ADDIS ABABA UNIVERSITY
FACULTY OF VETERINARY MEDICINE

EVALUATION OF SEMEN PARAMETERS IN ETHIOPIAN INDIGENOUS BULLS KEPT AT KALITI, ARTIFICIAL INSEMINATION CENTER, ADDIS ABABA, ETHIOPIA

BY
HUNDERRA SORI AYANA

JUNE, 2004
DEBRE ZEIT, ETHIOPIA

ADDIS ABABA UNIVERSITY
FACULTY OF VETERINARY MEDICINE

EVALUATION OF SEMEN PARAMETERS IN ETHIOPIAN INDIGENOUS BULLS KEPT AT KALITI, ARTIFICIAL INSEMINATION CENTER, ADDIS ABABA, ETHIOPIA

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

By
HUNDERRA SORI AYANA

JUNE, 2004
DEBRE ZEIT, ETHIOPIA

EVALUATION OF SEMEN PARAMETERS IN ETHIOPIAN INDIGENOUS BULLS KEPT AT KALITI, ARTIFICIAL INSEMINATION CENTER, ADDIS ABABA, ETHIOPIA

BY

HUNDERRA SORI AYANA

Board of Examiners Signature

1. Prof. Ph. Dorchies _____

2. Prof. Feseha Gebreab _____

3. Dr Wondwossen Abebe Gebreyes _____

4. Dr Giles Innocent _____

5. Dr Andy Catley _____

6. Dr David Barrett _____

Academic Advisors:

Dr. Shiv Prasad _____

Dr. Emiru Zewdie _____

DECLARATION

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

Name Hunderra Sori Ayana

Signature _____

Date of submission _____

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

Dr Shiv Prasad _____

Dr Emiru Zewdie _____

DEDICATION

To my parents, friends, relatives and others who have been working for my success.

ACKNOWLEDGEMENTS

My special thanks go to the Oromia Agricultural Bureau for sponsoring my study and giving me study leave permission.

I would like to thank my advisor Dr. Shiv Prasad for project design, scholarly advice, guidance, constructive comments, reference provision, careful correction and finalization of the thesis. I would also want to thank my Advisor Dr. Emiru Zewdie for his continuous support, advice, technical help, and material provision during my field work.

I also appreciate the contribution of the NAIC staff and management for allowing me to work on the semen of their animals and in their laboratory. The assistance and cooperation of W/ro Tewodida Mohammed, Ato Afework Worana, W/rt Assia Mussa, W/rt Workiye Abera, during the laboratory work and sample processing is also thankful. The contribution and assistance of workers of the bull stud deserve special thanks. I also like to extend my thanks to Dr. Netsanet Demeke and Dr. Deneke H/Mariam for material provision and constant advice during the study period.

I would like to thank Associate Dean for Research and Graduate Programs, Faculty of Veterinary Medicine for providing facilities, guidance and critical monitoring of the work during entire period of the work.

I also extend my sincere thanks to Dean, Faculty of Veterinary Medicine for extending overall facilities of the faculty and solving the problems arising at the time of need.

The biochemical tests were done at Arsho medical laboratory PLC. I would like to thank the workers of this laboratory. The spermatozoa dimensions were measured and spermatozoa were photographed in Parasitology and Pathology department of Faculty of Veterinary Medicine, Addis Ababa University. I want to thank the cooperation of the workers in this department in general and particularly W/rt Eskedar Hailegebrael for assisting me in photographing the spermatozoa. Thanks are also due to Ato Mulatu Kabeta who has assisted me in scanning the spermatozoa photographs. I also want to thank Dr. Getachew Mulugeta for providing micrometer and advice to use it.

I also want to thank Ato Girmay Medhin and Dr. Mathieu Lesnoff for their assistance in statistical works. The contribution of Dr Getachew Tilahum, Dr Gobena Ameni, Ato Mengistu Leggesse, and Dr Tamirat Degefa in this regards is also acknowledged. I also want to thank others who directly and indirectly assisted me during the entire program of the work.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ANNEXES

.

LIST OF ABBREVIATIONS

%	Percent
µl	Micro liter
µM	Micrometer
^{0}C	Degree Celsius
ACP	Acid Phosphatase
AI	Artificial Insemination
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
AV	artificial vagina
cm	Centimeter
df	degrees of freedom
EASE	Ethiopian Agricultural Sample Enumeration
E.C	Enzyme Commission
FSH	Follicle Stimulating Hormone
gm/dL	gram per deciliter
GnRH	Gonadotropin Releasing Hormone
GOT	Glutamic Oxalacetic Transaminase
GPT	Glutamic Pyruvic Transaminase
IFCC	International Federation of Clinical Chemistry
kg	Kilogram
km	Kilometer
LH	Luteinizing Hormone
LST	Local Standard Time
ml	Milliliter
mm	millimeter
mmol/L	milli mole/liter
MOA	Ministry of Agriculture
n	number of paired data
NAIC	National Artificial Insemination Center
ng/ml	nanogram per milliliter
nm	nano meter
NMSA	National Meteorological Service Agency

PAS	Periodic acid-Schiff
RH	Relative humidity
rpm	revolution per minute
SE	standard error (of mean)
U/L	International unit per liter

ABSTRACT

The semen of six indigenous breeds bulls (Barca, Boran, Horro and Sheko) which were kept at the National Artificial Insemination Center (NAIC), Kaliti, Addis Ababa, Ethiopia were evaluated for physico-morphological parameters (volume, color, mass and individual motility, concentration, total count, percentage live and morphologically normal, total morphologically normal and viable number), biochemical parameters (GOT, GPT, ACP, ALP, total protein) and hormonal assay (testosterone). Semen was collected for a thirteen week period from six indigenous bulls kept at the AI center. The collection was done once per week using an artificial vagina and semen samples were subjected to normal physico-morphological characterization and seminal plasma was separated and preserved at -20 ^{0}C until analysis.

The overall mean (SE) value for the indigenous bulls in the semen characteristics studied were volume 4.84 (0.20) ml, mass motility 3.15 (0.11), individual motility 68.72 (1.37) percent, concentration 1.54 (0.07) billion/ml, total count 7.35 (0.47) billion/ejaculate, viable number 5.10 (0.33) billion/ejaculate, total morphologically normal number 7.12 (0.45) billion/ejaculate, live percentage 79.73 (0.65), percent morphologic normal 94.70 (0.38), percent normal acrosome 96.99 (0.60), GOT 1530.91 (60.15) U/L, GPT 131.99 (9.36) U/L, ALP 3333.98 (608.84) U/L, ACP 8003.68 (716.06) U/L, total protein 7.38 (0.26) gm/dL, testosterone 2.84 (0.3) ng/ml, head abnormality 1.87 (0.15) %, mid-piece (body) abnormality 1.92 (0.22) %, tail abnormality 1.50 (0.18) %, total abnormality 5.29 (0.38) %, major abnormality 2.25 (0.19) %, minor abnormality 3.04 (0.28) %, acrosome defect 3.0 (0.60) %, narrow at base 0.37 (0.09) %, abnormal contour 0.12 (0.04) %, undeveloped form 0.74 (0.15) %, detached abnormal 0.21 (0.09) %, pear shaped 0.05 (0.02) %, small abnormal 0.05 (0.03) %, abaxial attachment 0.57 (0.11) %, other head abnormality 0.07 (0.03) %, spermatozoa head length 9.18 (0.03) μm, head breadth 4.61 (0.01) μm, mid-piece length 13.61 (0.02) μm, tail length 47.56 (0.1) μm.

The semen physico-morphologic and seminal plasma biochemical parameters observed in this study for the Ethiopian indigenous bulls are comparable to normal fertile bulls reported elsewhere based on the evaluation of fresh semen parameters.

Key words: Ethiopia/NAIC/Indigenous breeds/physico-morphological parameters/biochemical parameters/semen/spermatozoa

1. INTRODUCTION

Livestock are a vital source of economic and social support for millions of poor people throughout Africa. Ethiopia has been reported to have the highest number of livestock population in Africa (Tegegne, 1989; I. L. C. A., 1992). The contribution of livestock, however, does not commensurate with the number of animals or the extent of land resources used. The major contributing factors that have impeded the full exploitation of cattle potential in Ethiopia are seasonality and quality of nutrition, low genetic potential for production in the indigenous breed, the occurrence of diseases and parasites, poor livestock-management systems, and large socio-economic factors (Wagner, 1968; Morrison *et al.,* 1981; Tegegne, 1989; I. L. C. A., 1992). However, due to Ethiopia's diversified agro-climatic zones, the country has got a considerable potential for increasing livestock productivity (Mukasa-Mugerwa, 1989; I. L. C. A., 1992).

The majority of Ethiopia's cattle have been reported to be indigenous breeds and small non-descript Zebu types (Mukasa-Mugerwa, 1989) that are poor in major economically important traits but they have good adaptation to tropical climates (Tegegne, 1989). Selection and controlled breeding of superior animals has been found to increase productivity. The development and use of Artificial Insemination (AI) techniques have also revolutionized cattle production and genetic improvement, particularly in the dairy sector, in developed countries (Tegegne *et al*., 1995).

Reproductive performance is one of the major determinants of cattle productivity in any production systems. The contribution of the bull either through the natural mating or AI where each bull represents half of the genetic composition of its progeny (Blezinger, 1999) and many cows can be inseminated with the semen of a single bull (Faulkner and Pineda, 1980; Hafez, 1993), and its contribution in the production of meat and milk is of great importance which necessitates careful scrutinization of the productive and the reproductive traits of bulls before extensive use (Coulter and Foote, 1979). Genetic improvement could be achieved via the use of selected superior bulls in both natural mating and AI operations. However, emphasis on maximum selection for production traits and the fact that most studies on cattle reproduction concentrate on the female rather than the male have tended to minimize attention given to reproductive performance of bulls (Coulter and Foote, 1979). Failure of many bulls to consistently and efficiently breed has been reported to be associated with the

production of poor quality semen, seasonal changes in semen quality, high incidence of abnormal spermatozoa and problems in sexual behaviors that reduce the fertility of the bull (Roberts, 1971; Hafez, 1993; Blezinger, 1999). One of the few attempts that have been undertaken to improve cattle productivity in Ethiopia is cross-breeding the indigenous zebu (*Bos indicus*) with temperate (*Bos taurus*) breeds with the aim of mixing the high genetic potential productivity of the temperate breeds with merits of adaptability, better disease resistance and hardiness of the indigenous cattle (Gebrewold, 1984). In the situations where feed and other resources are scarce and the climate is potentially stressful for animals, live-stock improvement, particularly genetic improvements that aim at producing high yielding animals suffers from high costs in relation to the returns, which obliges the live-stock producer to choose for an intermediate yield that is better than the old, but not as high as possible, as a better option (Wiener, 1994). However in Ethiopia, there is insufficient information on the reproductive potential of zebu and crossbred bulls, and on the effect of both genetic and environmental factors on the bull reproductive parameters (Tegegne *at al*., 1995). One important factor which limits the reproductive performance and fertility of bulls have been reported to be the quality of semen they produce, which can be affected by numerous exogenous and endogenous factors (Hafez, 1993; Blezinger, 1999). Concerning the quality of semen in indigenous breeds and cross-bred bulls, the information available are not adequate to be utilized as the basis of genetic improvement that the country has aimed at. The main objective of the present study is therefore, to evaluate the physico-morphological and biochemical characteristics of semen of the indigenous bulls that were used for semen production at the National Artificial Insemination Center (NAIC), Kaliti, Addis Ababa.

2. LITERATURE REVIEW

2.1. Cattle Production in Ethiopia

Ethiopia is known to have a cattle population around 41.5 million (EASE, 2003), the majority of which, 99.4 %, are indigenous breeds with very few hybrids, (0.5 %) and exotics (0.1 %). Cattle production together with the production of other livestock sectors, has been known to be a very important and essential component of the agricultural sector contributing for meat, milk, cheese, butter, export commodities (live animals, hides, skins), draught power, manure, near-cash capital stock (EASE, 2003).

It has been known that the indigenous cattle breeds of Ethiopia have low productivity despite their special merit of overcoming harsh environmental conditions of the country; on the other hand the high performing exotic cattle can not cope with the harsh environment of the country (MOA, 1996). So improvement of cattle productivity without losing traits that are essential for survival has been proposed.

In Ethiopia few cattle breeds have been categorically identified and those identified are not adequately assessed and conserved (MOA, 1996). Therefore, proper identification, characterization and assessment of cattle breeds of the country should be considered so that implementation of improvement method is effected (MOA, 1996).

2.2. Indigenous cattle breed characterization

Cattle have been used by man as domestic animals for thousands of years, and through natural selection of the fittest they have developed greatly varying characteristics under the climatic and environmental conditions that exist in different parts of the world (Barber and Wood, 1976).

Throughout the world, food requirements have been known to favor the use of cattle that can produce milk and beef for human consumption. Africa has a great need to increase milk and meat production from cattle and perhaps has a great potential if specific constraints can be alleviated (Trail and Gregory, 1984). Climate and associated factors in most parts of Africa,

including nutrition, disease and parasite, environment, have been known to favor use of germ plasm with varying percentages of *Bos indicus* cattle because of their higher levels of adaptability to environmental stresses (Trail and Gregory, 1984).

Cattle in Africa has been known to be used for several purposes and in most cases can not be grouped into classes of beef, dairy and draft animals; in many situations the same animals have been known to be milked, used for draft, and finally slaughtered for consumption (Trail and Gregory, 1984).

Breed characterization in the tropics has been advised to be directed towards achieving effective selection among breeds either for use as straight breeds or for use in crossbreeding systems to utilize heterosis and complementarity, or for use as contributors to new breeds to simultaneously utilize heterosis and arrive at and maintain the most optimal breed composition (Trail and Gregory, 1984). The fact that a given trait requires the suitable environment which allows its expression (Barber and Wood, 1976) has been known to make simultaneous characterization of environment in which the breeds are evaluated necessary (Trail and Gregory, 1984). Furthermore, it is important to find the best breed of animal for each area, and not to try to make an animal adapt itself to unsuitable conditions (Barber and Wood, 1976).

Even though the response capability of *Bos indicus* breeds of cattle for both milk and meat production in tropical environments has been found to differ, and important differences in adaptability of *Bos taurus* breeds to tropical environments has been reported (Trail and Gregory, 1984), the exploitation of livestock possessing genetic resistance to diseases, has been given increasing consideration in livestock development programs. This is particularly true where conventional disease control measures are too costly, too complex to implement or as is common where drugs and vaccine are not available, as is common in developing countries for example *Bos taurus* breeds of W. Africa to African trypanosomes (Trail and Gregory, 1984).

The rapid genetic improvement in the tropics has been proposed to come through prior improvement of the environment (feeding, prophylaxis, shelter etc), then focusing on pre selection activities which are easy to set up (culling of unproductive females, castration of males which have visible faults), and finally on mass selection, at least with regard to the most heritable characteristics like conformation, speed of growth and adult size (Pagot, 1992).

In Ethiopia it is evident that all indigenous cattle in different parts of the country are well adapted to arid and harsh environment where they breed and play a significant role in rural economies of their respective area. Most of them have developed tolerance to heat stress, parasites and disease and are able to withstand to a great extent the seasonal lack of water and food and related adverse conditions characteristic to Ethiopia. But at the present well-defined selection programs are absent in the country (MOA, 1997), and the information on description of breeds in Ethiopia is either lacking or is not adequate to be utilized for sound programs of livestock conservation, selection and improvement.

2.3. Zebu bull traits

Even though the *Bos indicus* and *Bos taurus* breeds are known to have the same ancestral stock, their difference in some morphological and physiological traits have been documented (Vale-Filo *et al*., 1986): adaptation to the tropical area, larger and more numerous sweet glands, long legs, hard hooves, resistance to ecto-parasites, slower growth rate (reaching sexual maturity 12 to 18 months later than *Bos taurus*), lower feed conversion rate being some of the characteristic of the *Bos indicus*.

On the other hand, there has not been any observed difference between the genital tract of the zebu and the taurine breed bulls except in the size of some of the organs (Vale-Filo *et al*., 1986), where the testes in the adult zebu bulls has been known to be slightly smaller than those of taurine bulls, and the mean scrotal circumference for zebu bulls is 38.9 cm while the corresponding figure for the taurine bulls is 39.9 cm, but the consistency and size of the sigmoid flexure and accessory glands of the male genital tract have been found similar in both breeds. According to the same authors there has been an observed variation in testicular and preputial size among the zebu breeds, the smallest testicle being the feature of milk breed zebu types.

Even if the reaction of the zebu bulls to the cow has been found similar to the reaction of the taurine bulls, zebu bulls have been known to show libido and mating ability later in life than taurine bulls, and even then to show less libido than the latter (Vale-Filo *et al*., 1986) and have been known to take 10 to 20 minutes from manifestation of libido until completion of ejaculation, the corresponding figure for the taurine bull being 5 to 10 minutes (Vale-Filo *et*

al., 1986). The *Bos indicus* have also been known to have longer life span with bulls over 10 years of life having the ability to produce good quality semen (Vale-Filo *et al*., 1986).

2.4. Spermatogenesis

Spermatogenesis is the entire developmental process involved in the transformation of stem cell or spermatogonia into spermatozoon, the process which begins at the wall of seminiferous tubule and ends with the release of mature spermatozoa in the lumen of seminiferous tubule (Salisbury *et al*., 1978; Hafez, 1993; Stabenfeldt and Edqvist, 1993; Bearden and Fuquay, 2000). It involves mitotic proliferation, meiotic division, and differentiation of the haploid spermatid (Martin, 1985; Griffin and Wilson, 1992; Stabenfeldt and Edqvist, 1993). The primordial germ cells which migrate to indifferent gonad during embryonic stage forms gonocytes after several cell divisions and then differentiate to type A_0 (reserve stem cells) spermatogonia (at time just before puberty) from which other germ cells originate (Johnson, 1991; Hafez, 1993). Spermatogenesis has been known to start when spermatogonia undergo mitosis which ensures the formation of large number of germ cells and also maintains a supply of the germ cells (Stabenfeldt and Edqvist, 1993). The type A_1 (formed from type A_0 spermatogonia) spermatogonia divide progressively to form type A_2, and type A_3 spermatogonia, the latter divide again to form intermediate (I) spermatogonia. All of the spermatogonia could be identified through histological sections and have been recognized to provide the basis for proliferation of the germ line (Banks, 1986; Setchell, 1991; Hafez, 1993). The type A spermatogonia have been known to be large cells with large round nucleus containing finely dispersed chromatin and an eccentrically positioned nucleolus; which on mitotic division has been known to give rise to one stem cell and the other intermediate (I) spermatogonia. The latter have been known to be oval cells with oval nucleus containing peripherally positioned, coarsely clumped chromatin and two or three nucleoli. On the other hand, the type B spermatogonia have been known to be round and small with oval nucleus containing coarsely clumped chromatin and have been known to be formed from subsequent division of I spermatogonia, but subsequent division of B spermatogonia (once or probably twice) has been known to give rise to primary spermatocytes (16 primary spermatocytes from one type A spermatogonia) (Banks, 1986; Griffin and Wilson, 1992; Stabenfeldt and Edqvist, 1993). The tetraploid (Roberts, 1971) primary spermatocytes which have rather long lives (16 days in bulls) have been known to be large and consequently easily found and identified unlike the relatively small and diploid secondary spermatocytes which, owing to their rapid

conversion to spermatid, have been found difficult to find (Banks, 1986; Setchell, 1991). This series of cellular division, including the proliferation of the spermatogonia and meiotic divisions resulting in the haploid spermatids, have been termed as spermatocytogenesis and have been reported to take 45 days in bulls (Hafez, 1993). During spermatocytogenesis the primary spermatocytes duplicate their DNA and undergo progressive nuclear changes of prophase known as preleptotene, leptotene, zygotene, pachtene, and diplotene prior to dividing to give secondary spermatocytes. The resulting secondary spermatocytes divide again to give spermatids without further DNA synthesis (Hafez, 1993).

The spermatids at first formation are small, round cells with a spherical, centrally located nucleus that house granular chromatin, and the pale staining cytoplasm which contains numerous mitochondria, a compact Golgi close to the nucleus, some free ribosomes, two clearly visible centrioles and a chromatoid body (Martin, 1985). Once formed the round spermatids have been known to occupy the most developed, most numerous and largest layer of seminiferous tubular epithelium zone called zone of metamorphosis (Banks, 1986) and undergoes transformation into spermatozoa (spermiogenesis or metamorphosis) (Martin, 1985; Banks, 1986; Stabenfeldt and Edqvist, 1993; Hafez, 1993).

The spermatid maturation has been known to involve: formation of the tail to aid movement within the female reproductive tract, development of mitochondria to furnish energy during movement in the female reproductive tract, and development of an acrosome, an organelle which allows penetration of the oocyte, lose of much of the cytoplasm and disappearance of the cytoplasmic bridges (Stabenfeldt and Edqvist, 1993; Peters and Ball, 1995). Through Periodic acid- Schiff (PAS) reaction which stains deep red the developing acrosome components, four phases of developmental transformation of spermatids have been recognized (Salisbury *et al*,. 1978; Banks, 1986; Hafez, 1993) (Table 1).

Table 1. Developmental transformation of spermatid.

NO	Phases	Features
1	Golgi phase	Formation of PAS positive proacrosomal granules within the Golgi apparatus, the coalescence of the granules into a single acrosomal granule, adherence of the resultant acrosomal granule to nuclear envelope, and the early stage tail development at the pole opposite that of the adherence of the acrosomal granule. The proximal centriole migrates closest to the nucleus where it is thought to form the basis for attachment of the tail to the head.
2	Cap phase	Spreading of the adherent acrosomal granule over the surface of the spermatid nucleus until two-thirds of the anterior portion of the spermatid nucleus and development of axonemal components from elements of distal centrioles.
3	Acrosomal phase	Characterized by changes in the nucleus (condensation of chromatin into dense granules and reshaping of spheroidal nucleus into an elongated, flattened structure), the acrosome (the acrosome will also condense and elongate to correspond to the shape of the nucleus), and the tail (displacement of the cytoplasm to the caudal aspect of the nucleus and surrounds proximal aspect of the developing tail, development of manchette, chromatoid body, formation and going down of annulus, concentration of mitochondria near middle piece) of developing spermatids.
4	Maturation	Finalization of reshaping of the nucleus, acrosome and tail of the spermatids: progressive condensation of chromatin occurs to form fine homogenous material that uniformly fills the entire sperm nucleus, fibrous sheath and underlying nine coarse fibers are formed around the axoneme, the coarse fibers appear to be associated individually with the nine pairs of microtubules of the axoneme and are continuous with columns in the neck of the spermatid, the fibrous sheath covers the axoneme from the neck to the beginning of the end piece, the annulus moves to its place where it separates midpiece and principal piece, the mitochondria became tightly packed to a continuous sheath extending from the neck to the annulus, at the end stage the manchette disappears and the residual body forms which marks maturation of spermatid to spermatozoa.

The immature spermatozoon therefore consists of the head, neck, middle piece, principal piece and end piece, the whole structure being covered with plasma lemma (Banks, 1986). The head is covered by the head cap on the rostral and lateral surface and calyx on the lateral and caudal surfaces, the neck has a depression fossa in which the centriole is located and longitudinally segmented columns connect the head to tail, the middle piece consists of a flagellar core in a 9 + 2 arrangements, fibers that continues with those of the neck, a helical arrangement of mitochondria, and an annulus that is attached to the plasma lemma; the principal piece contains a flagellar core in a 9 + 2 arrangement, fibers of various sizes that end in this segment and a fibrous sheath. On the other hand, the end piece has been found to contain only the axoneme covered by the plasma membrane (Hafez, 1993).

According to Stabenfeldt and Edqvist (1993), in bull mitotic proliferation, meiotic division and spermiogenesis before spermatozoa are released into the lumen has been known to require 14, 28 and 22 to 23 days respectively. Similarly, Peters and Ball (1995) described approximate time for the whole process of spermatogenesis to be around 60 days. The daily sperm production in Holstein and Hereford bulls has been recognized to be 7.5 $x10^9$/ml and 5.9 x 10^9/ml respectively (Peters and Ball, 1995). Similar data for spermatozoa of indigenous Ethiopian bulls is lacking.

2.5. Transport of spermatozoa through excurrent ducts

It has been suggested that the passage of immature spermatozoa through the seminiferous tubule, rete testes, efferent ducts and into the epididymis is due to positive pressure in the tubule, which results from extrusion of formed spermatozoa into the lumen; negative pressure in the tubule, which results from absorption of water by the tubule linings and from ejaculation; ciliary action of the lining of efferent ducts and upper portion of the epididymis; and pressure caused by the normal movement of the animal that compresses the scrotum and thus its contents; once at epididymis by rhythmic contraction of the epididymis for movement along the epididymis (Salisbury *et al*., 1978). The oxytocin released at time of ejaculation, cause involuntary muscular contraction, and may also aid movement along the excurrent ducts. In the bull transport of spermatozoa through the epididymis has been known to take about one week (Salisbury *et al*., 1978), in the order of two to three days to pass via caput and corpus epididymis, and three to five days in the cauda epididymis (Peters and Ball, 1995). Furthermore, the transit time of spermatozoa through the caput and corpus epididymis is not

affected by the frequency of ejaculation unlike its passage via caudal epididymis (Peters and Ball, 1995). According to same authors, frequency of ejaculation was found not to affect the fertilizing capacity of spermatozoa owing to lack of much variation in transit time through the epididymis. On the other hand, maximum number of spermatozoa has been found in bulls rested for 7 to 10 days (Peters and Ball, 1995).

During their stay in the epididymis, the spermatozoa complete spermiogenesis (divest cytoplasmic droplets), acquire the capacity for independent motility, modification of the structural state of the sperm nuclear membrane involving change in the metabolic character, alteration in the nature of the surface of plasma membrane, and acrosome (become less permeable), and acquire the capacity to achieve optimum rates of fertility (Salisbury *et al*., 1978; Prasad and Sinha, 1985).

2.6. Biochemical composition of bovine seminal plasma

Seminal plasma is the secretion of the male accessory sex organs which is released at time of ejaculation and provides an ionically balanced and nutritive environment that is conducive to the survival of sperm within the female reproductive tract, and makes up semen with spermatozoa (Martin, 1985; Stabenfeldt and Edqvist, 1993). The fluid has been reported to contain nutrients, buffers and other substances that contribute to the transport and survival of gametes (Martin, 1985). The carrier and the protector functions of seminal plasma have been reported to be essential especially in the bull where ejaculation is intra-vaginal (Garner and Hafez, 1993). The same authors also described that seminal plasma is a composite secretion arising from a number of sources including the testis, epididymis, and the accessory glands of the male.

Even though the fact that spermatozoa from cauda epididymis can fertilize eggs without the addition of accessory glands secretion makes the real function of seminal plasma vague apart from its transport function, the unique biochemical markers in semen can be used as indicators of specific accessory glands functions (Garner and Hafez, 1993).

Fructose, sorbitol, citric acid, ascorbic acid, amino acids, proteins, potassium, sodium, lipids, phosphorylcholine and prostaglandins are found in seminal vesicle secretions; zinc which is mainly associated with the enzyme carbonic anhydrase and lactic dehydrogenase in semen. Fructose, citric acid, cholesterol, numerous proteins and free amino acids, and enzymes (including alkaline and acid phosphatases, proteolytic enzymes, glycosidase, aspartate aminotransferases), decapacitating factors and sperm coagulating antigens have been reported to have probable sources of prostatic gland origin (Faulkner and Pineda, 1980; Matin, 1985). The bulbo-urethral glands have been known to secrete fluid which is rich in GAG-glycosaminoglycans whose main purpose is lubrication of the tip of penis (Martin, 1985).

The accessory gland secretions account for 60-90 % of seminal plasma of which the greater part is of seminal vesicle origin. When added to seminal plasma, motility and metabolic activity of sperm is stimulated; furthermore, insemination of mature sperm has been known to give better fertility than spermatozoa from testis or from epididymis (Faulkner and Pineda, 1980).

Other constituents of seminal plasma which have not been localized to specific sites include: phosphate and bicarbonate which provides protection against the low pH of the female reproductive tract, phospholipids, antimicrobial constituents including seminal plasmin, immunoglobulin (IgA mainly), variety of hormonal substances including androgens, estrogens, FSH, LH, chorionic gonadotropin-like material, growth hormone, insulin, glucagons, prolactin, relaxin, thyroid releasing hormone and enkephalins (Martin, 1985; Garner and Hafez, 1993).

In summary, the seminal plasma is known to contain proteins, amino acids, enzymes, hormones, energy substrates, fatty acids, lipids, inorganic and organic substances, buffering agents and other miscellaneous constituents (White, 1980; Martin, 1985; Garner and Hafez, 1993; Bearden and Fuquay, 2000).

2.7. Hormonal regulation of the spermatogenesis

Normal testicular function, spermatogenesis and steroidogenesis, has been known to require hormonal stimulation by gonadotrophins, which in turn have been known to be controlled by pulsatile secretion of gonadotrophin releasing hormone (GnRH) from the hypothalamus (Faulkner and Pineda, 1980; Garner and Hafez, 1993; Peters and Ball, 1995).

Even though testosterone secretion from Leydig cells is known to be produced as early as 42 days of gestation (Peters and Ball, 1995), seminiferous tubules of juvenile males lack any response to gonadotrophins (Faulkner and Pineda, 1980) making factors which make the germinal cells sensitive to gonadotrophin stimulation obscure.

The cessation of spermatogenesis following hypophysectomy and its restoration following readministration of gonadotrophins (FSH, LH) or FSH and testosterone has made clear that the pituitary gland is essential to the function of seminiferous tubules (Faulkner and Pineda, 1980; Hafez, 1993; Peters and Ball, 1995).

It has been thought that the initiation of spermatogenesis is dependent largely upon concentration of testosterone and FSH; similarly, high concentration of testosterone, produced following LH stimulation of Leydig cells, is required to maintain spermatogenesis (Johnson, 1991; Garner and Hafez, 1993). The role of FSH being stimulation of Sertoli cells directly to stimulate their functions in support of germ cell development (Johnson, 1991; Peters and Ball, 1995). Testosterone has been recognized to induce differentiation of gonocytes to prespermatogonia and A-spermatogonia (Johnson, 1991).

The seminiferous tubules have been known to be under the regulatory activity of the hypothalamus and anterior pituitary, the secretion of LH and FSH by the latter controlled by the secretion of hypothalamic releasing factors, being essential for the development of seminiferous tubule (Banks, 1986). FSH, through its direct effect upon seminiferous tubule, has been known to initiate spermatogenic events (Banks, 1986), and to aid in completion of meiosis of the germ cells (through its influence on Sertoli cell activity) (Faulkner and Pineda, 1980; Stabenfeldt and Edqvist, 1993). The LH has been known to exert its effect via its tropic effect on Leydig cell and its effect on Leydig cell for production of testosterone: LH has been known to bind specifically to Leydig cell membranes and to activate the production of cyclic adenosine monophosphate (cAMP), the process which has been known to activate the enzyme

protein kinases which catalyze the phosphorylation of intracellular proteins and the mobilization of steroid precursors, mainly through the conversion of cholesterol to pregnenolone (Henricks, 1991; Stabenfeldt and Edqvist, 1993). Testosterone and FSH stimulate the synthesis of androgen binding protein (ABP) by the Sertoli cells (Banks, 1986; Stabenfeldt and Edqvist, 1993), the ABP, has been suggested to aid in stabilizing the concentration of androgens in the seminiferous tubules for use in both spermatogenesis and Sertoli cell function (Stabenfeldt and Edqvist, 1993).

Androgens, once secreted in pulsatile pattern by interstitial Leydig cells, by pulse release stimulation of LH from anterior Pituitary gland, have been known to diffuse to adjacent Sertoli cells, where it is essential for maintenance of spermatogenesis (Hafez, 1993), and to blood vessel where it act as a negative feed back at hypothalamus and pituitary to block release of additional LH (Garner and Hafez, 1993). The necessities of a relatively high level of testosterone for spermatid maturation have been recognized (Garner and Hafez, 1993). The myoid cells and epithelial cells of epididymis have been known to be dependent on androgens for their normal function (Garner and Hafez, 1993). The latter has been known to be met by the transport of androgens to the epididymis by androgen binding protein produced by Sertoli cells.

2.8. Semen collection

The success of AI depends on the collection of a relatively large number of potentially fertile spermatozoa from genetically superior sires (Garner, 1991). The routine collection of semen for AI in dairy and beef bulls (Faulkner and Pineda, 1980) is by using artificial vagina.

Appropriate and specialized facilities, equipment, and procedures have been used during collection of semen to prevent injury to the bulls and their handlers, to maximize the physiological responsiveness of the bull in producing the semen and to enhance the quantity and the quality of the semen that can be collected (Garner, 1991). The area for semen collection has been preferred to be clean, relatively quite, free of distractions and any other stressful procedures. There has been a report of increase in spermatozoa motility by 50 % through proper sexual stimulation of the bull (Salisbury *et al*., 1978).

Routine semen collection has been practiced by using artificial vagina in bulls (Faulkner and Pineda, 1980). This technique is simple in construction, furthermore it simulates natural copulation by providing suitable temperature, pressure, and lubrication that evoke ejaculation and yields a representative sample (Salisbury *et al*., 1978; Garner, 1991; Hafez, 1993). The artificial vagina consists of a firm cylindrical tube with a thin walled rubber lining which forms a jacket that is lined with warm water, a rubber funnel holding a collection receptacle which is attached to one end of the lined cylinder (Salisbury *et al*., 1978). Filling the jacket properly with warm water and lubrication and proper application of the artificial vagina has been reported to yield a high degree of success in the semen collection (Salisbury *et al*., 1978). Furthermore, the same authors recommended clean, sterile and dry parts of the artificial vagina to avoid contamination of the semen and disease transmission from one bull to other bull; cleanliness of the bull and the environment, adequate precollection sexual stimulation, and maintenance of libido by avoiding distraction, changing and moving the teaser and the environment. The ideal method of collection of semen has been advised to be safe for the sire and the collector, produces a seminal sample that is representative of normal ejaculate, is free of contamination and protected from thermal shock (Faulkner and Pineda, 1980).

Other techniques that have been employed for semen collection (but less frequently) are the electroejaculation (when the male refuses to serve the artificial vagina or when it fails to mount), and manual manipulation (by massaging ampullae and vesicular glands via the rectum) (Salisbury *et al*., 1978; Garner, 1991; Hafez, 1993). The quality of semen that is obtained by such techniques is relatively poor (Salisbury *et al.*, 1978; Hafez, 1993).

The temperature of the semen should be maintained at 37 ^{0}C by the protective cover on the collecting vial, and this temperature should be maintained until the initial dilution and during the evaluation of seminal quality (Faulkner and Pineda, 1980). The same author also recommended the need to keep all glasswares and seminal extenders at 37 ^{0}C.

2.9. Semen quality assessment

2.9.1. Significance

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 a definite correlation between testicular pathology, disease of the reproductive tract and accessory gland, and the semen characteristics and fertility has been found (Roberts, 1971; Faulkner and Pineda, 1980). The main goal of semen quality tests is to accurately predict semen fertility using rapid, inexpensive techniques (Gomes, 1977). Some tests like individual motility and morphologically normal percentage are known to have a significant positive correlation with fertility (Das *et al.*, 1999). The heritability estimates of semen quality and quantity traits are known to be within the range of 0.02 to 0.28 with heritability of motility being about 0.2 (Stalhmmar, 1995). Similarly, other researchers reported the heritability of some seminal attributes like semen volume, and spermatozoa concentration and total count to be 0.3 to 0.5 (Graffer *et al*., 1998).

Despite the significance of associating the correlation of fertility and semen quality characteristics (Faulkner and Pineda, 1980), the presence of the range of values for semen characteristics and the characteristics of the female animal population to be inseminated have been found to be some of the source of bias during estimating the correlation between fertility and seminal quality (Faulkner and Pineda, 1980). Another limitation of relating seminal quality to fertility has been associated to the fact that concerning the epididymal and testicular contributions, the value of seminal quality reflects past events unlike the secretion of accessory glands which are concurrent with ejaculation (Faulkner and Pineda, 1980).

No single measurements of seminal quality have been found as a reliable criterion for predicting fertility, that necessitates incorporation of many useful measurements of seminal characteristics within the limits of practicality (Faulkner and Pineda, 1980). Minimal tests that have been conducted for routine seminal analysis at AI include volume, concentration, percentage and rate of motility, but for research purpose additional measures of physical, chemical and metabolic characteristics of the semen have been recommended to be analyzed (Faulkner and Pineda, 1980). Attempts to diagnose infertility have been advised to include hormonal assays, microscopic examination of tissues obtained by testicular biopsy, chemical

analysis of semen to define testicular and accessory gland function, and special studies of sperm morphology and ultrastructure (Faulkner and Pineda, 1980).

2.9.2. Techniques

Semen has been reported to be the entire seminal discharge of the male during normal ejaculation (Roberts, 1971) and is known to consist of cellular elements, the spermatozoa, which are produced in the seminiferous tubules, and seminal plasma or the liquid portion of the semen produced by the secretion of the seminiferous tubules, ducti differentia and ampulla, seminal vesicles, prostate and bulbo-urethral glands (Roberts, 1971; Garner, 1991; Hafez, 1993). In bull semen sperm cells have been known to account for only 10 % of the semen.

The ideal method of evaluating the fertility of breeding male, other than its ability to produce pregnancy, is by the examination of its semen (Hafez, 1993). Reliable estimations of the semen quality require careful examinations of a minimum of three ejaculates collected at intervals of a few days (Hudison, 1972; Garner, 1991). Such an approach has been shown to give representative data on seminal quality of a particular animal (Hudison, 1972; Garner, 1991). Semen analysis technique has been recommended to be simple to perform, inexpensive, rapid and effective to process and preserve initial quality and fertility of semen, and to give important conclusions and standards for species of interest, a deviation from which has been recognized and correlated with fertility (Gomes, 1977; Garner, 1991; Hafez, 1993). The same authors also stressed the significance of conducting mixtures of several tests for selecting ejaculates that can be used for their higher fertility potentials, as the single test which can accurately predict the fertility of the individual ejaculates has not been found. Earlier reports by Roberts (1971), Faulkner and Pineda (1980) and Uwland (1984) indicated the necessity of associating semen evaluations with detailed clinical examinations of the animal. Semen samples should be tested for the various physico-morphological and biochemical characteristics including (Roberts, 1971; Uwland, 1984; Morrow, 1986; Garner, 1991; Hafez, 1993):

2.9.2.1. Appearance or color

It has been known that the bull semen which is concentrated has creamy, milky or creamy white and opaque color (Roberts, 1971; Hafez, 1993; Bearden and Fuquay, 2000). On the other hand, semen with few spermatozoa and that from animal with reproductive system infections has been reported respectively to be translucent and curdy in appearance (Hafez, 1993). Some bulls have been known to consistently produce yellow semen which is due to a harmless pigment known as riboflavin. However, the semen should be free from hair, dirt, urine, blood, faeces and other contaminants (Garner, 1991; Hafez, 1993; Bearden and Fuquay, 2000). The semen with the spermatozoa concentration of one million to 1.2 million per cubic millimeter or higher has been classified as creamy consistency while the spermatozoa with concentration of 5×10^5 to 6×10^5 per cubic millimeter has been classified as a thin milky consistency, and those with the spermatozoa concentration of less than 3×10^5 spermatozoa per cubic millimeter has been classified as watery, translucent or clear (Roberts, 1971).

2.9.2.2. Volume

The volume of the ejaculate is readily measured by collecting the sample directly into a graduated vial (Garner, 1991; Bearden and Fuquay, 2000), or by weighing the tubes after semen collection on top-loading balance, and later converting the reading into milliliter by using a computer program, the latter has been known to reduce error associated with visual reading of the tube specially when small volume or bubbles are found by 10 % (Bearden and Fuquay, 2000). The volume has been reported to decline when young bulls are used or when there is frequent ejaculation or incomplete or failure of ejaculation and in bilateral seminal vesiculitis (Roberts, 1971; Garner, 1991; Hafez, 1993). In summary a number of factors like season of the year, method of collection, and the sexual preparation of the bull have been known to affect semen volume. The normal volume of semen of the bull has been known to be within the range of 5 to 15 ml (Sorensen, 1979), 1 to 15 ml (Bhosrekar, 1990), 2 to 10 ml (Setchell, 1991), 5 to 8 ml (Hafez, 1993), 4 to 8 ml (Bearden and Fuquay, 2000) per ejaculate. Nevertheless, small volume, unless accompanied by a low semen concentration has also been reported to be harmless (Roberts, 1971; Uwland, 1984; Hafez, 1993). The ejaculate volume is known to be important in determining the dosage of the semen straw during semen processing (Peters and Ball, 1995).

2.9.2.3. Mass activity

Mass motility of spermatozoa is described to be the function of both concentration and motility (Rao, 1957; Roberts, 1971; Bhosrekar, 1990). In concentrated semen there is a pattern ranging from very slow to very rapid motion depending on the quality of the semen. This mass activity can be graded for instance, 0, +, + + and + + + which could be used to indicate no mass activity, slow (mild) waves and eddies, very strong (rapid) wave motion and eddies at the end of wave, and eddies respectively. Semen showing mass activity of at least + + is considered as good. Mass activity of the spermatozoa may also be graded 0 to 5 grades (Roberts, 1971; Bhosrekar, 1990; Coles, 1986). Where 0, 1, 2, 3, 4, and 5 have been known to represent immotile sperm, stationary bunting (weak rotatory movements), oscillatory movements with no wave motions or eddies, motions with slowly moving waves and eddies, spermatozoa movements exhibiting rapid waves and eddies and extremely rapid waves and eddies respectively.

2.9.2.4. pH

The pH of semen has been reported to be measured by pH paper, bromothymole blue or a pH meter (Roberts, 1971). The pH of bull semen is slightly acidic, about 6.7, and has been reported to rise ($\geq$ 7) when there is incomplete ejaculation, excessive use of the bull or in yearling bulls and in pathologic situation of the testis, epididymis, ampulla or seminal vesicle (Roberts, 1971; Arthur *et al*., 1989). Dense semen samples which possess excellent motility have been known to show lower values of pH (Arthur *et al*., 1989).

2.9.2.5. Sperm concentration and sperm count

Spermatozoa concentration refers to the number of spermatozoa per milliliter of semen (Hafez, 1993) and the sperm concentration of the bull has been known to range from 800 to 1200 (Sorensen, 1979), 300 to 2000 (Setchell, 1991), 800 to 2000 (Hafez, 1993), 1000 to 3000 (Bearden and Fuquay, 2000) million per milliliter of semen. According to Roberts, 1971, the concentration of the spermatozoa in the ejaculate of the fertile bull varies from 3 $x10^5$ to 2 x 10^6 per cubic millimeter, with an average of 8 $x10^5$. The sperm concentration of

fertile bulls range from 3 x10 ^{5} to 3 x 10 ^{6} /mm^{3}, and low sperm concentration has been known to be the feature of testicular hypoplasia and degeneration (Arthur *et al*., 1989).

Determination of sperm concentration has been reported important as it is the highly variable semen characteristics (Hafez, 1993). Various techniques like haemocytometer counts; turbidemeteric measurements, spectrophotometer, and electronic counting systems (Garner, 1991; Hafez, 1993; Bearden and Fuquay, 2000) have been used to determine the sperm concentration of the sample.

The sperm count has been known to be the total number of the spermatozoa in the ejaculate, and is calculated by multiplying the spermatozoa concentration of the semen by the volume of the ejaculate (Hafez, 1993). Semen collected with an electroejaculator has been known to have a lower concentration of spermatozoa than that collected with an artificial vagina, mainly due to excess accessory gland secretion in the former case (Roberts, 1971; Arthur *et al*., 1989; Hafez, 1993). In many infertile or sterile bulls, a rapid decline in sperm concentration between first, second and third successive ejaculates has been known which indicates poor spermatozoa reserves and reduced sperm cell production (Roberts, 1971). The concentration of spermatozoa has been reported to vary with sexual development and maturity of the bull, with feeding regimen, and with reproductive health of the testes, season of the year and geographical localities (Salisbury *et al.,* 1978).

2.9.2.6. Motility of spermatozoa

Motility of the spermatozoa has been defined as the percentage of sperm cells which are motile under their own power and progressively motile spermatozoa has been defined as those spermatozoa which are moving or progressing from one point to another in a more or less straight line (Bearden and Fuquay, 2000).

Spermatozoa are driven by a propulsive apparatus, the flagellum, which is equipped with contractile proteins strategically arranged in longitudinal organelles, the coarse fibers, and with associated subfilaments, and micro tubes, which provide the propulsive force necessary to overcome internal structural resistance and external viscous drag of extra cellular fluids (Hafez, 1993). Motility of spermatozoa at time of collection has been used commonly as a measure of the fertilizing ability of the sperm (Roberts, 1971; Bhosrekar, 1990). However,

spermatozoa have been found to lose their fertilizing capacity before they lose motility which puts motility estimation to be not necessary indicative of fertilizing capacity of the sperm (Hafez, 1993). In general, however, a definite correlation has been found between concentration, morphology, and motility of the spermatozoa; and the proportion of the total number of actively motile normal spermatozoa in the ejaculate has been found to show levels of fertility of the bull (Roberts, 1971). The low correlation between sperm motility and fertility has been suggested to result from the low accuracy and precision of the visual method to evaluate spermatozoa motility, influence of other attributes of sperm quality on fertility, the imprecision in measuring the fertility of the individual ejaculates, and the influence of many factors unrelated to semen such as parity of the female, season of the year, month the semen is used, and herd management (Hafez, 1993).

Several endogenous and exogenous factors (Roberts, 1971; Bhosrekar, 1990; Hafez, 1993) have been recognized to affect sperm motility. Excessive heat, and chemical or foreign agents, and sudden reduction in temperature during collection depress sperm motility. Normally 40-75 % (Sorensen, 1979; Hafez, 1993) and 50 to 80 % (Bearden and Fuquay, 2000) of bull sperm has been found motile, however, spermatozoa motility below 50 % (Roberts, 1971), 40 % (Bearden and Fuquay, 2000) has been known to be associated with low conception rate or poor fertility. Sperm motility has been known to reduce to one third to half of the normal following an increase in abnormal cells, dead cells, necrospermia or poorly viable cells which have been found to be associated with testicular degeneration (Roberts, 1971). Spermatozoa with morphologic abnormalities have been known to show no progressive motility (Bearden and Fuquay, 2000).

Even if several techniques have been devised to study, describe and quantify sperm motility, the simplest one used for general purpose involves a visual appraisal of the percentage of motile sperm and the quality of the motility of individual spermatozoa (Hafez, 1993), which enable us to determine the viable number of spermatozoa (Peters and Ball, 1995). Spermatozoa fertility has been reported to be dependent on the total number of actively motile spermatozoa (viable number) in the ejaculate.

2.9.2.7. Live/dead count

Differential staining to determine the proportion of live to dead cells is undertaken by supravital staining with stain mixture such as Nigrosin-Eosin (Salisbury *et al*., 1978; Hafez, 1993). By this technique dead cells stain red with Eosin against the dark Nigrosin background while the live cells exclude the stain (Hafez, 1993). Furthermore the results of these techniques correlate with visual estimates of progressively motile cells (Hafez, 1993). Commonly accepted standards for percentage of live spermatozoa in bull semen collected by artificial vagina and to be used for AI was given by Lindasay *et al*. (1982) to be 70 %, and 75 % by Sorensen (1979).

2.9.2.8. Spermatozoa morphology

Bull spermatozoa are known to have two parts: head and tail (Garner, 1991; Hafez, 1993). The head of spermatozoa is known to consist the nucleus as its most part consisting highly condensed chromatin (43 % of which is DNA), combined with Arginine rich strongly basic small proteins called protamins (57 %), and the anterior half of the head is covered by a double walled acrosomal cap, its intermediate part or equatorial segment covered with a very thin cytoplasmic layer and posterior part in which the chromatin concentration and staining ability are heaviest is covered by well developed membrane, post nuclear cap (Bhosrekar, 1990; Garner, 1991; Hafez, 1993). The acrosome, which covers the anterior part of the nucleus, has been recognized to consist of an inverted sack of membrane which contains a specific lipoprotein complex, including enzymes like hyaluronidase, acrosin, esterases, acid hydrolases, and other proteinases which are known to be involved in the fertilization process (Bhosrekar, 1990; Garner, 1991). Optimal fertility of the spermatozoa is known to depend on the structural and biochemical intactness of the acrosome (Sullivan, 1978). The base of the head of the spermatozoa is recognized to be concave, the implantation groove to which the tail is fastened (Bhosrekar, 1990).

The tail of the sperm consists of the neck, middle, principal and end pieces (Garner, 1991; Hafez, 1993; Bearden and Fuquay, 2000): the neck or the connecting piece forms a basal plate that fits in to a depression in the posterior surface of the nucleus (head), and this basal plate is continuous posteriorly with nine coarse fibers that project posterior throughout most of the tail; the region of the tail between the neck and the annulus has been known to be the middle

piece whose central core makes up the axoneme together with the entire length of the tail. The axoneme is composed of nine pairs of microtubules that are arranged radially around two central filaments, the latter (microtubules) are surrounded by nine coarse (dense) fibers, and numerous mitochondria have been found to peripherally cover the axoneme and its associated dense fibers of the middle piece. This mitochondrial sheath, which serves for the generation of energy for the sperm motility, has been found to be arranged in helical pattern around longitudinal fibers of the tail and terminates at the annulus. The principal piece, which continues posteriorly from the annulus and extends to near the end of the tail, has been recognized to be composed of centrally the axoneme and its associated coarse fibers, and externally a fibrous sheath has been found to give stability for its contractile elements. On the other hand, the end piece contains only the axoneme covered by the plasma membrane (Hafez, 1993).

The total length of bull spermatozoa has been reported to be 70-80 microns (Bhosrekar, 1990), 68-74 microns (Ortavant *et al*., 1969; Sullivan, 1978), and head and midpiece has been reported to cover 8-10 microns, neck has been known to cover 0.3 to 1.5 microns, principal piece has been known to cover 45-50 microns and the terminal piece has been known to cover 2-4 microns (Bhosrekar, 1990; Sullivan, 1978). Some authors described the midpiece length as 13 microns (Faulkner and Pineda, 1980) and 14.84 microns (Ortavant *et al*., 1969). The shape of the head of bull spermatozoa has been reported to be a thin toung-like plate with an evenly rounded anterior outline (Bane, 1982).

It has been known that the head of the spermatozoa contains half of the genetic material, the neck connects the base of the head of the spermatozoa to the mid-piece and consists the proximal centriole, the mid-piece surrounded by the mitochondrial helix which serve as metabolic center for the spermatozoa, the sperm tail provides motility, and the axial fiber bundle contains contractile fibers for motility, and courses longitudinally through the center of spermatozoa (Sullivan, 1978; Bhosrekar, 1990; Hafez, 1993).

Sometimes low motility estimates have been associated with improper handling of the semen rather than poor semen quality. For this reason, evaluation of semen as part of breeding soundness examinations, tend to emphasize sperm morphology assessments rather than motility (Hudison, 1972; Morrow, 1986; Garner, 1991).

2.9.2.8.1. Abnormal morphology of spermatozoa

Any morphological deviation from the normal structure of spermatozoon has been considered abnormal (Sullivan, 1978). Ejaculated semen has been reported to contain some morphologically abnormal spermatozoa (Faulkner and Pineda, 1980; Hafez, 1993) which has not been associated with lowered fertility until the proportion of abnormal spermatozoa exceeds 20 %. An increasing important morphologic assessment has been estimation of the levels of normalcy of the acrosome because of its obvious role in fertilization (Garner, 1991). Increased prevalence of abnormal spermatozoa has been found to be associated with reduced fertility (Lagerlof, 1934; Roberts, 1971; Sullivan, 1978; Arthur *et al*., 1989).

Table 2. Summary of the different morphological abnormalities of bovine spermatozoa**.**

NO	Head abnormalities	Body or mid-piece abnormalities	Tail abnormalities
1	Macro cephalic head	Swollen neck	Tightly coiled tails
2	Micro cephalic head	Kinked neck with coiling of midpiece	Double tails
3	Elongated narrow heads	Necked or filiform neck	Absent tails
4	Pyriform heads or pear shaped	Abaxial attachment of neck	Bent tails
5	Twisted, irregular shaped heads	Double middle piece	Broken tails
6	Round short heads	Kinked middle piece	Shortened tails
7	Abnormal (knobbed) heads	Cork-screw middle piece	
8	Nuclear membrane invagination	Loose or free middle piece	
9	Detached or free heads	Kinked necks	
10	Detached galea capitis and acrosome	Middle piece with cytoplasmic droplets	
11		Bent middle piece	

Adapted from Roberts, 1971

The detached or loose heads and detached galea capitis have been known to be abnormalities which possibly could occur due to excessive agitation or incorrect smearing and long term storage of spermatozoa respectively. High percentages of middle piece protoplasmic droplets have been observed in over used bulls due to rapid passage of spermatozoa through the

epididymis (Roberts, 1971). Cold-shock or osmotic shock due to the presence of water or urine in the ejaculate has been noted to cause bending of the tail at the junction of the tail and middle piece with or without a protoplasmic droplet (Roberts, 1971). Abnormalities of middle piece and tail have been reported to interfere with fertility by reducing sperm motility.

According to their significance as indicators of potential breeding soundness, spermatozoa abnormalities has been classified as (Table 3) major or minor abnormalities (Blom, 1972) where the major sperm defects have been recognized to be related to impaired fertility, or to abnormal condition in testes and epididymis while the minor sperm defects seem to be less important and call attention only when any minor defect exceeds 10 to 15 % (Blom, 1972).

Table 3. Classification of different morphologic abnormalities of bovine spermatozoa.

NO	Major defects	NO	Minor Defects
1	Undeveloped	16	Narrow heads
2	Double form	17	Small normal heads
3	Knobbed sperm defect	18	Giant heads and short broad heads
4	Decapitated sperm defect (Guernsey)	19	Free normal heads
5	Diadem defect	20	Detached acrosomal cap
6	Pear-shaped head	21	Abaxial implantation
7	Narrow at base	22	Distal droplet
8	Abnormal contour	23	Simple bent or coiled tail
9	Small abnormal heads	24	Terminally coiled tail
10	Free abnormal heads		
11	Cork screw sperm defect		
12	Other midpiece defect		
13	Proximal droplet		
14	Pseudo droplet defect		
15	Strongly coiled tails (Dag-defect)		

Source; Blom, 1972

High frequency of abnormal spermatozoa have been found to be associated with extremes of temperature, diseases including reproductive infections, congenital testicular hypoplasia, and advancing age of bulls (Sullivan, 1978). Accordingly appearance of tailless heads in the ejaculate has been known to be associated to early testicular degeneration and in some cases

of partial testicular hypoplasia (Sullivan, 1978). On the other hand excessive agitation and incorrect smearing, and aging after prolonged sexual rest have been recognized respectively to result in tailless head and detached galea capitis (Roberts, 1971).

Sperm abnormality, in specific ejaculates, besides its effect to impair fertility (Roberts, 1971; Peters and Ball, 1995), some abnormalities like knobbed acrosome (Friesian bull), tailless heads (in Guernsey bulls), and pseudo droplet defects have been recognized to be inherited (Roberts, 1971; Sullivan, 1978; Hafez, 1993).

Apart from morphological abnormality (Sullivan, 1978), spermatozoa with normal morphology might result in sub-fertility or infertility due to aneuploidy, chromosomal inversion, translocation, diploidy (abnormal gene content), nuclear physico-chemical defects which can be identified via cytogenetic or karyotyping studies.

The presence of 10 % or more of any single type of the above head, body or tail abnormalities has been reported to cause reduced fertility (Hancock, 1959; Roberts, 1971; Hafez, 1993). Following testicular degeneration, the percentage of dead and abnormal spermatozoa has been reported to increase (35-60 % in severe cases) (Roberts, 1971).

2.9.2.9. Biochemical analysis of seminal plasma for glutamic oxalacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), alkaline phosphatase (ALP), acid phosphatase (ACP).

Enzymes such as GOT, lactate dehydrogenase, cholinesterases and alkaline or acid phosphatases, etc (Roberts, 1971), proteolytic enzymes, phospholipases, transaminases like GOT, acid and alkaline phosphatases, ATPase, glycosidase, dehydrogenases, nucleotidases, DNases, hyaluronidase (Bhosrekar, 1990) have been recognized to be intimately related to the sperm cell and have been known to pass to seminal plasma when there is damage to the sperm cells. For this reason enzyme determination of semen plasma has been found essential in measuring the degrees of damage to the sperm cells before ejaculation and during the freezing process (Roberts, 1971). According to Bhosrekar (1990), enzymes in semen are mainly bound to sperm cells and their presence in seminal plasma has been known to be associated to leakage out of dead or damaged cells.

Following dilution, freezing, thawing of bovine semen, and cold shock reactions the activity of such enzymes have been reported to rise above their initial level in seminal plasma owing to increased cellular permeability which cause leakage of intracellular enzymes into the media concomitant to increased sperm abnormality and decreased sperm motility and viability (Mann and Lutwak-mann, 1981; Dhami and Kodagali, 1990). Similar situation have been known in fresh semen containing a high proportion of abnormal sperm (Mann and Lutwak-mann, 1981). Such damage to the sperm cells and leakage of vital enzymes has been known to lower post thaw recovery and fertility (Dhami and Kodagali, 1990).

Some previous researchers have reported the seminal plasma enzymes level as follows: Aguirre *et al*. (1988) reported the value of ACP in bovine seminal plasma as 1268.1 U/L, Reddi and Raja in 1980 reported ACP and ALP level in buffalo bull as 757.13 (47.43) BU/100 ml and 632.32 (3.04) BU/100ml respectively. Singhal *et al.* (1976) reported the GOT in crossbred bulls as 101.5 BU/100 ml, where as the GOT level in seminal plasma of crossbred bull was reported as 898.5 (38.36) BU/100 ml, and 385.25 BU/100 ml respectively by Saxena and Tripathi (1978) and Pandit and Garg (1983). Singhal *et al.* (1976) and Saxena and Tripathi (1978) reported GPT level in seminal plasma of crossbred bulls as 60 BU/100ml and 39.42 (4.99) BU/100ml respectively.

The information on level of enzymes in seminal plasma has some disagreements. Some described enzymes like GOT as purely intracellular (Bhosrekar, 1990) and its presence in seminal plasma indicates leakage out of the spermatozoa. Some others described epididymis as one source of GOT and GPT (White, 1980), ACP and ALP (White, 1980; Bhosrekar, 1990), for the latter (ACP and ALP), accessory gland has been known to be the other source (White, 1980; Martin, 1985). In both cases (GOT, GPT, ACP, ALP), it has been known that if the level increases in seminal plasma, in might be due to leakage out of the cells (spermatozoa) which is an indication of depressed metabolic activity of the spermatozoa (White, 1980).

2.9.2.10. Biochemical analysis of seminal plasma for total protein

The bovine seminal plasma has protein level of 3 to 8 gm/dL (Setchell, 1991) and an average value of 6.8 gm/dL (Faulkner and Pineda, 1980; Hafez, 1993). The seminal plasma contains proteins, amino acids and polypeptides (White, 1980; Hafez, 1993; Bearden and Fuquay, 2000). These proteins and lipoproteins have a protective action against the dilution effect by

preventing loss of intracellular constituents (Hafez, 1970). Similarly, recent reports on seminal plasma proteins described the presence of fertility associated proteins in the bovine seminal plasma (Bearden and Fuquay, 2000; Nauc and Manjunath, 2000). In contrast to the protective role of seminal plasma proteins more recent studies on bovine seminal proteins showed that these proteins facilitate capacitation (by binding to the spermatozoa surface) by promoting cholesterol efflux from the spermatozoa membrane and thus have deleterious effect on spermatozoa (Therien *et al*., 1998; Nauc and Munjunath, 2000). Spermatozoa membrane cholesterol has been known to have an important role in modulating membrane bilayer fluidity and stability (Therien *et al*., 1998; Nauc and Munjunath, 2000).

2.9.2.11. Hormonal analysis of seminal plasma for testosterone

Testosterone is known to be essential for spermatozoa maturation and survival in the male reproductive tract (Gunjam and Amann, 1976; Tuli *et al*., 1991). High concentration of testosterone and other androgens in semen are recognized to depress the spermatozoa metabolic rate, even though the concentrations found in the male tract have no permanent effect (Gomes, 1977; Bearden and Fuquay, 2000). It has been recognized that the increase in the metabolic activity of spermatozoa leads to the decrease in their survival rate (Hafez, 1970). So such depressing act by androgens might be beneficial to prolong the survival rate of the spermatozoa. On the other hand, testosterone has been found essential for the normal secretory function of epididymis and accessory sex glands (Salisbury *et al*., 1978; Faulkner and Pineda, 1980; Martin, 1985; Hafez, 1993) which are known to have an essential role in spermatozoa maturation and survival. The clear roles of androgens to the ejaculated spermatozoa need further clarification. Gunjam and Amann (1976) reported the seminal plasma testosterone level in bovine as 2.87 (0.55) ng/ml, and Tuli *et al.* (1991) found positive correlation between testosterone in the seminal plasma and mass motility ($r = 0.43$, $P < 0.01$) and non-significant correlation with semen volume, ($r = 0.12$, $P > 0.05$), and reported seminal plasma testosterone level of 1.41 (0.14) ng/ml in seminal plasma of buffalo bulls.

2.10. Factors affecting bovine semen quality

2.10.1. Age

Zebu cattle in general have been recognized to reach puberty 6-12 months later than the European breeds, the age at puberty for the latter being 12-14 months (Sane *et al.,* 1982; Hafez, 1993). Age at first breeding has been found to depend more on body weight than on age, and can be delayed by slow growth. Similarly it has been reported that testicular size, scrotal circumference and body weight are positively correlated with age, and the semen volume, quality and amount of mature spermatozoa have also been found to be positively correlated with testicular size and scrotal circumference (Coulter and Foote, 1979; Sane *et al*., 1982; Heinonen, 1989; Blezinger, 1999). If every parameter has been kept equal, best semen quality has been obtained from bulls which are a little under weight (Blezinger, 1999). Brito *et al*. (2002) in their study conducted to access effects of environmental factors, age and genotype on sperm production and semen quality in AI bulls in Brazil, reported an increase in sperm minor defects and decrease in sperm motility with an increase in bulls age. Bulls which are two years or younger have been known to have higher percentage of head abnormality (Chacon *et al*., 1999), on the other hand bulls which are older than seven years has not been recommended to use for natural mating as such bulls have reduced sperm production owing to higher incidence of lesions such as testicular senile fibrosis and tubular calcification (Caroll *et al.,* 1963; Kumi-Diaka *et al*., 1981; Rao and Bane, 1985), which decrease their fertility (Uwland, 1984).

2.10.2. Breed

Libido or sex drive and mating ability in the bull has been reported to be influenced by genetic factors, and lack of sexual desire has been found more common in some strains and breeds of cattle (beef breeds and *Bos indicus*) than others (Hafez, 1993). However, no association has been observed between libido and semen quality.

Some European breeds, such as Polled Herefords have been known to give good quality semen during winter and early spring when the weather is cool; on the other hand, Brahman types, bulls have been reported to have better semen quality during warmer months (Blenziger, 1999). But whether such difference is due to breed or seasonal difference needs

investigation. *Bos indicus* bulls have been reported to have a significantly higher sperm concentration (Table 5) and higher sperm morphologic defects than *Bos taurus* (Brito *et al*., 2002). Some authors reported live percentage in *Bos indicus* (Gir) as 80.13 (1.43) (Shelke and Dhami, 2001), and 71.85 (1.49) (Rana and Dhami, 2003), in Sahiwal as 72.22 (Ahsan *et al*., 2003), Friesian-Sahiwal cross as 74.22 (Ahsan *et al*., 2003), in Friesian bull as 87.35 (0.54) (Dhami *et al*., 1998) and Ongole bull as 82.17 (Veeraiah *et al*., 1999). Similarly, at different times different authors reported the total value of morphologic abnormalities as 15.54 (1.09), 27.15 (0.97), 15.41 (0.86), 11.74, 9.26 (0.43), 22.5 (1.4) in Gir (shelke and Dhami, 2001), Friesian x Sahiwal (Ahsan *et al*., 2003), Sahiwal (Ahsan *et al*., 2003), Ongole (Veeraiah *et al*., 1999), Friesian (Dhami *et al*., 1998), and Gir (Rana and Dhami, 2003) respectively. The percent normal acrosome was reported 92.33 in Ongole bulls (Veeraiah *et al*., 1999), and 84.8 (0.9) in Gir (Rana and Dhami, 2003), and the viable number of spermatozoa was reported as 4.9 billion in *Bos taurus* and 6.7 billion in Brazilian zebu (Brito *et al*., 2002).

Table 4. Some semen characteristics in different breeds of bulls

Parameter	Value reported[1]	Breed	Author
Volume (ml)	4.84 (0.16)	Gir	Shelke and Dhami, 2001
	5.62 (0.14)	F x Sahiwal	Ahsan *et al*., 2003
	3.64 (0.09)	Sahiwal	
	6.9 and 8.2	*Bos taurus*	Brito *et al*., 2002 @
	6.6 and 6.7	*Bos indicus*	
	4.96	F x Sahiwal	Andrabi *et al*., 2002
	3.81	Ongole	Veeraiah *et al*.,1999
	6.1	crossbred	Hector and Oscar, 1998
	5.12 (0.18)	Friesian	Dhami *et al*., 1998
	7.05	Zambian zebu	Omar, 1997
	3.43	Borgou	Adamou *et al*., 1996
Mass motility (0 – 5)	2.96 (0.13)	Gir	Shelke and Dhami, 2001
	1.25 (0.04)	F x Sahiwal	Ahsan *et al*., 2003
	1.36 (0.04)	Sahiwal	
	2.89	Ongole	Veeraiah *et al*.,1999
	3.43 (0.12)	Friesian	Dhami *et al*., 1998
	3.85	Borgou	Adamou *et al*., 1996
Initial motility (percentage)	67.87 (2.69)	Gir	Shelke and Dhami, 2001
	50.5 (0.93)	F x Sahiwal	Ahsan *et al*., 2003
	60.55 (0.33)	Sahiwal	
	55.0	F x Sahiwal	Andrabi *et al*., 2002
	76.53	Ongole	Veeraiah *et al*.,1999
	68.6	Dual purpose	Hector and Oscar, 1998
	79.33	Zambian zebu	Omar, 1997
	75.7	Borgou	Adamou *et al*., 1996
	71.5 (0.9)	Gir	Rana and Dhami, 2003
Sperm concentration (x 10^9/ml)	1.22 (0.04)	Gir	Shelke and Dhami, 2001
	1.2	*Bos taurus*	Brito *et al*., 2002
	1.65	*Bos indicus*	
	1.43	Ongole	Veeraiah *et al*.,1999
	0.08	Dual purpose, Mexico.	Hector and Oscar, 1998
	0.95	Friesian	Dhami *et al*., 1998
	1.09	Zambian zebu	Omar, 1997
	1.19	Borgou	Adamou *et al*., 1996
	1.61 (0.05)	Gir	Rana and Dhami, 2003
Total count (10^9/ejaculate)	8.2	*Bos taurus*	Brito *et al*., 2002
	11.4	*Bos indicus*	

@ indicates two values reported for two different years, [1]numbers in brackets indicate SE.

2.10.3. Nutrition

The importance of proper nutrition (adequate amount of vitamins and minerals, balanced amount of protein and energy) for good reproductive performance has been known (Blezinger, 1999). Balanced amount of protein and energy are required for production and physical activity associated with breeding (Blezinger, 1999).

Nutritional deficiencies, in the bull have been reported to delay the onset of puberty and depress production and characteristics of semen (Hafez, 1993). The negative effect of under feeding has been found to be more serious in young than adult bulls (Hafez, 1993).

Tegegne *et al.* (1992a) on their first study on Boran and Boran-Friesian cross bulls found a number of semen characteristics (at puberty) to be influenced by supplementary feeding. That is, in both genotypes, supplemented bulls had a larger semen volume and higher sperm concentration than the control bulls, and in Boran-Friesian crosses they observed a significant higher motility score and percentage of spermatozoa in supplemented than control bulls.

The restrictive effect of limited feed intake on the age at puberty and the rate at which mature sperm production is attained has been recognized to be due to primarily diminished gonadotropin output (Salisbury *et al.,* 1978; Hafez, 1993). It has been suggested (Mann and Lutwak-Mann, 1981) that underfeeding, protein malnutrition and certain hypovitaminosis some how interfere with the regulation of hypothalamo-pituitary axis thereby altering gonadal steroid production, besides which responses of accessory glands to testosterone has been found to reduce.

On the other hand, obesity and overfeeding have been known to reduce sexual function in a bull (Hafez, 1993; Blezinger, 1999). These have been associated with excessive fat deposition in the scrotum which may interfere with testicular thermoregulation (Blezinger, 1999), even though the degree of body fat that is required to adversely affect sperm production has not been well established, and needs more research.

2.10.4. Temperature and season

Climatic factors like ambient temperature; humidity, convection current, and solar radiation affect testicular function directly or through neurohormonal mechanism (Sane *et al*., 1982). Elevated body temperature during periods of high ambient temperature or pyrexia from disease has been noted to cause testicular degeneration and reduce the percentage of normal and fertile spermatozoa in the ejaculate (Hafez, 1993). Body cooling either via water sprinkling or fanning has been reported to decrease percentage of dead sperm, sperm abnormality and acrosome damage in Murrha Buffalo bulls (Mandal *et al*., 2002). Semen characteristics in Pakistan Livestock Research Station on crossbred Friesian and Sahiwal bulls showed higher semen volume from August to December, higher sperm motility from October to December and higher sperm abnormality during June and July (Andrabi *et al*., 2002). Igboeli and Rakha (1971) in their study concerned with assessment of seasonal changes in the ejaculate characteristics of Agoni (short horned zebu in Zambia) found poor quality ejaculates (low volume, high incidence of abnormal spermatozoa, and low fructolysis index) during a hot period. Chacon *et al*. (1999) also reported a significant positive correlation between abnormal tails and proximal cytoplasmic droplets and mean monthly temperature and rainfall.

Chronic heat treatment has been reported to reduce testosterone concentration and spermatid concentration while the integrity of Sertoli cells become compromised, which has been found to be followed by poor semen quality and a concomitant drop in motility (Egbounlike *et al*., 1985). On the other hand, a mild heat stress for a short period of time (48 hours) has been found to affect semen quality of a herd bull for as long as one and half months (www.selectsires). The effect of this factor, even if reported to be high in areas with high temperature and humidity, the expression of these effects has also been recognized to vary with genotype of the animal, season of the year, and management factors employed (Egbounlike *et al*., 1985; Morrow, 1986). Sperm production in tropical region has been known to be affected during hot season through reduction of feed intake resulting in reduction in body weight and testicular function (Egbounlike *et al*., 1985).

2.10.5. Scrotal circumference

Scrotal circumference has been recognized to be highly heritable, and to serve as an indicator of puberty, total sperm production, semen quality, pathologic condition of testes, potential sub

fertility or infertility of bulls, and consequently failure to select properly for this trait has the potential of decreasing fertility and production for the generations to come (Ott, 1986). The bulls with larger testes have been known to give larger volume of semen, larger sperm motility and percentage of normal spermatozoa (Ott, 1986). The scrotal circumference estimates for most yearling bulls has been recognized to be 32 to 36 cm; the average estimate being 26.1 cm (Coulter, 1986), and 27.9 cm (Ott, 1986) at puberty. As the scrotal circumference increases, the probability of having seminal quality which is acceptable increases until a scrotal measurement of 38.0 cm is attained but no bull with the scrotal measurement less than 30 cm has been know to produce semen of acceptable quality (Coulter, 1986).

2.10.6. Disease of testis, epididymis and accessory glands

Pathologic condition of testis, epididymis, and seminal vesicle has been recognized to interfere with fertility by disturbing spermatogenesis or sperm maturation, leading to abnormal semen characteristics or preventing the passage of spermatozoa from testes to urethra (Hafez, 1993; Blezinger, 1999). Some of such disease conditions include: testicular degeneration, orchitis, epididymitis and seminal vesiculitis (Hafez, 1993).

The seminal changes which have been reported during testicular degeneration include: increase in immature and abnormal sperm with normal motility, but as the disease progresses the ejaculate becomes thin and watery, due to low concentration of the spermatozoa, and there is appearance of giant cells, azoospermia or necrospermia in severe testicular degeneration (Hafez, 1993). When the bull suffers from inflammation of the testes (orchitis), the seminal changes that have been observed (Hafez, 1993) are asthenozoospermia, oligozoospermia, teratozoospermia, giant cells, white blood cells, and red blood cells with normal semen volume. Similarly, poor semen characteristics and contamination of semen with inflammatory cells have been reported as the main features observed when there is epididymitis (Hafez, 1993), as purulent exudation and lowered fructose content of the semen have been recognized as the main feature of seminal vesiculitis (Hafez, 1993).

2.10.7. Other factors

Other factors like frequency of service (ejaculation), poor semen collection technique, and cow to bull ratio, procedure of handling the semen during and after collection, analytical techniques, and variation among technicians, pharmacologic agents and normal physiologic variations have been recognized to influence the semen quality of the bull (Faulkner and Pineda, 1980; Hafez, 1993; Blezinger, 1999).

Steroid compounds like testosterone, testosterone ester, methyl testosterone, estrogens, progestational compounds, which were used as contraceptives (in human) or drugs like nitrofurantoin (in feed), irradiation, etc., has been known to affect sperm output and to reduce semen quality (Jackson, 1972). In this situation the drugs have been documented to affect the meiotic phase while the hormones affect both the meiotic and the spermiogenesis phase. Some drugs like anticancer drugs (tretamine) have been known to act on spermatid, testicular and epididymal sperm where they can bring sterility for about 5 weeks without bringing problem in sperm motility, and still other (like trimethyl phosphate) have been known to bring such functional sterility, and an irreversible genetic damage (Jackson, 1972).

3. MATERIALS AND METHODS

3.1. Methods

3.1.1. Study area

The study was conducted at the National Artificial Insemination Center (NAIC) which is located at Kaliti, Addis Ababa. The place is located at 38^0 45' 52'' East longitude and 8^0 54' 12'' North latitude. The center was established in 1981 and is the only semen producing center in Ethiopia where 80 % of the semen production is from exotic (mainly Holstein-Friesian, and Jersey breeds) and 20 % is from the indigenous breeds (Barca, Boran, Horro and Sheko).

3.1.2. Study animals

Among those bulls that are being utilized for the semen production at NAIC, six indigenous bulls (three Horro, one from each Boran, Barca and Sheko) were selected for this particular study. All the bulls (Figure 1) were kept intensively under the same management conditions being given 2 kg concentrate and 9 to 10 kg hay per day, mineral lick every 1.5 to 2 months during dry period (1.25 kg/bull) and green feeds at the time of availability. The bulls were weighed every month and the scrotal circumference was taken once during the study period. The information on the birth date of the bulls was obtained from the center.

During the study period data on daily and monthly minimum and maximum temperature, mean temperature, rainfall, and relative humidity at 0600LST, 1200LST and 1800LST were obtained from nearest station of National Meteorological Service Agency (NMSA), Addis Ababa which is about 10 to 20 km from the study site.

1. Barca Bull

2. Boran Bull

3. Horro Bull

4.Sheko Bull

5. Bull sexual stimulation around teaser, and cleaning the teaser

6. Mounting and preparation to collect semen

7. Semen collection into AV

Figure 1. Photographs of different study animals and semen collection procedures.

3.1.3. Study design

3.1.3.1. Sampling procedure

Six indigenous bulls (one Barca, one Boran, three Horro and one Sheko bulls) which were in use for semen production at the NAIC, Kaliti, Addis Ababa, Ethiopia were used for this study. Semen was collected on a weekly basis, once per week for thirteen weeks from first week of October to end of December following the optimal recommended procedures (Salisbury *et al*., 1978; Bhosrekar, 1990): the bulls were washed and groomed and the environment for semen collection cleaned and rinsed with water; besides general examination for any disease; proper and careful treatment of the bulls, like avoidance of distraction. The teaser bull was also cleaned prior to and during (in between) semen collection practices and used to stimulate the bulls and to allow a false mount and finally for semen collection. The bulls were adequately sexually stimulated by a well-acquainted handler and had two or more false mounts. In all of the cases semen was collected by using the artificial vagina, and only the first ejaculate was used for the study purpose and a total of 67 semen samples were collected and analyzed for physico-morphological analysis, from which only 62 samples were used to analyze for enzymes and protein tests, and 40 samples for testosterone analysis (Annex 3).

3.1.3.2. Examination of the collected sample

3.1.3.2.1. Physical examination of the semen and the spermatozoa

Immediately following collection, the semen was kept at 34 ^{0}C (Bhosrekar, 1990) in water bath (IMV, L'AIGLE, France) and examined grossly (for appearance, volume, and presence of foreign materials such as dust or pus), microscopically (for mass activity and individual motility, live/dead count and morphology of the spermatozoa) and concentration, sperm total count, viable number (percent motile multiplied by total count) of the spermatozoa following the recommended procedures (Salisbury *et al*., 1978; Roberts, 1971; Morrow, 1986; Bhosrekar, 1990; Garner, 1991; Hafez, 1993).

Gross examination was made as rapidly as possible after collection for the presence of any dust, hair, or any foreign body, and to note the color of the semen for each animal in each breed and was recorded as creamy, white, yellow, watery, or brown. The volume of the ejaculate from each animal in each group was recorded in graduated collection tubes to the nearest 0.1 ml.

A large drop of undiluted semen was placed on warmed slide (37 ^{0}C) and examined on a stage warmed thermostatically set (at 37 ^{0}C) phase contrast microscope (Nikon, Japan) at magnification of 100 times (100x) and will be scored for mass activity from 0 to 5 according to the intensity of the wave motion. The individual motility of sperm cells was estimated as a percentage by examining undiluted semen placed on warmed slide covered under warmed cover slip and then those sperm cells which exhibited progressive movements under stage warmed (37 ^{0}C) microscope at a magnification of 200x and scored 0 to 100 % according to the estimated percentage of spermatozoa which move in a progressive forward direction.

Sperm cell concentration was determined by using calibrated spectrophotometery (IMV, Technologies France) and Dipura (HAMLITON micro lab ®500B). The sperm concentration obtained was multiplied by the total volume of the ejaculate (to get total count of spermatozoa) the total count was multiplied by progressively forward moving percentages (individual motility) to get viable number of the spermatozoa (Brito *et al*., 2002).

For morphological examination of the spermatozoa, one milliliter of Hancock solution (Buffered formole saline) was kept in 34 ^{0}C in water bath to which one drop of fresh semen was added with warm (37 ^{0}C) Pasteur pipette and gently mixed for preservation of the spermatozoa. Later morphological abnormalities mainly head, mid-piece and tail defect, proximal and distal droplets were examined by placing small drop of the sample on clean grease free glass slide and covering by cover slide under phase contrast microscope (200x) (Hancock's technique). By this technique 500 spermatozoa were counted and the abnormalities were visualized and recorded as head, mid-piece (body) and tail abnormalities (Roberts, 1971; Salisbury *et al*., 1978). Characterization of head abnormality (including acrosome defect) was made on 500 spermatozoa stained by the William's technique (Williams, 1920; Bane, 1982) from the sample preserved by using Hancock solution (Annex 2).

The proportion of live spermatozoa was determined on smears prepared from fresh semen by the Eosin-Nigrosin technique during which 500 spermatozoa were counted under 1000x or oil immersion lens (light microscope). In this technique: two drops of mixed stain and one drop of fresh semen were mixed and smears were made on glass slide (by using spreader glass), allowed to dry in air, and finally examined under Bright field light microscope (1000x). Cells which accepted Eosin subsequently stain pink, against dark background of Nigrosin and were taken as dead. Similarly, sperm cells which exhibited such property to at least half of the sperm head were taken as dead. On the other hand, those sperm cells that didn't accept the stain and remained white were taken as alive. Sperm dimension measurements were done by measuring the head, midpiece and tail of the spermatozoa from those stained by the William stain technique (using Bright field microscope and micrometer) and five spermatozoa were measured from each slide and a total of 50 spermatozoa for each bull breed were measured.

3.1.3.2.2. Biochemical analysis of enzymes, total protein and testosterone in seminal plasma

Immediately following collection after samples for physico-morphological semen analysis were taken, the remaining ejaculate was centrifuged at 3000 rpm for 10 minutes and the supernatant, seminal plasma was separated from spermatozoa and preserved at -20 ^{0}C until later transported to laboratory (Arsho Medical Laboratory PLC., Addis Ababa) for enzymes (GOT, GPT, ALP, ACP), total protein and testosterone analysis.

3.1.3.2.2.1 Alanine aminotransferase (ALT/GPT) analysis

Analysis were carried out on Vitros 250 Chemistry system (Johnson Johnson, Ortho-clinical Diagnostic.Inc.100 Indiago Creek Drive Rochester, NY 14626-5101, USA) and the ALT activity was measured at 37 ^{0}C, using wavelength of 340nm approximately within five minutes, which is based on the optimized procedure of IFCC, method modified to 37 ^{0}C (Bergmeyer *et al*., 1985 Part 3). The Vitros Chemistry for ALT uses the Vitros ALT slide which is a dry, multi-layered, analytical element coated on a polyester support containing reactive ingredients like lactate dehydrogenase, (a porcine muscle, E.C.1.1.1.27); L-alanine; sodium α-ketoglutarate; nicotinamide adenine dinucleotide, reduced; sodium pyridoxal-5-

phosphate (P-5-P) and other ingredients like pigments, binders, buffers, surfactants, cross-linking agent, and stabilizers.

In these tests an 11 µl drop of the seminal plasma was deposited on the Vitros ALT slide and was evenly distributed by the spreading layer (containing the ALT substrate L-alanine and sodium α-ketoglutarate) to the underlying layers.

This method is based on the principle that ALT/ GPT catalyzes the transfer of an amino group from L-alanine to α-ketoglutarate forming glutamate and pyruvate:

$$\text{Alanine} + \alpha\text{-ketoglutarate} \overset{\text{ALT}}{\underset{\text{p-5-p}}{\rightleftharpoons}} \text{Glutamate} + \text{pyruvate}$$

The pyruvate generated in the deamination of the Alanine is converted to lactate by Lactate Dehydrogenase (LDH); while nicotinamide-adenine-dinucleotide reduced (NADH) is oxidized to nicotinamide-adenine-dinucleotide oxidized (NAD^+).

$$NADH + H^+ + \text{pyruvate} \overset{\text{LDH}}{\rightleftharpoons} \text{Lactate} + NAD^+$$

In the above reaction the rate of NADH oxidation is monitored by reflectance spectrophotometery and the rate of change in reflection density measured in a linear region is then converted to enzyme activity. The enzyme activity was given in units per liter (U/L). One U has been known to be the amount of enzyme which converts one micro mole of the substrate per minute (Tietz, 1987; Byrne, 1989).

3.1.3.2.2.2. Aspartate aminotransferase (AST/GOT) analysis

Analysis were carried out on Vitros 250 Chemistry system (Johnson - Johnson, Ortho-clinical Diagnostic.Inc.100 Indiago Creek Drive Rochester, NY 14626-5101, USA) and the AST activity was measured at 37 ^{0}C and wavelength of 670 nm, which is based on the optimized procedure IFCC, method modified to 37 ^{0}C (Bergmeyer *et al.*, 1985 Part 2).

The Vitros Chemistry for AST uses the Vitros slide which is a dry, multi-layered, analytical element, coated on a polyester support containing reactive ingredients like sodium aspartate; sodium α-ketoglutarate; sodium-pyridoxal-5-phosphate (P-5-P); sodium phosphate; 2-(3, 5-dimethoxyl-4-hydroxyphenyl)-4, 5-bis (4-dimethyl aminophenyl) imidazole (leuco dye);

pyruvate oxidase (aerococcus viridans, E.C.1.1.1.3); peroxidase (horse radish root, E.C.1.11.1.7) and oxalacetate decarboxylase (Pseudomonas sp.,E.C.1.10.3.3). Other ingredients include enzyme cofactors, pigments, binders, buffers, surfactants, stabilizers, dye solublizers, filter dyes, scavenger, and cross-linking agent.

In these tests an 11 µl of the diluted seminal plasma was deposited on the slide and was evenly distributed by the spreading layer to the underlying layers. This method is based on the principle that AST/GOT catalyzes the transfer of an amino group from L-aspartate to α-ketoglutarate in the presence of Pyridoxal-5-phosphate (P-5-P) forming glutamate and oxaloacetate:

$$\text{Aspartate} + \alpha - \text{ketoglutarate} \overset{\text{AST, P-5-P}}{\rightleftharpoons} \text{Glutamate} + \text{Oxaloacetate.}$$

The oxaloacetate generated in the deamination of the aspartate is converted to pyruvate and carbon dioxide by oxaloacetate decarboxylase. Pyruvate is then oxidized to acetyl phosphate and hydrogen peroxide by pyruvate oxidase.

$$\text{Oxaloacetate} \xrightarrow{\text{oxaloacetate decarboxylase}} \text{pyruvate} + CO_2$$

$$\text{Pyruvate} + \text{phosphate} + O_2 \xrightarrow{\text{pyruvate oxidase}} H_2O_2 + \text{acetyl phosphate}$$

$$H_2O_2 + \text{Leuco dye} \xrightarrow{\text{peroxidase}} \text{Dye}$$

The final reaction step involves the peroxidase catalyzed oxidation of a leuco dye to produce a colored dye. The rate of oxidation of leuco dye is monitored by reflectance spectrophotometery at 37 ^{0}C. The rate of change in reflectance density is measured over a linear region then converted to enzyme activity in units per liter (U/L). One U has been known to be the amount of enzyme that converts one micro mole of the substrate per minute (Tietz, 1987; Byrne, 1989).

3.1.3.2.2.3. Alkaline phosphatase analysis

Analysis was carried out on Vitros 250 Chemistry system (Johnson- Johnson, Ortho-clinical Diagnostic.Inc.100 Indiago Creek Drive Rochester, NY 14626-5101, USA) and the ALP activity was measured at 37 ^{0}C, and wavelength 400 nm, which is based on the optimized method of alkaline phosphatase (Tietz, 1987).

The Vitros chemistry system for ALP uses the Vitros ALP slide which is a dry, multi-layered analytical element coated on a polyester support containing reactive ingredients; p-nitrophenyl phosphate and other ingredients like p-nitrophenyl phosphate; 2-amino-2-metheyl-1-propanol (AMP) and magnesium sulphate and other ingredients like pigment, binders, buffers, surfactants, cross-linking agent and stabilizer.

In this assay an 11 µl of diluted seminal plasma was deposited on the slide and was evenly distributed by the spreading layer (containing the ALP substrate) to the underlying layers. The alkaline phosphatase present in the sample catalyzes the hydrolysis of the p-nitrophenyl phosphate to p-nitrophenol at alkaline PH, 37 ^{0}C and a light wave length of 400nm which takes approximately five minutes.

P-nitrophenyl phosphate $\xrightarrow{Mg^{+2}, AMP, ALP}$ P-nitrophenol + H_3PO_4

The p-nitrophenol that absorbs light at wavelength in the region of 400 nm, diffuse into the underlying layer, and it is monitored by the reflectance spectrophotometery. The rate of change in reflection density is then converted to enzyme activity measured in units per liter (U/L). One U has been known to be the amount of enzyme that converts one micro mole of the substrate per second (Tietz, 1987; Byrne, 1989).

3.1.3.2.2.4. Acid phosphatase (ACP) analysis

Analyses were carried out on SEAC ch 16 photometer (Italy) and the ACP activity was measured at 37 ^{0}C, using a light wavelength of 405 nm, which is based on the procedure of Hillmann calorimetric method (Hillmann, 1971). This method utilizes reactive ingredients obtained from Linear Chemicals S. P. Spain, Barcelona: where R1: 150 mmol/L citrate buffer at P^{H} 5.0; R2: 10 mmol/L α- naphtilphosphate and 6 mmol/L Fast Red TR. In this assay the working reagent was prepared by dissolving gently a tablet of R2 with one bottle of buffer reagent R1 after which 200 µl of the diluted (1:100) seminal plasma was mixed with 2.0 ml of working solution and then incubated for five minutes at 37 ^{0}C, at 405 nm, then initial absorbance was recorded then consecutively after one minute for three minutes from which the difference between absorbance per minute (change in absorbance/minute) was calculated and then finally multiplied by 750 and the dilution factor to get the total acid phosphatase in international units per liter (U/L).

This method is based on the principle that ACP catalysis the hydrolysis of α-naphtilphosphate liberating α-naphtol:

$$\alpha\text{-naphtilphosphate} \xrightleftharpoons{ACP} \alpha\text{-naphtol} + Pi$$

The liberated α-naphtol reacts with a chromogen forming a yellow azocompound:

$$\alpha\text{-naphtol} + \text{Fast Red TR} \rightleftharpoons \text{Azocompound}$$

The rate of formation of azocompound is proportional to the concentration of ACP present in the sample.

3.1.3.2.2.5. Total protein analysis

Analyses were carried out on SEAC ch 16 (Italy) and the total protein amount was measured at 37 ^{0}C, using a light path of 1cm and 540 nm wavelength, which is based on the BIURET calorimetric method (Peters, 1968). This method is based on the principle that proteins in the sample give an intense violet–blue complex with copper salts (II) in alkaline medium. The intensity of the color is proportional to the amount of total proteins present in the sample.

100 µl of the sample was mixed with 5.0 ml of the working reagent (prepared by mixing 15 mmol/L tartarate, 100 mmol/L sodium iodide, 15 mmol/L of potassium iodide and 5 mmol/L of copper sulphate, all from Linear Chemical S.P. Spain, Barcelona), and then incubated at 37 ^{0}C for 20 minute, 5 ml of the working reagent (as Blank) and a mixture of 100 µl of the standard and 5.0 ml of the working reagent (as standard) were also incubated in the same manner. After incubation of the test materials (Blank, standard, and mixture of the unknown) were kept at room temperature for 5 minutes after which the absorbance of unknown and the standard against the blank reagent at 540 nm was recorded. The total protein values were calculated by the formula:

$$\frac{\text{Absorbance of unknown}}{\text{Absorbance of standard}} \times \text{concentration of standard} = \text{gm/dL of total proteins}$$

3.1.3.2.2.6. Testosterone enzyme immuno assay

This assay was made using testosterone enzyme immuno assay kit (Linear Chemicals, S.L. 08390 Montgat Barcelona, Spain) that is based on the standard technique. The assay was made following the following procedure: the desired numbers of wells were secured in the holder after which 10 µl of the standard, specimens and controls was added into appropriate wells. Then 100 µl of testosterone-HRP conjugate reagent was added to each well. Lastly 50 µl of rabbit anti-testosterone reagent was dispensed to each well. The mixture was thoroughly mixed for 30 seconds and incubated at 37 ^{0}C for 90 minutes. After 90 minutes incubation the micro wells were rinsed and flicked with distilled or deionized water followed by the addition of 100 µl of TMB Reagent (coloring agent) into each well (which was mixed by gentle mixing for 10 seconds. Then it was kept (incubated) at room temperature (18-25 ^{0}C) for 20 minutes after which the 100 µl of stop solution was added to each well (to stop the reaction), gently mixed and visualized for the complete conversion of blue color to yellow color. Finally the absorbance (A) was measured at 450 nm with a microtiter well reader within 15 minutes, the mean absorbance (A 450) for each set of reference standards, controls and samples, calculated, standard curve constructed, and the corresponding concentration of testosterone in nanogram per milliliter (ng/ml) was determined.

This assay is based on the principle of competitive binding between testosterone in the test specimen and testosterone-HRP conjugate for the constant amount of rabbit anti-testosterone. In the incubation, goat anti-rabbit IgG-coated wells were incubated with 10 µl of testosterone standards, controls, sample, 100 µ1 testosterone-HRP conjugate reagents and 50 µ1 rabbit anti-testosterone reagent at 37 ^{0}C for 90 minutes. During this incubation, a fixed amount of HRP-labeled testosterone competes with the endogenous testosterone in the standard, sample, or quality control serum for a fixed number of binding sites for the specific testosterone anti-body. Thus the amount of testosterone peroxidase conjugate immunologically bound to the well progressively decreases as the concentration of testosterone in the specimen increases. Unbound testosterone peroxidase conjugate is then removed and the wells washed, and a solution of TMB reagent added and incubated at room temperature for 20 minutes, after which blue color is developed and further color development is stopped by adding 1N HCL and then absorbance is measured spectrophotometerically at 450 nm. In this assay the intensity of color formed is proportional to the amount of the enzyme (testosterone peroxidase) present and is inversely related to the amount of unlabeled testosterone. A standard curve is obtained by plotting the concentration of the standard versus the absorbance,

and finally concentration of testosterone in the sample and controls calculated from the standard curve.

3.1.4. Data analysis

For each parameter the mean value was calculated for weekly values in six bulls (pooled mean) which was then analyzed for its overall mean, median, minimum, maximum and standard error of semen characteristics by using the descriptive statistic, and bivariate correlation coefficient of those semen characteristics were analyzed using SPSS (2002) statistical package, and the 95 % confidence interval of semen characteristics were analyzed using STATA (2001) statistical package. Proportion calculation was used to see the proportion of different semen colors and to see the relation between the color of semen and its spermatozoa concentration. A single sample t-test was used to compare the mean values of the present semen parameters with the literature values. In case where there was missed value due to ejaculation of the bulls out of artificial vagina, the mean of other bulls whose ejaculate was successfully collected was used to find the mean parameters of the six bulls each week.

4. RESULTS

The study was conducted at the National Artificial Insemination Center (NAIC) Kaliti, Addis Ababa, Ethiopia from October to the end of December 2003 on six Ethiopian indigenous bulls.

4.1. Animal and weather data

The six indigenous bulls utilized for the study purpose were kept under the similar management conditions. The mean (SE) monthly body weight of the bulls during the study periods were 479 (10.7) kg, 550 (6.8) kg, 413 (3.2) kg, and 358 (3.8) kg for Barca, Boran, Horro and Sheko bulls respectively (NAIC). The information obtained from the same center showed that the average age of the Horro bulls during the study period was 4.0 years while the age of Barca, Boran and Sheko bulls during the study period was 9.0, 6.7, and 9.6 years respectively.

The weather data of the study period was obtained from near meteorological station (10 to 20 km) from National Meteorological Service Agency, Bole Station, Addis Ababa, Ethiopia (NMSA) and is given in Table 5.

Table 5. Mean meteorological data during the semen collection periods

Meteorological features	Months		
	October	November	December
Minimum temperature (^{0}C)	8.2	7.4	6.0
Maximum temperature (^{0}C)	23.7	23.6	23.3
Rainfall (mm)	0.2	0.0	1.1
RH at 0600LST (%)	58	56	66
RH at 1200 LST (%)	33	41	40
RH at 1800 LST (%)	36	38	42

Source NMSA

4.2. Results of semen quality analysis

The results of the semen analysis based on a once per week collection for 13 consecutive weeks for six Ethiopian indigenous bulls kept at Kaliti, and total 67 semen samples examined for physico-morphology, 62 samples for enzyme analysis and a random sample of 40 samples for testosterone are given as follows:

4.2.1. Results of semen physico-morphological analysis.

The over all mean (SE), median, minimum, maximum, and 95 % confidence interval value result of the semen physico-morphological analysis for the indigenous bulls is given in Table 6

Table 6. Summary statistic of semen physico-morphological characteristics of six indigenous bull.

Parameters	Mean (SE)[1]	95 % CI	Minimum	Maximum	Median
Volume (ml)	4.84 (0.20)	4.4-5.28	3.5	6.08	4.77
Mass motility (0-5)	3.15 (0.11)	2.92-3.33	2.45	3.7	3.17
Individual motility (%)	68.72(1.37)	65.73-71.71	57.5	75.00	68.9
Concentration (10^9/ml)	1.54 (0.07)	1.39-1.69	1.15	1.98	1.50
Total count $^+$	7.35 (0.47)	6.33-8.37	4.71	11.04	7.45
Viable number $^+$	5.1 (0.33)	4.38-5.82	3.25	7.4	5.17
TMN $^+$	7.12 (0.45)	6.12-8.12	4.53	10.47	7.11
Live percentage	79.73 (0.65)	78.31-81.15	75.93	85.31	79.46
MN (%)	94.70 (0.38)	93.86-95.54	92.42	97.47	94.81
Normal acrosome (%)	96.99 (0.60)	95.69-98.29	93.55	100.0	96.75

[1] Numbers in bracket indicate SE, CI = confidence interval, TMN = total morphologically normal number, MN = percent morphological normal, + = billion/ejaculate

Observations on semen color in Ethiopian indigenous bulls in this study (Table 7) showed that 77.61 % and 17.92 % of the semen collected during the study period had creamy and milky colors respectively, the remaining portion being yellow, brown and watery in equal proportion

(1.49 %). As it is given in Table 8, the creamy colored semen had spermatozoa concentration which ranged from 0.8 to 1.0 billion/ml to 2.3 to 2.5 billion/ml, the greatest part (36.5 %) being those which had spermatozoa concentration between 1.4 to 1.6 billion/ml. The largest proportion (41.7 %) of the milky semen had spermatozoa concentration which lies between 1.1 to 1.3 billion/ml. The yellow, brown and watery semen respectively had spermatozoa concentration of 0.8 to 1.0, 1.1 to 1.3 and less than 0.8 billion/ml.

Table 7. Proportions of different semen colors in six indigenous bulls

NO	Color	Unit	Frequency	%
1	Creamy	pcs	52	77.61
2	Milky	pcs	12	17.92
3	Yellow	pcs	1.0	1.49
4	Brown	pcs	1.0	1.49
5	Watery	pcs	1.0	1.49
Total		pcs	67	100.0

Table 8. Relation of semen colors and spermatozoa concentration in six indigenous bulls

NO	Colors	Spermatozoa concentration (10^9/ml)	Unit	Frequency	%
1	Creamy	0.8-1.0	Pcs	7	13.5
		1.1-1.3	Pcs	8	15.4
		1.4-1.6	Pcs	19	36.5
		1.7-1.9	Pcs	6	11.5
		2.0-2.2	Pcs	10	19.2
		2.3-2.5	Pcs	2	3.9
		Total	pcs	52	100.0
2	Milky	0.8-1.0	Pcs	3	25.0
		1.1-1.3	Pcs	5	41.7
		1.4-1.6	Pcs	2	16.7
		1.7-1.9	Pcs	2	16.7
		Total	pcs	12	100.1
3	Yellow	0.8-1.0	Pcs	1.0	100.0
4	Brown	1.1-1.3	Pcs	1.0	100.0
5	Watery	<0.8 (= 00.51)	pcs	1.0	100.0

4.2.2. Results of seminal plasma biochemical analysis in six indigenous bulls

The mean (SE), median, minimum, maximum, and 95 % confidence interval value in the seminal plasma GOT, GPT, ALP, ACP, total protein and testosterone level are given in Table 9.

Table 9. Summary statistic for seminal plasma biochemical analysis in six indigenous bulls

Parameters[2]	Mean (SE)[1]	95 % CI	Minimum	Maximum	Median
GOT	1530.91 (60.15)	1398.52-1633.30	1148.0	1824.0	1459.15
GPT	131.99 (9.36)	111.4-152.58	85.0	197.0	120.15
ALP	3333.98 (608.84)	1993.92-4674.03	1449.3	7964.3	2345.85
ACP	8003.68 (716.06)	6427.64-9579.73	4287.3	14120.0	7760.15
Total protein (gm/dL)	7.38 (0.26)	6.8-7.95	5.5	8.7	7.45
Testosterone (ng/ml)	2.84 (0.30)	2.18-3.51	1.3	4.6	2.6

[1] Numbers in bracket indicate SE, [2] all are in U/L unless specified, CI = confidence interval.

4.2.3. Results of different morphologic abnormalities of spermatozoa in six indigenous bulls

The mean (SE), median, minimum, maximum, 95 % confidence interval values of different morphologic abnormalities in different indigenous bulls of Ethiopian kept at Kaliti is given in Table 10. Some of the different spermatozoa morphologic abnormalities observed during study period were given in Figure 2.

Table 10. Summary statistic for different morphologic abnormalities of spermatozoa in six indigenous bulls

Type of abnormalities[3]	Mean (SE)[1]	95 % CI	Minimum	Maximum	Median
Head abnormality	1.87 (0.15)	1.54-2.20	0.89	3.05	1.87
Midpiece[2] abnormality	1.92 (0.22)	1.43-2.41	0.23	3.04	2.13
Tail abnormality	1.50 (0.18)	1.11-1.89	0.70	2.55	1.31
Total abnormality	5.29 (0.38)	4.45-6.13	2.53	7.58	5.15
Major abnormality	2.25 (0.19)	1.83-2.67	0.73	3.09	2.38
Minor abnormality	3.04 (0.28)	2.43-3.65	1.80	5.25	2.72

[1] Numbers in bracket indicate SE, [2] midpiece or body abnormality, [3] all are in percentage values, CI = confidence interval.

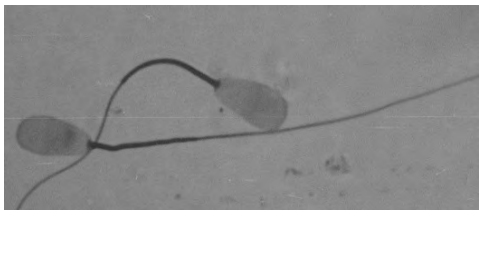
4.2.4. Results of different head abnormalities of spermatozoa in six indigenous bulls

In the study conducted to characterize the head abnormalities using the William stain, in the indigenous bull the mean (SE), median, minimum, maximum, and 95 % confidence interval values are given in Table 11.

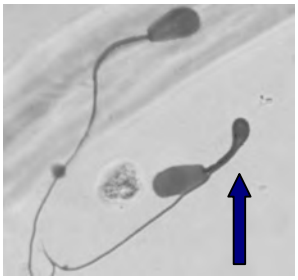
Table 11. Summary of different head abnormalities of spermatozoa in six indigenous bulls.

Head and neck abnormalities[2]	Mean (SE)[1]	95 % CI	Minimum	Maximum	Median
Acrosome defect	3.0 (0.60)	1.70-4.31	0.0	6.45	3.25
Narrow at base	0.37 (0.09)	0.18-0.56	0.0	1.11	0.28
Abnormal contour	0.12 (0.04)	0.03-0.20	0.0	0.45	0.07
Undeveloped form	0.74 (0.15)	0.42-1.06	0.0	1.85	0.50
Detached abnormal	0.21 (0.09)	0.00-0.42	0.0	1.25	0.12
Pear shaped	0.05 (0.02)	0.01-0.09	0.0	0.16	0.0
Small abnormal	0.05 (0.03)	-0.01-0.11	0.0	0.35	0.0
Abaxial implantation	0.57 (0.11)	0.34-0.80	0.04	1.16	0.53
Others	0.07 (0.03)	0.0-0.14	0.0	0.40	0.02

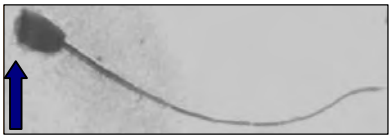
[1] Numbers in bracket indicate SE, [2] all are in percentage values, CI = confidence interval.



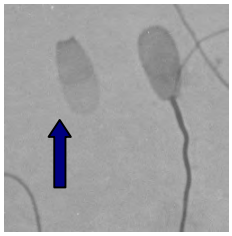
1. Normal spermatozoa



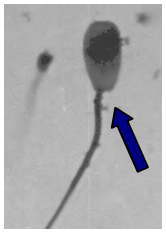
2. Bent Midpiece (arrow)



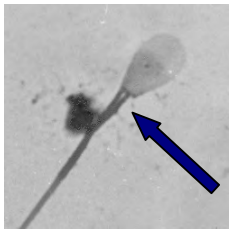
3. Abnormal acrosome (arrow)



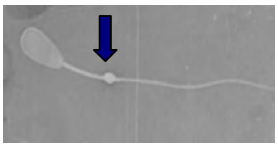
4. Detached head (arrow)



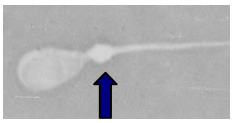
5. Abaxial implantation (arrow)



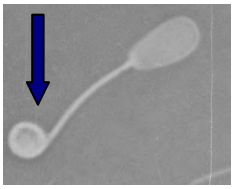
6. Double mid-piece (arrow)



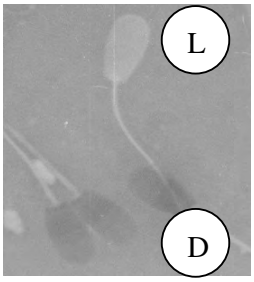
7. Distal droplet (arrow)



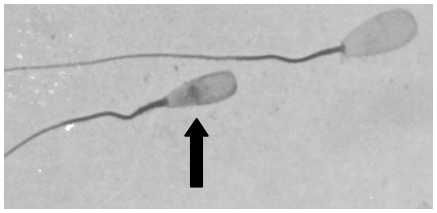
8. Proximal droplet (arrow)



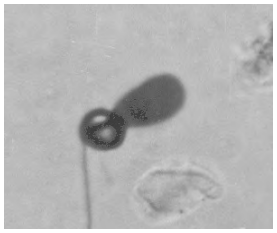
9. Terminally coiled tail (arrow)



10. Live (L), dead (D)



11. Elongated narrow head (arrow)



12. Dag defect

Figure 2. Some of the spermatozoa different morphologic abnormalities observed during the study period.

4.2.5. Results of spermatozoa dimensions in six indigenous bulls.

The mean (SE), median, minimum, maximum, and 95 % confidence interval values for spermatozoa dimensional measurement in the indigenous bulls are given in Table 12.

Table 12. Summary of spermatozoa dimensional measurements (µm) in six indigenous bulls.

Sperm	Mean[1]	95 % CI	Minimum	Maximum	Median
Head length	9.18 (0.03)	9.12-9.24	9.02	9.35	9.2
Head breadth	4.61 (0.01)	4.59-4.64	4.52	4.95	4.62
Midpiece length	13.61 (0.02)	13.56-13.64	13.47	13.77	13.61
Tail length	47.56 (0.10)	47.33-47.78	46.95	48.20	47.52

[1] Numbers in bracket indicate SE, CI = confidence interval.

4.2.6. Results of correlation analysis between seminal plasma biochemical parameters in six indigenous bulls.

On the basis of this study in indigenous bulls, the seminal plasma GOT level was significantly positively correlated with the level of GPT ($r = 0.70$, $P < 0.05$). The total protein level showed negative correlation with GPT and ACP but the correlation coefficients were small (Table 13).

Table 13. Pearson's coefficients of correlation between seminal plasma biochemical parameters in semen of six indigenous bulls.

	GOT	GPT	ALP	Total protein	ACP
GOT	1.0	0.70*	0.36	0.03	0.51
GPT		1.0	0.47	-0.09	0.52
ALP			1.0	0.09	-0.07
Total protein				1.0	-0.35
ACP					1.0

* $P < 0.05$, df = 10, n = 12.

4.2.7. Results of correlation analysis between seminal plasma biochemical parameters and different morphological abnormalities of spermatozoa in six indigenous bulls.

The correlation coefficient and the direction of linear relationship between GOT, GPT, ALP total protein, and different morphologic abnormalities in the indigenous bulls are tabulated (Table 14). There was significant inverse relation between ACP and midpiece (body) abnormality ($r = -0.70$, $P < 0.05$). Similarly there was significant inverse relation between major abnormalities and ACP ($P < 0.05$).

Table 14. Pearson's coefficients of correlation between the seminal plasma biochemical parameters and different sperm abnormalities in six indigenous bulls.

Biochemical parameters	Spermatozoa morphologic abnormalities[1]					
	Head abnormality	Midpiece[2] abnormality	Tail abnormality	Total abnormality	Major abnormality	Minor abnormality
GOT	0.19	-0.31	-0.15	-0.16	-0.27	-0.04
GPT	0.11	-0.51	0.09	-0.19	-0.43	0.02
ALP	0.06	-0.44	-0.57	-0.48	-0.43	-0.40
ACP	-0.26	-0.70*	-0.07	-0.51	-0.69*	-0.26

[1] * $P < 0.05$, n = 11, df = 9, [2] mid-piece or body abnormality.

4.2.8. Results of correlation analysis between spermatozoa motility and different morphological abnormalities of spermatozoa in six indigenous bulls.

As it is given in table 15, the spermatozoa individual motility showed negative correlation with different spermatozoa morphologic abnormalities (except with major spermatozoa abnormalities) except that the correlation coefficients are small, but the situation in case of correlation between the spermatozoa mass motility and different morphologic abnormalities is positive.

Table 15. Pearson's coefficients of correlation between the spermatozoa motility and different sperm abnormalities in six indigenous bulls.

Motility	Spermatozoa morphologic abnormalities[1]					
	Head abnormality	Midpiece[2] abnormality	Tail abnormality	Total abnormality	Major abnormality	Minor abnormality
MMT	0.10	0.26	0.37	0.36	0.60*	0.09
IMT	-0.32	-0.14	-0.02	-0.22	0.10	-0.37

[1] n =12, df = 10, MMT = mass motility, IMT = individual motility, [2] mid-piece or body .

4.2.9. Results of correlation analysis between the different semen physico-morphologic parameters in six indigenous bulls

The mass motility of spermatozoa of indigenous bulls showed significant positive correlation with live percentage ($r = 0.56$, $P < 0.05$) and strong positive significant correlation ($r = 0.82$, $P < 0.01$) with spermatozoa individual motility. Similarly the spermatozoa individual motility showed positive correlation with live percentage, percent morphologically normal, normal acrosome, viable and total morphological number of spermatozoa (Table 16).

Table 16. Pearson's coefficient of correlation between spermatozoa motility and different physico-morphologic parameters in six indigenous bull

Sperm motility	Semen physico-morphologic parameters[1]						
	Mass motility	Individual motility	Live percentage	MN%	Normal acrosome	Viable number	Tmn
MMT	1.0	0.82**	0.56*	-0.37	-0.29	0.46	0.18
IMT		1.0	0.45	0.21	0.08	0.52	0.15

[1] ** $P < 0.01$, * $P < 0.05$, $n = 13$, $df = 11$ except MN% and Tmn where $n = 12$, $df = 10$, Tmn = total morphologically normal number, MN% = percent morphologically normal, MMT = mass motility, IMT = individual motility.

5. DISCUSSION

In this study it was observed that 13.5 %, 15.4 %, 36.5 %, 11.5 %, 19.2 % and 3.9 % of the semen with creamy color had spermatozoa concentration of 0.8 to 1.0, 1.1 to 1.3, 1.4 to 1.6, 1.7 to 1.9, 2.0 to 2.2 and 2.3 to 2.5 billion/ml respectively. The observed milky colored semen also had spermatozoa concentration of 0.8 to 1.0, 1.1 to 1.3, 1.4 to 1.6, 1.7 to 1.9 billion/ml in 25 %, 41.7 %, 16.7 % and 16.7 % of the cases respectively. In case of creamy colored semen, this result agrees with former reports. It has been reported that the semen with spermatozoa concentration greater than or equal to 1.0 to 1.2 million/ml has been reported to have light creamy to creamy color (Roberts, 1971). Semen with spermatozoa concentration of 500,000 to 600,000 spermatozoa/ml has been reported to be milky, and that has spermatozoa concentration less than 300,000 spermatozoa/ml has been known to be watery (Roberts, 1971). The present observation of milky (with spermatozoa concentration ranging from 0.8 to 1.0 to 1.7 to 1.9 billion/ml) and watery semen (with spermatozoa concentration of 0.51 billion/ml) is higher than that reports of Roberts (1971). This variation might be associated to breed or due to subjectivity of the color determination technique.

In this study the Ethiopian indigenous bulls kept at Kaliti AI center had mean (SE) semen volume of 4.84 (0.20). This value is significantly lower ($P < 0.01$) than the semen volume reported in *Bos taurus* bulls (6.9 ml and 8.2 ml) in different years in Brazil and in *Bos indicus* in Brazil (Brito *et al*., 2002), the value for the latter being 6.6 ml and 6.7 ml in different years. The semen volume reported by Ahsan *et al.* (2003) in Sahiwal bulls (3.64 ml) is significantly lower than this value. The same author reported semen volume of 5.62 (0.14) in Friesian-Sahiwal cross which is significantly higher than the present value ($P < 0.01$). The semen volume reported by Shelke and Dhami (2001), that is, 4.84 ml lacks significant difference with the present value. Such variability between reports on semen volume might be attributed to difference in age, breed, nutritional status, geographic location, season of the year the study covers, method of the semen collection procedure and frequency (Caroll *et al*., 1963; Igboeli and Raka, 1971; Salisbury *et al*., 1978; Tegegne *et al*., 1992a; Hafez, 1993; Blezinger, 1999; Andrabie *et al*., 2000). However, the range of values given for semen volume in the literature (Bhosrekar, 1990; Setchell, 1991; Sorensen, 1979; Hafez, 1993; Bearden and Fuquay, 2000) agrees well with the present.

In this study the mean (SE) value for spermatozoa mass motility was 3.15 (0.11). Ahsan *et al*. (2003) reported the mass motility of spermatozoa in Friesian-Sahiwal cross and Sahiwal bulls

as 1.25 and 1.36 respectively which are significantly lower ($P < 0.01$) than the present value, and Veeraiah *et al*. (1998) reported the mass motility of spermatozoa as 2.89 in Ongole bulls which is significantly lower ($P < 0.05$) than the present observation. The mass motility of spermatozoa reported by Dhami *et al*. (1998) as 3.43 in Friesian bull and Adamou *et al*. (1996) as 3.85 in Borgou bull is significantly higher ($P < 0.01$) than the present value. On the other hand some researchers reported mass motility of spermatozoa as 2.96 (Shelke and Dhami, 2001) in Gir which does not have significant difference with the present value.

In this study it was observed that the mean (SE) individual motility of spermatozoa of indigenous bulls was 68.72 (1.37) %. The individual motility of spermatozoa reported by Ahsan *et al*. (2003) as 50.5 % and 60.55 % respectively in Friesian-Sahiwal cross and Sahiwal bulls, individual motility reported by Andrabi *et al.* (2002) as 55.0 % in Friesian-Sahiwal cross bulls are significantly lower ($P < 0.01$) than the present value. On the other hand, the individual motility of spermatozoa reported by Veeraiah *et al*. (1999) as 76.55 % in Ongole bulls, individual motility reported by Omar (1997) as 79.33 % in Zambian short horn zebu and individual motility reported by Adamou *et al.* (1996) as 75.7 % in Borgou bulls show strong significant difference with the present value ($P < 0.01$). The reports of Shelke and Dhami (2001) on individual motility of spermatozoa as 67.89 % in Gir and reports of Hector and Oscar (1998) in dual purpose Mexico bulls who reported individual motility as 68.6 % agree with the present value. It has been known than 40-75 % (Sorensen, 1979; Hafez, 1993) and 50-80 % (Bearden and Fuquay, 2000) of the semen of bulls has been found motile.

Present study found that the mean (SE) spermatozoa concentration of semen was 1.54 (0.07) billion/ml. This value is in line with spermatozoa concentration reported in *Bos indicus* in Brazil as 1.65 billion/ml (Brito *et al*., 2002), concentration reported by Veeraiah *et al*. (1999) in Ongole bulls and that reported by Rana and Dhami (2003) in Gir as 1.61 billion/ml. On the other hand the present value is significantly higher ($P < 0.01$) than spermatozoa concentration reported by Hector and Oscar (1998) in Mexican dual purpose bulls, Dhami *et al*. (1998) in Friesian bull, Omar (1997) in Zambian short horn zebu, and Adamou *et al*. (1996) in Borgou bull who reported spermatozoa concentration of 0.08, 0.95, 1.09, and 1.19 billion/ml respectively. Wide ranges have been known for normal fertile bull spermatozoa concentration as 800 to 2000 (Hafez, 1993), 1000 to 3000 (Bearden and Fuquay, 2000) million per milliliter of semen. The variability of spermatozoa concentration with different works report could be due to variation in genotype, nutrition, age, management, semen collection frequency and

technique (Caroll *et al*., 1963; Igboeli and Raka, 1971; Salisbury *et al*., 1978; Tegegne *et al*., 1992a; Hafez, 1993; Blezinger, 1999; Andrabi *et al*., 2002).

In this study the mean (SE) spermatozoa total count was found to be 7.35 (0.47) billion/ejaculate. This value agrees well with spermatozoa total count reported in *Bos taurus* in Brazil (Brito *et al.*, 2002) as 8.2 billion/ejaculate, but was significantly lower ($P < 0.01$) than the spermatozoa total count reported by same authors in *Bos indicus* as 11.4 billion/ejaculate. The latter variability might be attributed to various factors like age and management condition of the bull, season the study covers (caroll *et al*., 1963; Hafez, 1993; Blezinger, 1999). The present value agrees with the sperm total count set for the normal fertile bull (Bhosrekar, 1990; Setchell, 1991; Hafez, 1993; Bearden and Fuquay, 2000).

The spermatozoa mean (SE) live percentage was 79.73 (0.65) %. This value is in line with the spermatozoa live percentage reported by Shelke and Dhami (2001) in Gir as 80.13 %, but is significantly higher ($P < 0.01$) than live percentage reported by Rana and Dhami (2003) in Gir who reported the live percentage of spermatozoa as 71.85 %, and significantly higher than ($P < 0.01$) live percentage reported by Ahsan *et al.* (2003) in Sahiwal and Friesian-Sahiwal cross bulls who reported live percentage of spermatozoa as 72.22 % and 74.22 % respectively. The spermatozoa live percentage reported by Dhami *et al.* (1998) in Friesian bulls as 87.35 % and Veeraiah *et al.* (1999) in Ongole bulls as 82.17 % was significantly higher ($P < 0.01$) than the present value. However, the spermatozoa live percentage observed in these bulls agrees well with live percentage recommended for normal fertile bulls. A commonly accepted standard for percentage of live spermatozoa in bull semen collected by artificial vagina and used by AI was reported to be more or equal to 70 to 75 % (Sorensen, 1979; Lindasay *et al*., 1982).

The mean (SE) percent normal spermatozoa observed in this study were 94.70 (0.38). This value agrees well with the percentage morphological normal spermatozoa recommended for normal fertile bull. The proportion of ejaculated spermatozoa that contain normal spermatozoa of 80 % or more has been known not to be associated with lowered fertility (Faulkner and Pineda, 1980; Hafez, 1993).

The mean (SE) percent normal acrosome observed was 96.99 (0.60) %. This value is significantly higher ($P < 0.01$) than the value reported for normal acrosome by Veeraiah *et al*. (1999) in Ongole bulls and Rana and Dhami (2003) in Gir who have reported 92.33 % and 84.8 % respectively. The viable number of spermatozoa observed in these indigenous bull was

5.10 (0.33) billion/ejaculate. This agrees well with viable number of spermatozoa reported in *Bos taurus* as 4.9 billion/ejaculate but is significantly lower ($P < 0.01$) than the viable number of spermatozoa reported in *Bos indicus* as 6.7 billion/ejaculate in Brazil (Brito *et al*., 2002). Ejaculate characteristics of a bull in general has been known to be affected by a number of factors: age, genotype, nutrition, season the study covers, ejaculation frequency and method of semen collection, knowledge of the investigator (Caroll *et al*., 1963; Hafez, 1993; Blezinger, 1999).

The mean (SE) head abnormality, mid-piece (body) abnormality, tail abnormality, total abnormality, major abnormality and minor abnormality observed for these bulls were 1.87 (0.15) %, 1.92 (0.22) %, 1.50 (0.18) %, 5.29 (0.38) %, 2.25 (0.19) %, 3.04 (0.28 % respectively. Previous reports on spermatozoa abnormalities reported values which have strong significant difference with present values ($P < 0.01$) like total morphologic abnormalities of 15.54, 27.15, 15.41, 11.74, 9.26, 22.5 percents respectively in Gir (Shelke and Dhami, 2001), Friesian-Sahiwal cross (Ahsan *et al*., 2003), Ongole (Veeraiah *et al*., 1999), Friesian (Dhami *et al*., 1998) and Gir (Rana and Dhami, 2003). Such differences could be attributed to several factors which affect ejaculate characteristics. On the other hand, the present observed values were lower than the maximum recommended spermatozoa abnormality value for normal fertile bull. The spermatozoa of normal fertile bull has been recommended not to contain more than 20 % total abnormality, and individual head, mid-piece and tail abnormality of 10 % or more (Faulkner and Pineda, 1980; Hafez, 1993). In line with this the different head and neck abnormalities observed were appreciably low in Ethiopian indigenous bulls.

The mean (SE) seminal plasma levels of GOT, GPT, ALP, ACP, total protein and testosterone were 1530.91 (60.15) U/L, 131.99 (9.36) U/L, 3333.98 (608.84) U/L, 8003.68 (716.06) U/L, 7.38 (0.26) gm/dL, and 2.84 (0.30) ng/ml. This seminal plasma GOT level in these bulls is significantly higher ($P < 0.01$) than GOT level reported in crossbred bulls by Singhal *et al*. (1976) who reported seminal plasma GOT level as 545.1 U/L, but found lower than GOT level reported by Saxena and Tripathi (1978) in crossbred bulls who reported seminal plasma GOT level as 4825 U/L and GOT level reported by Pandit and Garg (1983) in crossbred bulls who reported the seminal plasma GOT level as 2068.8 U/L. The seminal plasma GPT level reported in seminal plasma of crossbred bulls by Singhal *et al.* (1976) and Saxena and Tripathi (1978) significantly differ ($P < 0.01$) from the present value who reported seminal plasma GPT levels as 322.2 U/L and 212 U/L respectively. The seminal plasma ALP and

ACP level reported by Aguirre *et al.* (1988) as 954.2 U/L and 1268.1 U/L respectively , ACP level reported by Reddi and Raja (1980) as 4065.8 U/L in buffalo bulls were significantly lower ($P < 0.01$) than the present value. The level of enzymes in seminal plasma varies based on the level of initial damage to the spermatozoa, or subsequent damages due to freezing or dilution (Roberts, 1971; Mann and Lutwak-Mann, 1981; Dhami and Kodagali, 1990). In this particular study the semen was not frozen or diluted prior to the sampling for enzyme analysis. The present value for seminal plasma total protein agrees well with the seminal plasma total protein reported by Hafez (1993), Faulkner and Pineda (1980) and Setchell (1991). Normally 3 to 8 gm/dL of the total protein has been known to be found in bovine seminal plasma (Setchell, 1991). The seminal plasma testosterone level observed in this study agrees well with bovine seminal plasma testosterone level reported by Gunjam and Amann (1976) as 2.87 ng/ml, but differs significantly ($P < 0.01$) from seminal plasma testosterone level reported by Tuli *et al*. (1991) and Javed *et al.* (2000) who reported the seminal plasma testosterone level as 1.41 and 0.97 ng/ml respectively. Testosterone level in serum of bulls has been found to vary with factors like age and level of sexual stimulation of bull (Salisbury *et al*., 1978; Faulkner and Pineda, 1980) but the cause of variability in seminal plasma testosterone level of bulls needs further study.

The spermatozoa have been known to have head and tail, the latter consisting of neck, mid-piece, principal piece and end piece (Roberts, 1971; Salisbury *et al*., 1978; Prasad and Sinha, 1985; Garner, 1991; Hafez, 1993; Bearden and Fuquay, 2000). The spermatozoa dimensional measurement results in this study agree well with reports of Bhosrekar (1990) and Sullivan (1978) on head length and tail length who reported the spermatozoa head length ranging from 8 to 10 μm and spermatozoa tail length ranging from 45 to 50 μm, but had strong significant difference in mid-piece length reported by Faulkner and Pineda (1980) and Ortavant *et al*. (1961) who reported the mid-piece length as 13 μm and 18.84 μm respectively. Whether spermatozoa dimensional measurements vary between breeds and the physiological implication of such variability needs further study.

The correlation analysis between the spermatozoa motility and different physico-morphologic parameters showed that as spermatozoa individual motility and live percentage significantly increases ($P < 0.01$ and 0.05 respectively), mass motility increases. Even if the coefficients were small, viable and total morphologic number of spermatozoa showed positive correlation with mass motility. The live percentage, percent morphologically normal, normal acrosome,

viable and total morphologically normal number of spermatozoa showed positive correlation with individual motility (though the correlation coefficients were small). This situation occurred as expected in that, according to the previous works it has been reported that live morphologically normal spermatozoa shows progressive motility (Bearden and Fuquay, 2000), and an increase in dead and abnormal cells decrease spermatozoa motility (Roberts, 1971). The negative correlation between the spermatozoa individual motility and different morphologic abnormalities observed in these bulls (though the coefficients of correlation were small) reflects such idea.

In the correlation analysis made to see the degree of linear relationship between the biochemical parameters, it was observed that as GOT increases, the GPT significantly increase ($P < 0.05$), ALP and ACP also non-significantly increase; as ACP increase, GPT also increase non-significantly. This positive correlation might be due to similarity of conditions that lead to the presence of these enzymes in seminal plasma and agrees with the reports of Dhami and Kodagali (1990) who reported positive significant correlation between such enzymes in buffalo bull. Total protein showed negative correlation with GPT and ACP which support the theory that proteins and lipoproteins prevent the loss of intracellular constituents of spermatozoa (Hafez, 1993). Lack of significant association might be due to the fact that the semen in these bulls was in physiological normal range, as it was seen from the physico-morphological parameters, making the enzyme level to lie within the normal range. It has been known that following a rise in abnormal or damaged spermatozoa in semen, levels of enzymes in semen increases (Mann and Lutwak-Mann, 1981; Bhosrekar, 1990; Dhami and Kodagali, 1990). In the present study the level of enzymes was negatively correlated with different morphologic abnormalities (in most cases) which disagree with the former idea. This situation might in part be associated with the very low level of different abnormalities observed in these bulls.

6. CONCLUSIONS AND RECOMMENDATIONS

This study evaluated the semen physico-morphological and biochemical characteristics of indigenous bulls kept at the Kaliti AI center. Based on the physico-morphological and biochemical parameters of fresh semen analyzed from these indigenous bulls, it was observed that most of the semen attributes lie within the normal level set for normal fertile bulls elsewhere in the world.

After completing preliminary studies it was found that the seminal attributes were encouraging with few abnormalities. However, due to constraints of low numbers of semen producing bulls, the present results need further validation in a larger bull population.

Currently the AI center is keeping these indigenous bulls mainly with the aim of genetic conservation, and to use their semen for such breeding activities as back-crossing (when the need arises). As the result the semen of these bulls is not used for extensive AI activities in the field which made getting retrospective data related to the non-return rate of the cows inseminated with the semen of these bulls difficult. Future studies should be planned which can address the field fertility of semen of these indigenous breeds in association with the laboratory results.

The national AI center should plan the way to develop these breeds side by side to the cross-breeding program with the exotic bulls for the genetic improvement of livestock in the country.

7. REFERENCES

Adamou, N., Diaye, M., Jondel, R., Adjovi, A. (1996): Borgou bull semen characteristics and potential use in artificial insemination. *Revenue d'Elevage et de Medicine Veternariredes pays Tropicaux*. **49** (2), 174- 177.

Aguirre, S. M., Capaul, E. G., Luca, L. D. Cinque, M., De-Luca-L, A. D. (1988): Total and none prostatic acid phosphatase and alkaline phosphatase activity in bull and Ram semen. *Veterinaria-Argentina*. **45** (5), 382-387.

Ahsan, U. H., Miraz, R. H., Zahid, I. A. (2003): Semen characteristics of crossbred (Friesian x Sahiwal) and Sahiwal young bulls maintained under subtropical conditions of Punjab. *Pakistan Veterinary Journal*. **23**(2), 100-102.

Andrabi, S. M. H., Naheed, S., Khan, L. A., Ullah, N. (2002): Semen characteristics of crossbred (Friesian x Sahiwal) bulls at Live-stock Research Station, National Agricultural Research Center. Islamabad: *Pakistan Veterinary Journal*. **22** (4), 181-187.

Arthur, G. H., Noakes, D. E. and Pearson, H. (1989): Veterinary Reproduction and Obstetrics. 6th ed. London: Bailliere Tindal, pp. 509-584.

Bane, A. (1982): The normal sperm morphology in domestic animals. Lecture note at postgraduate course, translated from Swedish by Settergren, I.

Banks, W. J. (1986): Applied Veterinary Histology. 2nd ed. USA: Williams and Wilkins, pp. 489-504.

Barber, J. and Wood, D. J. (1976): Live-stock Management for East Africa, London: Edward Arnold Pub. pp. 1-37.

Bearden, H. J. and Fuquay, J. W. (2000): Applied Animal Reproduction. 5th ed. Upper Saddle, New Jersey: Prentice Hall Inc., pp. 138-147.

Bergmeyer, H. U., Horder, M., Rej. R. (1985): Approved recommendation on IFCC method for the measurement of catalytic concentration of enzymes. Part 2. IFCC method for aspartate aminotransferase. *J. Clin. Chem. Clin. Biochem.* **24**, 297.

Bergmeyer, H. U., Horder, M., Rej. R. (1985): Approved recommendation on IFCC method for the measurement of catalytic concentration of enzymes. Part 3. IFCC method for alanine aminotransferase. *J. Clin. Chem. Clin. Biochem*. **24**, 481.

Bhosrekar, M. R. (1990): Semen Production and Artificial Insemination. 1st ed. India: BAIF Development Foundation. pp. 4-205.

Blezinger, S. (1999): Cattle Today: Many Factors Affect Bull Performance. http://www.cattletoday.com

Blom, E. (1972): The ultrastructure of some characteristic sperm defects and a proposal for a new classification of the bull spermiogram. Proc. VII. Simposio Internazionale di Zootechenia.Milana.

Brito, L. F. C., Silva, A. E. D. F., Rodrigues, L. H., Vieira, F. V., Deragon, L. A. G., Kastelic, J. P. (2002): Effect of environmental factors, age and genotype on sperm production and semen quality in *Bos indicus* and *Bos taurus* in Brazil. *Animal. Reproduction Science*. **70**, 181-190.

Byrne, E. A. (1989): Calculations in clinical Chemistry. In: Kaplan, L. A. and Pesce, A. J. (ed): Clinical Chemistry, Theory, Analysis, and Correlations. 2nd ed. St. Louis: C. V. Mosbay Company, pp. 29-39.

Caroll, E., Ball, L., Scott, J. (1963): Breeding soundness in bulls, *JAVMA*.**142**, 1105-1111.

Chacon, J., Pere, Z. E., Mueller, E., Soderquist, L., Rodríguez-Martinez, H. (1999): Breeding soundness examination of extensively managed bulls in Costa Rica. *Theriogenology*. **52**, 21-231.

Coles, E. H. (1986): Veterinary Clinical Pathology. 4th ed. Philadelphia: W. B. Sounders Company, pp. 261-266.

Coulter, G. H. and Foote, R. H. (1979): Bovine testicular measurements as indicator of reproductive performance and their relation to productive traits in cattle. *Theriogenology*. **11**, 297-311.

Coulter, G. H. (1986): Puberty and post puberal development of beef bulls. In: Morrow, D. (ed): Current Therapy in Theriogenology. 2nd ed. Philadelphia: W. Sounders Company. pp. 142-152.

Das, H. N., Verma, R. P., Ansari, M. R., Munjumdar, A. C. (1999): Assessing of the fertilizing potential in crossbreeding bulls by some current diagnostic approach invitro. *Journal of Animal Sciences*. **69** (1), 27-31.

Dhami, A. J. and Kodagali, S. B. (1990): Enzyme leakage and fertility of buffalo spermatozoa in relation to the quality of semen ejaculates and extenders. *Theriogenology*. **34** (5), 853-864.

Dhami, A. J., Greesh, M. Sahni, K. L. (1998): Seasonal influence on the quality and freezablity of semen of Friesian and Murrah buffalo bulls. *Indian Journal of Animal Reproduction*, **19**(1), 55-58.

EASE, Ethiopian Agricultural Sample Enumeration, (2003): Statistical Report on Farm Management Practice, Livestock and Farm Implements Part II, Results at the Country Level. Addis Ababa, Ethiopia, pp. 219-235.

Egbounlike, G. N., Tongun, V. A. and Agiang, E. A. (1985): Sperm production in ruminants in hot humid climate. *Wld. Rev. Anim. Prod.* **21**, 11-17.

Faulkner, L. C. and Pineda, M. H. (1980): Male reproduction. In: Donald, M. C. (ed.): Veterinary Endocrinology and Reproduction. 3rd ed. Philadelphia: Lea and Febiger, pp. 235-273.

Faulkner, L. C. and Pineda, M. H. (1980): Artificial insemination. In: Donald, M.C. (ed): Veterinary Endocrinology and Reproduction. 3rd ed. Philadelphia: Lea and Febiger, pp. 330-366.

Garner, D. L. (1991): Artificial Insemination. In: Cupps, P. T. (ed.): Reproduction in Domestic Animals. 4 th ed. San Diago, California: Academic Press Inc., pp. 251-274.

Garner, D. L. and Hafez, E. S. E. (1993): Spermatozoa and seminal plasma. In: Hafez, E. S. E. (ed): Reproduction in Farm Animals. 6th ed. Philadelphia: Lea and Febiger, pp. 144-164.

Gebrewold, A. (1984): Physiological reaction to biometeriological factors in Ethiopian zebu cattle and their cross with European dairy breeds in different environments in Ethiopia. Giessen University, W. Germany, PhD. thesis.

Gomes, W. R. (1977): Artificial Insemination. In: Cole, H. H. and. Cupps, P. T. (ed): Reproduction in Domestic Animals. 3rd ed. New York: Academic Press, pp. 257-284.

Graffer, T. Solbu, H. and Filseth, O. (1988): Semen production in artificial insemination bulls in Norway. *Theriogenology*. **30** (5), 1011-1021.

Griffin, J. E. and Wilson, J. D. (1992): Disorders of testes and the male reproduction. In: Wilson, J. D. and Foster, D. W. (ed): Williams Text Book of Endocrinology. 8th ed. Philadelphia: W. B. Sounders Company, pp. 799-852.

Gunjam, V. K. and Amann, R. P. (1976): Steroids in fluids and sperm entering and leaving the bovine epididymis, epididymal tissue, and accessory sex gland secretions. *Endocrinology*. **99** (6), 1618-1630**.**

Hafez, E. S. E. (1970): Reproduction and Breeding Techniques for Laboratory Animals. Philadelphia: Lea and Febiger, pp. 56-73.

Hafez, E. S. E. (1993): Reproduction in Farm Animals. 6 th ed. Philadelphia: Lea and Febiger, pp. 405-439.

Hancock, J. L. (1959): The morphologic characteristics of spermatozoa and fertility. *Internat. J. of fertil*. **4** (4), 347.

Hector, C. R., and Oscar, G. C. M. (1998): Reproductive aspects of crossbred bulls in a dual purpose cattle project in Mexico. In: Ostensson, K. and Vale, W. G. (ed): Animal Reproduction and Biotechnology for Latin American proceeding of a conference, pp. 33-38.

Heinonen, M. (1989): Artificial insemination in cattle. Monograph, Ministry of Agriculture, Addis Ababa, Ethiopia, pp. 21, 55-69.

Henricks, D. M. (1991): Biochemistry and physiology of the gonadal hormones. In: Cupps, P. T. (ed.): Reproduction in Domestic Animals, 4th ed. Sandiago, California: Academic press inc., pp.76-117.

Hillmann, G. Z. (1971): *Clin. Chem.Clin. Biochem*. **9**, 273.

Hudison, R. S. (1972): Symposium of reproduction in cattle and horse, Anim. vet. Soc. for study of breeding Soundness. American College of Theriogenology, Mimeo, Hastings.

Igboeli, G., and Raka, A. M. (1971): Seasonal changes in the ejaculate characteristics of Agoni (short horn zebu) bulls. *Journal of Animal Science*. **33** (3), 651-654.

ILCA. (1992): Future of livestock industries in East and South Africa. Proceeding of the Workshop Held at Kadoma Ranch Hotel, Zimbabwe.

Jackson, H. (1972): Chemical control of male contraception. In: Austin, C. R. and Short, R. V. (ed.): Reproduction in Mammals: Artificial Control of Reproduction. London: Cambridge University press, pp. 67-86.

Javed, M. T., Abrar, K., Mumtaz, A. (2000): Influence of season on seminal plasma testosterone and oestrogen in healthy and abnormal bulls and their relationship with other semen parameters. *Veternarski archive*. **70** (3), 141-149.

Johnson, L. (1991): Spermatogenesis. In: Cupps, P. T. (ed.): Reproduction in Domestic Animals. 4 th ed. San Diago, California: Academic Press, Inc., pp.174-206.

Kumi–Diaka, J., Nagaratnam, V., Ruwuaan, J. S. (1981): Seasonal and age related changes in semen quality and testicular morphology in tropical environment. *Vet. Rec*. **108**, 13-15.

Lagerlof, N. (1934): Morphologic studies on the changes in the sperm structure and in the testes of bulls with decreased or abolished fertility. (Trans. Title). *Acta. Path. microbiol. scand*. 19: (supp.). 254.

Lindasay, D. R., Entwistle, K. W., Winantea, L. (1982): Reproduction in Domestic Livestock in Indonesia. Australian Universities of International Development Program (AUIDP), Australia.

Mandal, D. K., Nagpaul, P. K., Gupta, A. K. (2002): Effects of Body surface cooling during hot dry and hot humid seasons on seminal attributes of Murrah buffalo bulls. *Indian Journal of Animal Sciences*. **72** (2), pp. 192-194.

Mann, J. and Lutwak-Mann, C. (1981): Male Reproductive Function and Semen, New York: Springerverlag, pp.19.

Martin, C. R. (1985): Endocrine physiology. New York: Oxford University press, Inc., pp. 571-612.

MOA, Ministry of Agriculture, (1996): Live-stock Breed development Subprogram (First draft).Addis Ababa, Ethiopia.

MOA, Ministry of Agriculture, (1997): National Livestock Development Program. Addis Ababa, Ethiopia.

Morrison, W. I., Murray, M. and Melntyre, W. I. M. (1981): Bovine trypanosomiasis, disease of cattle in tropics. In: Current Veterinary Medicine and Animal Science, pp. 469-497.

Morrow, A. (1986): Current Therapy in Theriogenology.2nd ed. USA: W. B. Sounders Company, pp.125-152.

Mukasa-Mugerwa, E. (1989): A Review of Performance of Indigenous Zebu (*Bos indicus*). ILCA Monograph 6, pp. 1-4.

Nauc, V. and Munjunath, P. (2000): Radioimmunoassays for bull seminal plasma proteins (BSP-A1/-A2, BSP-A3, and BSP-30-kilo-Daltons) and their quantification in seminal plasma and sperm. *Biology of Reproduction*. **63**, 1058-1066.

Omar, M. A. M. (1997): Source and breed variations in semen quality of South African bulls. *Egyptian Journal of Physiological sciences*. **21** (1). 13-19.

Ortavant, R., Courot, M., Hochereau, M. T. (1969): Spermatogenesis and morphology of spermatozoon. In: Cole, H. H. and Cupps, P. T. (ed): New York and London: Academic Press.

Ott, R. S. (1986): Breeding soundness examination of bulls. In: Morrow, A. (ed.): Current Therapy in Theriogenology. 2ed. Philadelphia: W. B. Sounders Company, pp. 125-135.

Pagot, J. (1992): Genetic improvement of tropical breeds. In: Animal Production in the Tropics. 1st ed. London and Basington: Macmillan Press LTD., pp. 293-418.

Pandit, R. K. and Garg, U. K. (1983): Freezablity, GOT release and fertility of crossbred bull semen. *Indian Journal of Animal Production*. **4** (1), 36-38.

Peters, T. J. (1968): *Clin. Chem.* **14**, 1147.

Peters, A. R. and Ball, P. J. (1995): Reproduction in cattle. 2nd ed. USA: Blackwell Science. Ltd., pp. 62-182.

Prasad, A. and Sinha, A. K. (1985): Essential of endocrinology and reproductive physiology. New Delhi: Allied publishers, pp. 121-147.

Rana, C. M., and Dhami, A. J. (2003): Sephadex filtration-measure improving the quality of Gir (*Bos indicus*) and Jafarabadi (*Bubalus bubalis*) bulls. *Indian Journal of Animal Sciences*.**73** (6), 607-611.

Rao, C. K. (1957): Effect of technique of staining on sperm morphology. The *British veterinary Journal*. **45**, 1376.

Rao, A. and Bane, A. (1985): Pathological condition of the genital organs of the normal fertile bulls. *Indian Veterinary Journal*. **62**, 242-256.

Reddi, N. M. and Raja, C. K. S. V. (1980): Seasonal variations in acid and alkaline phosphatase contents in surti buffalo bull semen. *Kerala Journal of Veterinary Science*. **11** (1), 48-50.

Roberts, S. J. (1971): Veterinary Obstetrics and Genital Disease, 2 ed ed. Indian: CBS publishers and Distributors, pp. 612-750.

Salisbury, G. W., Van Demark, N. L., Lodge, J. R. (1978): Physiology of Reproduction and Artificial Insemination of Cattle. 2ed. ed. San Francisco: W. H. Free man, pp. 385-479.

Sane, C. R., Luktuke, S. N. and Deshpande, B. R. (1982): A Text Book of Reproduction in Farm Animals. Bombay: Vargheese Publishing House, pp. 425-488, 494-504.

Saxena, V. B. and Tripathi, S. S. (1978): Studies on the physico-morphological attributes and preservability of semen of crossbred bulls. *Indian Journal of Animal Science*. **48** (12), 865-869.

Setchell, B. P. (1991): Male reproductive organs and semen. In: Cupps, P. T. (ed): Reproduction in Domestic Animals. 4th ed. San Diago: Academic Press, pp. 221-249.

Shelke, V. B. and Dhami, A. J. (2001): Comparative evaluation of physico-morphological attributes and freezability of semen of Gir cattle (*Bos indicus)* and Jafarabadi buffalo (*Bubalus bubalis*) bulls. *Indian Journal of Animal Sciences.* **71** (4).

Singhal, J. P., Kaker, M. L., Reirian, M. N. (1976): A note on enzymatic activity of semen transaminases in crossbred bulls. *H. A. U. J. Res.* **6** (2), 164-166.

Sorensen, Jr. A. M. (1979): Animal Reproduction Principles and Practices. New York: McGraw-Hill Publishing Company, pp. 85-233.

SPSS (2002): SPSS 11.5 for windows, standard version, SPSS. INC. http://www.spss.com.

Stabenfeldt, G. H. and Edqvist, L-E. (1993): Male reproduction process. In: Swenson, M. J. and Reece, W. O. (ed): Dukes' Physiology of Domestic Animals. 11th ed. Ithaca and New York: Cornell University press, pp. 771-786.

Stalhammar, E. M. (1995): Prospects of selecting Artificial Insemination bulls for fertility. Uppsala: *Swedish University of Agricultural Science*. No.116.

Stata (2001): Intercooled stata 7.0 for windows. 702 University Drive East: Stata Corporation. stata@stata.com

Sullivan, J. J. (1978): Morphology and motility of spermatozoa. In: Salisbury, G. W., Van Demark, N. L., Lodge, J. R. (ed): Physiology of Reproduction and Artificial Insemination of Cattle. 2ed ed. San Francisco: W. H. Free man, pp. 286-328.

Tegegne, A. (1989): Reproductive development and function in zebu and crossbreed cattle in Ethiopia. James Cook University of the North Queensland, Australia. PhD. thesis.

Tegegne, A., Entwist, K. W. and Mukasa-Mugerwa, E. (1992a): Nutritional influence on growth and onset of puberty in Boran and Boran-Friesian crosses bulls. *Theriogenology*. **35**, 1005-1016.

Tegegne, A., Kassa, T. Mukasa-Mugerwa, E. (1995): Aspect of bull production with emphasis on cattle in Ethiopia. In: Proceedings of Third National Conference of Ethiopian Society of Animal Production, ESAP. Proceedings, Addis Ababa, Ethiopia, pp. 43-55.

Therien, I., Moreau, R., Manjunath, P. (1998): Major proteins of bovine seminal plasma and high density lipoproteins induce cholesterol efflux from epididymal sperm. *Biology of Reproduction* **59,** 768-776**.**

Tietz, N. W. (1987): Fundamentals of Clinical Chemistry. 3rd ed. Philadelphia: W. B. Sounders Company, pp. 385-386.

Trail, J. C. M. and Gregory, K. E. (1984): Animal breeding in Sub-Saharan Africa: toward an integrated program for improving productivity. In: Simpson, J. R. and Evangelou, P. (ed): Livestock Development in Sub Saharan Africa constraints, prospects, policy: Boulder, Colorado: Westview Press Inc., pp. 107-122.

Tuli, R. K., Lohan, J. S., Singhal, S. P. (1991): Testosterone and progesterone hormone in buffalo bull seminal plasma and their correlation with seminal characteristics. *Indian Journal of Dairy Science*. **44** (9), 587-589.

Uwland, J. (1984): Possibilities and limitations of semen evaluation for the prognosis of male fertility. In: Courot, M. (ed.): The Male in the Farm Animal Reproduction, pp. 269-289. Boston: Martinus Nijoff.

Vale-Filho, V. R., Pinheiro, L. E. L. and Basrur, P. K. (1986): Reproduction in zebu cattle. In: Morrow, A. (ed): Current Therapy in Theriogenology. 2ed ed. Philadelphia: W. B. Sounders Company, pp. 437-442.

Veeraiah, G., Naidu, K. V., Rao, K. B. (1999): Studies on semen characteristics of Ongole bulls. *Indian Veterinary Journal*. **76** (6), 585-560.

Wagner, D. G. (1968): Dairy cattle management in Ethiopia. *HSIU College of Agriculture Experiment Station Bulletin* (Ethiopia). **55**, 299.

White, I. G. (1980): Secretion of male reproductive tract and seminal plasma. In: Hafez, E. S. E. (ed): Reproduction in Farm Animals. 4th ed. Philadelphia: Lea and Febiger, pp. 189-202.

Wiener, G. (1994): Animal Breeding, London and Basingstoke: Macmillan Education Limited, pp. 1-20.

Williams, W. W. (1920): *Cornell vet.*10, 87-94.

www.selectsire.

8. ANNEXES

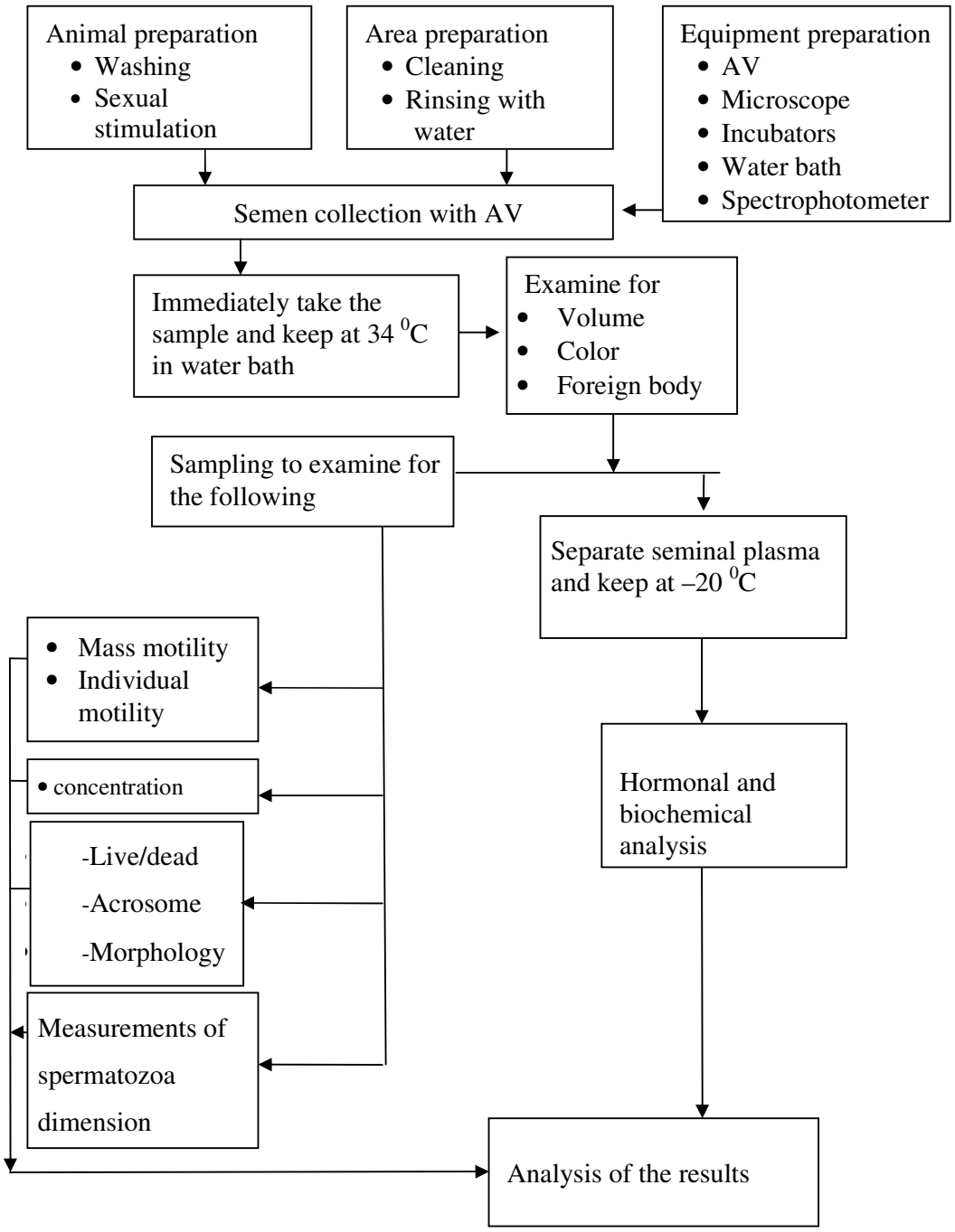
Annex 1: Procedure for the preparation of the artificial vagina (Salisbury *et al*., 1978)

1. Insert the inner liner into the cylinder
2. Turn the ends of the liner back over the cylinder
3. Fit the collection receptacle into the ends of the rubber funnel
4. Place the funnel over one end of the cylinder and the liner
5. Secure the connections between the receptacle and the funnel and between the ends of the liner and the cylinder with string or elastic bands or heavy cords.
6. Once the artificial vagina is assembled in the above manner, fill it with water at the proper temperature and pressure (Note the temperature of the water will vary with the air temperature, with delay between filling and collection, and with individual bulls. At the time of collection the temperature of AV between 42 ^{0}C and 44 ^{0}C is usually effective). If the external temperature is cold or if there is a chance of delay between filling of warm water and collection of the semen a temperature of 55 ^{0}C or higher is recommended.
7. Following addition of warm water to the water jacket at the proper temperature and pressure, the first 3 to 5 inches of the interior of the liner should be thinly coated with sterile lubricating jelly that is harmless to the spermatozoa.

Annex 2: Williams stain, staining technique

1. Make fresh semen smear (thin) on clean glass and allow it to dry,
2. Fix the dried smear on the flame,
3. Flood the fixed smear with absolute alcohol for 3-4 minutes,
4. Flood the smear with chloramine solution, for 1-2 minutes,
5. Wash the smear with distilled water and flood it (finally) with carbol fuchsin for 8 to 10 minute,
6. Wash with tape water, air dry, and examine under oil immersion objectives of the bright field microscope.

Annex 3: Schematic diagram showing procedures followed during the semen analysis



9. CURRICULUM VITAE

I. Personal Identification

Name	Hunderra Sori Ayana
Birth Place	Eastern Wellega Zone, Limu Woreda, Lomicha Kebele, Gasis.
Birth date	14/03/1975
Sex	male
Marital status	single
Religion	Christian
Denomination	Protestant
Nationality	Ethiopian
Profession	Veterinarian
Occupation	Field veterinary service

II. Contact Address

Western Shoa Zone, P.O.B. 212, Sebeta Post Office, email: sori@freemail.et

III. Education

Elementary -Limu elementary and Junior Secondary School, East Wellega Zone, Limu woreda, Galila town from 1979/80 to 1986/87 where I learned from beginning to 8th grade.

High school -Gida Senior Secondary School, Eastern Wellega zone, Gida woreda, Gida Ayana town where I continued my education from 9th grade and took the Ethiopian School Leaving Certificate Examination, from 1987/88 to 1990/91.

University - In 1991/92 I took my common course in Faculty of Science Addis Ababa University, Addis Ababa, Ethiopia. There after, from 1992/93 to the 1997/98 I took my study in Faculty of Veterinary Medicine, Debre Zeit, Ethiopia.

IV. Work Experience

Since1999 until I joined the MSc program in Faculty of Veterinary Medicine in 2002/2003, I was serving as field veterinarian in Oromia Regional State, West Shewa Zone, first for one year in Challiya woreda, there after in Ilu woreda in the same zone.

V. Language Skill

Oromo language ……. Read, write and speak

Amhara language…….. Read, write and speak

English language ……...Read, write and speak

VI. Technical papers

Sori, H. (1997): Bovine Clinical and Sub clinical Mastitis in Indigenous Zebu and Cross-bred Cattle in and around Sebeta. DVM. thesis, Addis Ababa University, Faculty of veterinary medicine, Debre Zeit.

Sori, H. (2003): Physiology of Estrus Cycle and Optimum Time of Insemination in a cow. A review. Faculty of Veterinary Medicine, Debre Zeit, 33pp.

VII. REFERENCES

Addis Ababa University, Faculty of Science, Registrar office.

Addis Ababa University, Faculty of Veterinary Medicine, Debrezeit, Registrar office.

Oromia Regional State, Agricultural office, Addis Ababa.

Sori Ayana: East Wellega zone, Limu woreda, Lomicha kebele.