



Sudan univerSity for Science & technology College of graduate studies

# Ovulation Induction in Dromedary She – camels by using two Different protocols During non Breeding Season

تحفيز الاباضه في النوق خارج موسم التزاوج بإ ستخدام نوعين من البرامج الهرمونيه

# Ву

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Dissertation Submitted to the college of graduate Studies for the Partial fulfillment of the requirements for the degree of (M Sc) in animal production

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2016

**Koranic Verse** 

قال الله تعالى :

بسم الله الرحمن الرحيم ( افلا ينظرون الى الابل كيف خلقت )

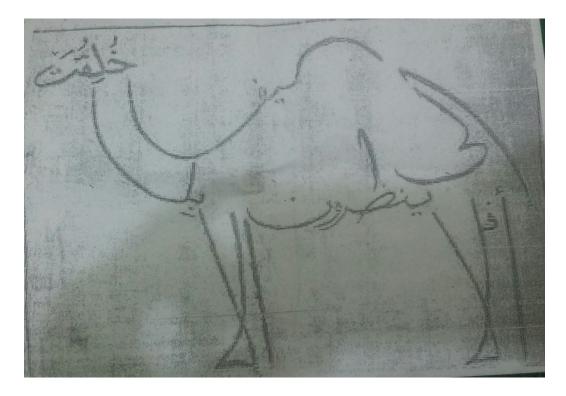
صدق الله العظيم

### سورة الغاشيه الايه 17

#### In the name of Allah , The gracious ,The Merciful

# **{DO THEY NOT LOOK AT THE CAMEL ,HOW THEY ARE CREATED}.**

Truth of Allah Almighty – {Al –Gashyia , Verse 17}.



# Dedication

I dedicate this work

# to my mother & father

# to my brother Hazim for their continued

# support

# to my husband

# Who illuminate my life and made it beautiful

# The gift from Allah



# My life partner

# for his encouraging and advising

**Really iam very lucky for being in my life** 

# Acknowledge

First thank to Allah for assistance , health , power and patience to accomplish this work. It is my pleasure to thank my father ,advisor , supervisor ,**Prof. Abdel Aziz Makkawi** may Allah bless him and gives him health and wellness .Also all thanks to safari institute for animal reproductive technology . I especially thank **Dr. Duria Alhag** for her great effort to make the success of the ultrasonography.

To my parent for encouragement and support and special thank to my brother who is the best adviser and supporter . I would like to thank **Nesreen Alheber** for her great effort to make this work a success .also all of thank to the manager of the farm **Osman** who helped us in animal securing. Also Iwould like to Thank the management of the Camel Research Center( CRC) for giving me the permission to conduct this research in the center .

#### Abstract

The study was conducted in the camel research center (CRC)( **Shambat** )to compare the effect of two hormonal protocols in ovulation induction in dromedary she- camel ( Camelus dromedarius ) during non breeding season. Number of eleven she – camels with varying age (6 – 17 years) and the average body weight from (250 - 350 kg) and one male used for mating were selected for this experiment. The experimental animals were divided into three groups : group (A) control group ( N =3), group (B), (N = 3) intravenous injection with 1500 IU HCG 10 ml group ( c),

(N= 5) intravenous injection with 1000 IU of FOLLIGON (PMSG). Ovulation was observed in all experimental groups .The proportion of ovulation (4/5, 2/3, 1/3) 80%, 67 % 33% PMSG, control and HCG respectively. The confirmation of the ovulation was done by ultrasonographic examination .Results of the present study demonstrated that there is a highly significant difference between HCG ,PMSG and control group in induction of ovulation in dromedary she – camel during non breeding season (P $\leq$  0.05) also there is significant difference in number and size of ovarian follicle in left and right ovary (P $\leq$  0.05). In PMSG group ovulation occurred after 48 h post injection .In HCG group the ovulation occurred after 5 days post injection .

The study concluded that for ovulation to occur the size of the mature ovarian follicle should reach size of 0.7 - 3cm.

5

خلاصه

اجريت التجريه بمركز ابحاث الابل (شمبات) لمقارنة تاثير المعاملات الهرمونيه على حث التبويض في النوق خارج موسم التزاوج . على عدد ( 11) من النوق من مختلف الاعمار تتراوح بين ( 6 – 17 ) ومتوسط وزن الجسم حوالي (250 – 350 كجم ) وواحد فحل للتلقيح. قسمت حيوانات التجربه الى ثلاث مجموعات : المجموعه ( أ ) تتكون من عدد 3 نوق تركت بدون معامله هرمونيه ( مجموعة التحكم ) والمجموعه التانيه ( ب ) بها عدد 3 نوق تمت ج ) بها عدد 5 نوق تمت معاملتها بااستخدام 5 مل من هرمون مصل مشيمة المرأه الحامل 1000 وحده دوليه عن طريق الحقن الوريدي لوحظت عملية التبويض في كل المجموعات التجريبيه وكانت نسبة التبويض (5/4 و3/2 و3/1) 80 % و%67و33% PMSG كنترول HCG. على التوالي. تم الكشف عن التبويض بااستخدام الموجات فوق الصوتيه . اظهرت النتائج ان هنالك فروق معنويه بين الثلاث مجموعات ( PMSG, Control and HCG) في حث التبويض في النوق تحت معنويه (P ≤ 0.05) وايضًا هنالك فروقات معنويه بين الثلاث مجموعات في حجم وعدد الحويصلات المبيضيه في المبيض الايمن والايسر تحت معنويه (P ≤0.05). توصلت الدراسه الى انه في المجموعه المعامله بهرمون PMSG التبويض يحدث بعد 48 ساعه من الحقن الوريدي اما المجموعه المعامله بهرمون HCG التبويض يحدث بعد خمسه ايام من الحقن الوريدي.

وتوصلت التجربه الى انه لحدوث التبويض في النوق يجب ان يتراوح حجم الحويصلات المبيضيه الناضحه بين (0.7 –3سم) .

#### list of contents

Koranic Verse	1
Dedication	7
Acknowledgments	7
Abstract	7
Arabic Abstract	7
list of Contents	17
Table of Contents	7
Chapter :Introduction & Objective	1-3
Chapter 2 : literature review	4- 25
2-1 – The Camel	4
2-1-1- Domestication Of Camel	5-6
2-1-2- Camel Classification	6-7
2-1-3- Camel Economic Importance	7 -8
2-1-4- Camel In Sudan	7 - 8
2-1-5-Camel Distribution &Number	9- 10
2-1-6- Camel Breeds Of The Sudan	10
2-1-7- Camel Reproduction	11 – 12
2-1-7-1- Camel Puberty	12
2-1-7-2-Camel Fertility & Infertility	13
2-1-7-3- Camel Breeding Season	13 - 14
2-1-7-4- Camel Steaming Up	15
2-1-7-5-Camel Estrus Cycle&	16- 19
Ovulation	

2-1-7-6- Follicular Waves Pattern	19- 20
2-1-7-7- Sexual Cycle Of Male	20-21
Camel	
2-1-7-8- Rutting Behavior	21-22
2-1-7-9- Copulation	22
2-1-7-10- Pregnancy Of Camel	23
2-1-7-11-Pregnancy Diagnosis	23-24
2-1-7-12- The Main Challenge	24-25
Facing Camel Reproduction	
Chapter 3 : Material & Methods	26-29
3-1- Site Of The Experiment	26
<b>3-2- Duration Of The Study</b>	26
3-3- Building & Housing	26
3-4- Herd Structure	27
3-5- Feeding Program	27
<b>3-6- The Experiment</b>	27
3-7- Hormonal Protocol	27
3-8- Ovarian Examination	28
3-9- Ultraonographic Examination	28
3-10- Mating Program	29
3-11- Statistical Analysis	29
Chapter 4 Results & Discussion	30-39
Conclusion & Recomondation	40
References	41- 49
Appendex	50 - 69

#### listoftables

r

Table	Title	No.
Table 1(a	The proportion of ovulation in she – camel effected by hormonal treatment (raw data)	32
Table 1(b	The proportion of ovulation in she – camel effected by hormonal treatment (statistical analysis)	33
Table 2(a)	The ovarian follicles size before injection ( raw data )	34
Table2(b	Number of ovarian follicles before and after injection (statistical analysis)	35
Table 3 (a	Number and size of the ovarian follicles after injection (raw data )	36
Table3(b	Number of right ovarian follicles before and after injection(statistical analysis)	37
Table 4	Size of left ovarian follicles before and after injection	38
Table 5	Size of right ovarian follicles before and after injection	39

#### **Abbreveiations**

- SAS\_= Statistical Analysis System (ver.9.3-1 issued 2016).
- CRD = Randamized Complete Design
- LSD0.05 = Least significant difference
- DMRT = Duncan's multiple range test
- SD = Standard deviation
- SE = Experimental stander error
- Sig = Level of significance (p-value)
- n.s = Not significant
- \* = significant (  $p \le 0.5$ )
- \*\* = Highly significant ( $p \le 0.01$ )

**Reference :** Montgomery , Douglas C.(2011). Design and Analysis of experiments (5<sup>th</sup> edn.) New York ; John wiely and sons .p.section 3-2.ISBN 9780471316497.

## **CHAPTER 1**

# Introduction

Camels are an important livestock species in the arid and semi arid zones in Asia and Africa . They contribute significantly to the livelihood of the pastoralists and agropastoralists living in the fragile environments of the deserts and semi deserts of Asia and Africa. Most camels surviving today are domesticated. (Fedewa et al - 2000).

Sudan has the second largest camel population in the world, estimated at nearly4, 700,000 (  $M \land R \land F - 2012$  )

They are well adapted to the local environmental conditions and can survive in zones which are prohibitive for other livestock species. They occupy a geographical zone in the north of latitude 14° N in the west and 16° N in the east (**Wilson -1984**).

The camels of Sudan belong to the species Camelus dromedaries (one humped), and are owned and raised by nomadic tribes. Camel herders migrate north in the wet season and south during the dry season. And the country is home to some of the most well-known camel nomads, the **Kababish**, **Shukria**, **Hadendowa** and others. <u>Tribal</u> groups in Sudan breed distinctive types of camels (**Mason and Maule, 1960**). Well-known among these are the <u>Anafi</u> and <u>Bishareen</u>, prized for their racing

and riding capacities, the **<u>Rashaidi</u>**, kababeesh , Hamari are strong transport camel with superior drought resistance, and the large whitish Lahaween, which gives high meat yields.

Camels in the Sudan are classified as pack (heavy) and riding (light) types according to the function they perform. These traits were probably developed as a result of selection applied by the various camel owning tribes. The Arab breed of camel is well suited for meat production and transportation. Camel milk is important at the subsistence level but is rarely marketed. The export of camels for slaughter -mostly to Egypt, but also to the Libyan Arab Jamahiriya and other countries, is an important source of foreign currency, which is not overlooked in a country with few roads and a harsh environment .**(Fadil 1986).** 

Camels are seasonal breeders and their reproduction is different as compared to other livestock. The reproductive efficiency of camels under their natural pastoral conditions is low. The reasons for this low reproductive efficiency include the short breeding season, the late age of reaching puberty and the long gestation period. For this reason The introduction of controlled breeding programs is very important and use of reproductive technologies such as artificial insemination , embryo transfer ,synchronization and ovulation induction in this species are very effective to increase reproductivety (Cooper et al - 1992).

12

# Objective

This study aimed to compare two hormonal protocols (HCG, PMSG) on ovulation induction in dromedary She – camel during non breeding season and to compare their effect on the success of the breeding program.

# CHAPTER - 2

### Literature review

## 2-1- The camel

The camel is an even-toed ungulate within the genus *Camelus*, bearing distinctive fatty deposits known as "humps" on its back. The two surviving species of camel are the <u>dromedary</u>, or one-humped camel (Camelus . dromedarius), which inhabits the Middle\_East and the Horn of Africa; and the bactrian, or two-humped camel (Camelus . bactrianus), which inhabits Central Asia. Both species have been domesticated; they provide milk, meat, hair for textiles or goods such as felted pouches, and are working animals with tasks ranging from human transport to bearing loads.

The "Camel" is also used more broadly to describe any of the six camellike mammals in the family <u>Camelidae</u>: the two true camels and the four New World camelids: the llama, alpaca, guanaco, and vicuña of South America (*Bornstein. (2010), ''llama''. (2012.*)

The term "camel" is derived via Latin and Greek (*camelus* and κάμηλος *kamēlos* respectively) from Hebrew or Phoenician *gāmāl*. (*Herper*, *Douglas*. 2012).

### **2-1-1- Domestication**

Most camels surviving today are domesticated (**Fedewa, Jennifer (2000**) **Walker, Matt (2009**). Along with many other megafauna in North America, the original wild camels were wiped out during the spread of Native Americans from Asia into North America, 12,000 to 10,000 years ago (**MacPhee, et.al (1999) Worboys, Graem(2010),** The only wild camels left are the Bactrian camels of the Gobi Desert.(**Ramet 2011**)

Like the horse, before their extinction in their native land, camels spread across the Bering land bridge, moving the opposite direction from the Asian immigration to America, to survive in the Old World and eventually be domesticated and spread globally by humans.

Dromedarius may have first been domesticated by humans in Somalia and southern Arabia, around 3,000 BC, the Bactrian in central Asia around 2,500 BC( Mukasa – Mugerwa - 1981, Scarre, Bulliet- 1990 Chris - 1993, , book google - 2016) , as at Shar-i Sokhta (also known as the Burnt City, Iran). (Hirst, Kris - 2014)

One-humped camel (*Camelus dromedarius*) survive in an arid environment where the supply of good quality forages is very limited. Its selective retention of feed particles in the fermentation chambers may be one of the many factors responsible for its adaptation to low quality feeds. Retention in the gut is related to the size of offered forage particles, and to eating and ruminating behaviour. (Heller *et al* 1986). Martin Heide's (2010) work on the domestication of the camel tentatively concludes that the bactrian camel was domesticated by at least the middle of the third millennium somewhere east of the Zagros Mountains in Iran , then moving into Mesopotamia in syria, and suggests that mentions of camels "in the patriarchal narratives may refer, at least in some places, to the Bactrian camel." while noting that the camel is not mentioned in relationship to Canaan (Heide & Martin. 2011).

#### **2-1-2-Classification of camel**

#### Classification

Camel

Dromedary camel,

Camelus dromedaries

Bactrian camel,

Camelus bactrianus

#### Scientific classification

Kingdom: <u>Animalia</u>

Phylum: <u>Chordata</u>

Class: <u>Mammalia</u>

Order: <u>Artiodactyla</u>

Family:	<u>Camelidae</u>	
`Tribe:	<u>Camelini</u>	
Genus:	Camelus	
	<u>Linnaeus</u> , <u>1758</u>	
Species		
Camelus bactrianus		
<u>Camelus dromedarius</u>		
<u>Camelus ferus</u>		
† <u>Camelus gigas</u> (fossil) <sup>[1]</sup>		
† <u>Camelus sivalensis</u> (fossil) <sup>[2]</sup>		
† <u>Camelus moreli</u> (fossil)		
<i>-</i> -		

(Falconer, Hugh-1868)

# **2-1-3-Camel Economic importance**

The versatility of the camel and its ability to survive and perform in the harsh arid and semi-arid areas of the world have earned it names such as "ship of the desert", while its strength and docility have been exploited for agricultural, transport and riding purposes. It is, however, the animal's unparalleled ability to convert the scanty resources of the desert into milk, meat and fiber for the pastoralists of tropical Africa and Asia that have gained it the most reputation. Camel herders give deserved recognition to their animals and **Sweet (1965)** wrote that the camel is the basic resource among the Bedouin camel herding tribes of northern Arabia, such as the Rwala, Shammar and Mutair. The wide array of functions and products

which the camel can provide is probably best summarized by **Bulliet** (1975), who wrote that the camel can be milked, ridden, loaded with baggage, eaten, harnessed to a plough or wagon, traded for goods or wives, exhibited in a zoo or turned into sandals and camel hair coats.

# 2-1-4-Camel in Sudan

Camels have regained recognition for their food-producing potential in arid and semi-arid areas of Sudan. their vital role in supporting human populations in some of the poorest and frequently drought-stricken areas of the world (**Hjort af Ornäs - 1988**). The devastating African drought in (**1984-1985**) demonstrated that camel ownership can give pastoralists a competitive edge and an excellent chance for survival. Whereas entire herds of cattle, sheep and goats succumbed to the arid conditions, camel populations survived relatively unscathed In parts of the rain-fed agricultural belt of the Sudan, current developments suggest that camels are indeed able to be integrated with crop cultivation systems. They can exploit efficiently the by-products of large-scale mechanized durra (sorghum) cultivation, and may even mitigate some of the ecological side-effects for which these monocropping schemes are known.

# 2-1-5- Distribution and numbers

The number of camels in 2012 are 18 million with 90% being dromedaries (**Dolby, Karen -2010**). Dromedaries alive today are domesticated animals (mostly living in the Horn of Africa, the Sahel, Maghreb, Middle East and South Asia). The Horn region alone has the largest concentration of camels in the world, (**Bernstein, William J** - **2009**) where the dromedarius constitute an important part of local nomadic life. They provide nomadic people in Somalia (which has the largest camel herd in the world)(**Mukasa - Mugerwa, E - 1981**), Ethiopia and Sudan with milk, food, and transportation. (**Abokor - 1987**).

The Bactrian camel is, as of 2010, reduced to an estimated 1.4 million animals, most of which are domesticated (**Fedewa, Jennifer - 2000**, **Dolby, Karen – 2010 , Denver Zoo – 2012**). The only truly wild Bactrian camels, of which there are less than one thousand, are thought to inhabit the Gobi Desert in China and Mongolia (**Hare, J** -2012).

The largest population of feral camels is in Australia. There are around 700,000 feral dromedary camels in central parts of Australia, descended from those introduced as a method of transport in the 19th and early 20th

centuries (Webster, George - 2010, Dolby, Karen – 2010 and Saalfeld Edwards - 2008).

A small population of introduced camels, dromedaries and Bactrians, wandered through Southwest United States after having been imported in the 1800s as part of the U.S. Camel Corps experiment. When the project ended, they were used as draft animals in mines and escaped or were released. Twenty-five U.S. camels were bought and imported to Canada during the Caribou Gold Rush . ( Mantz, John - 20 April 2006)

### 2-1- 6-Camel breeds of the Sudan

Sudan has the second largest camel population in the world, estimated at nearly 4,700,000( M A R F -2012) and the country is home to some of the most well-known camel nomads, the( **Kababish, Shukria, Hadendowa**) and others. Tribal groups in Sudan breed distinctive types of camels. Well-known among these are the (**Anafi and Bishareen**), prized for their racing and riding capacities, the (**Rashaidi**), a sturdy transport camel with superior drought resistance, and the large whitish Lahaween, which gives high meat yields. (**Mason and Maule – 1960,Salih - 1988**).

# 2-1-7-Camel Reproduction

Camel is a seasonal breeder and the reproduction is different as compared to other livestock .The reproductive efficiency of camels under their natural pastoral conditions is low. The reasons for this low reproductive efficiency include the short breeding season, the late age of reaching puberty and the long gestation period of 13 months. The introduction of controlled breeding programmes is important but several problems have to be considered. For example, estrous behavior is very vague and difficult to interpret, as it does not often relate to follicular development in the ovaries. In addition, all camelids are induced ovulations that normally ovulate only in response to mating, so alternative methods of inducing ovulation, such as injecting gonadotrophic hormones, have been investigated. The use of embryo transfer is becoming increasingly important but involves the necessity to super ovulate the donors and synchronize the recipients so that they ovulate preferably 24 h after the donor. Super ovulation can be achieved using exogenous gonadotrophins, although there is a high incidence of follicle luteinization before mating, of over stimulated ovaries and nonresponsive females. The development of AI in camels is complicated by the difficulty of collecting semen and the gelatinous nature of the semen produced. However, diluting semen in Green Buffer and inseminating a

minimum of 300 x 10(6) live spermatozoa has given encouraging results. The ability to control the follicular cycle of camels is leading to an improvement in reproductive efficiency. Although this report will not deal in depth with camel fertility, the reproductive characteristics of both male and female camels must be examined before the importance of camel milk for human nutrition in drought areas can fully be assessed. It is often stated that the most negative argument against camel breeding is their slow and uncertain reproductive rate (**Novoa**, **1970**). In some areas camel breeding is even considered to be too hazardous to be undertaken systematically (**Gast <u>et al.</u>**, **1969**).

# **2-1-7-1-** Camel puberty

The camels are sexually mature at 4 to 5 years of age (Mares-1954, Yasin and Wahid-1957,Evans and Powys - 1979;). although a 3-year old camel can be used for reproduction (Williamson and Payne- 1959 Novoa-1970 ,Leonard- 1894). In the male, full reproductive prowess is not developed until six years (Novoa, 1970) or even seven years (Hartley, 1979) of age. Domesticated vicugnas could reproduce at one year of age (Romero, 1927), but the fertility of both males and females at this age was low (Koford, 1957). Alpacas and vicugnas are not normally bred until they are at least two years old (Novoa, 1970).

# 2-1-7-2- Camel fertility and infertility

Fifty percent fertility, or even less, has been recorded (Keikin-1976, Yuzlikaev and Akhmedier - 1965). Repeated matings were often due to improper development of follicles (Barmintsev-1951). Injections of Pregnant Mare Serum Gonadotropin (PMSG) with an interval of 48–72 hours led to 100 percent calving rate (Yuzlikaev and Akhmediev- 1965). Anatomical abnormalities of the females are the main causes of infertility (Shalash and Nawito- 1963). Another cause of infertility is fetal death (Shalash- 1965, Tayeb-1953). This was suspected when a single fetus was born, but more than one corpus luteum was found. Infertility and slow breeding habits of the camel can be associated with poor feeding and management. It assumed to do better selection, disease control, and improved husbandry that could not only improve the standard of milk production, but could shorten the time for first calving, intercalving intervals and quality of the herds in general.

# **2-1-7-3-** Camel Breeding season

It was historically reported that breeding time of camels is in November and December (Leonard, 1894). However, camels, both male and female, are seasonal breeders (Yasin and Wahid, 1957), mating during the rainy, or cold season (Yagil and Etzion, 1980). Longer hours of daylight initiate the breeding season (Chen and Yuang, 1979). Musa (1969) described an all-year-round estrus in the female camel, but this was not found in any other publication.

The pattern of the reproductive cycle appears to relate to the harsh environment in which the camels live (Novoa, 1970). The calves are born in the months most suitable to quarantee their survival. The breeding season differs in various countries. In the region of Pakistan (Yasin and Wahid, 1957), China (Chen and Yuan, 1979), Egypt (Shalash, 1965) and Israel (Yagil and Etzion, 1980), the breeding season is from December to April. This is the period in which both males and females are fertile. In Somalia the male camel ruts in the spring from April to May, (Mares, **1954**). In India the breeding period is from November to February (Singh and Prakash, 1964). In Morocco the rutting season occurs in winter and spring. Both rutting season and consequent births coincide with adequate water and feed supplies. In Russia the domesticated Bactrian was found to be polyestrous, having estrus cycles all the year round (Bosaev, 1938). The wild camel in the Gobi desert, however was a seasonal breeder (Bannikov, 1945). The rut occurring between January and February. In the Sudan, Musa and Abusineina (1978) report the season as being from March to August.

# 2-1-7-4- Camel steaming up

Camels are traditionally reared in extensive area. Steaming up process is one of the food operations performed on the animal before the start of the breeding season of about 2-3 weeks. The primary purpose of which is to improve body weight and condition. In many arid countries too, we can observe a high development of intensive dairy farming on sub-urban areas. The influence of nutrition on milk increase and reproduction has been extensively investigated in conventional farming species and it is generally concluded that decreased nutrient intake reduces the growth of calves, delays the onset of puberty in heifers (Formigoni et al -1996) and increases the post-partum interval to conception in dams (Lalman et al-**2000**). In contrast, feeding to improve body condition before calving can increase both calf birth weight, its subsequent gains and reproduction efficiency of the dam (Houghton et al, 1990). In dromedary several productive and reproductive characteristics such as restricted breeding season, long gestation period, low daily body weight gain, and high calf mortality appear to be the major constraints to increase productivity of dromedary herds (Ismaïl, 1990). However, there is few and fragmented information on the effects of nutritional deficiencies on the dromedary performances at critical periods including puberty and parturition (Moslah, 1993).

#### **2-1-7-5-** Camel estrus cycle and ovulation

As previously mentioned, the female camel is a seasonal polyestrous animal. The period of estrus is easily recognizable by the animal's general restlessness, often aggressiveness in manner, and by swelling and discharge from the vulva (Yasin and Wahid – 1957, Yagil and Etzion-1980). The length of the estrus cycle is normally 2–3 weeks (Bodenheimer – 1954, Leonard - 1894), although in the Bactrian camel the period can extend to 30-40 days (Bosaev - 1938). The actual heat lasts for 3-4 days (Bodenheimer- 1954, Leonard- 1894), although 21 days was considered by Yasin and Wahid (1957) as being the period of heat. Synchronization of estrus in she – camel is based on the use of PMSG (yagil and etizon -1984, Elias et al. 1985 and Anouassi and Ali - 1991) HCG or LH (Nova -1970, Shalah- 1987, Elias-1990and Anouassi and Ali, 1990). GnrH together with LH or HCG hormones were also used for the same purpose ( Chen et al .1985 and Bono et al .1991 &b). Ovarian cycle activity was fully described by Musa (1979). He reports a 28-day cycles in which follicles mature in 6 days maintaining their size for 13 days, then regress in 8 days. There is no spontaneous ovulation in the camel (Chen and Yuan-1979, Musa-1979 Shalash- 1965), so that without mating there is no luteal phase. Manual stimulation of the cervix for 15 minutes did not induce ovulation,

although luteinization of the mature Graafian follicle occurred (**Musa** and Abusineina- 1978). The ovulatory activity of the left ovaries (50.2– 56.5%) is slightly higher than that of the right (43.5–49.8%), but left horn pregnancy is usual (98.2–100%). Multiple ovulations occur in 12.4– 18.6% of camels but twin births are very rarely, if ever, observed. Ovulation occurs 30–48 hours following copulation (**Shalash -1965**, **Chen and Yuan- 1979**), Injections of HCG lead to ovulation 24 hours later. Confirmation of ovulation was done by trans rectal ultrasonography as decribed by(**Skidmore,2011**).

The studies conducted to induce ovulation, Ovulation was induced 1-2 days post HCG injection into females having large follicles ( Aqrawal, Rai, Khanna ,1997). To increase the success rates of ovulation induction the follicle size diameter should be between 1.0 to1.7 cm. (Logman-2015).

The study of logman mentioned that the proportion of she camels that ovulated during 24-48 hours in respone to treatment were (6/7 vs 4/6 vs 0/6) in the GnRH, HCG and the control groups respectively, there are highly significant differences between the treated groups compared to the control group. but there are no significant differences between the GnRH and HCG groups ( $p \le 0.05$ ), (Logman-2015).

The study of Skidmore showed that 70%, 60% and 60% of them ovulated in response to mating, or treatment with buserelin or HCG, respectively. Skidmore et.al -1996). They Stated that without pregnancy there is no formation of a corpus luteum. The size of the corpus luteum depends on the ovarian activity (Musa and Abusineina - 1978). The corpus luteum was larger and lasted longer when mating occurred at the time of maximum follicular development. When mating took place later, the corpus luteum was smaller and disappeared in a short period of time. In Beersheva (Israel) research was carried out using radio-immuno assay of sexual hormones in the blood of the female. There was an increase in oestradiol activity from the beginning of December which ended toward the beginning of April. Surprisingly enough, there were also peaks of progesterone activity, although no male was present. The peaks in hormone activity were 23 days apart. From the middle of January there were peaks of estradiol accompanied by peaks of progesterone 2 days later, every 7 days. Twenty-four to 48 hours following mating, the luteinizing hormone (LH) appeared. The LH then declined steadily for 6 days and a second period peak even greater than the first, was found almost two weeks later. At that time, the progesterone levels were extremely high. The oestrogen levels were low, but both hormones showed peaks in activity every 4 days. LH activity was non-existent. These 4-day fluctuations continued almost right through the pregnancy.

While undertaking physiological research on body fluids and renal function, two camels aborted. In these camels the hormone activity declined to base-line levels. Estrus has been known to re-occur a day after calving (**Barmicev - 1939**). If the camel is well fed, estrus can occur within one month post partum (**Mares – 1954**), **Yasin and Wahid - 1957**). If the camel has no milk, then estrus occurs within 28 days (**Evans and Powys, 1979**). This means that with good feeding conditions camels can be mated as soon as the young calves start grazing. The <u>Lama</u> pacos have prolonged periods of estrus interrupted by short periods of anoestrus (**San Martin - 1961**).

### **2-1-7-6-Follicular Wave Pattern**

The rapid increase in human population in the developing countries has led to a high demand for meat production. The one-humped camel most probably, is a better provider of food in desert and semi-desert areas compared to cattle which are severely affected by heat and scarcity of water and feed. Efforts to improve the reproductive efficiency of the female camel are closely related to a better understanding of the follicular cycle. The exact mechanism regulating folliculogenesis in the female dromedary camel have only been partly ravealed. This review summarizes the valuable information and achievements obtained during the last years on follicular wave pattern, folliculogenesis and hormones secretion in the female dromedary camel. In addition, some Assisted Reproductive Techniques (ART) such as follicular wave synchronization, the induction of ovulation and super ovulation were also reviewed. (Ghazi, Basiouni - 2007).

### 2-1-7-7-Sexual cycle of the male camel

The male camel is a seasonal breeder, the season corresponding with that of the female (El Amin-1979), (Yagil and Etzion-1980). The male undergoes behavioral and hormonal changes during the rutting season (Chen and Yung- 1979), (Yagil and Etzion-1980). The male is normally docile and easily controlled, however, in the rutting season he can become so aggressive that he is dangerous and cannot be handled. He is extremely restless. He blows a balloon-like flap out of the side of his mouth which is called palatal flap (Charnot – 1963), (Yagil and Etzion-**1981**). Its appearance is accompanied by a roaring-gurgling sound. The lips are often covered with saliva. The glands between the ears secrete a dark, bad-smelling, watery secretion. This area is constantly grubbed against all objects in the surroundings, including grass mounds. The back legs are spread, and the tail is then beaten against the penis. Drops of urine are deposited on the tail and spread over the back. Eventually, the hindquarters have a strong urine odor. The rutting males readily attack

each other and timid males soon learn to keep away from the territory staked out by more aggressive males.

### 2-1-7-8-Rutting behavior

In the rutting periods there was increased secretion of androgens. The increase in hormone secretion was found in the blood (**Yagil and Etzion-1980**) and urine (**Charnot-1958**). Adenohypophysis (increased neurosecretory activity) also occurred (**Charnot and Racadot - 1963**, **Santini, 1964**). Secretions from the neck, poll and glands were also found to contain large amounts of androgens (**Yagil and Etzion - 1980**).

There were no changes in behavior when the blood and neck gland hormone levels were low. The female camel also has a palatal flap and neck glands (Leese - 1927), but these are dormant. This suggests the dependency of secondary sex characteristics on the androgens.

Male alpacas have no changes in appearance or behavior in the breeding season (Novoa - 1970). This is surprising as there is a strong similarity between the behavior and external appearances of the rutting camel and the in-musth Asiatic elephant (Jairudeen, et al. - 1972). The latter also becomes extremely aggressive, secretes from the head glands, plays with his penis and urinates onto the ground.

# 2-1-7-9- Copulation

There are many reports concerning the copulation of camels. These vary from being rarely observed (Mares - 1954) to the act being screened from humans by other members of the herd (Yasin and Wahid - 1957). But if not aided, courting and mating can be very violent. If the male selects a female and she is not willing to go down quietly when he approaches her, he will bite at her neck and eventually roughly force her to the ground. There the female utters her guttural protest, while the male first straddles her and then gently slides down until he is squatting on his back legs. Copulation lasts for about 15 minutes. This is accompanied by much gurgling and grunting. The male pushes forward continuously with many pelvic thrusts. The male gives about 7 ml of semen with an average of 615 million sperm per ml (Chen and Yuang, 1979). The male camel can mate at 3 years of age, but the optimal age to begin is between 4-5 years (Hartley – 1979, Mares -1954, Yasin and Wahid- 1957). At 6 years of age they are in full reproductive vigour. The best males are selected for breeding the rest are castrated or used as baggage camels (Mares - 1954). The male can breed for 7 years (Hartley-1979). The male dromedary can mate with 50 to 80 females a season, when he is in good condition (Leese - 1927, Yasin and Wahid- 1957). The Bactrian male mates with 10 females per season (Terentjev - 1951).

# 2-1-7-10-Pregnancy of camel

The right and left ovaries seem to function equally in the camel. In spite of this fact it is observed that 99 percent of pregnancy occurs in the left uterine horn. Anatomically the left horn is slightly bigger than the right one (**Musa & AbuSineina,1978 a;Wilson,1984; Arthur et al., 1985).** In the bactrian camel 96.49 percent of foeti were found in the left horn (**Chen & Yuen, 1984**).

The average gestation length is 390 + 2 days (Yagil, 1985) or 375 days (Arthur et al., 1985 a) and it is commonly stated as 12 to 13 months. In the bactrian camel the gestation length averages 402.2 + 11.5 days (Chen & Yuen, 1984 a).

# 2-1-7-11-Pregnancy diagnosis

Various methods could be employed to detect pregnancy in the camel. These include rectal palpation, ultrasonography,laboratory methods ,behaviour of the animal. (**Barminestiv 1951 & musa Abusineina-1976**) and estimation of blood progesterone (Skidmore et.al 1992).

#### 2-1-7-12-The main challenges facing camel reproduction

The reproductive efficiency of camels under their natural pastoral conditions is low(Novoa, 1970). The reasons for this low reproductive efficiency include the short breeding season, the late age of reaching puberty and the long gestation period of 13 months. The introduction of controlled breeding programmes is important but several problems have to be considered. For example, estrous behavior is very vague and difficult to interpret, as it does not often relate to follicular development in the ovaries. In addition, all camelids are induced ovulators that normally ovulate only in response to mating, so alternative methods of inducing ovulation, such as injecting gonadotrophin hormones, have been investigated. The use of embryo transfer is becoming increasingly important but involves the necessity to super ovulate the donors and synchronize the recipients so that they ovulate preferably 24 h after the donor. Super ovulation can be achieved using exogenous gonadotrophins, although there is a high incidence of follicle luteinization before mating, of over stimulated ovaries and non-responsive females. The development of AI in camels is complicated by the difficulty of collecting semen and the gelatinous nature of the semen produced. However, diluting semen in Green Buffer and inseminating a minimum of 300 x 10(6) live spermatozoa has given encouraging results. The ability to control the

follicular cycle of camels is leading to an improvement in their reproductive efficiency. The fertilization rate of camels is considered very low (**Novoa, 1970**).

#### Chapter 3

#### Material and method

#### **3-1-** Site of the experiment

This experiment was conducted in camels Research Center (CRC) Khartoum (**Shambat**) belonging to veterinary college of the university of Khartoum at latitude 15- 16 (°N) and longitude 31.5 - 34 (°E). The center was established in (**1982**) to explore the world ignored camels of the Sudan. It was started as research center but now it became training center in addition to research center.

#### **3-2-Duration of the study**

The experiment was started in the period from January and it continued for six mont until June 2016 when the temperature was  $37c^{\circ}$  (minimum) and  $41c^{\circ}$  maximum temperature .

#### **3-3 – Building & Housing**

The animals were housed in semi closed system. The farm was Divided into (4) pens with dimention  $11 \times 11 \text{ m}^2$ . Wall of the pens was made of steel . One of these pens was used for mature females used for the breeding program, the others for the calves, milking animal and one was allotted for the male.

#### **3-4** –Herd structure

The herd consisted of (30) animals from different breeds Anafi, bushari and Arabian breed divided into seven mature females with age from (6-12) years, five heifers age from (4-5) years, fourteen calves age from (2-4) years, three milking animals and one adult male (6) year.

# **3 - 5- Feeding program**

The herd was set for browsing shrubs and by products of the farm in the early morning in addition to dry forage which was given ad lib. Formulated camel rations consist of 20% dura,15 % ground nut cake , 50% bran , 5% lime stone and 10 % salt.

#### **3-6-** The experiment

A number of (11) she –camels with varying age from (5-17) years and average body weight between (250- 350 kg) were selected to study the effect of hormonal treatment on ovulation induction. One adult male was used for mating . The experimental animals were separated randomly to three groups and identified by neck tags.

#### **3-7- Hormonal protocol**

#### - Group (A) control group

In this group number of (3) animals were set without any treatment and used as control group .

- **Group** (**B**) – (**HCG**)

In this group a number of (3) animals were treated by (HCG) Human Chronic Gonadotrophin (CHORULON) 3000 IU intravenous injection 10 ml for each female applied as instructurcted by the manufacturer . **Procedure** : Each vial of freeze – dried powder for injection contains 1500 I.U Human Chronic Gonadotrophin (HCG). each vial of solvent contain 5 ml of the reconstituted solvent .powder for solution for injection (supplied with reconstitution solvent) according to instruction.

#### - Group (C) treated by PMSG (Folligon)

A number of (5) females were treated by PMSG (FOLLIGON )1000

I.U, 5 ml for each female as instructed by the manufacture.

**Procedure** : each vial contain freeze – dried powder for injection :gonadotrophin equine serum (PMSG) 1000 IU solvent for reconstitution according to instruction.

#### **3-8-** Ovarian Examination

Ultrasonographic examination was made after 48-72 hour post injection to check the status of the two ovaries and detect and follow folliclular growth , and incidence of ovulation .evaluation depend on measuring the follicle size.

#### **3-9-** Ultrasonographic examination

Animals were bound and given Anesthesia according to body weight (1ml for each 100 kg body weight) according to the method described by the lable to enable animal control. and ultraonographic examination was made to measure the follicles size when the animals were in sitting position .The tail was raised and after wearing hand gloves the rectum evacuated from feces to facilitate entering the linear probe after using lubricant gel .The linear probe reflect the state of the two ovaries through image that appear in the monitor.

#### **3-10** - Mating program

Mating of the experimental animals was decided two days after injection program .The experimental animals were mated in sitting position twice a day , in the morning and evening . The adult male detect estrus female , run with her and bite her in her neck to force her to sit on the floor . The duration of mating take about 10-15 minute .

# **3-11-** Statistical Analysis Techninque Abbreviation and Reference

#### {A}Technique

Data generated were subjected to SAS ver.9.3-1 issued 2016) .Two factor Randomized complete design RCD was assessed for ovarion follicles number and size ; and one factor Randomized complete design RCD was assessed for proportion of ovulation in she camel and then means were separated according to DMRT.

#### CHAPTER 4 Results & Discussion

**Results table (1) The proportion of ovulation in she - camel affected** by hormonal treatment

on %
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, D
, D

The present study illustrates that ovulation was occurred in all groups of the experimental animals(Table 1), and the proportion of ovulation was (4/5, 2/3, 1/3) 80%, 67%, 33% PMSG, control and HCG respectively. In the control group ovulation occurred in the right ovary, 48 hour after mating. This agreed with the finding of **Chen and Yuan**-(**1979**), **Shalash** (**1965**) who found that ovulation occurred after 30 - 48 hours as response to mating stimuli. **Table** (**1**)

Also the present study indicated that ovulation can also be induced by HCG administration .Similar results were reported by Anouassi et .al (1992).

In HCG group one of three females treated with HCG came into estrous after 5 days post injection . This disagreed with the results reported by **Vyas (2011)** who reported that the ovulation did not occur up to 96 hours ,and also disagreed with (**logman 2015& martin 1961**) who reported that ovulation was occurred 24-48 hours post HCG injection. This disagreement might due to the small size of the experimental animals. and agreed with the results of **Aqarwal et.al (1987)** who reported that ovulation occurred on day 5 post HCG . when ovulation occurred in the left ovary, the experimental animal was exposed to the male and mated successfully. The proportion of ovulation in HCG group was about 33 % which was lower than reported by (**Skidmore 1996**) who found that generally the proportion of ovulation at response to HCG was 67 %. This might be due to the small follicles size than the standard (1-1.9) as described by **Skidmore (1996)**.

In PMSG group ovulation occurred in the right ovary. In addition to the obvious estrus sign after 2 day post injection of PMSG, mature follicles were palpated and hence mated successfully. The best results were obtained by PMSG (**pregnant mare serum gonadotrophin**) 4/5 according to number of ovulated she – camel. Such results agreed with the finding by **Yagil and Etizon** – (**1984**) who reported that PMSG was used for estrus synchronization in she - camel during non breeding season.

# **Results table (2) The ovarian follicles size before**

#### injection

Animal number	Number of follicles		Follicl	es size
	Tomcies			
CONTROL	Left	Right	Left	Right
NO. 4	-	2	-	3 cm
NO. 12	1	2	0.5 cm	3 cm
NO. 13	-	-	-	-
HCG				
NO. 6	3	3-5	0.5 cm	0.5 cm
NO. 7	-	-	-	-
NO. 11	3	2	1.7 – 2 cm	1.3- 1.5 cm
PMSG				
NO. 5	2-3	3-5	0.7 cm	0.7 cm
NO. 8	-	2	-	1.6 cm
NO. 9	1	2	1.2 cm	1.1 cm
NO.10	-	-	-	-
NO . 24	-	-	-	-

Results(**Table 2**) in the present study illustrate that the best ovulation ratio before the hormonal treatment occurred when the follicles size measured about 0.7 –3cm.**Table (2)** This finding disagree with the results of ( **Logman 2015 & Skidmore 1996**) who reported that to increase the success rate of ovulation the follicle size should measure about (1- 1.7) cm.

Animal number	Number of		Follic	les size
	folli	cles		
CONTROL	Left	Right	Left	Right
NO. 4	-	2	-	3 cm
NO. 12	1	2	0.5 cm	3 cm
NO.13	-	-	-	-
HCG				
NO. 6	3	3-5	0.5 cm	1.3- 1.4 cn
NO. 7	-	-	-	-
NO. 11	3	2	2.7 cm	1.5 -1.7 cn
PMSG				
NO. 5	2-3	3-5	0.7 cm	0.7 cm
NO. 8	-	2	1.7 – 1.9	2 cm
			cm	
NO. 9	1	2	1.1 cm	1.3 -1.4 cm
NO. 10	-	2	-	1.7 – 2 cm
NO. 24	3	1	0.7 cm	1.5- 1.7 cm

Results table (3) the number and size of the ovarian follicles after injection

Also in the results (Table 3) the present study illustrate that the number of ovarian follicles in the control group, the right ovary

was bigger than the left ovary and the average size of follicle (3cm). Table (2)&(3)

In HCG group number of ovarian follicles in the left ovary before and after injection was more than the right ovary and size of ovarian follicles after injection was more than size of ovarian follicles before injection . This agreed with **Shalash - (1965)** who reported that the ovulatory activity of the left ovary is slightly higher than that of the right .

In PMSG group the maximum number of ovarian follicles was found in the right ovary and the average size before injection was 2.9 cm and it was increased to 3.8 after injection of PMSG. This is quite acceptable since PMSG is an analogue to FSH in stimulating the follicular growth.

Results table (4) Proportion of ovulation (%) in She-camel in the control and treated groups .

Treatment	Ovulation (%)
Control	67.00 <sup>b</sup> ±0.25
НСС	33.00° ±0.19
PMSG	80.00 <sup>a</sup> ±0.37
P-value	0.004**

Values are mean±SD.

Mean(s) bearing different superscript(s) in a column are significantly different (P $\leq$ 0.05) according to DMRT.

The statistical analysis showed that there were highly significant differences between the three groups in the induction of ovulation (P $\leq$  0.05) such results were similar to those obtained by (**Skidmore2011 & logman 2015**). Table (4)

# **Results table(5) : Number of left ovarian follicles before and after injection.** (statistical analysis)

Treatment	Before injection	After injection
Control	0.33 <sup>d</sup>	0.33 <sup>d</sup>
	$\pm 0.58$	±0.58
HCG	2.00 <sup>a</sup>	2.00 <sup>a</sup>
	±1.73	±1.73
PMSG	1.00 <sup>c</sup>	1.60 <sup>b</sup>
	±1.73	±1.82
P-value	0.041*	

Values are mean±SD.

Mean(s) bearing different superscript(s) in a column are significantly different (P $\leq$ 0.05) according to DMRT.

Treatment	Before injection	After injection
Control	1.33 <sup>cd</sup> ±1.15	1.33 <sup>cd</sup> ±1.15
НСС	2.33ª ±2.52	2.00 <sup>b</sup> ±2.00
PMSG	1.60° ±1.67	2.20 <sup>ab</sup> ±1.09
P-value	0.036*	

# **Results table (6)Number of right ovarian follicles before and after injection**

Values are mean±SD.

Mean(s) bearing different superscript(s) in a column are significantly different ( $P \le 0.05$ ) according to DMRT.

The data analyzed also showed that the results in table (5)& (6)reflected significant differences in the number of the ovarian follicles in the left and right ovaries before and after hormonal injection .

Treatment	Before injection	After injection
Control	$0.17^{cd} \pm 0.29$	0.17 <sup>cd</sup> ±0.29
НСС	0.83 <sup>b</sup> ±1.04	1.07ª ±1.44
PMSG	0.38° ±0.55	0.88 <sup>b</sup> ±0.69
P-value	0.047*	

Results table (7): Size (cm) of left ovarian follicles before and after injection (statistical analysis).

Values are mean±SD.

Mean(s) bearing different superscript(s) in a column are significantly different ( $P \le 0.05$ ) according to DMRT.

**Results table (8): Size (cm) of right ovarian follicles before and after injection. (statistical analysis)** 

Treatment	Before injection	After injection
Control	2.00 <sup>a</sup> ±1.73	2.00 <sup>a</sup> ±1.73
HCG	0.67° ±0.76	1.03 <sup>bc</sup> ±0.91
PMSG	0.68 <sup>c</sup> ±0.70	1.56 <sup>b</sup> ±0.54
P-value	0.049*	

Values are mean±SD.

Mean(s) bearing different superscript(s) in a column are significantly different (P $\leq$ 0.05) according to DMRT.

The results in table (7) & (8) reflected significant differences in the size of follicles size in both right and left ovaries before and after injection. Such results disagree with the finding of (Logman 2015 & Skidmore1996). This disagreement might be attributed to the body size of the experimental animals or environmental condition .

#### **Conclusion & Recommendation**

#### **Conclusion**:

\* Ovulation can be induced in dromedary she – camel during non breeding season by Folligon PMSG & HCG.

\* PMSG ( **pregnant mare serum** ) gave best and fast result to induce ovulation in addition to large follicle number .

\* To increase the success rate of a ovulation, follicle size should be 0.7 - 3cm.

#### Recommendation

- More studies should be conducted using PMSG in ovulation induction of dromedary She –camel during non breeding season .
- The low reproductive efficiency in she camels can be improved by better understanding of the reproductive cycle and increased use of assisted reproduction techniques such as ovulation induction artificial insemination and embryo transfer .

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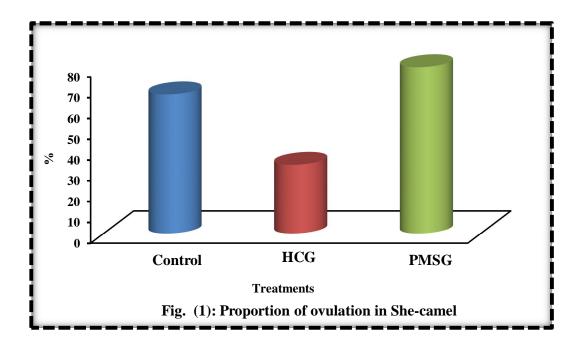
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# Appendix

Fig (1)





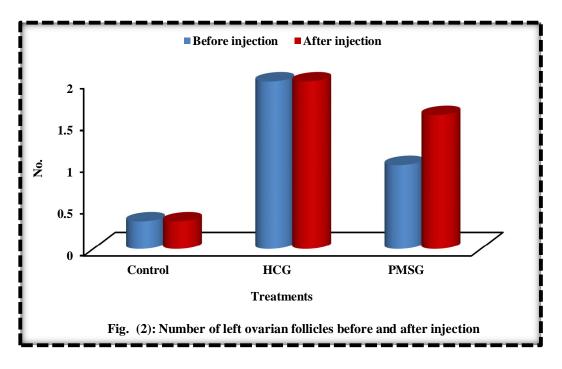
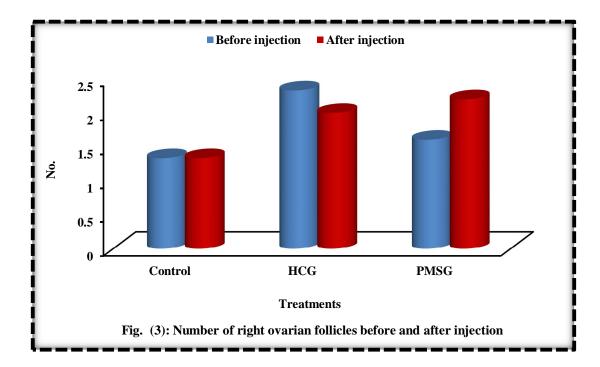
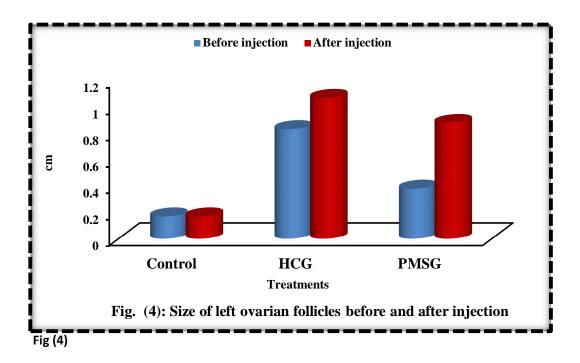
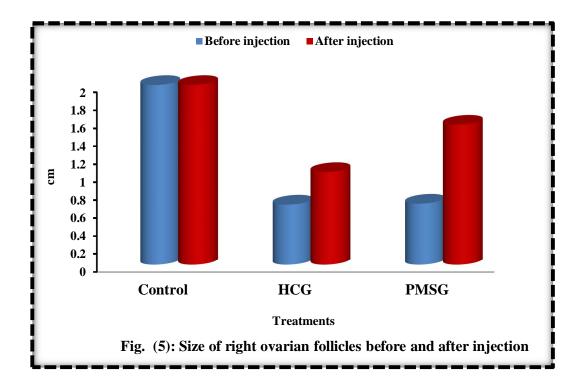


Fig (3)







# Housing of the experimental animals



Plate (1) illustrate the type of housing of the experimental animal

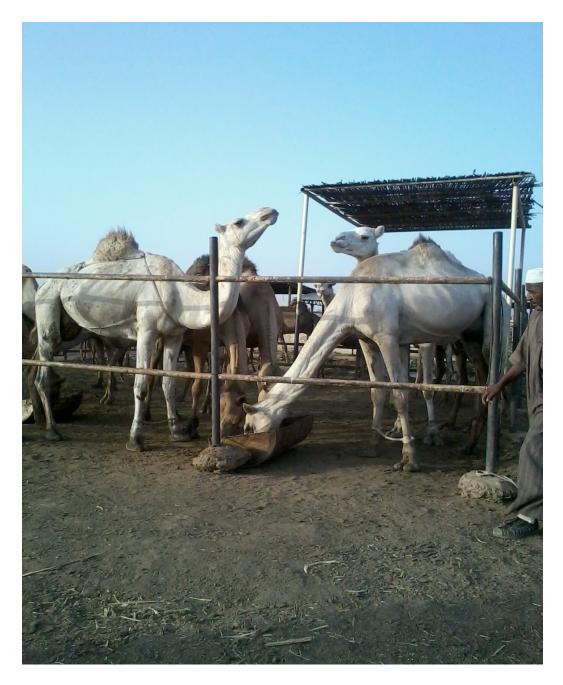


Plate (2) illustrate the housing for the experimental animal

# Anesthesia in camel



# Plate (3) illustrating anesthesia administration of the treated animal

# **Rectal palpation**



Plate (4) illustrating rectal palpation of she - camel

# Ultrasonografic examination



plate (5) illustrating ultrasonografic examination of treated she – camel

# **Feces evacuation**



Plate (6) illustrating feces evacuation before rectal

palpation

# Ultrasonographic equipment



Plate (7) illustrating ultrasonographic equipment used

for ovarian examination.

#### Camel age names



Plate (8) illustrating camel age name

#### Male rutting behavior (palatal flap)

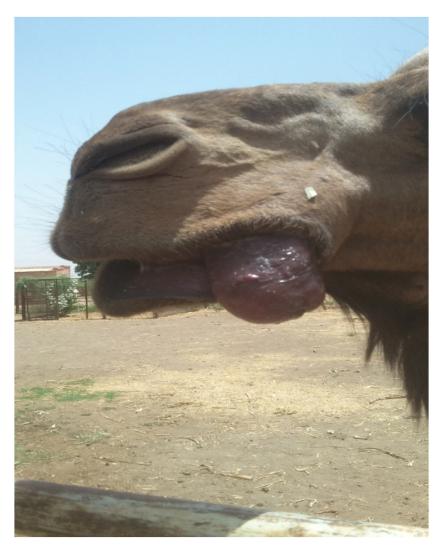


Plate (9) illustrating male rutting behavior

# **Endrogen secretion**



Plate (10) illustrating hormonal secretion from the androgenous gland at the back of the head

# **Estrus detection**

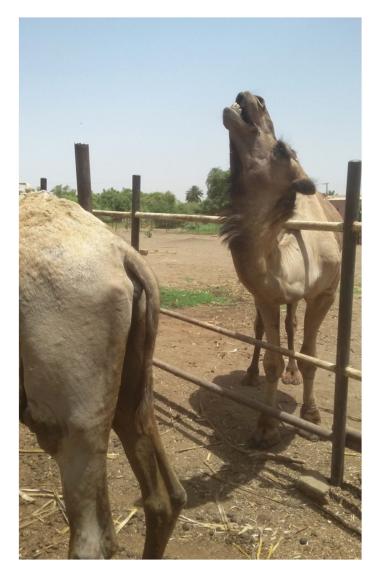


Plate (11) illustrating estrus detection in camel

# Mating in camel



Plate (12) illustrating mating behavior in camels

# Scientifict Name : (HCG) Human Chronic gonadotrophin Commercial Name : Chorulon



Plate (13) illustrate (HCG ) hormone using in the experiment

# Scientifict Name : (PMSG) Pregnant mare Serum Comercial Name : FOLLIGON

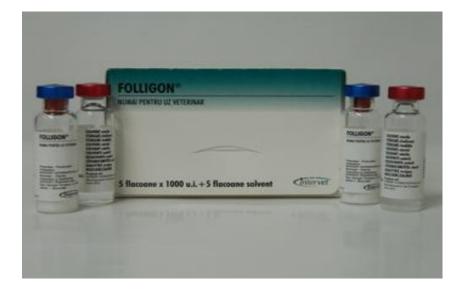


Plate (14) illustrate (PMSG ) hormone using in the experiment