ADDIS ABABA UNIVERSITY

FACULTY OF VETERINARY MEDICINE

ULTRASONOGRAPHIC STUDIES ON OVARIAN DYNAMICS AND ASSOCIATED ESTRUS MANIFESTATIONS OF JENNIES UNDER CONTROLLED MANAGEMENT

BY

HASSEN KEBEDE HASSEN

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SCHOOL OF GRADUATE STUDIES IN VETERINARY OBSTETRICS AND GYNAECOLOGY

JUNE, 2008

DEBREZEIT, ETHIOPIA

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A thesis submitted to the School of Graduate Studies of Addis Ababa University in Partial Fulfillment of the Requirements for the Degree of Master of Science Veterinary Obstetrics and Gynecology

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ACKNOWLEDGEMENTS

I am very grateful to my advisor, Dr. Alemayehu Lemma for his strict and meticulous follow up in each step of implementation of the study project and finalization of this thesis. The contribution laid by Professor Merga Bekana in commenting and correction of this thesis as well as encouragement of my research endeavors was also invaluable.

I am also very grateful to office of school of graduate studies for every offer I have received both morally, materially and financially, with out which I couldn't have been able to achieve majority of my dreams during my stay at Addis Ababa University. The financial contribution by SIDA SAREC II project was also of paramount importance for harmonious completion of project work from providing the ultrasound machine to coverage of different expenses needed for my study period.

I would like to thank staff members of International Donkey Protection Trust specially Dr. Ayele Gizachew and Dr .Tesfaye Merga for their unreserved efforts in management of jennies used as well as provision of reference materials needed for the study.

My special thank is also for Ato Hica Waktole. Ato Yoseph Chernet , Ato Mekonnen Sorsa, and Ato Taye Dinku for their unreserved efforts and ever willingness to assist me during the actual study which takes greater part for sweetness of the long study follow up.

ABBREVIATIONS

AAEP	American Association of Equine Practice
AC	Anovulatory cycle
AD	Animal day
BCS	Body Condition Score
CL	Corpus Luteum
DD	Day of divergence
DE	Day of Emergence
FAO	Food and Agricultural Organization
FVM	Faculty of Veterinary Medicine
hCG	Human Chorionic Gonadotropin
IOI	Interovulatory Interval
LH	Leutenizing hormone
NMSA	National Meteorological Agency
OC	Ovulatory Cycle

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ABSTRACT

Serial ultrasonographic study was conducted on nine Jennies aged 5-14 years from December 2007 to April, 2008 with the objective of characterizing ovarian follicular activities and estrus manifestations under controlled management. The jennies were housed, fed on hay and concentrate, regularly watered and were not working during the study. Ovarian follicular activity was determined from number and size distribution of follicles, length of interovulatory interval, growth rate of preovulatory follicles, diameter of follicles at the onset of estrus and incidence of ovulation. Estrus manifestations were characterized using length of estrus and estrous cycle. The mean (± SD) number of follicle detected per ovary was 5.45 ± 2.25(range 1 -16) with sizes ranging from 2.88mm to 44mm.The mean (± SD) size of follicle encountered at the onset of estrus was 25.9 ± 3.7 mm (range 20.9 – 34.4) while that of the preovulatory follicles at -1 day before ovulation was 36.81 ± 3.78mm. The mean (± SD) interovulatory interval, estrus and estrous cycle length were 25.4 ± 3.6; 7.85± 2.99 and 24.22 ± 7.43 days respectively. The mean (± SD) growth rate of the preovulatory follicle after day of divergence, which fell on -8 day before ovulation was 1.89 ±0.33mm/day ($P<0.001$). Follicles were known to grow in cohorts constituting two or more waves. Serum progesterone profile followed the same patterns of ovarian dynamics with peak values being detected during mid luteal phase. Serum progesterone assay revealed blood progesterone profiles of <1.0 ng /ml during estrus and up to 11ng /ml during mid - luteal phase which has shown the presence of ovarian cyclicity through out the study period with patterns following follicular dynamics. Majority (82.14%) of the estrus manifestations were significantly ($p<0.005$) associated with ovulations. Body condition was positively correlated ($r = 0.52$, $P< 0.001$) with the diameter of the preovulatory follicle and negatively with the number of follicles per ovary ($p<0.01$). Estrus length was also found to be positively correlated to size of preovulatory follicle at onset of estrus ($r = 0.412$, $p<0.05$) and negatively correlated to interovulatory interval ($r = -0.548$, $p<0.05$). Ovarian follicular dynamics in jennies are generally equine prototype with subtle differences. In contrast to previous reports of reproductive activity under traditional management systems, this study confirmed as improved management can also improve the ovarian activity. However, it still remains to be verified as to see the relative and individual effects of management factors on follicular activity.

Key words: Estrous cycle / Jennies / ovarian follicular activity / ultrasonography / progesterone

1. INTRODUCTION

Ethiopia, with estimated 5.2 million heads, owns one of the largest donkey populations only next to China (Starkey and Starkey, 2000; FAOSTAT, 2003). The percapita distribution is 27 donkeys for every 100 people (FAO, 1989), which is probably one of the highest ratios in the world.

There is little doubt that the donkeys have not only been most used but also most abused animals in history. In spite of the fact that they have spent hundreds of years as hard working friend of man, relatively little attempt has been made to study any aspect of its biology. In Ethiopia, unlike their wide-ranging roles in the national economy, donkeys are among the most neglected species of livestock with very little or no interference in improving their productivity in terms of breeding, nutrition and health management. On the contrary, little or no complaints have been raised against their reproductive failures compared to cattle where exaggerated losses result from poor management schemes. The question arises whether donkeys are resistant to severe environmental conditions such as nutritional derangements to behave normally in their reproductive performance or else parameters are not worried about while the impact still existed.

For instance, numerous observations confirm that in almost all cases donkeys are left to forage for themselves when not working. For most of the time they generally maintain good body condition even without supplementation with the exception of the months of March and April (Feseha and Awoke, 1995). Body condition is known to play an important role in influencing the ovarian activity in many domestic species. The interval to the second postpartum ovulation is increased in mares with poor body condition and the level of leutenizing hormone (LH) is decreased during foal heat (Henneke *et al*., 1984; Hines *et al*, 1987).

Several studies have been conducted in Ethiopia on the socioeconomic importance and management practices common in different study areas of Ethiopia. Outstanding examples are studies on foaling season in Debre Birhan area (Wilson, 1991); breeding, parturition and age at maturity around Gondar and Dire Dawa (Mohammed and Teketel, 1992; Feseha and Yoseph, 1996; Fesseha and Awoke, 1995), management and utilization (Fisseha *et al*., 2004). Research needs of Ethiopian donkeys have also been reviewed and published by different scholars (Alemu *et al*., 1997). As often constrained by the prevailing traditional donkey management system, scientific studies on the general biology are limited in Ethiopia. More specifically, apart from field studies by Alemayehu, *et al.*, 2004 and Lemma, 2006; relatively little has been objectively addressed pertaining to basic physiologic parameters of reproduction under controlled management. Future implementation of controlled breeding or intervention schemes on genetic improvement necessitates the availability of detailed information on the physiology of ovarian dynamics.

There fore the objectives of the present study were to:

1. Determine ovarian follicular dynamics under controlled management.
2. Study behavioral manifestations associated with the ovarian follicular activities.

2. LITERATURE REVIEW

2.1. Reproductive anatomy and physiology of equines

The reproductive tract of equines consists of the vulva, vagina, the uterus and the fallopian tubes. There exists extensive literature on the reproductive anatomy of mares but since information is lacking for jennies, extrapolated knowledge from the mare has been used more often. Generally the sizes of individual organs are believed to be proportionally smaller in jennies due to the size of the animal. The cervix of the mare is firm enough to be palpated and used as a point of orientation during rectal examination. During anestrus, it may be closed or partially dilated. However, as the serum progesterone concentration increases during diestrus, the cervix contracts and becomes blanched and centrally situated in the vaginal lumen. As estrus approaches, the external cervical opening becomes moist under the effect of increasing serum estrogen and the cervix becomes relaxed. These changes, combined with teasing results, history and findings by ultrasound help to establish the reproductive stage (Lofstedt, 2001).

The equine uterus is a hollow muscular organ and it is more flaccid than those of ruminants and pigs. It is also known to be bipartite where the uterine body is more extensive than the horns. The average diameter of the uterus (8 - 12cm at the body and 3cm at the mid horn) has been known to vary, depending on age, breed, parity and stage of sexual activity. It has been found to be larger in older and multiparious animals, and to temporarily increase in size during estrus and pregnancy (Morel, 2003). The endometrium of both mares and jennies has longitudinal folds. The appearance and size of these folds vary depending on the stage of reproductive function and therefore have a diagnostic significance in ultrasonographic assessment of the reproductive status during estrous (Pycock, 2002; Lemma *et al*, 2006). The gestation period in both species has been reported to vary between 330 and 400 days (Mcdonnel, 1998 ; Lôfstedt, 2001).

Equine ovaries are bean shaped structures situated near the kidney and more laterally apposed by the mesovarium part of the broad ligament. In the mare, during the non-breeding season, the ovaries become small, hard or firm in consistency and are inactive. During the breeding season, however, the ovaries increase in size and become softer to touch (Löfstedt, 2001). Older and multiparous mares tend to have larger ovaries up to l0cm (Alemayehu, 2004). Ovulation is spontaneous and occurs at the ovulation fossa, a structure unique to equids. It consists of a depression, lined with the germinal cells destined to develop into ova, at the free, ventral edge of the ovary (Rowlands and Weir, 1984).

2.1.1. Breeding and sexual maturity

As with most other taxa, tropical-zone equids are less strictly seasonal than are their temperate-zone cousins. Photoperiod is known to be the most important environmental cue for seasonal reproduction in temperate regions (Ginther, 1992). However, even in the tropics, seasonal birth peaks suggest that nutritional factors related to rainy seasons are important (Churcher, 1993, Alemayehu, 2004). In Ethiopia, various observations suggest that donkeys are able to breed all year round. However, high foaling periods were registered in the Debre Birhan area in the months of March, April, June, July and August (Wilson,1991). In Gondar, that breeding as well as parturition periods were noted to coincide in most of the cases with the onset of the rainy season (May/June). In Awassa most foaling were observed to occur in February and March (Mohammed and Teketel, 1992). Reports from a more objective studies has also indicated seasonal pattern of follicular activity coinciding with the rainy seasons with most probable implication on influence of nutrition rather than photoperiod (Alemayehu, 2004).

Donkeys reach mature weight at about 2 - 3years of age. The average weight of donkeys (both males and females), transporting goods into Debre Birhan, was approximately 105 kg and 124 kg in central Ethiopia (Wilson, 1991; Alemayehu, 2004). Breeding age for female donkeys has been reported as 4 – 5 years in Hawassa and 3 years in central Ethiopia (Alemayehu, 2004).

2.1.2. Estrus, estrous cycle and ovulation

External manifestation of sexual receptivity or rejection by jennies occurs upon visual and tactile senses with a jack. Occasionally, vocalization of the male only, without visual contact, is enough to stimulate jennies to show overt signs of estrus. The first overt and most characteristic estrous sign observed in jennies has bees indicated as persisting or intermittent opening of the mouth (jawing) with ears depressed against the extended neck. Others include positioning, lifting of the tail, winking and standing to be mounted (Henry *et al*., 1998). Under pasture management system, when the number of jennies in estrus per day increased due to estrus synchronization, spontaneous mounting between jennies might happen. The first sign of impending estrus shown by jennies bred at pasture was indicated as gradual move to the vicinity of the male. About one day before showing overt signs of estrus, jennies start to spend most of the day in the vicinity of the male. Once in estrus, besides staying near the male, the jennies started to repeatedly approach and tease male (Henry, *et al.*, 1991).

Estrous behaviors of domestic equids also include increased frequency of urination and separation from others. The tail may be deflected to one side or held straight out from the perineum, and rhythmic eversion of the clitoris occurs during but also independently of urination. The facial expression of adult estrous domestic horse mares is characterized by a slightly lowered head, ears held back and to the side, and relaxed facial muscles. However, the facial expression of estrous zebra and donkey mares may include the retraction of the lips to expose the incisors, often accompanied by a high-pitched vocalization.Younger horse mares may snap at the approach of a stallion, a behavior commonly seen in mature as well as young estrous donkeys (Clayton *et al.* 1981)

Estrous cycles of equids are of moderate length relative to other mammals, ranging between 19 and 35 days. However, compared to other large domestic livestock, the estrus phase is longer in *equids* , ranging from several days to a week, with a mode of 5 or 6days (Asa, 1996). Diestrus in the donkey is indicated to be longer than in the horse (Carluccio *et al.* 2008):

2.2. Management and reproduction in donkeys

Donkeys can consume dry matter equivalent to 2 - 2.5% of their live weight per day, and larger horses can eat greater on assumption that they are given the time to eat it. Compared to the horse, the donkey is better at digesting roughage feeds and so will tend to digest a greater proportion of the dry matter than the horse given the same roughage diet which is suggested to the proportions of the micro-organisms in the hind-gut are different, with those in the donkey being better at cellulose digestion. Other suggestions also indicate the proportionately larger digestive tract in relation to live weight in the donkey. In the past it was suggested that the fact that donkeys seem to "do better" than ponies, was because donkeys had lower requirements for food than horses per unit live weight (as much as 25% in some reports). Comparative studies of energy and protein requirements of donkeys and ponies have not shown any differences of this magnitude. Therefore it seems likely, in the absence of other evidence, that at least part of the reason why donkeys do better than horses on the same feed is their greater ability to digest roughage feeds. This is advantageous when a fixed amount of food is fed, where horses cannot take advantage of their generally higher voluntary intakes of roughage (Pearson, 2005).

Numerous observations in Ethiopia have confirmed that in almost all cases donkeys are left to forage for themselves when not working. For most of the time they generally maintain good body condition with the exception of the months of March and April (Alemayehu,2004). Feeding of donkeys is entirely based on grazing on communally owned grasslands as well as roadsides that are overgrazed and where growth of forage is poor. Donkeys, whose feeding is often neglected, survive due to their tremendous capacity to utilıze foods of low quality (Fisseha *et al.*, 2004).

Although research reports are lacking on specific impact of nutritional management on reproductive parameters in donkeys, the effect of nutrition and body condition on seasonal reproduction has been described in temperate breeds of mares. Mares, which receive supplementary diet of concentrates, ovulate earlier than mares without supplementation. The anovulatory period is shorter in mares, which gain weight during early spring. An additive effect of nutritional supplementation and artificially photoperiod has also been observed. The interval to first ovulation is longer in mares with condition score of less than 5.0 (scale from 1 to 9) compared to mares with score above 5.0. A high-energy intake shortens the interval to first ovulation in transitional mares with a low level of body fat but does not benefit mares in moderate or fat body condition (Daels, 2006).

In Ethiopia, from studies conducted in central highlands ,most common type of housing system of donkeys was a stable yard (unroofed and often constructed from thorny wood) while a barn or a house (in some instances with incomplete wall and door) was used for horses. Adult donkeys are kept together with other animals in the same yard, irrespective of the season, reproductive or health status while horses are kept separately in most cases. Foals are kept in separate barn built for newborn animals (Alemayehu, 2004).

2.3. Ultrasonography of reproductive organs

A successful application of ultrasonic imaging techniques requires an understanding of the basic mechanisms, appropriate scanning procedures (Squires *et al*, 1988). Diagnostic ultrasound waves are classical sound waves operating at frequencies far above normal audible frequencies generally at a range of 1-10 MHz (Powis, 1986; Rantanen and Ewing, 1981).The high frequency waves are formed by vibration of specialized man made crystals having piezoelectric properties that are housed within probes or transducers .The sound waves then propagate through the soft tissue which when they encounter tissue interfaces, reflect it back as echo. Echoes returning from tissue acoustic interface (echo texture) are then received by the transducer and converted to electrical impulses. These impulses are displayed on an oscilloscope screen as a cross-section of the tissue where each portion is represented by shades of grey, ranging black to white, depending on the amount and intensity of reflecting sound beam (Powis, 1986).

Liquids (follicular fluid, fluid in the yolk sac) that do not reflect sound waves (non-echogenic or anechoic) appear black on the screen. Dense tissues (fetal bone, cervix, corpus luteum) reflect much of the sound beam (hyper echogenic or hyper echoic) appear white on the screen (Pierson *et al*., 1988). The ability to monitor ovarian dynamics and early detection of pregnancy using ultrasonography has bestowed new insights into animal reproduction for both diagnostic and research purposes. The more recent uses were demonstrated in different species of large domestic animals such as donkeys (Meira *et al*., 1995; Blanchard *et al*., 1999; Lemma *et al*., 2006).

The development of real-time imaging in the late 1970's made the science of ultrasonography adaptable for the study of the internal reproductive tract via transrectal route (Pierson, 1988). Diagnostic ultrasound is now accepted as the most non-invasive technique for imaging soft tissues and accurate assessment of the size, shape, position and texture of soft tissues in donkeys. Its current application in equine reproduction includes monitoring follicular changes; predicting and confirmation of ovulation; evaluation of corpus luteum; estimating the stage of estrous cycle; diagnosing ovarian irregularities and pathology; detection of early pregnancy; twins and embryonic death; evaluation of uterine pathology and evaluation of testes and accessory sex glands in male (Squires *et al*., 1988; Griffin and Ginther, 1992; Meira *et al*., 1998; Vanderwall, 2000). A 5 MHz transducer has been known to be more suitable for evaluating the ovaries but lower frequencies (3 or 3.5 MHz) are recommended to view larger structures like large fetuses (Ginther and Pierson, 1984; Squires *et al*. 1988; Pierson *et al*. 1988).

The ultrasonic anatomy of the ovaries has been described in previous reports (Pierson *et al*. 1988 ; Ginther, 1995;Lemma *et al*., 2006). Follicles appear as black (nonechogenic) circumscribed structures that vary from spherical to irregular in shape. Irregular shapes have been attributed to compression between adjacent follicles or other structure. The diameters of non-spherical follicles can be estimated by adjusting the irregular shape to an approximately equivalent circular form (Ginther. 1995).

Follicles as small as 2 to 3mm can be detected with a high frequency transducer and the diameter of preovulatory follicles are known to reach more than 30 mm in Jennies (Meira *et al*, 1995; Blanchard *et al*, 1999; Alemayehu, 2004). Using transrectal ultrasonic B-mode (grey-scale) imaging in the mare, the granulosa is identifiable as anechoic band enclosing the antrum (Ginther , 1995). The two layers of theca can be assumed on the basis of position, but the boundary between the theca externa and interna is indistinct. The inner boundary of the theca interna can be located as a result of its close apposition to the basement membrane of the granulosa. In the initial equine B-mode ultrasonographic study (Pierson and Ginther, 1985), granulosa thickness increased as the interval to ovulation decreased. The mean scores increase for echogenicity and thickness of the granulosa during the 24 h before ovulation was also described (Carnevale, 1998). In addition to an increase in echogenicity of the granulosa, prominence of an anechoic band in the expected area of the theca layers increased daily and progressively in the period 3 to 1 days before ovulation (Gastal *et al.* 1998).

The number and size of follicles on a given ovary are known to vary widely, depending on the stages of the estrus cycle and on season in both species in temperate regions. A recent description of the growth pattern of follicles in mares as determined by ultrasonography shows that ovarian follicles grow in more than two waves (Ginther, 1995; Evans, 2003; Ginther, 2000). Profound breed differences in wave patterns during estrous cycle have been reported. In some breeds (quarter horses and ponies) usually only one major wave develops in late diestrus and ends in ovulation. In other breeds (thoroughbreds), a secondary major wave frequently develops in early diestrus and the dominant follicle may be anovulatory or ovulatory. The pattern of follicular waves has not been well described for jennies (Lemma *et al*, 2006).

Ultrasonic follicular data in equine can be processed using four methods: a grouping method, an identity method, a mathematical or non-identity method, and a tier method to characterize the follicle wave pattern. The first uses grouping of follicles into diameter categories and requires daily records of diameters of each follicles. The tier method utilizes combined records of daily follicle data from both ovaries and ranking them from the largest to the smallest without identity of individual follicles; the follicles are then divided into tiers of six follicles per tier in decreasing size order for analysis. The identity method requires records of day-to-day identity of individual follicles while the non-identity (mathematical) method requires profiles of the three largest follicles per ovary without regard to the daily identity. The last method has advantages over the other three because a day-to-day identity of follicles is not required and only few follicles have to be measured in each ovary (Ginther and Bergfelt, 1992; Ginther, 1995).

Ultrasonographically detectable changes occur in the equine preovulatory follicle as ovulation approaches, and significant progressive changes have been reported for wall thickness as well as for follicle diameter. Thickness and echogenicity of the follicular wall reportedly increased within 24 h before ovulation and a shape change occurred 12 h before ovulation (Carnevale *et al.*, 1988; Ginther, 1995). The combination of diameter, shape changes, and thickening of the wall appeared to be valuable for assessing preovulatory-follicle status (Pierson and Ginther, 1985). Recently, cellular and vascular histologic changes in equine follicles prior to ovulation were described (Kerban and Sirois , 1997).

Size of preovulatory follicles has been used as accurate as any other method in determining the time of ovulation. The disappearance of a previously detected preovulatory sized follicle has been considered as an indication of recent ovulation (Ginther, 1995; Griffin, 1995; Squires *et al.*, 1988; Townson and Ginther,1989a). The subsequent formation and identification of a corpus luteum also confirms ovulation. The success of accurate detection of ovulation varies, depending on the frequency of ultrasonic examination. It has been shown in mares that no ovulation would pass undetected if scanning is performed every 3 days after the preovulatory follicle reached ≥30 mm in diameter until the end of estrus (Ginther, 1995).

The corpus luteum (CL) is generally detectable with a 5 MHz transducer for 16 - 17 days after ovulation (Griffin, 1995; Townson and Ginther, 1989b). Two distinct luteal morphologies have been described both in jennies and mares: a uniformly echogenic and a centrally non-echogenic corpus luteum. About 50% of corpora lutea are known to be uniformly echogenic throughout their period of detectability and the remaining *50%* develop a centrally located non-echogenic area, representative of a blood clot (Lemma *et al.*, 2006; Ginther, 1995). No functional differences have been found between these two morphologies. Echogenicity or brightness of CL becomes greatest during the first two days of its formation. Subsequently, echogenicity decreases until the end of the luteal phase (Griffin and Ginther, 1992).

2.4. Control of estrous cycle

The major physiological events associated with reproductive activity in mare are endocrine changes which in turn govern and drive the other physiological changes as well as behavioral activity. Endocrinological control of the estrous cycle is governed by the hypothalamic- pituitary-gonad (ovaries) axis. Overriding the whole of this control mechanism is the effect of photoperiod changes; decreasing day length causing estrous cycle to cease and increasing day length causing the cycles to occur. (Morel, 2003).As the day length increase inhibition of the axis removed, allowing gonadotropin releasing hormones to be produced by the hypothalamus and driving LH and FSH production by pituitary. Not only does the anterior pituitary have a direct effect on ovarian functions by stimulating folliculogenesis, follicular maturation, ovulation and corpus luteum formation, but also the ovary has an effect upon hypothalamus and anterior pituitary through estradiol, produced by maturing follicle and through progesterone produced by corpus luteum formed after ovulation of follicles (Arthur, 2002).

In non-pregnant mares, prostaglandin F_2 (PGF2α) secreted from the endometrium between days 13 and 16 after ovulation, induces regression of the corpus luteum. Releases of PGF2α precede the decline of plasma progesterone concentrations by three to four hours (Stabenfeldt *et al.*, 1981). Artificial intervention in one of the components will result in a change or modification in physiological cyclicity of the system.

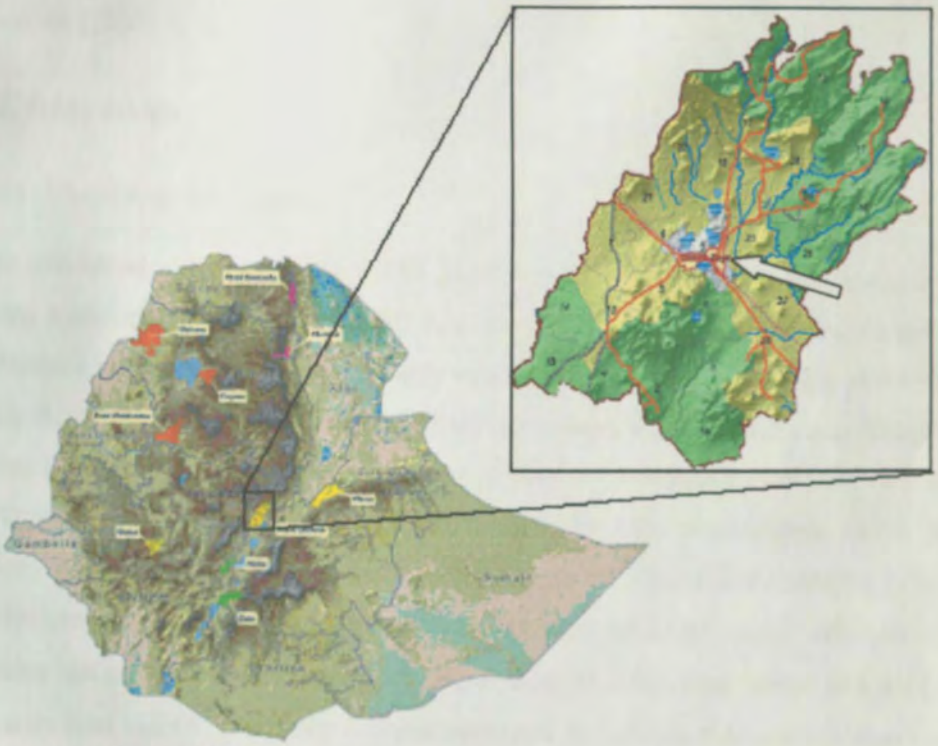
Native PGF2α and its analogs have been used in the mare to manipulate ovarian function for several purposes, but mainly as a luteolysin to induce and/or synchronize estrus for breeding (Nie *et al.*, 2003). Prostaglandins impact on ovarian, uterine, placental, and pituitary function to regulate reproduction in female livestock. They play important roles in ovulation, luteal function, maternal recognition of pregnancy, implantation, and maintenance of gestation, microbial-induced abortion, parturition, postpartum uterine and ovarian infections, and resumption of postpartum ovarian cyclicity. Prostaglandins have both positive and negative effects on reproduction; they are used to synchronize estrus, terminate pseudo pregnancy in mares, induce parturition, and treat retained placenta, luteinized cysts, pyometra, and chronic endometritis (Weems *et al.*, 2006).

The use of the combined steroids (progesterone and estradiol) has also been indicted as the most accepted method for maximum control of estrous cycle in prompting to assume its cyclicity (Loy *et al.*, 1981; Varner *et al.*, 1988; Lofstedt, 1988).

3. MATERIALS AND METHODS

3.1. Study area

The study was conducted in central Ethiopia at Debre-Zeit, FVM. It is located at $8^{o}48^{'}$ $0^{"}$N and 38^{0}58'60"E at an altitude of 1990 m.a.s.l. Mean minimum and maximum temperatures are 16^{o}C (60.8^{O}F) and 27^{O}C (80.6^{O}F), respectively. Annual rainfall and mean relative humidity records are in the order of 825mm (32.5 inch) and 52%, respectively. The climate is characterized by a bimodal rainfall with the short rainy season occurring from March to May, preceded by the long dry season from October and February. The long rainy season occurs from June to September (NMSA).



Source: ILRI (2005)

Figure 1: Map of Ethiopia indicating the study site (Debrezeit)

3.2. Study animals

A total of nine non-pregnant Jennies aged 5 to 15 years body and weight from 110 to 125kg (Svendson,1997)(Annex I) were used. All the Jennies were maintained on hay, wheat straw and natural pasture supplemented with 0.5kg wheat bran during the night. The jennies had access to water *ad libitum*. The Jennies were treated against the common internal and external parasites with Ivermectin (Vermic®-centrovet Ltda. Chile) at a rate of 1ml / 50kg at the start of the study and twice during the succeeding period until the end of the study. The jennies were synchronized using Lutalase® (5mg/IM Dinoprost tromethamine, Pharmacia and Upjohn Co. Ltd. USA) twice at 14 days interval (Glazer *et al*., 2004). Body condition was scored according to Pearson and Quassat, (2000).

3.3. Study design

3.3.1. Ultrasonographic study

The ultrasound scanner (Mindray, DB-3300, Vet Digital Ultrasonic Diagnostic Imaging System, Hong Kong) equipped with a 5 MHz real time linear-array transrectal transducer. A gloved and lubricated hand was used to completely evacuate feaces before scanning to prevent interference with the transmission and reception of the ultrasound waves. Mild tranquilization was applied using intravascular tranquilizer reconstituted from a combination of 0.2mg (0.2ml) Butorphanol tartarate (Turbogesics®-Fort Dodge Animal Health Ltd. Southampton SO30 4QH, UK) and 0.6mg (0.6ml) Romifidine (Sedivet ®-Boehringer Ingelheim Ltd. Labiana Life Sciences S.A. Barcelona, Spain) during the first week of scanning to allow muscle relaxation of rectum and induce analgesia. Scanning was then begun from bladder, then uterus and until eventually the ovaries were visible. The probe was moved to and fro, rotated side ways to view each ovary.

Ovarian follicular activity

Jennies were serially scanned at every other day basis for 52 days. The number of follicles in each ovary were counted and recorded. Follicular diameters were measured using the internal electronic caliper and the data was grouped in to size categories (small = 2-10mm, medium = 11-20, large > 20mm) (Squires *et al*. 1988; Lemma *et al*., 2006). The first three largest follicles in each ovary were measured and recorded to manage the follicular data according to Ginther

(1995). Average diameters were taken when follicles assumed irregular shape mostly obvious prior to ovulation. The number and size of follicles detected in each ovary were counted and recorded.

Incidence of ovulation

Ovulation was confirmed with the sudden disappearance of the preovulatory follicle, ultrasonic demonstration of the corpus luteum and an increase in the level of serum progesterone above 1ng. Follicular data was normalized to a mean of 25 days (Ginther, 1995) and the day of wave emergence was accordingly determined (DE). The day at which the dominant follicle assumed the maximum growth differing from the subordinate follicles was considered Day of Divergence (DD). The interovulatory interval (IOI) was determined as the interval between two successive ovulations. Cycles ending in ovulation were taken as ovulatory cycles (OC) while those with follicles of size above 25mm (anovulatory follicle) and not ending in ovulation were considered as anovulatory cycles (AC). The number of ovulations and anovulatory cycles was summarized to determine the relative incidence of ovulation with in each month of the study period. All waves appearing until the emergence of the dominant ovulatory follicles are considered to constitute the total number of waves in a cycle.

3.3.2. Progesterone assay

Blood sample was collected through jugular venipuncture into non-heparinized tubes twice a week and serum was harvested for progesterone assay. The serum was stored at -20^{0}C until assayed. Plasma progesterone a level was measured by indirect competitive ELISA technique (Ovucheck ® plasma ELISA kit, Biovet Inc, Canada). Values above 1ng were considered to show the presence of ovulation when the corpus luteum was simultaneously observed through ultrasonography.

3.3.3. Observational studies

Jennies were allowed to come in contact with a jack for 30 minutes before each of scanning to detect the presence of estrus. The number of days each jenny was observed for heat signs was quantified as animal day (AD). Jennies were observed for a total of 119 animal days in which a total of 551 estrus signs were recorded. The length of time from the onset of one or more obvious signs of estrus to the onset of the next similar sign was considered as one estrous cycle (Donald, 1981). Behavioral manifestations were observed and scored for their intensity and length of manifestation as early (when less than 25% of the common signs appear, overt (greater than the 75% of the common manifestations commence) or subsiding (when jennies kick the jack, show signs of refusal but do show jawing). This information was later on used to study the correlation between follicular activities as determined by the size of ovulatory follicles and estrus.

3.4. Statistical analysis

All data and observations were stored in Microsoft Excel and all computations and comparisons for each variable interaction was performed using SPSS for Windows (SPSS version 15.0, 2006,Chicago, USA). Follicluar data was normalized to the mean cycle length of 26 days to determine the day of wave emergence. The non-identity (mathematical) method previously described for mares was applied to present the data in characterizing follicular waves (Ginther, 1995). Accordingly, individual waves of each ovary were depicted from measures of the first three largest follicles of each ovary. Later, a composite plot was made to determine the day of divergence of the ovulatory follicle from the subordinates. The growth rate of ovulatory follicle after the day of divergence was calculated (regressed) from normalized follicular data. General linear model was used to see the interaction between body condition score with follicle size and number, age, interovulatory interval, interval between onset of heat and presence of significant interactions were assessed. Associations between categorical variables like spherical/irregular shape assumption before ovulation was analyzed using x^2 test. Values of $P<0.05$ was considered as cut off value for statistical significance. In describing the temporal occurrence of behavioral manifestations among total observations, percentages were used. The time of ovulation in ovulatory cycles and last detected peak before atresia in anovulatory cycles was taken as reference point. Follicular data was digitally stored for later analysis of number and size distribution of growing follicles. Mean, range and standard deviation were used to describe the variation in each parameter. Graphs and tables were used as a statistical tool to summarize different variables.

4. RESULTS

4.1. Ultrasonographic study

4.1.1. Ovarian follicular activity

Follicles of different diameters ranging from 2.88 to 44 mm were detected. One up to sixteen follicles (n =936, 5.45 ± 2.25; mode = 6) were detected per ovary. As many as 10 follicles of different sizes were detected and visible in a single scan field (Figure 2). Follicles appeared on the gray scale ultrasonic image as black areas assuming different shapes varying from spherical to roughly triangular. Corpus luteum appeared as white circumscribed area with centrally hyper echoic area (Figure 3).

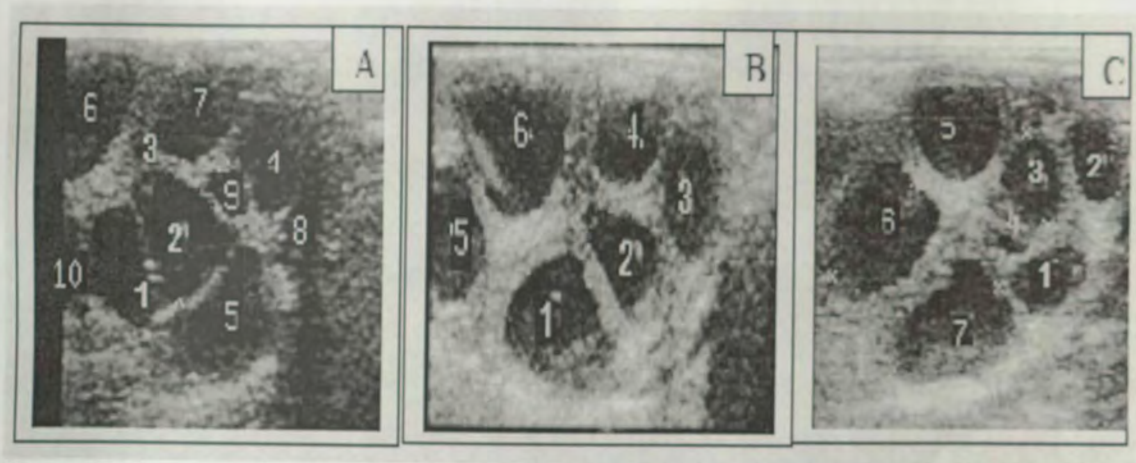


Figure 2: Ultrasonic images showing population of A (10), B (6) and C (7) follicles detected per scan field.

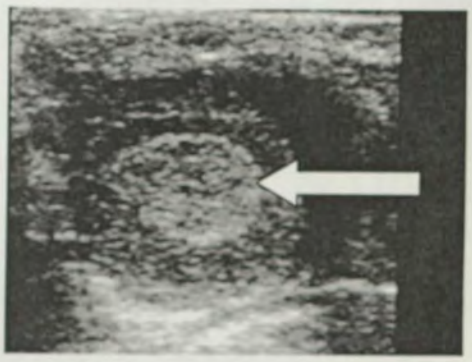


Figure 3: Appearance of corpus luteum of age 4 days after ovulation.

Distribution of follicular population revealed the relative abundance of medium sized follicles (Figure 4). The number of follicles found in each ovary generally decreased as the size of the follicles increased ($p<0.005$). No statistically significance difference was observed in the number and size distribution of follicles between the left and right ovaries.

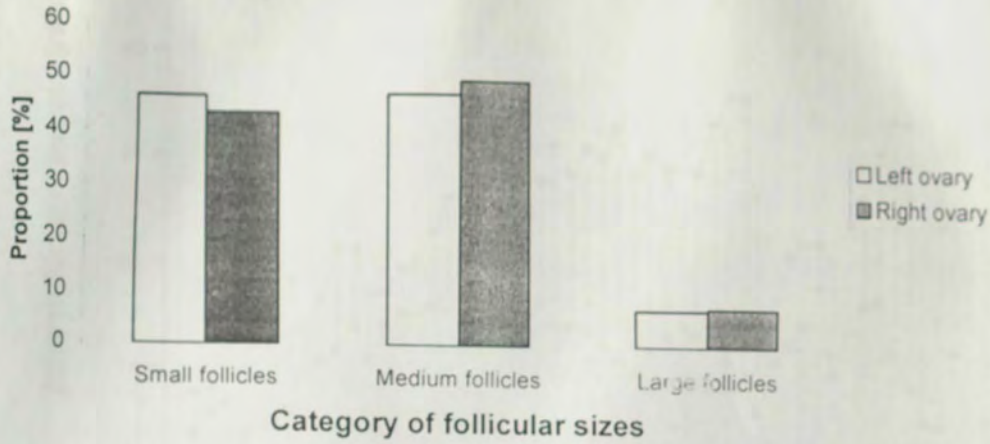


Figure 4: Population of follicles of different size categories encountered during the study period (N=4950).

The mean (±SD) size of the first largest follicle per animal was 22.3±7.9 (N=450). The growth pattern of dominant follicle is depicted in figure 5 and 6. The mean (±SD) diameter of the dominant follicle at time of the onset of estrus was 25.9±3.7mm (range=20.9-34.35 mm). Mean (±SD) observed number of follicular waves was 2.4±1.54 per each cycle. The average growth rate of ovulatory follicles after day of divergence was 1.89±0.33mm/day (Figure 6). The estimated mean diameter of the first largest follicle at the anticipated time of wave emergence was 17.88 ± 4.6mm (Figure 6).

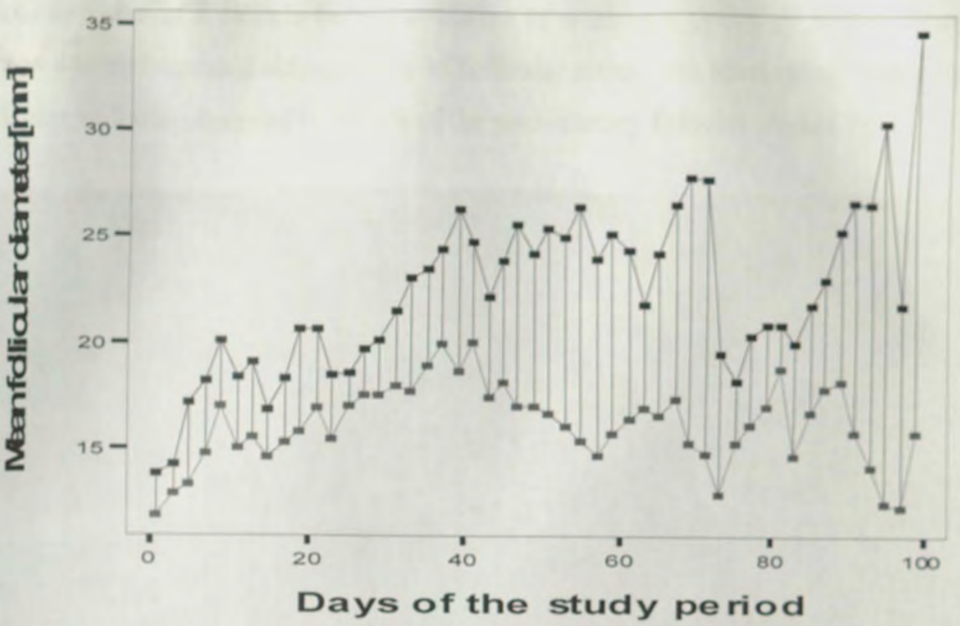


Figure 5: Growth pattern of the dominant and subordinate follicles through out days of the study period bars showing mean differences.

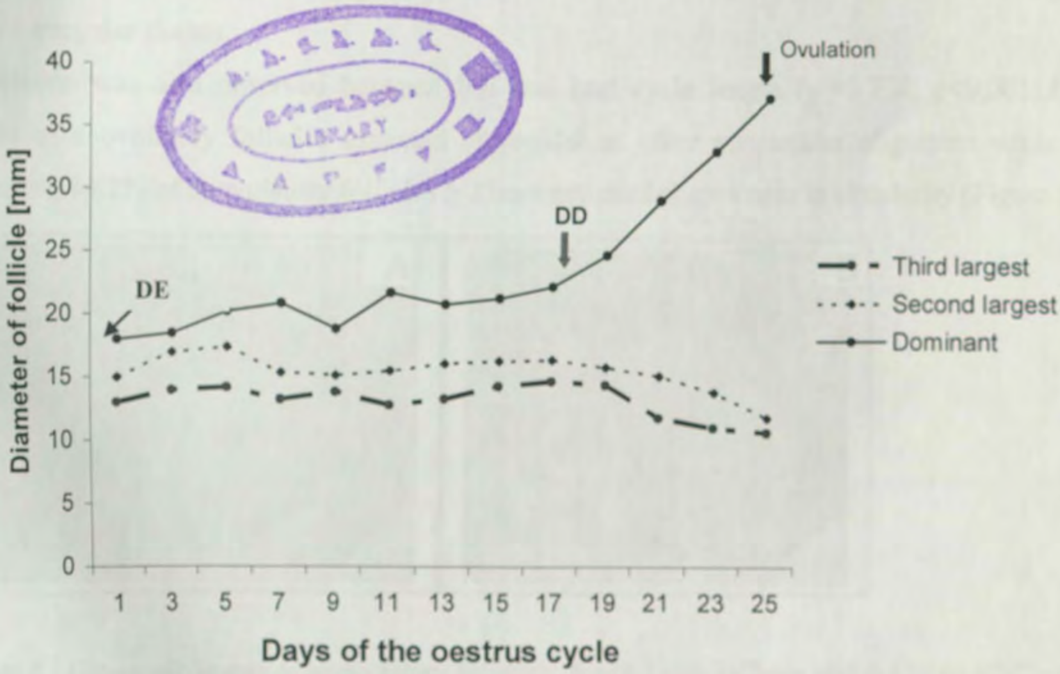


Figure 6: Growth pattern of the first three largest follicles and the day of divergence taken from mean diameters of first, second and third follicle.

4.1.2. Incidence of ovulation

The maximum size of a follicle before ovulation in ovulatory cycles, or atresia in anovulatory cycles was 44mm. Increased echogenicity of follicular membrane (thickening) and irregularity of the follicular wall was observed in 68.9% of the preovulatory follicles (Figure 7).

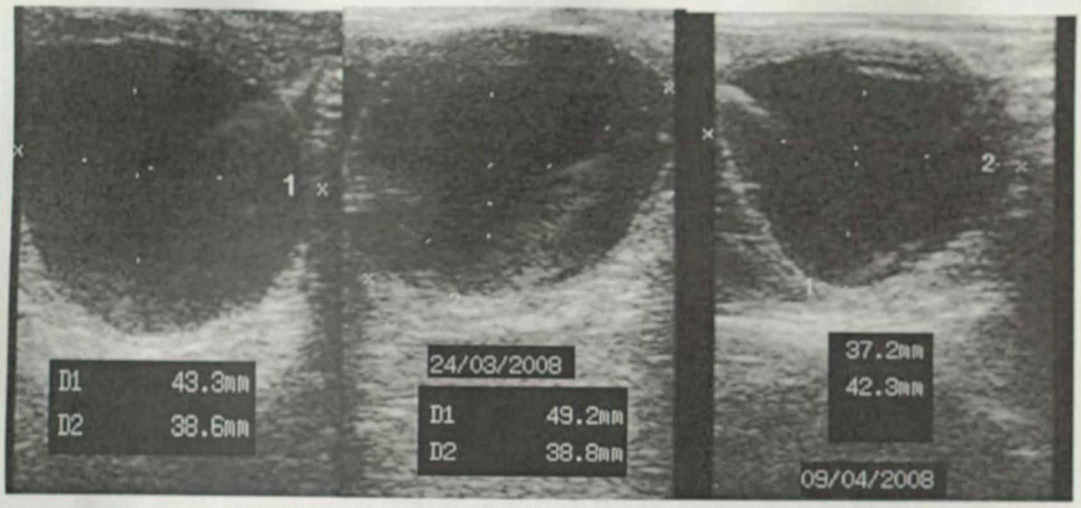


Figure 7: Ultrasonic image of a preovulatory follicle taken one day before ovulation assuming irregular shapes.

Correlation was also observed between IOI and heat cycle length ($r = 0.734$, $p<0.005$).Only 15.8% of anovulatory follicles assumed ellipsoidal or other noncircular alignment while the remaining 84.2% of anovulatory follicles ≥ 25mm assumed shapes near to circularity (Figure 8).

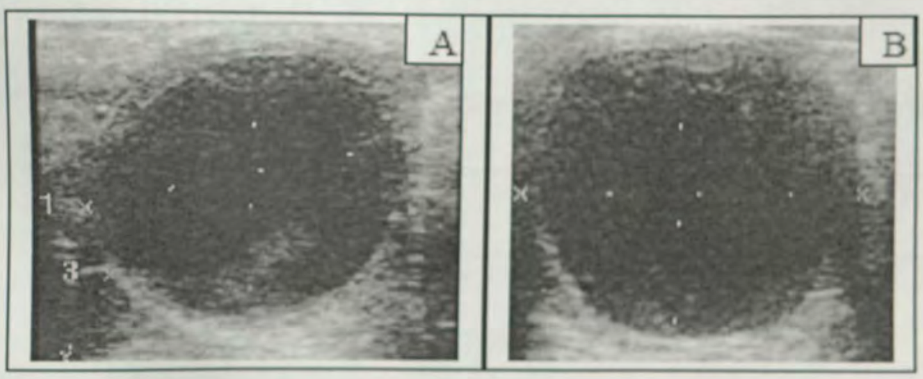


Figure 8 : Ultrasonic image of anovulatoty follicles: A (34.1+36.3)/2mm and B (36.6+37)/2 mm; both taken three days before last day of atresia.

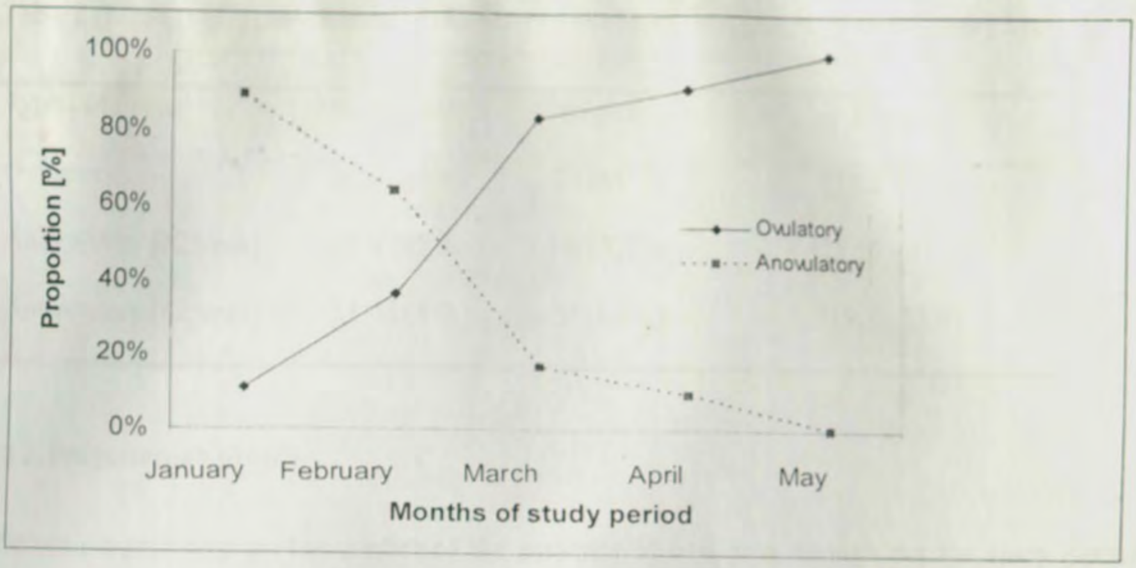


Figure 9 : Incidence of ovulatory and anovulatory cycles with five months in the study period.

There has been a significant difference in proportion of ovulatory cycles in between each months of the study period being lowest at start of the study and highest in April (P<0.001). In January 11.1% to 90.9% in April (Figure 9).The incidence of ovulation (single) was 56% from the left ovary and the remaining came from the right ovary (Table 1). Mean (±SD) interovulatory interval was 25.4 ± 3.6days Nearly 12 % of interovulatory intervals were out layers from the normal range (≤ 15 days).Although a large follicle of size 41.2 mm was detected to be anovulatory , there was a significant difference between mean sizes of ovulatory and anovulatory follicles (P<0.01)(Table 2).

Table 1: Frequency of ovulatory and anovulatory cycles in left and right ovaries

Side involved	Ovulatory cycles	%	Anovulatory cycles	%	Total	%
Right ovary	16	44.4%	10	55.6%	26	48%
Left ovary	20	55.6%	8	44.4%		52%
Total	36	(66.7%)	18	33.3%	54	(100%)

Table 2: Mean (±SD) peak follicular diameter of ovulatory and groups of anovulatory cycles.

Type of follicle	Mean(± SD)	Frequency	Range
Ovulatory	36.81±3.8	29(61%)	[25.2 – 44]
Anovulatory [≥25mm]	29.87±5.3	13(27.7%)	[25.15 - 41.2]
Anovulatory [≤25mm]	21.48±1.9	5(11.3%)	[19.3 - 23.9]

4.2. Progesterone profile

Serum progesterone profile confirmed the presence of ovulation through out the study period with relatively fewer ovulations during the December and January. A considerable individual variation was observed in the serum levels in different cycles with in the animal. The highest serum Progesterone level was detected at mid luteal phase was 11ng /ml and lowest being observed at around ovulation was <1ng/ml. The pattern of serum progesterone level closely followed the presence of active corpus luteum. No statistically significant difference was observed between serum progesterone concentration and body condition score of individual jennies.

4.3. Observational studies

4.3.1. Estrus synchronization

Among jennies injected with PGF2α for synchronization, 66.6% were observed with estrus signs with in 72 hours after first injection $PGF_2\alpha$ and all the remaining observed estrous cycles through out the study periodi were seen with in one week interval. Immediate adverse effects of colic like symptoms were also imminent on 77.7 % of experimental Jennies administered with in 3 hours post administration. Symptoms like flank looking (71%), increased frequency of raising and lying (42.85%) and frequent urination (85.7%) were observed during PGF2α treatment.

4.3.2. Estrus and estrous cycle

All the study jennies were observed for a total of 119 AD. The mean duration of estrus manifestation after monitoring 27 jenny estrus periods was 7.85±2.99 days (range = 3-16 days). The most frequent behavioral signs observed were standing to be mounted, vocalization, positioning, winking of vulva and clitoris, increased frequency of urination, jawing, salivation, chasing of the male, flehman reaction and mounting of other jennies. The relative incidence of each of the major behavioral signs is indicated in Table 3. Estrus length was also found to be positively correlated to size of preovulatory follicle at onset of estrus ($r = 0.412$, $p<0.05$) and negatively correlated to interovulatory interval ($r = -0.548$, $p<0.05$).

Table 3: Estrus signs of jennies during 119 animal day observation.

Estrus sign	Frequency in jenny day	Percentage
Standing to be mounted	119	100%
Jawing and depression of the ear against the neck	119	100%
Positioning	61	51.30%
Chasing the male	29	24.2%
Winking of the vulva and clitoris	99	83.19%
Mounting of other jennies	18	15.20%
Courting of the jack	41	34.5%
Vocalization	52	43.70%
Flehman reaction	13	10.9%

Nearly one fourth (24.7%) of all estrus signs (551 Jenny estrus sign observation) were observed with in three days before the decline from peak follicular growth in anovulatoty cycles and three days before anticipated ovulation time in ovulatory cycles (Table 4). Overt signs of estrus was associated with peak follicular growth ($P<0.001$). The mean (±sd) length of estrous cycle was 24.22 ± 7.4 days. Out of the total estrus periods, 82.14% were associated with ovulatory follicles while the remaining 17.86% of estrus periods end up with anovulatory/atretic follicles. Only 2.5% (N=551) of the jenny day estrus manifestations were observed for days beyond ovulation.

Table 4: Relative incidence of estrus signs during the 119AD observation with reference to day of ovulation or after peak follicular diameter

Heat sign	Days from ovulation/peak preovulatory growth								
	-15	-13	-11	-9	-7	-5	-3	-1	+1
Standing to be mounted	1.7%	1.7%	4.2%	5.9%	17.7 %	21%	21.9%	21.9%	4.2%
Jawing	1.7%	1.69%	4.2%	5.9%	17.7 %	21%	21.9%	21.9%	4.2%
Positioning	1.6%	1.64%	3.3%	6.6%	18.03%	23%	24.6%	23.3%	0
Chasing the male	0	0	3.5%	6.9%	10.35%	20.7%	24.1%	34.5%	0
Winking of vulva and clitoris	1%	1%	1%	8.1%	17.2%	21.2%	24.2%	26.3%	0
Mounting of other jennies	5.6%	0	5.6%	11.2%	16.8%	22.4%	27.8%	11.2%	0
Courting the jack	2.4%	2.4%	4.9%	7.3%	17.1%	26.8%	29.3%	9.8%	0
Vocalization	0	1.9%	3.8%	5.8%	13.5%	21.2%	28.9%	19.2%	5.8%
Flehman reaction	0	0	0	7.7%	23.1%	7.7%	46.2%	7.7%	7.7%

4.3.3. Body condition score and ovarian dynamics

Preovulatory follicle size was found to be statistically correlated with body condition scores ($r = 0.522$, $P < 0.001$). Mean body condition score at group level was more or less maintained constant after period of two month acclimatization except last month of the study period (April). Preovulatory follicle size was found to be positively correlated to body condition score of individual animals ($r = 0.522$, $P < 0.001$). Incidence of ovulation, size of preovulatory follicle and interovulatory interval were significantly different from the rest of study periods. The highest ovulation rate 10/11(90.9% follicles attaining >25mm) ($P<0.005$) and the largest mean monthly preovulatory follicle size (37.81 ±1.83) ($p<0.001$) was observed

5. DISCUSSION

5.1. Ovarian follicular activity

The number of follicles detected per ovary at a time was 5.45 ± 2.25 ranging from 1-16. Similar observations of 1-13 follicles were made in jennies under traditional management (Lemma, 2006a). The current experimental study observation has revealed 89 % of ovulations occurred when the size of preovulatory follicle attained ≥ 33.5 mm sizes. Reports of several study observations were in line with present study findings. The size ovulatory follicle in jennies was indicated for size ≥25–30 mm (Henry *et al.*, 1987; Purdy, 2002). The overall mean of the first biggest follicle encountered in this study (22.3±7.9) was more or less similar to findings in previous studies on jennies under traditional management in both seasons (Alemayehu, 2004).In this study the growth of preovulatory follicle after day of divergence (1.89±0.33mm) was similar to the previous findings of rate of follicular growth at time of estrus 2.3 ±0.53 (intervals overlap) (Alemayehu ,2004).Certain indicators of follicular activity like size of preovulatory follicle at wave emergence and the day of divergence ,size of preovulatory follicle before ovulation, average number of follicles detected per ovary observed in similar studies undertaken in previous studies were generally lower compared to measurements observed in this study.

Under the present observation, 33.3% of the overall cycles (including ovulations in May), and 39% of cycles up to April were anovulatory. Of the anovulatory cycles observed up to April , 11.3% of them were having largest follicle below 25 mm size and 27.7 % being anovulatory after achieving preovulatory size. Higher proportions of anovulatory cycles were observed in previous studies at the same annual period in traditionally managed donkeys by Alemayehu (2004).The differences might have been most probably due to absence of work load and improved nutritional management followed in this study trial. Anovulatory phases have been highlighted as both physiological and pathological events commonly seen in equine. Ovulation failure (anovulatory follicles) is indicated as a normal physiologic event for the mare during the spring and fall transition periods. Anovulatory follicles of up to quite large sizes have been reported to occasionally occur during the physiologic breeding season (Ginther, 1992).Anovulatory follicles persisting for up to 2 months with abnormal estrus behavior and prolonged interovulatory intervals (Meyers, 1995) have been found documented. The cause of ovulation failure has been suggested to be endocrine in nature, either from a lack of sufficient pituitary gonadotropin stimulation to induce ovulation or from insufficient estrogen production from the follicle itself for preovulatory LH surge (McCue, 2000).

In the current study observations, 56% of the overall ovulatory follicles were from left ovary. Neither of the ovulatory cycles were bilateral nor multiple at the same time. The present finding of slightly greater ovulations in left ovary (Jennies) was in agreement with the findings indicated for equines in Arthur, 2002.

Under the current study, 11.8% of interovulatory cycles were with very short intervals which might have been resulted due to premature destruction of the corpus luteum (luteolysis). The most common cause of premature luteolysis in the mare has been associated to endometritis resulting inflammation of the endometrium and resulting synthesis and release of prostaglandins to cause luteal regression (McCue, 2000).

Day of divergence in diameter between the dominant follicle and subordinates estimated from regression of standardized follicular data was eminent when dominant follicle attain size of 24mm.This finding was almost in agreement with the findings in mare starting at 22.7mm reported in mares (Ginther *et al*., 2005).

5.2. Progesterone profiles

The present assay findings of serum progesterone profile has shown peaks >11ng/ml and levels of progesterone below 1ng/ml that has indicated the existence of cyclicity via out the study period. The trend encountered on serum progesterone was in line with the activity or life span of active corpus luteum. However no significant difference observed in serum progesterone levels between each jennies in relation to body condition scores in the study period. These findings were in agreement with previous study finding of <1ng-11ng/ml (Carlucio *et al*.,2008). Plasma progesterone concentrations of jennies ranging from 2.2 ng /ml (estrous cycle day 7) to peak levels >9 ng/ml (days 12–18) then dropping to <1ng/ml right before ovulation have been reported (Carluccio *et al*., 2005).Although the upper limits of progesterone concentration could not be taken with out uncertainty due to cross reaction indicated with other steroid blood components, its usefulness for indication of cyclicity is of paramount importane.

5.3. Estrus and estrous cycle

Under the current study, mean ± SD observed estrus length was 7.85±2.99, ranging from 3 to16 days while the observed estrous cycle length was 24.22 ± 7.4 days ranging from 7-34 days. Similar reports of varying estrus cycle length ranging from 20–40 days (Blanchard *et al.*, 1999; Ferlding,1988 ; Pugh, 2002; Purdy,2002) were encountered. Estrus duration of ranging 5-10 days, with ovulation 5–6 days after the onset of estrus (Vandeplassche *et al.*, 1981; Ferlding, 1988; Purdy, 2002) were reported. Extreme short cycle length observation of 7 days might be associated with premature regression of corpus luteum which is indicated as in cases of extreme short interovulatory intervals.

Behavioral estrus signs of standing to be mounted, mouth opening, jawing and ears back, winking of vulva and clitoris, positioning through back ward movement, vocalization kicking of jack to trigger mounting, chasing male when failed to mate, mounting of Jennies when jack is absent, Flehman like manifestations were observed. Similar observations of behavioral estrus characterized by mouth opening and closing (occasionally with the neck extended), chomping with salivary dribbling, winking, urinating were reported (Clayton *et al.*, 1981;Ferlding, 1988;Alemayehu,2004) which were similar to current observation. Manifestations of homotypical (female like) estrus signs o several heterotypical (male like) sexual behaviors (42.88%) such as mounting, herding/chasing, teasing and /or Flehmen response were documented on pastured jennies (Henry *et al.*, 1998). Although interovulatory interval was interchangeably used with estrous cycle, the interval between onsets of successive heat episodes was used for estrous cycle to include heat cycles observed in anovulatory cycles.

5.4. Body condition score and ovarian dynamics

In this study, the changes in preovulatory follicle size, proportion of ovulatory cycles in each of the study periods as well as estrus manifestation were significantly correlated,increased in last months of the study period. There was also variation in the above variables between jennies of different BCS. Although the effect of feeding regime on changes in body condition scores could not be appreciated directly due to gradual change, the changes in parameters used for characterizing ovarian dynamics were reported to vary with respect to body condition scores in individual animals. These changes in both ovarian as well as general reproductive parameters following seasonal nutritional availability were shown in several of the previous study reports in donkeys (Alemu *et al.*, 1997). The positive correlation between higher BCS and increased follicular activity (both in number and diameter of follicles) was also reported in for different livestock species, such as horses (Gentry and Thompson, 2002; Godoi *et al.*, 2002), cattle (Waltner *et al.*, 1993; Dominguez, 1995) and sheep (Towhidi *et al.*, 2003; Wettemann *et al.*, 2003). More recently, results from field studies on Jennies has indicated the positive correlation between number and size of ovarian follicles (both growing and pre ovulatory follicles) with seasons of nutritional abundance, higher follicular activity between March and September (wet seasons) being apparently associated with the relatively better body condition during the same period (Lemma *et al.*,2006a). High rates of conceptions were induced in cattle by stimulation of follicular growth via favorable nutritional conditions after rains (Falvey and Chantalakhana, 1999). This indicates the presence of proportional seasonal fluctuations in the individual energy status that have direct bearing on ovarian activities. As observed through different studies of tropical animals, follicular activities of jennies in this study is generally a function of the body condition which is indirectly influenced by seasonally varying quality and quantity of forage resource (Churcher, 1993; Thatcher and Hansen, 1993).The energy-consuming heavy work falling on the dry season can further affect follicular activity by increasing the nutritional requirement, primarily for energy, and by reducing the time available to eat. This seems to have exerted an extra energy challenge for normal ovarian activities (Dominguez, 1995; Oussaid *et al.*, 2000) that was not compensated by the traditional supplementation regime.

The correlation between the increase in the number and diameter of follicles during the period of increasing rather than decreasing BCS is attributed to the effect of positive energy balance. This agrees with similar findings in ewes (Rhind and McNeilly, 1998) and mares (Gentry and Thompson, 2002). Follicular activity and body condition during transition between seasons Improvement in ovarian activity during transition is most likely the result of the combined effects of sudden change in feed type, improved nutritional status and change of management (the relative rest from the heavy work during the dry season). Hughes *et al.* (1980) reported that ovarian activity leading to the growth and ovulation of follicles is stimulated in mares that turn from dry feed to fresh grass in spring. Similar conditions have also been observed in cattle (Falvey and Chantalakhana, 1999; Paterson *et al.*, 2003). The fact that both the number and largest diameter of follicles decreased when the animals entered the next dry season from the previous wet season further shows that drop in the body condition also results in suppression of the ovarian activity.

In mammals, nutrition exerts a significant influence on reproductive function through changes in body weight and condition (Downing and Scaramuzzi, 1991) affecting processes of follicular development and then ovulation rate (Scaramuzzi *et al.*, 2006).

Marked effect dietary energy intake on reproductive functions in a number of species has been highlighted. Body condition is a function of dietary feed intake. Improving the body condition of cows and ewes at mating for increasing pregnancy rates, reducing the interval between parturitions and overall increase of ovarian activity was significantly implicated. Furthermore requirement of a minimum level of body fat for adequate reproductive performance was indicated (Frisch, 1980). Studies in mares entering the breeding season or foaling in low body condition was also reported for having prolonged post partum intervals, reduced conception rates and required more cycles per conception than mares entering the breeding season in better body condition scores (Henneke, 1981). More recently, indications of increased ovarian activity, in goats, either by short-term or long-term nutritional inputs mediated by mechanism acting at a local level, through changes in insulin in a non LH-dependent manner was highlighted (Meza-Herrera, *et al*, 2008). Short-term fasting of mature ewes during diestrus results in increased serum concentrations of progesterone and a delayed pre-ovulatory surge release of LH and reduction in total numbers of follicles (Alexander *et al.*, 2007).

Restriction of dietary energy to 40% of maintenance levels for 13–15 days suppressed ovarian follicular development and resulted in anovulation in 60% of beef heifers (Mackey *et al.*, 1999). Short-term feed deprivation depressed secretion of estradiol and LH in Syrian hamsters (Morin, 1986), LH, testosterone, and glucose in rhesus monkeys (Cameron *et al.*, 1993; Cameron, 1994), LH in prepubertal gilts (Foxcroft and Cosgrove, 1994), and milk-fed ovariectomized prepubertal lambs (Foster and Olster, 1985; Foster *et al.*, 1989). Limited feed resources was also indicated to reduce reproductive efficiency to an extent dependent upon the degree of feed restriction (Mackey *et al.*, 2000) and reproductive status at the time of feed restriction (Smith, 1988). Ovulation rate was decreased in protein-restricted (Smith, 1988) and fasted ewes (Killeen, 1982).

5.5. Heat synchronization

Under the current study, 5mg/kg was used based on the recommendations of the manufacturer .It was observed to induce heat signs (though not overt as natural one) was observed with in 72 hours of administration. This observation was in agreement with previous reports of 2-5 days after $PGF_2\alpha$ treatment with doses 1.25-10mg (Barker, 2006).Immediate signs of colic like symptoms were observed in the present study post administration of $PGF_2\alpha$ which could be attributable to exaggerated effects in inducing myometrial contraction. Activity of prostaglandins on uterine musculature has been highlighted in different study observations. Natural prostaglandins (PGF-2α) also known to produce potent myometrial activity (Pashen *et al*., 1982; Ley *et al*., 1989) in mare ; synthetic prostaglandins increasing both frequency and amplitude of uterine contractions in sows (Langendijk, 2003).Transient sweating and colic-like symptoms have been frequently seen commencing 5 to 10 minutes after normal recommended dosage administration in mares (Wüstenhagen *et al*., 2002; Bruce, 2006). Side effects may be due to constriction of the smooth muscle of the body, which includes muscle under the skin and also uterus (hence colic-like symptoms as a result of cramping). Side effects were avoided using 0.1-0.5mg given twice at 24 hours interval with good desired clinical effects (Irvine *et al*., 2002; Handlera *et al*, 2004).

6. CONCLUSIONS AND RECOMMENDATIONS

The present study findings have shown ovarian dynamics in jennies as prototype and typical to parameters previously described for the equines and studies on the same species under traditional management system. Both ultrasonographic findings and plasma progesterone levels revealed the presence of cyclicity and ovarian follicular activities during the months from December to April. Overt estrous manifestations, as opposed to early and subsiding signs, were found to be good indicators of imminent ovulation. Improved follicular activities as measured by higher incidence of ovulation and larger sizes of preovulatory follicle were clearly remarkable cues for the effect of exemption from heavy work, poor feeding and housing systems prevalent in the traditional management system. The initial period of the study characterized by irregular follicular activity was most probably as a result of the shift from the previous management system and time for acclimatization. Parameters of ovarian dynamics considered in this study like the day of divergence, diameter of preovulatory follicle, diameter of the dominant follicle at the day of divergence and mean number of follicles were slightly different from previous reports with most probable implication of the effect of improved management. However it still remains to be answered how the individual components of management factors were involved in changing the ovarian follicular activity. Based on these observations, the following recommendations are forwarded.

- As this study was carried out in one unique season, further studies involving all the seasons are required to verify the effect of season on follicular activity under controlled management.
- Although the animals were under known management system, the individual and relative effects of each factors such as work load and nutritional status on ovarian follicular dynamics have not been ruled out which necessitate further research to be undertaken in order to validate this aspect by controlling individual factors at a time.
- In the traditional equine production system, donkeys mate at first opportunity aggravating conditions of the resource poor owners with respect to space and feed resources. Therefore it is time for some of the extension works to be introduced in controlling reproduction. In this regard knowledge gathered through such studies as determination of heat signs selection of breeder, optimal management skill like the case in any other species must be transferred to donkey owners for implementation.

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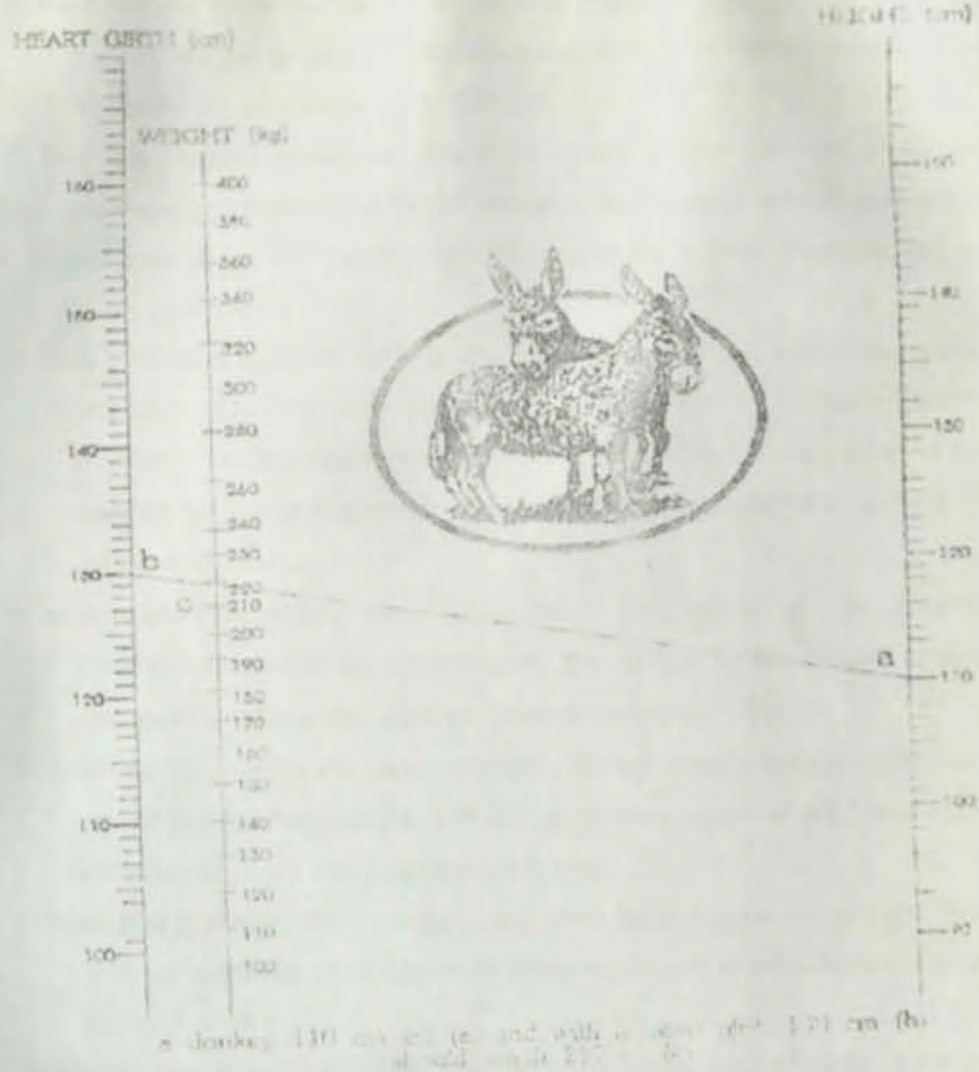
8. ANNEX

Annex 1: Heart girth Nomogram



(Source: Svendson. 1997)

Annex 2: Body condition scoring

1- **Poor**: Animal extremely emaciated. Spinous processes, ribs, tailhead, and point of hip and point of buttocks project prominently; bone structure of withers, shoulders, and neck easily noticeable; no fatty tissue can be felt.

2- **Very thin**: Animal emaciated. Slight fat covering over the base of spinous processes; transverse processes of the lumbar vertebrae feel rounded; spinous processes, ribs, tailhead, and point of hip and point of buttocks prominent; withers, shoulders, and neck structures faintly discernible.

3- **Thin** : Fat built up about halfway on the spinous processes; transverse processes cannot be felt; slight fat cover over ribs; spinous processes and ribs easily discernible; tailhead prominent, but individual vertebrae cannot be identified visually; point of buttocks appear rounded but easily discernible; point of hip not distinguishable; withers, shoulders, and neck accentuated.

4- **Moderately Thin**: Slight ridge along back; faint outline of ribs discernible; tailhead prominence depends on conformation, but fat can be felt around it; point of hip not discernible; withers, shoulders, and neck not obviously thin.

5-**Moderate** :Back is flat (no crease or ridge); ribs not visually distinguishable but easily felt; fat around tailhead beginning to feel spongy; withers appear rounded over spinous processes; shoulders and neck blend smoothly into body.

6- **Moderate to fleshy:** May be slight crease down back; fat over ribs spongy; fat around tailhead soft; fat beginning to be deposited along the side of withers, behind shoulders, and along the sides of neck.

7- **Fleshy**: May have crease down back; individual ribs can be felt, but there is noticeable fat between ribs; fat around tailhead soft; fat deposited along withers, behind shoulders, and along neck.

8- **Fat** Crease down back; difficult to feel ribs; fat around tailhead very soft; area along withers filled with fat; area behind shoulder filled with fat; noticeable thickening of neck; fat deposited along inner thighs.

9- **Extremely Fat** : Obvious crease down back; patchy fat appearing over ribs; bulging fat around tailhead, along withers, behind shoulders, and along neck; fat along inner thighs may cause them to rub together; flank filled with fat (Source: Pearson and Quasat, 2000)

Annex 3: Age estimation in donkeys on tooth eruption (Svendson,1997)

Age	Changes in dentition
2.5years	Permanent central incisors erupt
3.5 years	Permanent lateral incisors erupt
5 years	Permanent corner incisors just in wear, they erupt at 4.5 years
6 years	Teeth oval infundibulum
8 years	Dental star now appearing in the central incisors
11 years	Infundibulum of central and meddle incisors becoming shallow Incisors quite long and dental star apparent in all teeth
14 years	Pronounced triangular shape infundibula further back on the tooth and wearing out
15-20 years	Infundibulum disappeared from central incisors, triangular shaped teeth

9.CURRICULUM VITAE

Name:	Hassen Kebede Hassen
Nationality:	Ethiopian
Date of Birth:	26/07/1975
Place of Birth:	Woldelulo
Woreda	Tehuledere
Zone	South Wollo
Religion:	**ISLAM**
Language:	Amharic: Speaking and writing
	English: Speaking and writing

Educational Background:

1982-1988:	Primary education at Woldelulo elementary school
	School certificate award, Woldelulo
1989-1990:	Sulula Primary and Junior Secondary School
1991-1994:	Hayk Senior Secondary high school
	Leaving Certificate Award,
1995-2000:	Addis Ababa University. Faculty of Veterinary Medicine.
	Degree of Doctor of Veterinary Medicine
2006-2007:	Addis Ababa University .School of Pharmacy. Addis Ababa
	Master of Science Degree in Pharmacology
	(Regular program).
2006-to Date:	Addis Ababa University Faculty of Veterinary Medicine
	Master of Veterinary Science in Tropical Veterinary Obst. & Gyna.
	(Summer program)

Employment Background:

September 2001- January 2004: BoARD Metema Wereda District Veterinarian

February 2004 - December, 2006: Head of Livestock Production and Veterinary Service

January 2006 - June, 2006 BoARD District Officer

Other Skills and Experiences:

- Computer literate: Word and Excel
- Participatory rural appraisal
- Planning and assessment of community based projects

Project Experience

Integrated Livestock Development Project (ILDP) 2003-2006

United Nation Capital Development Fund (UNCDF) 2003

Improving Productivity and Market Success (IPMS) 2004-2005

Research Experience:

- A survey on health status of horses in the highlands of North Gondar - DVM thesis
- survey on major ethnoveterinary practice in north western lowlands of Amhara region
- Assessment of trypanocidal drug resistant *Trypanosoma congolense* isolates and survey on trends in trypanocidal drug management practices in selected districts of Western Amhara Regional state, Ethiopia, Msc – thesis
- Ultrasonographic studies of ovarian dynamics and associated estrus manufestations of jennies under controlled management

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SIGNED DECLARATION SHEET

I, the under signed, declare that the thesis is my original work and has not been presented for a degree in any University.

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Date of submission 25/06/2008

This thesis has been submitted for examination with our approval as University advisors.

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