

INTRODUCTION

Throughout the world's vast dry land areas, mobile pastoral animal husbandry permits the utilization of scarce natural vegetation for food production and thus guarantees survival and income for a limit human population engaged in nomadic pastoralism . Yaqoob and Nawaz (2007) mentioned that due to the rapid growing of human population, shrinking of the cultivated areas, and environmental changes, thus reducing the fodder production areas for the cattle, sheep and goat, and also adversely affected the production potential of these animals. Under this situation and these circumstances we must search for other available source for production. Camels, due to their unique adaptation to the dry land conditions, are considered as a cornerstone of many African pastoral economies. Also they added that rearing camel is the best option for more production and the proper utilization of the vast dry lands.

The one hump camel (*Camelus dromedarius*) is an indispensable animal which contributes effectively both as a source of food and welfare to people who live in arid lands or living under tough environments. For these reasons the camel is considered as an animal for food security, and because of its unique biophysical characteristics and its importance in the socio-economic development of arid and semi-arid zones. Furthermore camels are frugal in their needs, and high in the production of milk, meat, work and wool. In the past, the primary use of camel was for the transport of goods and passengers in the desert and semi-desert areas, while wool, milk, skin and meat were by-product (Williamson and Payne, 1987). Knoess(1979) and Khanna and Rai (1993) they considered that camel seemed to be a very important animal for its productivity under desert conditions.

There are different classification systems for camels (Blanc and Ennesser, 1989; Kohler, 1993). The Sudanese camels are classified according to their use as packing and riding camels, the former include Arabi camels with well-developed hindquarter, large hump, rigid body, relatively short neck and large head, and heavy bones and muscles. The riding camels include Bishari and Anafi camels, with relatively small head and ears, alert eyes, a fine and short neck, strong and fine shoulders, a very deep chest and well-sprung ribs to the back. Sudanese camels mainly found in the central, Butana area, and the western region, Kordofan and Darfur states (El Khider, 2002).

World stock of Dromedaries of approximately 17 million and other 2 million Bactrians were recorded by (Shwartz,1992). He also recorded fifteen million dromedaries occur in Africa and Middle East and 2 million in India and Pakistan. Recent camel's population in Sudan is estimated to be 4.77 million (MOARF, 2013). It ranks second after Somalia in the world.

Fortunately the camel is the most capable animal surviving and producing under desert conditions compared with other productive farm animals, as previously mentioned, with some attention, camel is a highly productive animal either with bedwines or farmers in newly reclaimed areas. But one of the most important factors affecting camels' productivity is its low reproductive efficiency. The maintenance of high level of reproduction in camels will be very important for both raising its profitable production and giving high opportunity for selection and genetic improvement (El-Wishy, 1987). Skidmore (2003) noted that the reproductive efficiency of camels is low due to the short breeding season and the late puberty age. She also mentioned that the development of AI in camels is complicated by the difficulty of collecting semen and the gelatinous nature of the

produced semen. However, diluting semen and inseminating females has given encouraging results. One of the major importance for the application of AI technology in camels is that, although the male camel is a seasonal breeding animal, the she camel is sexually active all over the year and during the non-breeding season of the male (Vyas *et al*, 2004). But Al Qarawi (2005) reported other factors resulting to low fertility include age at first calving, low libido of male thereby reducing breeding opportunities and late postpartum oestrus. Yagil (2006) mentioned that the interest of camel's reproductive processes started with the appearance of their economic benefits.

Many producers and authors had utilized and studied many methodologies to improve the efficiency of camel reproduction including the use of reproductive hormones to control and regulate the oestrus cycle and ovulation (Skidmore, 2003; Yaniz *et al*, 2004). The artificial insemination (AI) is considered as one of the most important, and the fastest way in the modern technology, for the application of genetic improvement, through the breeding programs of farm animals. This is carried out by introducing and extending the best genetic criteria of selected camels into numerous female animals (Adams *et al*, 2009; El-Hassanien *et al*, 2010; Skidmore, 2011). While Morton *et al* (2008) reported that development of A.I will facilitate the more widespread use of superior sires, increasing the welfare of the males and reducing the risk of spread of communicable diseases and pests. Moreover, the use of A.I will result in genetic gain, which would be unlikely to occur using conventional breeding programs.

Progress on artificial insemination (AI), semen preservation and related techniques in camelids has been slow in comparison to other livestock species. As

there is relatively limited information in the literature on anatomy and physiology of male and female camels (Morton *et al*, 2008).

In order to identify the way it goes for success of the artificial insemination in camels, it is a must to understand the male and female reproductive system anatomy in different species (Dromedary, Bactrian and Lama). This subject was studied by many authors (Chen *et al*,1985; Smuts and Bezuidenhout,1987; El-Wishy, 1992; Zhao, 1995; Casas *et al*,1996). Therefore, a good knowledge of the camel reproductive physiology is the key element to good management, to understand the causes of infertility, and to find out its treatment, especially for males as it represent half of the genetic profile of the herd.

The increasing in the human population pressure and declining per capita production of food in Africa precipitated an urgent need to develop previously marginal resources, such as the semi-arid and arid rangelands, and to optimize their utilization through appropriate livestock production system among which Camel production is certainly the most suitable. Despite the fact that Camels are major component of the agro-pastoral systems in vast pastoral areas in Asia and Africa, little known about their production potential and production systems compared to other domestic animals (Mehari *et al*, 2007).

It is therefore necessary to look into ways of how to improve the production of this neglected species, so with the present study was undertaken to contribute to the better understanding of factors influencing reproduction in Camels, and to make available knowledge suitable for immediate dissemination and application by herders in order to reduce oestrus cycle losses and to enhance stability of pastoral husbandry and management systems.

The overall objective was to contribute and explore sounding the depth of camels with reference to its reproduction behavior, which haven't been carried out by enough scientific research, achieving the meaning of the Quranic verse precious:

{DO THEY NOT LOOK AT THE CAMELS, HOW THEY ARE CREATED?} - Truth of Allah Almighty – (Al-Gashya, verse 17).

The specific objectives are:

- (1) To monitor the ovulatory response following ovulation induction regimes in the dromedary She – camel during non-breeding season.
- (2) To determine the effect of the hormonal treatments on induction of ovulation.
- (3) To characterize the sex steroid hormones concentrations as a response following ovulation induction regimes in the dromedary camels.
- (4) To evaluate the success rate of applying the artificial insemination with fresh collected semen and natural mating under different ovulation induction regimes during non-breeding season.
- (5) To contributes to improving of reproductive efficiency, and to reduce the time, effort ,and cost.

1.1. General Taxonomy

Camels belong to the order of *Artiodactyla*, suborder *Tylopoda*, and family *Camelidae*. This family contains two genera; *Camelus* and *Llama* (El Khider, 2002). There are two camel species: *Camelus bactrianus* (the tamed, two – humped camel; the wild form is referred to as *Camelus ferus*) and *Camelus dromedaries* (one-humped camel, the dromedary) (Ouis, 2002).

The genus *Camelus* consists of *Camelus dromedarius*, dromedary camel (one hump), and *Camelus bactrianus*, Bactrian camel (two humps). Both species are also known as old world camelids. The genus *Lama* consists of four species, namely, *Lama glama* (llama), *L. pacos* (alpaca), *L. guanicoe* (guanaco), *L. vicugna* (vicuna) , they are known as new World camelids (lamoids) (El-Bahrawi, 2005; NRCC, 2011).

The term dromedary is derived from the Greek word "Dromados" (run) and in the strict sense is used for riding camels (Higgins, 1986). Also he mentioned that *C. dromedaries* gets its name from the Geek word “dpomados ” which meaning running, and *C. bactrainus*, the Bactrian camel named after the area of Bactriana in Central Asia.

Camels of the Old and New World belong to the family Camelidae in the ruminant suborder of the order *Artiodactyla*. They are usually separated from the other ruminants into the infraorder *Tylopoda* (Pad – footed) because their hooves are replaced by callous pads ending in claws. In the Old World there are two types of camels, that are known today as Camelus dromedarius (dromedary) and Camelus bactrianus (Bactrian camel), while in the New World the camelidae are

represented by two domestic species, *Lama glama* (Llama) and *Lama pacos* (alpaca), and two wild species, *Lama guanicoe* (Guanaco) and *Vicuna Lama vicugna* (Sumar, 1983).

Taxonomically, the family Camelidae is the only family within Tylopoda, which is one of three suborders of the order Artiodactyla (Zeuner, 1963). The family Camelidae contains only three genera. The Old World genus is *Camelus*, comprising two species, *Camelus dromedarius*, or the one – humped camel of Arabia and Africa, and the *Camelus bactrianus*, or two - humped camel of Northern Asia. In the New World are found the genus *Lama*, with three species, and the genus *Vicugna*, with but a single species. Larson and Ho (2003) reported they are pseudo-ruminants and have several unique features: they walk on pads rather than hoofs, do not have horns or antlers, and their red blood cells are oval shaped.

1.2. Historical Background

Camels appear to stem from common ancestors in North America some 16 million years ago, some migrated across the Bering Strait to Siberia and others across the Central American Isthmus, the one - humped camel (*Camelus dromedarius*) occupies and is well adapted to hot arid desert environments from India in the East to Mauritania and Mali in the West. The two - humped camel or Bactrian camel (*Camelus bactrianus*) is found in the cold desert and semi – arid areas from the Caspian Sea across Central Asia to Manchuria (Sumar, 1983).

Yagil (1985) reported that camels evolved in North America from the upper Eocene period, throughout the Tertiary period, and into the Pleistocene period,

altogether a time span of forty millions years. During the Eocene the camel's ancestor *Protylopus* was small as a rabbit, while the bigger (about three meters over the shoulder) *Aepycamelus* and *Titanotylopus* of the Pleistocene spread to Asia, Africa, Europe, and America.

One of the problems of localizing the original area of camel domestication is the fact that archaeologists tend to take an interest in settlements, while camel husbandry is a nomadic phenomenon which is considerable more complicated to trace.

Bulliet's (1975) argument for localizing the area of domestication to the South Arabian coast is by reference to a tradition saying that wild camels mated with the camels of Jinns » (A jinn is a mythical spiritual being in Arab folklore, and also mentioned in the Quran.), these camels were supposed to have lived in Southern Arabia, in the legendary land of Wabar » (Wabar (or Ubar) is one of the lost legendary cities of Southern Arabia, where exactly the city of the 'Ad.

The domestication of the camel occurred relatively recently compared with other animals, such as, Sheep (1000 B.C), Goats (8000 B.C), Pigs (6500 B.C) and Cattle (5000 B.C) (Planhol and Rognon,1970). While Schwartz and Dioli (1992) mentioned that they have been domesticated about 3000 years ago. Graham, (1996) added that camel was domesticated around 2500 – 3000 B.C.

The total camels' population in the world is estimated to be about 26 million according to the Food and Agriculture Organization (FAO, 2011).

1.3. The Benefits or Importance of Camels

Camels are an important livestock species in the arid and semi-arid zones, it contributes significantly to the livelihood of the pastoralists and agro-pastoralists living in the fragile environments of the desert and semi-desert of Asia and Africa (Ishag, 2011). While Kamal (2008) and Meiloud *et al* (2011) indicated that, camel is one of the best adapted animals of the desert, a source of milk, meat, and wool and is used for transportation. One-humped camel (*Camelus dromedarius*) is the only animal that can exist for several weeks without water and remaining to provide human with meat and milk (Yagil, 1982). It is unique producer of food in the arid and semi-arid zones of the World (Glimore, 1981; Marai *et al*, 2002). In the arid and semi-arid regions of the World, specially East Africa and the Middle East, where a total of 15 million Dromedaries are found (Schwartz, 1992; FAO, 1995), Camels are still the main sustainable agricultural resources for millions of pastoralists. In these countries camels are primarily used for the transport, production of milk (Knoess, 1979; Schwartz, 1992; Köhler-Rollefson, 1994) and meat (Qureshi, 1986) as a source of finance (Camel export) and for the provision of skin and wool, where Bactrian camels are found (Williamson and Payne, 1987). Furthermore, the camel is used for racing and tours in some parts of East Africa and Middle East. The camel is the only domesticated animal that can thrive in the extremely harsh arid environments. It is therefore necessary to look into ways of how to improve the production of this neglected species.

The camel (*Camelus dromedarius*) is found in desert and appears thriving there.

Data collected over the years have shown the ability of this animal to survive long period without water (Schmidt-Nielsen, 1964; Mcfarlane, 1965; Maloiy, 1972;

Yagil *et al*, 1976). During the periods when camels were subjected to water deprivation they continued to eat normally (Yagil *et al*, 1976). Camels provide mankind with a range of products and services, e.g. wool, meat, milk and the power to resist draught (Schwartz and Dioli, 1992). The unique adaptability makes camels ideal for exploitation in many pastoral systems in the arid and semi-arid areas of Africa (Yagil, 1985; Schwartz, 1992; and Wilson, 1998). With the growing and increase of importance of medicinal and nutritional values of camel milk worldwide, there is an urgent need to exploit potentials, as it adapted to harsh conditions (Amasaib *et al* 2013). The ability of the Camelidae to go for long periods without water and live on thorny and high-fiber diets, stand high altitudes, and extreme temperatures make them one of the few families well adapted for food and agricultural production under harsh semi-desert environment. The desert areas are characterized by scarcity of food resources necessary to maintain life of man, animals and plant. In those critical situations, camels play a major role in providing nourishment and livelihood for mankind, at times when other livestock classes cannot survive. The camel possesses certain unusual and unique physiological and morphological features that enable it to thrive in extremely arid environment, like: long eye lashes which protect their eyes from desert sand, control the opening and closing their nostrils, the height of the body which allows the camel to stand high above the hot sand, the mechanism of heat loss, and the fluctuated body temperature (Gihad,1995). Wilson (1984) mentioned that, 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 to the North of latitude 14°N in the West and 16°N in the East.

1.4. Classification of Dromedary Camels

Unlike other conventional farm animal species, the camel has not been subjected to intensive selection to perform certain physiological functions such as milk or meat production. The selection criteria used by nomads were more oriented towards ensuring survival in a harsh environment, speed, and other aesthetic characteristics that vary from one tribe to the other (Ishag, 2011).

Camels can be grouped into Mountain Camels and Plains Camels, with the first category subdivided into Baggage (Pack) and Riding Camels, and the latter category subdivided into Desert and Riverine Camels (Leese, 1927; Novoa and Wilson, 1992; Köhler-Rollefson, 1993). While Gillespie (1962) reported that the Camels are mainly classified into two types: Pack and Riding. Arabian Camels can be classified according to tribes, color and regions. Djemali and Al-Hadrami (1991) mentioned that these classifications assign little importance to the main products (Milk and Meat). Wardeh (2004) mentioned that a new classification divides Camels into four major classes: Beef, Dairy, Dual purpose and Race Camel. This classification is based on the fact that the Camel is a major component of the agro – pastoral systems in Asia and Africa.

1.5. Camel in Sudan

1.5.1. Historical Background

The history of the Dromedary Camel in Sudan is even more obscure. It's believed to have entered the Sudan from Egypt. A specimen of Camel hair rope of the old

kingdom was found at Fayum in Upper Egypt, dating about 2980-2475 B.C, indicating that the animals had moved south by that period. In the Sudan, the oldest evidence is a bronze figure of camel with saddle found at Marawi, and estimated between 25-15 B.C (Adison, 1934; Robinson, 1936).

Tracking of historical trends in the Sudan is difficult because the lake of reliable data (Romet, 2001).

Probably the camels entered the Sudan through the following routes:

- (1) North West Africa route during 4th to 6th Century.
- (2) Egyptian route.
- (3) Red Sea route (most recent) (Salman, 2002).

1.5.2. Camel Population and Distribution

Sudan considered as one of the largest camel populated countries in the world, it ranks second after Somalia in the world. Sakr and Majid (1998) mentioned that, Sudan is rated the second in the numbers of camel population in the World. They reported also, camels constitute 22 % of the animal biomass in Sudan and 26.3 % of the numbers of camel in the Arab World. Recent camel's population in Sudan estimated to be 4.77 million, it constitutes 4.5 % from the total livestock population in Sudan which is estimated to be 105 million (MOARF, 2013).

The majority of this number is kept by migratory pastoralists "Abbala" in arid and semi-arid zones of Sudan, where camel pastoralists prevail with limited resources in subsistence production systems. These camels are spread in a belt that extends between latitude 12°N-16°N, this belt is characterized by an erratic rainfall of less than 350 mm (Wardeh, 1989). Agab (1993) mentioned that camels in Sudan are concentrated in two main regions: The Eastern states, where camels are found in the Butana plain and the Red Sea mountains, between latitude 14° N to 16° N longitude 33-36° E, and Western regions (Darfour and Kordofan). Table 1.1 shows the distribution of camels in the different states of Sudan, growth rate of camel's

herd in Sudan is 1.4 % (Babiker, 1988).

Table 1.1: Distribution of Camel by states

States	Camel Numbers	Camel Population %
Kassala	696381	14.59
El Gadarif	345565	7.24
Red Sea	289244	6.06
Northern	49639	1.04
River Nile	115507	2.42
El Gezira	124575	2.61
Khartoum	6682	0.14
White Nile	35789	0.75
North Kordofan	1251958	26.23
South Kordofan	536008	11.23
North Darfour	597102	12.51
South Darfour	88468	1.85
East Darfour	72383	1.52
West Darfour	237218	4.97
Center Darfour	194261	4.07
Sennar	117893	2.47
Blue Nile	14319	0.30
Total	4773000	100

Source: MOARF. Information center (2013).

1.5.3. Camel Classification

Camels in the Sudan and elsewhere are classified as pack (heavy) and riding (light) types according to the function which they perform (Gillespie, 1962). The Riding camel has received more attention and has undergone intensive selection (Ishag, 2011). The following classification is based on conformation and tribal ownership:

(1) Pack Camel

It comprises 90 % of the total number of the camels in Sudan. It is characterized by Large, heavily built body, with capacity for developing a relatively large hump and includes the following types:

(A) Arabi Camel: It is sandy, gray, large, heavily built animal with well-developed hump. Widely distributed in the Sudan due to its good performance as work animal. Arabi camel is subdivided into three types.

I. Light Pack: Found in the East of the Nile in the area of the Red Sea bred by Hadandwa, Beni Amir and Al-Amarar tribes.

II. Big Arabi: Spread in Butana region bred by Shukria, Bataheen and Lahaween tribes.

III. Heavy Arabi: It is characterized by its heavy weight, big hump, long neck, big head, Roman nose, heavy bones, usually with long hair on the hump and shoulder (Al-Khoury,2000). Size wise it is known to be the largest camel in the Sudan. It spreads in the desert and semi-desert areas west of the Nile River. This type includes Kababish Camel in Northern Kordofan.

(B) Garbawy and Fiesani Camels: These are found mainly in Northern Darfour State (Zayed *et al*, 1991).

(C) Rashaidi Camel: This type is pinkish-red in color, slightly shorter, not quite as heavy as Arabi camel types and less numerous. They are mainly herded by Rashaida nomads in the Eastern Sudan. Some Rashaidi camels are owned by tribes who share the same ecological zone such as Shukria, Bataheen and Lahaween tribes (Al-Amin, 1979).

(2) Riding Camels

This type its conformation is the best suited for riding and selected mainly for its speed. It is lighter in body weight, and characterized by small head and ears, long and fine shoulders, very deep chest and well-muscled quarter. It is mainly bred in the Northern-East of the country between River Nile and the Red Sea, includes the following types:

(A) Anafi Camel: It is the fastest subtype; it has long legs, white body color,

small hump, and long narrow head. They are bred by Rufaa, Kenana, Shukria and Kawahla tribes.

(B) Bishari Camel: These camels reared by Bisharien, Al-Amarar, Hadandwa and Beni Amir in Kassala and Red Sea regions. They are very famous for their racing ability (Wardeh, 1989). These animals are stronger and slightly larger than Anafi type. Al-Khoury (2000) described the Bishari camel as having short and strong legs, fine and thin skin and white to yellow colour.

1.6. Reproduction in Camels

Low reproductive performance of dromedary camel has become the major impediment in multiplication and genetic improvement of this species (Wani and Nowshari,2005). Camels reproduction is handicapped or displayed by different constrains as semen characteristics, long gestation period, late sexual puberty and maturity, limited breeding season and the mechanism of Oestrous cycle and ovulation of she- camel (Deen, 2008; EL-Hassanien *et al*,2010). Skidmore (2013) indicated that the reproductive performance of camels is generally regarded to be low (40%), this could be due to the late age of reaching puberty (3 - 4 years), the long gestation period (13 months) and the relatively high incidence of abortions and non-conceptions possibly attributed to poor nutrition, poor management, limited breeding opportunities for the females due to seasonality of breeding. Opportunities to improve reproductive efficiency of dromedary camels are limited, the continued use of traditional methods of management adds difficulties to the practical improvement of its reproductive efficiency, freezing semen and artificial insemination (AI) could be employed to overcome some of those problems (Zhao *et al*,1994). Wani *et al*, 2008 indicated that assisted reproductive technologies such as artificial insemination (AI), in vitro fertilization (IVF), embryo transfer (ET), and cryopreservation of gametes allows exchange of genetic materials between

population without the need to transport animals, and it also eliminates problems of behavioral incompatibility, overcomes physical conditions that limit breeding, and reduces opportunities for disease transmission.

1.6.1. Breeding Season & Seasonality of Camels

Dromedary seasonal sexual behavior is very variable due to the wide geographical distribution of camels, but generally it is related with the period of low humidity, low temperature and increased rainfall (Gombe and Oduor-Okelo, 1977; Yagil and Etzion, 1980). Khan and Kohli (1972) noted that onset of the rutting season may be affected by the type of management and the individuality of males. However, males that are loose in a herd tend to come into rut earlier and remain in season a longer time than confined males. Camels breed only during certain times of the year and, therefore, are considered as seasonally polyestrous animals (Basiouni, 2007). The natural mating season and most conceptions occur in all areas at time of the year when the follicular wave is longest (Wilson, 1998). Briefly, the Camel can be described as a seasonal breeder with a marked peak in sexual activity. Occasionally, and particularly in specific climatic zones, a lesser peak also occurs. Both males and females show low levels, at least physiologically of breeding activity throughout the year. The breeding peak, therefore, can be considered facultative rather than required. The variation in the timing and length of the season clearly demonstrate that local environmental factors trigger off the start of increased sexual activity. What the exact factors are, whether they are climatic or nutritional?. Table 1.2 brings together most of the data on breeding season that are available together with climatic data at the onset of the breeding season and nutritional status (Wilson, 1984).

Table No. 1.2: Geographical variation in the Breeding Season of Camels with some known or inferred climatic and nutritional data at the onset of Breeding (Wilson, 1984).

Male in Rut	Females in Heat	Area	Source	Climate	Nutritional Status
Nov - Mar		India	Matharu, 1966	Day length Decreasing then Increasing	'Depending on level of nutrition' but not specified
Nov - Feb		India	Singh and Prakash, 1964		
Oct - Mar		India	Khan and Kohli, 1972		
Dec - Feb		India	Joshi, Viyas, Pareek, 1978	Increasing Day length	
Dec - Mar		Pakistan	Yasin and Abdul Wahid, 1957		
Mid Jan - End May		Turkest	Abdunazarov, 1970	Very cold becoming warm: rapidly increasing day length	
Jan - Feb		Iran	Islamy, 1950		
Mar - Apr		Egypt	Abdel Raouf and El Naggar, 1964	Increasing Day length: Warm to Hot	Short growing season, rising plane
Mar - May		Egypt	Shalsh and Nawito, 1964		
Spring		Sudan	Osman and El Azab, 1974		
Nov - Apr		Tunisia	Burgemeister, 1975	Day length Decreasing then Increasing: Rain; Cool to Warm	Fairly Good
Dec - May		Morocco	Charnot, 1963a	Day length Decreasing then Increasing: Cool to Hot	Fairly Good
Nov - Apr		Morocco	Charnot, 1965		
Aug - Sept		Mali	Swift, 1979	Day length Decreasing; Rain	Good
Feb - Mar		Mali	Swift, 1979	Day length Increasing; Warm to Hot	Depends on Winter conditions
June		Somalia	Leese, 1927	Day length static	Good
Sept - Nov		Somalia	Leese, 1927	Day length slowly Decreasing	
June - Sept		Australia	McKnight, 1969	Day length Increasing	

Females show higher breeding activity during winter and spring than summer and autumn seasons. Changes in food supply, mineral supplementation and photoperiod have been suggested to be responsible for not only productive

performance but also for seasonal breeding pattern of female camel (Onjoro *et al*, 2006; and Fatnassi *et al*, 2014). Ahmad *et al* (2007) mentioned that under the sub-tropical conditions of Pakistan, the camels appear to be seasonal breeder, with higher activity during the peak (November-April) than the low (May-October) breeding season. And he also mentioned that previous reports showing a clear breeding season in many countries where clear changes in photoperiod and/or food supply occur throughout the year. Hence, it may be hypothesized that camels may have the potential to breed all year around, but are prevented to do so by environmental cues. This view is supported by the observations that ovarian activity did not cease completely during summer and autumn seasons. Whether photoperiod or food supply is the key factor inducing seasonality in camels remains to be investigated. According to Sghiri and Draincourt (1999), food supply may be more important than day light duration. The complex interactions between photoperiodic and nutritional effects on the reproductive functions of camels warrant further investigation (Ahmad *et al*, 2007).

1.6.2. Puberty

In the field of reproduction, the scientific literature has been enriched in the last decade by introducing new methods for the study of hormonal profiles and correlation findings with behavior and direct observation of ovarian function (Tibary and Memon, 2004). Studies on puberty in the female dromedary are based on a few clinical and field observations (Wilson, 1986; Rai *et al*, 1991). The onset of sexual activity in the female starts as early as 2–3 years of age (Arthur *et al*, 1985). But in most management systems dromedaries are not bred until they almost reached their mature physical size at 4 years of age, resulting in an age at first calving of 5 years or more (Beniwal and Chaudhry, 1984). Several factors can

affect the onset of puberty, such as adequate nutrition, body weight, photoperiod, temperature, and water availability (Wilson, 1989). But nutrition and adequate growth seem to be the most important factors. It was observed in male dromedary camels that first sexual signs of the onset of puberty started between 3-5 years of age and sexual activity can continue until 20 years age (Sharma and Vyas, 1981; Yagil, 1985 and Khanna *et al*, 1987). While it has been noted in bactrian camels Chen *et al* (1980); Chen and Yuen, (1984) that puberty is generally reached by 4 years and decline after 15 years of age or may continue until 20 years old.

1.6.3. The Oestrus Cycle

Definition of the Oestrus cycle in Camelidae is different from that used in other species. This is due to the induced nature of ovulation in these species. In fact, the use of the term “Oestrus cycle” may not be very appropriate for these species because the cyclic ovarian activity and oestrus behavior is largely dependent on the presence or absence of ovulation triggering stimulus. In the absence of mating there is a succession of follicular waves with highly variable rhythm (Tibary and Memon, 2004).

1.6.3.1. The Follicular Wave

The regular and recurring hormonally controlled sequence of events which culminates in the spontaneous release of an ovum (or of ova) is known as the oestrus cycle. The term oestrus cycle, properly used, thus refers to animals which are spontaneous ovulators, this type of ovulation being the normal in the majority of animals. In a few mammals including cats, rabbit and the camel, rupture of the follicle does not occur spontaneously, coitus being required to induce release of the ova, in these animals the Neuro-Endocrine reflex involving the initiation of Luteinizing hormone release is delayed until coitus occurs. This type of cycle,

involving reflex or induced ovulation is more properly known as a follicular wave (Wilson, 1984). While Basiouni (2007) mentioned that camels are induced ovulators, normally they only ovulate in response to the stimulus of mating (neuroendocrine reflex) in which the initiation of the preovulatory LH surge from the pituitary gland is delayed until the coitus occurs. Therefore, if ovulation is prevented by the absence of mating, mature follicle(s) will regress and a new wave of follicles will start to grow again. Follicles grow and have a period of maturity during which time ovulation can occur from these follicles, and then regress again if ovulation is not induced. It is, therefore, more accurate and appropriate to describe the cyclical changes in the camels ovarian follicular dynamics as a 'follicular wave pattern' rather than an oestrous cycle (Basiouni, 2007 and Skidmore, 2013). Earlier attempts to describe this follicular wave pattern in dromedaries were based on post-mortem examinations and rectal palpations in a small number of animals. Results of these studies reported the lifespans of the dominant follicle (i.e. from when follicles first appear to when follicles regress) to range from 17 to 23 days in India (Joshi *et al*, 1978), 24 days in Egypt (Wilson, 1984) and 28 days in the Sudan (Musa and Abusineina, 1978), and waves of follicular development were longer at the beginning and end of the season (19–22 days) than during the middle (12–15 days) (Elias *et al*, 1984). Moreover, the introduction of techniques such as Ultrasonography and Laparoscopy allowed repeated examinations on the same animal and led to a better understanding of the follicular wave dynamics in these species (Bravo and Fowler, 1986; Adam *et al*, 1989; Bravo *et al*, 1990; Adams *et al*, 1991; Bourke *et al*, 1992). Recently, Fatnassi *et al*, (2014) reported that follicular growth occurs in regular waves during the breeding season. Where waves of follicular growth, maturation and atresia occur constantly in both ovaries (Manjunathaa *et al* 2012).

1.6.3.2. Phases of the Follicular wave

In the spontaneous ovulators (sheep, goat, and cattle) the oestrus cycle occurs in four distinct phases known as pro-estrus (follicular growth period), estrus (when female accept coitus), meta-estrus (when the corpus luteum is developing) and di-estrus (when the corpus luteum is developed, activated and finally degenerated) ((Nawito *et al.*1967). Van Teinhoven (1968) and Hafez (1974) added, in another classification these four phases were divided into two main phases: follicular phase (estrogenic phase) and luteal phase (progesterone phase). In induced ovulators and specifically in the camel, there are also four distinct phases but the normal terminology is not appropriate (Nawito *et al.*1967). The four phases of the follicular wave in Camel are:

1. Mature follicular stage, equivalent to oestrus or heat. The camel should not be considered to be in continuous oestrus in spite of the fact that ovarian maturity is follicular. Unlike the rabbit, which will accept the male at any time, the female camel will accept the male only during the mature follicular stage. There is no normal luteal phase.
2. The atretic follicular stage commences after a varying period of time if mating does not occur. Atresia is probably due to degeneration and phagocytosis of the granulosa of the follicles or to the extravasation of blood and the formation of the blood follicles.
3. The non-follicular stage.
4. The growing follicular stage (Wilson, 1984).

But Skidmore (2011) observed that these waves of follicular activity vary greatly between camels, but are indicated by periodic increases in the number of follicles

present in the ovaries from which one would grow to become the dominant follicle while other follicles regressed. She also added each follicular wave can be divided into three distinct phases: the growth phase (10.9 ± 3 days), mature phase (7.6 ± 4.2 days) and regression phase (11.9 ± 4.2 days).

1.6.3.3. External signs of Oestrus or Heat

Anatomical, physiological and behavioral signs of heat are evident, the intensity of heat varies both individually and seasonally. Skidmore (2013) indicated that oestrus behavior in camels is highly variable, and signs of oestrus are difficult to relate to follicular activity in the ovaries and cannot be used reliably to decide timing of breeding in camels. Camels in heat become restless, bleat continuously and associate with the male, the tail is lifted and flapped and they urinate little and often. The lips of the vulva swell and open and close irregularly. There is usually more or less copious emission of mucus, foul smelling to humans but presumably a powerful and attractive olfactory stimulus for the male camel. Vaginal examination reveals that it is pink colored and moist, although the degree of wetness decreases as heat progress. The cervix is moist and relaxed. Rectal examination will reveal that the uterine horns are turgid at the beginning of heat – although not so much as in the cow – and Graafian follicles but not corpora lutea can be felt on the ovary (Joshi *et al* 1978). Several authors have described signs of sexual receptivity or oestrous behavior in dromedary camels such as chasing and mounting other females, restlessness, swelling of the vulva, straddling the hind legs and urination, vaginal mucus discharge and receptivity to the male (Novoa, 1970; Wilson, 1984). When displayed, oestrous behavior lasts on average 4–6 days but can vary from 1 to 21 days and can be observed up to 7 days after ovulation (Musa and Abusineina, 1978).

1.6.4. Rutting Behavior

It was observed in male dromedary camels that first sexual signs of the onset of puberty started between 3-5 years of age and sexual activity can continue until 20 years age (Sharma and Vyas, 1981; Yagil, 1985 and Khanna *et al.*, 1987). While it has been noted in bactrian camels Chen *et al* (1980); Chen and Yuen (1984) that puberty is generally reached by 4 years and decline after 15 years of age or may continue until 20 years old. One of the most important signs is the secretion of the poll gland which is tarry dark with a very strong smell. The poll gland situated dorsally on the neck just behind the camel head. Marie (1987) noted that poll gland weight can increase from 40-100g in non- breeding season to 200-240 g in rutting season.

Sexual behavior is also characterized by exteriorization of the soft palate, but this has only been described in Dromedaries not in Bactrian camels. The protrusion of the soft palate occurs all day long at intervals of 15-30 minutes and is accompanied by loud gurgling and roaring sounds. The protrusions become more frequent with increased excitement such as the presence of other males and females (Khan and Kohlli, 1972; Sharma and Vyas, 1981).

1.6.5. Ovulation

The mechanism by which ovulation is initiated has been used to classify mammals as either spontaneous or induced ovulators, based on the biological process that triggers release of gonadotropin-releasing hormone (GNRH) and initiates the ovulatory cascade, mice, cattle, horses, and pigs are considered spontaneous ovulators because ovulation occurs at regular intervals as a result of increasing systemic concentrations of estradiol from growing dominant follicles, which stimulates GNRH secretion from neurons in the hypothalamus, which in

turn elicits a surge release of LH from the anterior hypophysis (Espey and Lipner, 1994). In contrast, ovulation in the induced ovulators such as camelidae does not occur at regular intervals, but rather in response to a copulatory stimulus. Spontaneous ovulation was detected by ultrasonography and rectal palpation in 5% of dromedary camels (Nagy *et al*, 2005) and 4–8% of llamas and alpacas (Adams, 2007).

The term ‘reflex’ ovulator is often synonymously used with ‘induced’ ovulator because of the perception that ovulation occurs as a response to the stimulation of sensory nerves in the vagina and cervix by the penis during copulation (Fernandez-Baca *et al*, 1970). In contrast to the concept of a direct neural stimulus, there is increasing evidence for the presence of a biochemical substance in seminal plasma that acts in an endocrine fashion to elicit pituitary LH release and ovulation (Chen *et al*, 1985). Previously, mentioned that dromedary female camels are known as induced ovulators, which need different treatments for induction of ovulation (Skidmore *et al*, 2013). Moreover Skidmore (2013) mentioned that ovulation must be induced before insemination. However, mechanical stimulation of the cervix which triggers ovulation in species such as cats and rabbits, does not induce ovulation in camels (Musa *et al*, 1990, 1992). There are various stimuli that induce ovulation in family camelidea, but the most common mechanism is mating by an intact or vasectomized male (Fernandez-Baca, *et al*, 1970), however, the mechanical stimulation only does not stimulate ovulation in all family camelidae (EL-Hassanien *et al*, 2010). Ovulation can be induced in Dromedary and Bactrian camel by a single injection of LH, GnRH or human chorionic gonadotropin (hCG) (Chen *et al*, 1985; Marie and Anouassi, 1987; Anouassi *et al*, 1992; Skidmore *et al*, 1996 ; Vyas and Sahani, 2005; Ismail *et al*, 2007; Moghiseh, *et al*.2008; Rawy, 2011; and Derar *et al*, 2014).

1.6.6. Hormonal control of the ovarian cycle

In vertebrates, reproduction is primarily controlled by the hypothalamic - pituitary - gonad(HPG) axis and, the structure of this endocrine pathway is highly conserved in jawed vertebrates (gnathostoma). The hypothalamic neuroendocrine system regulates synthesis and release of the gonadotropins, follicle-stimulating-hormone (FSH) and (LH), from the pituitary, which in turn stimulate gonadal development, in particular via the induction of sex steroid synthesis. Sex steroids feedback to the hypothalamus and the pituitary, thereby regulating gonadotropin synthesis and release (Kanda *et al*, 2011; Thackray *et al*, 2010). In addition, non-steroidal feedback regulation of gonadotropins by FSH-stimulated gonadal inhibins contributes to the synchronization of the HPG axis at all stages of the life cycle (de Kretser *et al*, 2002; Thackray *et al*, 2010).

1.6.6.1 Gonadotropin Releasing Hormone (GnRH)

This known and named for its role as the final common signaling molecule used by the brain to regulate and control reproduction in all vertebrates. The GnRH decapeptide is synthesized by neurosecretory cells in the hypothalamus and secreted into portal vessels, to be transported to the pituitary gland where it stimulates secretion of luteinizing hormone (LH) and follicle stimulating hormone (FSH) from pituitary gonadotrophs (Fernald and White, 1999). Harris (1950) mentioned that GnRH was predicted to exist in 1950, but since the hypothalamus contains minuscule amounts of GnRH, and the peptide has modified N – and C – termini, it was difficult to isolate. Consequently, its structure was not determined until 20 years later (Matsuo *et al*, 1971; and Burgus *et al*, 1972). Its act via G-protein coupled receptors (gonadotropin - releasing hormone receptors, GnRH-R). To date, several molecular forms of GnRH and GnRH-R have been identified in vertebrates. In most species, two forms (three in some fish) of GnRH are present,

one that is hypophysiotropic, stimulating gonadotropin release from the pituitary, and one that plays a neuromodulatory role in the central nervous system (CNS). The hypothalamus forms an interface between the CNS and the endocrine system, integrating internal (nutrition, metabolism), and external factors (e.g., temperature, photoperiod, pheromones). Thus, the hypothalamus is triggered by several factors of the CNS and peripheral hormones to maintain physiological homeostasis by regulating pituitary release of tropic hormones, which control the activities of peripheral endocrine glands (Thackray *et al*, 2010).

1.6.6.2 Human chorionic gonadotropin (hCG)

This glycoprotein hormone used primarily to induce ovulation in mares, the biological action of this human-origin hormone is due to its inherent luteinizing hormone (LH) type activity. A surge of natural or endogenous LH from the anterior pituitary during estrus (heat) causes final maturation and ovulation of the dominant follicle. Exogenous hCG essentially mimics the role of endogenous LH.

1.6.7 Control of Ovulation

Commercial and scientific interest in camels is rapidly increasing, especially in racing and milking camels in the Middle East, thus it has become desirable to develop methods for manipulating the follicular cycle in females to maximize reproductive efficiency throughout the relatively short breeding season. Because camels are an induced ovulating species, the ability to control ovulation is of fundamental importance (Skidmore, 2011).

Alternative methods to induce ovulation in dromedaries have been investigated and whereas mating to a vasectomized male, intramuscular injection of seminal plasma or injection with LH-like gonadotrophic hormones (such as GnRH or hCG) will induce ovulation (Chen *et al*, 1985; Marie and Anouassi, 1987; Musa *et al*, 1993). Manual stimulation of the cervix or intrauterine injection of water or

cloprostenol will not induce ovulation (Anouassi *et al*, 1992; Sheldrick *et al*, 1992). In addition, due to the relatively common occurrence of the larger than typical anovulatory follicles it is important to determine the optimum time of the follicular wave cycle to treat the camel to insure that the follicle is most receptive to any such therapy. Studies have been conducted using Gonadotrophin Releasing Hormone (GnRH) or gonadotrophic hormones to induce ovulation in female camels with follicles of varying sizes. Camels were treated with either 20g of the GnRH analogue, buserelin, or 3000 i.u. of human Chorionic Gonadotrophin (hCG) when the follicles ranged from <0.9 cm to >3.0 cm in diameter (Skidmore *et al*, 1996a). The results indicated that both GnRH and hCG would reliably induce ovulation in 85% of camels if the follicle measured between 1.0 and 1.9 cm in diameter but decreased sharply to 12.5% if the follicle size increased to 2.0–2.9 cm and failed to induce ovulation when dominant follicles were either <0.9 cm or >3.0 cm in diameter. Although there can be ovulations from follicles of 1.0 cm the oocytes from these follicles tend not to fertilize and the most desirable ovulation and fertility results were obtained when the dominant follicle measured between 1.3 and 1.7 cm in diameter at time of ovulation (Skidmore, 2011).

1.7. Natural Mating Behavior

Several investigations Khan and Kohli, (1972); Yagil and Etzion, (1980); Chen *et al*, (1980); Chen and Yeun, (1984); Rai *et al*, (1988), and Tibary and Anouassi, (1997) described male mating behavior as the male mounts the female in a couch position in which the female sits in recumbence and the male squats behind her with his hind legs flexed and his forelegs extend on both sides of the female. Additionally, either female lies down spontaneously or the male forces her to lie down then the male mounts the female. Erection is only achieved after female is mounted for a total copulation time of about 10-20 minutes, with ejaculation

occurring three or four times during that period. The straight body of the camel accompanied by an extension of the neck usually give an indication of the significant occurrence of ejaculation. El Bahrawy (2005) observed that mating takes place with the female kneeling on the ground then the male approaches from behind and squats upon it for a mating duration lasting between 7 to 15 minutes followed by slow intermittent ejaculation. Taha-Ismail (1988) noted that the male camel copulated the female in a sitting position, after mounting, the male starts pelvic thrusting to locate the vulva with his penis, total copulation time ranges from 10 to 22 minutes (average 13 minutes) to give 3 to 4 ejaculates, through each the male rests for few moments and resume copulation. Rai *et al.*, (1988) reported that for copulation of Bikaneri male camel, mating duration ranged between 3-25 minutes with average of 5.5 minutes, while an average of 3 was reported for bactrian camels in China (Chen *et al.*, 1980; Chen and Yuen, 1984). Billah and Skidmore (1992) noted that the time for full ejaculation was 4 to 23 minutes and each ejaculate was composed of two to five squirrels at intervals, some minutes apart. Moreover, the ejaculation process usually comes in fractions with periods of rest in-between until ejaculation is completed and the process usually lasts 5 to 10 minutes (Musa *et al.*, 1992).

1.8. The Artificial Insemination (A.I)

Artificial insemination (AI) is considered one of the most important and fastest tool in the modern technology for the application of genetic improvement, through the breeding programs of farm animals (Durrant, 2009). But has not been developed as a routine method for breeding camels, compared to its widespread application in other farm animals (Wani *et al.*, 2008). In addition, assisted reproductive technologies such as artificial insemination (AI), in vitro fertilization

(IVF), embryo transfer (ET), and cryopreservation of gametes allow exchange of genetic materials (such as milk, meat, wool production, and racing ability) between population without the need to transport animals, and it also eliminates problems of behavioral incompatibility, overcomes physical conditions that limit breeding, and reduces opportunities for disease transmission. They also continued that utilization of this technology is, however, dependent on the availability of the viable and functional spermatozoa, this necessitates the need for an optimal diluent and proper storing condition for the spermatozoa to maintain its quality and fertilizing ability for longer period. Among many reasons for the lack of AI technology in dromedaries are the viscosity of ejaculated semen, which needs to be liquefied before its evaluation, its low post liquefaction motility and lack of suitable semen extenders, which can maintain its viability for a prolonged period. The use of AI has been reported in camelids although insemination trials are rare, this could be because of the difficulties involved in collecting because of their copulatory behavior and refusal to serve the artificial vagina, as well as handling the semen due to the gelatinous nature of the seminal plasma (Wani *et al*, 2008 and Skidmore *et al*, 2013).

The use of AI as a breeding technique has been reported in camelidae since the 1960s with the first camelid offspring from AI being reported in a Bactrian camel in 1961(Elliot, 1961). However, it has only been during the last 25 years that this technique has started to be used more frequently as interest grows in trying to improve genetic traits such as milk, meat and wool production as well as racing ability in the Middle East. A major advantage of artificial insemination is that it can be used to increase the overall reproductive efficiency of the species however, some essential prerequisites are required before an artificial insemination programs can begin. Firstly the male camel has to be trained to the use of an artificial vagina

(AV) so that semen can be collected, and then it has to be diluted in a suitable extender to maximize the use of each ejaculate. Secondly, as camels are induced ovulators, ovulating only when mated, ovulation has to be induced in each female camel that is to be inseminated.

In the dromedary camel several authors have studied semen preservation and insemination Anouassi *et al.*, (1992); Bravo *et al.*, (2000); Skidmore and Billah, (2006a), but the majority of studies report low post-thaw motilities and few, if any, pregnancies with AI of chilled or frozen semen (Deen *et al.*, 2003). This could be because of the difficulties involved in collecting semen from male camels and that the ejaculates are of low volume, low sperm concentration and are highly viscous. The spermatozoa are entrapped within this viscous seminal plasma which makes the handling, diluting and cryopreservation difficult (Bravo *et al.*, 2000; Deen and Sahani, 2000).

1.8.1. The Advantages of Artificial Insemination (A.I)

Artificial insemination has many advantages as it allows more efficient use of genetically superior males by inseminating more than one female with a single ejaculate, thereby reducing the number of females the male has to mate, and increasing his number of offspring. Secondly, the use of AI would eliminate the need to transport the male or female animal to the stud, as it is much easier to transport semen, and this would reduce the costs and risks of transporting valuable animals. It would also reduce the risk of disease and infection, as all contact between male and female is prevented, and the semen can be treated with antibiotics before insemination. Thirdly, it helps eliminate behavioral problems as often a male camel will refuse to mate a particular female, or get aggressive towards her, so AI would eliminate the risks of injury. In addition, AI allows for the preservation of semen, either for 24 hrs by chilling, which enables the semen to

be transported between farms/countries for insemination in other breeding herds, or for many years by deep freezing, which could extend the reproductive lifespan of the camel even beyond its death. Finally, AI can also help manage cross breeding programs between different species of camelidae as it eliminates problems associated with size, behavior and living in different habitats (Skidmore *et al.*, 1999).

1.8.2. Collection of Semen

Semen collection in camels presents many difficulties mainly due to the nature of their copulatory behavior i.e. mating in sternal recumbence, lengthy ejaculation throughout copulation (from 5 to 20 min) and the highly viscous nature of the semen itself. The main techniques used these days for collecting semen from camels are the artificial vagina AV or, more rarely, electro-ejaculation (Skidmore *et al.*, 2013).

For collection by AV a modified bull vagina (30 cm long, 5 cm internal diameter) has given the best results (Bravo *et al.*, 2000). However, if the semen makes contact with the rubber liner this can have an adverse effect on sperm motility, thus a shortened AV is more popular allowing the semen to pass directly into the collection flask. Alternatively, an additional disposable plastic inner liner may be inserted to avoid contact with the rubber material although the authors have found that this is not accepted well by the males. The AV is prepared by filling with water at 55–60 °C to give an internal temperature of 38–40 °C and pressure inside the AV, to stimulate ejaculation, is obtained by blowing air between the inner liner and outer rigid wall. A clear, glass water-jacketed (35–37 °C) semen vessel is attached to the apex of the internal latex rubber liner to keep the semen warm during the lengthy ejaculation process and enable visualization of ejaculation. Observation of natural mating suggested that the highly mobile urethral process

of the camel penis may need to gain entry to the cervix to stimulate ejaculation during the extended copulatory process. For this reason, a foam imitation cervix of about 8 cm in length is placed inside the AV and the entrance of the AV is lubricated with jell before use. To collect the semen a sexually receptive female is first teased by the male to make olfactory contact and get him aroused before the bull is lead up behind the sitting female. As soon as the bull has sat down on the female and makes a few thrusts, the operator grasps the bull's sheath and directs his penis into the AV, holding it there by manual pressure at the base of the scrotum. The bull makes several thrusts, interspersed by periods of rest, until ejaculation is complete and during this time care must be taken to ensure the penis stays inside the AV otherwise the ejaculate will become contaminated with sand and dirt introduced by the penis. The ejaculate is usually expected in fractions and this whole process can take between 5 and 10 min, although it may occasionally last for 20 min or longer (Rai *et al.*, 1988). It is very difficult to ascertain when ejaculation has been completed so the collection procedure is usually continued until the male stands up. As the collection procedure can be rather prolonged it can be advantageous to add about 1–2 ml of extender to the collection vessel before the collection (Skidmore *et al*, 2013).

1.8.3. Semen Evaluation

Once collected the semen has to be evaluated and the following parameters should be noted: **Volume:** The volume of the ejaculate can be measured directly from the graduated collection tube and it can vary from 2 to 10 ml as there is great variation between males and even between ejaculates from the same male(Skidmore, 2013)

Colour: The colour of the ejaculate can vary from a greyish translucent colour, if the ejaculate is predominantly the gelatinous seminal plasma fraction and not very concentrated, to a creamy white colour as the concentration of spermatozoa increases (Skidmore, 2013).

Viscosity: One of the main characteristics of camelid semen is its high viscosity which makes handling and estimation of the spermatozoa parameters difficult. This viscosity is usually attributed to the presence of mucopolysaccharides from secretions of the bulbourethral gland or the prostate, but the degree of viscosity depends on the individual male and on the quantity of the gel fraction in the ejaculate. According to some authors the semen will partially liquefy if stored at 25–37 °C for 10–20 min (Abdel- Raouf and El-Naggar, 1976; Musa *et al.*, 1993) but other studies show it can take up to 8 h (Tibary and Anouassi, 1997a). Evaluation of the viscosity can be estimated by measuring the strand formed between the glass slide and a pipette.

Sperm concentration: After an aliquot of the semen is diluted 1:100 or 1:200 in formal citrate the concentration can be measured using a haematocytometer. There are few reports on the normal range of sperm concentrations in camels but it is suggested to be between 200 and 300 × 10⁶ ml⁻¹ (Anouassi *et al.*, 1992).

Motility: Motility is best estimated by placing a drop of diluted semen (1:1 in a suitable extender) on a pre-warmed slide and examining it under the microscope. The initial motility can be very low depending on the viscosity of the semen but increases as the semen liquefies, thus a true evaluation can only be made after the semen has liquefied. Other methods of determining sperm motility such as computerized semen motility analyzers have been used (Al-Qarawi *et al.*, 2002) but tend to be difficult in camelids due to the gelatinous nature of the semen.

Sperm viability: Sperm viability is assessed by exclusion of dyes such as Eosin-

nigrosin, Eosin-fast green or Propidium iodide. Stained smears are made by placing 1–2 drops of Eosin-nigrosin stain and semen on one end of a slide, then the drops are mixed and smeared forming a thin film. Live sperm have an intact membrane and exclude the stain, while the stain penetrates dead sperm with non-intact membranes. Live sperm are pink and dead sperm purple (Skidmore, 2013).

Sperm morphology: Assessment of sperm morphology is made using smears prepared and stained on a microscope slide. A minimum of 100 spermatozoa are then examined under high power magnification (1000×) and assessed as follows: normal, abnormal heads (large, small, tapering, pyriform, vacuolated, double head), abnormal midpiece, (distended or irregular, abnormally thick/thin), abnormal tails (short, multiple, broken, coiled, absent, bent, presence of cytoplasmic droplets) (Skidmore, 2013).

1.8.4. Semen Extension

Several extenders have been used for dilution of freshly collected camel semen (Sieme *et al.*, 1990) such as skimmed milk-glucose extender (Kenney *et al.*, 1975), Androhep (Waberski *et al.*, 1989), and lactose-egg yolk (Anouassi *et al.*, 1992), and Green buffer egg yolk (I.M.V. L'Aigle, France; Skidmore *et al.*, 2000). Most of these extenders contain an energy source (glucose or fructose), a protein for cold shock protection (lipoprotein from egg yolk or casein from milk), a buffering system and antibiotics. Once collected the semen is diluted in a ratio of 1:1–3:1 (extender: semen) depending on the concentration of the ejaculate, with warmed (30–35 °C) extender added slowly to the semen. It is better to then allow the semen to liquefy before insemination to aid better mixing of the semen with the extender and to allow more accurate assessments of concentration and motility. More recently, further studies have compared the extender INRA-96 (I.M.V.) with Green

Buffer. Results showed that whereas motility was higher after dilution in Green Buffer (67%) compared with INRA- 96 (59%), membrane integrity was higher after dilution in INRA-96 (65%) compared with Green Buffer (56%). However, sperm viability and acrosome integrity were similar for both buffers and pregnancy rates were unaffected by diluent, Green Buffer (34%) and INRA-96 (34%) (Morton *et al.*, 2010). However, the progress in semen preservation and related techniques in the camelidae family has been slow in comparison to other livestock species, this could be partly be due to the lack of proper semen extender to maintain the viability of spermatozoa for short and long term (Abdoon *et al*, 2013)

1.8.5. Method and optimum number of spermatozoa to inseminate

Cervical insemination: Fibroscopic evaluation of the camel's cervix before and after mating has shown that semen is deposited partly intra-uterine and partly intracervical (Tibary and Anouassi, 1997b). Therefore in AI the semen is generally deposited directly into the uterus, just cranial to the cervix, by means of a manually guided bovine insemination catheter passed through the relatively short, straight camel's cervix. Pregnancy rates of 50% have been achieved after insemination of 300×10^6 live spermatozoa (Bravo *et al.*, 2000) or as few as 100×10^6 (Anouassi *et al.*, 1992) directly into the uterine body. However with a short, open cervix that occurs during oestrous there can be a considerable loss of spermatozoa, due to backflow of semen through the cervix, when it is deposited just into the body of the uterus. Therefore subsequent studies investigated whether better results may be obtained if the semen is deposited at the tip of the horn rather than the body of the uterus.

Deep Uterine insemination: In other species it has now been proposed by several authors that semen be deposited at the tip of the uterine horn based on the fact that the major preovulatory sperm reservoir maybe at the uterotubal junction (UTJ)

rather than the body of the uterus or cervical canal (Hunter, 1988). The advantage of deep uterine insemination is that the semen is deposited nearer the UTJ and thus should further reduce the number of spermatozoa needed for successful fertilization (Lopez- Gatius, 2000). Insemination of camels however is easier as the cervix is shorter and straighter and the uterus less coiled, therefore it is simpler to pass a catheter through the cervix and guide it up the uterine horn per rectum.

Another study was therefore carried out to investigate more closely the optimum number of spermatozoa needed and preferred site of insemination (Skidmore and Billah, 2006a). A total of 40, 80 or 150×10^6 motile spermatozoa were deposited either just through the cervix into the uterine body or at the tip of the uterine horn ipsilateral with the ovary containing the dominant follicle and the results are shown that although a pregnancy rate of 53% or 43% could be achieved after insemination of 150×10^6 motile spermatozoa into the body of the uterus or at the tip of the horn respectively, a pregnancy rate of 40% could also be achieved when a reduced number of only 80×10^6 spermatozoa were deposited at the tip of the uterine horn. However, as only one camel inseminated with 40×10^6 spermatozoa at the tip of the horn conceived it would suggest that perhaps 80×10^6 is the minimum number of sperm needed to establish a pregnancy by deep intrauterine insemination.

1.8.6. Timing of insemination in relation to ovulation

As mentioned before that Skidmore (2013) indicated oestrus behavior in camel is highly variable, and the signs of oestrus are difficult to relate to follicular activity, and it cannot be used to decide timing of breeding. She added that Ultrasonography of the ovaries is therefore regarded as the most reliable method to monitor follicular growth, determine the correct time for mating, and to mate in the correct stage of the follicular cycle to maximize the chances of ovulation and conception. One of the most important aspects of insemination in camelidae is its timing in

relation to ovulation. The interval from mating to ovulation is not precisely known in camels although based on clinical observations most authors have suggested it is between 32 and 40 h after mating in dromedaries (Marie and Anouassi, 1986, 1987; Tibary and Anouassi, 1996) and 30–36 h in Bactrian camels (Xu *et al.*, 1985). However, in a more recent study where the ovaries of dromedary camels were scanned at regular 2 h intervals from 20 to 36 h after induction of ovulation with 20 g GnRH (i.v.), the majority of camels ovulated between 26 and 30 h after injection (Skidmore and Adams, unpublished data). This more rapid ovulatory response could be because the GnRH injected intravenously is absorbed more quickly by the pituitary thus causing a more rapid LH preovulatory peak. To investigate the most appropriate time to inseminate the camels a study was carried out where females were inseminated with 150×10^6 live spermatozoa either at the same time or 24 h after the GnRH injection. The results indicated that whereas 53% of camels conceived if they were inseminated 24 h after GnRH injection, only 36% conceived if they were inseminated at the same time as the GnRH injection (Skidmore and Billah, 2006b). This would suggest that to maximize the chances of conception after AI the camel should be inseminated 24 h after GnRH injection.

The present study conducted from August 2011 to August 2014, and for the field experiments a lot of camel farms were visit, a lot of camel herd owners was contact for example: Major general Mahgoub Hassan Saad's camel farm, which located in Khartoum Bahri (Al Ezba), Major general Dr. Al Aas's camel farm (Al Kadaroo), Camel research center, which belong to U of K, in Khartoum Bahri (Shambat), Mr. Ahmed Bahlas's camel farm in Portsudan, Mr. Ibrahim Ahmed, (Jabal Awlia), Mr. Abdel Hadi, the owner of Al Amera for camel milk (Soba), and Mr. Al Kabbashi (West Omdurman). Due to different reasons, sometimes technical and sometimes logistic reasons it could not possible to continue the field experiments in above camel farms.

2.1. Area of study

The field experiments were conduct at the Camel Reproduction Centre (CRC), Nakhlee, which located 48 km East the center of Dubai, UAE. This Centre was established in 1989 at the request of His Highness General Sheikh Mohammed bin Rashid Al Maktoum, Crown Prince of Dubai and Minister of Defense for the UAE. The center comprises of different laboratories containing state of the art equipments and facilities which help to do research on camel reproduction. The activities in (CRC) include:

- Collection, analysis and storage of Semen.
- Artificial Insemination (A.I) with fresh, cooled or frozen Semen.
- Development of Low dose Uterotubal Insemination Techniques.
- Collection and transfer of fresh and frozen / thawed embryos.
- Genetic studies on hybrid between New and Old World Camelids.
- Control the ovarian cycle of the camel.
- Studies on the Physiology of pregnancy.
- Infertility investigation and treatments.

While the lab experiment for hormonal assay was conducted at the College of Medical Laboratories, in Sudan University of Science and Technology (SUST), which is located in Western Khartoum (Al Mogran) - Republic of Sudan.

2.2. Experimental Animals

A total of 32 mature Dromedary she – camels, aged 6-8 years (average weight between 500-600 kg) and four adult males were used, and they were identified by Neck Tags (Necklace neck), housed at the Camel Reproduction Centre, Dubai, UAE, during the Non breeding season. Nineteen (19) animals were used in the first experiment divided into three groups as follows:

Group (A) set as a treatment group (N=7).

Group (B) set as a treatment group (N=6).

While group (C) set as control group without any hormonal treatment (N=6).

Thirteen (13) animals were used in second experiment divided as follows:

Group (D) inseminated artificially (N=7)

Group (E) inseminated naturally (N=6).

2.3. Management & Housing

The animals were housed in groups in fenced pens, each of 2 - 4 m² area. They were fed a diet of commercially formulated camel rations mixed concentrates and Lucerne hay twice a day to provide their requirements. Water was permanently available, and exposed to natural day length and ambient temperatures. (Figure No: 2.1).



Figure No: 2.1
The Housing System in (CRC)

2.4. Experiment (1)

2.4.1. Hormonal Protocols for Ovulation Induction

Ovulation was induced by two hormonal protocols as follows:

Group (A):

Protocol: GnRH {GONAVET Veyx® 10 ml}, each 1 ml contain Gonadorelin – [6-D-Phe]. Batch No: 12J252 from Veyx – Pharma GmbH D-34639 Schwarzenborn, Germany.

Procedure: Each she - camel were intramuscular injected with 2 ml.

Group (B):

Protocol: CHORULON® {Chorionic Gonadotropin 1000 I.U} with 5 vials as diluents (Sterile Phosphate Buffer water 10 ml). Batch No: A 037A02 – Code No: 022219 from Intervet INC, Millsboro, DE 19966 Holland.

Procedure: Each she - camel were intravenous injected with 3 ml.

Group (C):

Protocol: Water for Injection 100 ml

Batch No: 124051, Hameln Pharmaceuticals LTD, UK.

Procedure: Each she - camel were intramuscular injected with 1 ml.

2.4.2. Ovarian Examination

The ovaries of every camel were examined on alternate days throughout the defined experimental periods (0 hrs. = time of injections, 48 hrs = after 48 hrs) to detect and follow the follicles growing and the onset of ovulation. Also she camels were examined in (Week 1 = after 7 days, Week 2 = after 14 days) to identify the presence and persistency of the corpus luteum on the same ovary that had previously held the dominant follicle.

Confirmation of ovulation was done by Trans rectal Ultrasonography as described by (Skidmore *et al*, 1992).

Procedure: The females were restrained in a standing position in crush, the perineal region was cleaned very well and the tail was lifted and rolled with gauze. The rectum was evacuated from the feces after had been wearing the long gloves and putting the lubricant gel, similar to the standard technique which used in Cattle. Then the linear probe (array transducer) passed through the rectum and placed in contact with the bottom of the rectum to reflect the images from the ovary interface, which displayed in the monitor. An ALOKA (Model Prosound 2) real time scanner with a 5 / 6 MHz linear array transducer (Hitachi Aloka Medical, Ltd, Tokyo, Japan) was used. All follicles ≥ 0.5 cm in diameter and corpus luteum were monitored, counted and measured using the internal electronic calipers and photographed using a Sony video printer (Model UP 860CE) (Al Carmal Ltd, Dubai, UAE) interfaced with the scanner. Data recorded on the scanning worksheet. (Figure: 2.2).



Figure No: 2.2

ALOKA real time scanner
(Trans rectal Ultrasonography)

2.4.3. Blood sampling

Blood samples for serum preparations were collected in non-heparinized tubes from the jugular vein. Blood (8 ml) were recovered at alternate days throughout the defined experimental periods from all experimental animals by jugular venipuncture using a vacutainer tube. They were kept at room temperature (19-23°C) for 1-2 hours before being centrifuged at 3000 for 5 minutes. Using the centrifuge machine (ROTINA 380) – Type: 1701 - Year: 2010 - Made in Germany.

The serum was decanted and stored at - 20°C until subsequent assay for Progesterone, Estrogen, FSH and LH, by Enzyme Linked Immunosorbent Assay (ELISA) test.

2.4.4. Hormones assay

Estrogen, Progesterone, FSH and LH levels in serum samples were determined by the ELISA (Sunrise produced by TECAN, Type: Sunrise, REF: F 039300 SN: 03930005626, Made in Austria) and the analysis was done by the software called Magellan (Document part No: I 117519 - 2013 – 12, Version No: 4.2). The method (17 .mth, End point, Workspace, Magellan, v 5.03,.....) at a wave length of 450 nm. A kit (Biorex diagnostic, ESTRADIOL, PROGESTERONE, Biorex diagnostic ltd, United Kingdom) was used to measure Estrogen, Progesterone levels. A kit (Fortress diagnostic, FSH, LH, fortress diagnostic ltd, United Kingdom) was used to measure FSH, LH levels.

2.4.4.1. Assay Procedure

All samples and reagents were left to attain room temperature before used. Any micro - well strip which not being used was removed from the frame and stored in the refrigerator for later use. (Appendix).

2.4.4.2. Assay Protocol

Calculated microliters of each sample from the 32 camels according to the type of hormone (25, 50, 50 and 50 microliters) for the Estrogen, Progesterone, FSH, and LH respectively, and standards (containing different levels of diluent) were dispensed into assigned wells of a micro plate coated with purified anti-hormone antibodies. This was followed by adding different reagents and solutions, and incubated for a certain period. Then, finally the absorbance was read in each well at 450 nm (using a reference wavelength of 620-630 nm to minimize well imperfections) using a micro plate reader (Sunrise produced by TECAN, Type: Sunrise, REF: F 039300 SN: 03930005626, Made in Austria), analyzed by a software called Magellan (Document part No: I 117519. 2013, Version No: 4.2). (Appendix).

2.5. Experiment (2)

2.5.1. Ovarian Examination

This was done by the same principles, protocols and procedure which mentioned in the experiment (1).

2.5.2. Semen Collection

Semen was collected from dromedary bulls using a receptive female and artificial vagina (AV) at 8:00 to 8:30 am as described by (Bravo *et al*, 2000). Briefly, olfactory contact was made by leading a receptive female past the male's pen. The female was then restrained in sternal recumbence. The Bull was then lead up from behind to the sitting female with the operator on the left side of the female. Then the male allowed mounting the female and made a few thrusts, the operator grasped the sheath and the penis was deflected into the AV and the male allowed copulating. The AV consisted of a modified bull artificial vagina (30 cm in length with a 5 cm internal diameter, Minitüb, Tiefenbach, Germany) with a rubber AV

liner (Minitüb, Tiefenbach, Germany) a cervix-like structure, and a camel collecting glass (IMV Technologies, L'Aigle, France) filled with water heated between 55-60°C which gave an internal temperature of 38-40°C. A clear, glass water jacketed (35-37°C) semen vessel was attached to the apex of the cone shaped internal latex rubber liner to enable visualization of the ejaculate.

2.5.3. Semen Assessment and Evaluation

Semen was assessed immediately after collection, and maintained at 37°C.

Semen liquefaction was achieved by leaving the ejaculate in a water bath at 37°C for 1-2 hours. The semen samples were used to assess the quality. Each ejaculate was assessed for the following characteristics:

2.5.3.1. Semen Volume:

The volume of semen was noted from the graduated collecting tube immediately.

2.5.3.2. Semen Color:

The color of the camel semen is Greyish-White depending on the ratio of the gelatinous fraction, which is grey, to the sperm rich fraction, which is white, this was done by the naked eye.

2.5.3.3. Semen pH:

Digital pH meter was used to determine the semen pH immediately after the collection.

2.5.4. Sperm Assessment and Evaluation

2.5.4.1. Motility:

Sperm motility was examined by using a double phase-contrast microscope (Nikon), with warm stage (Theater) adjusted at 37°C. 100 microliter were added

from the semen diluted with extender to 900 ml Extender, after gentle mixing 10 microliter were used and put the drop in the middle of the counting chamber to observe the motility at the power 40 X.

2.5.4.2. Concentration:

Assessment of concentration was done as follows: 900 ml PBS (Phosphorus Buffer Solution) were added to 100 ml semen (with extender), after gentle mixing a drop was put in the middle of the counting chamber and counted at the power 10 X in 4 fields each field consist of 25 squares = 100 squares).

2.5.4.3. Sperm Morphology (Abnormalities) / Sperm Viability:

CASA system (CASA system = Computer-Assisted Semen Analyzer »). Its unique software (IMV Technologies, L'Aigle. France), which have the following benefits:

1. Automated morphology.
2. Interactive illumination check.
3. Magnificent screen layout: Control to initiate analysis, quick selection of analysis setup, live image area, results updated in real time.
4. Dose calculation: Concentration – Extender volume – Number of doses.
5. Automated heated stage
6. Fluorescence
7. Redesigned graphical user interface
8. Intuitive navigation
9. Multilanguage
10. High speed digital camera: Superior image quality, seamless image capture.
11. Unlimited storage of predefined analysis setups.
12. Built in database with customizable reports.

13.Fast: 8 seconds / 10 fields (30 images / field).

Procedure: One drop of the semen was put and run the system.

2.5.5. Semen Extension:

As soon as semen was collected and directly transported to the (CRC) lab, and it maintained at 37°C in the water bath, semen was immediately extended with Green Buffer with 20 % (v:v) egg yolk added (which prepared by adding 8 ml Green Buffer (IMV Technologies, L'Aigle, France) to 2 ml egg yolk. Semen was diluted at a ratio of 1:1 & 1:2 (semen: extender), depending on the concentration of the ejaculate. The extender warmed by a water bath to 37°C, and then added slowly to the semen.

2.5.6. Method and Timing of Insemination

Insemination was carried out with the female in a standing or sitting position, in the latter case the female was well restrained. The perineal region cleaned very well and the tail lifted and rolled with gauze. The semen deposited (248 / inseminate, 2 ml / animal) into the body of the uterus (Intrauterine) via insemination gun passed through the vagina and the cervix, with one hand in the rectum holding the Cervix, similar to the standard technique which used in Cattle. The time of insemination was 24h after the hormonal treatment with GnRH. While the control group left to mated naturally, by allowed the male mounting the female which sited in recumbence after the male approached and squats behind her with his hind legs flexed and his forelegs extended on both sides of the female. The mating duration between 7 to 15 minutes.

2.6. Statistical analyses

The data were analyzed using SPSS statistical software version 16 for windows (SPSS, 2015). The experiment parameters which include, hormones profile of Estrogen, Progesterone, FSH and LH concentrations, the Follicles size differences between groups and within groups were compared by One way ANOVA, all values expressed as means \pm SD.. While the multiple differences in Estrogen, Progesterone, FSH and LH between groups and within groups were compared by multiple comparison LSD. The incidence of Ovulation and in the second experiment the incidence of pregnancy differences were compared by 2 x 2 crosstab (Chi square test).

The results are shown that in table (3.1) there are no any significant differences between all groups in the follicle size (Diameter cm) in 0 hours to be sure that there was no effect or interaction made by the follicles on the hormonal profile or the parameters. Ultrasonic measurement of the follicle size is shown in Fig (3.1).

The proportion of she camels that ovulated during 24-48 hours in response to treatments were (6 / 7 vs 4 /6 vs 0 / 6) in the GnRH, hCG and the control groups, respectively, are shown in table (3.2) and Fig (3.2). While table (3.3) shown that 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 ($P \leq 0.05$).

Serum estrogen concentration (pg / ml) did not differ significantly ($P \leq 0.05$) between all groups in 0 hours and Week 2. In contrast with 48 hours and Week 1 which were significantly higher ($P \leq 0.05$) between the treated groups (GnRH and hCG groups) compared with control group. While there was no significant difference between the treated groups throughout the alternate periods (Table. 3.5).

Serum progesterone concentrations (ng / dl) did not differ significantly ($P \leq 0.05$) in the all groups in 0 hours and Week 2. While the serum progesterone concentration significantly higher ($P \leq 0.01$) in the GnRH group compared to the hCG and the control group respectively in 48 hours. Week 1 showed that the serum progesterone concentration significantly higher ($P \leq 0.01$) in the hCG group compared to the GnRH and control groups respectively (Table. 3.6).

Serum FSH concentration (miu / ml) in table (3.7) shown that there is a significant difference ($P \leq 0.05$) between the control and GnRH groups compared with the hCG group in 0 hours. The results in 48 hours show that there higher significant difference ($P \leq 0.01$) between the control group compared with GnRH, and hCG

groups. While results in Week 1, and Week 2 show that there are no significant differences between all groups ($P \leq 0.05$).

Results of LH serum concentration in the present study in table (3.8) are shown that zero levels throughout the alternate periods in all groups.

Results of the second experiment are shown in tables (3.9 to 3.11) as follows:

The results of semen assessment and evaluation for the males which were use in the artificial insemination are shown in table (3.9).

In the present study table (3.10) show that there are no significant differences between the two groups in the follicle size (Diameter cm) in 0 hours to be sure that there was no effect or interaction made by the follicles on the parameters (Fig.3.7). While table (3.11) shown that there is no statistically significant differences between the Artificial insemination (A.I) and the Natural Mating (N.M) groups ($P \leq 0.05$) in the onset or occurrence of pregnancy.

Experiment (1):

Table 3.1: Follicles Size (Diameter cm) in 0 hours in different groups in she - camels

Groups	N	Means \pm SD	P. Value
GnRH	7	1.46 \pm 0.05	0.45
hCG	6	1.50 \pm 0.06	
Control	6	1.43 \pm 0.14	

* N = Number of Animals

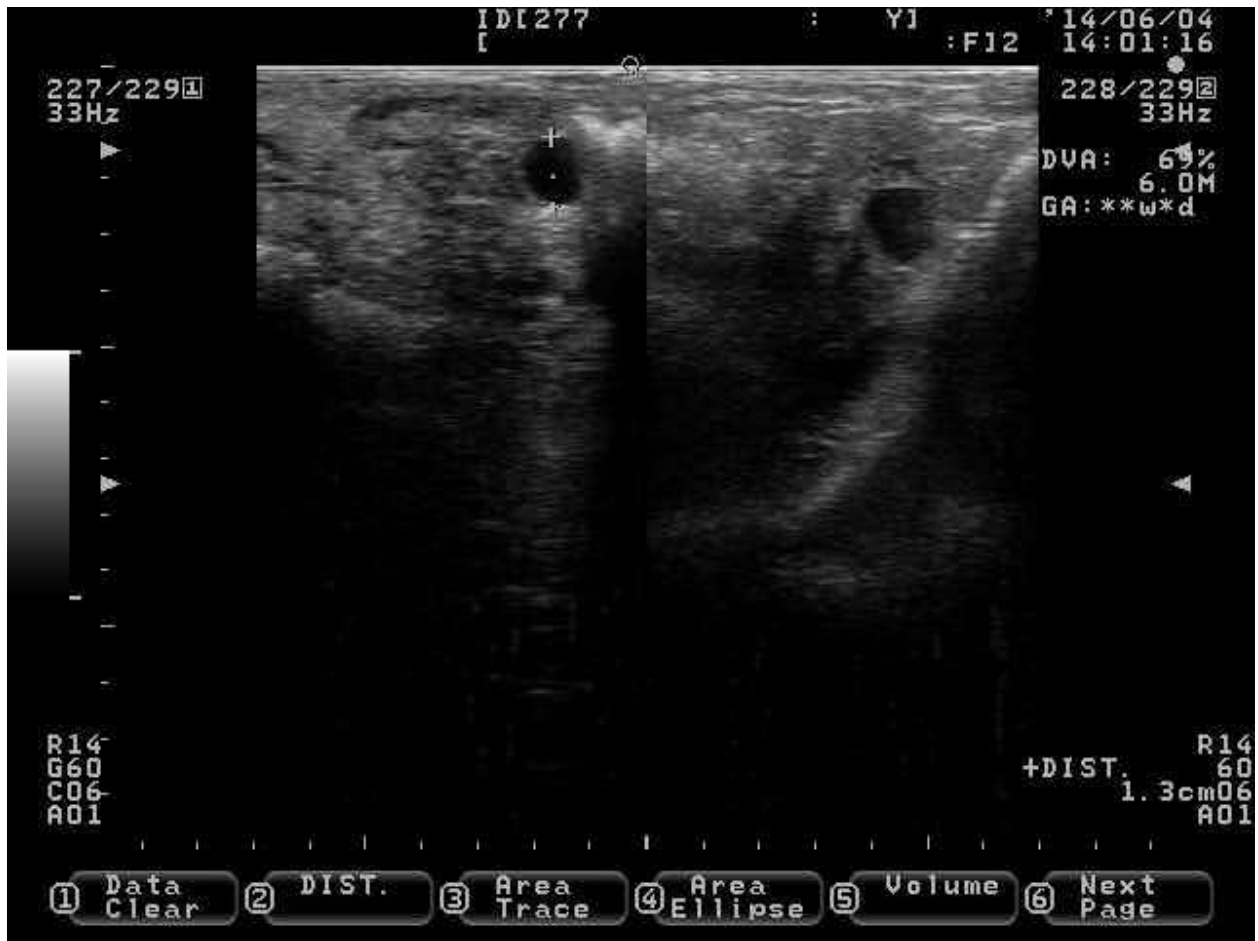


Figure No: 3.1
Measurement of the follicle size (Diameter cm) by ultrasonography

Table 3.2: Response (%) to the ovulation induction protocols during 24 - 48 hours
In She – camels injected by hormones within groups

Groups	Ovulated	Ovulation %
GnRH	6 / 7	85.7 %
hCG	4 / 6	66.6 %
Control	0 / 6	0 %

Table 3.3: Ovulation Onset during 24-48 hours in She – camels injected by
hormones

Groups	Pearson Chi – Square (χ^2)	df	P. Value
GnRH vs hCG	0.66	1	0.42
GnRH vs Control	9.55	1	0.002
hCG vs Control	6.00	1	0.014

df = degree of freedom

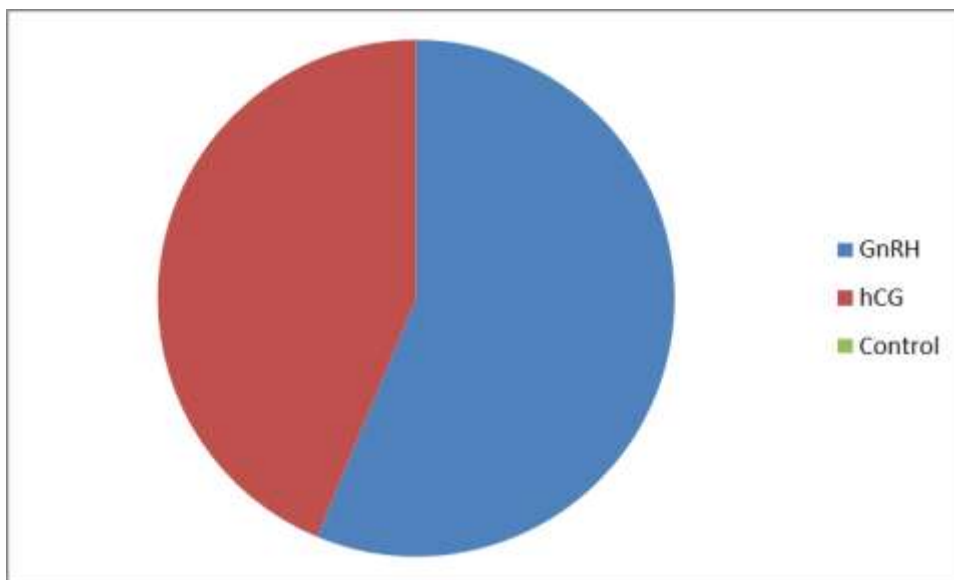


Figure No: 3.2
Ovulation induction in she – camels injected by hormones
Within groups

Table 3.4: Response to the ovulation induction protocols during 24 - 48 hours in She – camels injected by hormones between groups

Groups	N	Ovulated	Ovulation %
GnRH	7	6 / 19	31.6 %
hCG	6	4 / 19	21.1 %
Control	6	0 / 19	0 %

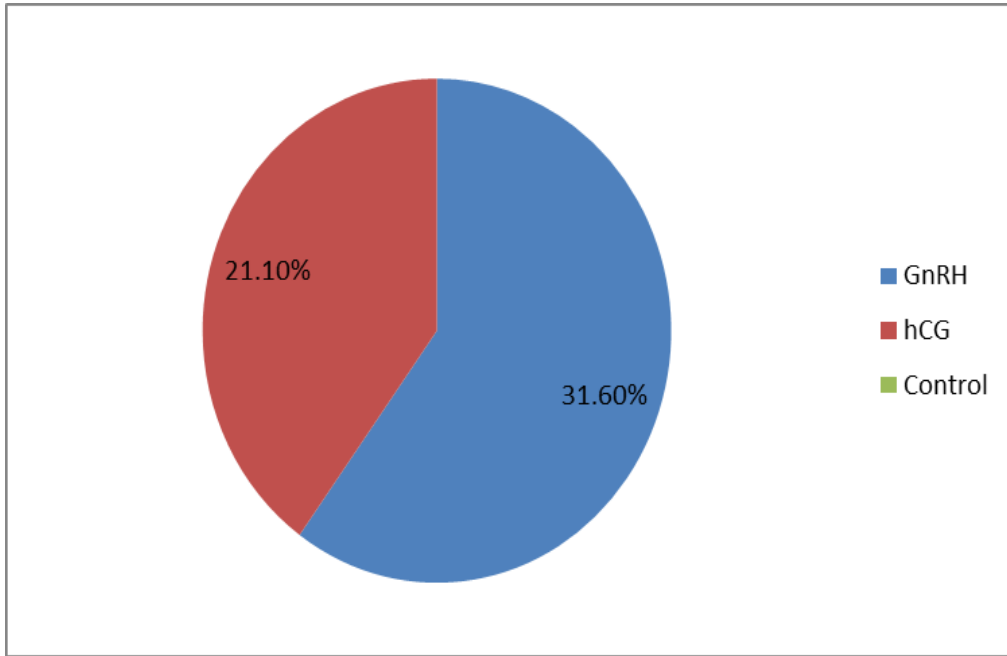


Figure No: 3.3
Ovulation induction in she – camels injected by hormones
between groups

Table 3.5: Estrogen Concentration (pg / ml) (Means \pm SD) during alternate periods in She - camels

Groups	N	E2 Con. 0 hr	E2 Con. 48 hrs.	E2 Con. Week 1	E2 Con. Week 2
GnRH	7	74.69 \pm 17.83	77.37 \pm 12.77 ^a	67.24 \pm 6.04 ^a	104.31 \pm 42.62
hCG	6	67.36 \pm 20.38	72.56 \pm 13.32 ^a	72.35 \pm 24.45 ^a	106.71 \pm 76.88
Control	6	57.27 \pm 8.52	53.87 \pm 20.02 ^b	48.49 \pm 7.62 ^b	58.13 \pm 15.11
P. Value		0.196	0.039	0.030	0.202

- * N = Number of Animals E2 Con = Estrogen Concentration
- * 0 hr = the day of Injection 48 hrs = after 48 hours from treatment
- * Week 1 = after 7 days from treatment Week 2 = after 14 days from treatment
- * Different superscript letters within the same column means significant differences ($P \leq 0.05$)



Figure No: 3.4
Estrogen Concentration (pg / ml) (Means) during alternate periods in She – camels

Table 3.6: Progesterone Concentration (ng / dl) (Means \pm SD) during alternate Periods in She - camels

Groups	N	P4 Con. 0 hr	P4 Con. 48 hrs	P4 Con. Week 1	P4 Con. Week 2
GnRH	7	0.86 \pm 0.29	1.15 \pm 0.21 ^a	1.64 \pm 0.06 ^b	1.44 \pm 0.76
hCG	6	0.97 \pm 0.29	0.89 \pm 0.12 ^b	4.19 \pm 3.15 ^a	1.03 \pm 0.26
Control	6	0.86 \pm 0.18	0.76 \pm 0.15 ^b	0.82 \pm 0.09 ^b	0.77 \pm 0.12
P. Value		0.69	0.003	0.011	0.073

- * N = Number of Animals
- * P4 Con= Progesterone Concentration
- * 0 hr = the day of Injection 48 hrs = after 48 hours from treatment
- * Week 1 = after 7 days from treatment Week 2 = after 14 days from treatment
- * Different superscript letters within the same column means significant differences ($P \leq 0.05$)

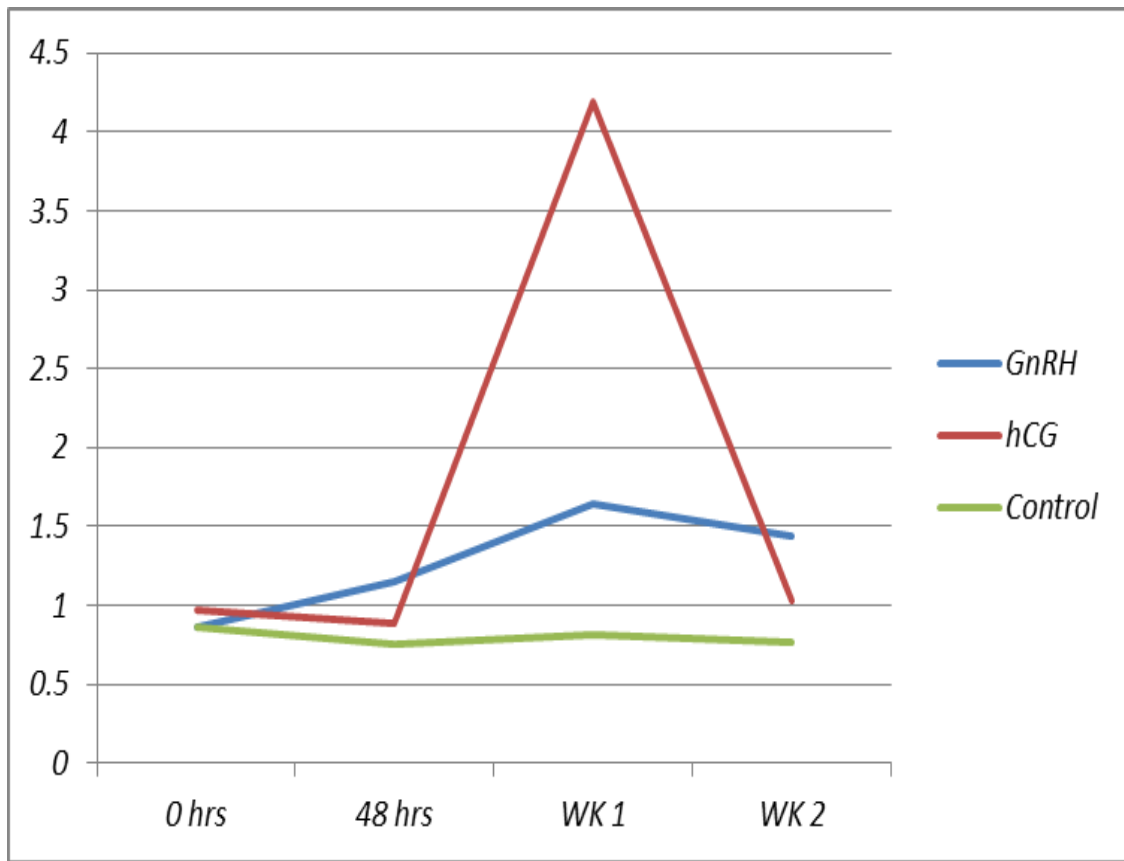


Figure No: 3.5
 Progesterone Concentration (ng / dl) (Means) during alternate
 Periods in She - camels

Table 3.7: FSH Concentration (miu / ml) (Means \pm SD) during alternate periods in She - camel

Groups	N	FSH Con. 0 hr	FSH Con. 48 hrs	FSH Con. Week 1	FSH Con. Week 2
GnRH	7	0.22 \pm 0.10 ^a	0.00 \pm 0.00 ^b	0.06 \pm 0.03	0.00 \pm 0.00
hCG	6	0.00 \pm 0.00 ^b	0.00 \pm 0.00 ^b	0.40 \pm 0.15	0.00 \pm 0.00
Control	6	0.33 \pm 0.19 ^a	0.40 \pm 0.20 ^a	0.00 \pm 0.00	0.52 \pm 0.11
P. Value		0.032	0.015	0.077	0.11

- * N = Number of Animals * FSH Con= FSH Concentration
- * 0 hr = the day of Injection 48 hrs = after 48 hours from treatment
- * Week 1 = after 7 days from treatment Week 2 = after 14 days from treatment
- * Different superscript letters within the same column means significant differences ($P \leq 0.05$)

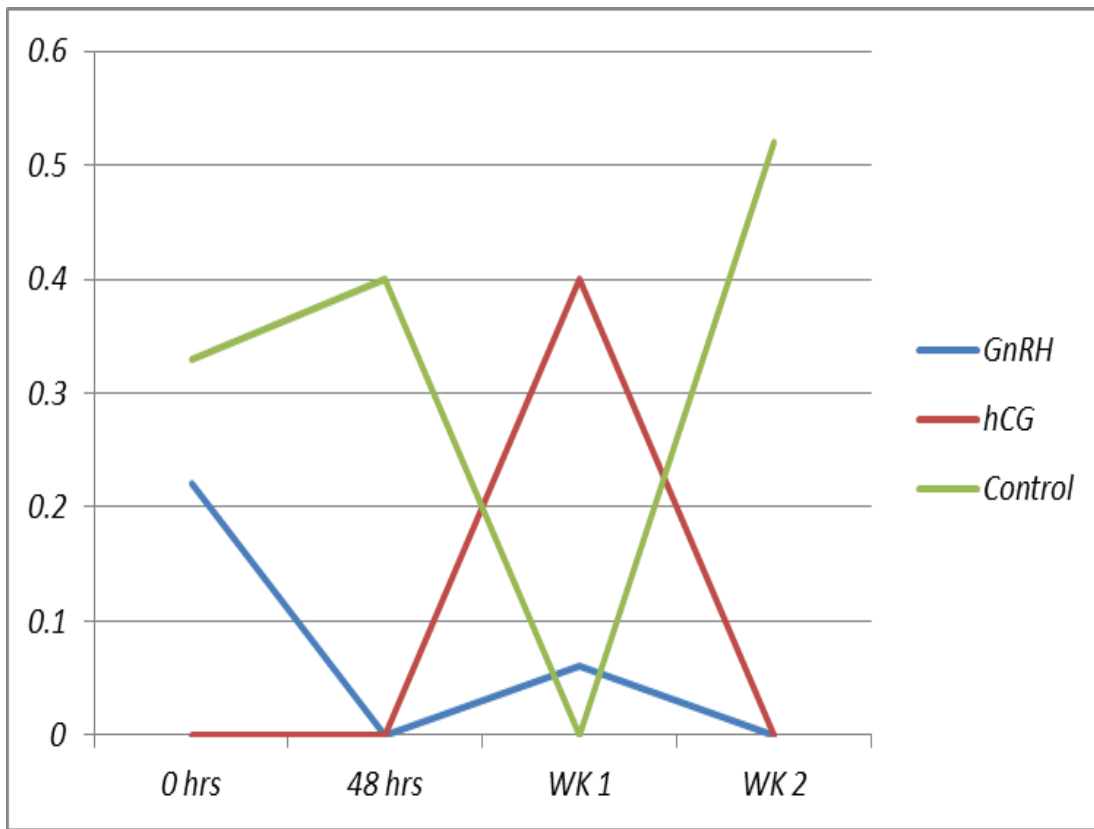


Figure No: 3.6
 FSH Concentration (miu / ml) (Means) during alternate periods
 in She – camel

Table 3.8: LH Concentration (miu / ml) (Means \pm SD) during alternate periods in She – camel

Groups	N	LH Con. 0 hr	LH Con. 48 hrs	LH Con. Week 1	LH Con. Week 2
GnRH	7	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00
hCG	6	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00
Control	6	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00

* N = Number of Animals

* LH Con = LH Concentration

* 0 hr = the day of injection

* 48 hrs = after 48 hours from treatment

* Week 1 = after 7 days from treatment * Week 2 = after 14 days from treatment

Experiment (2):

Table 3.9: Semen Assessment & Evaluation for the male which used in A.I

Characteristic	Result
Semen Volume	2.5 – 3 ml / Ejaculate
Semen Color	Greyish-White
Semen pH	7.27
Motility	45.5 – 70 %
Concentration	205 x 10 ⁶ ml ³

Table 3.10: Follicles size (Diameter cm) in 0 hours in different groups in she - camel

Groups	N	Means \pm SD	P. Value
A.I	7	1.46 \pm 0.43	0.63
N.M	6	1.37 \pm 0.14	

* A.I = Artificial Insemination N.M = Natural Mating

* N = Number of Animals

* Different superscript letters within the same column means significant differences ($P \leq 0.05$)

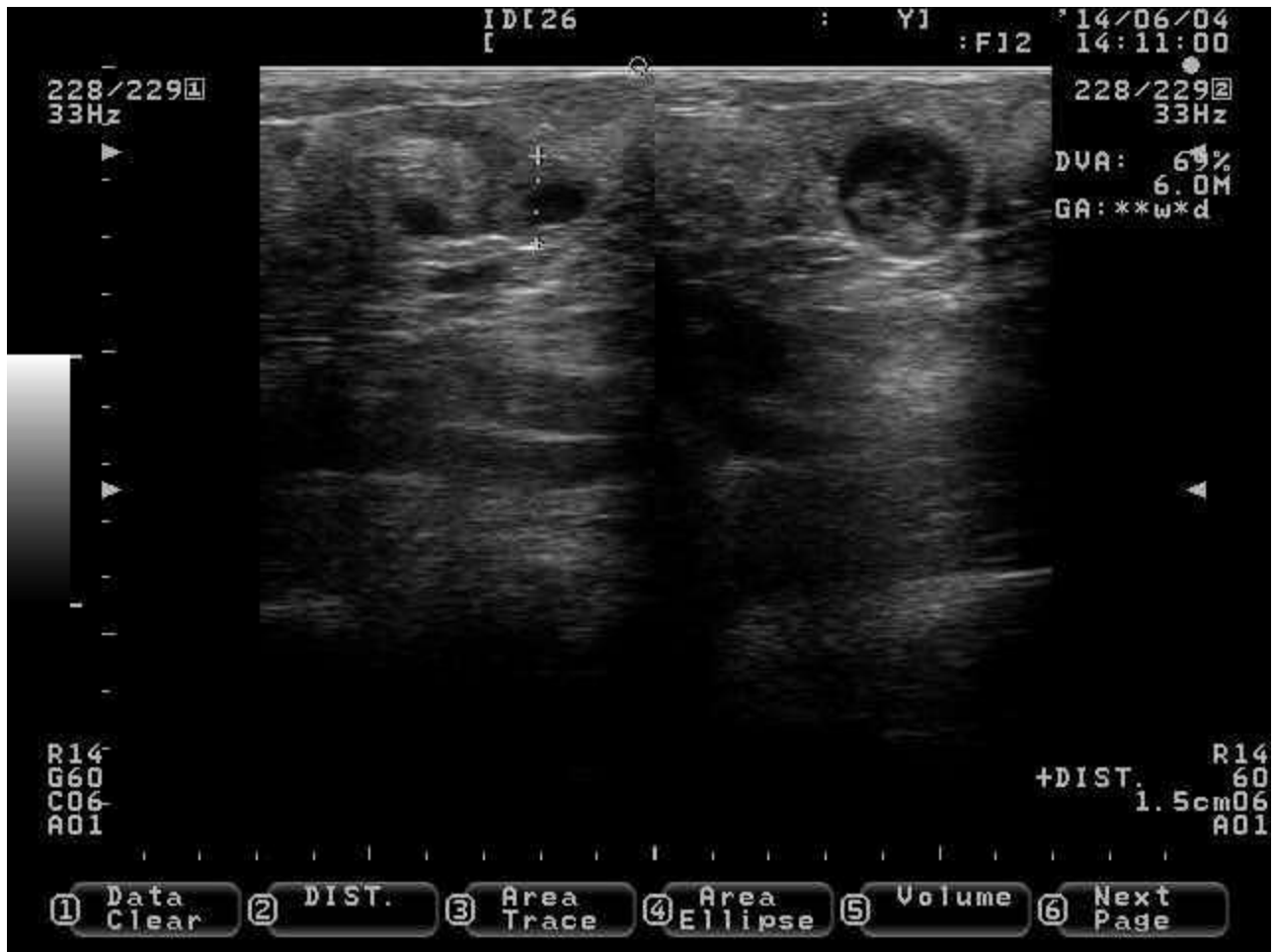


Figure No: 3.7
Measurement of Follicle size (Diameter cm) by Ultrasonography

Table 3.11: Pregnancy occurrence in different groups in She - camels

Groups	Pregnancy (%)	Pearson Chi – Square χ^2	df	P. Value
A.I	1 / 7 (14 %)	1.500	1	0.22
N.M	3 / 6 (50 %)			

* A.I = Artificial Insemination N.M = Natural Mating

The results of this study concerning ovulation induction presented in tables (3.2 to 3.4) are consistent with those of previous study of ovulation induction in dromedary camel done by Skidmore (2011), and supported the hypothesis that camels are induced ovulators and thus normally only ovulate in response to mating. In the absence of mating, ovarian follicles tend to regress after a period of growth and maturity, this was observed by Chen *et al*, (1985); Marie and Anouassi (1986); Cristofori *et al*, (1989); Manjunathaa *et al*, (2012); and Derar *et al*, (2014). While Skidmore *et al*, (1996); Tibary and Anouassi, (1996) and Tibary *et al*, (2007) reported that in the absence of mating or other ovulatory stimuli (i.e. GnRH or hCG treatment) there is a succession of overlapping follicular waves with variable rhythm showing three phases: growth, maturation and regression.

Ovulation can also be reliably induced using hormonal protocols. From the present study findings the ovulation occurred during 24 to 48 hours in response to the hormonal protocols, Gonadotropin Releasing Hormone (GnRH), and Human Chorionic Gonadotropin (hCG). These results showed that the proportion of She camels that ovulated during 24-48 hours were (6 / 7 vs 4 / 6 vs 0 / 6) in the GnRH, hCG and the control groups, respectively. The statistical analysis showed that there was highly significant difference ($P \leq 0.01$) between the GnRH and hCG compared with the control groups, respectively. Similar results were observed by a number of authors Anouassi *et al*, (1992); Sheldrick *et al*, (1992); and Skidmore, (2011). Different results were observed by Vyas (2011), he mentioned that ovulation did not occur up to 96 hours.

The present study also indicated that ovulation may be induced 24–48 hours following treatment with (GnRH), which in conformity with Bono *et al*, (1985); Anouassi *et al*, (1994); and Silva *et al*, (2012), or its analog like Busereline which

reported by Cooper *et al* (1992); McKinnon *et al* (1992); Musa *et al*, (1993) and Skidmore *et al* (1995). But the present study is not come in conformity with Vyas (2011), who reported that ovulation did not occur up to 96 hours after treatment with Buserelin administration.

The present study reported that ovulation can be also induced by an administration of (hCG). Similar results were observed by Anouassi *et al*, (1994). The present results agrees with San Martin, (1961); and Adams *et al*, (2009) who mentioned that injections of (hCG) lead to ovulation 24 hours later in Lama pacos.

The present study indicated that there was no significant difference ($P \leq 0.05$) between the GnRH and hCG in inducing the ovulation 6/7 (85 %) and, 4/6 (66 %) respectively, which agrees with results of Skidmore *et al* (1996). Similar results were observed by Rato *et al* (2006a), they mentioned that the proportion of mice that ovulated was similar among GNRH, hCG groups, suggesting that the hypothalamo – hypophyseal axis was functional and that the ovaries were capable of responding. The results of this study agrees with Ratto *et al* (2006b), they found that no differences were detected among groups (mated, LH, and GnRH) in ovulation rates.

Results of the present study supported the hypothesis that peripheral concentrations of estrogen increased with increasing of the follicle diameter until the follicle reaches 1.0 to 1.9 cm in diameter, which agrees with 1.0 to 1.7 cm in diameter, mentioned by Skidmore (2011). While Anouassi and Tibary (2013) reported that the follicles become responsive when they reach 9 mm which is equal 0.9 cm.

The mean \pm SD of the concentrations of serum Estrogen (pg / ml) presented in this study were increased significantly ($P \leq 0.05$) before the ovulation which was

due to the growing of the follicles, from 74.69 ± 17.83 to 77.37 ± 12.77 , 67.36 ± 20.38 to 72.56 ± 13.32 in the GnRH, hCG groups compared to the control group respectively. Similar results were observed by Skidmore (2011). She mentioned that concentrations of Estrogen increased with the increasing of the diameter of the follicles. Hegazy *et al* (2004), in their results revealed level of Estrogen from 1.24 to 67.23 pg / ml. But the present findings disagree with Ismail *et al*, (2008), they noticed that the Estrogen concentrations were nearly constant throughout the experimental period. Such disagreement might be due to the she camels individual response, during breeding season.

Table No: 3.6 showed that the mean \pm SD of the concentrations of serum Progesterone (ng / dl) after ovulation significantly ($P \leq 0.05$) increased slowly and then raised steadily after the first week of the ovulation, from 1.15 ± 0.21 to 1.64 ± 0.06 , 0.89 ± 0.12 to 4.19 ± 3.15 in the GnRH, hCG groups compared with the control group respectively. This agrees with findings of Skidmore, (2011) who mentioned that progesterone concentrations remain low for the first 3-4 days after ovulation and then rise steadily to a peak of around 2.7 ng / ml on day 8 or 9 before falling sharply again on days 10-11 to reach mean values of 0.5 ng / ml by days 11 or 12, and if the female is not mated then the serum Progesterone levels remain low all the time. The results agree with Ismail *et al* (2008) and Nagy *et al* (2005), they found an increase in the Progesterone concentration after the treatment with GnRH. And also agrees with Anouassi and Tibary (2013), who reported that progesterone levels start to increase 2–3 days after ovulation and reach high levels (>2 ng/ml) by day 5 after ovulation. While Ulloa-Leal *et al* (2014) reported that it will be at day 6 after ovulation. Such results might explain the harmony of the hormonal regulation pattern.

In the present study the increase of mean serum Progesterone concentrations of the experimental camels coincided with the decrease of serum estrogen concentration at the same periods. This may indicate the changing of the follicular structures into luteal structures as a result of GnRH and hCG treatments, this agrees with the findings of Ismail *et al* (2008).

In the present study, Serum FSH concentration (miu / ml) (Table No: 3.7) showed low levels. This because the activity of the pituitary gland was lesser in summer season (Non Breeding season), as reported by Ismail (1987); Akral, and Khanna (1995); and Hegazy *et al* (2004). Those authors reported that the pituitary activity corresponded to the breeding season. The decrease of FSH may be due to the increase of ovarian steroid hormones (Estrogen and Progesterone) as a consequence of the feedback mechanism.

LH serum concentration (miu / ml) in the present study (Table 3.8) show zero level, this is attributed to the nature of LH, which is release as surge just 1 – 2 hours after ovulation. This finding is in agreement with Skidmore (2003).

Results on the natural and artificial breeding of camels are very controversial, depending on the seasonality, and the applied technique. The results of this study which presented in table (3.10) expressed no significant differences in pregnancy occurrence ($P \leq 0.05$) between the artificial insemination (A.I) and the natural mating (N.M) 1 / 7 (14 %), 3 / 6 (50 %). These results although disagree with the findings of Bravo *et al* (1997), but it comes in agreement with the results of many authors Fernandez-Baca *et al* (1970); Xu *et al* (1985); Williamson and Payne (1987), who reported insignificant differences between A.I and N.M in camels. The result of this study could be attributed to the poor quality of semen as expressed in table 3.8. There may be many reasons for the reduced semen volume

obtained in the present study including age of the animals, agro climatic conditions, number of collection per week, the collection procedure, the rutting season, and the average concentration of the spermatozoa per ejaculate (sperm concentration of the semen). The last one is affected by multitude of factors such as individual bulls, frequency of service, intensity of sexual excitement, season, evaluation process and the status of liquefaction of semen before evaluation. From the other side, the report of Novoa (1970) that the fertilization rate in camel was low (37-53 %) as compared with that of other domestic livestock might be a reason for such discrepancy between A.I and N.M. The present results agree with Hegazy *et al* (2004), who reported that, the incidence of inactive ovaries during May, August and October, might be responsible for the failure of conception rate. This may be attributed to the higher temperature associated with adverse nutritional status of the animals during the summer season.

Conclusions

From the present study it could be concluded that:

1. Ovulation can be induced in non-breeding season by using hormonal protocols.
2. To increase the successes rates of ovulation induction the follicle size diameter should be between 1.0 to 1.7 cm.
3. No significant differences between GnRH & hCG in inducing ovulation.
4. The hormonal analysis of Estrogen, progesterone, FSH, and LH in case of follicular ovarian wave indicated a large individual variation.
5. No significant differences between the Artificial insemination and the Natural mating in pregnancy onset during non – breeding season.
6. To the best of our knowledge the available literature indicates the absence of any previous study on Pituitary ovarian axis in female camels, and also no base line data could be traced for FSH and LH levels.

Recommendations

1. No available data could be detected for FSH and LH levels. It was noticed that these hormones could cross-react with those of human being using ELISA technique, however, the results needs further investigation.
2. No significant differences between GnRH & hCG so it is recommend to use the cheapest one.
3. No differences between Artificial insemination and Natural mating in pregnancy occurrence during non – breeding season, so this study recommend more trails and experiments on A.I technique and semen characteristics.

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Plate 1: Determine the Follicle size



Plate 2: Blood collection from the Jugular vein



Plate 3: Serum separation & Preservation



Plate 4: Semen collection



Plate 5: Semen evaluation by naked eye



Plate 6: Semen evaluation (Laboratory)

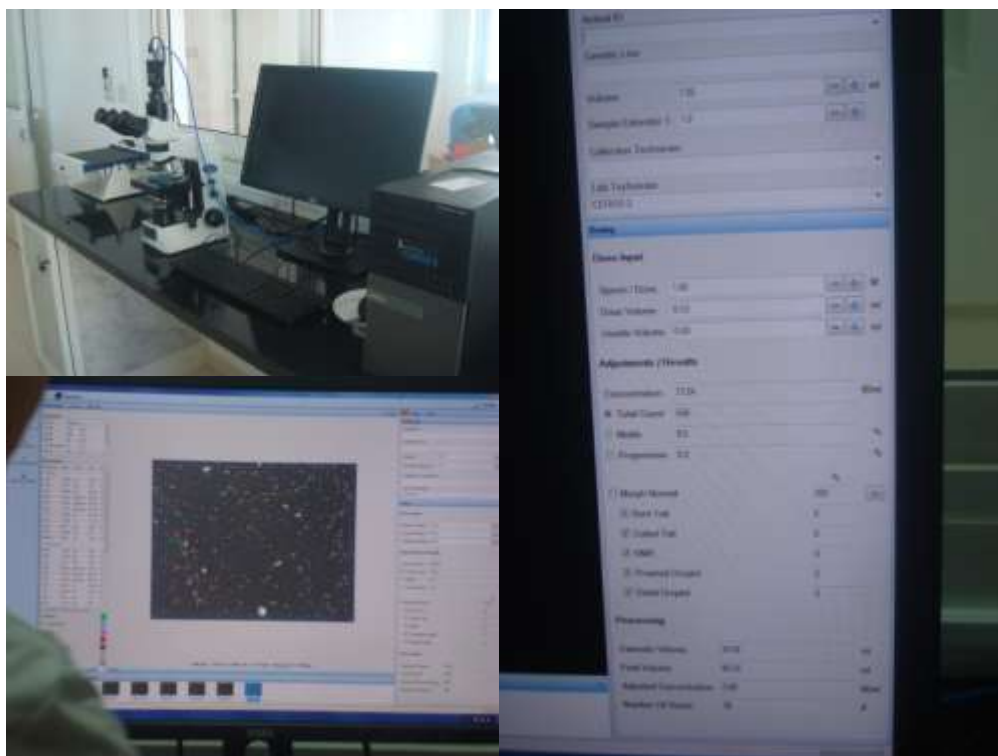


Plate 7: CASA system for semen analysis



Plate 8: Artificial Insemination



Plate 9: Prepare samples & kits for ELISA technique



Plate 10: Some ELISA steps & software