected at every 10 days for measuring serum progesterone level using ELISA test. Uterine swabs were collected from all camels by double guarded culture swab for bacterial isolation and antibiotic sensitivity testing. Recovered she-camels with mature follicle (12–18 mm) were induced for ovulation using 0.021 mg of buserelin acetate and allowed to mate naturally. The results revealed that all camels (100%) showed abstinence (erection and curving of her tail, raising head and refusing the male) and there is no significant difference in diameters of corpus luteum (CL) (2.45±0.15, 2.47±0.29 cm and 2.43±0.15, 2.43±0.30) and serum progesterone levels (2.45±0.30 and 2.67±0.28 ng/mL) between two groups at day 0 and 10, respectively. At day 20 and 30, the diameters of CL were significantly ($p\leq 0.05$) decreased in the group II (1.35±0.29 and 0.74±0.24 cm) than those in the group I (1.74±0.09 and 1.19±0.07 cm). There was higher significant difference ($p\leq 0.05$) between progesterone level in the group I (1.23 ± 0.16 ng/ml) and group II (0.92± 0.37 ng/ml) at day 30. Complete receptivity was 0% and 50% in the group II and group I, respectively. In the group II, the maximum diameter of dominant follicle was 1.33 ± 0.42 cm at day 30, while in the

group I was reached 1.38 ± 0.30 cm at day 40. The most isolated bacteria were *Escherichia coli* (70%), *Staphylococcus haemolyticus* (25%) (sensitive to 100% gentamycin), *Clostridium perfringens* (10%),*Pasteurella pneumotropica* (5%) and *Brevundimonas diminuta* (5%) (sensitive 100% to ampicillin).The pregnancy rate was higher in the group II than those in the group I (60 and 20 %, respectively). In conclusion, this study showed that two doses of 1.25 mg or three doses of 0.75 mg $PGF_{2\alpha}$ can be treated persistent CL in the dromedary she-camels. The most common organisms caused endometritis were *Escherichia coli* and *Staphylococcus haemolyticus* and can be treated by gentamycin. Strong positive correlation of the CL diameter with serum progesterone level was found significant (r= 0.93). High pregnancy rate (60%) can be obtained after treatment of persistent CL using 1.25 mg $PGF_{2\alpha.}$

المستخلص

أجريت هذه الدراسة لتقييم فعالية جرعات مختلفة من البروستاجلاندين في علاج الجسم الأصفر المستمر وللكشف عن انواع البكتريا المسببة لالتهاب بطانة الرحم في اناث الابل وحيدة السنام. قسمت عدد 20 من الإبل التي لها تاريخ لمرض ضعف الخصوبة بشكل عشوائي وعلى قدم المساواة لمجموعتين خلال موسم التكاثر. المجموعة الأولى: تلقت 3 مل (0.75ملغم) والمجموعة الثانية تلقت 5 مل (1.25ملغم) من الكلوبروستينول عن طريق الحقن العضلي بواقع 3 جرع كل 10 ايام. إجري فحص الموجات فوق الصوتية عبر المستقيم وتقييم التقبل الجنسي للجميع في يوم القبول وكل 10 أيام (الأيام 0 و 10 و 20 و 30 و 40). بالتزامن مع فحص الموجات الصوتية ، جمعت عينات الدم كل 10 أيام لقياس مستوى البروجسترون في السيرم. جمعت مسحات من الرحم من جميع الإبل للزراعة البكتيرية واختبار الحساسية للمضادات الحيوية. احثت عملية الاباضة في اناث الابل المستشفية والتي تحمل جريب ناضج (18–12 ملم) باستخدام استيت البسرلين (0.021 ملغم) وسمح لها بالتزاوج الطبيعي. أوضحت النتائج أن جميع الإبل (100٪) أظهرت الامتناع عن الجماع (الانتصاب والانحناء لذيلها ، ورفع الرأس ورفض الذكر) في اليوم 0 واليوم 10 مع عدم وجود اختلاف معنوي في قطر الجسم الأصفر (2.47±0.29 ,2.45±0.15سم و 2.43±0.30 and 2.43±0.15 سم) ومستويات بروجسترون المصل ((2.67±0.28 and 2.45±0.30 نانوغرام / مل) في كلتا المجموعتين في اليوم 0 و 10 علي التوالي. في اليوم 20 و30، نقص قطر الجسم الاصفر بشكل معنوي في المجموعة الثانية (0.74±0.24 and 1.35±0.29) مقارنة بالمجموعة الاولي 0.751.74±0.09 and (1.19±0.07 سم). هنالك اختلاف معنوي في مستوي البروجستيرون في المصل بين المجموعة الاولي (0.16 ± 1.23 نانوغرام / مل) والمجموعة الثانية (1.250.92±0.37 نانوغرام / مل) في اليوم 30. وكان التقبل الجنسي الكامل بنسبة 0% و50% في المجموعتين الاولي والثانية علي التوالي. في المجموعة الثانية، كان اعلي قطر للجريب الناضج (0.42 ± 1.33 سم) في اليوم 30 بينما كان قطره في المجموعة الاولي يساوي 0.30 ± 1.38 سم في اليوم 40.أكثر أنواع البكتيريا المعزولة كانت الإشريكية القولونية (70٪) تليها المكورات العنقودية الدموية (25٪). بينما تم عزل الباستوريلة النيموتوربيكا(*Pasteurella pneumotropica)* والبرفينديموناس ديمنيوتا *(Brevundimonas diminuta)* مرة واحدة فقط (5٪ لكل واحدة). تم عزل المطثية الحاطمة *(Clostridium perfringens)* مرتين فقط (10٪). وكانت الإشريكية القولونية والمكورات العنقودية الدموية حساسة للجنتاميسين (100٪). وكانت الباستوريلة النيموتوربيكا و البرفينديموناس ديمنيوتا و المطثية الحاطمة حساسة للأمبيسيلين (100 ٪). كان معدل الحمل أعلى (P =

0.068) في المجموعة الثانية (1.25ملغم) من المجموعة الاولي (0.75ملغم) (60 و 20٪ على التوالي). في الختام ، كانت جرعة واحدة من الحقن العضلي من 0.75 أو 1.25 ملغم من عقار الكلوبروستينول غير فعالة للحث على تحلل وعلاج الجسم الاصفر المستمر. كانت الإبل بحاجة إلى ما لا يقل عن ثلاث جرعات من 0.75 ملغم كلوبروستينول أو جرعتين من 1.25 ملغم من الكلوبروستينول لاستردادها. كانت أكثر أنواع البكتيريا المعزولة والمسببة لالتهاب بطانة الرحم هي الإشريكية القولونية تليها المكورات العنقودية الدموية وكلاهما كانت حساسة للغاية للجنتامايسين. هنالك ارتباط ايجابي معنوي (r= 0.93) مابين قطر الجسم الاصفر ومستوي البروجستيرون في الدم. يمكن الحصول علي معدل حمل عالي بعد علاج الجسم الاصفر المستمر باستخدام 1.25 ملغم من عقار كلوبروستينول.

INTRODUCTION

The camel is an important animal in many areas of the world, especially in Arab countries. The low fertility rates in camels constitute an obstacle in camel reproduction and hence in camel production. To increase offtake rate in any population of camels, one has to improve the fertility rate in that population. Such an improvement may be necessary to convince camel owners to trade young camels which might then be conditioned for better meat quality (Sieme et al.,1990).

The total population of dromedaries is about 1.6 million animals within the Arabian Peninsula, 53% of which are found in Saudi Arabia (Abdallah and Faye 2012). Bedouins prefer camel meat and milk to other types of meats and milks. Camel meat is considered to be superior to and healthier than other types of meat because of its higher protein and lower fat content; it is recommended for the prevention of cardiovascular disease and atherosclerosis because it lowers cholesterol levels in the blood. It may protect against tumors because it contains unsaturated fatty acids such as linoleic acid. Camel meat can also be used to cure exhaustion and fatigue because it contains more glycogen than other types of meat (Kadim et al., 2008).

Camel milk is considered to be a complete food source that can sustain a person through a typical day. It maintains its quality and texture for 12 days. It is a rich source of proteins with potential antimicrobial activities that are not found, or found only in small amounts, in other types of milk. Milk of camel is lower in lactose, short-chain fatty acids and cholesterol but higher in water (89.6%), prolactin, vitamins (especially C and B1), volatile acids (especially linoleic and polyunsaturated), minerals (potassium, magnesium, iron, copper, manganese, sodium, and zinc) and immune-globulins than other types of milk (Morton, 1984, Wernery, 2007).

Despite the above-mentioned benefits of camel meat and milk, camel production is still not undertaken on a commercial scale. The reproductive efficiency of dromedary camels is generally considered to be low. Birthing rates rarely exceed 40% in nomadic herds or 70% in more intensive herds (Tibary and Anouassi 1997; Tibary et al., 2005 and Kaufmann, 2005).

The persistent corpus luteum in female camels cause reproductive disturbances and infertility, in addition of causing the female camels to show false signs of pregnancy (Tibary and Anouassi, 1997). The presence of active luteal tissue on the ovary leads to the anovulatory state where growing follicles of consecutive waves proceed to dominance but fail to ovulate. This is due to the negative feedback of progesterone on LH release (Youngquist and Threlfall, 2007). Persistent corpus luteum is mostly associated with pathological conditions of the uterus such as pseudopregnancy (hydrometra) pyometra, mummification and maceration. If these previous pathological conditions exist for months or even longer, the corpus luteum becomes centrally located in the ovary and difficult to be palpated and treated (Noakes et al., 1991). In camel, the corpus luteum is usually palpated at pregnancy; however, some camels show corpora lutea in a non-pregnancy state following embryonic death and pyometra (Waheed et al., 2009). Persistent corpora lutea can be treated by injection of luteolytic dose of PGF2α (Tibary and Anouassi, 1997).

Uterine inflammation results in diminished fertility and is a considerable barrier to camel production, often resulting in significant economic loss. Uterine inflammation has been described as the most commonly encountered form of infertility in dromedary camels (Tibary and Anouassi, 2000). Inadequate clinical trials comparing the efficacy of different treatments for endometritis have been performed in the camel. Additionally, the efficacy of antibiotics should be evaluated periodically

because resistant strains of bacteria can arise owing to the indiscriminate use of antibiotics (Vekateswaran and Rajeswar 1991).

Objectives:

1. To evaluate the efficiency of different doses of $PGF_{2\alpha}$ (cloprostenol) for treatment of persistent CL in dromedary she-camel.
2. To determine the number of doses of cloprostenol ($PGF_{2\alpha}$) required for regressing persistent CL.
3. To monitor the most common bacteria causing endometritis and determine the best antibiotic for treatment.
4. To determine the best dose of $PGF_{2\alpha}$ which achieved high pregnancy

CHAPTER I
LITERATURE REVIEW

1.1. Camel population:

It is difficult to exactly determine the number of camels in the world, firstly, because it is mainly an animal of nomadic people and pastoralists who are moving frequently, and secondly, because camels are not usually subjected to obligatory vaccination. So, an exhaustive census for the camels is quite difficult (Faye, 2004). Morton (1984) reported that approximately 94% of the estimated world's camel population was thought to be one-humped or dromedary camels. The two-humped Bactrian camel comprises 6%and is primarily in Asia (Mukasa-Mugerwa, 1981). Seventy percent of the world's camels are located in the tropics, a majority of them in Sub-Sahara Africa. Five adjoining countries-Somalia, Ethiopia, Kenya, Sudan, and Djibouti have about 84% of African camels and 60% of the world's camels. (Morton, 1980)

Sudan is an agricultural country with the largest population of livestock in the Arab world and is the second in Africa after Ethiopia. In 2000, there about 37.1 million heads of cattle, 46.1 million heads of sheep, 38.5million heads of goats and 3.1 million heads of camels (Anonymous, 2000)

In 2001, the total camel population was 19 million of which 17 million were Dromedaries (*C. dromedarius*) and 2 million were Bactrian camels (*C. bactrianus*) (Farah, 2004). According to FAO statistics the world population of camels is about 20 million animals, 15 million camels live in Africa and 5 million in Asia (Clements, et al., 2002).

In 2008, Arab Organization for Agricultural Development reported that Arab countries had more than 15 million camels at the end of 2008 and

more than 70% of them are concentrated in Sudan and Somalia. The number was estimated to be around 7.13 million in Somalia and about 4.4 million in Sudan. Saudi Arabia had the fourth largest camel wealth with around 869,000 heads. It was followed by the UAE which had nearly 378,000 camels (AOAD,2008). Also, Hare (2008) reported that majority of camels are dromedaries (more than 15 million), most of them are in Somalia (7 million), Sudan (4 million), Ethiopia and Kenya. During the last decade, camels' population in Saudi Arabia decreased from 426,015 in 1997 to 260,000 heads in 2007 (Abdallah and Faye, 2012). Disturbances in reproductive efficiency might be a factor in the progressive decline in the number of camel populations in this country (Ali et al., 2010 B).

The world camel population is increasing regularly with a yearly growth of 2.1% (Kamuanga et al., 2008). Officially, the total number of camels in the world was around 27 million heads (Faye, 2014).

1.2. Economic importance of camel:

The camel, known as "ship of the desert" is an old habitant to the desert where water and food are scarce and ambient temperature is high (Chaudhary and Akbar, 2000). The camel has great tolerance to high temperatures, high solar radiation and water scarcity. It can survive well on sandy terrain with poor vegetation and may chiefly consume feeds unutilized by other domestic species (Kadim et al., 2008). The camels inhabit the most extreme climates on the globe and their process of multiplication is determined by the availability of food and protection for the newborn (Abebe, 2016). Konuspayeva (2007) reported that large and small camels play an important role in arid lands or high mountains for milk, meat, wool and energy production.

1.2.1. Camel milk:

The camel milk production occupies a tiny place (<1%), but finally, the dairy potential of camel appeared higher than that of the cow reared

under the same climatic and feeding conditions. In Ethiopia, the Afar farmers rearing simultaneously cattle and camel get on average 1 to 1.5 liters of milk with Afar zebu against 4 to 5 liters with Dankali camel (Richard and Gerard, 1985). Camels in UAE usually provide about 39.50 thousand metric tons of milk, 1200 thousand tons of meat, 800 tons hides and 120 tons of hair annually (Al-Ani 2004).

Camel milk is considered to be a complete food source that can sustain a person through a typical day. It maintains its quality and texture for 12 days and is a rich source of proteins with potential antimicrobial activities that are not found, or found only in small amounts, in other types of milk. Camel milk is lower in lactose, short-chain fatty acids and cholesterol but higher in water (89.6%), prolactin, vitamins (especially C and B1), volatile acids (especially linoleic and polyunsaturated), minerals (potassium, magnesium, iron, copper, manganese, sodium and zinc) and immune-globulins than other types of milk (Morton 1984 and Wernery 2007).

1.2.2. Camel meat:

Camel meat is considered to be superior to and healthier than other types of meat because of its higher protein and lower fat content; it is recommended for the prevention of cardiovascular disease and athero-sclerosis because it lowers cholesterol levels in the blood. Camel meat may protect against tumors because it contains unsaturated fatty acids such as linoleic acid. Camel meat can also be used to cure exhaustion and fatigue because it contains more glycogen than other types of meat (Kadim et al. 2008).

1.2.3. Camel power:

The camel can be used also for packsaddle, draught and race (Pacholek et al., 2000). As a pack animal, camel is able to walk at 4-5 km/h for 10 hours with 150 to 300 kg on the back. Extreme values with 400-500

kg are reported in Pakistan. In Niger, the weight of the packsaddle is between 200 and 250 kg. The pack camel could transport this charge for 30 to 35 days, walking 60 km each day (Pacholek et al., 2000)

Camel is considered a source of big profit, especially those that take place in the beauty and races competitions (Khalaf, 1999). The Camel Racing Federation was established in 1992 in UAE, for example, the race area in Al Wathba (Emirate of Abu Dhabi) are annually traded more than one billion dirhams between buying and selling. Therefore, camel racing is the sport of choice in the UAE.

Camel represents a great legacy for their owners and plays a very important role in the economy and social life of Bedouins and pastoralists in different localities in the world (Zaher et al., 2017). Historically, camels were a dependable source of not only transport but also food and milk. Arabs were proud of the number of camels they possessed; the camels were given as a bride's dowry among the Bedouin tribes (Almathen et al., 2016). The scientific community plays an essential role for considering camel under three aspects underlying the importance of camelids, (milk, meat, power). The rapid increase in human population in the developing countries has led to a high demand for milk and meat production. The one-humped camel most probably, is a better provider of food in desert and semi-desert areas compared to cattle`s which are severely affected by heat and scarcity of water and feed (Khalaf, 1999).

1.3. Reproductive physiology of camel:

The breeding capacity is of major importance to camel owners, the decrease in reproductive performance affects negatively on camel industry. Regular monitoring of camel herd for breeding problems and/or infertility is considered a major part of herd health management (Kaufmann, 2005). In general, the reproductive efficiency of camels under their natural pastoral conditions is low. The reasons for this low reproductive efficiency

compared to the other domesticated species include the short breeding season, the late age of reaching puberty, the long gestation period of 13 months and long interval between birth (Skidmore, 2011). Additionally, estrus behavior is very vague and difficult to interpret, as it does not often relate to follicular development in the ovaries (Skidmore, 2003).

Camel is seasonal polyestrous. Camels generally do not come in heat in the summer season. The breeding activity starts in the cold months with short days which extended from November to April in UAE(Atakan,2016). The duration of estrus cycle varies from 14-22 days and the duration of heat is for 3-4 days (Atakan et al. 2016). The follicles take 2-3 weeks (interval between emergences up to be matured) and tend to be longer at beginning of breeding season (Elias et al. 1984). Follicular wave recruitment is followed by a period of follicular growth of 3-6 follicles until the establishment of one or two dominant follicles (Tibary and Anouassi, 1996).

The onset of sexual activities in the female camel marks the beginning of puberty and it has been found to start as early as 2-3 years of age (Molash, 1990; Tibary and Anouassi, 1997). However, they are usually not bred until they reach their physical maturity at about 70% of their adult body weight at 3-4 years of age (Al-Hozab, 1999). The follicular waves vary between camel but can be divided into three phases: i) growth phase, ii) the mature phase, iii) regression phase (Skidmore et al,.1996). Also, the follicular wave was divided into two phases: i) non-breeding phase, ii) the breeding phase. It was considered the growth phase and regression phase as one phase and called it non-breeding phase because of overlapping between these two phases. Most females that have regressed follicles of last follicular wave have growing follicles of the next follicular wave (Swelum et al., 2015: Swelum et al., 2018 a)". Regular ultrasonographic examination of the ovaries show that the follicle can ovulate when they reach minimum

of 10mm, length of phase (6 days) for follicular growth and maturation, all follicles that are larger than 25mm in diameter not able to ovulate (Anouassi et al,.1994).

Anouassi et al. (1994) reported that the important point that the ovulation in these species is not spontaneous but induced by coitus (naturally) or by hormone. Wilson (1984) reported that ovulation occurs 32 to 40 hours after copulation under the influence of LH. Chen et al. (1985). reported that ovulation can be induced by the seminal plasma, but not by the spermatozoa, and the incidence of ovulation after insemination was 87%. Most of the females (66%) had ovulated by 36 hours after insemination and the rest by 48 hours, as after natural service. There is an ovulation-induced factor exist in the seminal plasma of the dromedary camel and the effects of seminal plasma on ovulation is similar to the effect of GnRH. (Atakan et al., 2016).

In Bactrian camels, ovulation can be induced by deep intravaginal deposition of whole semen or sperm-free seminal plasma as well as by i.m. injection of semen or seminal fluid (Zhao et al., 1990). In dromedaries, ovulation is induced by mating with an intact or vasectomized male, but manual stimulation of the cervix or intrauterine injection of whole semen, seminal plasma, water or the prostaglandin F analogue(cloprostenol) does not stimulate the release of sufficient LH from the pituitary to cause ovulation (Sheldrick et al., 1992).

The corpus luteum (CL) starts developing 2-4 days after the ovulating stimulus as a spherical, echogenic mass that grew from a diameter of 0.7 ± 0.2 cm on day 3 to 2.2 ± 0.1 cm by day 9. The CL can be palpated per rectum easy between day 8 and 10 after mating (Elias et al., 1984)". If conception has taken place, then after 15 to 25 days the she camel, especially when approached by a male or handled by an attendant, shows cocking of the tail but if she has not conceived then cocking of tail is

not seen (Rathore, 1986). Pregnancy could be diagnosed at 18 days, by ultrasonography, or later if diagnosed by rectal palpation of the uterus. If the maternal recognition of the pregnancy does not occur, CL is completely regressed after 13 days (Arthur, 1985)". It exhibited a secretory life-span of 8.5 ± 0.5 days, as reflected by the serum progesterone profile. Progesterone concentrations remain low (<1 ng / ml) then start to rise and reach 2 ng/ml by day 6 post-mating then decline to basal level (1 ng/ml) by day 15-17 in absent of conception (Skidmore, et al., 1992)

Progesterone concentrations remain low (< 0.5 ng/ml) for the first 3-4 days after ovulation and then rise to reach a mean peak of 2.6 ng/ml on day 8 (Skidmore et al,. 1996). The serum concentration of progesterone represents a balance between the production by the CL and the metabolism in the liver (Diaz et al. 2002). If ovulation does not occur, the dominant follicle continues to grow then starts to regress, some non-mated camels develop follicles > 25 mm in diameter (cyst-like follicles). These non-ovulatory cyst-like follicles do not appear to affect fertility and other smaller follicles may continue to grow normally (Tinson and McKinnon, 1992). Thus, presence of a corpus luteum cannot be used to confirm cyclicity in camels. She-camel with ovaries presenting follicles more than 5 mm in diameter were considered as having active ovaries (Sghiri and Draincourt, 1999).

During pregnancy, progesterone level in the camels confirm that these animals depend on ovarian progesterone throughout their pregnancy, level of progesterone remain above 2 ng/ml from the initial dictation with CL until shortly before parturition (Tibary, Anouassi, 1997). All pregnancies are carried on the left horn whether the ovulation occurred on the left or the right ovary (Tibary and Anouassi, 2000). The placenta of camelids is epitheliochorial, and the pregnancy is located in the left uterine horn in 98% of the cases (Arthur,1985). The length of gestation is 398 ± 13

and 372 ± 11 days in camels carrying male and female fetus, respectively. The level of progesterone hormone throughout pregnancy was fluctuated between 4 and 5 ng/ml except for a slightly lower value (2.5 ± 0.27) at 9 to 10 months of gestation. On an average, the camels carrying a male fetus had higher progesterone levels (5.13 ± 0.69 ng/ml) than those carrying female fetus (3.45 ± 0.20) (Agarwal et al., 1987). The pregnancy lasts about 13 months and the uterine involution is completed within 40 days after parturition and the period of resumption of the ovarian activity after parturition is variable (Monaco et al, 2015). The progesterone concentration reaches its peak after ovulation and falls immediately before parturition (Skidmore et al., 1996)

1.4. Infertility in the dromedary she-camel:

The fertility is defined as the ability of the male and female to produce viable germ cells, mate and conceive and subsequently give birth (Mukasa-Mugerwa, 1981). Reproductive diseases and infertility in the female can be placed in one of 4 categories of complaints: 1)Failure of the female to become pregnant (Repeat Breeding Syndrome),2)Failure to maintain pregnancy after breeding and conception (early embryonic death, fetal loss or abortion),3) Failure to complete breeding because of physical or behavioral problems (intromission difficulties, refusal of the male) and4) observed abnormalities in the genitalia (abnormal conformation or lesions of the vulva and perineum or abnormal vaginal discharge,) (Tibary and Anouassi, 2000). Reproductive failure may be due to management errors as presenting of dromedary females which had no follicular structures on the ovaries or only follicles smaller than 9 mm for breeding, breeding with a young male, overuse of males and lack of verification of intromission during copulation (Tibary and Anouassi, 2000).

Early pregnancy loss is probably one of the most important factors result in the reduction of reproductive efficiency in camels. Recognizing

the occurrence and incidence of embryonic loss may be instrumental in application of new reproductive technologies to increase service rate in a herd and reduce embryonic loss in camels (Pratap et al., 2012). The percentage of early embryonic death (before day 45) in the dromedary camel is ranged from 8 to 32% (Tibary and Anouassi, 2000). Pratap et al.(2012) recorded 6.9% early abortion including the pregnancy loss during embryonic stage and early fetal stage in multiparous, whereas no early fetal loss was recorded in primiparous camels.

1.4.1 Abnormal ovarian structures:

1.4.1.1Ovarian cysts:

In camels, various cysts may develop in / and around the ovaries. While some cysts are incidental findings at post-mortem, others are associated with fertility disturbances. Intra ovarian cysts include anovulatory Graafian follicles, cystic corpora lutea and cystic rete. Para-ovarian cysts are derived from mesonephric tubules, paramesonephric ducts, uterus and the mesosalpinx (Maclachlan and Kennedy, 2002).

Ovarian cysts are described according to the structure involved and their appearance. Cysts are classified as follicular cysts, luteal cysts, cystic corpora lutea or hemorrhagic cysts according to their histological and physical characteristics. Pathologic ovarian cysts may be follicular or luteal and may be single or multiple on one or both ovaries. Follicular cysts are thin-walled, fluid-filled structures associated with low plasma progesterone levels. Luteal cysts are partially luteinized fluid-filled structures that result in higher plasma progesterone levels (Peek, and Divers, 2008). Only follicular cysts have been described in bactrian camels and are associated with infertility (Tibary and Anouassi., 1997).

1.4.1.2. Ovulation failure and follicular cyst:

The cystic ovary condition is not well documented as in cattle or other domestic animals. In fact, the term "cystic ovaries" does not always apply to *Camelidae* because a large proportion (30 to 40%) of females develops some form of follicular cyst if not bred because the ovulation in these species is induced (Tibary and Anouassi, 1996; Tibary et al., 2005). If ovulation does not occur, some non-mated camels develop follicles > 25 mm in diameter (cyst-like follicles). These non-ovulatory cyst-like follicles do not appear to affect fertility and other smaller follicles may continue to grow normally (Tinson and McKinnon, 1992). Kaufmann (2005) reported that ovulation failure might be caused by inadequate LH release in response to copulation and the camels showed the clinical symptoms of repeat breeding and refuse mating with ovarian cysts. Therefore, ovarian cysts (5.3%) and ovarian inactivity (3.6%) did not represent major infertility problems in camels (Ali et al., 2010 b).

1.4.1.3. Cystic corpus luteum and luteal cyst:

The terms for cystic corpus luteum can often be confused with those for luteal cysts, though the first is a normally functional structure and the latter a pathological condition. Because of this, the contemporary term “corpus luteum with a cavity” has been suggested to replace the classical term cystic corpus luteum (Chuang et al., 2010). A cystic corpus luteum in a cow is defined as “luteal tissue initiating from a corpus hemorrhagicum and containing fluid in a central cavity greater than 7 mm in diameter (Chuang et al., 2010). Because cystic corpora lutea are found in cows that are normally cycling or pregnant, they are considered to be a normal stage or variation of CL development (Kahn, 2010).On the other hand, the luteal cysts are an extension of follicular cysts such that the non-ovulatory follicle is partially luteinized spontaneously or in response to hormonal therapy (Statham and Thorne, 2009).The Luteinized ovarian cyst and persistent

corpus luteum in female camels cause reproductive disturbances and infertility, in addition of causing the female camels to show false signs of pregnancy (Tibary and Anouassi, 1997). The presence of active luteal tissue on the ovary leads to the anovulatory state where growing follicles of consecutive waves proceed to dominance but fail to ovulate. This is due to the negative feedback of progesterone on LH release (Youngquist and Threlfall, 2007). Anovulatory follicles do not have any negative effect on newly emerging follicular wave and ovulation of a new follicle is possible even in the presence of this structure. However, some of these anovulatory follicles can become luteinized producing enough progesterone to induce decrease in uterine tone and rejection of male. Luteinized cyst can also be the result of partial luteinization of follicle following breeding (Tibary and Anouassi, 1997).

A high proportion of camels (75% 6/8) with ovarian cysts evidenced cocking behavior for prolonged periods (more than 2 months) and a high plasma progesterone (<1.5ng/mL) (Quzy et al., 2013). Sonographically, cysts showed hyperechogenic streaks in an anechogenic lumen in 75% (6/8) of the camels whereas in 25% camels (2/8) the ovarian cysts evidenced anechogenic structure with a thick echogenic wall (Quzy et al., 2013).

1.4.1.4. Persistent corpus luteum:

Corpus luteum that persists on the ovary beyond day 20 without pregnancy is considered pathologically persistent. The presence of active luteal tissue on the ovary leads to the anovulatory state where growing follicles of consecutive waves proceed to dominance but fail to ovulate. This is due to the negative feedback of progesterone on LH release. (Borman et al., 2004).

Shalash (1964) reported that persistent corpora lutea are rare in the female *Camelidae*. However, the condition has been suspected on the basis

of prolonged elevated plasma progesterone levels with absence of pregnancy (Adam et al, 1989). Persistent corpus luteum is mostly associated with pathological conditions of uterus such as pyometra, mummification and maceration. If these previous pathological conditions exist for months or even longer, the corpus luteum becomes centrally located in the ovary and difficult to be palpated and treated (Noakes et al., 1990).

In camel, high progesterone concentrations is rarely due to persistent corpus luteum but rather to the luteinization of hemorrhagic follicles (Tibary., Anouassi, 2000). The condition of persistent corpus luteum has been suspected in llamas on the basis of prolonged high plasma progesterone level. In some cases of camels, progesterone remained higher than 2 ng/ml for 15 days or more in absence of mating and corresponded to luteinized anovulatory follicles which tend to have very slow regression (Tibary and Anouassi, 1997). In camel, the corpus luteum is usually palpated at pregnancy; however, some camels show corpora lutea in a non-pregnancy state following embryonic death and pyometra (Waheed et al., 2009).

1.4.2. Infectious causes of infertility:

Out of 447 female camels examined for reproductive disorders, the major causes of infertility were endometritis and metritis (57.1%). Vaginal adhesions were the second important infertility problem (16.1%), while ovarian cysts (5.3%) and ovarian inactivity (3.6%) did not represent major infertility problems (Ali et al,.2010 b). Waheed et al. (2009) stated that the major four infertility problems were pyometra, repeat breeder, endometritis and mucometra. The highest frequency of repeat breeders was found during winter, whereas the number of endometritis cases was significantly higher

during autumn. The infectious causes are classified into bacterial, viral and parasitic infection.

The problems of reproduction in the camel are not extensively investigated as in other animal species specially the bovine. Endometritis and metritis were found in mild, moderate, and severe degrees in frequencies of 117/255 (45.9%), 77/255 (30.2%), and 61/255 (23.9%), respectively (Ali et al., 2010b). Chronic metritis accompanied with adhesions of the uterus (not freely movable) to the broad ligaments.

There are miscellaneous causes of infertility including anomalies of the genital tract (1.1%), hydrosalpinx (0.5%), and vaginal tumors (0.5%). Early embryonic mortality seems to be high in the camel although two and three corpora lutea were found; the reason for these high prenatal losses is still open for more investigation. (Ali et al., 2010 b).

The uterus of female dromedaries could be the site of acquired pathologies which seriously affect female fertility such as endometritis, pyometra and mucometra or be the reason for repeat breeding (Tibary and Anouassi, 2000). Factors that interfere with ova transport in the bursa (i.e. bursitis) and uterine tube (i.e. salpingitis; occlusions) or impair semen viability and transport (uterine adhesion, endometritis, obstruction of the utero-tubal junction) may be the cause of repeat breeding (Tibary., Anouassi, 2000). Uterine infection should be suspected in any animal with a history of repeat breeding or early embryonic death. Diagnosis is confirmed by the results of clinical examination. Examination of the perineum and vulva may reveal mucopurulent discharge. In some cases, the base of the tail may present dried flakes of vaginal discharge. Rectal palpation and ultrasonography may in some cases reveal a thickened uterine wall and various amount of fluid (Tibary and Anouassi., 1997). Unlike cattle, there is dearth of information regarding infective pathogens colonizing the genital tracts in camels. (Mshelia et al. 2014).

Uterine culture yields a wide variety of non-specific micro-organisms including; *Corynebacsderium pyogenes*, *E. coli, β-haemolyticStreptococci*, *Staphylococcus sp*., *Klebsiella pneumonie* and *Aspergillus spp., Mucor sp*. (Wernery and Wernery, 1992).

A total of 160 genitalia of camels and cows were investigated in Maiduguri, north-eastern Nigeria to compare bacterial isolates and the antibacterial susceptibilities of some of the isolates. *Streptococcus (Str.) pyogenes* (31%), *Escherichia (E.) coli* (24%) and *Staphylococcus (S.) aureus* (20%) were the most common vaginal bacterial isolates in camels; while *E. coli* (73%), *Str. pyogenes* (18%) and *S. aureus* (11%) were the most frequent isolates in the cows. Of the 78 uterine isolates recovered in this study, *E. coli* was the most prominent in camels (8%) and cows (54%). The overall weight of genital infection in all camels and cows examined was highest ($P < 0.05$) with *E. coli* (79%), but there was no difference ($P > 0.05$) between vaginal and uterine bacterial isolates from camels and cows in this study, (Mshelia et al. 2014).

The animals suffered from puerperal infections of the genital tracts are subsequently characterized by low reproductive performance (Sheldon et al.,2006).

A total of 54 female camels with a history of conception failure were examined through trans-rectal palpation, ultrasonography, and vaginal explorations. Animals were categorized according to type of uterine infection (endometritis n=26 animals) and (metritis n=28 animals). Several types of both Gram-negative and Gram-positive bacteria were isolated from diseased animals. Presence of bacteria was detected in samples (87.5 %) in cases of endometritis in contrast to (92.5%) from metritis cases. Several microorganisms were isolated from infected camels. The microorganisms associated with endometritis were identified as *Staphylococcus aureus*, 16 isolates (40 %), *Corynebacterium spp*., 8 isolates (20 %), *E. coli*, 6 isolates

(15 %) and *Salmonella spp.* 5 isolates (12.5 %). In metritis cases the isolated bacteria were identified as *Corynebacteriumspp*.12 isolates (30 %), *Proteus spp*. 10 isolates (25 %), *Klebseiella spp*. 8 isolates (20 %) and *Salmonella spp*. 7 isolates (17.5 %) (Nabih and Osman, 2012).

The bacteria colonizing the genital tract of the female camel (*Camelus dromedarius*) have been shown to be the major causes of reproductive disorders in this species. (Ali et al., 2010 a).

Cephapirin, a first-generation cephalosporin, and oxytetracycline satisfy most of the criteria for the treatment of endometritis (Noakes et al., 1991). Oxytetracycline can be an irritant; thus, it stimulates the inflammatory response and uterine defensive reactions and promotes poly-morphonuclear leukocytes (PMN) infiltration to the uterine lumen and the regeneration of uterine tissue. Therefore, it can be a very useful antibiotic for the treatment of endometritis, especially in cases of chronic endometritis. Nevertheless, it is not used by certain practitioners because it leaves residues in the milk. In contrast, cephapirin leaves no residue in milk, and it achieves concentrations in the endometrial tissue above the minimal inhibitory concentration (MIC) of sensitive bacteria for at least 24 hours after a single treatment (Adams, 2001).

1.5. The negative impact of persistent CL on the owners:

Showing symptoms of pregnancy in female camels with no true conception are a real problem which is primarily harming the owner for the following reasons:

1. Owners are always keen to deal with their pregnant female camels with excessive and intensive care and they are keen to feed them a special type of feed at a higher cost usually.
2. The owner must wait for 13 months to discover that the camel is not pregnant and consequently, major loss of expenses for shelter, food, medicines and care would occur.

3. If the camel shows signs of pregnancy (false pregnancy signs), the owner waits for about 13 months and consequently will lose the present breeding season and the next.

4. When the animal shows clinical signs of pregnancy (false pregnancy signs) veterinarian is restricted from giving many medicines as many medicines are forbidden to use in cases of pregnancy. So, it will affect treatment of many disease conditions when the camel shows signs of pregnancy.

5. There is also a negative impact in case of sale or purchase of the camel, where there is a big difference between the price of the pregnant and non-pregnant camel.

CHAPTER II

MATERIALS AND METHODS

2.1. Study area and design:

The present study was conducted during the breeding season (October 2016) at the Artificial Insemination and Embryo Transfer section in Animal Production Division, Animal Wealth Sector of Abu Dhabi Agricultural and Food Safety Authority (ADAFSA), in the Emirate of Abu Dhabi, UAE. Location coordinates are: Latitude 24.47ºN and longitude 54.37ºE, Zone 40R, Elevation 7 m.

2.2. Materials:

2.2.1. Experimental animals:

A total of twenty dromedary she-camels were used in this experiment. Their age is between 6-10 years and weighing between 400 - 600 kg. These animals were delivered because of infertility problems. All camels were Arabians which are characterized by golden-brown color, long legs and big feet, a dropped-down nose and lower lip, big, thick eyelashes, a fit body, and soft dark hair on its hump. And originally came from Southern Oman.

2.2.2. Animal housing and feeding:

The animals were housed in open yard (8 x 8 meters) shaded by slopped shades of 5-8 m high; This shade was oriented east to west and made by reed mats. The camels were fed Alfalfa hay mixed with rhodes grass (7 kg/ head twice daily) to meet their requirement according to National Research Council (NRC). Drinking water was provided *ad-libitum* to all camels.

2.3. Methods:

2.3.1. Case history and data collection.

The owners of camels complained from the conception failure (1-3 years) despite showing typical signs of pregnancy, such as tail lifting, anestrus, the posture of standing with high head and dribbling of urine in the presence of male camels. History regarding the camels were collected from the owners and recorded. Age of the camels, according to the owner's data and dentition, was recorded. Also, number of parities, age at first calving and time of last calving were recorded.

2.3.2. Rose Bengal test:

Blood samples (5 ml) were collected by jugular vein puncture into vacutainer tubes without anticoagulant (Greiner Bio-One, Kremsmünster, Austria) immediately after receiving the animal for detection of *Brucella sp* using rose bengal test (RBT). Benga test (zoetic/ France) is a rapid slide agglutination test (RSAT) for the serological diagnosis of brucellosis caused by *Brucella melitensis*, *B. abortus* and *B. suis*. The inactivated and concentrated suspension of *B. abortus* antigen that has been stained with rose bengal and dispersed in an acid buffer allows for the detection of Brucella antibodies (figure 2.1).

The blood samples were centrifuged for 3 minutes at 3000 rpm. After that, one drop of the serum was placed on the test plate and well mixed with one drop of the brucella antigen (a stained *B. abortus* suspension at pH 3.6–3.7) and was shacked for 4 minutes using plate shaker. Then, the test plate was observed under light for presence of agglutination which indicates positive result of brucellosis. The collected sera were lapelled and stored at-20ºC until used. (Maymona et al., 2013).

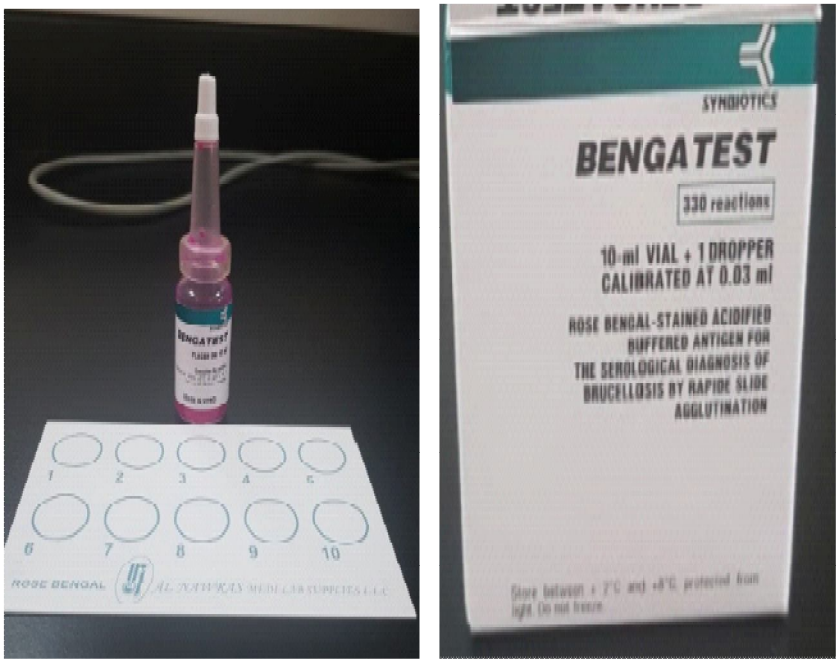

Figure 2.1: Rose Bengal Test (BENGA TEST)

2.3.3. Vaginal examination:

Camels were restrained inside steel chutes in standing position. The hind limbs were tied together using a nylon rope and the tail was restrained by tying to the chute in upward direction. Animals were sedated by 2.5 ml xylazine 2% (Xyl-M2, VMD Livestock pharma, Arendonk, Belgium). Faces were removed from the rectum using a gloved lubricated hand. Vagina examination was performed using speculum for inspection of the mucus in the vaginal wall. The vaginal wall was palpated via passing lubricated gloved hand per vagina.

2.3.4. Ultrasonographic scanning:

Trans-rectal ultrasonographic examinations were performed for all camels every 10 days, starting from the day of admission (day 0) until day 40, in the standing position using real-time ultrasound machine equipped with 5 MHz a linear-array transducer (UST-5820-5C, SSD Pro-sound 2, ALOKA, Co., Japan) (figure 2.2) to examine the cervix, uterus, ovaries and fallopian tubes. The uterus was examined for abnormal contents, size and

wall thickness. Measurements of follicles and corpus luteum were taken electronic calipers.

The follicular wave was divided into two phases: the breeding (ovulatory) phase and the non-breeding (non-ovulatory) phase. The breeding (ovulatory) phase is defined as the period of fertile ovulation in which the ovaries have mature ovulatory follicles (12-18 mm) with/without the presence of other sized follicles. The non-breeding (non-ovulatory) phase is defined as the period in which the ovaries have no mature ovulatory follicle and may have follicles ≤11 mm or ≥19 mm. According to the ultrasound examination results, the percentages of camels belonging to the breeding phase and the non-breeding phase were calculated at each examination.

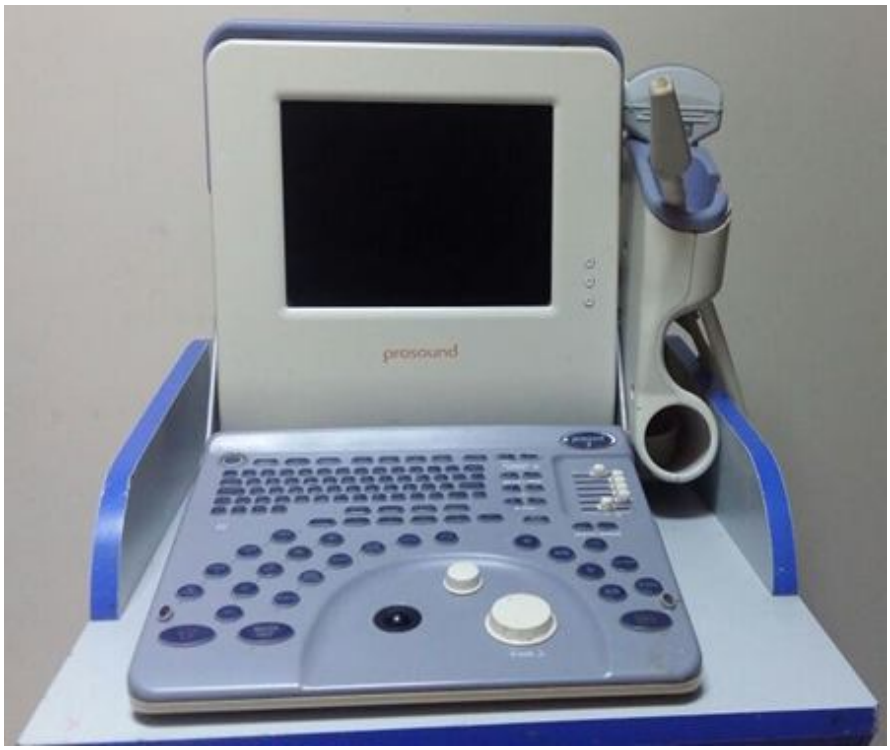

Figure 2.2: Ultrasonic machine

2.3.5. Assessment of sexual receptivity:

The behavior signs of she-camels in the presence of male camel were assessed. The sexual receptivity of the females was evaluated by

approaching to the male and check the acceptance every 10 days (days 0, 10, 20, 30 and 40) using a camel-teaser (without mating) during the period of the experiment until natural mating day. 40% (4/10) expressed good signs of receptivity for up to 5 days, became quiescent for 2-3 days and again started to show sexual behaviors. The sexual receptivity was graded to: abstinence, incompletely receptive, and completely receptive. (Mahla et al., 2015).

2.3.6. Progesterone assay:

Blood samples (5 ml) were collected from jugular vein into plastic vacutainer tubes without anticoagulant at the day of admission and every 10 days after $PGF_{2\alpha}$ injection. The samples were centrifuged for 3 minutes at 3000 rpm to separate serum. The progesterone level was measured using an ELISA test (eProCheck® 2.0, Labstock Micro-Services /Ireland) (figure 2.3). The analysis was done according to the manufacture guidelines.

2.3.7. Bacterial culture and sensitivity test:

Uterine swabs were collected from all camels by double guarded culture swab (Equivet Company, Denmark) (figure 2.4) for bacterial culture and antibiotic sensitivity testing according to Barrow and Feltham (1999). The cervix was opened by the finger of examiner's hand (cervix in camel is weak and opened easily) because it will be closed due to progesterone effect. Then, swab samples were preserved in amies clear medium (R&D, China) and transported cooled to the laboratory within 3 hours after collection (figure 2.5).

The swab was gently squeezed against the wall of tube in order to remove excess fluid. After that it was used to streak the agar plate for a lawn of growth. Sheep blood, MacConkey and sabouraud agars were used (figure 2.6). After the streaking completed, the plate was allowed to dry for 5 minutes. Then, antibiotic discs were placed and gently pressed into the surface of the agar using flame sterilized forceps or inoculation loop (figure

2.7). The antibiotic discs which used in the current study were ampicillin, gentamycin, penicillin, amoxycillin, oxacillin, neomycin, oxytetracycline, chlortetracycline, chloramphenicol, polymixin, streptomycin, furazolidin, trimethoprim-sulphamethoxazole, enrofloxacin, tetracyclin and Co-trimoxazole. The inoculated plates were carefully inverted and incubated for 24 hours at 37° C. After incubation, a metric ruler was used to measure the diameter of the inhibition zone for each antibiotic. The obtained measurement from the individual antibiotics was compared with the standard table to determine the sensitivity zone (whether the tested bacterial species was sensitive or resistant to the tested antibiotic). The exact range of the diameters is well documented and standardized.

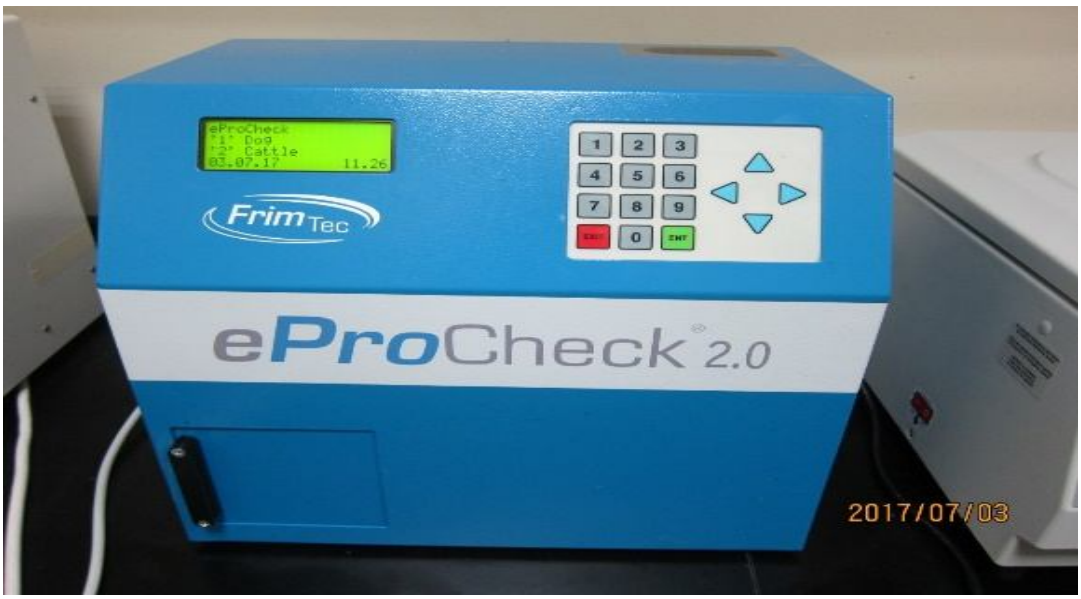

Figure 2.3: ELISA test device for measurement of progesterone

Figure 2.4: Double guarded culture swab for bacterial culture

Figure 2.5: Swab (CITOSWAB) with amies clear medium

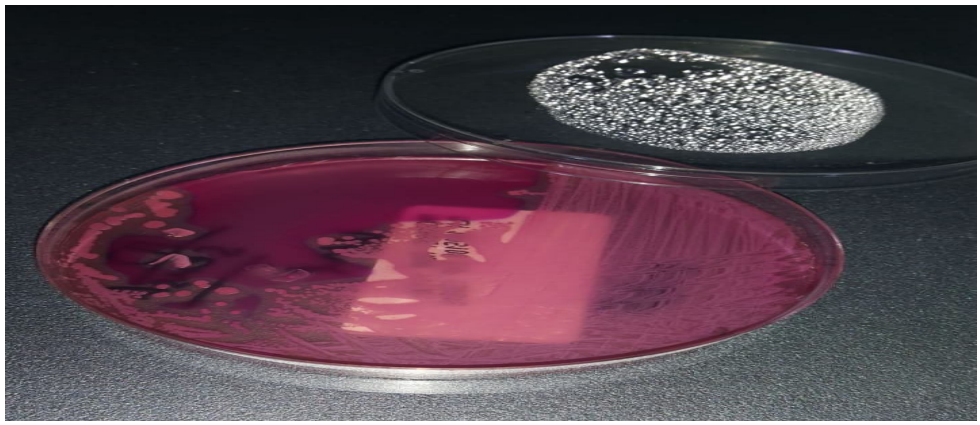

Figure 2.6: Culturing of bacteria in the blood agar.

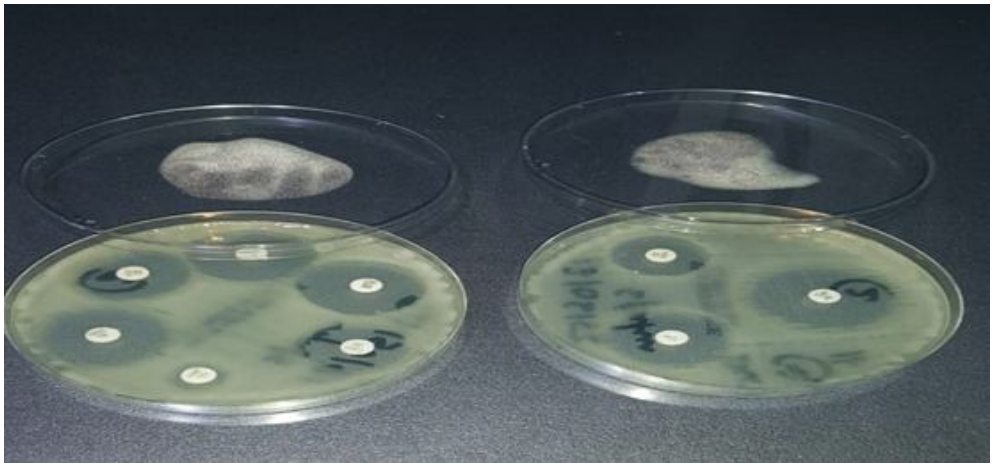

Figure 2.7: Antibiotic sensitivity tests.

2.3.8. Prostaglandin treatment regime:

Camels which had high level of progesterone (>1.5 ng/ml), with persistent CL on the ovary, at the day of admission (day 0) and day 10 were subjected to $PGF_{2\alpha}$ treatment. These animals were considered have infertility and divided equally into two groups each consists of 10 animals. First group (n=10): received 3 ml (0.75 mg) of cloprostenol i/m (Oestrophan, 0.25 mg/ml injection solution , Bioveta, Czech Republic) at day 10, 20, and 30 and called group I. Second group :(n=10) received 5 ml (1.25 mg) of Cloprostenol i/m and called group II.

After collecting the uterine swab, uterine douching was performed (everyone day from $PGF_{2\alpha}$ injections) using 120 ml of lotagen (1.728 g Policresulenum as Polycondensate of m-cresolsulfonic acid and formaldehyde at the mass ratio of 14:1) (Bioveta, Czech) and the Bovivet disposable uterine catheter (Kruuse, Denmark) with a 60 ml disposable syringe. Excess faeces were removed from the rectum using gloved lubricated hand. Vulva and perineal region of the camel were cleaned using water and the area was wiped and dried using a clean tissue paper. Gloved lubricated hand was passed into the rectum and the uterine catheter was introduced into the uterus through the vagina and cervix. Based on the antibiotic sensitivity test results, the recommended doses of gentamycin 10% (as sulphate) 100 mg/ml (GENTAJECT, FATRO/Italy), ampidexalone (Ampicillin trihydrate 87,00 mg per 1 ml and dexametazon 25mg per 1 ml) (Maravet/ France) or teramycin (LA 20% 200mg oxytetracycline per ml) (Pfizer/ brazil) were administered parenterally.

2.3.9. Mating of recovered animals:

Ten days after the treatments, all camels were re-examined ultrasonographically to evaluate their recovery from persistent CL and/or endometritis. Only recovered camels, with mature follicles (12–18 mm), were mated naturally after induced ovulation using 0.021 mg of buserelin

acetate (5ml Receptal, MSD). Pregnancy was diagnosed using ultrasonography on day 20 post-mating and confirmed on day 40.

2.4. Statistical analysis:

Data were analyzed using SPSS (Statistical Package for Social Sciences) version 16 (2007). The differences in the percentages achieved in each treatment group (group I and group II) were calculated by the Chi-square test. The level of significance was tested at a 0.05 or 0.01 level of probability. The progesterone hormonal levels were analyzed using analysis of variance (ANOVA) and presented as mean ± standard deviation. The correlation between CL diameters and progesterone levels was determined using person correlation analysis (Rocha et al., 2019).

CHAPTER III

RESULTS

3.1. Sexual receptivity:

Sexual receptivity of she-camels is presented in figure 3.1. At day 0 and 10, all camels (100%) showed abstinence (erection and curving of the tail, raising head and refusing the male) (figure 3.2). Ten days after first dose of $PGF_{2\alpha}$ (day 20), 50% and 80% of she-camels showed incomplete receptivity in group I and group II, respectively (Figure 3.3). Half of animals (50%) from group II showed complete receptivity at day 30, while only 30% of camels from group I accepted the male at day 40 (figure 3.4.).

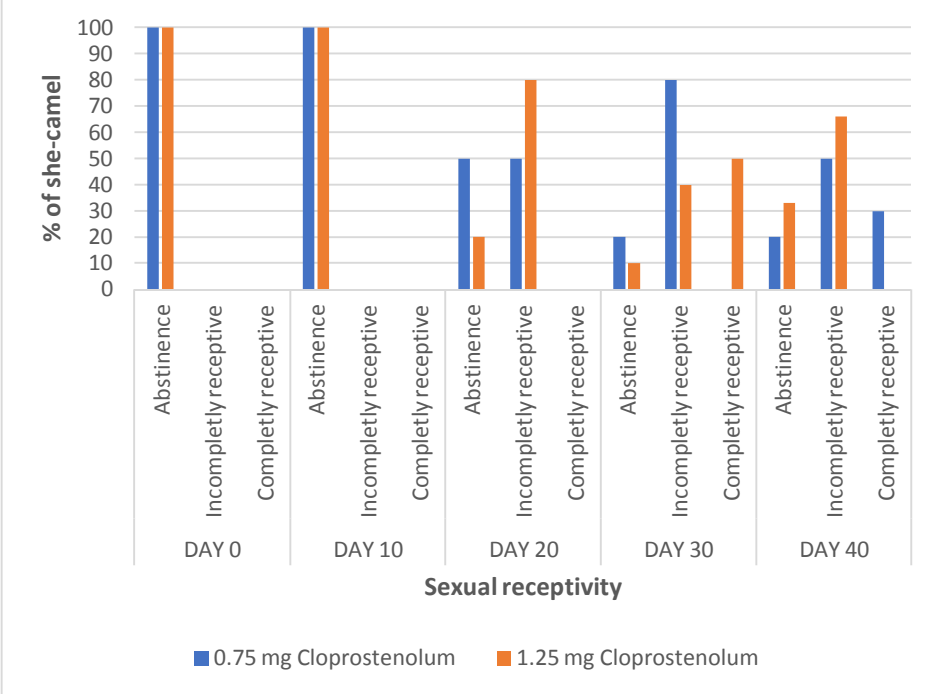

Figure 3.1: Sexual receptivity in the she-camels

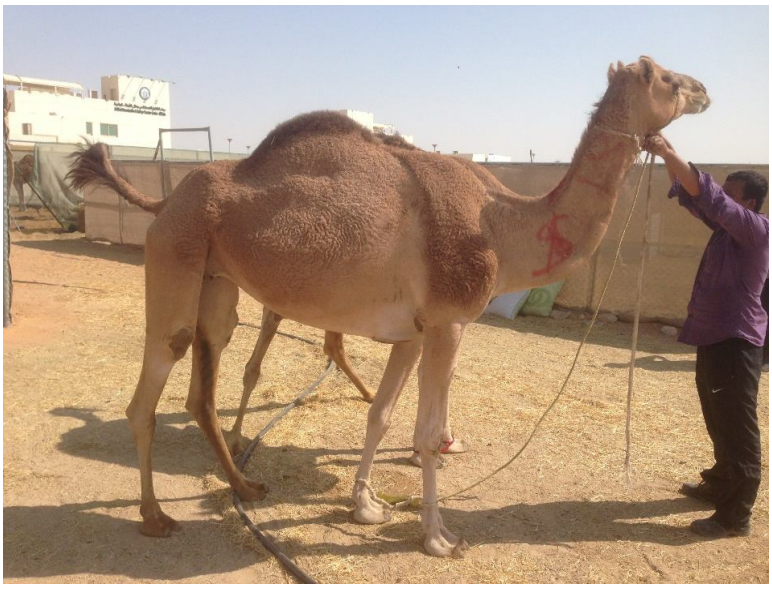

Figure 3.2: The she-camel showing abstinences (erection and curving of the tail).

Figure3.3: The she-camel showing incomplete receptivity.

Figure 3.4: The she-camel showing complete receptivity.

3.2. Measurements of CL diameters:

The diameters of CLs before and after treatment by different doses of $PGF_{2\alpha}$ are presented in Figure 3.5. The diameters of CLs were almost the same at day 0 (2.45±0.15 and 2.47±0.29 cm) and day 10 (2.43±0.15 and 2.43±0.30 cm) in group I and group II, respectively. The diameters of CLs were significantly ($p \leq 0.05$) decreased in both groups at day 20 (1.74±0.09 and 1.35±0.29 cm) (Figure 3.6) and day 30 (1.19±0.07 and 0.74±0.24 cm). At day 40, the diameters of CLs were decreased in group I (0.83±0.05 cm), while no significant difference in CLs diameters (0.68±0.06 cm) in group II were observed. The diameters of CLs significantly ($p \leq 0.05$) decreased in group I than in group II at day 20 and 30.

3.3. Measurements of follicles diameters:

The diameters of dominant follicle before and after treatment by different doses of $PGF_{2\alpha}$ are presented in Figure 3.7. No changes in the diameters of dominant follicles were recorded at day 0 (0.60±0.17 and 0.80±0.54 cm) and day 10 (0.60±0.16 and 0.80±0.54 cm) in group I and group II, respectively. In the group II, the maximum diameters of dominant follicles were observed at day 30 (1.33 ± 0.42 cm) (Figure 3.8) and then

decline at day 40 (1.20 ± 0.26 cm). In the group I, the follicles diameters were gradually increased until reach maximum value (1.38 ± 0.30 cm) at day 40. No significant difference in the diameter of dominant follicle was observed between both groups.

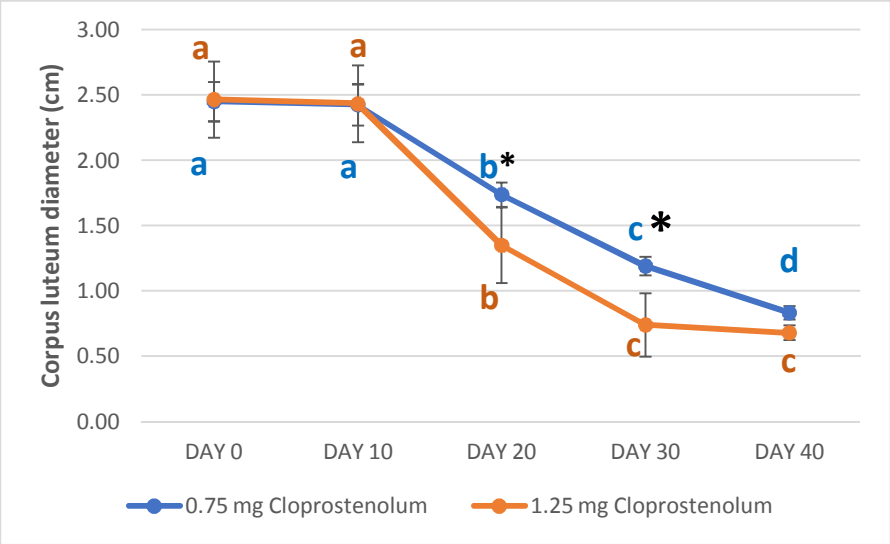

Figure 3.5: The diameters of CL before and after treatment by different doses of $PGF_{2\alpha}$. ($p \leq 0.05$)

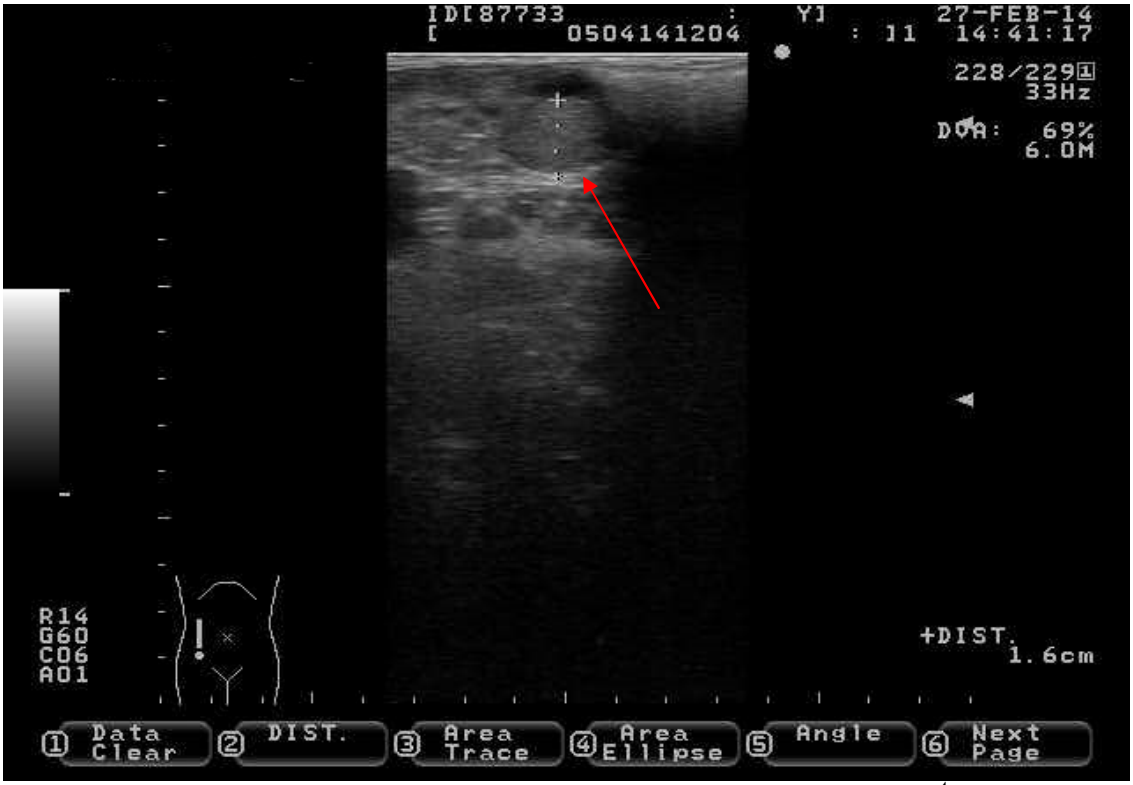

Figure 3.6: Typical CL image at day 20 (10 days post to 1st dose of PG 0.75)

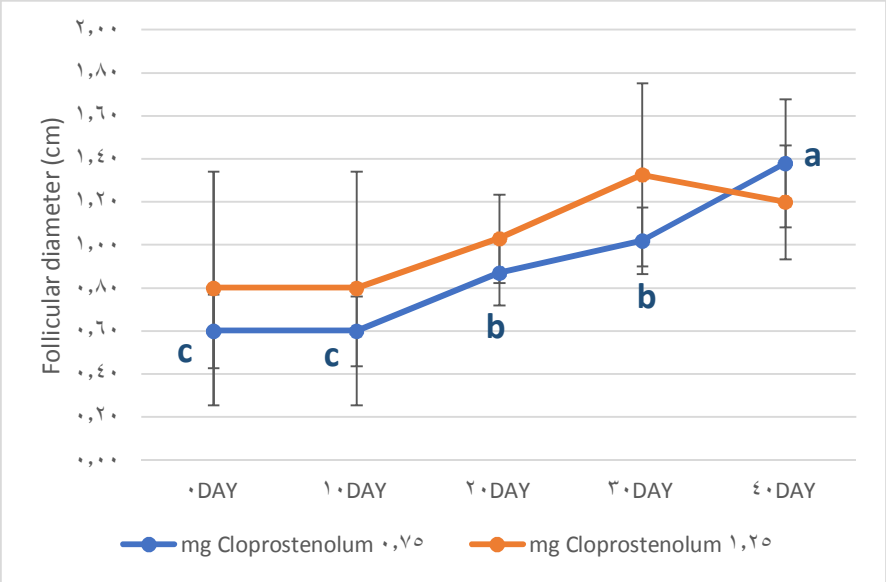

Figure 3.7: The diameters of follicles before and after treatment by different doses of $PGF_{2\alpha}$.

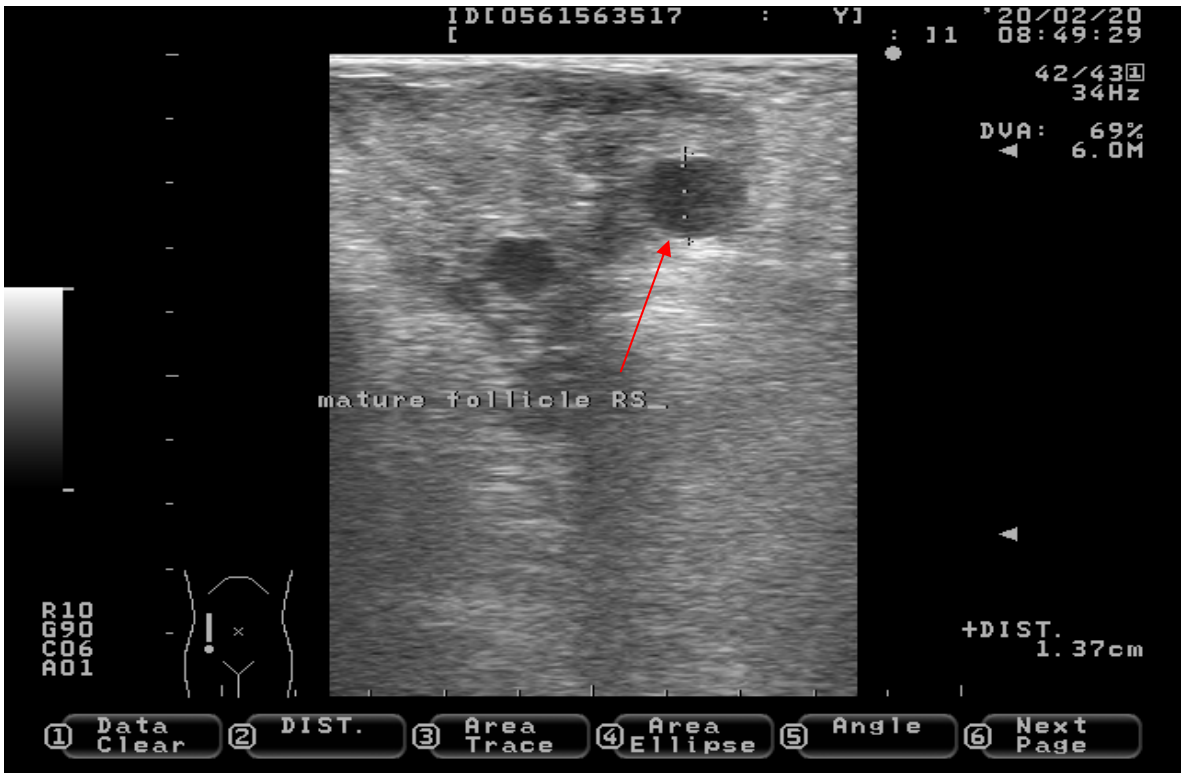

Figure 3.8: Mature dominant follicle at day 30 (10 days post to 2nd dose of PG 1.25)

3.4. Responsiveness of she-camels for breeding:

The percentages of she-camels in the breeding and non-breeding phases before and after treatment by different doses of $PGF_{2\alpha}$ are presented in Figure 3.9. At day 0 and 10, all camels were in the non-breeding phase in both groups. At day 30, the percentage of camels in the breeding phase were significantly ($p \leq 0.05$) higher in the group II (70%) than the group I (20%). At day 40, the percentage of camels in the breeding phase was significantly ($p \leq 0.05$) higher in group I (80%) than group II (33%).

3.5. Assessment of serum progesterone level:

Measurement of serum progesterone level before and after treatment by different dose of $PGF_{2\alpha}$ is presented in Figure 3.10. At day 0 and 10, the serum progesterone levels were the same (2.45±0.30 and 2.67±0.28 ng/ml) in the group I and group II, respectively. At day 20 and 30, the serum progesterone levels declined in both groups (1.73±0.21 and 1.50±0.34 ng/ml) and (1.23 ± 0.16 and 0.92± 0.37 ng/ml), respectively. There was higher significant difference ($p \leq 0.05$) between progesterone level in the group I and group II at day 30. At day 40, the serum progesterone level was significantly decreased in the group I (0.84±0.10 ng/ml); while no significant decrease was observed in the group II (0.91±0.04 ng/ml).

3.6. The correlation between the CL diameter and serum progesterone concentration:

The correlation between the CL diameter and serum progesterone concentration is presented in Figure 3.11. Strong positive correlation between the CL diameter and serum progesterone concentration was reported ($r = 0.93$).

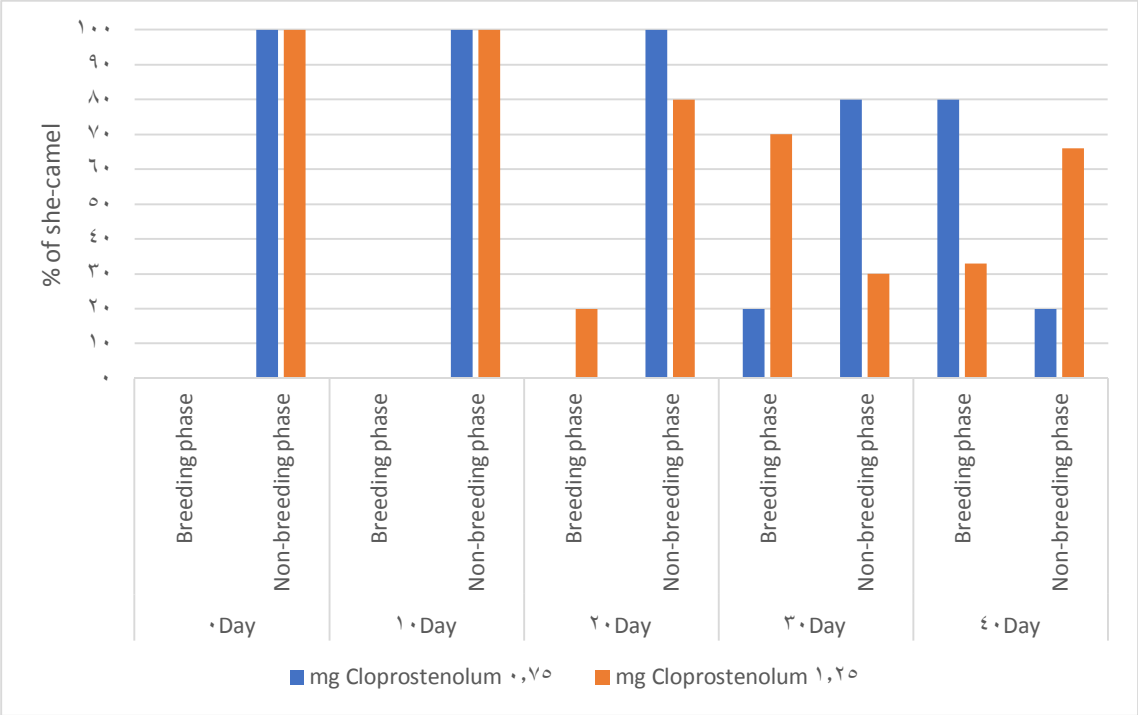

Figure3.9: The percentages of camels in breeding phase before and after treatment by different doses of $PGF_{2\alpha}$.

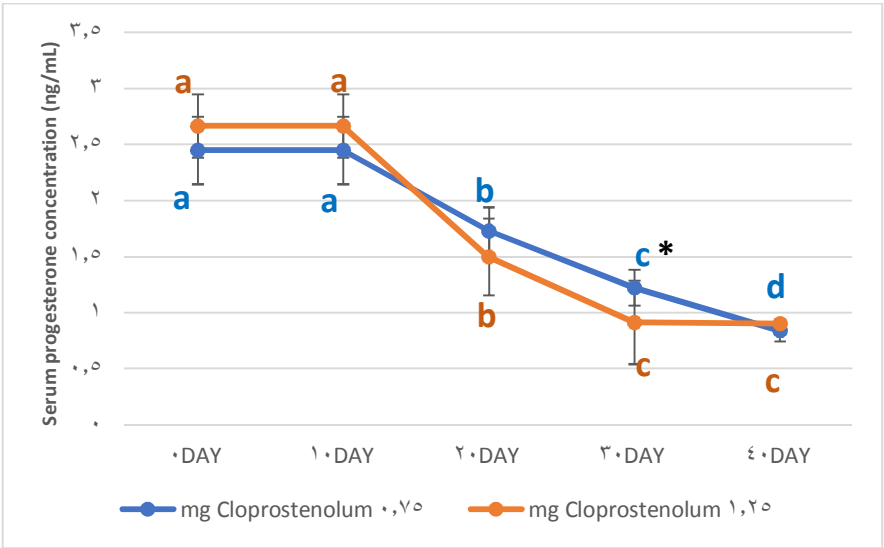

Figure 3.10: The serum progesterone level before and after treatment by different doses of $PGF_{2\alpha}$($p \leq 0.05$)

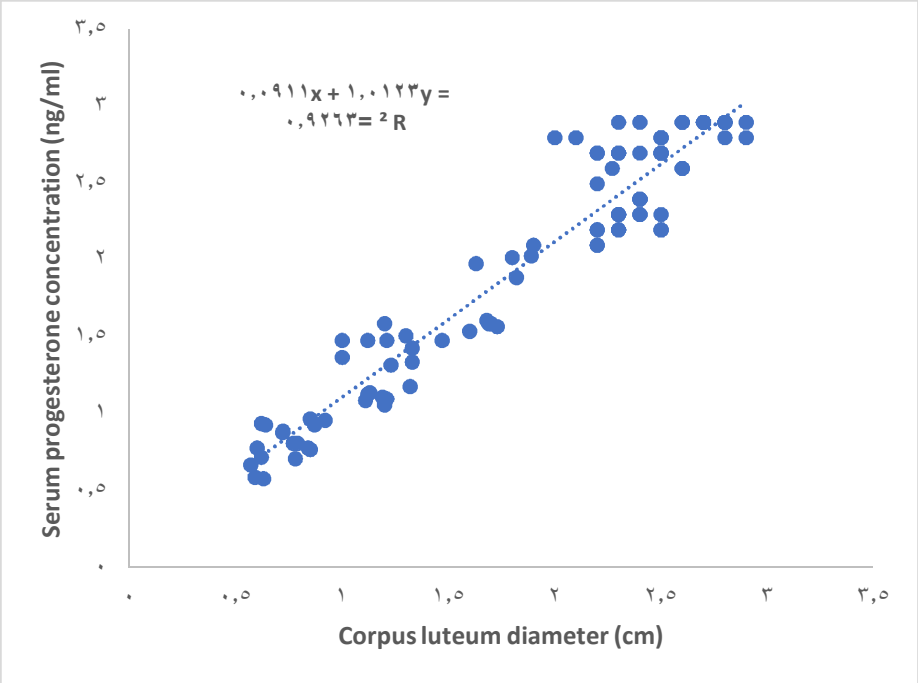

Figure3.11: The correlation between the corpus luteum diameter and serum progesterone concentration.

3.7. Bacterial isolation and sensitivity test:

The bacterial isolation from the uterus was presented in figure 3.12. The most isolated bacteria were *Escherichia coli* by 70% (55% *Escherichia* coli plus 15% combination of *Escherichia* and *Staphylococcus haemolyticus*) followed by *Staphylococcus haemolyticus* 25% (10% *Staphylococcus haemolyticus* and 15% Combination of *Escherichia* and *Staphylococcus haemolyticus)*. While, *Pasteurella pneumotropica* and *Brevundimonas diminuta* were isolated one time (5% for each one). *Clostridium perfringens* was isolated two times (10%). The results of antibiotic sensitivity test for *Escherichia coli* and *Staphylococcus haemolyticus* are presented in figure 3.13. *Escherichia coli* and *Staphylococcus haemolyticus* were (100%) sensitive to gentamycin. The results of antibiotic sensitivity test for *Pasteurella pneumotropica*, *Brevundimonas diminuta* and *Clostridium perfringens* are presented in

table 3.1. *Pasteurella pneumotropica*, *Brevundimonas diminuta* and *Clostridium perfringens* were (100%) sensitive to ampicillin.

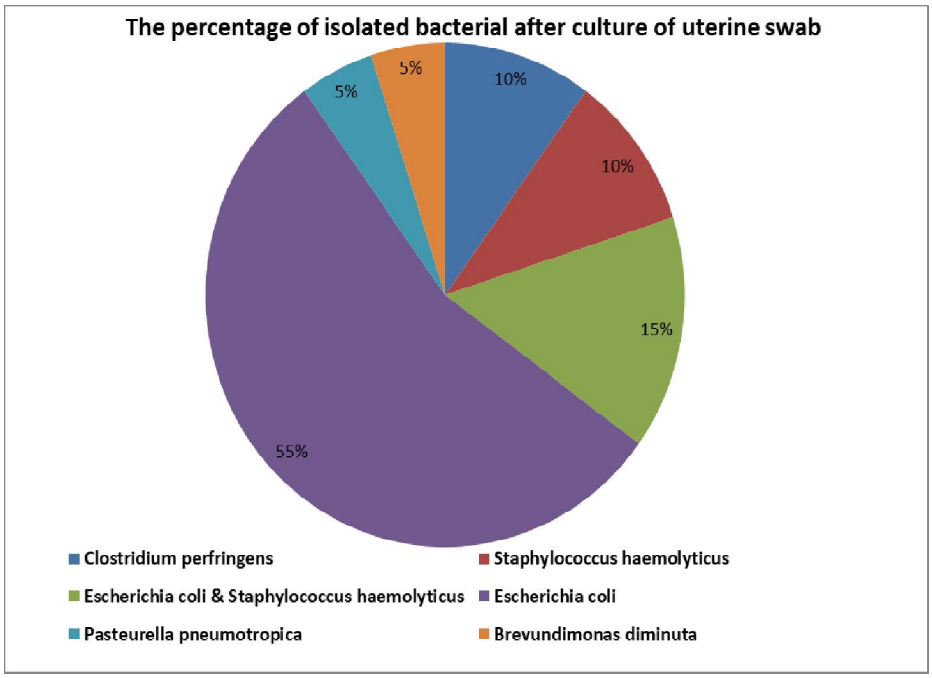

Figure3.12: The bacteria isolated from the uterus.

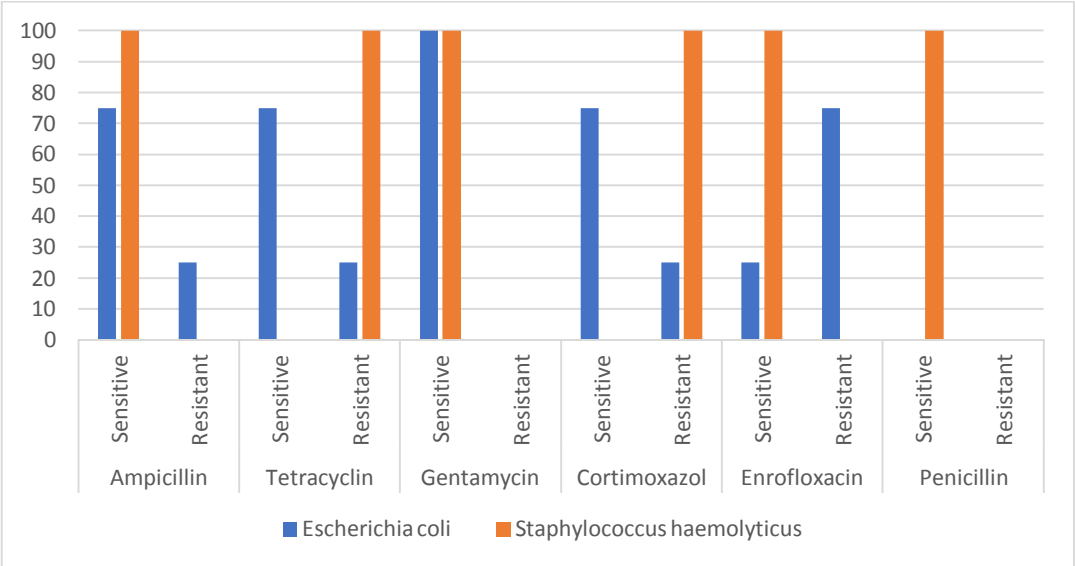

Figure3.13: The antibiotic sensitivity test for *Escherichia coli* and *Staphylococcus haemolyticus*.

Table 3.1. The antibiotic sensitivity test for *Brevundimonas diminuta, Clostridium perfringens,* and *Pasteurella pneumotropica.*

SN	Antibiotics	*Brevundimonas diminuta*	*Clostridium perfringens*	*Pasteurella pneumotropica*
1	Ampicillin,	100%	100%	100%
2	Gentamycin	80%	0%	50%
3	Penicillin	40%	30%	70%
4	Amoxycillin	40%	40%	60%
5	Oxacillin	80%	80%	0%
6	Neomycin	100%	0%	0%
7	Oxytetracycline	70%	0%	70%
8	Chlortetracycline	80%	0%	80%
9	Chloramphenicol	60%	40%	70%
10	Polymyxin	80%	0%	0%
11	Streptomycin	70%	0%	0%
12	Furazolidone	70%	60%	70%
13	Sulfamethoxazole / trimethoprim	0%	0%	100%
14	Enrofloxacin	50%	40%	60%

3.8. The pregnancy rate of recovered camels:

The pregnancy rate of camels after treatment is presented in figure 3.14. The pregnancy rate was 20% and 60% in the group I and group II, respectively. A significant difference was found between both groups.

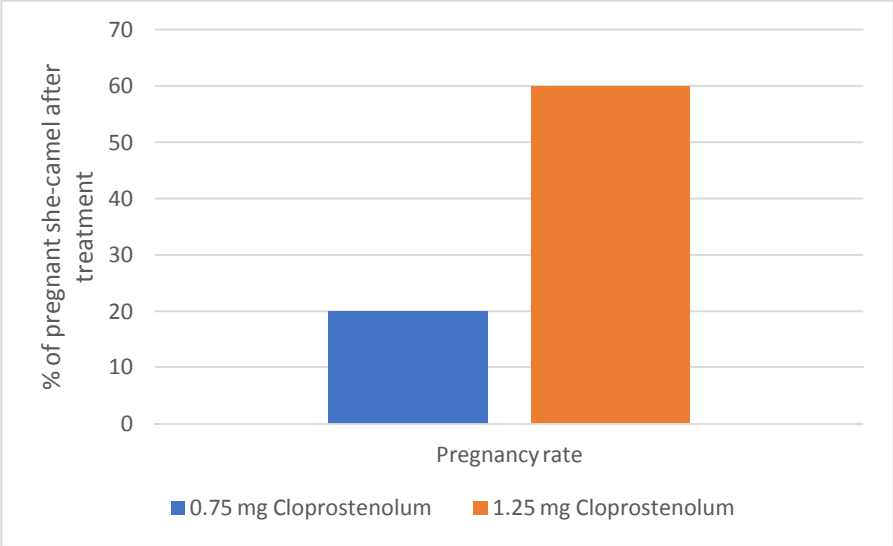

Figure3.14: The pregnancy rate of camel after treatment by different doses of $PGF_{2\alpha}$.

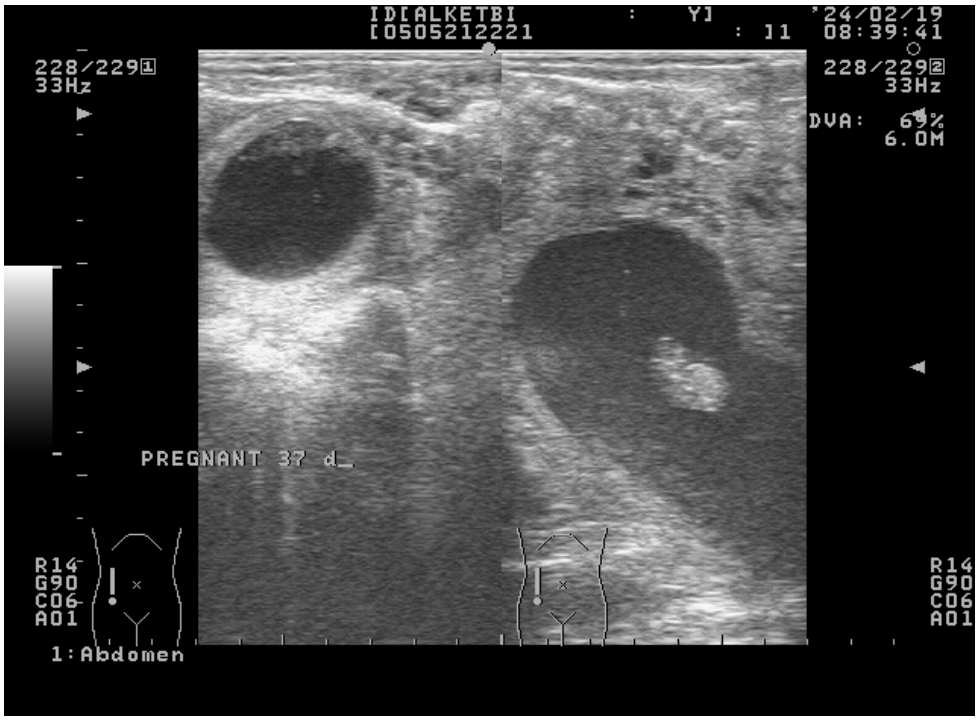

Figure 3.15: Fetus at 37days of gestation after treatment with PG 1.25

CHAPTER IV

DISCUSSION

Persistent corpus luteum is a major problem in the camel industry as it causes financial loss to farmers. In the presence of persistent corpus luteum, the owners wait for more than one year to find that the camel is not pregnant as most owners rely on external signs of pregnancy like tail lifting and urination which is usually shown after 12 to 17 days of successful breeding. In the UAE camel owners are interested in breeding their female camels to specific high genetic camel males. Subsequently, the desired male breeds hundreds of females every breeding season and subjected to get infection from infected female which in turn increases the chance for cross-infection of the uterus accompanied by persistent of CL.

In the present study, all camels (100%) showed sexual abstinence (erection and curving of her tail, raising the head and refusing the male) as all of them had at least one CL diagnosed at day 0 and at day 10 and this result come in agree with those obtained by Shalash and Nawito. (1964) ; Tibary and Anouassi, 2000) , who mentioned that the corpus luteum was considered persistent when it was evident on different subsequent sonographic examinations without evidence of pregnancy and tail cocking. In addition to Sghiri and Draincourt. (1999) who reported that the presence of CL for a prolonged period leads to a non-cyclic ovary and abstinence situation.

Our results were also similar to that reported by Tibary and Anouassi. (1997), who mentioned that persistent corpus luteum in female camels cause reproductive disturbances and infertility. The female camels will show false signs of pregnancy.

Our results revealed that all camels in both groups (group I and group I) had CL measured (2.45±0.15 and 2.47±0.29 cm) respectively. Different result was reported by Elias et al. (1984) who measured the CL 2.2±0.1 cm and this may be due to the effect of climate or breed.

In the present, all CLs could be palpated, clearly detected by ultrasound examination, and associated with uterine infections according to sensitivity test. This result comes in agree with Waheed. (2009) who stated the corpus luteum is usually palpated at pregnancy, however, some female camels show corpora lutea in a non-pregnancy state following embryonic death and pyometra.

The dimensions of CLs in the present study were higher than that mentioned by Chuang et al. (2010) who reported that a cystic corpus luteum (CL) is defined as "luteal tissue initiating from a corpus hemorrhagicum and containing fluid in a central cavity greater than 7 mm in diameter".

In the current study, when we found that she- camel was ready for breeding, she gets closer to male pen, chasing and mounting the others females. Our results are similar to mentioned by Mahla et al. (2015) who found that the following signs for the female in estrus followed the male, came near and remains in close proximity, restlessness, get excited in presence of male, jumps, runs and try to get close to male or other females. The measurements of dominant follicles in the current study found to be (1.33 ± 0.42 cm) in group II and, (1.38 ± 0.30 cm) in the group I, respectively. Our findings are similar to that reported by Skidmore et al. (1996); Manjunatha et al. (2012), who reported that the diameter of the mature follicle that is optimum for mating is 1.0-2.0 cm.

In the present study, all camels were in the non-breeding phase in both groups after 10 days later to first dose of $PGF_{2\alpha}$ treatment and our findings disagree with Quzy et al. (2013) ,who mentioned that $PGF_{2\alpha}$ could easily regress the CL, and mature follicles were seen on day 8 of treatment and Ismail et al. (1998), who reported that mature ovulatory sized follicles are present on the ovaries within 4 to 5 days of treatment.

Our study revealed that the level of serum progesterone in all 20 camels were high (2.45±0.30 and 2.67±0.28 ng/ml), and all of them were infertile these results come in accordance with Youngquist and Threlfall. (2007) who mentioned that the presence of active luteal tissue on the ovary leads to the anovulatory state where growing follicles of consecutive waves proceed to dominance but fail to ovulate due to the negative feedback of progesterone on LH release.

In our study, the serum progesterone levels were (2.45±0.30 and 2.67±0.28 ng/ml) in the group I and group II, respectively. This agree with Tibary and Anouassi, (1997), who reported that the level of progesterone remain above 2 ng/ml from the initial dictation with CL. Conversely, our results disagree with Quzy et al. (2013) who found that a high proportion of camels (75% 6/8) with ovarian cysts evidenced cocking behavior for prolonged periods (more than 2 months) and a high plasma progesterone (<1.5ng/mL). This slightly difference could be attributed to climatic differences.

The present study also agreed with Adam et al. (1989) who found that the persistent CL is suspected when there is a prolonged elevated plasma progesterone level in the absence of pregnancy.

The present study at 20 and 30 days later to administration of $PGF_{2\alpha}$, the serum progesterone levels declined in both groups (1.73±0.21 and 1.50±0.34 ng/ml) and (1.23 ± 0.16 and 0.92± 0.37 ng/ml), respectively.

These results come in accordance with that obtained by Skidmore. (2005), who found that there is a sharp decline in progesterone level after luteolysis in camel.

In the present study there is a strong positive correlation between CL diameter and serum progesterone concentration. Our results agree with that obtained by Nagy et al. (2005), who mentioned that luteal diameter and serum progesterone concentration were positively correlated ($r = 0.71$, $P < 0.001$), but there was a significant difference between morphological and functional development of the CL in dromedaries. Similar results were also obtained by Lüttgenau et al. (2011), who found that low plasma progesterone levels are accompanied by reduced luteal size and increased size of the dominant follicle.

All camels in our present study had uterine infection with several types of bacteria isolated from the uterus similar results were found by Noakes et al. (1990).Persistent corpus luteum is mostly associated with pathological conditions of the uterus. The present findings also agreed with Werny and Kumar. (1994) who reported that the uterine infections were considered to be the most common cause of reproductive failure in female camels.

In the present study the most commonly isolated bacteria were *Escherichia coli* (70%) followed by *Staphylococcus haemolyticus* (25%). *Pasteurella pneumotropica* and *Brevundimonas diminuta* (5%) and *Clostridium perfringens* (10%). The results partially disagreed with Ali et al. (2010 a) who reported that the most frequently isolated bacteria from female camels with uterine infections were *Arcanobacterium pyogenes*, *Streptococcus pyogenes*, and *Staphylococcus aureus*, *Corynebacterium, E. Coli* and *Proteus*. Nabih and Osman. (2012) found that the most isolate bacteria

were high percentage of *Corynebacterium* and *Proteus sp*. followed by *Klebseilla sp.*, while *Salmonella sp*., was the lowest isolates.

Based on sensitivity test, the most effective antibiotic is gentamycin followed by ampicillin then oxytitracycline combined with uterine douching of loutagen. This was in agreement with Nabih and Osman (2012) findings that gentamycin I/M injection seems to be more efficient in treating female camels with endometritis in combination with acriflavine intra uterine wash in addition to Swelum et al. (2015) who reported that recovery was higher in cephapirin-treated animals (87.5%) but in the current study 80% from isolated bacteria were sensitive to the gentamycin and 55% from isolated bacteria were sensitive to oxytetracycline, fairly compatible with the result of Swelum et al. (2015) who reported that 66.7% of animals treated by oxytetracycline showed good result. Oxytetracycline is a broad-spectrum antibiotic that is active in mucopurulent and anaerobic environments.

In the present study, $PGF_{2\alpha}$ was used basically to treat the persistent CL Persistent corpora lutea can be treated by injecting luteolytic dose of PGF2α. (Tibary and Anouassi, 1997; Skidmore, 2005).

In the present study, the pregnancy rate was higher (p= 0.068) in group II than group I (60 and 20 %, respectively) and these findings come in accordance with Powers et al. (1990); Wernery and Kumar (1994), who reported pregnancy rates after endometritis treatment vary from 30 to 60% while in another study, the conception rates obtained after endometritis treatment with acriflavine 0.1%, lotagen 4% and gentamicin (300 mg/100 ml) were 58.9%, 49.3% and 42.5%, respectively (Ali et al. 2010 a).

CONCLUSION AND RECOMMENDATIONS

1. Treatment of persistent CL in dromedary camel requires two doses (i/m injections) of 0.75 mg or three doses 1.25 mg of $PGF_{2\alpha}$.
2. The most isolated bacteria causing endometritis in she camel were *Escherichia coli* followed by *Staphylococcus haemolyticus* and can be treated using gentamycin.
3. Positive correlation was found between CL diameter and progesterone level.
4. The owners should be advised not to depend upon tail lifting only as a sign of pregnancy in the camel and should be advised to confirm the pregnancy using ultrasonography.
5. Vets & lab technicians should not be confirmed the pregnancy based on the progesterone level.
6. Further studies should be conducted to evaluate the effect of higher doses of $PGF_{2\alpha}$ for treatment of persistent CL.

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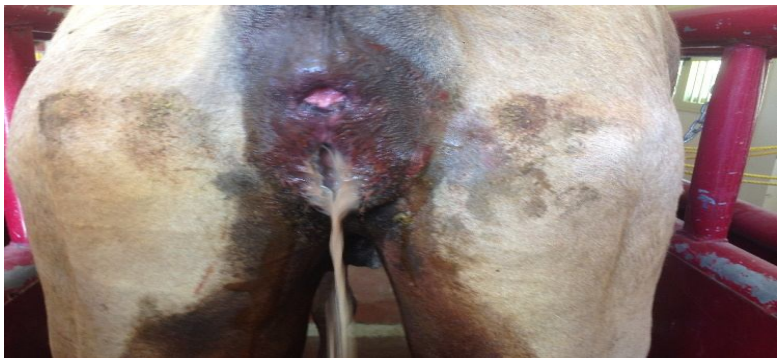
APPENDICES

Appendix A: images of camels

1. Controlling the animal



2. Uterine infections (pyometra)

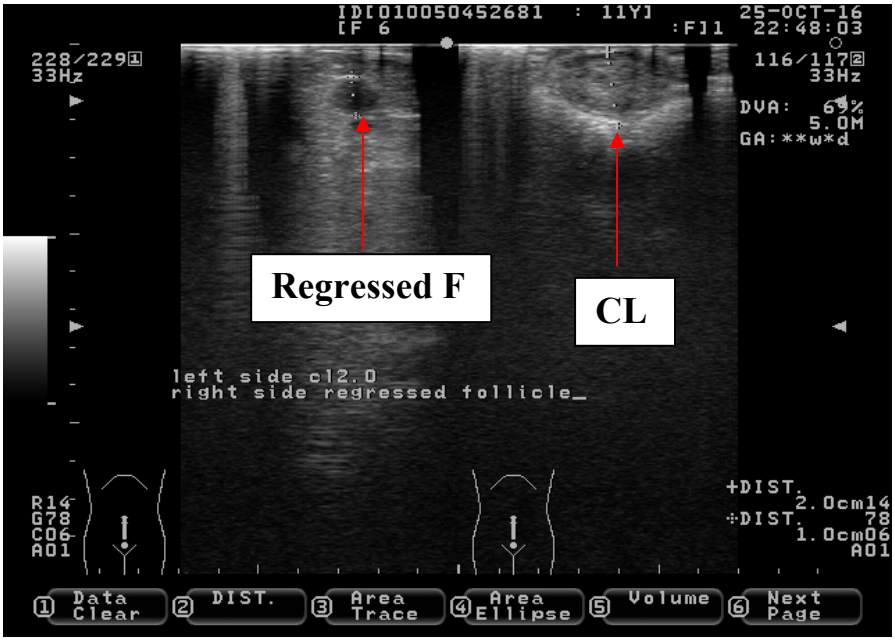
Appendix B: kits, hormones and drugs

1. ELISA kits

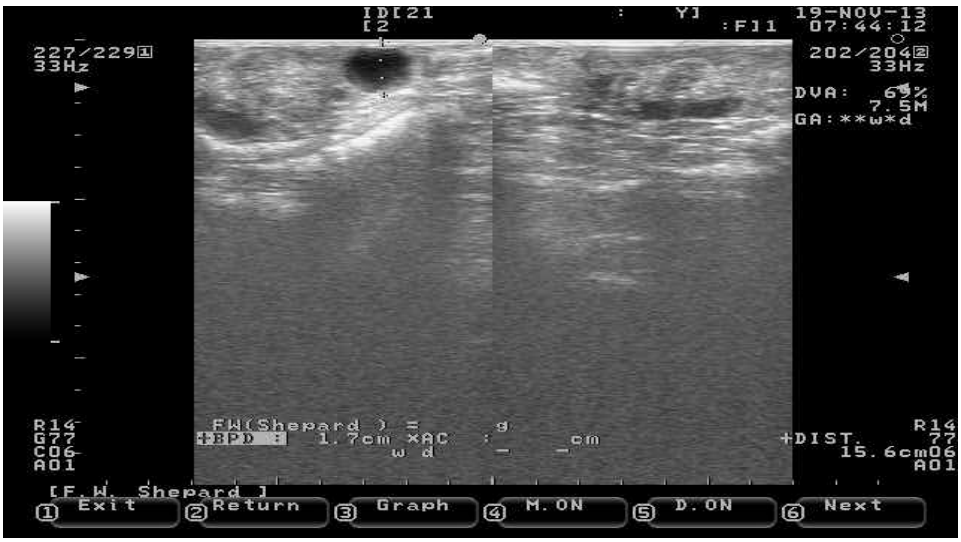
2. Cloprostenol and Lotagen

3. Antibiotics

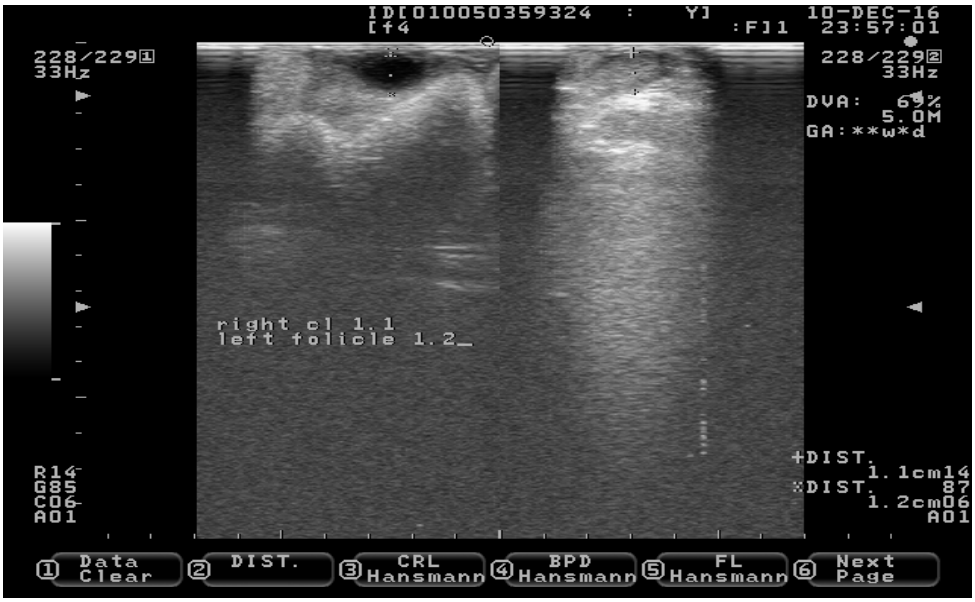
Appendix C: Different ultrasound images



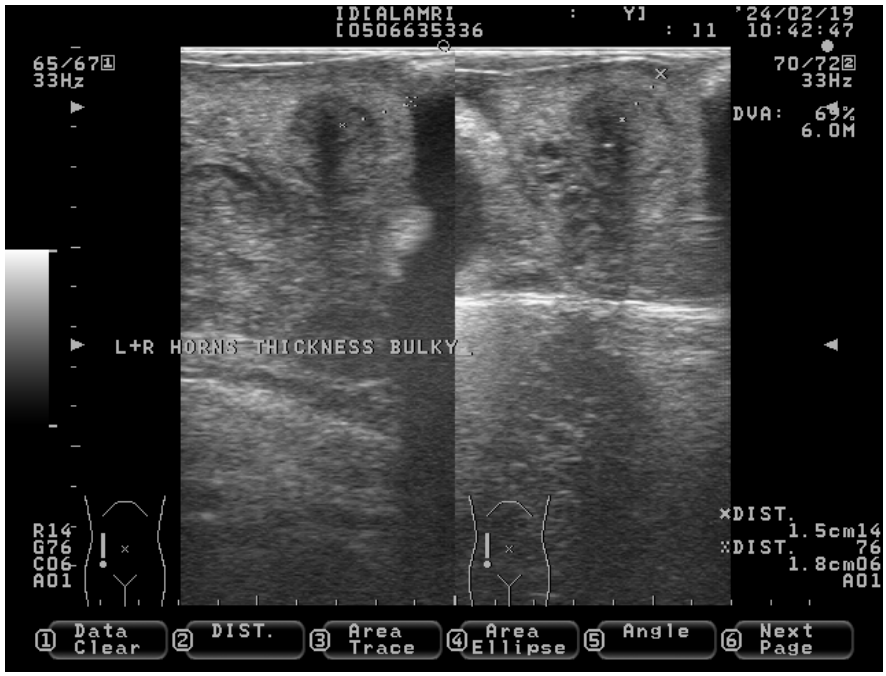
1. CL in the left ovary & regressed follicle in the right ovary



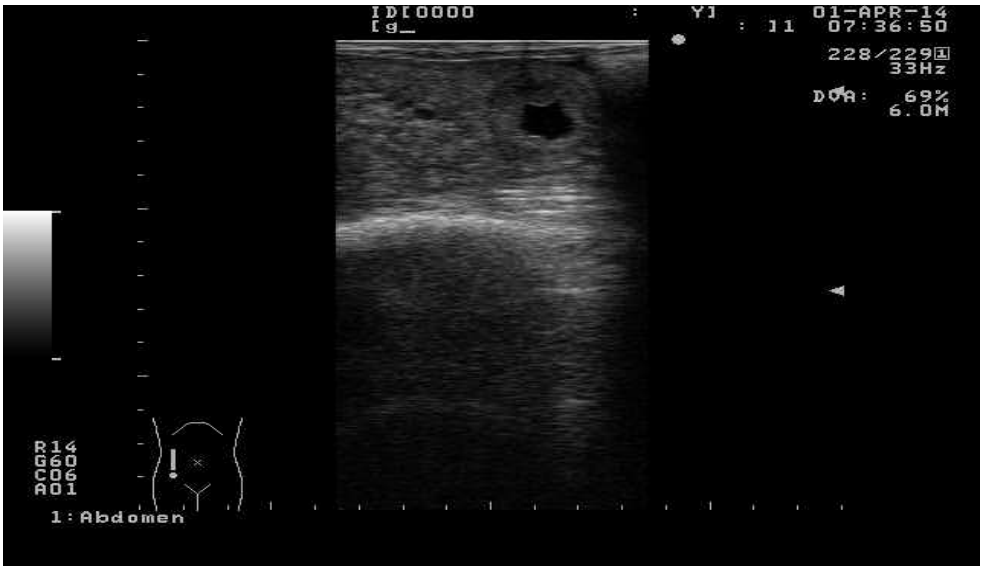
2. Mature dominant follicle at day 30 (10 days post to 3^{d} dose of PG 0.75)



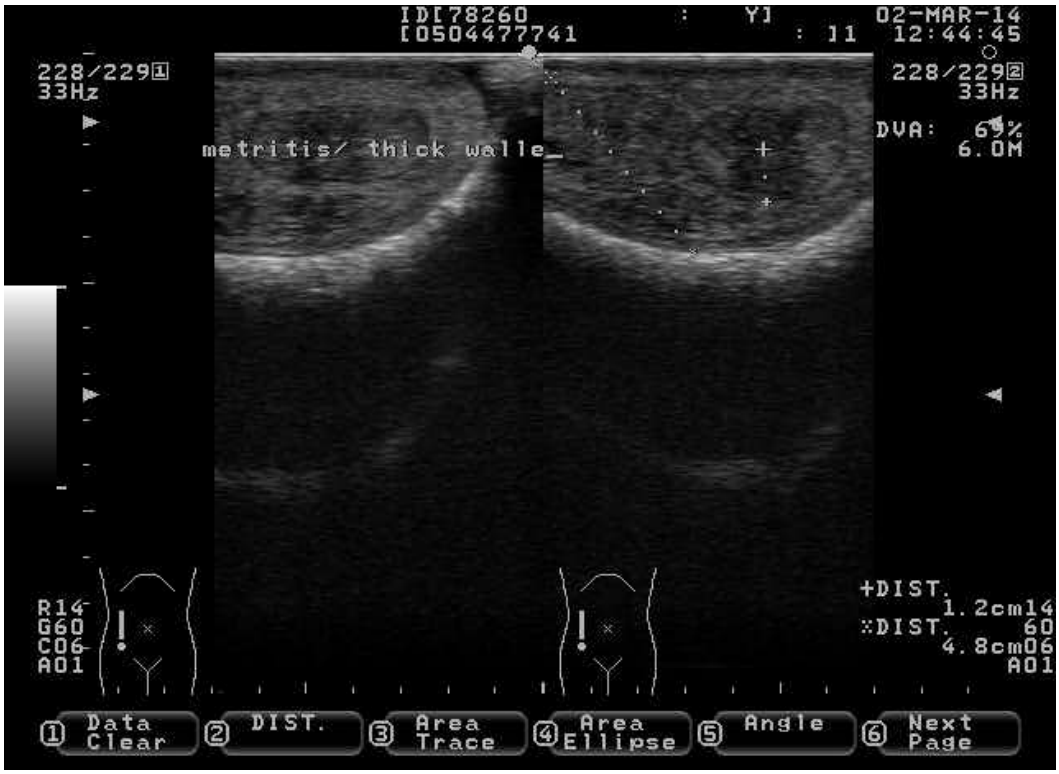
3. Mature dominant follicle at day 30 (10 days post to 2nd dose of PG 1.25



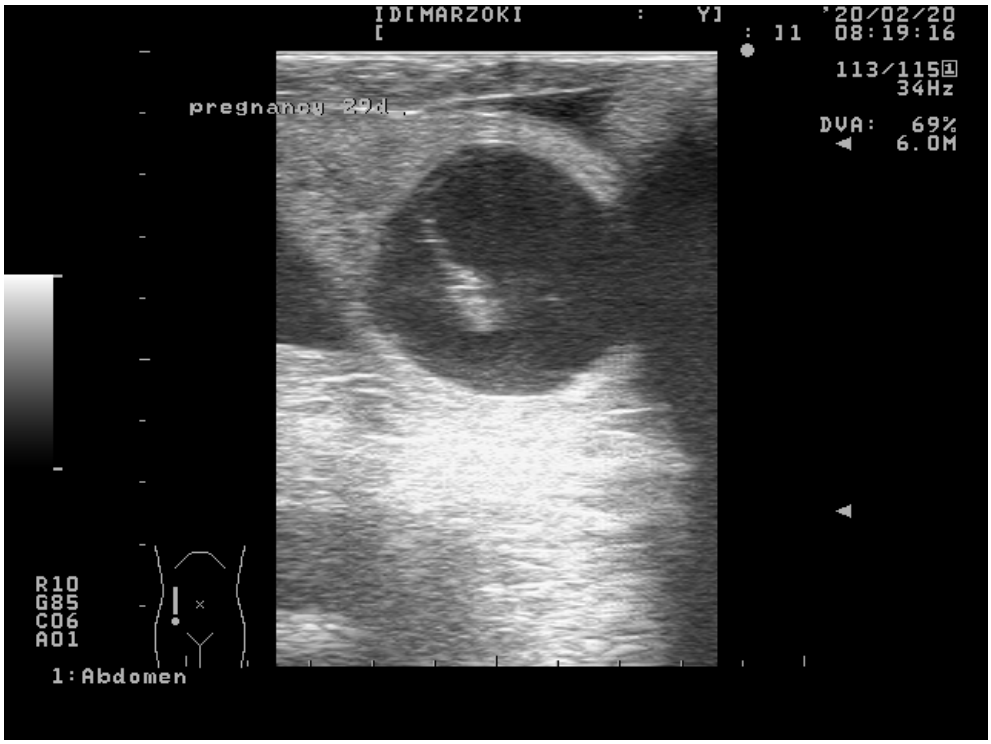
4. Thickness in the uterine wall



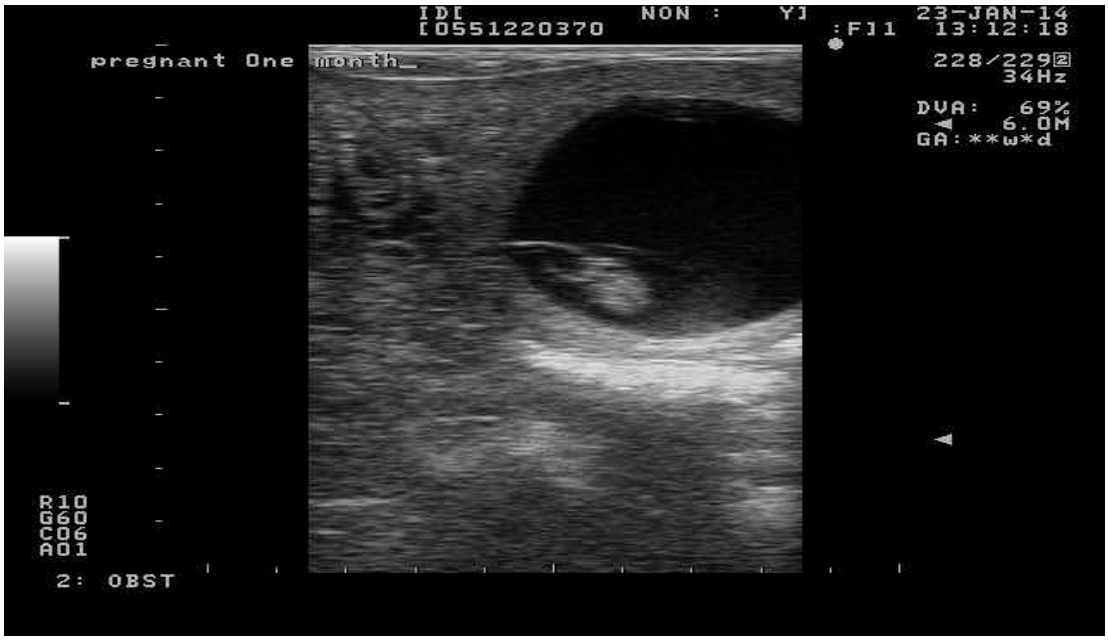
5. Fluid inside the Horne (metritis)



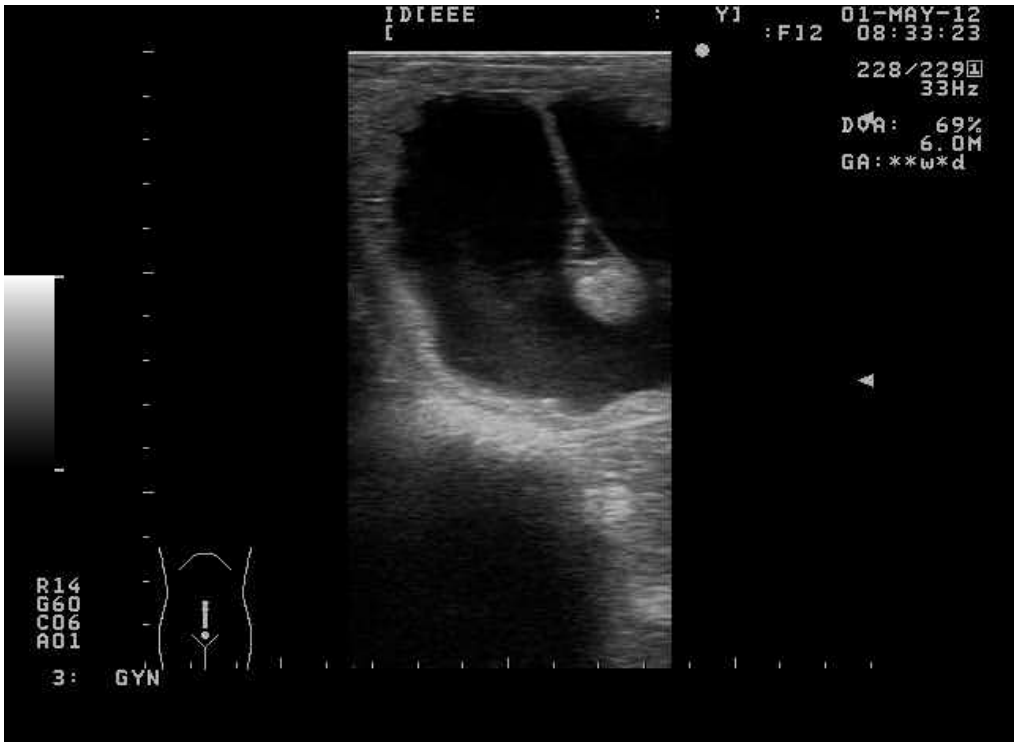
6. Thickness in the uterine wall (metritis)



7. Pregnancy 29 days after treatment with $PGF_{2\alpha}$



8. Pregnancy 30days



9. Pregnancy 35days