Awe
3875
2009

ADDIS ABABA UNIVERSITY

FACULTY OF VETERINARY MEDICINE

Study on Breeding Soundness and Fertility of Senar Jackass *(Equus asinus)* in North Gondar, Amhara Region, Ethiopia

BY

AWEKE TSEGA ABERRA

JUNE 29, 2009

Debre Zeit

ETHIOPIA

ADDIS ABABA UNIVERSITY

FACULTY OF VETERINARY MEDICINE

Study on Breeding Soundness and Fertility of Senar Jackass *(Equus asinus)* in North Gondar, Amhara Region, Ethiopia

A thesis submitted to the School of Graduate Studies of Addis Ababa University in partial fulfillment of the requirement for the Degree of Master of Veterinary Science in Veterinary Obstetrics and Gynecology.

BY

AWEKE TSEGA ABERRA

JUNE 29, 2009

Debre Zeit

ETHIOPIA

Study on Breeding Soundness and Fertility of Senar Jackass *(Equus asinus)* in North Gondar, Amhara Region, Ethiopia

BY

AWEKE TSEGA ABERRA

Boards of External Examiner **Signature**

1) Dr. Alemu Yami ....
2) Dr. Adugna Tolera ....
3) Dr. Kassahun Awgichew ....

Academic Advisors

Dr. Alemayehu Lemma ....

Prof. Merga Bekana ....

ACKNOWLEDGEMENT

In appreciation for the great efforts toward making me a better student, my thanks go to my advisor Dr. Alemayehu Lemma who also provided me with most of the research equipment. His patience and understanding encouraged my interest in learning, and his motivation compelled me to reach my goals as a graduate student. Thanks, also to my co-advisor Prof. Merga Bekana, for his support in the correction of this manuscript.

I wish to express thanks to Drs. Tsegaw Fente, Desalegn Mengesha, and Shimelis Dagnachew, Ato Bidir Zegeye, Ato Tadegagni and staff of University of Gondar, Faculty of Veterinary Medicine, they were providing me with the necessary laboratory space and a place for the experimental Donkeys.

I also would like to extend my gratitude to my fellow graduate students for their invaluable support and advice. I would also like to thank North Gondar Department of Agricultural and Rural Development, the Sustainable Natural Resource Management Program of North Gondar, and Dabat Woreda Agricultural and Rural Development Office for their provision of vehicle during my research period.

A special thanks is extended to my wife (W/ro Amaru Anagaw), my brothers: Tadesse, Mogessi, Werkineh, Manahilo, Asmamaw and Hibreworki Birhane Tsegazeab; my sisters: Abeba, Atsede and Abebechi Tsegazeab; and my children: Eyobi and Dagimawi Aweke), who together have been behind me from the beginning. Without their love, encouragement, and support, I would never have attained my goal of becoming a graduate student of Addis Ababa University, Faculty of Veterinary Medicine.

Finally, I would like to extend my thanks to Addis Ababa University, Faculty of Veterinary Medicine for overall support during the two years Masters programme.

ABREVIATION

AI	ARTFICIAL INSEMINATIN
AV	Artificial vagina
CBPP	Contagious Bovine Pleuro Pneumonia
CPA	Cryoprotective Agents
CSA	Central Statistic Authority
DIC	Differential interference contrast
DOA	Department of Agriculture
eCG	Equine chrionic gonadotrophin
EHV	Equine Herpes Virus
ET	Embryo Transfer
EVA	Equine Viral Arthritis
HOST	Hypo-osmotic Swelling test
PPR	Peste des Petitis Ruminants
TB	Tuberculosis

TABLES OF CONTENTS

PAGE

ACKNOWLEDGEMENT I

ABREVIATION II

LIST OF TABLES V

LIST OF FIGURES VI

LIST OF ANNEX VII

ABSTRACT VIII

1. INTRODUCTION 1

2. LITERATURE REVIEW 4

2.1 Donkey breeds and breeding methods in Ethiopia. 4

2.2. Advantages of artificial insemination (AI) in equine breeding 4

2.3. Breeding soundness evaluation in equine 5

2.3.1. Reproductive anatomy and physiology of the equine 6

2.3.2. Selection of the male and fertility prediction 9

2.4. Semen collection 11

2.4.1. The artificial vaginal 11

2.4.2. Preparation of the jackass and the semen collection area 13

2.4.3. Frequency of semen collection 14

2.5. Semen evaluation and handling 14

2.5.1. Gross evaluation of semen 16

2.5.2. Microscopic evaluation of semen 17

2.5.3. Other tests of semen quality 18

2.6. Semen processing and storage 19

2.7. Insemination of the females and pregnancy diagnosis 20

2.7.1. Ultrasound as a tool for pregnancy diagnosis in jennies and mares. 21

3. MATERIALS AND METHODS 23

3.1. Description of the study area 23

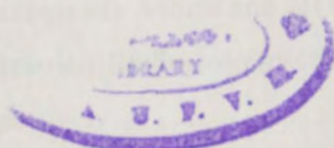
3.2. Study animals 24

3.3 Study Design 25

3.3.1. Evaluation of the phenotype and the sexual behavior of the study jacks 25

3.3.2. Collection and evaluation of semen 26

3.3.3. Teasing, collection and semen handling........27
3.3.3. Fertility assessment........28
3.4. Statistical analysis........29
4. RESULT........30
5. DISCUSSION........38
6. CONCLUSION AND RECOMMENDATION........41
7. REFERENCE........42
ANNEXI........51
CURRICULUM VITAE........55
DECLARATION LETTER:........60



LIST OF TABLES

Table 1: Description of donkey types present in Ethiopia. 4

Table 2: Summary of advantages and disadvantages of the most commonly used artificial vaginas in equines 12

Table 3: Summary of results of semen analysis for different domestic animals 15

Table 4: Summarized phenotypic measurement of studied Senar jackasses and randomly observed Senar Jackass. 30

Table 5: Copulatory sequence of semen collections [n=160] of Senar jackasses 31

Table 6: Correlation between total and gel-free semen, and concentration of spermatozoa in 160 semen collections. 33

Table 7: Relationships between total semen volume and mass motility, percent of live spermatozoa, percent morphologically normal and pH of semen sample 34

Table 8: Summary of the ultrasonic findings on inseminated jennies and mares at day 28 and day 28 of post insemination, respectively. 37

LIST OF FIGURES

PAGE

Figure 1: Map of the study area in North Gondar, Amhara Region, Ethiopia 23
Figure 2: A partial display of laboratory equipment used for semen evaluation in donkeys 26
Figure 3: The distribution of sex drive among the study jacks during semen collection (n=160) 32
Figure 4: Plot of progressive motility of spermatozoa 35
Figure 5: Live: Dead spermatozoa picture and Morphological analysis 36
Figure 6: Intra-vaginal AI in jennies, (left panel) and intra-vaginal AI in horse (right panel) 36
Figure 7: Ultrasonographic picture 28 day's old embryo of donkey (left) and horse (right) 37

LIST OF ANNEX

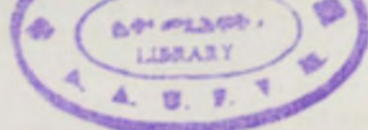
Annex 1: Sexual behaviour measurement formate 51
Annex 2: Gross semen evaluation 51
Annex 3: Microscopic semen evaluation........ 52
Annex 4: Phenotypic characterization senar donkey 53
Annex 5: Mounting without penile Erection Annex 6: The Missouri model equine AV unassembled and assembled from left to right used in the study with 53
Annex 7: Schematic representation of the testis and associated epididymis. The tunica albuginea (a)' seminiferous tubules (b) and interstitial tissue; rete testis (f); efferent ducts (c); epididymis forms a single duct that spans up to 45 m in length and consists of an initial segment (d). a caput (or head; e). a corpus (or body; g). and a cauda (or tail; h). which, in turn. connects with the ductus deferens (i)........ 54

ABSTRACT

This study was carried out with an objective of evaluating the breeding soundness, fresh and chilled semen, and fertility of Senar jackasses. The physical characteristics such as the body weight, height, body condition, and age of the jacks was measured and recorded. The characteristics of sexual behavior as determined by score of the sex derive, and the time taken for erection, first mount and intromission were assessed in the presence of estrous jennies and recorded before semen collection. Semen was then collected using Missouri model artificial vagina. The semen was subjected to both gross and microscopic evaluation procedures after which the fertility of the semen was determined by inseminating jennies and mares of known fertility. The body weight of the jacks range between180 and 332 kg, with mean body condition score of 7. The mean (± SD) of height at wither was 118 ± 12.39 cm while the heart girth measured 131.8 ± 27.62 cm. The mean (± SD) scrotal circumference [cm], scrotal length [cm] and width [cm] were 25.08 ± 3.35, 16.8 ± 2.39 and 9.28 ± 5.67, respectively. Jacks mounted on average for 4.61 times before developing full erection. The mean (± SD) time taken for first erection, first mount, and total time taken for ejaculation were 12.11 ± 7.15, 13.45 ± 7.01, and 23.06 ± 7.75 minutes, respectively. Most of the time (88.1%) during the collection, the jacks took less than 30 minutes with a sex derive score ≤ 2 to complete semen collection. The longest time to ejaculation was 58 minutes. The mean (± SD) score of sex drive, number of ejaculatory thrust, number of mount without and with erection, were 23.03 ±7.78, 4.28 ± 0.6, 4.61 ± 1.92 and 1.9 ± 0.88, respectively. The most prominent sexual behaviors of the jacks during courtship were vocalization, sniffing of the vulva, flehmen reaction, mounting with or without erection, naso-nasal contact, and herding. Vocalization usually occurred immediately after visual marking of the female. Vocalization was more intense in the presence of a competing jack in the vicinity of an estrous female. In 91.5% of the collection, the color of the semen was creamy white. The mean (± SD) total and gel-free semen volume [ml], gel volume [ml], and semen concentration [billion/volume] were in the order of 61.1±12.6ml, 50.3±12.3ml, 10.8±2.8ml, and 7.4±0.1 billion/volume, respectively. On the other hand, the mean (±SD) mass motility [%], live percent [%], and morphologically normal [%] were 93.5±2.4; 94%, 89.0±2.9, respectively. There was a significant correlation between total semen volume and gel-free semen volume ($r = 0.98$; $p<0.05$); between total semen volume and sperm concentration ($r = 0.65$; $p<0.05$). When semen volume was

categorized in to five intervals and analyzed, there was a significant deference ($p<0.01$) in spermatozoa concentrations among the volume groups. The highest spermatozoa concentration (0.685 billion/ml) was found in animals giving 100 ml total semen. The mean (± SD) of the longevity spermatozoa was measured at different older age from 15 minutes, 30 minutes, 45 minutes, 60 minutes and 120 minutes were 87.71 ± 3.67, 81.81 ± 4.5, 75.25 ± 5.9, 68.09 ± 7.46 and 50.28 ± 6.78, respectively. Sex drive did not affect any of the semen parameters. Pregnancy rate of the chilled semen after per-vaginal artificial insemination was 58.3% in Abyssinian jennies (n=12), 40% in Senar jennies (n=5) and 64.7% in mares (n=17).

Keywords: Artificial insemination, breeding, fertility, semen, jacks, Gondar



1. INTRODUCTION

Ethiopia has the largest equine population in Africa with estimated 6.1 million heads; over 69.24% of this number accounts for donkeys (CSA, 2004). But the tropical donkeys have received little from planner, extension and research workers. One reason for this may be a lack of knowledge on many aspects of this animal (Fielding and Krause, 1998). Various attempts have been made to classify African asses into breeds. In fact, many authors confirm that there are little recognized differentiations among the donkeys of Africa. This is probably due to a lack of breed characterization studies (Payne and Wilson, 1999). In Ethiopia, the basic stock is sometimes considered to be poor because of the random breeding that is most common and highly prevalent breeding method. In addition the information gap on the reproductive characteristics of donkeys is immense and the donkeys are frequently assumed to be physiologically similar to horses though contrary to the available scientific facts (Tefera, 2002).

Generally, four donkey types are believed to exist, unevenly distributed in all agro-ecological zones. The small-scale farmers and the highlands have the largest share with 2 - 3 animals per family and with female donkeys being most common (70%). Breeding ages were reported to be between 4 5 years for places like Adea, Bereh, Awassa, Gondar and Dire Dawa (Gebreab *et al.*, 2003; Lemma *et al.* 2006). Senar donkeys are one of the donkey types found in the Northwestern and central part of Ethiopia well known for their performance in mule production. According to Gebreab, *et al.*, (1997). Senar donkeys have better body conformation than other donkey types currently present in Ethiopia because mainly due to their relatively larger body size. Mules are reputed for their fitness and survival in harsh environmental conditions than the parents (Tefera, 2002). Due to their unique sexual features Senar male donkeys are expensive and are not easily accessible in all areas of the country. Moreover, anecdotal and unpublished information suggest that the work performance of Senar donkeys is nearly twice that of the most common Abyssinian types. They adapt easily in different agro-ecology, where they themselves and their hybrids are used frequently as a means of transporting goods, farm products, building materials, charcoal, fuel and wood, water for livestock and household use.

With the current livestock production system, the ever increasing competition between animals and human for resource is particularly sever in the central highlands of Ethiopia. Therefore, replacing the large number with few best performing animals would alleviate the tremendous stress both on the environment and human living condition. Donkeys are known to play a great role in the livelihood of the resource poor farmers. While they represent an indispensable transport system in areas whose landscape comprise rough terrain to accommodate motorized vehicles, efforts made to improve donkey's performance or even understand their biology is largely ignored. Application of biotechnologies particularly artificial insemination (AI) is of paramount importance in this regard, impacting both needs of improving performance and easing the environmental degradation at once.

Although the history of AI, which is the first generation of reproductive biotechnology, in Ethiopia goes back to 1938 (Heinonen, 1989), no attempt has ever been made in Ethiopia that has one of the largest Equine populations in Africa. There is virtually no effort made to improve equine breeding that are the powerhouse of the agricultural sector. At natural mating, the average fertile stallion and jack ass ejaculates 2-12 billion and 3.3-18 billion spermatozoa directly into the body of the uterus, respectively. Yet fewer than 100 spermatozoa pass through the uterotubal junction to reach the site of fertilization to give a per cycle conception rates of 60 – 70% (Allen *et al*., 2001). Hence, AI and breeding soundness evaluation of donkeys are needed to improve the reproductive performance, work performance through crossing, and produce mules that are more adaptive population.

Laboratory assessment of sperm quality is an essential procedure in many aspects of assisted reproduction in domestic species. The choice of adequate parameters by reproducible, fast, and sensitive methods is of increasing demand (Gadea *et al.* 2004). Donkeys tend to be more fertile than horses, having an average conception rate of 78% while mares average 65%. (Hagstrom, 2004). Expanded use of frozen semen in the equine industry is dependent on simplified breeding strategies. However, mares bred with frozen semen are often examined 4-6 times/day and inseminated immediately before or within 6hrs post-ovulation because of lower survivability of frozen-thawed spermatozoa in the reproductive tract (Squires *et al*., 2003). Recent studies (Samper, 2005) have shown that deep insemination in to the horn ipsilateral to the ovary with the

pre-ovulatory follicle results in 80% of the sperm remaining in that oviduct with-higher conception. With the technique becoming more and more refined, it is not uncommon to have selected stallions breed over 500 mares during a 6 month breeding season in many developed countries (Samper, 2005). On the other hand, with the apparent differences from horses, the efficient application of AI in donkeys, require an understanding of their peculiar behavioral and semen characteristics. In Ethiopia, there have been limited studies conducted regarding Abyssinian jennies (Lemma et al., 2006). Therefore, the objectives of the present study were:

1. To carry out breeding soundness and semen evaluation of Senar donkeys
2. To perform fertility evaluation of chilled Senar donkey semen

2. LITERATURE REVIEW

2.1 Donkey breeds and breeding methods in Ethiopia.

The common donkey (*Equus asinus asinus*) was domesticated some 5000 years ago (Protsch and Berger, 1973), from a race of the African wild asses *Equus asinus africanus* (Nubian wild ass) and *Equus asinus somalicus* (Somali wild ass) of Ethiopia and other parts of North East Africa (Payne and Wilson, 1999). Based on average height at withers four types of donkeys are recognized in Ethiopia: Jimma, Abyssinian, Ogaden and Sennar (Gebreab *et al.*, 1997).

Table 1: Description of donkey types present in Ethiopia.

Donkey Type	Height at withers [cm]	Other descriptions
Jimma Donkey	95-100	Head is large, ears short, grey colour
Abyssinian Donkey	86-102	Predominantly grey, widespread
Ogaden Donkey	103	Tall with heavy bones
Sennar Donkey	100-114	Large, 'better' body conformation

Source: Gebreab *et al.*, 1997

Except for the hand mating in mule production with Senar donkeys, the most prevalent method of breeding in Ethiopia is natural and uncontrolled where animals mate randomly at first opportunity in the filed (grazing land). There is also no proper selection.

2.2. Advantages of artificial insemination (AI) in equine breeding

A number of newer techniques are available to improve efficiency of reproduction, but most require a meticulous attention and competent technical expertise such as forced ovulation, estrus control, cooled or frozen semen, AI and embryo transfer (Daels, 2003). There are basically three breeding methods currently in use in equines: pasture breeding, hand breeding and AI (Blanchard, *et al.*, 2001). In pasture breeding the stallion is turned out to pasture or kept in a paddock with one or more mares. This requires minimum labor however; there are obvious

disadvantages like injury to stallion or mares, overuse of stallion or breeding of only easy mares, and inaccurate record keeping. Jennies, like mares, can be pasture or hand bred (Blanchard, *et al*., 1999). If natural breeding systems are used, the jenny should be mated the second day of estrus, and then at 48hr intervals until the end of standing heat (Fielding, 1998). The advantages of AI far outweigh its disadvantages. The major advantages are genetic improvement; controls of venereal diseases; improved record keeping; more economic than natural service when genetic merit is considered; safe to eliminating injury during natural service and availing geographical restrictions (Bearden *et al*., 2004; Davies, 2008).

AI has also the advantages of safer usage which minimizes injury from the male, has less contamination to the uterus, ensures insemination with viable and adequate numbers of sperm, promotes timed insemination of both normal and none "receptive" females, and reduce the cost of the transport of the females. It is also safe for male individual and prevents its overuse. The other advantages in the male are more mares can be bred with each ejaculate; ensures the use of a stud for breeding even if sick, injured or deceased with frozen semen. The known drawbacks of AI are that it is labor extensive, moderately expensive and requires skilled, experienced veterinary professional. Ex-copula semen collection using Imipramine/xylazine has been attempted with very limited success rate in donkeys (Sghiri *et al*., 2006). However, the use of frozen semen has been an important tool for maintaining this species in some parts of the world where they are kept as pet animals (Oliveira *et al*., 2006).

2.3. Breeding soundness evaluation in equine

Sexual behavior of jacks is known to be slightly different from that of the horse with regard to the time to erection and mounting. Jacks seem to have a slower copulatory response especially when breeding mares for mule production (Tibary *et al*., 2006). Breeding soundness evaluation in donkeys is performed in a more similar fashion to horses. However, several parameters such as testicular size, semen volume and total sperm per ejaculate are significantly higher in the jack when compared with the stallions (Tibary, 2005). Reproductive evaluations are necessary prior to purchase and as routine procedure prior to each breeding season or if a problem is suspected (Thompson, 1994). Pre-copulatory behavioral sequences include naso-nasal contact; nibbling and

or sniffing of the head, neck, back of the knee, body, flank, perineum and tail. Olfactory investigation of voided urine or feces; and flehmen are also very common features. Often these precopulatory sequences are evaluated as part of the mating behavior that is finally scored to give an estimate of the breeding soundness. Mating behavior should be evaluated because a male that scores well on other fitness criteria may not have high libido. The male should be observed in the presence of females that are in estrus. The evaluation may also include time to the first copulation, time to repeated copulation, number of copulation until sexual exhaustion, and time needed to recover from sexual exhaustion (Bearden *et al.*, 2004). Achieving an erection at a distance from jennies is one important characteristic of breeding behavior jacks. Spontaneous erection and masturbation occur at the same rate as in the horse with an episode of every 90 min (Tibary *et al.*, 2006).

The selection criteria for reproductive competence in the male equines are similar to those of female and can be listed as history of the male, temperament and libido; age; general body conformation; reproductive tract examination; semen evaluation; chromosomal abnormalities; blood sampling; infections and general stud management (Davies, 2008). The evaluation of semen quality is useful in predicting the fertility of sperm donors and is of great importance in maximizing reproductive efficiency, either under natural breeding conditions or in programs of assisted reproduction (Colenbrander *et al.*, 2003; Rodriguez *et al.*, 2001). Further, it is a useful tool in clinical diagnosis of subfertile animals. Conventional evaluation techniques have based on the subjective assessment of semen parameters such as motility, morphology and semen volume or concentration (Verstegen *et al.*, 2002).

2.3.1. Reproductive anatomy and physiology of the equine

A good understanding of the reproductive anatomy and physiology and the control mechanisms involved therein is an essential prerequisite for studying the applied aspects of semen collection, evaluation and storage in equine. The equine male's reproductive tract consists of testes, epididymis, penis and accessory sex glands (Dyce *et al.*, 1987). The testes are the site of spermatozoa production, and the primary site for androgenic hormone production hence they play a central role in the reproductive function of both jacks and stallions. The scrotum in equines is relatively short and non-pendulous compared to ruminants. The positioning of the testes outside

the main body cavity is necessary to maintain a testes temperature of 35 - 36°C, which is a requirement for optimal production of spermatozoa. Within the outer scrotal skin layer lays the tunica dartos, a muscle fiber and connective tissue layer, whose contraction aids in drawing the testes up towards the body. Seminiferous tubules consist of germinal cells at different stages of development and sertoli or nurse cells. The interstitial tissue of the testes is made up largely (21 - 57%) of Leidig cells responsible for the production of male androgen (Stabenfeldt *et al*., 1996).

At full erection, the penis in equines generally doubles in size to 80 - 90cm in length and 10cm in width. At ejaculation, the glans penis triple in size, forcing open the cervix to allow sperm deposition directly into the uterus and preventing initial leakage of semen from the female (Davies, 2008). The jack, like the jenny has to the mare, has many reproductive similarities to the stallion. However, some differences are noticeable in the testes and penis of the jack which tends to be larger compared that of the stallion (Kreuchauf, 1984; Pugh, 2002). Jack's reproductive organs are larger than that of the stallions. This is also true for ampulla, seminal vesicles and bulbourethral glands. During erection and ejaculation, the glans penis presents a more pronounced dilation. The scrotum is also more pendulant and testicles and epididymis are larger than in horses but present similar anatomical appearance. The epididymis consists of long and highly convoluted tubules measuring up to 45 meters in length and weighing up to 250gm each in a mature stallion while the testis weighs on average 276gm in jackass (Lemma and Derresa, 2009). The accessory glands responsible for production and secretion of the majority of the fluid component of semen termed seminal plasma are situated between the end of the vas deferens and the root of the penis. The vesicular glands which are paired, multi-lobed glands are the ones responsible for the production of the gel-like fraction of equine semen. The ampullae's glands are paired out folding of the vas where it meets the urethra. They do have a considerable contribution to both the pre-sperm and sperm-rich fractions (Davies, 2008).

A detailed comparison between the testes of donkeys and mules indicated that structures such as the leydig cells and sertoli cells are quite similar in morphology and function (Neves *et al*., 2005), demonstrating that the seminiferous tubules of mules might be able to sustain complete spermatogenesis of normal spermatogonia from donkeys hence with role as a desirable natural recipients for germ cell transplantation from donkeys (Chiarini-Garcia *et al*., 2009). The

ultrasonic features of the testicles are characterized by the presence of a large central vein and more prominent cauda epididymis and these features are more or less similar in both the jack and stallion (Tibary *et al.*, 2006).

Spermatogenesis is an extremely complex process in equines that involves germ cell proliferation, germ cell differentiation, and programmed germ cell death or apoptosis (Varner, 2007). This lengthy process, 57 days in the stallion which is slightly shorter than the 61 days in the bull, controlled by vast array of messengers acting through endocrine, paracrine and autocrine ways. Spermatozoal production occurs, like in other species of domestic animals, within the seminiferous tubules (Annex 7). Both ends of these highly coiled tubules open directly into the rete testis, such that the both cellular and non-cellular products of the seminiferous tubules are excreted into the rete testis and delivered to the excurrent duct system (Varner, 2007). The seminiferous epithelium consists of germ cells in various steps of development intermingled with sertoli cells that serve to provide structural support and a nurturing source to the germ cells.

Structurally, equine sperm consist of three areas: the head, the mid-piece and the tail with three distinct functions (Davies, 2008). The head is mainly made up of nuclear material, containing the haploid number of chromosomes. The head of the sperm has a double membrane: an outer cell membrane and an inner nuclear and acrosomal membrane. The mid-piece of the sperm contains a high proportion of mitochondria, organelles within the cell that produce energy. The mid-piece is, therefore, often termed the power plant of the sperm, providing the energy for metabolism and to drive the tail. The tail is made up of series of fibrils, equivalent to those found in the major muscle blocks of the body. According to Amann and Graham, (1993); Davies, (1999), stated that using the energy provided by the mid-piece, the tail is whipped from side to side, driving the sperm movement in a wave-like motion.

Most mammalian spermatozoa are incapable of in vivo fertilization on existing situation in the testis. The cells must undergo considerable post-testicular remodeling within the epididymis to acquire this ability. Spermatozoa in the epididymis are intrinsically capable of motility, (Johnson *et al.*, 1980) but do not exhibit motility until released from the epididymis. A threshold level of cyclic adenosine monophosphate (cAMP) is present in spermatozoa within the cauda epididymis.

Observable changes in spermatozoa during epididymal maturation, based on light microscopic analysis include acquisition of flagellar movement within the corpus epididymis, followed by a progressive pattern of spermatozoal motility within the cauda epididymis, and translocation and shedding of the cytoplasmic droplet (Varner, 2007).

Under natural conditions, regulation of spermatozoal motility occurs at three critical points: epididymal reservoir where there is suppression of motility; ejaculation where there is activation of motility; and oviductal reservoir that induce hyper activation of motility. Activated motility is considered necessary for propelling sperm into the oviductal reservoir (Varner, 2007). Specific motility inhibiting proteins have been identified in rat cauda epididymal fluid that, when removed, allow initiation of motility. A pH-dependent inhibitory factor has been reported in bulls (Carr and Acott, 1984). Although such inhibitory factors may exist, it is possible that sperm motility may simply be suppressed by the acidic pH of the epididymal environment. The pH of bull cauda epididymal fluid is 5.5 while that of the stallion and jackass are 6.9-7.7 and 7.6, respectively (Holt and Harrison, 2002; Gastel *et al.*, 1996). Caudal epididymal fluid of bulls, rams, boars, and stallions does not contain measurable quantities of bicarbonate which is known to be a key factor for spermatozoal motility in these species (Holt, and Harrison, 2002). However, bicarbonate is present at fairly high concentrations in seminal plasma and may be higher in seminal plasma of stallions than other mammals.

The stallion, like the mare, is a seasonal breeder in the temperate zone but tends to show a less-distinct seasonality around the tropics. Unlike the female, if the males are given enough encouragement, they are capable of breeding all year round even in temperate region (Davies, 2008). Sperm production, unlike ova production, is a continual lifetime process and is not governed by cyclical hormonal changes and as with ovulation (Davies, 2008). As with the female, the reproductive activity of the male equine can be divided into physiological and behavioral changes. The physiological changes are following hormone patterns such as LH and FSH; inhibin and activin, prolactin and oestrogen that are associated with reproductive activity and endocrinological control of male's reproduction. Behavioral changes depend on testosterone, the prime drive of male sexual behavior especially that associated with mating (Davies, 2008).

2.3.2. Selection of the male and fertility prediction

When a male equine has been accepted as a sire, it is necessary for regular monitoring of semen parameters at least once in a year. Regardless of the performance criteria used for selection, stock should also be selected on reproductive competence. However, all too often, such criteria are not considered, with potentially serious consequences for the individual breeder and the equine breed as whole (Davies, 2008). Regardless of the type of equine intended for breeding, reproductive competence and the ability to produce healthy offspring with minimal danger to the life and well-being of the dam is of prime importance. Equines, unlike other farm livestock, have been selected primarily for performance ability, often at the expense of reproductive competence. As a result, there are many potential reproductive problems that the breeder should be aware of in selecting both the female and the male (Davies, 2008). A stallion's reproductive temperament and willingness to cover termed as libido partly determines the reproductive potential. A male equine with a low libido will need to mount the female several times before ejaculation, taking up to 20 or more minutes to cover. It may show initial interest very reluctantly or may fail completely (Davies, 2008). The number of mounts per ejaculation and the time between actual intromission and ejaculation are good indicators of libido. The number of mount per ejaculation should be as near to one as possible and the time between intromission and ejaculation a matter of seconds (Thompson, 1994).

A variety of information should be recorded and analyzed for frequent evaluation of fertility. The protocol involves the collection of five daily ejaculates that are checked for the following indices: general parameters (gel free volume, gel volume, pH), sperm quantity (sperm concentration, total number in the ejaculate), and sperm quality (percent of total motile sperm at collection before and after dilution and after survival at 40°C, vitality estimated at collection, abnormal forms). Aspects of stallion behavior (mounting delay, number of mounts before ejaculation, total time of collection) are also measured (Pawlak *et.al.*2001). Proven repeatability of an accurate measurement of the different parameters in 5daily ejaculates is obtained in higher number of ejaculate (Davies, 1999). Every ejaculate of semen will contain some morphologically abnormal spermatozoa. The expected range of 8% to 10% has no adverse effect on fertility. If the accumulated total abnormal spermatozoa exceed 25% of the total in an ejaculate, reduced fertility can be anticipated (Bearden *et al.*, 2004). Abnormal sperm can be classified under abnormal head

(primary defect), abnormal tails (tertiary defect) and abnormal cytoplasmic droplets (secondary defect).

In summary, primary defects such as detached heads, head shape abnormalities, double heads, no mid-piece, rudimentary tails, highly coiled tails, and mid-piece abnormalities are thought to originate during spermatogenesis and so occur within the testis. Secondary defects such as tail abnormalities such as kinks, bends, coils or swellings, detached heads and tails, and protoplasmic droplets originate during the transport of the spermatozoa and then semen through the epididymis, vas deferens and urethra. Tertiary defects like loss of acrosome, fraying or thickening of the mid-piece, slight bending of the tail, detachment of spermatozoan heads and bursting of spermatozoan heads may result from inappropriate handling post ejaculation (Ricketts, 1993).

2.4. Semen collection

2.4.1. The artificial vaginal

The methods of semen collection in equines include the use of sponges, condoms; dismount samples and artificial vaginas (AV). Semen needs to be collected in an environment which closely mimics the female's vagina, but which eliminates the potentially detrimental effect of natural secretions. The aim is to encourage ejaculation and collect semen by minimizing any collection effect on the sample and on subsequent fertilization rates (Davies, 2008). There are three major types of AV, the Cambridge, the Nishikawa and the Missouri Models, that satisfy these criteria in slightly different ways (Table 2). Variables for any AV are temperature, pressure and liner. Initial internal temperature should be between 45 and 50^{0}C. On colder days this may necessitate filling with water at up to 70°C. Most disposable liners are made of plastic which some stallions dislike. Using an internal rubber liner may be preferable but will require cleaning and disinfection of the liner after use (Shepherd, 2008).

Extra manual stimulation may be required at the base of the penis or at the glans penis in some individuals. Both stallions and jacks need an initial training to ejaculate in to an AV but once accustomed to this procedure collection is much easier either on a dummy or jump mare.

Standard equine collection equipment can be used in donkeys. Both Prudy (2005) and Lemma and Derresa, (2009) have exclusively and successfully used the 16" Missouri AV model. In both reports, the jacks were initially teased to verify the jennet in good standing heat prior to introduction for collection. The internal water temperature of approximately 115 ^{0}F (47^{0}C) often suffice as in stallion.

Table 2: Summary of advantages and disadvantages of the most commonly used artificial vaginas in equines

Types of equine AV	Advantages	Disadvantages
Missouri (Annex 6)	lightweight and easy to manoeuvre, assemble and clean; much easier use in jacks, a low risk of water contamination; extra stimulation of the glans penis is possible	Not accepted by all stallions, depends on penis size
Cambridge/Hannover	Accepted by most stallions.	Heavier to handle.
Colorado	Well accepted by most stallions	Heavy to handle (requires 8 l of hot water).
The Nishikawa model or Japanese AV	lightweight and thus easy to manoeuvre	shorter in length than other types of AV available
The Hannover AV	lighter in weight and easier to manipulate as compared to Cambridge AV	-
The Roanoke model	lightweight and thus easy to maneuver, also recommended for jacks	relatively high risk of water leakage and contamination of the sample collected
Open ended	Can collect sperm rich fraction of ejaculate only	Careful timing required.

Source: Shepherd (2008) and Davies (1999.)

Experienced jacks usually need five to thirty minutes to complete the breeding act, while most stallions complete the act in ten minutes or less. Some jacks may take hours to cover a female or never complete the breeding act at all (Hagstrom, 2004). The jennet will be noticed to "jaw"

when in estrus and approached by the jack. The jack should not be allowed to bite the jennet, or to stay on her back until he has achieved a full erection. The collection process with donkeys is much different from that with full size equines due to the different reproductive behavior of the donkey (Purdy, 2005).

2.4.2. Preparation of the jackass and the semen collection area

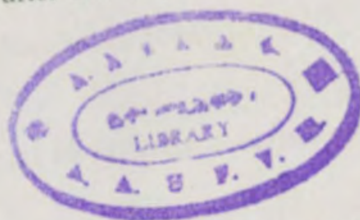
It is easy to collect semen from a jack, even one which is used to only field breeding using a jump jennet in estrus. It is easy to train jacks and jennets if they are handled properly. This means that the handlers and collector must be patient and must allow for the normal cautious behavior of donkeys when confronted with new situations. As always, grain is an excellent motivator when bringing donkeys into unfamiliar areas. The collection process with miniature donkeys is much different from that with full size equines due to the different reproductive behavior of the donkey. A jack may take as long as 30 minutes to achieve a full erection (Purdy, 2005).

The preparation of the jackasses prior to the use of the AV is the same as for natural covering and in accordance with the guidelines set for minimal contamination (Prudy, 2005). The penis has to be thoroughly washed to remove the smegma, debris and the bacteria therein (Samper, 1995). Even though the act of mounting a mare is a natural instinct, some stallions adapt very readily to the use of a jump mare, dummy and AV. However, it is most important that the stallion should be trained to perceive the process as a pleasurable event and is happy to continue. Unpleasant psychological or painful physical associations with the use of the AV may permanently reduce the libido, not only for AV collection but also in natural covering (Davies, 1999). Initially the presence of a teaser female well in to estrus is required regardless of whether the stallion is to be trained to use a dummy or a jump mare or both.

In order for the male to ejaculate successfully, adequate sexual stimulation is required. Evidence suggests that the quality of the semen sample collected may be affected by the method and length of sexual stimulation. Ionata *et al*. (1991) demonstrated that teasing a stallion for 20min prior to ejaculation resulted in a significant increase in the total volume of semen collected, mostly due to a greater volume of the gel-free fraction. There was also a reduction in the number of mounts required per ejaculation, though the concentration of spermatozoa remained lower.

2.4.3. Frequency of semen collection

The frequency of collection that a male equine can tolerate without detrimentally affecting semen quality is highly variable. Research evidence suggests that instead of a single collection per day regularly over the breeding season, double collections that are 1hour apart give better results (Davies, 1999). The first ejaculate in double collections taken on alternate days is reported to produce the best quality semen. However, pooling of these two samples resulted on average with higher spermatozoa motility, a lower incidence of spermatozoa abnormalities and a higher percentage of live spermatozoa before and after freezing than single daily collections (Davies, 2008).



2.5. Semen evaluation and handling

Semen evaluation is primarily carried out to give an indication of the fertilizing capacity of spermatozoa produced. This may be carried out as part of an assessment process prior to purchase, as a routine management practice at the beginning and at set stages throughout the breeding season, or to assess the male's suitability for inclusion in an AI program (Varner, 2007). As a routine part of an AI programme, all stallions and jacks should have a full assessment of their semen sample. This enables picking of any problem that might have occurred since the last breeding season. There are numerous variables that can affect spermatozoa quality (Davies, 2003). Once a stud has been accepted on an AI programme for a particular season, then a further evaluation may be used for assessing sperm motility, concentration and morphology of spermatozoa prior to its extension for insemination or storage (Varner, 2007). In all procedures involving the handling of live semen samples, it is imperative that a constant temperature of 37 - 38°C is maintained throughout to avoid cold or heat shock (Varner, 2007). All tests should be carried out on aliquots removed from the sample. It is best that all tests which rely upon live spermatozoa, such as live: dead ratio and motility, are carried out as speedily as possible (Davies, 2008).

Table 3 shows the semen volume collected by artificial vagina greatly varies among different animals. It ranges from 15 - 60ml in tropical donkeys, 12.5 - 50 ml in small breeds and miniature donkeys and from 25 to 250 ml in large breeds (Prudy, 2005, Lemma and Derresa, 2009). In some breeds (Catalonian, Poitou) the gel fraction may be very copious. Spermatogenic efficiency increases until 6 years of age then plateau (Tibary *et al*., 2005; Tibary, 2005). The total number of spermatozoa in the ejaculate ranges from 5 to 18 billion per ejaculate per ejaculate. Total progressive motility and normal morphology are generally above 70% in normal healthy males. As in the horse, abaxial attachment of the spermatozoa tail is normal in donkeys. Sperm abnormalities of donkeys are also similar to those described in horses Prudy (2005) and Tibary (2005). Objective evaluation of donkey sperm motion parameters using computer assisted motility analysis has also been described (Tibary *et al*., 2006).

Table 3: Summary of results of semen analysis for different domestic animals

Semen parameters	Donkeys	Horse	Cattle	Sheep	Swine
Semen Volume [ml]	12.5 - 250	60 - 80	2-10	1	250
Sperm concentration (Billion/ml)	1.3 - 10	0.15	1-1.2	2	0.2
Total sperm (Billion)	3.3 - 18	9	4-7	3	45
Motile sperm [%]	65 - 90	70	65-70	75	60
Live percent [%]	80-90	80-90	65	70	55
Morphologically normal sperm [%]	69 - 92	70	80	90	60
Useful sperm per ejaculate [$x10^9$]	5- 11	Less	1.04-5.2	0.75	27.5

Source: Hagstrom (2005); Purdy (2005); Lemma and Deresa (2009)

2.5.1. Gross evaluation of semen

Semen could be evaluated grossly for volume, appearance, osmolarity, and pH. Equine semen, unlike in other domestic species, contains a gel fraction. Gross evaluation begins by estimating both the total and gel-free volume using a graduated measuring device. As soon as the semen sample has arrived in the laboratory, the first process is to filter the sample to remove the gel fraction, detritus and sloughed epithelial cells (Davies, 2008; Varner, 2007). Once the semen is filtered, its color and consistency should be assessed. A good sample appear milky white in color, though it may range from watery to creamy, depending on the spermatozoa concentration within the sample. Abnormal colors may indicate contamination with urine, blood or pus (Rigby *et al.*, 2001). The osmolarity of equine semen as measured by an osmometer ranges between 290 and 310 mOsm (Davies, 1999). Values of the seminal fluid pH are in the order of 6.9 and 7.7, though some authors suggest a tighter range of 7.35 - 7.7. In general, pH levels tend to be higher in the second in successive collection of ejaculates. This change in pH is mainly due to a reduction in epididymal secretions, after depletion of reserves during the first ejaculate, and a lower concentration of spermatozoa.

During its life, the spermatozoon experiences considerable changes in its environment, most notably during maturation within the epididymis and at ejaculation. During epididymal transit, an uptake of osmolytes from epididymal secretions takes place, and spermatozoa acquire the ability to regulate cell volume (Yeung *et al.* 2004). Within a given species, sperm volumetric response to changing osmotic conditions shows a high level of heterogeneity. Some ejaculates develop several peaks under isotonic, hypotonic, and hypertonic conditions. Whereas, the differences under hypotonic or hypertonic conditions can be mostly attributed either to ongoing cell damage (plasma membrane degeneration), the changes under isotonic conditions remain poorly understood. Spermatozoa corresponding to the different populations obviously differ in functional status, mostly in their cytosolic ion content and the functionality of their membrane channels. It has recently been demonstrated that the occurrence of additional subpopulations under isotonic conditions is associated with an uncontrolled ion uptake from the external medium and is related to a deficiency in the signaling pathways that control cell volume (Petrunkina *et al.* 2005*b*, 2007).

2.5.2. Microscopic evaluation of semen

Extender dilution rates between 1:1 and 1:4 are recommended before microscopic evaluation. The examination could be performed to evaluate motility, longevity, concentration, morphology, live: dead ratio, cytology and for presence of microorganisms (Table 2). The semen sample is assessed for progressive motility at 15 min intervals for the first hour, followed by hourly assessments. The concentration of spermatozoa within a sample is one of the most important parameters and could be determined using a haemacytometer or a spectrophotometer. Concentration of spermatozoa not only indicates usability of the sample but also the reproductive capability of the male and the number of females that can be covered from the ejaculate (Varner, 2007).

The percentage of motile spermatozoa, and in particular those showing progressive motility, is a good indicator of the number of viable spermatozoa. Some authors report that there is a very good correlation (r 0.63) between progressive motility and morphologically normal spermatozoa (Varner, 2007). Assessment of motility over a period of time and at different temperatures: 37 - 38^{0}C, 22^{0}C and 5^{0}C; may be carried out to give an indication of the length of time over which acceptable levels of motility are maintained (Samper, 1995). However, fertilization success cannot be attributed solely to the absolute number of viable, motile, and morphologically normal spermatozoa inseminated into the female but more especially to their functional competence (Petrukina *et al*., 2007).

Spermatozoa morphology is commonly assessed by microscopic examination either as unstained semen sample fixed in buffered formal saline, or as stained, often with eosin–nigrosin. On average 200 spermatozoa must be examined for a reasonably accurate estimation of the proportion of normal to abnormal spermatozoa. Other stains include Indian ink, Eosin-aniline blue, Bromophenol blue-nigrosin, Giemsa, Wright stains; and fluorescence. Every ejaculate of semen will contain some 8% to 10% morphologically abnormal spermatozoa with no adverse effect on fertility. However, fertility will be reduced if the total abnormal spermatozoa exceed 25% of the total in an ejaculate (Bearden *et al*., 2004). Primary defects such as detached heads, head abnormalities, double heads, abnormalities or absence of mid-piece, rudimentary tails, and

highly coiled tails originate during spermatogenesis. Separation of the head and tail, as it occurs during fertilization, is sometimes seen in heat-damaged semen (Bearden *et al.*, 2004). Secondary defects such as tail abnormalities (kinks, bends, coils or swellings) and cytoplasmic droplets originate during the transport of the spermatozoa through the epididymis, vas deferens and urethra. Tertiary defects like loss of acrosome and bursting of spermatozoan heads may result from inappropriate handling during semen processing (Ricketts, 1993).

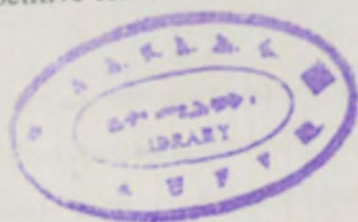
A much more accurate and quantifiable method for assessing the live: dead ratio may be achieved by differential staining, which can also be used in morphology assessments. The live: dead ratio gives an accurate indication of spermatozoan viability, and often values greater than 50% are expected for fertile stud used in AI (Davies, 1999). Cytological evaluation of a semen sample is particularly advantageous if the stud has a history of haemospermia or genital tract infections. Blood cells, leucocytes and erythrocytes, as well as primary spermatogenic cells, can be identified. On the other hand, semen samples may contain some non-pathogenic or have bacteriospermia. Virus isolation from the sperm-rich fraction is normally possible in stallions shedding the virus. However, it is not practical to isolate viruses from a semen sample. Equine herpes virus (EHV) and equine viral arteritis (EVA) may be shed in the semen of infected or previously infected studs (Squires *et.al*, 2003). Other organisms such as mycoplasma may be isolated from semen samples but no significant work has been carried out on the incidence or implications of mycoplasma contamination.

2.5.3. Other tests of semen quality

After collection, the collecting vessel must be carefully removed from the AV and the semen placed in an incubator at $38^{O}C$ and then evaluated as soon as possible. In addition to the basic semen evaluation parameters, Cytology of the semen samples revealed the presence or absence of blood cells, leucocytes and erythrocytes can be identified in semen sample using haematoxylin and eosin or wrights stain, and viewed under a haemacytometer (Davies, 2008).

Other semen quality tests include bacteriological analysis, functional tests like biochemical analysis, membrane integrity tests, flow cytometery, filtration assay, hypo-osmotic stress test, cervical mucus penetration test, oviductal epithelial cell explant test, zona-free hamster ova

penetration assay, heterospermic insemination and competitive fertilization, hemizona assay and DNA analysis (Davies, 2008; Petrunkina *et al*., 2007).).



2.6. Semen processing and storage

Sugar-containing diluents were most commonly used to preserve semen with a per cycle pregnancy rates varying between 13 and 44%, and the number of females inseminated per trial ranging between 5 and 116. A successfully preserved spermatozoon must survive the uterine environment, be transported to the oviduct, be maintained there until the oocyte arrives, and be prepared to penetrate the oocyte and be able to produce a viable embryo. For most species it means that the spermatozoon must undergo capacitation. Fertility trials are the only way to assess the whole process of capacitation but it is easy to see that many of the steps involved are membrane-related and most likely dependent on the protein components of the membrane or presence of seminal plasma during cooled storage (Crabo, 2001; Jasko *et al.* 1991; Pruitt *et al.* 1993). Therefore, semen is usually extended to concentrations between 25 and $50x10^6$ spermatozoa/ml to reduce the proportion of seminal plasma (Magistrini *et al.* 2000; Squires, *et.al*, 2003). Sperm motility largely remains the main criterion upon which success or failure of the freezing procedure is evaluated in the laboratory (Seime, 2001; Katila, 2001).

Spermatozoa cells can be damaged either by the formation of intracellular ice crystals when cooling takes place too rapidly or by chemical toxicity or osmotic stress as well as mechanical damage brought about by phase separation of solution and crystal growth. Hence cooling rate or freezing velocity is an important regulatory factor during cryopreservation (Mazur, 1984; Holt, 2000; Watson 2000). The first equine pregnancy using sperm cryopreserved in glycerol was reported in 1957 and glycerol is still considered the optimal cryoprotectant for equine sperm (Davies, 2008).

2.7. Insemination of the females and pregnancy diagnosis

Artificial insemination is a viable technique for use in ponies, miniature horses, and miniature donkeys. Standard equine equipment may be used to collect, evaluate, transport, and inseminate semen in these species. Limited experience exists with fresh cooled and immediate post collection semen insemination in miniature donkeys (Purdy, 2005). Despite being infertile, the female mule (molly) and female hinny do cycle, although it is typically extremely variable and erratic. Similarly, though the male mule (john) and male hinny do not produce spermatozoa though they produce testosterone and thus display stallion-like behavior (Hagstrom, 2004). Many donkey breeders are frustratingly aware of one peculiarity of jacks, which is the added time needed for them to achieve erection and ejaculation compared to horses.

The method used for inseminating mares requires special emphasis on cleanliness, because the hand is placed in the vagina with a finger through the cervix (Bearden *et al.*, 2004). A 50ml syringe with a volume of semen containing 500,000,000 motile sperm is connected to a plastic inseminating catheter. The catheter is passed through the cervix, and the semen is deposited in the body of the uterus. Insemination is performed on the second to fourth days of estrus, and if the mare is still in estrum on fifth or sixth days, insemination should be performed again. If the stallion semen is of good quality and fertile, insemination need only be performed every 48 to 72 hours, since spermatozoa retain their fertilizing capacity for up to 4-6 days in the mare (Robert's, 2002).

The collection process with miniature donkeys is much different from that with full size equines due to the different reproductive behavior of the donkey (Purdy, 2005). Donkeys tend to be more fertile than horses, having an average conception rate of 78% while mare's average 65%. Furthermore, multiple ovulations are much more common in donkeys than horses. Jennets have a longer cervix than mares; however, it is smaller in diameter. This, along with the fact that the donkey cervix protrudes into the vagina farther than in horses, makes AI more difficult than in mares (Hagstrom, 2004). The method used for inseminating Jennet's is the same as for mares; the difference is deposition is made into the uterine horn with the largest follicle side. Alternate

techniques are being researched, including use of cervical forceps to hold the cervix rigid in position.

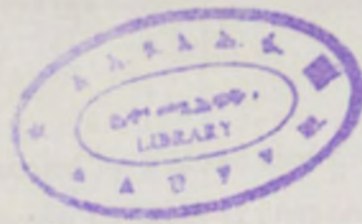
Donkeys tend to be more fertile than horses, having an average conception rate of 78% while mare's average 65%. Furthermore, multiple ovulations are much more common in donkeys than horses. The method used for inseminating jennets is the same as for mares; the difference is deposition is made into the uterine horn with the largest follicle side. However, jennets have a longer and smaller or narrower cervix than mares. This, along with the fact that the donkey cervix protrudes into the vagina farther than in horses, makes AI more difficult than in mares (Hagstrom, 2004). Alternate techniques are being researched, including use of cervical forceps to hold the cervix rigid in position.

Pregnancy diagnosis is necessary for management and husbandry reasons. The early diagnosis of pregnancy is particularly important to diagnose the presence of single or multiple conceptuses, embryo mortality, and pseudopregnancy that may compromise the fertility of a mare (Gary, 2005). Pregnancy diagnosis in equine have been performed on the basis of absence of subsequent oestrus, clinical examination, measurement of concentrations of progesterone, equine chorionic gonadotrophin (eCG), and placental oestrogens and ultrasound examination (Gary, 2005; LeBlanc, 2006). Stud personnel and owners commonly use absence of subsequent oestrus as an initial screening method. Optimum time for clinical examination depends on the experience of the clinician, time of year, and value of the mare. Repeat examinations are recommended up to 40 days because after this time, pregnancy failure is rarely followed by a fertile estrus (Bearden *et al.*, 2004).

2.7.1. Ultrasound as a tool for pregnancy diagnosis in jennies and mares.

Ultrasonographic scanning has resulted in an increased knowledge of the dynamics of early pregnancy in most domestic animals. It is the safest and more accurate method of pregnancy diagnosis in equines. The examination becomes much easier to perform once some basic landmarks and procedures are learned. A 5MHz linear probe gives a very satisfactory result for reproductive tract examinations in donkeys (Lemma *et al.*, 2006; Purdy, 2005). The modern method of pregnancy diagnosis is ultrasonographic examination from day 10 up to day 60. The

technology has revolutionized the study of early pregnancy in the mare. Transrectal examination of the donkey uterus and ovaries is most often performed by attaching the ultrasound probe to a ¾ inch diameter extension arm of approximately 14 inches in length. The ultrasound probe is further lubricated with external application of lubricant before introduction into the rectum. It is initially advanced through the anal sphincter in a 45-degree upward direction to allow for the tilt of the donkey pelvis (Purdy, 2005). The ultrasonic features of a gravid uterus in jennies are similar to mares. The early embryo termed the embryonic vesicle has a quite similar appearance as the follicles appearing as a spherical anechoic structure surrounded by a hyperechoic uterine wall. As the embryo develops, the fetal membranes are also visible (Lemma *et al.*, 2006). Pregnancy diagnosis could be established in equine as early as Day 10 using ultrasound.



3. MATERIALS AND METHODS

3.1. Description of the study area

The study was conducted in North Gondar, Northwestern part of Ethiopia. It is divided into three major agro-climatic zones: highland, mid-highland and lowland. The altitude ranges from 4620 meters in the Semein Mountain in the North to 550 meters in the west. The rainfall varies from 880 mm to 1772 mm with a monomodal distribution, while the minimum and maximum temperatures are in the order of -10^{0}C in the highland and 44.5^{0}C in the West. The area is also characterized by two seasons, the wet season from June to September, and the dry season from October to May. The fertility assessment study was mainly carried out around Dabat in North highland while much of the semen collection and evaluation was carried out in Gondar city which is part of the mid-highland. The natural habitat of the Senar donkeys is however, in the Northwestern lowland around Metema (Figure 1).

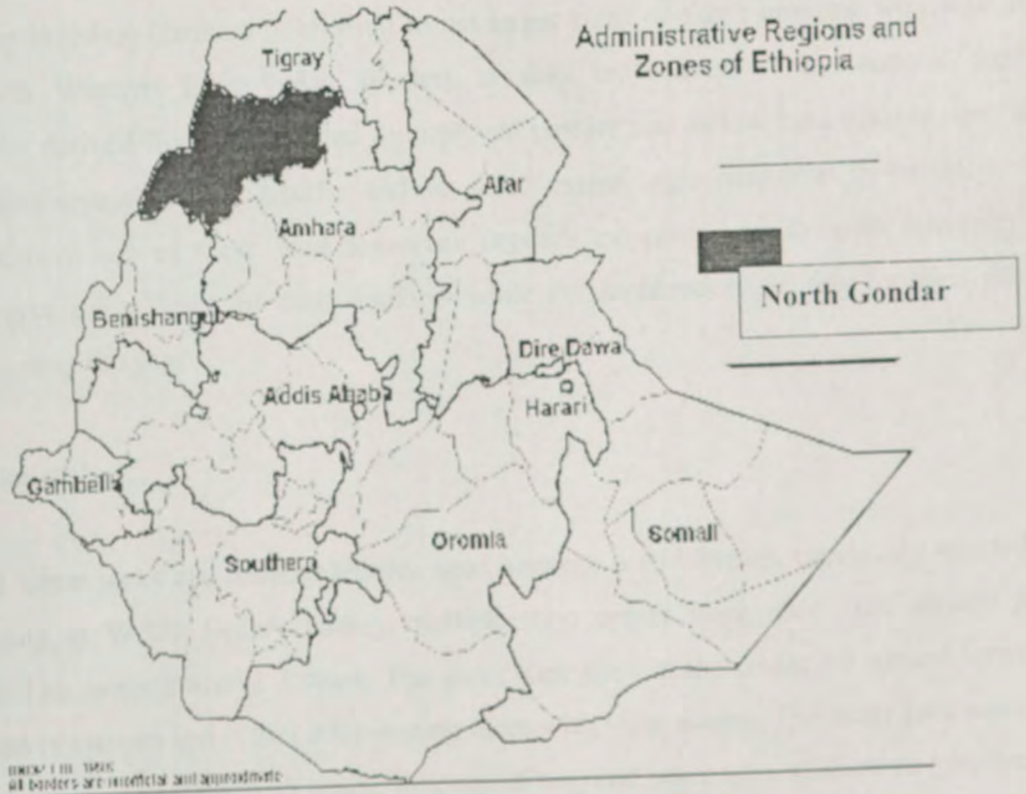


Figure 1: Map of the study area in North Gondar, Amhara Region, Ethiopia

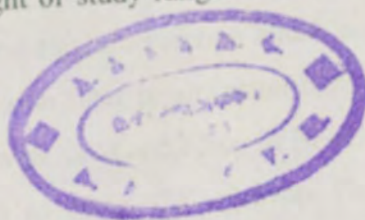
The farming system of the study area is characterized by a mixed crop-livestock production system. Transhumance, from the highlands to western lowlands, is practiced as an important strategy to secure grazing resources for the highland livestock during the dry season of the year. In the case of the lowlands, crop farming is not as intensive as high and mid-highland areas and livestock has larger contributions to the farmer's livelihoods (DOA, 1999). According to the 2002 report of the regional agricultural department, there are 2.873 million cattle, 1.65 million sheep, 0.879 million goats, 0.467 million equines, and 2.9 million human population. The herd size varies greatly, and it ranges from 2.11 to 7.15 animals (high and mid altitudes) to around 65 in the lowlands.

In the Northwestern lowland, donkey crossing with selected descendents of the Kessella Nubian wild asses is a very common practice, while mule production from Sudan Senar donkeys and local horses is practiced in the highlands. Mating, in almost all cases, except for the crossing, is natural and uncontrolled and hence usually associated with year-round foaling. In rare cases deliberate out breeding (farmers interesting to get larger sized donkeys crossing with wild asses around North Western Ethio-Sudan border) is also undertaken. The livestock feed is predominantly derived from the rain fed unimproved pasture and fallow land grazing, hay, crop residues, non-conventional foodstuffs, and to some extent, agro-industrial by-products. The extent of relative use of these feed resources depends on proximity to town however; the lowlanders give grain feeds for their donkeys while the highlanders do this for their ridding horses throughout the year.

3.2. Study animals

A total of 5 Senar jacks and 5 Senar jennies aged between 6 and 8years, previously selected for cross breeding at Wekin Senar Donkey multiplication center were used. The studied jacks generally had an average age of 7 years. The jacks were used in the center for natural service to produce donkey crosses and mules after mating them with local horses. The study jack asses and jennies were freely provided with ample amount of hay and were also allowed to graze in the field from 8:00 AM to 11:30 AM before noon and from 3:00 to 5:30 PM. The donkeys were

supplemented with 5kg concentrate feed (combination of bean barn 50%, whole maize 10%, lentil bran 20%, salt 1%, and oat 18%). Water was provided *ad libitum*. All the studied donkeys were vaccinated against African Horse Sickness and Anthrax before introduction to stable. They were also dewormed using Fenbendazole at 7.5mg/kg (Fencur, Ashesh and India). The study jacks had an average BCS of 8 while the jennies had 6 based on the method described in Pearson and Quasat (2000). The average body weight of study ranged between 180kg and 332kg on a Nomogram scale



3.3 Study Design

The study consists of two stages with the first stage involving phenotypic, morphometric and sexual behavioral evaluation of the male animals, while the second stage consist semen evaluation and fertility trials of the semen in jennies and mares. Both the jennies and mares were selected based on their previous history of fertility and each received 1ml natural prostaglandin (Clorprostenol, Pharmacia and Upjohn company, USA) for induction of estrus. All the females were first inseminated after Day 3 post injection when an over estrus was apparent and presence of a preovulatory follicle was detected. The insemination was repeated after 48 hours. The jennies and mares were scanned after 4 weeks post estrus to determine pregnancy (Prudy 2005 and Lemma *et al*., 2006) using a 5MHz linear array real time ultrasonography (Mindary, Hong Kong).

3.3.1. Evaluation of the phenotype and the sexual behavior of the study jacks

The phenotypic evaluation was performed by measuring the scrotal circumference, scrotal width and length of testes. Their body morphometric characteristics such as the body weight, height, heart girth, head tail length, and body condition were determined according to the method described in Pearson and Quasat (2000). Evaluation of the sexual behavior was carried out using score of the sex drive on 1-3 scale (1= strong sexual desire and first erection within 10minutes of teasing, 2 = moderate sexual desire and first erection within 10 - 20minutes, and 3= poor sexual desire and first erection later than 20minutes). Other parameters such as the time to erection [minutes], and length to semen collection [minute], the number of mount (with or without

erection), and number of thrust per ejaculation, and the interejaculatory interval [minute] were also recorded (Lemma and Derresa, 2009).

3.3.2. Collection and evaluation of semen

During semen collection, analysis and fertility assessment different materials such as Missouri AV, equitainer-72hr, water bath, haemacytometer, microscope slides and cover slips, pipettes, beakers, heating stage, portable pH meter, nylon filter paper, water bath thermometer, calibrated semen collection bottle, and digital camera were used according to Bearden *et al*., (2004) (Figure 2). Moreover, chemical and reagents such as eosin 1%, nigrosin 5%, sodium citrate 2.9%, formalin10%, sterile saline solution, immersion oil, distilled water, non-ionized water, and KY-Jelly were also employed. Semen was extended either using a pre-made bovine semen extender or skimmed milk (92^{0}C heated and fat free). The insemination procedure employed the use of plastic catheter, disposable sterile syringe (50ml), detergent and rectal glove.

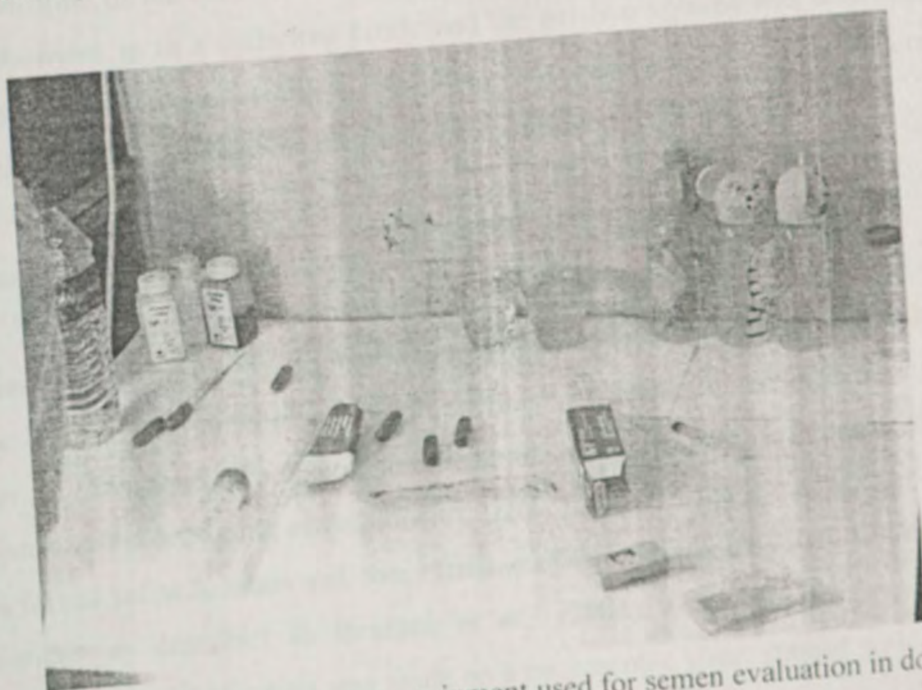


Figure 2: A partial display of laboratory equipment used for semen evaluation in donkeys

3.3.3. Teasing, collection and semen handling

The teasing, semen collection and evaluation were conducted twice per week with an interval of 48hrs between two collections in all jacks. All of the activities were performed during morning time starting from 9:30AM – 12:00AM. A total of 160 semen collections were carried out on biweekly basis and evaluation was performed for each collection according to the procedure of Prudy, 2005.

3.3.2.2. Gross evaluation

After the semen was collected it was immediately taken to the lab and kept in water bath that is preheated to 37^0C and was kept there throughout the evaluation procedures. The color of the semen was visually determined and recorded. The pH of the semen was also determined using a PH meter according to Bearden *et al*., (2004). The total volume of the semen was read directly from the graduation of the collection tube and recorded immediately. Then, semen was filtered using a sterile nylon in to a collecting bottle and the gel-free volume was determined directly from the reading of the graduation on the bottle. The volume of the gel was determined by subtracting the gel-free volume from the total volume (Robert's, 2002).

3.3.2.3. Microscopic evaluation

A thick smear of fresh semen was prepared immediately after collection and was examined under microscope for the determination of sperm motility according to the procedure described in (Bearden *et al*., 2004) and recorded as percent motile. Aliquots were then prepared from whole semen to perform the remaining evaluations. A 1:200 dilution of the semen aliquot was prepared with a 5% formal saline solution and concentration of the spermatozoa was determined using in haemacytometer as described in Bearden *et al*., (2004). Concentration was expressed as Billion/ml. A thin smear of semen was made on a microscopic slide, stained with eosin-nigrosin stain and 200 spermatozoa were counted to determine viability of spermatozoa. The result was expressed as live/dead percent. Morphology was further evaluated using the staining methods of Bearden, *et al*., 2004. Abnormal sperm count was conducted on every ejaculate of semen collected that revealed the quality semen.

The morphology of at least 200 spermatozoa was assessed from fresh sample to determine abnormality which was then expressed as percent abnormal according to (Hidalgo *et al*., 2005).

3.3.3. Fertility assessment

Fertility assessment was performed for both fresh and chilled semen sample after applying semen extender at a rate of 20 billion spermatozoa/ml using skimmed milk and egg yolk to which glucose and antibiotic were added (Chenier *et al*., 1998). The semen was also extended alternatively with a pre-made bovine semen extender (Andromed, MINITUB Abful-und laborteknik GmBH and Co.Kg, Germany) with the same dilution rate. For chilled semen, the semen was stored in Equitainer-72hrs (Agtech, Inc, USA) and aliquots were withdrawn from extended and preserved semen and kept at 37^0C to perform semen evaluation at different times in point according to Hidalgo *et al*., (2005). Sperm progressive motility was evaluated before cooling at 15 min intervals for the first one hour, followed by hourly assessments for 2 hours to determine the longevity. The semen was then evaluated again after 24hr. and 48hrs. The number of viable sperm was calculated using the following formula as described in Nunes *et al*., (2007):

Number of viable spermatozoa/ml = Number of Spermatozoa in 25 squares X 10 X dilution rate X 1000

Semen Concentration = Number of Viable Spermatozoa/ml X Gel-Free Volume, adopted from Bearden *et al*., 2004.

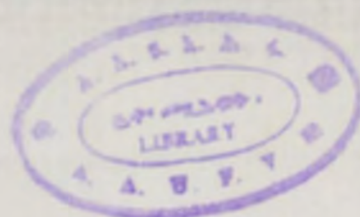
Spermatozoa fertilizing ability was afterwards determined by inseminating 15 jennies (5 Senar jennets, 10 Abyssinian jennets) and 10 mares that were given 1ml single injection of PGF (Lutalase, Pharmacia, USA) prior to insemination. The exact volume of semen concentration used to inseminate equines is 500,000,000 motile sperm per insemination according to the method described in Bearden *et al*. (2004). Insemination was carried out on the second and fourth day of estrus that was repeated after 48hrs.

The technique used for jennets and horses were similar. The Jennet's and horse's tail were first wrapped and fresh or chilled semen preserved in the equatonner constituted to 37°c evaluated the presence of progressive motile spermatozoa and mixed with skimmed milk as extender for insemination. The perivulvar area was cleaned with soap and water and the soap thoroughly rinsed off and the area is allowed to dry. A sterile long glove and insemination gun, and 50 ml all-plastic syringe are used for insemination. The inseminator carried a plastic catheter into vagina while wearing sterile glove and one finger inserted into the cervix and then the catheter passed under it into the uterine body. The semen filled 50ml one billion spermatozoa syringe are attached to the catheter and the semen was delivered with a gentle push. Artificial insemination was given for 5 Senar jennet (female donkey), 12 Abyssinia type jennet and 17 mares (female horse). The insemination techniques performed using standard equine equipment as indicated in Figure 6. Inseminations of the animals studied were performed only once using chilled semen at the first estrous cycle. No second insemination was conducted.

Ultrasonographic scanning was performed systematically starting from the cervix and reaching to the uterus and uterine bodies according to the previous research (Lemma *et al.*, 2006) and jennies and the mares were examined for pregnancy using ultrasound (5MHz, linear array, Mindray, Hong Kong) after Day 40 post insemination.

3.4. Statistical analysis

All collected data was stored in Microsoft Excel data sheet. The statistical analysis was performed using SPSS for windows (Version-15) and STATISTICA for windows (version 6, Statsoft, USA). The data was summarized using descriptive statistics. Differences among variables were measured using ANOVA and GLM. Correlation between variables was calculated using Pearson correlation (r). Values >0.26 were considered as showing presence of biologically meaningful correlation. P-value was held at 0.05 to show presence of significance.



4. RESULT

The morphometric characterization of the studied Senar jackasses as shown in Table 4. Senar jackass was bigger in body size than the Abyssinian donkey. Observation can be seen from Table 4. the phenotypic measurement of the studied jackasses were general within the normal range of 110 – 140 cm for height at wither and 23 – 27 cm for scrotal circumference, the scrotal width and scrotal length ranged from 8.5 – 10.8 and 14 – 20 cm, respectively.

Table 4: Summarized phenotypic measurement of studied Senar jackasses and randomly observed Senar Jackass.

Measurement [cm]	Study jacks (n=5)	
	Mean (±SE)	Range
Height at wither	118±5.54	110 - 140
Chest Girth	131±12.35	110-178
Ear Length	28.9± .51	27-30
Head To Tail Length	149.2± 3.48	141-158
Neck Length	62.2± .200	62-63
Tail Length	63.8± 2.75	56-73
Foreleg Height	83.8± 1.11	82-88
Scrotal circumference	25.08±1.43	23-27
Scrotal width	9.28±2.53	8.5-10.8
Scrotal Length	16.8±1.07	14-20

The results of the observational study on sexual behavior are summarized in Table 5. Full erection time length ranged from 3 – 41 minutes leading to intromission. The number of mount without and with erection varied from 1 -10.

Table 5: Copulatory sequence of semen collections [n=160] of Senar jackasses

MEASURES	160 donkey time		
	Mean(± SD)	Minimum	Maximum
No of mount without erection [count]	4.62±1.93	1	10
No of mount with erection [count]	1.9±0.88	1	10
Total time to first erection [minutes]	12.11±7.15	2	40
Total time to full erection[minutes]	13.45±7.02	3	41
Time taken for intromission[minutes]	22.06±7.75	5	57
Total time to ejaculation[minutes]	23.03±7.78	6	58
Number of thrust[counts]	4.21±0.61	2	5

Pre-copulatory behavioral sequences observed include naso-nasal contact; nibbling and or sniffing of the head. neck, back of the knee, body, flank, perineum and tail; olfactory investigation of voided urine or feces; and flehmen. Fast erection was observed at a distance from Jennies or one of the important characteristic of breeding behavior of Senar jack asses. The most prominent sexual behavior was vocalization, sniffing of the vulva. flehmen reaction, mounting with or without erection, and naso-nasal contact.

The results of the observational study on sexual behavior are summarized in Table 5. The study jacks tended to take more mounts without erection before they actually had erection. Once a jack gets erection, then it took on average less than two minutes to reach full erection that eventually led to intromission. However. jacks even after getting full erection they tended to stay longer nearly the total time required for full erection before intromission.

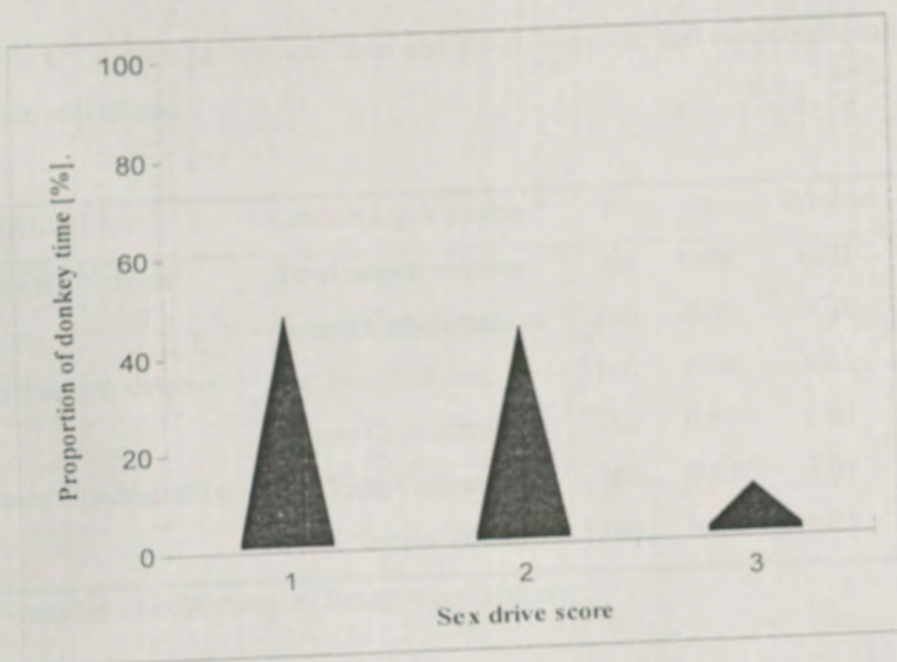


Figure 3: The distribution of sex drive among the study jacks during semen collection (n=160)

Most of the time of teasing donkeys had a very good sex drive of 89.5%. Time required for full erection was less than or equal to 20 minutes. Figure 3 presents the time required for full erection in overall 160 collection time.

Based on the study conducted on Senar Jackass gross semen evaluation revealed that range, mean and standard deviation of 160 sample examined during the study, the total semen volume, Gel-free volume, Gel volume, pH, semen concentration, mass motility, live: Dead, morphologically normal, morphologically abnormal and colour, respectively, 25-100ml, 61.09±12.58; 15-85ml, 50.33±12.29; 4-20ml, 10.79±2.77; 6.54-7.71, 7.36±0.13; 6.54-68.48, 25.68 ±8.1; 85-95%, 93.46±2.38; 82:18-94:6; 89.13:10.86±2.32; 80-94, 89.04±2.94; 6-20, 10.96 ±2.9 and the colour of semen was 91.5% creamy white, 2.5% milkfish and 6% deep creamy white.

There was significant correlation between Gel-free volume, Total semen volume and Semen concentration of 160 samples of Senar Jackass semen as indicated in Table 6.

Table 6: Correlation between total and gel-free semen, and concentration of spermatozoa in 160 semen collections.

VARIABLE	Correlating Variable	N	r	P value
Gel Free Volume	Total semen volume	160	0.98	0.01
	Semen Concentration	160	0.65	0.01
Total semen volume	Gel Free Volume	160	0.98	0.01
	Semen Concentration	160	0.65	0.01
Semen Concentration	Gel Free Volume	160	0.65	0.01
	Total Semen Volume	160	0.65	0.01

N= number of collection, r= Pearson correlation

LIBRARY

The result reported on Table 6 indicated that the total semen volume and gel-free semen volume were significantly correlated to sperm concentration ($p<0.01$, $r= 0.65$, $r= .65$, respectively). These is also true for the strong correlation between gel-free volume and total semen volume ($p<0.1$, $r=0.98$ level).

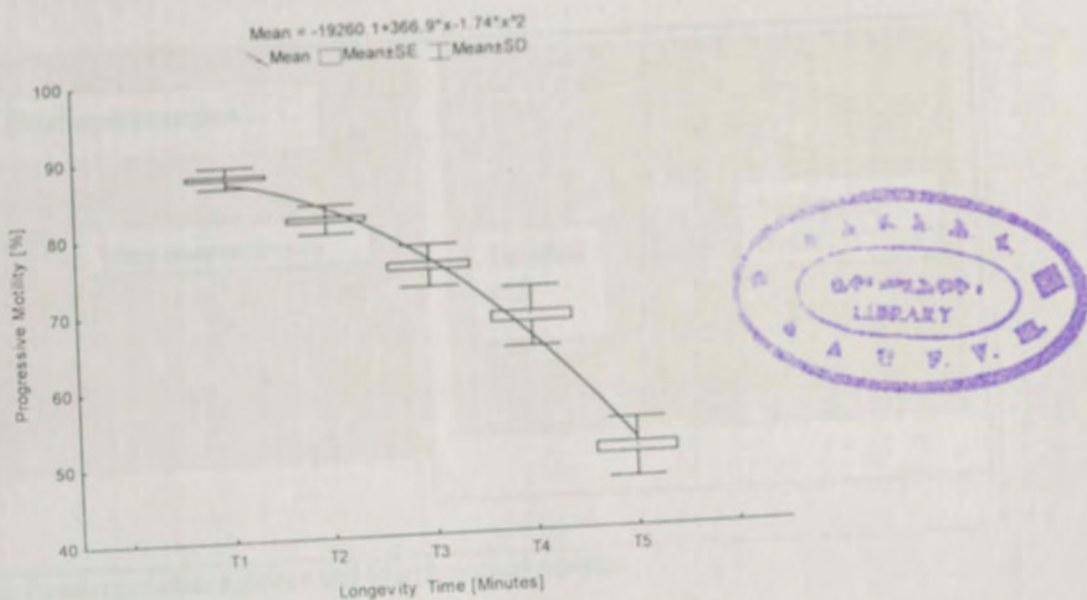
The result reported on Table 7 clearly show that grouped total semen volume given the mean and standard deviation of mass motility, percent of live spermatozoa, percent morphologically normal and pH, obtained that at $p<0.05$ there was no significant difference in each group.

Table 7: Relationships between total semen volume and mass motility, percent of live spermatozoa, percent morphologically normal and pH of semen sample

VARIABLE	Total semen volume interval [ml]	N	Mean	Std. Deviation	P-value
MASS MOTILITY	25 - 40	8	94.38	1.77	0.262
	41 - 55	56	93.39	2.36	
	56 - 70	63	93.10	2.61	
	71 - 85	31	94.19	1.87	
	86 - 100	2	92.50	3.54	
PERCENT OF LIVE SPERMATOZOAN	25 - 40	8	89.00	2.45	0.371
	41 - 55	56	89.29	2.32	
	56 - 70	63	88.79	2.50	
	71 - 85	31	89.65	2.16	
	86 - 100	2	88.50	2.12	
PERCENT OF MORPHOLOGICALLY NORMAL	25 - 40	8	89.00	2.45	0.839
	41 - 55	56	89.13	2.59	
	56 - 70	63	88.81	3.11	
	71 - 85	31	89.42	3.44	
	86 - 100	2	88.50	0.71	
pH	25 - 40	8	7.33	0.17	0.05
	41 - 55	56	7.36	0.10	
	56 - 70	63	7.38	0.12	
	71 - 85	31	7.32	0.19	
	86 - 100	2	7.23	0.01	

Semen concentration in different groups of total semen volume shows a significant difference at the level of $p<0.05$. The total semen volume above or equal to 56ml is usually accepted that has larger semen concentration. And semen pH also shows a significant difference between groups of total semen volume at the level of $p<0.05$, which means the larger the volume indicated that the semen pH is alkaline.

The mass motility of spermatozoa was above 90% in all semen samples collected in the present study. There was effect of seminal fraction on longevity of semen as defined here in ($p<0.05$) that examined at different older age from 15 minutes, 30 minutes, 45 minutes, 60 minutes and 120 minutes. During the study period, it was designed that without using extender measuring longevity of spermatozoa every 15 minutes for the first four times and the fifth one after 2hrs from the time of collection Figure 4.



LIBRARY

Figure 4: Plot of progressive motility of spermatozoa at different longevity observation time (n=160)

Figure 5 shows the total morphologically normal, abnormal sperm and types of abnormality like bented tail, coiled tail, head defect and mid-piece defect were, respectively, 89.04± 2.94, 10.96± 2.94, 4.49± 2.35, 2.77± 1.34, 1.93± 0.71and 2.37± 1.34. The morphological study of the 160 samples revealed that abnormalities observed were bented tail, coiled tail, head defect and mid-piece defect, 99.38%, 96.88%, 90.63% and 86.88%, respectively.

The mean value with standard deviation of the overall morphologically abnormal spermatozoa of the 160 sample were 10.97±2.98 SD. Morphology was further evaluated using the staining methods of Bearden, *et al.*, 2004. Abnormal sperm count was conducted on every ejaculate of semen collected that revealed the quality of semen. Morphological abnormalities were recorded bended tail, colied tail, head defect and mid-piece defect. Bended tail, coiled and head defect, was observed in one sample at the level of r= .413- correlation is significant at the of 0.01 and r=.197- correlation is significant at the level of 0.05, respectively; and the presence of coiled tail indicated that the presence of bented tail, head defect and mid-piece defect with the correlation coefficient r= .413, r=321 and r=.205, significance at the level of 0.01, 0.01 and 0.05, respectively.

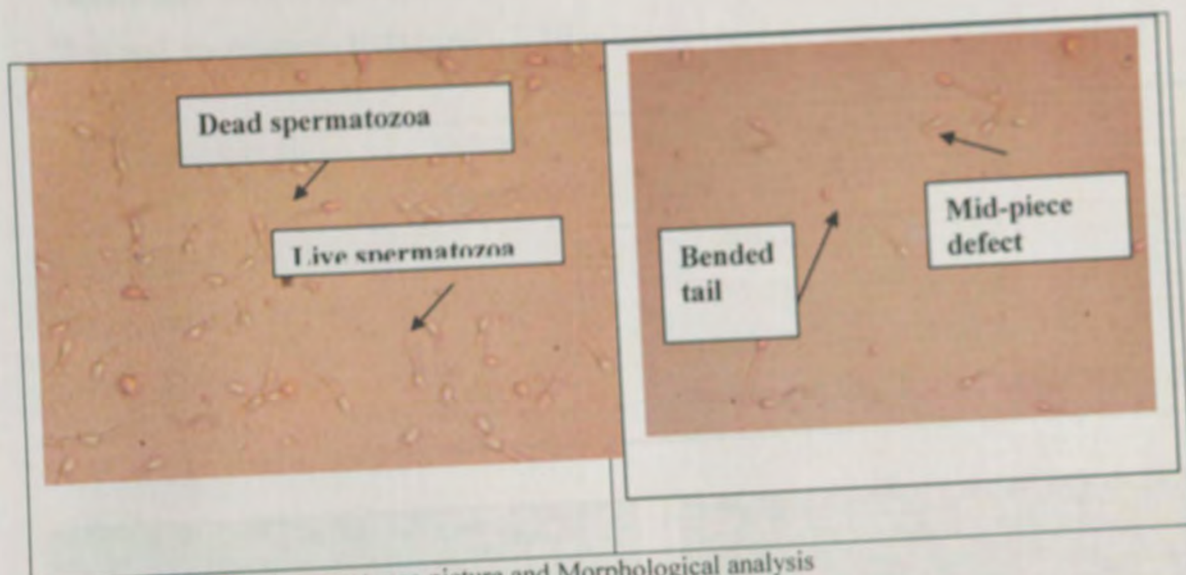


Figure 1: Live: Dead spermatozoa picture and Morphological analysis

As can be seen from Table 8, the number of insemination was 5 in Senar jennies, while 12 and 17 in Abyssinian jennies and mare, respectively.

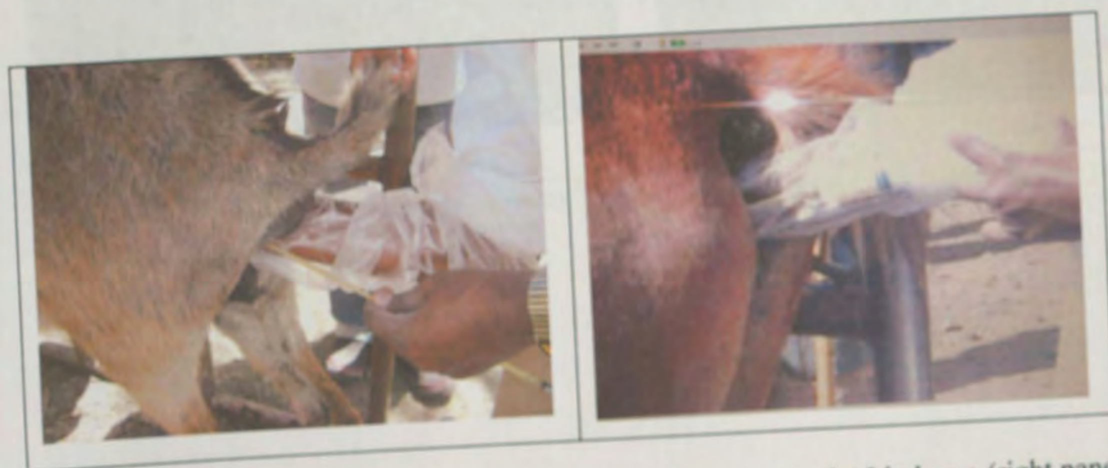
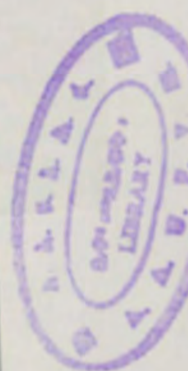


Figure 2: Intra-vaginal AI in jennies, (left panel) and intra-vaginal AI in horse (right panel)



The overall pregnancy rate after first insemination (Table 8) was 58.8% (20/34). The pregnancy rate for 5 Senar jennies, 12 Abyssinian jennies and 17 mares were 40, 58.3 and 64.7%, respectively. Pregnancy was confirmed by direct ultrasound scanning and observation of fetus within the gravid uterus (Figure 7).

Table 8: Summary of the ultrasonic findings on inseminated jennies and mares at day 28 and day 28 of post insemination, respectively.

Animals inseminated	Number inseminated	Ultrasound PD	
		Pregnant	Not-pregnant
Senar jennies	5	2 (40%)	3
Abyssinian jennies	12	7 (58.3%)	5
Mare	17	11 (64.7%)	6
Total	34	20 (58.8%)	14 (41.2%)

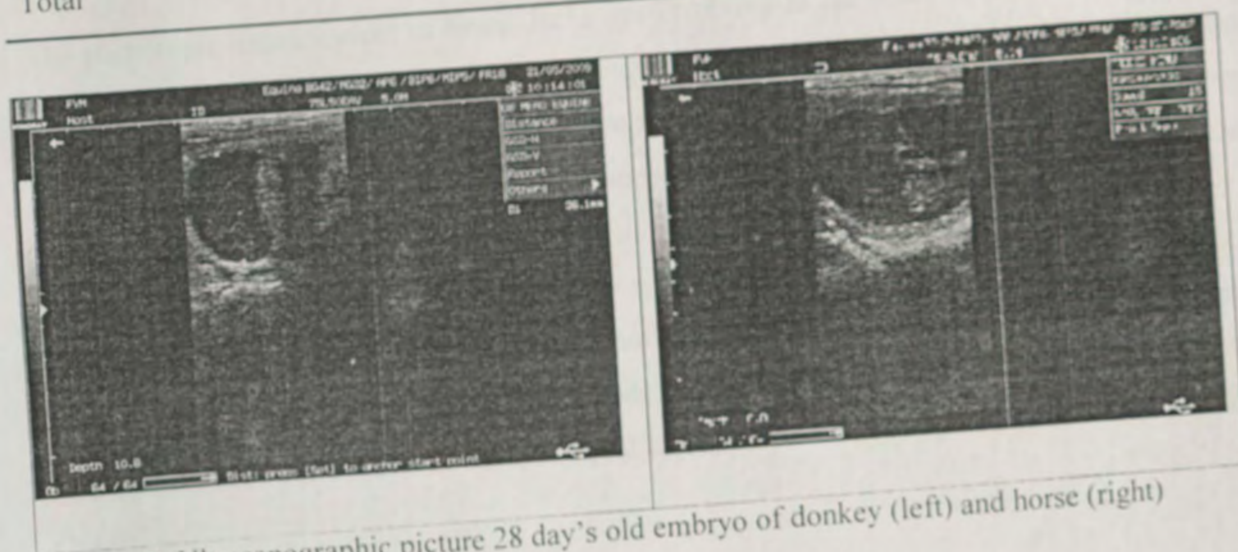


Figure 7: Ultrasonographic picture 28 day's old embryo of donkey (left) and horse (right)

5. DISCUSSION

The present shows major breeding soundness evaluation parameter of Senar Jackass as determined from observable sexual behavior, gross and microscopic semen evaluation, and fertility assessment by using short term cooling semen for artificial insemination. The results are generally in agreement on donkeys and also closely similar to the result of stallions (Davies, 2008). The study substantiate the assessment of sex drives is used as preliminary selection of Senar Jackass for further evaluation of semen and fertility test by AI or natural breeding.

The phenotypic measurement of Senar Jackasses involving in the study revealed that height at wither, chest girth, ear length, head to tail length, neck length, tail length, foreleg height and scrotal length in cent meter in larger body size than that of the local Abyssinian donkey. From these we can decide that Senar Jackasses are appropriate for mule production and the also in the agreement with Tefara, (2002) and Gebreab *et al.*, (1997).

Sexual behavior is slightly different from the horse with regard to time of erection and mounting. The sex drive of our study jackasses were exhibiting time taken for first erection of 89.5% of donkey collection time was less than or equal to 20 minutes in the agreement of Tibary et al., (2006).

The present study characterized for the first time the sexual behavior of Senar jackasses which are the same with the present result reported from other types of donkeys studied (Pugh 2002 and Purdy 2005) in the mean number of mount without erect, number of mount with erection, total time taken for the first erection and total time taken for ejaculation were 90% of the collection time were similarly accomplished within 30 minute length of time.

Some strong and obvious sexual behavioral responses like vaginal sniffing and flehmna's reaction were observed, similar to those reported by Gastal et al., (1996). The current finding on time to erection mean 12.11 ±7.15) was similar to earlier reports which ranged from 10 to 16 minutes (Henry *et al.*, 1991; Gastal *et al.*, 1996).

The mean length to semen collection (23.03 minutes) in the present study is less than the 30 minutes recorded by Gastal *et al*., (1996) and the 10 to 33 minutes reported by Kreuchauf (1984). The mean length of time for semen collection in Senar Jackasses did not show significant difference suggesting that time does not be a factor affecting semen volume.

Results of the semen analysis in the present study are generally similar to previous reports for donkeys (Blanchard *et al*., 2001; Prudy 2005; Vidament *et al*., 2007) and horses (Mello *et al*., 1999; Griggers *et al*., 2001; Varner, 2007). The mean total semen volume, gel-free volume, pH, color and sperm concentration of the ejaculates 61.09±12.58; 50.33±12.29; 10.79±2.77; 7.36±0.13; 93.8% creamy white, 2.5% milkfish and 6% deep creamy white and 25.68 ±8.1 billion does not included within the range of previous reports by Henry *et al*., (1991) and Prudy (2005). Gel volume of total semen collected during the study period was with the range of 6-20ml, this result is coincided with the report of Mello *et al*., 1999, and he explained that gel may be present in some ejaculate but apparently in lower proportion than that found in horses. The total semen volume recorded in this study is agreed with Prudy, 2005.

Although the hemacytometer is often regarded as the gold standard for determining spermatozoal concentration of ejaculates and is the system by which some of the photometric techniques are calibrated, the coefficient of variability was greatest for the hemacytometer (Rigby *et al*., 2001).

The color of the total semen collected in the present study period is creamy white above 90% which is similar with Prudy (2005).

The fertility assessment carried out by using 24 hrs chilled Senar Jackass semen, that resulted in 58.8% pregnancy rate with the range of 25-75% in horses as has been reported (Samper, 2005; Amann and Pickett, 1993; Vidament *et al*., 2007). However, the present study used only one estrous cycle, whereas other worker used an average of 3 breeding cycles. In these work the fertility rate was different between Senar jennet, Abyssinian jennet and mare, in that one cycle insemination resulted in successful pregnancy rate of 58.8% which is far more successful than the previous intrauterine insemination result 22.2% of Pickett *et al*., (1987). This is because of agro-ecological, managemental and types of donkeys.

The present study showed that fertility is similar when mares and Abyssinian Jennies are inseminated with cooled Senar Jackass semen preserved bovine semen extender and extended inseminated with skimmed cow milk, thus complementing previous results obtained on semen evaluation (Fayrer-Hosken and Caudle, 1989; Oba *et al.*, 1993; Pickett, 1993; Mello *et al.*, 1999; Griggers *et al.*, 2001; Blanchard *et al.*, 2001, Prudy 2005, Vidament *et al.*, 2007), and on fertility obtained after insemination of mares for mule production (Vidament, *et al.*, 2007; Ferreira and Henry, 1992) and motility is maintained in cow skim milk extender at 5°C for 24hrs which is the same techniques applied by Tibary *et al.*, 2008. In the present study the one cycle donkey cooled semen insemination result is much better than that reported by Oliveira *et al.* (2006) was none and 40%, for donkey and mare, respectively.

6. CONCLUSION AND RECOMMENDATION

From the present study it can be concluded that the breeding soundness evaluation result of Senar Jackass was found that fit for further crossing to get mule and improving the existing Abyssinian donkey. Senar Jackass evaluating its fertility, therefore, sensitive tests must be made of specific sperm parameter and sexual behavior in the regular period of time. Overall, it is clear that semen samples must be subjected in parallel to several different tests, selected on the basis of the criteria outlined in the study design. In order to provide the highest level of fertility prediction from such tests, their outcome should be subjected to multiparametric analysis.

Artificial insemination is a viable technique for use in Jennies and Mares. Standard equine equipment can be used to collect, evaluate, transport, and inseminate semen in this species. Limitation has been seen artificial insemination in Jennies and timing of insemination for Jennies and Mares are successfully managed using Clorprostenol. Pregnancy diagnosis is possible in using standard equine ultrasound equipment and a probe extender. In general, as with all investigative work, the more tests that are carried out, the better the overall assessment would gave more accurate the result. AI in equine is intended to increase biological and economic efficiency. This had been confirmed also in this work.

Therefore, the following recommendation are suggested for due consideration.

- There is a need to have standard equine open nucleus breeding center for research and development program at national level.
- Further studies should be carried out for appropriate comprehensive cryopreservetive techniques of equine spermatozoa.
- Nation wide phenotypic and genotypic characterization of equine breeds.
- Comparative studies of semen characterization with fertility assessment on different breeds, and using fresh, chilled or frozen semen for artificial insemination in both of donkey and horse family.

7. REFERENCE

Allen, W.R., Tiplady, C., Wilsher, S., Lefranc, A.C. and Morris, L.H.A. (2001): Videoendoscopic Low-Dose Uterotubal Insemination in the Mare; Proceedings of the Second Meeting of the, European Equine Gamete Group, Havemeyer Foundation Monograph Series No. 5, Pp.29.

Amann, R.P and Graham, J.K. (1993): Spermatozoal function. In: McKinnon, A.O. and Voss, J.L. (eds) Equine Reproduction. Lea and Febiger, Philadephia, Pp. 715-745.

Blanchard, T.L., Brinsko, S.P., Varner, D.D., Rigby, S.L. and Hurtgen, J. P. (2001): Evaluation of Testicular Size and Function in 1-3Year Old Stallions. Proceedings of the Annual Convention of the American Association for Equine Practitioners, **47**, 232-35

Bearden, H.J., Fuquay, J.W. and Willard, S. (2004): Semen and Its Component; and Insemination in mare. Applied Animal Reproduction, Sixth edition, Pp.174-185, 233, 310-313.

Carr, D.W. and Acott, T.S. (1984): Inhibition of bovine spermatozoa by caudal epididymal fluid: I. studies of a sperm motility quiescence factor. *Biol Reprod*; **30**, 913–925.

Chenier, T., Merkies, K., Leibo, S., Plante, C. and Johnson, W. (1998): Evaluation of Cryoprotective Agents for Use in the Cryopreservation of Equine Spermatozoa; Depts. Of Population Medicine (Chenier, Plante, and Johnson), Animal and Poultry Science (Merkies), and Biomedical Sciences, Animal Biotechnology and Embryology Laboratory (Leibo), University of Guelph, Guelph, Ontario N1G 2W1, Canada.

Chiarini-Garcia, H., Alves-Freitas, D., Barbosa, I.S. and Alemeida, F.R.L.C. (2009): Evaluation of the seminiferous epithelial cycle, spermatogonial kinetics and niche in donkeys (Equus assinus). *Anim.Reprod.Sci.*, doi: **10**.1016/j.anireprosci.2008.12.019.

Colenbrander, B., Gadella, B.M. and Stout, T.A.E. (2003): The predictive value of semen analysis in the evaluation of stallion fertility. *Reproduction in Animals*, **38**, 305-311.

Crabo, Bo. G. (2001): Physiological Aspects of Stallion Semen Cryopreservation, Proceedings of the Annual Convention of the AAEP, AAEP PROCEEDINGS / Vol. **47** / 2001.

CSA. (2004): Agricultural sample census, livestock sample census year 2003 by Central statistics agency, Addis Ababa, Ethiopia.

Daels, P.F. (2003): New technics of artificial insemination in the mare, Proceedings of the Belgian Equine Practitioners Society - BEPS, 2003, Brussels, Belgium, BEPS (Ed.) International Veterinary Information Service, Ithaca NY (www.ivis.org), 2003; P1004.1103.

Davies, M.C.G. (1993): Equine Reproductive Physiology, Breeding and Stud Management, 1st edn. Farming Press, Ipswich, UK.

Davies, M. C. G. (1999): Equine Artificial Insemination Institute of Rural Studies, University of Wales, Aberystwyth, UK Publication Date: May 1999; Pp: 416.

Davies, M.C.G. (2008): Equine Reproductive Physiology, Breeding and Stud Management, 2nd edn. Farming Press, Ipswich, UK.

DOA. (1999): Zonal agricultural activities and livestock production and health report submitted to the regional government Buero of Agriculture.

Dyce, K M., Sack W O. and Wensing, C. J. (1987): The male reproductive organ of Horse; Text Book of Veterinary Anatomy: Pp.535-541.

Fayrer-Hosken, R.A. and Caudle, A.B. (1989): Stallion fertility evaluation; part III. *Equine Practice*. **11**, 30-34.

Ferreira, M.F.L. and Henry, M. (1992): Effect of cooling rate and seminal extenders on sperm longevity and fertility in donkeys. In: Proceedings of 12th International Congress on *Animal Reproduction*, vol. **3**, The Hague, pp. 1406-1408.

Fielding, D. (1991): The number and distribution of equines in the world. In: Fielding, D. and Pearson, R.A. (1991). (Eds) Donkeys, mule and horse in tropical agricultural development. Proceedings of an International Colloquium, 3rd -6th September 1990. pp. 62-66.

Fielding, D. and Krause, P. (1998): Donkeys. The Tropical Agriculturalist series. Macmillan CTA.

Gadea, J., Selles, E. & Marco, M.A. (2004): The predictive value of porcine seminal parameters on fertility outcome under commercial conditions. *Reproduction in Domestic Animals*. **39**, 303-308.

Gary, C. W. (2005): Fertility and Obstetrics in the Horse, Third edition, Blackwell Publishing, pp 72-84.

Gastal, M.O., Henry, M., Beker A.R., Gastal, A.L. and Goncalves, A. (1996) Sexual behavior of jacks: Influence of ejaculatory frequency and season. *Theriogenology*. **46**, 1171-80

Gebreab, F., Alemu, G., Frew, K., Abule, I. and Ketema, Y. (1997): An overview of donkey utilization and management in Ethiopia. Workshop Reader of an International Meeting held 5th -9th May 1997. Debre Zeit, Ethiopia. Animal Traction Network fot Eastern and Southern Africa (ATNESA).

Gebreab, F., Gebre Wold, A., Kelemu, F., Ibro, A. and Yilma, K. (2003): Donkey utilisation and management in Ethiopia.This paper is published in: Starkey P and Fielding D (eds), *Donkeys, people and development*. A resource book of the Animal Traction Network for Eastern and Southern Africa (ATNESA). ACP-EU Technical Centre for Agricultural and Rural Cooperation (CTA), Wageningen, The Netherlands. 244p. ISBN 92-9081-219-2. CTA and Neda, The Netherlands supported this publication. For details of ATNESA and its resource publications see http://www.atnesa.org.

Griggers, S., Paccamonti, D.L., Thompson, R.A. and Eilts, B.E. (2001): The effect of pH, osmolarity and urine contamination on equine spermatozoa motility. *Theriogenology*. **56**, 613-622.

Hagstrom, D.J.M.S. (2004): Donkeys are Different; An Overview of Reproductive Variations from Horses, Equine Extension, University of Illinois. Pp4-5.

Heinonen, M. (1989): Artificial Insemination of cattle in Ethiopia. Ministry of Agriculture. Pp21-103.

Henry M, McDonnell SM, Lodi LD, Gastal EL (1991): Pasture mating behavior of donkeys (*Equus asinus*) at natural and induced oestrus. *Journal of Reproduction and Fertility*. **44**, 77–86.

Hidalgo, M., Rodriguez, I., Dorado, J., Sanz, J. and Soler, C. (2005): Effect of Sample Size and Staining methods on Stallion Sperm Morphometry by the sperm Class Analyzer; *Vet Med-Czech*. **50**, 24-32.

Holt, W.V. (2000): Basic aspects of frozen storage of semen, *Anim. Reprod. Sci.* **62**, 3-22.

Holt, W.V. and Harrison, R.A. (2002): Bicarbonate stimulation of boar sperm motility via a protein kinase a-dependent pathway: between-cell and between ejaculate differences are not due to deficiencies in protein kinase A activation. *J Androl*; **23**, 557–565.

Ionata, L.M., Anderson, T.M., Pickett, B.W., Heird, J.C. and Squires, E.L. (1991): Effect of supplementary sexual preparation on semen characteristics of stallions. *Theriogenology* **36**, 923–937.

Jasko, D.J., Lein, D.H. and Foote, R.H. (1990): Determination of the relationship between sperm morphology classifications and fertility in stallion: 66 cases (1987-1988). *Journal of the American Veterinary Medical Association*, **197**, 389-394.

Jasko, D.J., Moran, D.M., Farlin, M.E. and Squires, E.L. (1991): Effect of seminal plasma dilution or removal on spermatozoal motion characteristics of cooled stallion semen. *Theriogenol.* **35,** 1059-1067.

Johnson, L., Amann, R.P. and Pickett, B.W. (1980): Maturation of equine epididymal spermatozoa. *Am J Vet Res*; **41**, 1190–1196.

Katila, T., Karlsson, M, Reilas, T. Andersson, M.; Kaikkonen R.and Koskinen, E. (2001): Semen preservation. *Havemeyer Foundation Monograph Series No.* **5**, 3-5.

Kreuchauf, A. (1984): Reproductive physiology in the jackass. *Animal Research and Development*, **20**, 51-78

LeBlanc, M.M. (2006): Reproduction - Clinical Cases, Rood and Riddle Equine Hospital, Lexington, KY, USA. Publisher: American Association of Equine Practitioners, Lexington KY. Internet Publisher: International Veterinary Information Service, Ithaca NY (www.ivis.org), Last updated: 6-Dec-2006; P53108.1206.

Lemma, A., Schwartz, H.J. and Bekana, M. (2006): Application of ultrasonography in the study of the reproductive system of tropical jennies (*Equus asinus*). Tropical Animal Health and Production, **38**, 267-274.

Lemma, A., Bekana, M., Schwartz, H.J. and Hildebrandt, T. (2006): The Effect of Body Condition on Ovarian Activity of Free Ranging Tropical Jennies (*Equus asinus*). *Journal of Veterinary Medicine*, **53**, 1-4.

Lemma, A. and Derresa, B. (2009): Study on Reproductive Activity and Evaluation of Breeding Soundness of Jacks (*Equus asinus*) in and Around Debre Zeit, Ethiopia, Livestock Research for Rural Development online publication (in press).

Magistrini, M., Chanteloube, P. and Palmer, E. (2000): Influence of season and frequency of ejaculation on production of stallion semen for freezing. *Journal of Reproduction and Fertility. Supplement.* **35**, 127–133.

Mazur, P. (1984): Freezing of living cells: mechanisms and implications. *Am. J. Physiol.* **247**, 125-142.

McKinnon, A.O. and Voss, J.L. (1993): Equine Reproduction. Lea and Febiger, Philadelphia, London, pp. 465-471.

Mello, S.L.V., Henry, M., Souza, M.C. and Oliveira, S.M.P. (1999): Effect of split ejaculation and extenders on longevity of donkey semen preserved at 5°c. *Arq.Bras.Med. Vet. Zootec.*Vol.**52** n.4 Belo Horizonte Aug.2000.

Meira, C., Ferreira, J.C.P., Papa, F.O. and Henry, M. (1998): Ultrasonographic Evaluation of the Conceptus from days 10 to 60 of pregnancy in jennies. *Theriogenology*, **49**, 1475-1482.

Neves, E.S., Chiarini-Garcia, H. and Franca, L.R. (2002): Comparative testis morphometry and seminiferous epithelium cycle length in donkeys and mules. *Biol.Reprod.***67**, 247-255.

Neves, E.S., Chiarini-Garcia, H. and Franca, L.R. (2005): Ultra structural observation of the mule testis indicates normal function of somatic cells. *Anim.Reprod.* **2**, 263-271.

Nunes, D.B., Zorzatto, J.R., Costa e Silva, E.V. and Zuccari, C.E.S.N (2007): Efficiency of short-term storage of equine semen in a simple-design cooling system: *Animal Reproduction Science.* **104**, 434-439.

Oba, E., Bicudo, S.D., Pimentel, S.L., Lopes, R.S., Simonetti, F. and Hunziker, R.A. (1993): Quantitative and Qualitative Evaluation of stallion semen. Revista Brasileira de *Reproduca Animal*. **17**, 57-74.

Okamura, N., Tajima, Y. and Soejima, A. et al. (1985): Sodium bicarbonate in seminal plasma stimulates the motility of mammalian spermatozoa through direct activation of adenylate cyclase. *J Biol Chem*; **260**, 9699–9705.

Oliveira, J.V., Alvarenga, M.A., Melo, C.M., Macedo, L.M., Dell'Aqua, J.A. and Papa, Jr.F.O.(2006): Effect of cryoprotectant on donkey semen freezability and fertility. *Anim.Reprod.* **94**, 82-84.

Pawlak, M. And Tischner, M. (2001): Some Observations On The Puberty Of Stallions After Long Term Administration Of hCG, Havemeyer Foundation, Monograph Series No 5.pp. 15.

Payne, W.J.A. and Wilson, R.T. (1999): An Introduction to Animal Husbandry in Tropics. 5th ed. Pp. 546-575. Longmans, UK.

Pearson, R.A. and Quassat, M. (2000): A guide to live weight estimation and body condition scoring of donkeys. UK, J. Thomson Colors Printers Ltd. 21p.

Petrunkina, A.M., Harrison, R.A.P., Tsolova, M., Jebe, E. & Töpfer-Petersen, E. (2007): Signalling pathways involved in the control of spermatozoal cell volume. *Reproduction* **133,** 61–73.

Petrunkina, A. M., Waberski, D., Günzel-Apel, A. R., and Töpfer-Petersen, E. (2007): Determinants of sperm quality and fertility in domestic species. *Reproduction* **134,** 3-17.

Petrunkina, A.M., Jebe, E. & Töpfer-Petersen, E. (2005): Regulatory and necrotic volume increase in boar spermatozoa. *Journal of Cellular Physiology.* **204,** 508–521.

Pickett, B.W., Squires, E.L. and McKinnon, A.O. (1987): Procedures for collection, evaluation and utilization of stallion semen for artificial insemination. Anim. Reprod. Lab. Bull. No. 3. Fort Collins: Colorado State University.

Pickett, B.W. (1993): Collection and evaluation of stallion semen for artificial insemination. In: McKinnon, A.O. and Voss, J.L. (eds) Equine Reproduction. Lea and Febiger, Philadelphia, London, pp. 746-789.

Protsch, R. and Berger, R. (1973): Earliest radiocarbon dates for domesticated animals. *Science.* **179**, 235-239.

Pruitt, J.A., Arns, M.J. and Pool, K.C. (1993): Seminal plasma influences recovery of equine spermatozoa following *in vitro* culture (37°) and cold-storage (5°). *Theriogenol.* **39,** 291.

Pugh, D.G. (2002): Donkey Reproduction. Proceedings of the Annual Convention of the AAEP, **48**, 113-114.

Purdy, S.R. (2005). Artificial Insemination for Miniature Donkeys. In: Veterinary Care of Donkeys, Matthews N.S. and Taylor T.S. (Eds.). International Veterinary Information Service, Ithaca NY (www.ivis.org).

Ricketts, S.W. (1993): Evaluation of stallion semen. *Equine Veterinary Education,* **5**, 232–237.

Rigby, S., Varner, D.D., Thompson, J.A., Love, C.C., Brinsko, S.P. and Blanchard, T.L. (2001): Measurement of Sperm Concentration in Stallion Ejaculates Using Photometric or Direct Sperm Enumeration Techniques.**47,** AAEP PROCEEDINGS.Pp. 236-238

Robert's, S.J. (2002): Insemination in mare; Veterinary Obstetric and Genital Disease, Second edition, Pp.742.

Rodriguez, I., Sanz, J., Perez, C., Felipe, M., Dorado, I. and Hidalgo, M. (2001): Fertilidad in vivo del esperma congelado-descongelado. Jornadas de Investidacion, Cordoba, Spain, pp, 317-321.

Samper, J.C. (1995): Stallion semen cryopreservation: male factors affecting pregnancy rates. Proceedings of the Society for Theriogenology. San Antonio Texas, pp. 160–165.

Samper, J.C. (2005): Stallion semen cryopreservation: male factors affecting pregnancy rates. *Proceedings of the Society for Theriogenology*. San Antonio Texas, pp. 160–165.

Sghiri, A., Tibary, A. and El Idrissi, R. (2006): Behavioral Response To Imipramine/Xylazine treatment in jackass, 9th World Equine Veterinary Congress, Marrakech. In Press.

Shepherd, C. (2008): How to' Collect a semen sample. Proceedings of the 47th British Equine Veterinary Association Congress 2008 - Liverpool, United Kingdo

Sieme, H., Arav, A., Klus, N. and Klug, E, (2001): Cry preservation Of Stallion Spermatozoa Using A Directional Freezing Technique, Havemeyer Foundation Monograph Series No. 5 pp. *6-8.*

Squires, E.L., Barbacini, S., Necchi, D., Reger, H.P. and Bruemmer, J.E. (2003): Simplified Strategy for Insemination of Mares with Frozen Semen, 49th Annual Convention of the American Association of Equine Practitioners, 2003 - New Orleans, LA, USA. (Ed.). Publisher: American Association of Equine Practitioners, Lexington KY. Internet Publisher: International Veterinary Information Service, Ithaca NY (www.ivis.org), 21-Nov; P0654.1103.

Stabenfeldt, M J and Reece W O. (1996): Male reproductive process; Duke's Physiology of Domestic Animals, eleventh edition, Pp.665-677.

Tefera, M. (2002): Phenotypic and reproductive characteristics of donkeys in Ethiopia. Proceeding on 4th International Colloquium on Working Equines. Recent Development in Breeding, Reproduction and the Environment, pp. 322-326, Hama, Syria.

Thompson, D.L. (1994): Breeding management of stallion: Breeding soundness evaluation. Journal of Equine veterinary Science, **14**, 19-20.

Tibary, A. (2005): Pathologie genital / Infertilite. In Reproduction Equine Tome II: L'Etalon. Tibary, A. & M.Bakkoury (eds), Actes Edition, IAV Hassan II, Maroc, pp185-356.

Tibary, A., Bakkoury, M., Anouassi, A. and Sghiri, A. (2005): Examenet evaluation de l'aptitude a larc production. In Reproduction Equine Tome II: L'Etalon, Tibary A & M. Bakkoury (eds). Actes Edition edition, IAV Hassan II, Maroc, pp167- 174.

Tibary A., Sghiri A., Bakkoury, M. and Fite, C. (2006): REPRODUCTIVE PATTERNS IN DONKEYS. Male reproduction, Proceedings of the 9th International Congress of World Equine Veterinary Association, Jan. 22 - 26, 2006 - Marrakech, Morocco.pp, 313-315.

Tibary, A., Sghiri, A. and Bakkoury, M. (2008): Reproduction. The Professional Handbook of the Donkey, 4th edi, pp. 314-334.

Turner, T.T. and Giles, R.D. (1982): A sperm motility-inhibiting factor in the rat epididymis. *Am J Physiol*; **242**, R199–R203.

Varner, D. (2007): From a Sperm Eye View-Revisiting Our Perception of This Intriguing Cell; Proceeding of the 53rd Annual Convention of the American Association of Equine Practitioner, December 1-5, 2007, Orlando, Florida.

Varner, D.D., Schumacher, J., and Blanchard T.L. (1991): Diseases and management of breeding stallions. Goleta, CA: American Veterinary Publications, 1991.

Verstegen, J., Iguer-Ouada, M. and Onelin, K. (2002): Computer assisted semen analyzed in andrology research and Veterinary practice. *Theriogenology*, **57**, 149-179.

Vidament, M., Vincent, P., Martin, F.X., Magistrini, M. and Blesbois, E. (2007): Differences in ability of jennies and mares to conceive with cooled and frozen semen containing glycerol or not. *Journal of Animal Reproduction Science* (Article in press), 32p

Vidament, M., Vincent, P., Martin, F-X., Magistrini, M. and Blesbois, E. (2008): Differences in ability of Jennies and mares to conceive with cooled and frozen semen containing glycerole or not. *Animal Reproduction Science*. **112**, 22-35.

Usselman, M.C. and Cone, R.A. (1983): Rat sperm are mechanically immobilized in the cauda epididymis by "immobilin," a high molecular weight glycoprotein. *Biol Reprod*; **29**: 1241–1253.

Wilhelm, K.M., Graham, J.K. and Squires, E.L. (1996): Comparison of the fertility of cryopreserved stallion spermatozoa with sperm motion analyses, flow cytometry evaluation and zona-free hamster oocyte penetration. *Theriogenology*, **46**, 559–578.

Watson, P.F. (2000): The causes of reduced fertility with cryopreserved semen. *Anim. Reprod Sci*. **60**, 6.

Yeung, C.H., Anapolski, M., Setiawan, I., Lang, F. & Cooper, T.G. (2004): Effects of putative epididymal osmolytes on sperm volume regulation of fertile and infertile c-ros transgenic mice. *Journal of Andrology,* **25,** 216–223.

Zaneveld, L.J.D. (1982): The epididymis. In: Zaneveld LJD, Chatterton RT, eds. ***Biochemistry of mammalian reproduction.*** New York: John Wiley and Sons; 37–63.

ANNEXI

Data Collection Sheets

Annex 1: Sexual behaviour measurement formate

ID	SEXUAL BEHAVIOUR MEASUREMENT							
	NOMWOE	NOMWE	TTFFE	TTFFUE	TTFI	NOT	TTFEJ	ICI

NOMWOE=Number of Mount without Erection; NOMWE= Number of Mount with Erection; TTFFE= Time Taken For the First Erection; TTFFUE=Time Taken For Full Erection; TTFI= Time Taken For Intromission; NOT= Number of Thrust TTFEJ= Time Taken For Ejaculation; ICI= Intercopulatory Interval

Annex 2: Gross semen evaluation

ID	GROSS SEMEN EVALUATION						
	TSV	GFV	GV	pH	Color	S.C IN BILLION	

TSV= Total Semen Volume; GFV= Gel Free Volume; GV= Gel Volume; pH= Color S.C= Semen Concentration

Annex 3: Microscopic semen evaluation.

ID	MICROSCOPIC EVALUATION OF SEMEN															
	MM %	L	D	MN %	MA %	% BT	% CT	HD%	MPD%	PM-15''-1	PM-15''-2	PM-15''-3	PM-15''-4	PM 2 Hr	PM 24 Hr	PM 48 Hr

MM=Mass Motility; L: D= Live: Dead Ratio; MN %= Morphologically Normal; MA%= Morphologically Abnormal; % BT= Percent of Bented Tail; % CT= Percent of Coiled Tail; HD%= Percent of Head Defect; MPD%= Percent Mid-Piece Defect

PM= Progressive Motility, Hr= Hours

Annex 4: Phenotypic characterization senar donkey

I.D	MEASURABLE VARIABLES									
	HAW cm	CG cm	EL cm	HTL cm	NL cm	TL cm	FLH cm	SL cm	VC	
1										
2										
3										
4										

HAW = Height At Wither   CG = Chest Girth   NL = Neck Length

EL = Ear Length   HTL = Head Tail Length   FLH = Front Leg Height

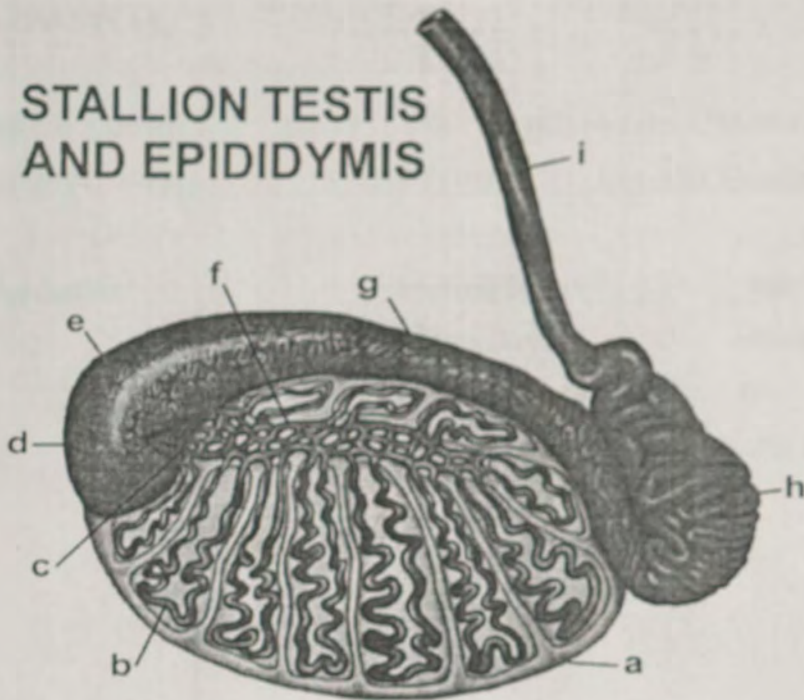
NL = Neck Length   TL = Tail Length

FLH = Front Leg Height   SL = Scrotal Length

VC = Ventral Abdomen Color

Annex 5: Mounting without penile Erection

Annex 6: The Missouri model equine AV unassembled and assembled from left to right used in the study with



Annex 7: Schematic representation of the testis and associated epididymis. The tunica albuginea (a)' seminiferous tubules (b) and interstitial tissue; rete testis (f); efferent ducts (c); epididymis forms a single duct that spans up to 45 m in length and consists of an initial segment (d), a caput (or head; e), a corpus (or body; g), and a cauda (or tail; h), which, in turn, connects with the ductus deferens (i).

Source: Varner, 2007.

CURRICULUM VITAE

AWEKE TSEGA ABERRA Tel-+251-058-1113382, Mobile-+251-0918-776595

Fax-+251-058-1116553 or +251-058-1110801, P.O.Box-180, Gondar, Ethiopia

Personal Information

Marital Status	Married
Nationality	Ethiopian
Age	42

Place of Birth: Gondar, North Gondar, Amhara, Ethiopian

Qualifications

1995 - Doctor of Veterinary Medicine.

2001 – Continuing Professional Development Course Organized by The Donkey Sanctuary Working Worldwide In Ethiopia from April 20-27/2001.

2002 – Attending and Contributed the Fourth International colloquium on Working Equine from 20-26 April 2002, Hama, Syria.

2002- Participatory Rural Appraisal (TOT) training by Winrock International Form July 23 to August 3, 2002.

2002 – Training on Applied Sampling Methods, Socio-Economic and Agricultural Research Data Analysis and Interpretation from March 9-13, 2002; ILDP.

2003 – Training on Standard Computer of MS-Window 98, MS-Word 2000, MS-Excel 2000, and MS-Access 2000; by 3B INFORMATION TECHNOLOGY CENTER.

2007 – Trainer in Leech control using Endodi S strain E-44 organized by Ethio Agri Ceft and Amhara Region BOARD and Food Security Office.

Education

1972 – 1978:- Primary Education in Gondar Meseret Elementary School.

1979 – 1986 – Secondary Education in Fasilades Comprehensive Secondary School, Gondar. Award Ethiopian secondary school leaving certificate exam.

1987 – 1994 Studying Veterinary Medicine at Addis Ababa University, Faculty of Veterinary Medicine, DebreZeit, **Ethiopia** (DVM).

Professional Experience

May, 1996 – March, 2000: District Veterinary Officer, Alefa Takusa District, in North Gondar Zone, Amhara Region, Ethiopia.

April, 2000 – April, 2001: District Veterinary Officer, Dabat District, in North Gondar Zone, Amhara Region, Ethiopia.

April, 2001 – April, 2002; District Agricultural Office Head, Dabat District, in North Gondar Zone, Amhara Region, Ethiopia.

April, 2002 – April, 2003; District Livestock Development and Veterinary Service Desk Head, Dabat District, in North Gondar Zone, Amhara Region, Ethiopia.

April, 2003 – October, 2004; District Agricultural and Rural Development office Vice Head, Dabat

District, in North Gondar Zone, Amhara Region, Ethiopia.

October, 2004 – October, 2007; Working as Animal Health Expert in Austro – Ethiopian Cooperation Supported Integrated Livestock Development Project, Gondar, North Gondar Zone, and Amhara Region, Ethiopia.

Member of the Ethiopian Veterinarians Association (EVA).

Languages Amharic and English.

Paper and publication

Donkeys in North Gondar. Socioeconomic importance, Management and Health Constraints. DVM Thesis, 1995.

Prophylactic Medicament of donkey milk for whooping cough infants (human), a paper presented 4th International Colloquium on Working equine, April 16-22/2002, Hama, Syia.

Overview of Leech control through Endod Application in North Gondar, Workshop on Control of Leeches using Endod, April 3/2006, Sheraton Addis.

Study on Breeding Soundness and Fertility of Senar Jackass (Equus asinus), in North Gondar. MSc

Thesis for partial fulfillment of Veterinary Obstetrics and Gynecology, June/2009.

References

Professor Fesseha Gebreab (BSc, DVM, and MVSc.); the Donkey Sanctuary, Donkey Health and Well fair project, Faculty of Veterinary Medicine, Addis Ababa University. P.O.Box – 34, DebreZeit, Ethiopia.

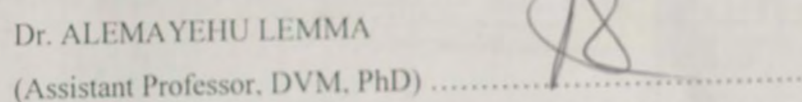
Professor Merga Bekana (DVM, PhD); Dean the Faculty of Veterinary Medicine, Addis Ababa University and Professor in Veterinary Obstetrics and Gynecology, P.O.Box – 34, Tel – 011-4339933.

Dr. Alemayehu Lemma (DVM, PhD); instructor in Veterinary Obstetrics and Gynecology, Faculty of Veterinary Medicine, Addis Ababa University, P.O.Box – 34, Tel – 011-4338917; Mobile - +251-0911-312252.

DECLARATION LETTER:

This thesis is my original work, has not been presented for a degree in any other University and that all sources of material used for the thesis have been duly acknowledged.

AWEKE TSEGA ABERRA ....



Dr. ALEMAYEHU LEMMA
(Assistant Professor, DVM, PhD) ....

Dr. MERGA BEKANA
(Professor, DVM, PhD) ....