ADDIS ABABA UNIVERSITY
FACULTY OF VETERINARY MEDICINE

STUDY ON SEMEN QUALITY AND FIELD EFFICIENCY OF AI BULLS KEPT AT THE NATIONAL ARTIFICIAL INSEMINATION CENTER

WUBET SINSHAW

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DEBRE ZEIT, ETHIOPIA

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Thesis submitted to the Faculty of Veterinary Medicine, Addis Ababa University, in partial fulfillment of the requirements for the Degree of Master of Science in Tropical Veterinary Medicine.

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BY

WUBET SINSHAW

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DEDICATION

This paper is dedicated to my beloved family; my wife**,** W/ro Birtukan Atinkut, my daughter, Lydia Wubet and my son, Natnael Wubet

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ABSTRACT

A laboratory based semen quality study was carried out at the National Artificial Insemination Centre, located at southeast outskirt of Addis Ababa, with the objective of evaluating the semen quality of AI bulls of the Centre. In addition, a field study was carried out to evaluate AI efficiency at field level in Girar Jarso Wereda of the North-Shewa Zone of the Oromia Regional State. The study was undertaken from October 2004 to April 2005. 16 AI bulls were included for the semen quality at the NAIC. A total of 93 postpartum dairy cows and heifers which came to the veterinary clinic in Girar Jarso wereda to get AI service were included in the sample to study AI field efficiency using the semen of 8 bulls among the 16 involved in the semen quality study. Semen was collected from each of the 16 bulls included in the sample once per week for eight weeks using artificial vagina and evaluated for volume, color, concentration, mass activity, individual motility, morphological abnormalities and percentage of alive spermatozoa. According to the results, the average volume ranged from 6.18 ml in the Friesian bulls to 7.18 ml in the crossbred bulls and the average concentration ranged from 1143.1 million/ml in crossbred animals to 1270.3 million/ml in Friesian bulls. Comparable means of mass activity score (3.17 in crossbred and 3.18 in Friesian bulls) and individual motility (71.67 % in crossbred and 71% in Friesian bulls) were recorded. The average proportion of total morphological defects was 18.17% in crossbred bulls and 16.10% in Friesian bulls. The proportion of alive cells was 74.67% in crossbred bulls while it was 76.60% in Friesian bulls. The results of independent t-test to see the effect of different factors on semen quality parameters indicated that only age ($p<0.05$) and scrotal circumference ($p<0.01$) of bulls had significant effect on semen volume. The results of Pearson's correlation analysis showed that mass action had positive correlation with individual motility ($p<0.01$) and concentration ($p<0.05$).

In the field study of the efficiency of AI, a minimum of 10 cows were served by the semen of each of the 8 bulls included in the field efficiency trial. The inseminated animals were checked for pregnancy by rectal palpation after 60 days of first

insemination. The results revealed that the overall pregnancy rate was 55.9% and only body condition score of cows or heifers affected pregnancy rate significantly ($p<0.05$).

Key words: Semen quality, AI, Bulls, Post partum Cows, Heifers, Field efficiency

1. INTRODUCTION

The world human population is increasing from time to time and there is a trend of increasing urbanization and elevated income that consequently leads to an increase in demand for food specially proteins of animal origin. This phenomenon is also happening in Ethiopia where there is a high population of livestock with low productivity levels, which could not meet the increasing demand for foods of animal origin like meat, milk and milk-products. For instance, from 3,720,000 local milking cows, a total of only 774, 000 metric tones of milk is produced annually, with an average yield of 206kg of milk per animal per lactation (NAIC, 1995).

To meet the increasing demand for milk and milk products, improvement of the productivity of dairy cattle through appropriate breeding programs, intensification of the diary production systems and development of market infrastructures are crucial steps (Peters, 1991; Zumbach and Peters, 2000). In improving the productivity of dairy cattle, the use of high producing dairy breeds for crossbreeding has been widely accepted with the aim of combining the superior performance of specialized dairy breeds with the superior adaptability of local stock. However, the delicate balance between the genetic performance ability and adaptability requires due attention and is determined by the degree of exotic inheritance (Peters, 1991).

A successful breeding program requires an effective and sustainable method of transferring genetic materials from one population to another. This can be performed through either natural service (NS) or reproductive technologies including artificial insemination (AI), embryo transfer, ovum pick-up, in-vitro maturation and fertilization and cloning. However, AI is the most practical reproductive technologies to be used in developing countries (FAO**,** 1995).

The use of natural service also remains wide spread even in areas where AI has proven to be efficient (Wattiaux, 1998). In Ethiopia, bull service for dairy improvement is being

practiced as the only available means for rural smallholder farmers and as an option to AI by urban and peri-urban dairy production systems (Bittner *et al*., 2000). In most of the cases, however, the use of bulls for breeding by individual farmer is not controlled and bulls are used indiscriminately without preceding selection practices (Bittner *et al*., 2000).

Artificial Insemination has been defined as the introduction of semen into the female tract without contact between the male and female (Cupps, 1991, Beeman, 1999). It is a very fast way of genetic propagation of improved bulls, if all necessary techniques and infrastructure are available. Historically, the first accounts of AI is dated back to 1780 when Spallanzani, an Italian physiologist, experimented with the dog and successfully inseminated a bitch by injecting fresh semen directly into the uterus. Pups were born after a normal gestation period. In France, AI has been used to overcome sterility in 1890. Later on, Ivanovi undertook AI in horses in Russia at the turn of the century and in cattle and sheep in 1928 (Sorensen, 1979). The Russians were training technicians in the use of AI before 1914 and by 1938 many thousands of cattle and horses and millions of sheep were being successfully bred. Cattle AI cooperatives were established in1938 in Denmark and in the USA, whilst the first centre for AI were established in 1942 in Britain (Arthur *et al*., 1992).

In Ethiopia, AI was first introduced in 1938 by Italians primarily for breeding horses. Since then, AI has been playing a significant role in Ethiopia. The activity was interrupted for sometime and later on initiated again by the British Over-seas Development Program through the use of fresh semen. The Dairy Development Agency (DDA) and Chilalo Agricultural Development Unit (CADU) also applied AI widely and effectively in the urban and rural areas, respectively. According to NAIC (1995), other institutions that have been using AI sporadically for different purposes include the former Wolaita Agricultural Development Unit (WADU), Alemaya Agricultural College and Institute of Agricultural Research (IAR).

Today, there is only one AI Centre in Ethiopia with a national mandate of production and distribution of semen and liquid nitrogen, training of AI technicians and dairy milk recording. However, some of the reports have suggested that AI activities have been unsatisfactory in the country in that its activity is limited to the capital and a few major cities due to a number of technical, financial, infrastructure and managerial constraints (Tegegn, *et al* 1995; Kelay, 2002). More than 92.5% of the smallholder dairy farmers in Selale area, a rural zone located within 110 km radius from the capital are dependent on natural service while about 75% of the urban dairy farmers could get AI service (Kelay, 2002). However, most of the previous works were preliminary and addressed semen quality and service efficiency issues separately.

The objectives of the present study are therefore,

- To evaluate the semen quality of AI bulls of the National Artificial Insemination Centre and compare semen quality between bulls;
- To evaluate AI field efficiency and
- To determine factors affecting AI field efficiency

2. LITERATURE REVIEW

2.1. Dairy production in Ethiopia

Four major dairy production systems have been recorded in Ethiopia. These include the lowland pastoral dairy production system, rural highland smallholder dairy production system, urban, peri-urban small-scale dairy production system and large-scale dairy production system (Kelay, 2002; Ketema and Tsehay, 1995).

2.1.1. Lowland pastoral dairy production system

Even though information on both absolute numbers and distribution vary, it is estimated that about 30% of the livestock population are found in the pastoral areas. The pastoralist livestock production system supports an estimated 10% of human population and covers 50-60% of the total area mostly lying at altitude ranging from below 1500 m.a.s.l. Pastoralism is the major system of milk production in the lowland. However, because of the rainfall pattern and relative seasonal shortage of feed, milk production is low and highly season dependent (Ketema and Tsehay, 1995; Kelay, 2002). Lowland pastoral dairy production system totally depends on the random type of natural service (Kelay, 2002).

2.1.2. Rural highland smallholder dairy production system

The Ethiopian highlands possess a high potential for dairy development. In the highland areas, agricultural production system is predominantly subsistence smallholder mixed farming, with crop and livestock husbandry typically practiced within the same management unit. There are two types of production systems, traditional system that is based on indigenous breeds and market-oriented system that is based on crossbred dairy cattle. The household mainly consumes the milk produced in the traditional system while

most of the milk is sold to generate income in the market-oriented system (Kelay, 2002). In this system, the traditional system uses random type of natural mating, while market-oriented system uses mainly hand mating (bull is stationed and cows in heat are brought to get service) by bull and AI to some extent.

2.1.3. Urban and peri-urban small-scale dairy production system

This system is developed in and around major cities and towns, which are located mainly in the highlands of Ethiopia where there is a high demand for milk. The system comprises small and medium size dairy farms based on crossbred and exotic dairy cattle. In this system, farmers are using AI extensively but also bulls as alternatives in times of AI failure (Ketema and Tsehay, 1995; Kelay, 2002).

2.1.4. Urban and peri-urban large scale dairy production system

Urban and peri-urban large scale dairy production system is a specialized dairy farming practiced by the state and very few individuals on commercial basis. Most of the large-scale dairy farms are concentrated in and around Addis Ababa and keep high grade or exotic pure breed stock and the predominant mating method is AI. The urban, peri-urban dairy farms produce only 2% of the total milk production of the country (Ketema and Tsehay, 1995; Tegegn *et al*., 2000; Kelay, 2002).

2.2. Artificial insemination

Artificial Insemination is a globally accepted method of breeding cattle and is a fast and easy means of exploiting the genetic potential of proven male animals. Zumbach and Peters (2000) estimated that a bull could inseminate as many as 1820 cows by deep frozen semen. However, AI application in developing countries is limited. Chupin and Schuh (1992) showed that the average AI application per year per country in Africa is

30.6 thousand, which is far below the values for Asia, Latin America and Near East countries (Table 1).

Table 1. AI coverage, semen production and AI application in different parts of the world

Region	AI coverage (% of cattle population)	Semen production (Straw/year/country)	AI application	
			Per year	Per inseminator
Africa	< 2	57,787	30,637	369
Ethiopia	NA	35,545	20,649	312
Asia	3-12	1,314,246	377,215	543
Latin America	5-6	367,006	308,127	841
Near east	4.5-14	442,987	110,675	801

NA= not available

Source: Chupin and Schuh (1992) and NAIC (1995)

According to information released from the National Artificial Insemination Centre in Ethiopia, the average AI annual applications for the years from 1984 to 2000 was 20.6 thousand, which is below the average value for Africa. Another issue that deserves mentioning is the cost of production of semen in the NAIC which is about 14 birr per straw which is very high as it is compared with the price per service, (2 birr for rural areas and 5 birr in the urban areas) (NAIC, 1995).

2.2.1. Artificial Insemination efficiency

As described by Barrett (1974) four factors determine AI efficiency. These factors are heat detection skill, fertility level of the herd, semen quality and inseminator efficiency. In general, low efficiency levels are reported in developing countries for AI. The pregnancy rate in Africa does not exceed 45% and the conception rate to first service was

48% in zebu cows kept at the Ministry of Agriculture Ranch in Ethiopia (Mukassa-Mugrewa, 1989). Data compiled and released by the NAIC revealed that the highest value for pregnancy rate attained in seventeen years due to AI was 44% (Table 2).

Table 2: Total inseminations, calves born, pregnancy rate and NSPC due to AI in Ethiopia

Year	Total inseminations	Pregnancy rate	Calves born	NSPC
1984	4,860	38,1	1,852	NA
1985	5,755	38,1	2,193	1.80
1986	11,349	38,1	4,325	1.60
1987	10,861	19,7	2,139	1.60
1988	16,900	43,9	7,424	2.87
1989	19,697	38,1	7,505	2.48
1990	20,695	38,1	7,888	2.50
1991	29,590	25,5	7,543	2.80
1992	16,280	38,1	6,205	2.40
1993	22,026	38,1	8,395	2.06
1994	21,707	33,8	7,341	2.20
1995	26,442	29,2	7,718	NA
1996	25,824	42,5	10,984	NA
1997	26,232	30,2	7,928	NA
1998	32,679	32,9	10,771	NA
1999	32,999	31,5	10,401	NA
2000	33,550	30,0	10,072	NA

NA= not available NSPC = Number of service per conception

Source: NAIC (1995)

2.2.2. Advantages and disadvantages of AI

2.2.2.1. Advantages of AI

- AI offers the best genetic improvement possible since AI bulls are derived from a very top and most heavily researched herds (Schuh, 2000)

- AI is delivered to eliminate the transmission of venereal diseases and genetic defects since we can have a control over the quality and safety of semen before it is applied into the cow (Schuh, 2000)

- Less number of bulls are kept for a very large number of cows and can be carried out in the absence of bulls (Kelay, 2002)

- Enables accurate recording of information such as breeding dates, pregnancy rates, inter-oestrus intervals and days to first service that can be used to monitor fertility **(**Webb, 1992**;** Schuh, 2000**)**

2.2.2.2. Disadvantages of AI

- Heat detection depends on farmers, which may not have the skill to do so. (Schuh, 2000).

- Requires a well-organized infrastructure including all weather roads, telephone, electricity, etc (Schuh, 2000; Tegegn *et al*., 1995).

- High cost of training and transportation of technicians (Schuh, 2000).

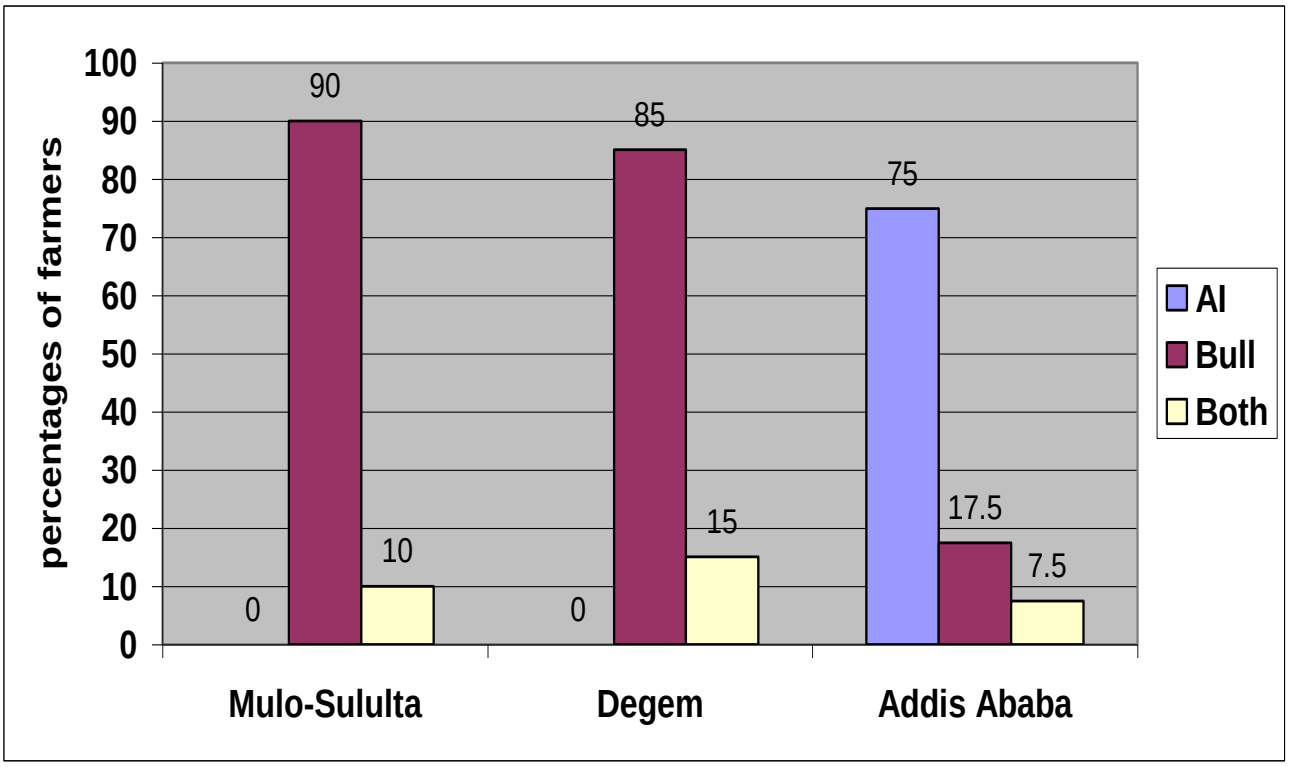
- Inseminators' inefficiency due to inadequate training of technicians or lack of experience of technicians. (Schuh, 2000; Beeman, 1999; Webb, 1992; Chenoweth, 2000).

- Difficulty in assessing the conception rate of cows when farmers are not cooperative in bringing cows after AI failure (Schuh, 2000).

2.2.3. The use of AI and natural service in diary production in Ethiopia

Over 99% of the cattle herd in Ethiopia are indigenous cattle and are owned by small holder subsistence farmers who are far away from the privilege of using AI. It is fair then, to say that nearly all farmers managing indigenous herd and a significant proportion of farmers owning crossbred dairy cattle in rural Ethiopia are dependent on natural service (Anteneh, 2003). A report by Kelay (2002) indicated that most of the farmers in Selale area (85-90%), a region located very close to the capital and where farmers kept crossbred dairy cattle, were using only natural mating due to the unavailability of AI service (Figure 1). Similar findings were reported by Abdinasir, (2000) for Arsi area for same type of farmers, who indicated that 85.7-91.7% of the farmers were using only natural mating. However, natural service in rural Ethiopia is not preceded by selection of bulls for their genetic merit except few cases where there is intervention by governmental and non-governmental organizations. Selection of bulls for genetic improvement purpose has been started in Ethiopia with the introduction of exotic breeds of dairy cattle into the country by development oriented institution. An example is the Small-holder Dairy Development Project (SDDP) that was operating in the highlands of Ethiopia that distributed crossbred dairy cows and pure breed exotic bulls to farmers on loan basis accompanied by an extension package of improved feeding practices (SDDP, 1999). In the urban areas it is AI, which is the dominant mating methods (75%), but there are still significant portions of farmers who depend on natural service solely (17.5) and as an alternative (7.5%) (Figure1). Furthermore, AI was said to be unreliable with regard to timely service by most of the farmers using AI (93%) (Kelay, 2002).

Figure 1. Patterns of mating practices in rural and urban areas in Ethiopia



Source: Kelay (2002)

2.3. Management of breeding bulls

2.3.1. Housing breeding bulls

Breeding bulls should be separately kept from the herd and from each other to protect them from disease transmission and fighting trauma. Mature bulls must be kept in individual pens and should be provided with individual runs adjacent to their pens (Payne, 1992). For mature large breed dairy bulls the stall should have a minimum surface area of 3.7 by 4.3 meters. According to Morrow (1986), bulls less than 3 years old can generally be housed in cow facilities, including tie stalls, box stalls or loose housing.

2.3.2. Feeding breeding bulls

Bulls kept for breeding have to get a balanced feed in order to keep its body condition constantly fit for breeding. Overfeeding or underfeeding is undesirable for breeding, causing lean and fatty body conditions, respectively. Although inconsistent, there are reports indicating quantity and quality of feed supplied to breeding bulls affects percentage motility, motility score and sperm concentration (Tegegn *et al.,* 1994; Mathvron *et al* 1998; Tozser *et al.,* 2000). In addition, severe under nourishment may cause irreversible testicular damage in young bulls and decreased sperm production in mature bulls while obesity due to excessive feeding can temporarily reduce breeding performance and sperm quality and cause laminitis (Arthur *et al*., 1992; Sprott *et al*., 1997).

2.3.3. Health care of breeding bulls

Breeding bulls should be protected from any diseases in general and venereal diseases in particular. In many situations, natural service bulls fail to participate in the health program designed for the cows. Bulls used for natural service should be provided with the same health care as cows. Venereal diseases such as vibriosis and trichomonosis are an important consideration when using natural service bulls (Sprott *et al*., 1997).

2.4. Fertility assessment of breeding bulls

Fertility assessment refers to repeated and regular examination of bulls for libido, serving capacity, morphological soundness and semen quality. Libido is defined as sexual desire while serving capacity is the ability to complete the act of mating. Both these characteristics differ among bulls and are distinctly different components of fertility (Chenoweth, 2000). There are reports indicating that libido and serving capacity are influenced by genetic heritage, age, social effects, bull to female ratio, nutritional effects and vary among sires of the same breed (Chenoweth, 2000). Unfortunately, libido and

serving capacity do not correlate well to other fertility parameters. Bulls may possess quality semen, but may be unacceptable breeders because of lack of desire or mating ability (Sprott, *et al*., 1997). Moreover, a bull should be fit morphologically to continue as a breeding bull. Therefore, morphological soundness examination should be performed annually on all breeding-age bulls. The examination should include the following (Sprott *et al.*, 1997; Wattiaux, 1998; Chenoweth, 1990; Jensen, 1979):

- Visual assessment of eyes, teeth, feet, legs and external genitalia;
- Internal palpation of accessory sex organs like ampullae, , vesicular glands, prostate gland
- Scrotal symmetry and position and condition of scrotal skin, and
- Determination of scrotal circumference

2.4.1. Scrotal circumference

Measuring scrotal circumference (SC) is a crucial part of the breeding soundness evaluation (BSE) and has been determined to be the measurement that best predicts the output of sperm cells for bulls when multiple collections by artificial vagina (AV) are not available. The measurement technique involves the use of a circular tape. This measure is useful because there is a correlation between the SC and the volume of semen-producing tissue that the bull posses. Since SC increases with the age and weight of the bull, it must be interpreted in light of the bull's age (DeeWhittier, 2000; Tegegn, *et al*., 1994)

Table 3. Minimum recommended scrotal circumference for Holstein-Frisian bulls

Age	SC (cm)
< 15 month	30
>15 < 18 months	31
>18 <21 months.	32
>21 < 24 months	33
> 24 months	34

Source: DeeWhittier (2000)

2.4.2. Semen collection and evaluation

2.4.1.1. Bull training and handling for semen collection

Although semen could theoretically be collected by using an AV, in most cases, the difficulty in training bulls to use this system makes it impractical. Successful semen collection with an AV depends on the bull being comfortable around people and they need to be trained to use the AV. However, bulls are big and dangerous and personnel safety should be emphasized constantly (DeeWhittier, 2000). Most bulls in AI centers have a nose ring installed as a valuable and humane means of physical control. Bulls undergoing semen collection should be haltered and one should never tie a bull up by their nose ring if startled, then can rip it out which is not only quite traumatic but yields an animal that may be exceptionally difficult to control. "False mount" is an effective way to sexually stimulate the bull. Providing two false mounts with two minutes of active restraint and one additional false mount maximizes sperm cell numbers. Final preparations are made to the AV between the second and third false mounts. Proper construction of the AV is important to avoid damaging the bull's penis and to avoid stressing sperm cells. During collection, the person handling the AV must remain aware of where their feet are relative to the bull. As the animal ejaculates, it is common for him to jump forward. To avoid foot injury, collectors should wear boots with steel toes, (DeeWhittier, 2000; Rouge, 2002; Webb, 1992).

2.4.1.2. Semen collection

Semen can be collected by using different methods including AV (Roberts, 1986), Electro Ejaculator and by physical massage of the ampullae and vesicular glands through rectum (Webb, 1992). AV is simple in construction and it stimulates natural copulation by providing suitable temperature, pressure and lubrication that evoke ejaculation and yield representative sample (Hafez, 1993). The AV consists of a firm cylindrical tube with a thin-walled rubber lining. The jacket formed is filled with warm water. A rubber funnel connected to a collection receptacle is attached to one end of the cylinder. When

the jacket is properly filled and the AV lubricated and properly applied, this method of semen collection is highly successful (Webb, 1992).

The other techniques that have been employed less frequently for semen collection are the electro-ejaculator and manual manipulation. Electro-ejaculator is used when the bull refuses to serve the AV or when it fails to mount and the quality of semen obtained by electro-ejaculator and manipulation of ampullae and vesicular glands is relatively poor (Hafez, 1993**;** Arthur *et al*., 1992; Rouge, 2002; Webb, 1992; DeeWhittier, 2000).

In AI centers, semen is typically collected 2 or 3 times per week per bull, with 2 or 3 ejaculates per collection day. An experienced veterinarian or reproductive physiologist should determine semen quality. An examination of the reproductive tract may indicate possible abnormalities in semen quality (DeeWhittier, 2000; Rouge, 2002; Sorensen, 1979).

2.4.1.3. Semen evaluation

Semen examination has great diagnostic value in determining the cause, severity and degree of testicular or accessory gland pathology or infertility, as well as being of value in estimating the fertility of the male. As definite correlation between testicular pathology, disease of the reproductive tract and accessory gland, the semen characteristics and fertility has been found useful (Roberts, 1986). The most important semen parameters that shall be considered and their respective recommended values (for adult Holstein-Frisian bulls) are presented in (Table 4).

Table 4. Semen parameters with standard values

Semen parameter	Recommended values
Ejaculate volume	5 ml (range 1-15 ml)
Sperm concentration	1200 million/ml (range 300-2500 million/ml)
Total sperm per ejaculate	4-5 billion
Progressive motility	> 30%
Morphological abnormality	< 30%

Source: Rouge (2002)

Color and volume evaluation

It is important to note the appearance of any semen sample, particularly to identify abnormal conditions. It is normal for the color of an ejaculate to vary among individuals. Some bulls, for instance, ejaculate distinctly yellowish semen, which is due to the presence of the harmless riboflavin pigment, wile most have light, cream colored semen indicating good concentration. Good sample will appear creamy. Fair samples of semen will appear thin and have a grayish cast. It looks like skimmed milk and has no swirling motion. Poor sample will have a very thin appearance because only a few sperm count (Cupps, 1991; Hafez, 1993).

Semen from most species is homogenous in consistency, and the presence of flakes or small clumps usually indicates an inflammatory process in the reproductive tract of the male. There are huge differences among species in sperm concentration and ejaculate volume (Table 4). There is also considerable variability in these parameters among males of a single species, (Rouge, 2002; Roberts, 1986; Hafez, 1993; Cupps, 1991).

Motility of spermatozoa

Sperm motility is usually assumed to be the percentage of sperm that are progressively motile. A progressively motile sperm swims briskly forward in a relatively straight line, as opposed to moving in circles. When analyzing motility, it is important to know whether the semen sample has been abused in any way**.** Exposure to heat, cold, any kind of residue on collection equipment, or the wrong PH or osmolality of an extender can adversely affect motility. Motility is also affected by periods of sexual inactivity; males that have not ejaculated for prolonged periods often have poor motility on the first ejaculate, but much better motility for a second ejaculate collected soon thereafter (Rouge, 2003).

Spermatozoa concentration

The most common means of determining sperm concentration is to simply count sperm under a microscope with the aid of a hemocytometer. Prior to counting sperm, they need to be killed to prevent movement (Rouge, 2002). A spectrophotometer has been used as an alternative technique to determine sperm concentration. A spectrophotometer measures the amount of light absorbed by a sample, and the more sperm are in the sample, the more light is absorbed. By generating a standard curve of absorbance versus sperm numbers, one can quickly and accurately measure sperm concentration without directly counting them (Rouge, 2002)

Morphological assessment

An important part of any breeding soundness examination is an evaluation of sperm morphology. In the most fundamental case, the size and shape of the head, midpiece and tail are examined**.** Additional information can be gained by evaluating integrity of the acrosome and sperm membranes (Rouge, 2003; Sorenson, 1979).

The results of a sperm morphology examination are reported as percent normal. It is always the case that some sperm from an ejaculate are morphologically abnormal, such as

bent tails or misshapen heads but when that fraction becomes excessive, fertility may decrease. It is also useful to sub classify the abnormal population into the types of abnormality observed. Two types of classification schemes are commonly used. The most basic type of classification scheme differentiates primary and secondary abnormalities (Rouge, 2003). Primary defects are the more severe and are thought to originate while the sperm was still within the somniferous epithelium of the testis. Secondary defects are less serious and thought to arise during passage thought the epididymis or by mishandling after ejaculation (Rouge, 2003; Sorenson, 1979; Hafez, 1993).

2.5. Semen processing and storage

The semen ejaculated and collected should be diluted or extended before freezing and storage. The main reason for extending (diluting) semen is to increase the number of females serviced from one ejaculation. A normal ejaculate from a dairy bull will contain 5 to 10 billion sperm, which can be used to inseminate 300 to 1000 cows if fully extended. There are several good semen extenders (Webb, 1992; Hafez, 1993). Those made from egg yolk or pasteurized, homogenized milk are two of the most widely used. A good extender not only adds volume to the ejaculate but favors sperm survival and longevity. Dilution rate depends on quality of the ejaculate, number of sperm cells, percent alive and mobility. As few as 12 million sperm per insemination have given good conception rates. Penicillin and streptomycin are also added to semen extenders. These antibiotics inhibit bacterial growth and reduce danger of spreading diseases such as vibriosis (Webb, 1992; Hafez, 1993).

The discovery that bull semen could be successfully frozen and stored for indefinite periods has revolutionized AI in cattle. In 1949, British scientists discovered that addition of glycerol to the semen extender improved resistance of sperm to freezing. Glycerol acts to remove water from the sperm cell prior to freezing and prevents the formation of cellular ice crystals which would damage the sperm. There are two methods of freezing and storing semen, dry ice and alcohol (100^{o}F) and liquid nitrogen (-320^{o}F). Liquid

nitrogen is preferred because there is no evidence of fertility deterioration with age. Fertility gradually declines in semen stored in dry ice-alcohol. Frozen semen can be stored indefinitely, if proper temperature is maintained. A recent report told of a calf born from frozen semen stored for 16 years. Fresh, liquid semen can be successfully stored for 1 to 4 days at 40^{o} F (Webb, 1992).

2.6. Heat detection and timing of insemination

2.6.1. Heat detection

Cows commonly show estrus at an average of 21 days (20 days for a heifer). The actual average length in lactating dairy cows is 23 days. After insemination, if a cow is pregnant, corpus luteum will not regress to insure production of progesterone and to maintain pregnancy. Conversely, if a cow is not pregnant after insemination, the corpus luteum regresses and the progesterone level remains at a basal level and the cow returns to estrus approximately 20-23 days after insemination. Therefore, if a cow is observed in estrus after insemination, it can be concluded that she is not pregnant (Moreira and Hanson, 2003). Errors in heat detection have a substantial effect on the length of the breeding period, conception rates, days open and calving intervals (Moreira and Hanson, 2003; Marcinkowski, 2004). Two to three times 30-minute intensive observation periods per day can enable observers to achieve high rates of efficiency. A number of heat detection aids have been developed to improve heat detection efficiency and eliminate the subjectivity associated errors with observation (Table 6). However, visual observation remained as the most practical and economical method in tropical countries (Marcinkowski, 2004; Roberts, 1986).

Table 5. Heat detection rates using various methods

Heat detection method	Percentage detected
Watched 24 hours per day	89
KaMaR heat mount detectors	87
Visual observation, 3X per day (Dawn, Noon and Evening)	86
Continuous videotape	81
Visual observation, 2X per day (Dawn and Evening)	81
Use of marker animals	75
Chalked tail heads	71
Two trained dairymen (at milking)	50
Herdsman (at milking)	50
Casual observation	43

Source: Marcinkowski (2004)

2.6.2. Timing of insemination and techniques of insemination

A frequent question concerning AI is at what time during estrus cows should be bred for greatest chance of conception. Since estrus may last from 10 to 25 hours, there is considerable latitude in possible time of insemination. Much research works have indicated that conception rate is lower when cows are bred prior to mid-estrus or later than 6 hours after cessation of estrus (standing heat in this case). Success in insemination timing is dependent upon a good heat detection program (Beeman, 1999; Webb, 1992; Chenoweth, 2000). It is recommended by Webb (1992) that cows detected to be in heat in the morning should be inseminated in the same day while those showing heat signs in the afternoon should be inseminated in the morning of the next day for better efficiency.

The technique of inseminating a cow is a skill requiring adequate knowledge, experience and patience. Improper AI techniques can negate all other efforts to obtain conception. Semen must be deposited within the tract of the cow at the best location and at the best time to obtain acceptable conception rates. 60% conception rate is common for

experienced inseminators. It is about the same as if the cow was mated with a bull naturally (Webb, 1992)

2.7. Pregnancy diagnosis

According to Frederico and Hansen (2003), there are several methods of pregnancy diagnosis in cattle and other animal species. Some of them are:

- Rectal Palpation:
- Milk Progesterone Test
- Blood Progesterone Test
- Pregnancy specific proteins
- Ultrasound

Rectal palpation is the most practical and commonly used method. The technique allows palpation of pregnancy through the rectal and uterine walls for fetal membranes, amniotic vesicle, cotyledons and fetus. Rectal palpation is probably the most commonly used method for pregnancy diagnosis (Frederico and Hansen; 2003; Roberts, 1986).

An experienced technician can determine pregnancy 35 to 40 days after insemination. Several palpable structures are indicative of pregnancy. Due to accumulation of fluids within the pregnant uterine horn, one of the initial signs of pregnancy is a difference in size of uterine horns (i.e., uterine asymmetry). It is also possible to feel the slipping of the chorioallantoic membrane (fetal membrane) along the greater curvature within the uterus (i.e., membrane slip). As pregnancy progresses, it becomes possible to feel the presence of the fetus within the pregnant horn. After about day 150, the fetus is too far forward in the body cavity to palpate the entire fetus although fetal structures can be palpated.

Beginning at about day 90, it becomes possible to feel the placentomes, which are the structures formed by the union of maternal caruncles and fetal cotyledons by which the placenta is attached to the uterus. Beginning at day 120 of pregnancy, one can palpate vibrations and pulsing in the uterine artery. This phenomenon is called fremitis and is caused by the large increase in blood flow through the uterine artery (Frederico and Hansen, 2003).

Rectal palpation has the advantage of being an accurate, fast, relatively cheap method that is less labor intensive as compared to the previous methods. Nonetheless, training is necessary and the examination should be conducted by a veterinarian or by an experienced herdsman. The main disadvantage of rectal palpation is that it cannot be performed until later in gestation than some other methods. Some experienced veterinarians are able to determine pregnancy by palpation as early as 35 days after insemination, but usually rectal examinations take place between 45 and 60 days after insemination to increase the accuracy of the examination (Frederico and Hansen, 2003, Roberts, 1986)

3. MATERIAL AND METHODS

3.1. The study area

3.1.1. National Artificial Insemination Center

Semen quality study was carried out at the National Artificial Insemination Centre (NAIC) located at southeast outskirt of Addis Ababa. Addis Ababa, the capital city of Ethiopia, lies between 8^o 55' and 9^o 07' north and 38^o 40' and 38^o 50' east situated in the center of a well drained plateau and surrounded by hills and mountains. The latitude is between 2000 meters and 3000 meters above sea level. The rainy season of Addis Ababa extends from Mid-June to Late-September with annual average rainfall of 1000mm. The average annual daily temperature is 16^oC. The city covers an extensive area of 216 km^2 and has a population of more than 3 million. According to a census undertaken by the Addis Ababa Regional Agricultural Bureau (1996) a total of 5,167 small, medium and large dairy farms are found and 34,649,450 liters of milk is produced annually. A total of 37,426 local and crossbred cows are available within the region out of which 20% are indigenous and 46.5% are crossbred.

The NAIC was established in 1981 under the Animal and Fisheries Resources Development Main Department (AFRDMD) of the Ministry of Agriculture with the mandate of organizing and coordinating AI activities, as well as directing the breeding program at a national level. The center is involved in semen and liquid nitrogen production, provision of regular group and individual training on AI technicians and farmers and dairy recording system. The AI field service is carried out by inseminators who were trained by the Center and assigned at the Agricultural Offices of the different regions of the country. Semen is collected from exotic (Friesian and Jersey), crossbred and zebu (Boran, Fogera, Barka, Horro and Arsi) bulls maintained at Kaliti and Asela farms. The center produced about 211,000 doses of semen and 209,000 liters of liquid

nitrogen in 11 years time (1984-1994). The center has trained 259 inseminators from 1984 to 2000. Although the information collected is not complete and consistent, the center also has a dairy recording system to evaluate the performances of AI bulls, to give technical and managerial advice to dairy farmers, to provide reliable and up-to date information on identifying bull dams to select breeding bulls of high pedigree records and later perform progeny testing. Currently, the Centre has 36 bulls, which are either Friesian, Jersey or crosses of exotic and indigenous breeds (NAIC, 1995).

3.1.2. Girar Jarso Wereda

The evaluation of AI efficiency at field level was carried out in Girar Jarso Wereda of the North-Shewa Zone of the Oromia Regional State. The Zone is an area located 110 km northwest of Addis Ababa. In the Zone, there are 12 weredas/districts from which Girar Jarso Wereda is selected purposely considering the better relative availability of AI activity. The Zone covers 117,4500 ha of land from which 40% is crop land, 25% is grazing land, 13% is forest and bush area, 7% is construction area and 15% is unproductive land. The human population in the area is 1,242,366. There are 210, 215 households in the region that practice predominantly mixed farming. The cattle population is about 1,113,200. Fourty two percent of the area is highland that is suitable for crop cultivation and livestock husbandry and the herd structure is characterized by a higher number of cows. The lowland area constitutes 35% and farming activities represent the largest part of the small holders' income. The herd structure in the lowland areas is dominated by oxen. Selale has 2 annual rainy seasons: from February-May (short rainy season) and from June-October (long rainy season) (Tittarelli, 1990).

3.2. Study population

Breeding bulls kept at the NAIC and postpartum dairy cows and heifers owned by smallholder farmers in the selected wereda were the study population. Bulls kept at the Centre were housed intensively with well-constructed, clean and concreted individual pen about the size of $12m^2$. They were fed according to their weight but on the average, about

9kg of hay and 2.5 kg of concentrates were supplied daily for each bull. In addition, mineral licks and green grass were supplied when they are available and water is given adlibitum. There is a veterinary clinic in the center which undertakes regular follow up and checks for some venereal diseases like brucellosis and other health aspects as necessary. None of the bulls were found positive for venereal diseases and disease history of the bulls indicate that they were treated for minor abnormalities like inter-digital wound, skin abrasions and joint callous.

3.3. Study design

The study was undertaken from October 2004 to April 2005. A cross sectional type of study was carried out to evaluate semen quality of AI bulls at the NAIC and AI field efficiency in Girar Jarso Wereda.

3.3.1. Sampling procedure

The sampling frame was the list of all breeding bulls kept in the NAIC and postpartum dairy cows as well as heifers in Girar Jarso Wereda. The sampling units were individual bull, postpartum dairy cows and heifers. All the reproductive age Friesian and crosses of Friesian and local bulls (n=16) in the Center, which were by then used for semen collection, were included in the sample to study semen quality. A total of 93 postpartum dairy cows and heifers, which came to the veterinary clinic in Girar Jarso wereda to get AI service were included in the sample to study AI field efficiency.

3.3.2. Data collection

3.3.2.1. Observational study on morphological parameters

The identification number, age, body weight and genotype of bulls kept by the NAIC were recorded at the beginning of the study. The management system prevailing in the centre (housing, feeding and health management systems) was also recorded. Bulls were examined for external morphological fitness. Testicular parameters (visual inspection, palpation of the testis and measurement of scrotal circumference) were also measured. Indicative parameters considered for external morphological fitness were masculinity and defects of feet, leg and external genitalia. Regarding testicular parameters the scrotum was visually observed and palpated for some physical abnormalities like pendulousness, skin adhesion, outer skin lesions, epididymis and spermatic cord abnormalities and constricted neck. In addition, scrotal circumference of each bull was measured using a measuring tape. Body condition score of bulls taken according to Rodenburg (1996).

3.3.2.2. Semen collection and quality evaluation

Semen was collected from each of the 16 bulls once per week for the period of eight weeks. During semen collection, bulls were evaluated for their libido, in the presence of teaser bull and were also evaluated for their ability to mount, seek, intromission and thrust.

Before starting semen collection, all the necessary materials were kept ready. The slide, slide covers and graduated collecting tubes were incubated at 37^0C. The microscope stage was maintained at 37^0C using installed stage heater and the water bath was kept at the same temperature as the stage (Rouge, 2002).

The AV was filled with warm water (42-48^0C) and air until it forms a "V"shape and then lubricated with Vaseline. AV was fitted with the graduated collection tube and covered with plastic or leaser covering sheet to protect the collected semen from sunlight. The

collection area was prepared before the start of each semen collection. The area was cleaned, rinsed with water to avoid dusting and the teaser bull was also be well restrained and cleaned with water, soap and brush to protect semen contamination with faeces and hair (Rouge, 2002). Bulls were hold by attendants with nose-ring and allowed to be sexually stimulated for some minutes and exercise one or two false mounts. During collection, the collector stands at the right side of the teaser bull, holding the AV with his right hand and held the prepuce of the bull and inserted the penis in to the AV, which was kept parallel to the anal area of the teaser. As soon as the bull ejaculated, the semen was taken to the laboratory and put in the water bath for quality check, processing and storage. The collected semen was evaluated for volume, color, presence of foreign material (blood, hair, pus and others), mass activity, motility, concentration, morphology and proportion of dead/alive (Annex 1) (Cupps, 1991; Rouge, 2003). Concentration was evaluated using Spectrophotometer (Annex 2).

3.3.2.3. Semen processing, storage and transportation

After sample collection and evaluation of semen from the 16 bulls, the semen collected from eight bulls, four Friesians and four crosses of Friesian and local bulls were further processed for field efficiency trial. Semen that passed the evaluations for color, mass activity, motility, concentration, morphology and dead/alive was diluted with half of the total sodium citrate and egg yolk diluents or extenders. The diluted semen was kept for one hour for gradual cooling up to 4^{o}C. The semen was then diluted with the remaining half extender and 2.56% of glycerol (4^{0}C) was added to protect intra-cellular ice formation (as cryoprotectant). The diluted semen was put for about 3-4 hours in the automatic filling and sealing machine, which automatically fills and seals the straws. The 0.25ml capacity French straws which, were filled with semen of $30x10^{6}$ spermatozoa/ml were placed on the nitrogen vapor rack and then transferred into the liquid nitrogen at -197^{0}C. Semen was transported to the site of field trial using nitrogen-containing cylinder filled with liquid nitrogen into which straw-containing tubes were put. During field insemination work, some straws were checked for the sperm motility after thawing at 37^{0}C. A straw was thawed at 37^{0}C water bath and cut on both ends with scissors and one

end was closed with the finger. A drop of semen was put on a warm microscope slide, covered with warm cover slide and put on the warm microscope stage. Thaw motility was appreciated under 40x microscope objective.

3.3.2.4. Insemination of postpartum cows and heifers

A total of 93 animals (68 cows and 25 heifers) were served by AI. A minimum of 10 cows were served by the semen of each of the 8 bulls included in the field efficiency trial. Postpartum cows or heifers diagnosed to be in heat by farm owners or farmers were brought to the veterinary clinic in the wereda for AI service. Then after, heat signs were confirmed by rectal palpation according to Roberts (1986). Insemination was performed after thawing of the straws or semen for 20-30 seconds at 34-37^{0}C water bath was made. The thawed straw was then cut with the scissors at the sealed end and put in the insemination gun or catheter. A sterile, disposable plastic sheath was put over the catheter containing the thawed semen and inserted into the vagina and then guided into the cervix by means of a gloved hand in the rectum. The inseminating catheter was passed through the spiral folds of the cow's cervix into the uterus. Part of the semen was deposited just inside the uterus and the remainder in the cervix as the catheter was withdrawn, (Chenoweth, 2000; Webb 1992). While the services were carried out, heat signs observed by farmers, time of heat detection, time of insemination and other information regarding individual cows coming to service were recorded (Annex 3). In addition, body condition score of cows taken according to Rodenburg (1996) (Annex 4).

3.3.2.6. Pregnancy diagnosis

Inseminated cows and heifers were examined for pregnancy by rectal palpation after 60 days post-service according to the methods described by Roberts (1986) (Annex 5). Cows or heifers, which come to heat after first insemination, were considered as non-pregnant.

3.3.3. Data analysis

All data collected during the whole course of the study period were entered in to Microsoft Excel program (Version 6.0, 2000). Descriptive statistics like proportions, means, standard errors and standard deviations were processed using SPSS (release 11.5, 2002) statistical package. The package was also used to compare the data of two genotype groups with independent t-test. STATA (7.0, 2001) statistical package was used to analyze effects of different factors on conception rate using logistic regression.

4. RESULTS

4.1. Results of observational study at the NAIC

4.1.1. Physical fitness

In all the 16 bulls examined physically, there were no major physical abnormalities. Some physical and pathological abnormalities recorded were, inter-digital wound on 5 bulls (4 Friesians and 1 Cross (Friesian x Barka)), wounds on dewclaws on 1 bull (Cross of Friesian and Barka), traumatic penal wound on 1 bull (Cross of Friesian and Boran), pendulous testis on 1 bull (Friesian) and 1 bull with both inter-digital wound and wound on dewclaws (Friesian).

4.1.2. Bull attributes

Out of the 16 bulls studied 37.5% (n=6) were crosses of Frisian with locals (three 75% crosses of Friesian and Boran and three 50% crosses of Friesian and Barka) and the remaining 10 bulls were Friesians. All the bulls were at the age of breeding with the range of 19 months to 85 months. The average age of the bulls was 43.4 months (SD= 19.8). The body condition score of each bull was scored from 3 to 4 (annex 5). The scrotal circumference value ranged from 30-44cm. The average scrotal circumference of the bulls was 36.8cm (SD= 3.4).

4.2. Descriptive results of semen quality of NAIC bulls

The main colors identified were creamy, yellowish, milky, watery and bloody. The overall number of bulls with respect to the colors were 1 (6.25%) watery, 6 (37.5%) creamy, 6 (37.5%) milky, 1(6.25%) bloody and 2 (12.5%) yellowish. The color distribution by breed is indicated in Table 6. No watery and bloody semen was recorded

for Friesian bulls and no yellowish semen was observed in crossbred bulls. Most of the semen samples were either milky or creamy in both cases.

Table 6. Proportions of semen colors among the two breeds of bulls

Color types	Crossbreed (%) N=6	Friesian (%) N=10
Watery	16.6	0
Milky	33.3	40
Creamy	33.3	40
Bloody	16.6	0
Yellowish	0	20

N= number of observations

The semen quality values for the 8 collections were pooled into one mean for each bull. The mean and standard deviations of semen quality parameters for the two breeds of bulls in the NAIC are presented in Table 7. In general, the crossbred bulls had better ejaculate volume and motility, while better results were recorded for Fresian bulls for the other characteristics (concentration, mass activity, morphological defects and percentage of live spermatozoa).

Table 7. Means and standard errors of semen traits in crossbreed and Friesian bulls

Semen characteristics	Cross breed bulls N=6	Friesian bulls N=10
Ejaculate volume (ml)	7.18 (0.69)	6.77 (0.82)
Mass activity (score:1-5)	3.17 (0.23)	3.18 (0.11)
Individual motility (%)	71.67 (1.67)	71.00 (2.33)
Sperm concentration (million cells/ml)	1143.10 (69.54)	1270.30 (87.38)
Percentage of major defects	3.50 (0.56)	2.86 (0.49)
Percentage of minor defects	14.67 (2.23)	13.24 (1.34)
Percentage of alive cells	74.67 (3.61)	76.60 (1.83)

4.2. Factors affecting semen quality

4.2.1. Effect of breed on semen parameters

Results of independent t-test (Table 8) revealed that breed difference had no significant effect on semen parameters ($p>0.05$). Crossbreed bulls had higher values of semen volume while Friesian bulls had better values in the rest of the parameters. Results of Man-Whitney test to test the effect of breed of bulls on scores of mass activity revealed that breed had no significant effect on mass activity ($p>0.05$).

Table 8. Results of independent t-test analysis to see effect of breed of bulls on semen Parameters

Semen parameters	Breed group	Mean (SE)	t-value	DF	P-value
Volume (ml)	Crossbreed	7.18 (0.70)	0.35	14	0.734
	Friesian	6.77 (0.82)			
Concentration (million/ml)*	Crossbreed	3.05 (0.03)	0.88	14	0.396
	Friesian	3.09 (0.03)			
Alive cells (%)	Crossbreed	74.67 (3.61)	0.55	14	0.603
	Friesian	76.60 (1.83)			
Morphologically normal cells (10)	Crossbreed	79.67 (1.33)	2.00	14	0.071
	Friesian	83.90 (1.46)			

* Values indicated here are log-transformed, SE= standard error, DF= degree of freedom

4.2.2. Effect of age on semen parameters

Independent t-test analysis showed that age had significant effect only on semen volume ($p<0.05$) (Table 9). Semen volume and alive cells percentage were higher in bulls older than 4 years of age than those less than 4 years of age. Although it was not significant, bulls older than 4 years of age had also better spermatozoa concentration and proportion of morphologically normal cells. The association between bull age and semen volume is depicted in Figure 1. Results of Man-Whitney test to determine the effect of age of bulls on scores of mass activity revealed that age had no significant effect on mass activity ($p>0.05$).

Table 9. Results of independent t-test analysis to see effect of age of bulls on semen parameters

Semen parameters	Age group	Mean (SE)	t-value	DF	P-value
Volume (ml)	4 &less years	5.96 (0.64)	2.63	14	0.02
	>4years	8.53 (0.68)			
Concentration (million/ml)*	4 &less years	3.06 (0.02)	0.97	14	0.35
	>4years	3.11 (0.05)			
Alive cells (%)	4 &less years	77.60 (2.35)	1.33	14	0.42
	>4years	73.00 (2.08)			
Morphologically normal cells (10)	4 &less years	81.30 (1.54)	1.16	14	0.27
	>4years	84.00 (1.57)			

* Values indicated here are log-transformed, SE= standard error, DF= degree of freedom

4.2.3. Effect of scrotal circumference on semen parameters

Volume is found (Table 10) to be highly affected ($p<0.01$) by scrotal circumference of bulls while the other parameters were not significantly affected ($p>0.05$). Bulls with a scrotal circumference of greater than 35cm had better semen volume than those with ≤ 35cm. Bulls with larger scrotal circumference had also better spermatozoa concentration while those with smaller value of scrotal circumference group had better percentage of alive cells and morphologically normal cells, although the results were not significant. The association between scrotal circumference and semen volume is shown in Figure 2. Results of Man-Whitney test to determine the effect of scrotal circumference of bulls on scores of mass activity revealed that scrotal circumference had no significant effect on mass activity ($p>0.05$).

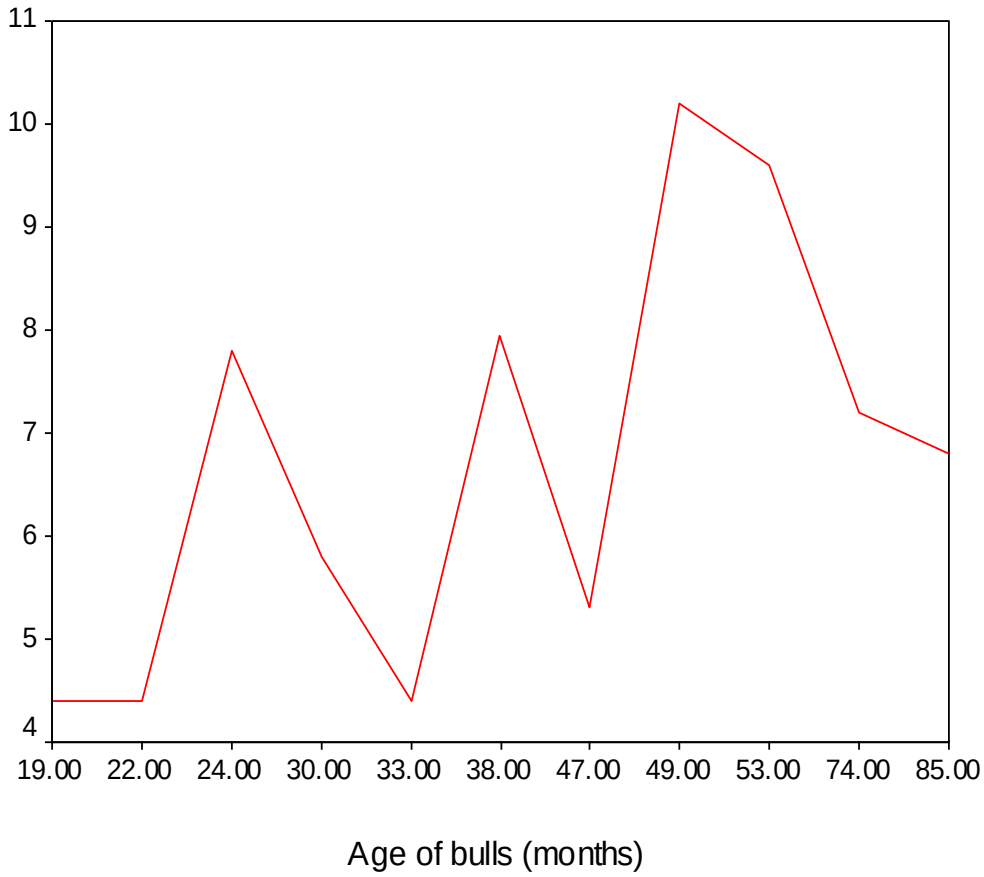


Figure 2. Graph showing the association between age of bulls and semen volume

Table 10. Results of independent t-test analysis to see effect of scrotal circumference of bulls on semen parameters

Semen parameters	Scrotal circumference	Mean (SE)	t-value	DF	P-value
Volume (ml)	≤ 35cm	4.62 (0.66)	3.84	14	0.002
	>35cm	7.97 (0.50)			
Concentration (million/ml)*	≤ 35cm	3.04 (0.03)	1.12	14	0.280
	>35cm	3.10 (0.03)			
Alive cells (%)	≤ 35cm	77.80 (1.60)	0.74	14	0.470
	>35cm	75.00 (2.39)			
Morphologically normal cells (10)	≤ 35cm	83.00 (2.72)	0.39	14	0.700
	>35cm	82.00 (1.21)			

* Values indicated here are log-transformed, SE= standard error, DF= degree of freedom

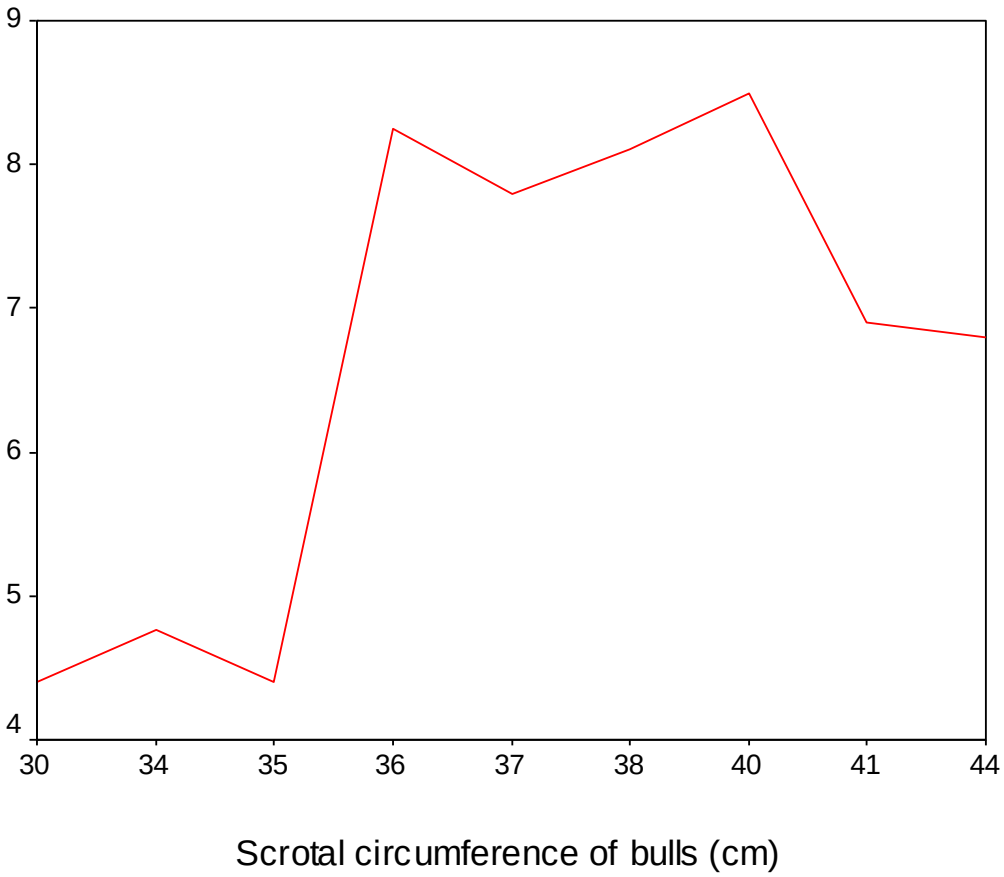


Figure 3. Graph showing the association between scrotal circumference of bulls and semen volume

4.3. Relationship between the different semen traits

The results of bivariate Pearson's correlation analysis (Table 11) indicated that scores of mass activity were positively correlated with individual motility ($p<0.01$) and spermatozoa concentration ($p<0.05$). All the other parameters used did not show significant association ($p>0.05$). There were tendencies of positive correlations between mass activity and percentage of alive spermatozoa and, volume and spermatozoa concentration.

Table 11. Results of Pearson's correlation analysis between different semen parameters bulls

Parameters	Results	VOL	INM	CON	ALP	MN
MA	Pearson Correlation	-0.063	0.669**	0.454*	0.370	0.154
	P-value	0.408	0.002	0.039	0.079	0.285
VOLU	Correlation coefficient		0.002	0.393	-0.194	-0.160
	P-value		0.496	0.066	0.235	0.277
INM	Correlation coefficient			0.232	-0.027	0.291
	P-value			0.193	0.460	0.137
CON	Correlation coefficient				0.178	0.200
	P-value				0.255	0.228
ALP	Correlation coefficient					-0.037
	P-value					0.446

** p< 0.01, *p<0.05, N=16, MN= morphologically normal, CON= concentration, INM= individual motility, MA= mass activity, VOL= volume, ALP=alive percentage

4.4. Results of study on field efficiency of AI bulls

4.4.1. Description of the dairy cattle attributes

A total of 93 dairy cattle (heifers or cows) were inseminated with semen collected from 8 of the 16 bulls studied. Twenty dairy cattle were local breeds (21.5%) while 4 (4.3%), 28 (30.1%) and 41(43%) of them were with 25%, 50% and 75% or above exotic blood mainly of Friesian, respectively. The age of the dairy cattle varied from 24 months to 85 months, their mean age being 53.6 months (SD=19.73). More than half of the dairy cattle (n=56, 60.2%) were non-lactating and the remaining (n=37, 39.8%) of the dairy cattle were lactating. Most of the dairy cattle (n= 65, 69.9%) were with a body condition score of 4 and 5 and the rest were (n=28) with less than 4 of body condition score. 26.9% (n=25) of the dairy cattle were heifers and the higher proportion (n=68, 73.1%) was made of cows.

4.4.2. Factors affecting field efficiency of AI

An overall first service pregnancy rate of 55.9%, was found under field condition. A univariate logistic regression was carried out to determine the effects of factors including

age of bulls, breed of bulls, scrotal circumference of bulls and mass action of semen of bulls. The result of the analysis (Table 12) indicated that none of the factors mentioned above had significant effect on the pregnancy rate of AI bulls ($p>0.05$). Better pregnancy rates were recorded for older bulls (older than 4 years), Friesian bulls and bulls with smaller scrotal circumference ($\leq$ 38cm) and higher mass action scores (>3).

Table 12. Results of univariate logistic regression to analyze effects of bull attribute on pregnancy rate

Factors	Group	N	Number pregnant (%)	CI (95%)	P-value	OR
Age of bulls	≤ 4 years	50	31(62.0)	0.26-1.34	0.204	0.59
	> 4 years	43	21(48.8)			
Breed of bulls	Friesian	45	24 (53.3)	0.84-1.22	0.908	1.01
	Crosses	48	28(58.3)			
SC of bulls	≤ 38 cm	62	36(58.1)	0.32-1.83	0.555	0.77
	> 38 cm	31	16(51.6)			
Mass action score of semen	≤ 3	45	22(48.9)	0.76-3.98	0.188	1.74
	> 3	48	30(62.5)			

N= number of observations, OR= odds ratio, SC= scrotal circumference

Univariate logistic regression was also carried out to determine the effects of cow or heifer attributes and time of insemination on pregnancy rate of AI bulls. The results (Table 13) revealed that only body condition score affected pregnancy rate significantly ($p<0.05$). Cows or Heifers with body condition score of greater than 3 had significantly higher pregnancy rate (63.1%) than those with body condition score lower than 3 (39.3%). The other factors considered had no significant effect on pregnancy rate ($p>0.05$) including parity number, breed and lactation status. Better pregnancy rates were recorded in cows than heifers, local breeds than crosses, non-lactating animals than lactating and for inseminations done within 12 hours after the detection of heat.

Table 13. Results of univariate logistic regression to analyze effects of cow attribute on pregnancy rate

Factors	Group	N	Pregnancy Rate (%)	CI (95%)	P-value	OR
Parity	Heifers	25	15(60.0)	0.84-1.28	0.733	1.04
	Cows	68	37(54.4)			
Breed of cows/heifers	Local	20	11(55.0)	0.39-2.84	0.926	1.05
	Cross	73	41(56.2)			
BC of cows/heifers	≤ 3	28	11 (39.3)	1.06-6.56	0.037	2.64
	> 3	65	41 (63.1)			
Lactation status	Lactating	56	31(55.4)	0.41-2.18	0.894	0.94
	Non-lactating	37	21(56.8)			
Time of insemination*	≤ 12 hours	32	20 (62.5)	0.28-1.59	0.355	0.66
	> 12 hours	61	32 (52.5)			

N= number of observations, OR= odds ratio, BC= body condition score, * time after the detection of sign of heat

5. DISCUSSION

Bulls kept at the centre are housed intensively, with well-constructed, clean concrete individual pen about the size of $12m^2$. This housing structure is very close to the suggestion of Payne (1992) that mature large sized dairy bulls require a minimum surface area of $15.9m^2$. The feeding regime for bulls at the NAIC (feeding according to their body weight with an average value of about 9kg of hay and 2.5 kg of concentrates) goes along with the recommendation of Arthur and Robert (1987), that states mature dairy bulls should be fed primarily with any good quality hay along with a limited amount of haylage or silage if available, plus concentrates. Feed about 1.5% of the body weight as air dry hay. It has been found out that overfeeding or underfeeding are undesirable for breeding, that is quality and quantity of feed supplied to breeding bulls affects percentage motility, motility score and sperm concentration (Tegegn *et al.*, 1994; Mathvron *et al.*, 1998; Tozser *et al.*, 2000). In addition, Sprott *et al.* (1997) has indicated that under-nourishment may cause irreversible testicular damage in young bulls and decreased sperm production in mature bulls.

The age of bulls studied was from 19 to 85 months. According to Owen Rae, (1999), these bulls are at the breeding age, because, he has recommended that the older bulls (>8years old) should be culled. On the other hand Lee et al., (1998) and IIRR (2000) have suggested that bulls can be used for up to 4 years and should be replaced or switched with other bulls to avoid inbreeding. Parry and Patterson (2001) have put that semen quality of an individual bull changes over time. According to their work, individual bulls showed an increasing number and size of fibrotic foci in the parenchyma (6% young bulls to 43% older bulls) of testicular tissue from the age range of 9-520 weeks

The scrotal circumference range found in this study (30-44cm) agrees with the recommendation of DeeWhittier (2000) (table 3).

In the present study, the mean semen volume of the individual bulls (16 studied bulls) ranged from 2.9-10.6ml and the overall mean is 6.925ml. The mean semen volume found in this study is a bit higher than the findings of Rouge (2002) and Hundera (2004) who reported semen volume of 5ml and 4.84ml, respectively. However, the volume range (2.9-10.6) is narrower than the one indicated by Rouge (2002) (1-15ml) and wider than the result of Hudera (2004) (3.5-6.1ml) and Tegegn *et al.,* (1994) (3.4-5.0ml). The result of Kelay (2002) is very close to this finding. He showed that the semen volume in 5 years time in the NAIC ranged from 6.82-9.19ml.

About 66.6% of the semen of crossbreed bulls and 80% of the semen of Friesian bulls had either milky or creamy color in this study. This goes along with some recommendations, which suggest that bull semen has to have creamy to milky color (Roberts, 1971; Sorenson, 1979; Hafez, 1993; Cupps, 1991). Watery color indicates lower spermatozoa concentration (Hundera, 2004). Yellowish color, which was seen in two bulls in this study, is described by some authors as normal (Roberts, 1971 and Sorenson, 1979). They justified it to be because of the presence of the harmless riboflavin pigment which is a heritable character in some breeds like Holstein, Hereford and Guernsey.

The scores of mass activity varied in this study from 2.6-3.9 and the overall mean is 3.2. Hundera (2004), and Tegegn *et a.,* (1994) reported very close results in local Ethiopian bulls, in crosses add Friesians with the range of 2.5-3.7 and 2.4-3.0. The overall mean individual motility was found to be 71.25% in this study which is close to the report of Zhang (2000) (70-85%) and Hundera (2004) (68.72%). Rouge (2002) and Parkinson (2004) stated that the percentage progressive motility should be greater than 30%. Greater than 70% motility is considered as very good by Owen Rae (1999).

The minimum and maximum concentration of spermatozoa in this study was found to be 0.853 and 1.602 billion cells/ml of semen, respectively. This result is in agreement with the reports of Zhang (2000); Rouge (2002); Hundera (2004) and Kelay (2002) who reported ranges of 0.75 – 1.6 billion cells/ml, 0.3-2.5 billion cells /ml, 1.15-1.98 billion

cells/ml and 1.14-1.39 billion cells /ml, respectively. However, the results of this study are by far higher than the reports of Tegegn *et al.,* (1994) (0.164-0.589 billion cells /ml in Boran and 0.164-0.303 billion cells /ml in crosses of Boran and Frisian. The overall mean of concentration (1.223 billion cells/ml) also agrees with results of Hundera (2004) and Rouge (2002) who reported 1.54 billion cells/ml and 1.20 billion cells/ml of semen concentrations. But our result is higher than the findings of Igboeli and Rakha (1971) (0.518 billion cells /ml) and Tegegn *et al*., (1994) (0.213 billion cells /ml in the rainy season and 449 billion cells /ml in dry season).

The mean percentage of alive cells found in this study (75.9%) is close to the report of Hundera (2004). He reported that mean live spermatozoa percentage being 79.73%. The overall mean percentage of morphologically normal spermatozoa was 82. These figures is not out of the recommended morphological normal cells, which states that the spermatozoa of normal fertile bull has not to contain more than 20% total abnormality (Hafez, 1993). In addition, Rouge (2002) and Parkinson (2004) suggested that the morphological normal cells should be greater than 70%. The result of this study is in agreement with the results of rainy and cold seasons of Igboeli and Rakha, (1971) who reported 73.12%, 83.23 and 61.10% morphologically normal cells in the rainy, cold and hot seasons, respectively. However, the present result is a bit lower than the finding of Zhang (2000) (90-94.9%). The overall average major defect of the whole bulls was 3.1%. This finding is similar with the result of Nagy *et al*., (1999) (3.9%) but disagrees with the finding of Hundera, (2004) who indicated that the mean major defects are 2.25%.

In this study only age ($p<0.05$) and scrotal circumference ($p<0.01$) of bulls affected significantly semen volume. This result can be explained by the findings of DeeWhittier (2000) who reported that there is a correlation between the scrotal circumference of bulls and the volume of semen-producing tissue that the bull posses. The effect of age on semen volume in this study could be explained by the positive relationship between age of bulls and scrotal circumference of bulls. Many reports confirmed that scrotal circumference increases with age (Tegegn *et al.*, 1994; DeeWhittier, 2000; Sprott *et al*., 1997; Wattiaux, 1998; Chenoweth, 1990; Jensen, 1997; Parkinson, 2004). Lack of

association between breed of bulls and semen volume and individual motility in this study goes along with the report of Tegegne *et al.*, (1994).

The total pregnancy rate of this single insemination trial was 55.9%. This seems higher when compared with the results of Keith *et al.* (2005); Mukassa-Mugrewa, (1989) and Mukasa-Mugerwa *et al*., (1991) who reported pregnancy rates of 53.8%, 48% and 47.5%, respectively after single insemination. The present result, however, is lower than ADAS/IGER (2001) researchers report. These researchers found out a pregnancy rate as high as 70.9%. It was reported by some researchers that 60% conception rate in single insemination is common for experienced inseminators which is about the same as if the cow was mated with a bull naturally (Beeman, 1999; Webb, 1992; Chenoweth, 2000)

This study revealed that the body condition of female animals was the only factor significantly affecting pregnancy rate after first AI service. ADAS/IGER (2001) researchers indicated that pregnancy rate increases with the improvement of body condition score of cows. This can be supplemented by the findings of Kassa and Tegegn, (1998) who reported that body condition score affected the duration from calving to resumption of ovarian activity.

6. CONCLUSIONS AND RECOMMENDATIONS

The semen quality parameters of bulls of the National Artificial Insemination Centre are in general within acceptable ranges recommended by previous workers. Among many factors age and scrotal circumference of bulls are the most important factors affecting semen volume. In the field AI efficiency evaluation, body condition of cows or heifers was the only factor found to be important in determining pregnancy rate.

From these conclusions, the following recommendations are forwarded:

- Bulls of appropriate age and scrotal circumference should be kept as breeding stock in the center
- Farmers or farm owners should be well aware of the importance of improved management, feeding and health care of their cows or heifers which could have direct or indirect impact on body condition of the animals
- A more extensive study considering a number of factors should be carried out in the different dairy production systems in the country

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8. ANNEXES

Annex 1. Procedures of semen quality evaluation

- Volume is measured carefully by graduated collecting tube which is fitted to the AV Color is subjectively evaluated as creamy, milky, watery, yellowish

- To grade mass activity, a drop of semen is put on a warm (37^0C) slide and then placed on a microscope stage which is also kept at the same temperature as the slide. The semen can be observed under 20x objective lens of the microscope. Based on its wave and eddies formation and its degree of cloudiness, semen can be graded (Rouge, 2003). The speed of the wave can indicate us the motility of the sperm cells and the color or cloudiness indicates the concentration of the spermatozoa in the semen.

- A drop of semen is placed on warm slide, covered by a warm cover slip (to make thinner microscopic field and to protect it from drying) and observed under 40 x microscope objective to see individual spermatozoa motility. The individual motility rate is registered from 0-100% according to the estimated percentage of spermatozoa, which move in a progressive forward motion (Rouge, 2003).

- Morphology of the spermatozoa can be evaluated using the technique called "Hancock's technique". A buffered formole saline (Hancock solution) was used as a preservative of the sperm cells. A drop of semen is put in one milliliter of Hancock solution, which is kept in a water bath (37^0C), with warm pipette and gently mixed. A drop of this mixture is put on a microscope slide and covered with a cover slip and examined under phase contrast microscope (200x) for morphological abnormalities like head, mid-piece and tail defects and proximal and distal droplets. For this technique from 350-500 cells should be observed per ejaculate (Cupps, 1991).

- Eosin-Nigrosin technique was used to see the proportion of dead and alive sperm cell.

    - Have microscope slides and eosin-nigrosin stain prewarmed to body temperature.
    - Pipette a drop of stain onto the end of a slide, then pipette a small droplet of semen next to the stain.
    - Place the edge of another slide into the drops of stain and semen - rock that slide back and forth a few times to mix the sperm and stain, then smear the second slide across the surface of the first.
    - Dry the slide rapidly by placing on a warming plate or waving it back and forth in the air.
    - Examine using a bright field microscope (typically using a 40-100X objective lens).
    - The Eosin-Nigrosin stain produces a dark background on which the sperm stand out as lightly colored objects. Normal live sperm exclude the eosin stain and appear white in color, whereas "dead" sperm (i.e. those with loss of membrane integrity) take up eosin and appear pinkish in color, *(Rouge, 2002).*

Annex 2. Procedures of using a spectrophotometer to measure sperm cell concentration

Parts of spectrophotometer

a) **Diluter –**

- Open its own program and select the " bull semen" program, among many of the semen programs
- Order to wash (washes its system with saline water)
- Order to suck the diluent to the level of 0.96 ml (leaving 0.04ml for semen)
- Provide semen to suck the amount of 0.04ml
- Then it mixes the semen with the saline diluent and pours it in the glass prism.

b) **Photometer**-

- Select the part "measure ready"
- Put the diluted semen containing prism in the photometer
- Press the "ok" button to measure the concentration of spermatozoa.
- It displays, on the screen, information about concentration of sperm cells/ml, volume of extender required, ejaculate volume, working does provided before, number of straws required.

c) **Printer**-

- It prints all the information displayed on the photometer.
-

Annex 3. Individual cow or heifer information sheet

1. Owner's name----------

2. Woreda----------kebele----------

3. Cow ID. number.----------

4. Breed----------

5. Exotic blood level----------

6. Age----------

7. BCS ----------1------ ----------2----------3----------4----------5------

8. Present health status----------

9. Calving date----------

10. Parity----------

11. First service date----------

12. Number (No.). Of services from first service----------

13. Date of last service----------

14. No. Of services in previous calvings----------

15. Lactating status : ----------lactating, ----------dry, ----------heifer

16. Pregnancy diagnosis: ----------positive, ----------negative

17. Type of previous matings: ----------AI, ----------Natural mating

18. Last calving: ----------unassisted, ----------dystocia, (mild, medium, sever)

19. Reproductive history (last gestation period), normal/live calf, stillbirth, abortion, vaginal/ uterine prolapse, retained fetal membrane, endometritis, milk fever, other problems

20. Heat detection method----------

21. Time heat observed----------

22. Time of insemination----------

23. Bull identification----------

Annex 4. Body condition scoring

Body condition is a reflection of the body fat reserves carried by the animal. Cattle should be scored by both looking at, and handling the backbone, loin and rump areas. Since the pin bone, hip bone, the top of the backbone, and the ends of the short ribs do

not have muscle tissue covering them, any covering you see or feel is the combination of skin and fat deposits (Rodenburg, 1996).

Condition Score 1

This cow is emaciated. The ends of the short ribs are sharp to the touch and together give a prominent shelf-like appearance to the loin. The individual vertebrae (spinous processes) of the backbone are prominent. The hook and pin bones are sharply defined. The thurl region and thighs are sunken and in-curving. The anal area has receded and the vulva appears prominent.

Condition Score 2

This cow is thin. The ends of the short ribs can be felt but they and the individual vertebrae are less visibly prominent. The short ribs do not form as obvious an overhang or shelf effect. The hook and pin bones are prominent but the depression of the thurl region between them is less severe. The area around the anus is less sunken and the vulva less prominent.|

Condition Score 3

A cow in average body condition. The short ribs can be felt by applying slight pressure. The overhanging shelf like appearance of these bones is gone. The backbone is a rounded

ridge and hook and pin bones are round and smoothed over. The anal area is filled out but there is no evidence of fat deposit.

Condition Score 4

A cow in heavy condition. The individual short ribs can be felt only when firm pressure is applied. Together they are rounded over with no shelf effect. The ridge of the backbone is flattening over the loin and rump areas and rounded over the chine. The hook bones are smoothed over and the span between the hook bones over the backbone is flat. The area around the pin bones is beginning to show patches of fat deposit.

Condition score 5

A fat cow: The bone structure of the top line, hook and pin bones and the short ribs is not visible. Fat deposits around the tailbone and over the ribs are obvious. The thighs curve out, the brisket and flanks are heavy and the chine very round.

Annex 5. Pregnancy diagnosis in the cow

External indications

- Determination of service or AI date

- Cession estrus cycle
- Changes in udder size in prim Para animals
- Observation of fetal movements after 6 months
- Auscultation of fetal movement or heart beat from 6 to 7 months of gestation (only by experienced vets.)

Internal indications

Rectal method of manual manipulation

The rectal method of manual manipulation is the most economic of pregnancy diagnosis, but requires skilled professional and honesty.

Procedures

- Proper protective closing
- Removal of rings, watches, finger nails
- Lubrication of the hand
- Removal of fecal materials from the rectum
- Perform systematic palpation (location of the cervix, retract the cervix, location of the uterus)

Causes of rectal hemorrhage

- Forced manipulation
- manipulation of extended rectum
- Sharp finger nails
- Manipulation for longer period

Key indicators

The first 2 months

- Asymmetry of the two uterine horns
- Pronounced fluctuation in the pregnant horn
- Thin uterine wall

3 to 4.5 months, in addition to the above mentioned ones.

- Fetal bump
- Enlarged uterus
- Cotyledons and some part of the fetus

4.5 to 6 months

- The uterus is down to the abdominal cavity
- Cervix over the brim of the pelvic cavity

6 to 9 months

- Large head and fetal legs can be palpated very easily in the pelvic cavity (Roberts, 1986)

9. CURRICULUM VITAE

I. Personal data

Name: Wubet Sinshaw Desta

Age: 40

Marital status: Married

Place of Birth: Amhara National Reginal State, West Gojam Zone, Ethiopia.
Language (speaking and writing): Amharic and English.

II. Educational background

1972-1976: Birakat primary school
1977-1978: Merawi junior secondary school
1979-1982: Tana-Haik comprehensive secondary school
1983-1988: Higher education, Addis Ababa University, Faculty of Veterinary Medicine

III. Work experience

1989-1995: In Gambebella National Regional state as Awraja veterinarian, veterinary officer of the Region, team leader of the Regional Agricultural office (vet. section)
1996-1997: Amhara National Reginal State, as field veterinarian of the North Gondar agricultural office.
1998-2003: Veterinary expert in Bahir Dar Regional veterinary laboratory.
2003-2005: Postgraduate study, Addis Ababa University, Faculty of Veterinay Medicine.

IV.Research activities

Study on the prevalence of Hydatidosis in Robe Municipal abattoir (1988).
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10. SIGNED DECLARATION SHEET

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

Name: Wubet Sinshaw
Date of submission: June 15, 2005

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

Dr Kelay Belhu: _____

Dr. Merga Bekana: _____