INTRODUCTION

According to the Food and Agriculture Organization (FAO) of the United Nations, there were an estimated 924 million goats in the world (FAO, 2014). The majority (86%) of them found in the low income countries (Davendra and Solaiman, 2010). Goats are bred in a wide range of production systems and have considerable economic importance in many regions (Fatet *et al.*, 2010). They are used for poverty alleviation in rural areas; as they have an excellent reproductive qualities, low maintenance requirements and highly adaptable to bad environmental conditions (Greyling, 2010). Goats are among the most fertile domestic species and are exploited for multiple purposes including meat, milk production and skin for leather (Holtz, 2005; Smith and Sherman, 2009).

Sudan possesses over 100 million head of livestock population, among this population; goats estimated to be about 31 million head (MOAR, 2012). This population figure puts the Sudan as a leading livestock producer in Africa and Arab countries (Elrasheed *et al.*, 2010).

Pregnancy diagnosis in goats is a primary factor for improving the reproductive performance in herds and achieving high economic efficiency (Karadaev, 2015). Reliable technique for early detection of pregnancy aids in culling or rebreeding of barren does, however; inability to detect early pregnancy can result in economic losses due to longer kidding intervals (Ishwar, 1995).

Variable methods have evolved over the years for pregnancy diagnosis. Choice of which method depends on the stage of gestation, availability of equipment, number of animals to be examined, desired accuracy, expenses and need for immediate results (Dawson, 1999; Goel and Agrawal, 1992). Methods such as non return to estrus, abdominal ballottement, hormonal assay, vaginal biopsy, rectal abdominal palpation, x-rays and laparotomy have limited utility or have been abandoned in small ruminants (Karen *et al.,* 2001; Greyling 2010). Non return to estrus may be of physiological and pathological causes; thus it may give false positive results. On the other hand, abdominal ballottement in goats is limited to late gestation (Purohit, 2010). Other methods such as rectal abdominal palpation, vaginal biopsies and laparotomy are invasive techniques; thus limits their uses as a routine method of pregnancy diagnosis.

Hormonal assay (P4) is costive, time consuming, and sampling should be taken at a fixed time after breeding (Ishwar, 1995).

B-mode real-time ultrasound has been used in large scale to monitor reproductive status in small ruminant, it is considered simple, non invasive, non-time consuming and reliable method in detecting pregnancy, estimating litter size and viability, and determining gestational age (Karen *et al.*, 2001; Santiago-Moreno *et al.*, 2005; Azevedo *et al.*, 2007; Godfery *et al.*, 2010). It represents a technological breakthrough that has revolutionized knowledge of reproductive technology (Medan and Abd El-Aty, 2010). It also permits a close relationship between research and practice, as research outcomes can be directly applied to the field (Gonzalez-Bulnes *et al.*, 2010). Ultrasound has become the most efficient diagnostic tool for managing reproduction which considered the backbone in animal production chain (Gonzalez-Bulnes *et al.*, 2010; Greyling, 2010). It is useful in diagnosis of pathological conditions which reduce fertility and reproductive performance in females such as hydrometra, pyometra, ovarian cysts and metritis (Hesselink and Taverne, 1994; Gonzalez-Bulnes *et al.*, 2010).

Pseudopregnancy also called hydrometra is a pathological condition of the uterus characterized by accumulation of aseptic fluid in the presence of a persistent functional corpus luteum (Hesselink, 1993a; Hafez and Hafez, 2000). It has been reported in small ruminants by many researchers worldwide (Tasal *et al.*, 1995; Yotov *et al.*, 2009, Ahmed *et al.*, 2010; Murugavel *et al.*, 2013; Khan *et al.*, 2015). It considered a major cause of infertility in goats (Lopez Junior *et al.*, 2004). The etiology and pathophysiology of the condition has not well understood (Wittek *et al.*, 1997; Purohit and Mehta, 2012). It is always associated with high plasma progesterone levels, anestrous and variable degrees of abdominal distension (Noakes *et al.*, 2009). In temperate areas, the incidence of hydrometra varies between 3.0 and 20.8%, however, reports of this pathological condition from tropics are scarce (Hesselink, 1993a; Lopez Junior *et al.*, 2004). In the Sudan only two case reports have been published regarding hydrometra (Ahmed *et al.*, 2010; Almubarak *et al.*, 2016), however the prevalence and associated risk factors are not reported.

The objectives of the present study were to:

1. Diagnose pseudopregnancy in goats in Khartoum State using real-time ultrasonography.

2. Treat the affected animals and follow the subsequent performance.

3. Estimate the prevalence of pseudopregnancy and identify the associated risk factors in goats in Khartoum State.

CHAPTER ONE

LITERATURE REVIEW

1.1. Goats

Goats are among the first ruminants to be domesticated and archaeological evidence suggests a long association between humans and goats (Gordon, 2004). Domestic goats (*Capra hircus*) are distinct species in the family Bovidae (Hafez and Hafez, 2000). They support a variety of socioeconomic functions throughout the world (Solaiman, 2010) and proved to be useful to man throughout decades due to their multiple productivity, small size, and non-competiveness with him for food (Abdel Aziz, 2010). Goats can thrive in a variety of climates, ranging from extremes of tropical rainforests to dry deserts (Gordon, 2004). Worldwide interest in goats has continued to increase dramatically during the last decade due to their importance in agricultural systems in low-income countries and increased demand for their products in developing countries (Smith and Sherman, 2009).

1.1.1. Goats population in the Sudan

In the Sudan goats estimated to be about 31 million head (MOAR, 2012). Four local breeds are known in the Sudan; Nubian, Desert, Nilotic and Tagger; in addition to their mixed crosses with exotic breeds (Ibrahim, 2000).

Foreign breeds have been imported into the country for genetic improvement of the local goats and in consequence improve the nutritional status of poor families, increase their incomes and increase milk supply (Ishag *et al.*, 2012). These include; Toggenburg, Anglo-Nubian, Saanen (temperate breeds) and Damascus (Middle East). Saanen proved to be an adequate breed and crosses with selected indigenous Nubian goats produced milk yield as high as 4.5 kg/day (HCENR, 2014).

1.1.2. Reproductive characteristics of goats

The most important factor determining lifetime production performance in goats is puberty. In females, puberty is defined as the stage when the doe starts to exhibits regular cyclic activity which relates to body weight and age of the doe at first standing estrus (Greyling, 2010). In the tropics, goats reach sexual maturity at 4-6 months (Tsegye, 2009), however, breeding in does should be delayed until the animal has attained 60-70% of its mature body weight (Greyling, 2010). In temperate zone, goats are seasonally polyestrous, however in the tropics indigenous goats tend to breed throughout the year (Matthews, 2009). The length of estrus cycle is 20-21 days with duration of estrus phase ranging between 24-48 hours and varies with age, breed, presence of the male and season. The gestation length is about 150 days; but variation occurs between breeds and individuals (Hafez and Hafez, 2000).

1.1.3. Reproductive efficiency

Capacity to reproduce is one of the most important production traits for goats. High rates of conception, kidding, and weaning are all important (Greyling, 2010). Hafez and Hafez, (2000) stated that reproductive efficiency depends on fertility rate (proportion of does mated that conceive), fecundity (kidding rate), and prolificacy (relative number of live offspring produced in a given interval). Goats are not usually managed as individuals; therefore total herd reproductive performance is important (Greyling, 2010). Following successful parturition, rebreeding should be done between 45 and 90 days post kidding to obtain three to four kids in two years. However, subfertility and/or infertility decline the desired performance (Jannat, 2014).

1.1.4. Infertility in the female goat (doe)

Infertility is defined as inability to produce viable young within specific time characteristics for each species (Hafez and Hafez, 2000). Under normal circumferences tropical goats should give birth at least three times in two years, therefore; kidding interval should not exceed 8 months (Tsegye, 2009). Goat breeds in temperate regions tend to breed only seasonally with the breeding season being from July/August to November/December in Northern hemisphere (Solaiman, 2010). Fertility is reduced at the beginning as well as the end of the breeding season, during hot weather, in young and old females, in under nourished or overly fat females, in forages with high estrogen contents, stress and diseases (Hafez and Hafez, 2000). Gonzales-Bulnes *et al.*, (2010) reported that failure of the animal to conceive has many causes either at the ovarian level (follicular and luteal cysts) or at the uterine level (hydrometra, metritis, endometritis and pyometra).

1.1.4.1. Pseudopregnancy

Pseudopregnancy or Hydrometra is an anoestrus condition in which fluid accumulates inside the uterus with persistence of corpus luteum (CL) and absence of foetus and/or placentomes (Pieteres and Taverne, 1986; Hesselink and Taverne, 1994; Hafez and Hafez, 2000). It is considered as a very important pathological condition because it represents one of the main causes of temporary infertility in dairy goats (Souza *et al.*, 2013) and it is the most common uterine pathology affecting fertility at uterine level (Kornalijnslijper *et al.*, 1997a; Gonzalez-Bulnes *et al.*, 2010). The definite etiology is still unknown (Hesselink and Elving, 1996; Purohit and Mehta, 2012). It is always associated with high plasma progesterone level secreted by a persistent functional CL, cessation of cyclical activity and variable degree of abdominal distension (Noakes *et al.*, 2009). The condition is incidentally found during routine pregnancy diagnosis of mated animals and in unmated non-cycling does both outside and inside the breeding season (Taverne *et al.*, 1995; Wittek *et al.*, 1997). The role of prolactin in the development of hydrometra in goats has been investigated and disturbances in either luteotrophic or luteolytic mechanism during the ovarian cycle can play a crucial role in the etiology of hydrometra (Taverne *et al.*, 1995). On the contrary, Kornalijnslijper *et al.* (1997b) reported that an increased plasma prolactin level is not related to the pseudopregnancy.

In temperate areas, the incidence of hydrometra in goats varies between 3.0 and 20.8% (Hesselink, 1993a). Some authors claimed that the incidence of hydrometra increases with high milk production, old dams and sometimes may be accompanied by fetal death (Hesselink, 1993a; Wittek *et al.*, 1998; Moraes *et al.*, 2007). Hesselink and Elving (1996) reported that incidence of hydrometra in goats was 10.4% during four oestrus seasons in the Netherlands. Their study suggested a genetic influence in the occurrence of hydrometra. Lopez Junior *et al.*, (2004) reported prevalence of 30.4% in Saanen goats raised in Brazil. They reported that pseudopregnant animal will remain unproductive without treatment or spontaneous voiding. In the tropic areas reports of hydrometra are scarce (Lopez Junior *et al.*, 2004).

Diagnosis of pseudopregnancy can easily be done by ultrasonography (Hesselink 1993a; Taverne *et al.*, 1995). The sonographic diagnosis is based upon recognition of fluid in the uterus in the absence of fetus and placentomes (Hesselink and

Taverne, 1994). The image corresponds with anechogenic areas which may traversed by hyperechogenic lines representing the thinly stretched uterine walls. Sometimes hyperechogenic dots originating from desquamated endometrial cells may be observed (Gonzalez-Bulnes *et al.*, 2010).

The term "Cloudburst" is used to describe the voiding of large volumes of fluid from the uterus as pseudopregnancy is terminated (Noakes *et al.*, 2009). Administration of prostaglandin analogues or oxytocin successfully leads to luteolysis and the cloudburst (Pieteres and Taverne, 1986; Moraes *et al.*, 2007, Purohit and Mehta, 2012). Both natural (5 - 10 mg) and synthetic (125 to 250 µg) $PGF_{2\alpha}$ hormones cause regression of the corpus luteum and emptying of the uterus (Smith and Sherman, 2009). The breeding performance was significantly decreased and it can be improved by second injection of $PGF_{2\alpha}$ given 12 days after cloudburst (Hesselink, 1993b; Wittek, *et al.*, 1997).

1.1.4.2. Metritis and endometritis

Metritis is the inflammation of all layers of the uterus characterized by suppurative exudates, hemorrhage and necrosis (Chauhan, 2010). It usually follows an abnormal parturition especially where there has been severe dystocia and affected animals show local and general symptoms such as toxemia, septicemia, anorexia and vaginal discharge (Noakes *et al.*, 2009). Sonographically; metritis is characterized by a little anechoic uterine content and an increase in the thickness of the uterine wall (Gonzalez-Bulnes *et al.*, 2010).

Endometritis, which implies inflammation of the endometrium, results from pathogens that contaminate the uterus at insemination or as a consequence of parturition or postpartum. It has a profound effect upon the fertility of the animal and characterized by presence of white or whitish-yellow mucopurulent vaginal discharge (Noakes *et al.*, 2009). Fluid accumulations which are detectable inside the lumen of a non-pregnant uterus can be indicative for chronic endometritis. The amount of the secretion can vary considerably. In many cases, a fluid filled lumen can only be detected in short segments of the uterus. In severe endometritis cases, both uterine horns can be distended to several centimeters along their entire length. The fluid caused by inflammation contains floccular echoes and the endometrium become hyperechoic (Kahn, 2004).

1.1.4.3. Pyometra

Pyometra is an acute or chronic suppurative inflammation of the uterus resulting in accumulation of pus (Chauhan, 2010). Pyometra may occur as a sequel to endometritis or metritis (Vegad and Katiyar, 1998). The incidence of pyometra in goats is low and if present; the doe will be febrile and anorectic. There may be a vaginal discharge with a foul odor. The previous history usually includes dystocia, fetal maceration and retained placenta (Rischen and Riese, 1982). Sonographically, pyometra characterized by a distended uterus which is filled with pus. Within the secretions of pyometra there are usually intensive reflections. The echogenicity of the fluid depends on the relative amount of cellular material in the secretions. A snow-storm-like image is typical (Kahn, 2004).

1.1.4.4. Fetal death

Fetal viability can be assessed during ultrasound scanning. The presence of fetal movement or heartbeat indicates a live foetus. The fetal heartbeat can be detected on day 35 by transabdominal and earlier by transrectal ultrasound (Matthews, 2009). Ultrasound scanning of a dead embryo or fetus revealed absence of embryonic or fetal movement, cessation of heart beats, decrease in fetal size, and an apparent decrease in the amount or complete absence of fetal fluid, followed by close attachment between the uterine wall and fetal parts. Moreover, ill defined shape and disorganization of placentomes observed during scanning indicate fetal death (Samir *et al.*, 2016).

1.1.4.5. Ovarian cysts

Cysts arise as a result of anovulation of a Graafian follicle. Under normal circumferences, anovulation is followed by either atresia or luteinization, after which the follicle undergoes regression. In cystic ovarian disease, the follicle increases in size and persists, for at least 10 days (Noakes *et al.*, 2009). Evidence and description of ovarian cysts in small ruminants, in contrast to the cow, are scarce. Gonzalez-Bulnes *et al.*, (2010) reported that the incidence in small ruminants varies between 5-8%. Traditionally, ovarian cysts have been classified as either follicular or luteal cysts. Follicular cysts are thin-walled and have little or no luteal tissue in the cyst wall. It is common to find multiple follicular cysts. Luteal cysts are thick-walled and usually single and have a large quantity of luteal tissue in the cyst wall (Noakes *et al.*, 2009). A clinical history of nymphomania and irregular estrous cycles may indicate the presence of cystic follicles in goats (Rischen and Riese, 1982). However, the luteal cyst usually results in cessation of cyclical activity as the structure functions as a persistent corpus luteum (Noakes *et al.*, 2009). Follicles are sonolucent fluid-filled structures that appear as black circular sacs during scanning (Matthews, 2009). Follicular cysts appear the same as follicles but with a diameter equal or more than 10 mm and with little or no luteinization. On the other hand, luteal cysts are fluid-filled ovarian structures with a diameter of 10 mm or greater and with a thick luteal wall up to 3 mm wide, indicating luteinization (Gonzalez-Bulnes *et al.*, 2010).

1.2. Ultrasonography

1.2.1. Sound wave definition

Sound is a mechanical energy transmitted by pressure waves in a material medium; it cannot propagate through a vacuum (Zagzebski, 1996).

1.2.2. Spectrum of sound waves

The acoustic waves are classified according to their frequency. 20 Hz - 20 KHz is an audible sound in which human beings can hear within this range. A sound wave whose frequency is greater than 20 KHz is termed ultrasound. Diagnostic ultrasound utilizes 1-20 MHz frequency (Zagzebski, 1996).

1.2.3. Production and detection of sound waves

The source of sound in medical ultrasound is the piezoelectric transducer (probe) which considered the most important part of the machine (Zagzebski, 1996). Ultrasound transducer uses piezoelectricity; a principle discovered by Pierre and Jacquas Curie in 1880s. Piezoelectric materials have the unique ability (dual action) to respond to the action of an electric field by changing shape and generating electric potential when compressed (Zagzebski, 1996).

1.2.4. Transducer arrays

Diagnostic ultrasound in veterinary medicine employs different types of transducers (Kahn, 2004). Linear probe generates a rectangular imaged-shape. It is preferred for transrectal ultrasound examinations because their shape permits safer manipulation in the rectum (Gayrard *et al.*, 2010). Convex (curvilinear) probe generates a trapezoid-imaged shape field (Zagzebski, 1996). Mechanical sector probes produce a fan shaped image that is very narrow at the surface and expands with depth; thus allows viewing of large structures located deep in the body and producing images through a narrow acoustic window (Gayrard *et al.*, 2010).

1.2.5. Principal echo display modes

There are three echo display modes, A-mode, M-mode and B-mode (Merritt, 2011).

1.2.5.1. Amplitude modulation (A-mode)

A-mode (A-scan, amplitude modulation) is a one-dimensional examination technique in which a transducer with a single crystal is used. The echoes are displayed on the screen along a time (distance) axis as peaks proportional to the intensity (amplitude) of each signal. The method is rarely used today, as it conveys limited information, e.g. measurement of distances (WHO, 2011). This mode has special use for ophthalmic examinations and other applications requiring precise length or depth measurements (Nyland *et al.*, 2002).

1.2.5.2. Brightness modulation (B-mode)

In brightness mode, display echo signals are electronically converted to intensity-modulated dots on the screen. The brightness of the dot is proportional to the echo signal amplitude. The B-mode display is used both in generating M-mode traces and in gray scale two dimensional imaging (Zagzebski, 1996). To generate a two-dimensional (2-D) image, multiple ultrasound pulses are sent down a series of successive scan lines, building a 2-D representation of echoes arising from the object being scanned. When an ultrasound image is displayed on a black background, signals of greatest intensity appear as white; absence of signal is shown as black; and signals of intermediate intensity appear as shades of gray (Merritt, 2011).

Real-time B- mode ultrasound produces the impression of motion by generating a series of individual 2-D images at rates of 15 to 60 frames per second. Now it is the major method for ultrasound imaging throughout the body. Real-time ultrasound permits assessment of both anatomy and motion. When images are acquired and displayed at rates of several times per second, the effect is dynamic. Because the image reflects the state and motion of the organ at the time it is examined, the information is regarded as being shown in real time (Merritt, 2011).

1.2.5.3. Motion modulation (M-mode)

M-mode displays echo amplitude and shows the position of moving reflectors. M-mode imaging uses the brightness of the display to indicate the intensity of the reflected signal (Merritt, 2011). M-mode tracings usually record depth on the vertical axis and time on the horizontal axis (Nyland *et al.*, 2002). Currently, the major application of M-mode display is evaluation of the rapid motion of cardiac valves, cardiac chamber and vessel walls (Merritt, 2011).

1.2.6. Sonographic echo texture (echo pattern, echogenicity)

Echo is the usual term for the reflected or back-scattered parts of the emitted ultrasound pulses that reach the transducer. For each echo, the intensity and time delay are measured at the transducer and electronically processed to allow calculation of the distance travelled. The displayed results form the basis of diagnostic ultrasound images (WHO, 2011). Changes in scattering amplitude from one region to another result in brightness changes on an ultrasound image and therefore are useful in delineating abnormal structures (Zagzebski, 1996).

The terms hyperechoic and hypoechoic are used in clinical imaging to describe structure on B-mode. Hyperechoic regions result from increases in the ultrasound scattering level compared to the surrounding tissue. Hypoechoic refers to the opposite condition, where the scattering level is lower than in the surrounding tissue (Zagzebski, 1996). Anechoic structures such as follicles, amniotic and allantoic fluid and urine appear black and highly echogenic structures such as bone appear white. The structures with intermediate echogenicity are represented in shades of gray (DesCôteaux *et al.*, 2010).

1.2.7. Ultrasound safety and bioeffects

Ultrasound has provided a wealth of knowledge in diagnostic medicine and has greatly impacted medical practice, particularly obstetrics. It remains one of the fastest-growing imaging modalities because of its low cost, real-time interactions, portability and apparent lack of biologic effects (bioeffects). No casual relationship has been established between clinical applications of diagnostic ultrasound and bioeffects on the patient or operator (Fowlkes and Holland, 2011). However, all diagnostic methods based on interactions of physical energy with biological tissues are associated with potential risks for patients (Hlinomazova and Hrazdira, 2005). Although there have been no proven adverse effects associated with obstetrical ultrasound, ultrasound exposure should be as low as reasonable achievable (ALARA). Increasing the amplitude of the sound waves rapidly increases the energy delivered to the patient and, theoretically, increase adverse bioeffects (Lieu, 2010). Ultrasonography is highly operator dependent, requiring skills and knowledge (RCR, 2014). The sonographer's responsibilities include maximum benefit of the diagnostic capability of ultrasonography, the knowledge of what to look for, and the competence to interpret the ultrasonographic findings based on the understanding of the physiology and pathological changes of the examined organs (Pinto *et al.*, 2013). Thus, non biological risks based on incorrect interpretation of ultrasound images have also been considered (Hlinomazova and Hrazdira, 2005).

1.2.7.1. Thermal effects

With diagnostic ultrasound, some of the acoustic energy transmitted into the tissue is scattered back in the direction of the transducer, termed backscatter, which allows a signal to be detected and images made. Energy also is lost along the propagation path of the ultrasound by absorption. Absorption loss occurs substantially through the conversion of the ultrasound energy into heat. This heating provides a mechanism for ultrasound-induced bioeffects. The rate of temperature increase in tissues exposed to ultrasound depends on a several factors, including spatial focusing, ultrasound frequency, exposure duration, and tissue type (Fowlkes and Holland 2011). The energy absorbed is therefore higher with stationary ultrasound emitters (transducer fixed, e.g. Doppler, TM-mode) than with scanning methods (transducer moved during examination, e.g. B-scan). Furthermore, the thermal effect is reduced by convection, especially in the bloodstream (WHO, 2011). The potential of causing adverse biologic effects due to tissue heating is of particular concern with longer pulse durations and higher pulse repetition frequency "PRF" used with Doppler ultrasonography than with other ultrasound operating modes (Nyland *et al.*, 2002). It is especially important to limit exposure with pulsed or continuous wave Doppler instruments, in which the beam is "on" a higher percentage of the time, sometimes at greater intensity levels, than with conventional ultrasound imaging because of the potential for tissue heating when the thermal index exceeds 1 (Nyland *et al.*, 2002; Bly *et al.*, 2005).

1.2.7.2. Non thermal effects

Non thermal effects have also been described, but there is disagreement about the relevance with current diagnostic practices. Acoustic cavitation which defined as the formation of bubbles in an ultrasound field is the non thermal phenomenon of most concern (Nyland *et al.*, 2002). However, mechanical effects from ultrasound are less important in the absence of gas bodies as in the situation with obstetric ultrasound (Bly *et al.*, 2005).

1.2.8. Methods of reproductive ultrasonography in goats

Ultrasonography in goats can be applied via the rectum (transrectal) or transabdominal through the skin (Kahn, 2004; Karen *et al.*, 2014; Raja-Khalif *et al.*, 2014*).* Recently transvaginal approach was also introduced (DesCôteaux *et al.*, 2010; Koker *et al.*, 2012; Enginler and Gurbulak, 2014). Linear, sector and convex probes can be used with the frequency of 3.5, 5 and 7.5 MHz as probe options in small ruminants (Enginler and Gurbulak, 2014). The decision of which method to be used depends on: the diagnosis to be made, type of the available probe and working conditions during the examination (Kahn, 2004). All methods have been used in monitoring the reproductive status in goats for different physiological and pathological conditions (Hesselink and Taverne, 1994; Matthews, 2009).

1.3. Prostaglandins

Prostaglandins are tissue hormones known as autocoids. They are classified according to their structure and function into four groups (A, B, E and F). Out of these, $PGF_{2\alpha}$ and prostaglandin E are the most important ones that have direct effect on reproductive organs and fertility (Dudhatra *et al.*, 2012). They are normally produced by the enodmetrium (Vegad and Katiyar, 1998).

Reproduction in small ruminants can be controlled by several methods developed in the last decades. Some of these involve the administration of exogenous hormones such as progesterone, prostaglandins or their analogues which modify the luteal phase of the cycle (Abecia *et al.*, 2012). Prostaglandin $F_{2\alpha}$ is the most potent luteolytic agent for small ruminants and it can be used during the breeding season for oestrus synchronization (Amiridis and Cseh, 2012). In addition, it has been used widely for induction of parturition and treatment of pathological conditions such as retained placenta, hydrometra and luteinized ovarian cysts (Moraes *et al.*, 2007; Matthews, 2009; Dudhatra *et al.*, 2012). Unlike sheep where placenta produces four times progesterone (P4) than CL, the goat depends on corpus-luteum-derived progesterone throughout pregnancy and thus susceptible to prostaglandins throughout the pregnancy (Matthews, 2009).

A single dose of $PGF_{2\alpha}$ is efficient in termination of abnormal pregnancies such as mummified fetus, hydroallantois and pregnancy toxemia (Purohit *et al.*, 2012; Chauhan *et al.*, 2014). Both natural and synthetic $PGF_{2\alpha}$ (Cloprostenol) cause regression of the corpus luteum and emptying of the uterus in case of hydrometra (Smith and Sherman, 2009). Injection of 250 µg of Cloprostenol given at 12 days apart has also proved efficient (Reddy *et al.*, 2014). For heat induction, the application of Cloprostenol in two doses of 125 µg given 13 days apart was easy and has the merit of being applied to a large flock with minimal labor and time in comparison to other heat induction protocols (Jubara, 1996).

CHAPTER TWO

MATERIALS AND METHODS

2.1. Study design, duration and location

A cross sectional study was performed in the period from March 2015 to February 2016. The present study was carried out at the Veterinary Teaching Hospital, College of Veterinary Medicine, Sudan University of Science and Technology (SUST), Hilat Kuku, Khartoum North. The Veterinary Teaching Hospital received goats from various areas of Khartoum State for routine pregnancy diagnosis; therefore it has been selected for the present study.

Khartoum State lies in central Sudan in the semi-arid zone between latitude 15° 32.799′N and longitude 32° 32.0166′ E in an area about 28.165 square kilometers. The meteorological data during study period was obtained from meteorological authority (Table 2.1).

2.2. Sample size calculation

378 female goats were used in the present study. The sample size was calculated using the formula: $n = (1.96)^2 P_{exp} (1 - P_{exp}) / d^2$ given by (Thrusfield 2007) where:

n = required sample size

P_{exp} = expected prevalence

d = desired absolute precision

Based on a previous prevalence (30.4%) reported by Lopez Junior *et al.* (2004), with (95%) level of confidence and (5%) desired absolute precision.

2.3. Determination of age and body condition

Age was determined according to the date of birth (if known), otherwise by using dentition formula reported by Ebert and Solaiman (2010). All goats included in this study were put into general health status categories as: poor, moderate and good according to Smith and Sherman (2009).

2.4. Ultrasound scanning

2.4.1. Animal preparation and positioning

Area of scanning (which extends from one side of the udder to the other side and 15 cm cranial to the udder (Goddard, 1996) was clipped and shaved carefully using manual clippers. Animals were turned on their backs (dorsal docubitus) on especially designed table.

2.4.2. Scanning technique

Transabdominal ultrasonography (Figure 2.1) was used in the present study. Sagittal, parasagittal, cross and cross oblique sections were taken to ascertain accurate diagnosis.

2.4.3. Machine and image recording

A real-time scanner (Pie Medical, Easote, The Netherlands) equipped with dual frequency (3.5-5) MHz transabdominal curvilinear transducer was used for the study. Images were stored in a memory card attached to the scanner and later were printed in thermal papers (Sony corporation, type 1, Normal, UPP-110S, 1-7-1, Konan, Minato-KU, Tokyo, Japan) using video graphic printer UP-895EC (Sony- Japan).

2.4.4. Ultrasound fine-needle guided aspirates

Uterine fluid was taken under sterile condition with the guidance of ultrasound probe so as to record the physical properties of the fluid.

2.5. Questionnaire

A well-designed questionnaire was prepared to gain knowledge in the occurrence of 12 hypothesized risk factors such as age of the dam, breed, general body condition, parity number, milk yield, previous reproductive performance, type of oestrus, type of insemination, rearing system, type of feeding, locality and season. Detailed information about the owners of the goats such as name, address and telephone number were also recorded (Appendix).

2.6. Treatment

Goats which diagnosed as having pseudopregnancy received 125 µg (0.5 ml) i/m injection of Cloprostenol (Estrumate®, Schering-Plough, Germany). The goats were reassessed by ultrasound and/or follow-up with animal's owners for the response to treatment and subsequent fertility.

2.7. Ethical approval

The study was approved by the research committee of the College of Veterinary Medicine, Sudan University of Science and Technology.

2.8. Statistical Analysis

The collected data were entered into an Excel spreadsheet (Microsoft Office Excel, 2007). Statistical analysis was performed using the Statistical Packages for Social Sciences (SPSS) version 22.0 (SPSS Inc, Chicago, USA). Descriptive statistics of the examined variables were obtained. Univariate analysis using chi square test (χ^2) was done to determine whether pseudopregnancy prevalence differed significantly between the levels of selected risk factors. A p-value ≤ 0.25 was considered significant.

Multivariate analysis using the logistic regression model was done to assess the strength of association between the potential risk factors and the occurrence of pseudopregnancy. A p-value ≤ 0.05 was considered significant.

Table 2.1: Climatic conditions during study period March 2015-February 2016*

Month	Temperature °C		Total rainfall (mm)	Relative humidity%
	Max.	Min.		
March	38.7	24.1	0.0	14
April	38.2	23.8	0.0	9
May	42.0	28.9	0.0	15
June	41.8	29.2	TR	20
July	40.9	28.7	0.4	27
August	38.3	26.4	28.9	41
September	39.6	27.0	13.9	37
October	38.4	31.1	29.3	27
November	36.0	21.6	0.0	21
December	29.5	15.6	0.0	23
January	28.5	14.9	0.0	22
February	33.0	17.5	0.0	20

Note: Max = Maximum Min = Minimum mm = millimeters TR = Trace

** Obtained from Meteorological Authority- Khartoum Station.*

Figure 2.1. Transabdominal ultrasound scanning

CHAPTER THREE

RESULTS

3.1. Sonographic pregnancy diagnosis

In the present study, out of 378 goats examined, 158 (41.8%) were diagnosed as pregnant based on the demonstration of fluid-filled gestational sac with fetal parts and/or placentomes (Figure 3.1). Out of the 158 pregnant goats, 152 (96.2%) were diagnosed as having viable conceptus. Fetal viability was indicated by the presence of fetal heart beats and/or movements (Figure 3.2). Six (3.80%) goats were diagnosed as having dead fetuses due to absence of fetal heart beats and movements (Figure 3.3 a, b)

178 (47.08%) goats were non-pregnant. Forty (10.6%) goats were diagnosed as pseudopregnant. Pseudopregnant goats showed compartmentalized fluid-filled uterus without fetal parts and placentomes. 40% of goats which diagnosed as having pseudopregnancy showed bilateral abdominal distention (Figure 3.4 a, b). Few of them showed development of the udder (Figure 3.5). Absence of oestrus cycle in the pseudopregnant goats varied between 2-5 months.

Out of the 40 pseudopregnant goats, 33 (82.5%) were diagnosed as having hydrometra; showing anechoic compartmentalized fluid-filled uterus (Figure 3.6 A). seven (17.5%) goats was diagnosed as having mucometra with hypoechoic compartmentalized fluid-filled uterus (Figure 3.7 A). Physical examination of the uterine fluids was documented. A colorless, water-like fluid was seen in case of hydrometra (Figure 3.6 B) and a mucoid slightly thick creamy fluid was seen in case of mucometra (Figure 3.7 B).

Two goats out of 378 (0.52%) were diagnosed as having pyometra; the uterus was distended with hypoechoic contents with highly echogenic debris and sediments which may alter the general health of the goat (Figure 3.8).

3.2. Prevalence and associated risk factors of pseudopregnancy

The prevalence of pseudopregnancy in Khartoum State was found to be 10.6 %. Forty (10.6%) out of 378 does were pseudopregnant (Table 3.1). 12 hypothesized risk factors were studied to assess their association with the occurrence of pseudopregnancy in goats. Seven (7) potential risk factors were documented, which include: breed, general body condition, age of the dam, type of estrus, milk yield, previous reproductive performance and type of feeding. Four factors include: season, locality, parity number and rearing system were considered of no risk. One risk factor (type of insemination) was not subjected to statistical analysis because all animals in the present study were naturally mated.

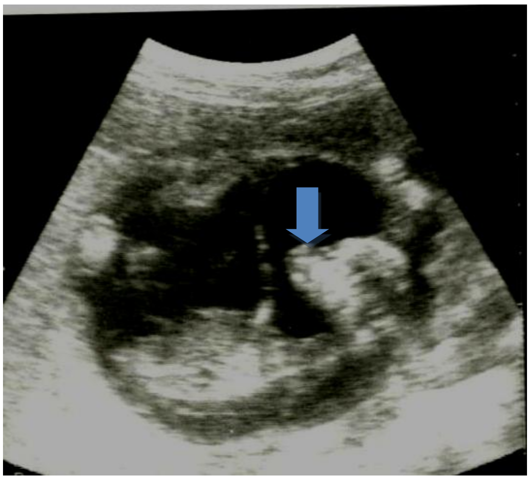

Figure 3.1. Fetus (arrow)

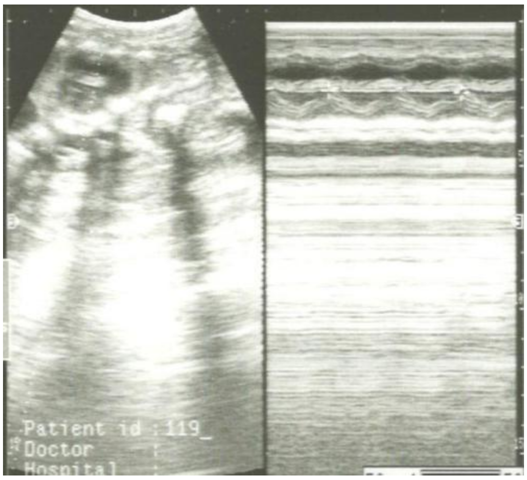

Figure 3.2. Viable fetus (heart beats shown in M-mode)

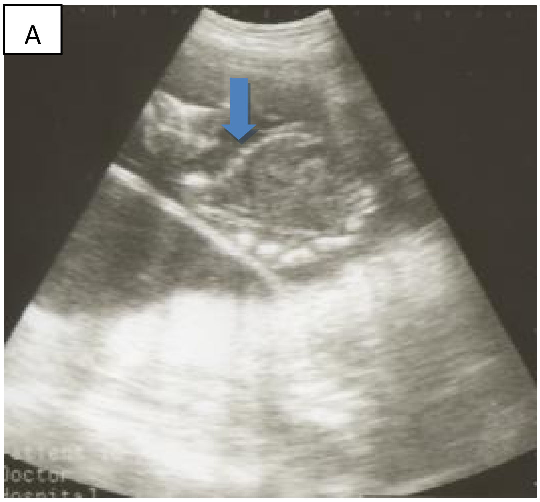

Figure 3.3. Dead fetus (arrow)

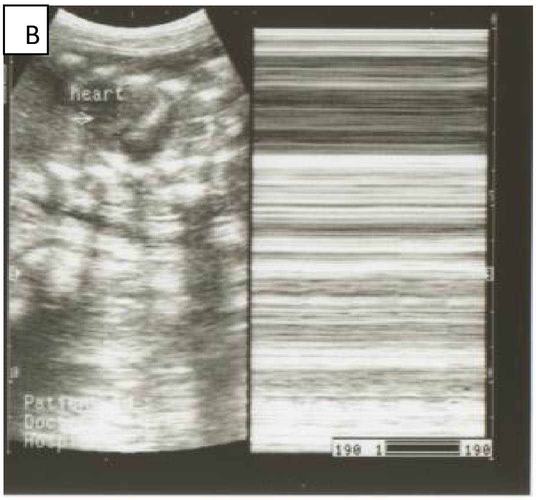

Figure 3.3. Dead fetus (M-Mode)

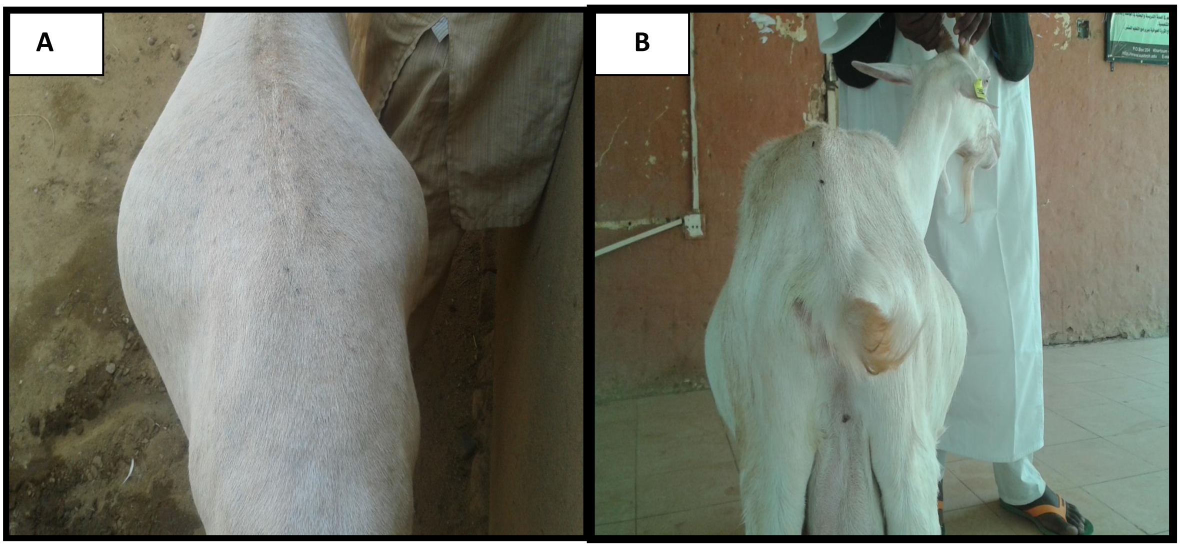

Figure 3.4. Bilateral abdominal distention

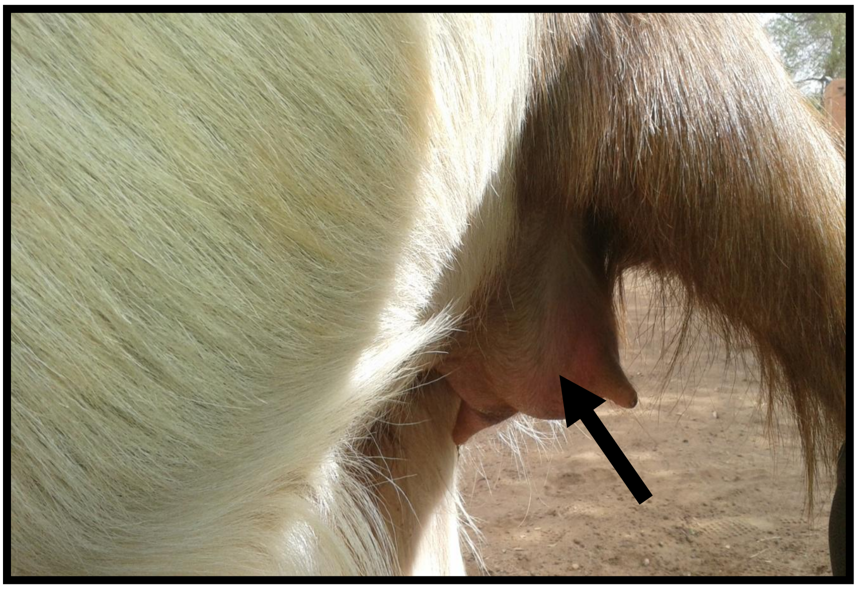

Figure 3.5. Udder development in pseudopregnant nulliparous goat

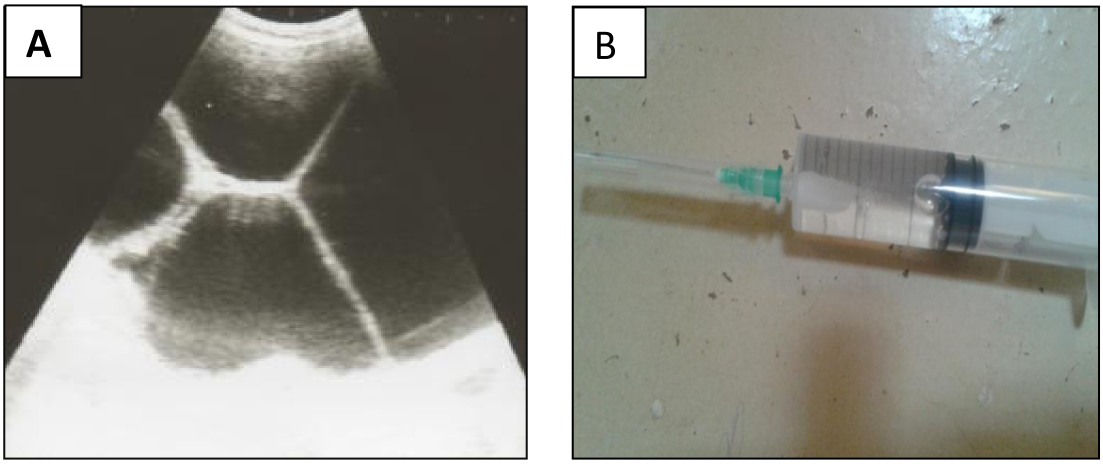

Figure 3.6. Hydrometra: Sonographic image (A) and physical appearance (B)

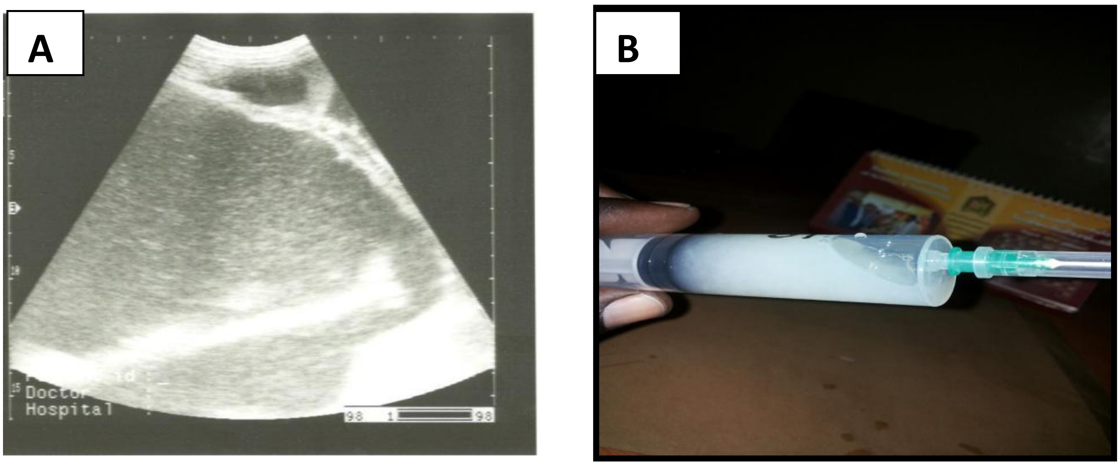

Figure 3.7. Mucometra: Sonographic image (A) and physical appearance (B)

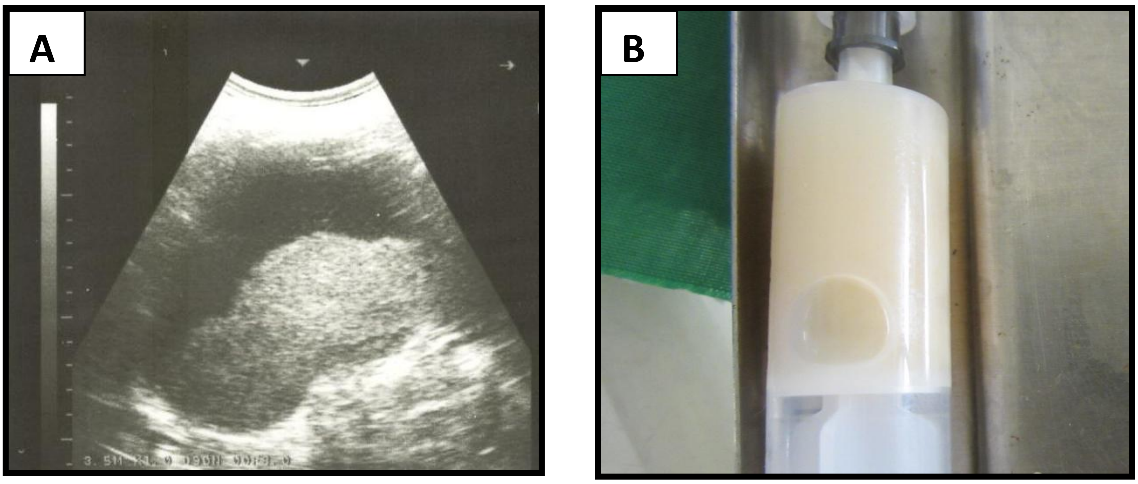

Figure 3.8. Pyometra: Sonographic image (A) and physical appearance (B)

3.2.1. Breed

Results of the present study showed that there was a significant association between pseudopregnancy and breed of the goat (χ^2 = 12.627; P-value = 0.082) (Table 3.2). The higher frequency of pseudopregnancy was found in Saanen breed. Fifteen percent (34/226) of the examined Saanen goats were found positive. While 2 (3.1%) of the Damascus goats and 2 (7.1%) of Damascus × Saanen were positive. Out of 33 Nubian and 13 Desert goats examined, only one positive case was diagnosed for each breed.

3.2.2. General body condition

Significant association (χ^2 = 5.974; P-value = 0.05) was found between the prevalence of pseudopregnancy and the general condition of the dam. Out of 292 goats with good body condition, 37 (12.7%) were diagnosed as pseudopregnant. 3 (3.7%) out of 82 goats of moderately body condition were diagnosed as pseudopregnant. Pseudopregnancy was not diagnosed among goats with poor general condition (Table 3.2).

3.2.3. Age of the dam

Significant association (χ^2 = 11.760; P-value = 0.0129) was observed between age of the dam and pseudopregnancy. The prevalence of pseudopregnancy was increased with age. Higher percentage 30.8% (4/13) and 33.3% (3/9) were recorded in goats above 6-8 years and > 8 years respectively (Table 3.2).

3.2.4. Type of estrus

Highly significant association (χ^2 = 12.794; P-value = 0.000) was reported between the type of estrus and pseudopregnancy. Six (37.5%) out of 16 animals with induced estrus were found to have pseudopregnancy (Table 3.2).

3.2.5. Milk yield

Results of the present study showed significant association between pseudopregnancy and milk yield (χ^2 = 5.951; P-value = 0.114). Out of 111 goats with 0 milk yield, 11 (9.9%) were found positive. Among 74 goats with daily milk yield 0.5-3 pounds, 4 (5.4%) positive cases were diagnosed. Out of 104 goats yielding >3-6 pounds/day, 10 (9.6%) positive cases were observed. Total number of goats yielding above 6 pounds/day was 89. Among those, 15 positive cases were found (16.9%). (Table 3. 2)

3.2.6. Previous history

Significant association (χ^2 = 13.397; P-value = 0.020) was found between the previous history of the dam and pseudopregnancy. 21/221 (9.5%) were diagnosed among goats with no previous reproductive problems. While 8/26 (30.8%) had a previous history of pseudopregnancy. Considering history of previous abortion only one goat was diagnosed as having pseudopregnancy (3.3%) (Table 3. 2).

3.2.7. Type of feeding

Type of feeding was found mixed for 364 goats. Out of them 40 (11%) were found pseudopregnant. Among 14 goats nourished on green fodder, none of them was found positive. Significant association was found between pseudopregnancy and type of feeding (χ^2 = 1.721; P-value = 0.190) (Table 3.2).

3.2.8. Season

Total number of goats examined during summer was 100. Among those, 8 does were pseudopregnant (8%). In autumn, 136 goats were examined. Among those, the positive cases were 19 (14%) and out of 142 does examined during winter, 13 positive pseudopregnant goats were observed (9.2%). No significant association (χ^2 = 2.661; P-value = 0.264) was found between pseudopregnancy and season of the year (Table 3. 2).

3.2.9. Localities

No significant association (χ^2 = 7.66; P-value = 0.264) was found between pseudopregnancy and locality (Table 3. 2). The results showed that pseudopregnant goats were found in all localities of Khartoum State. 40 goats were diagnosed as positive for pseudopregnancy. The frequency for each locality was: Karari (12), Shargalneel (8), Khartoum (7), Umbada (5), Omdurman (2), Bahri (4) and Jabal Awlia (2).

3.2.10. Parity number

No significant association was found between pseudopregnancy and parity numbers (χ^2 = 0.351; P-value = 0.767). The total number of multiparous goats examined during this study was 188. Out of them 22 (11.7%) were pseudopregnant. Among 112 nulliparous goats, 11 (9.8%) were pseudopregnant while, among 78 primiparous goats, 7 (9%) were diagnosed pseudopregnant (Table 3.2).

3.2.11. Rearing system

No significant association (χ^2 = 1.593; P-value = 0.451) was found between pseudopregnancy and rearing system. 40 out of 365 (11%) goats raised in closed system were pseudopregnant. Number of goats raised under open and mixed system was 3 and 10 respectively. Neither of them was found positive for pseudopregnancy (Table 3. 2).

3.2.12. Type of insemination

Statistical analysis was not done for type of insemination because all animals in the present study were naturally mated.

3.3. Results of multivariate analysis

Seven potential risk factors include: breed, general condition, age, type of estrus, milk yield, previous history and feeding type, which were found to be significantly associated with pseudopregnancy in the univariate analysis (P-value≤0.25) were subjected to the logistic regression. In multivariate analysis, none of these factors was found significantly (P-value≤0.05) associated with pseudopregnancy in goats with the exception of breed (P-value = 0.018) and type of estrus (P-value = 0.019). (Table 3.3)

3.4. Treatment with $PGF_{2\alpha}$

A single dose of Cloprostenol 125 μg (Estrumate®, Schering-Plough, Germany) resulted in termination of pseudopregnancy in all positive cases. The cloudburst was occurred 2-3 days following injection. The owners of the goats were advised to breed their goats at the next estrous cycle.

3.5. Subsequent fertility

Out of 40 treated goats, only 4 (10%) have recurrent pseudopregnancy. Intramuscular injection of 125 µg Cloprostenol (Estrumate®, Schering-Plough, Germany) was administered. Out of 4 goats only two get pregnant as evident by ultrasonography. The other two goats were diagnosed again as pseudopregnant.

Table 3.1: Prevalence of pseudopregnancy in 378 goats examined in Khartoum State from March 2015-February 2016

Result	Number of goats (frequency)	Percent %
Negative	338	89.4
Pseudopregnant	40	10.6
Total	378	100.0

Table 3.2: Univariate analysis of association of potential risk factors with pseudopregnancy in goats using the Chi-square test

Factor	No. examined	No. positive (%)	df	χ2	P- value
Season					
Summer	100	8 (8)			
Autumn	136	19 (14)	2	2.661	0.264
Winter	142	13 (9.2)			
Locality					
Karari	116	12 (10.3)			
Umbada	38	5 (13.2)			
Omdurman	18	2 (11.1)			
Shargalneel	123	8 (6.5)	6	7.66	0.264
Bahri	37	4 (10.8)			
Khartoum	30	7 (23.3)			
Jabal Awlia	16	2 (12.5)			
Breed					
Saanen	226	34 (15)			
Damascus	64	2 (3.1)			
Nubian	33	1 (3)			
Desert	13	1 (7.7)			
Anglonubian	4	0 (0)	7	12.627	**0.082***
Toggenburg	4	0 (0)			
Damascus×Saanen	28	2 (7.1)			
American	6	0 (0)			

Table 3.2: Univariate analysis of association of potential risk factors with pseudopregnancy in goats using the Chi-square test (*continued*)

Factor	No. examined	No. positive / (%)	df	χ2	P- value
General body condition					
Poor	4	0 (0%)			
Good	292	37 (12.7%)	2	5.974	**0.050***
Moderate	82	3 (3.7%)			
Age					
0.5-2 Y	161	14 (8.7)			
> 2 y - 4 y	156	14 (9)			
> 4y- 6y	39	5 (12.8)	4	11.760	**0.019***
> 6y-8 y	13	4 (30.8)			
> 8 y	9	3 (33.3)			
Type of estrus					
Natural	362	34 (9.4)			
Induced	16	6 (37.5)	1	12.794	**0.000***
Milk yield					
0	111	11 (9.9)			
0.5 – 3	74	4 (5.4)			
> 3-6	104	10 (9.6)	3	5.951	**0.114***
> 6	89	15 (16.9)			
Parity					
Nulliparous	112	11 (9.8)			
Primiparous	78	7 (9)	2	0.351	0.767
Multiparous	188	22 (11.7)			

Table 3.2: Univariate analysis of association of potential risk factors with pseudopregnancy in goats using the Chi-square test (*continued*)

Factor	No. examined	No. positive / (%)	df	χ2	P- value
Previous history					
Normal	221	21 (9.5)			
Pseudopregnancy	26	8 (30.8)			
Abortion	30	1 (3.3)			
Dystocia	1	0 (0)	5	13.397	**0.020***
Still birth	1	0 (0)			
No history	99	10 (10.1)			
Rearing system					
Open	3	0 (0)			
Close	365	40 (11)	2	1.593	0.451
Mixed	10	0 (0)			
Type of feeding					
Green fodder	14	0 (0)			
Mixed	364	40 (11)	1	1.721	**0.190***

**P≤0.25 was considered as significant; df = degree of freedom.*

Table 3.3: Multivariate analysis of association of potential risk factors with pseudopregnancy in goats using Logistic Regression

Factor	No. tested	No. positive (%)	Exp B (OR)	95% CI for Exp B		p-value
				Lower	Upper	
Type of estrus						
Natural	362	34 (9.4)	Ref.			
Induced	16	6 (37.5)	6.185	1.355	28.226	**.019***
Breed						
Saanen	226	34 (15)	Ref.			.358
Damascus	64	2 (3.1)	.160	.035	.730	**.018***
Nubian	33	1 (3)	.395	.042	3.702	.416
Desert	13	1 (7.7)	2.961	.271	32.325	.373
Anglo Nubian	4	0 (0)	.000	.000	.	.999
Toggenburg	4	0 (0)	.000	.000	.	.999
Damascus x Saanen	28	2 (7.1)	.460	.094	2.254	.338
American	6	0 (0)	.000	.000	.	.999

**P≤0.05 was considered as significant; C.I. = confidence interval; Exp (B) = exponent B, representing the odds ratio.*