TESTICULAR GROWTH AND SEMEN QUALITIES OF WEST AFRICAN BULLS

BY

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A

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DEDICATION

This thesis is dedicated to the fond memory
of a brilliant cousin, electronics engineer

DR. TAI ADEBANJO

(1944-1978)

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ABSTRACT

INTRODUCTION

There is an urgent need to increase the productivity of domestic livestock in the West African sub-region, particularly in Nigeria, in order to be able to meet the increasing demands of a rapidly growing population for dietary animal protein. While considerable progress in animal production has been made in several parts of the world, most of the livestock in West Africa continue to be husbanded by nomadic pastoralists whose time-worn methods are good but outdated and becoming increasingly unable to meet present day demands.

Livestock productivity can be improved to some extent through the provision of adequate nutrition, veterinary care and the adoption of good husbandry practices. However, it is the inherent quality or genotype of an animal which is the ultimate determinant of its performance in a given environment. It is therefore essential for optimum breeding schemes to be organized, preferably on a regional scale, for sustained genetic improvement of West African cattle and other domestic livestock.

Artificial insemination remains the most widely applied technique for the rapid proliferation of 'superior' genes within cattle populations throughout the world. A serious constraint on the large-scale adoption of this method in West Africa has been the lack of experimental data on the reproductive physiology of the local cattle breeds.

The aims of the present study were to investigate spermatogenesis and semen quality in indigenous West African bulls. Basic information obtained from this study is expected to be useful in the planning of progeny testing and artificial insemination schemes for West African cattle breeds.

The thesis is presented in six parts. Parts I and VI consist of the Literature Review and the General Discussion respectively. The experimental work is in four parts. The work reported in Part II deals with Testicular Size in West African Bulls. The third part is on Growth, Testicular Development and Spermatogenesis in Bunaji Bulls. Work on Semen Quality of Sokoto-Gudali and Bunaji Bulls is presented in Part IV while Part V reports on the *In Vitro* Storage of

Indigenous Bull Semen. The review of the literature in Part I follows in general the same order in which the experimental work is presented.

PART I

REVIEW OF THE LITERATURE

1.1. DEVELOPMENT OF THE TESTIS

Prenatal

In mammals and birds, two types of cells are distinguishable in the sex cords from early embryonic life. These are the large cells and the small cells, labeled by many authors as primordial and indifferent cells. There was controversy as to whether one or two cell-types are present in the fetal testis and it was also disputed whether the large cells are primordial cells that give rise to the definitive germ cells (Santamarina and Reece, 1957).

In the goat, Yao and Eaton (1954) found that the large primordial germ cells, which seemed to the authors to be remnants of the embryonic large cells, had disappeared by 60 days of age, when the first primary spermatocytes were seen. The latter were produced by the division and growth of the epithelial germ cells after birth. Similarly, Santamarina and Reece (1957) found that the sex cords of a cattle fetus of 3 months already

had the large and small cells which behaved as different entities throughout fetal life till about the second month after birth. Subsequently the large or primordial germ cells decreased gradually in number from the first month of postnatal life, disappearing almost completely by 70 days of age. The small cells lined along the basement membrane of the tubules gave rise to the germinal epithelium.

A more detailed account of prenatal development of the testis was recently given by Asdown and Hancock (1974) citing Gier and Marion (1970). Differentiation of the gonads of the male embryo follows the arrival of the primordial germ cells which migrate to the gonadal ridge in the bull fetus about day 26. The primordial germ cells are carried into the medulla by the primary sex cords which are formed from the coelomic epithelium and provide the element from which the germinal epithelium is formed.

Postnatal

The testes enter the scrotum half-way through fetal life (Ashdown and Hancock, 1974). The seminiferous tubules of the calf testes are filled with intratubular matrix from early fetal life to about 4 months of age (Santamarina and Reece, 1957). In Swedish Red-and-White bulls, the tubules of the testes in new-born animals are small, solid, and widely separated (Abdel-Raouf, 1960). From about 5 to 5½ months, the tubules enlarge and the matrix dissolves to form the lumens (Santamarina and Reece 1957).

From birth till maturity the bull testis undergoes a twenty-fold increase in weight, while the diameter of the seminiferous tubules increase approximately five-fold. In the study by Santamarina and Reece (1957), a 90-day Holstein-Friesian fetus had both testes weighing 0.5 g, while the seminiferous tubules had an average diameter of 34.3μ; in a 180-day fetus the values were 1.5 g and 43.5μ respectively. Subsequent values were 8.0 g and 42.5μ in a 1-day old calf and 19.0 g and 50.1μ in a 45-day old calf of the same breed. A group of 5 to 10-year old zebu bulls had either testis weighing between 139-209.7 g,

with seminiferous tubules ranging in diameter from 167-224μ (Kumi-Diaka, 1976).

In the developing bull testis, primary spermatocytes are observable in the seminiferous tubules by 24 weeks, spermatozoa in the tubules by 32 weeks and in the cauda epididymis by 40 weeks, appearing in the ejaculate by 42 weeks (Ashdown and Hancock, 1974). Qualitative development of the testes in Swedish Red-and-White bulls was complete by the 40th week after birth (Abdel-Raouf, 1960).

1.2. PATTERN OF GROWTH

Postnatal growth of the testis is curvilinear in cattle, sheep and goats. An initial period of rapid growth is superseded after puberty by a period of minimal growth. At sexual maturity, growth has virtually ceased while testicular degeneration may occur in very old animals.

In Merino rams, testicular weight is closely related to body weight but varies widely within any particular age group (Watson, Sapsford and McCance, 1957). General histological characteristics of the testes closely parallel their weight. The cross-sectional area of the seminiferous tubules increase directly with the relative increase in testicular weight until the latter attains minimum weight of 300 g. Likewise, the weight of the epididymides is more closely related to testicular weight in Hampshire and Southdown rams. However, the authors found that the diameter of seminiferous tubules increased in a curvilinear manner when compared with age, body weight or testicular weight. Breed differences were also found in the rate of increase in cellular types in

relation to age, bodyweight and testicular weight. In goats, the testis, epididymis, vesicular gland and penis exhibit identical patterns of growth, while the growth of the seminiferous tubules is closely correlated with testicular weight (Yao and Eaton, 1954).

Testicular weight and volume of Holstein bulls increased in a curvilinear manner from birth to 9 months of age (Macmillan and Hafs, 1969). By contrast, testicular length and diameter increased linearly over the same period.

Scrotal circumference is highly correlated to testicular weight and volume in bulls (Willett and Ohms, 1955; Hahn, Foote and Seidel, 1969) and has therefore been used to follow testicular growth in live animals. The relationship between scrotal circumference and age in bulls is curvilinear, with scrotal growth virtually complete by 60 months of age (Hahn, Foote and Seidel, 1969; Coulter, Larson and Foote, 1975). In one study by Almquist and co-workers (1976), a 32 per cent increase in scrotal

circumference of Charolais bulls was found from puberty (about 41 weeks) to 2 years of age. Three-fourths of this increase occurred between puberty and 65 weeks of age.

Many investigators have reported high correlations between scrotal circumference, age and liveweight (Hahn, Foote and Seidel, 1969; Fagerlin, Carroll and Brinks, 1971; Ball et al., 1974; Coulter, Larson and Foote, 1975). Recently however, Morstin and Jasiorowski (1976) reported a lack of significant relationship between testicular weight or size, and liveweight, age or ancestry of beef bulls fattened for slaughter either at a progeny test station or commercial feedlot. The result obtained by the latter investigators is probably due to the narrow range in the ages (12-16 months) and weights (nearly all bulls were close to 450 kg) of the animals used in the study. Considerable within-age-group variation has been found by other workers (Hahn, Foote and Seidel, 1969; Coulter, Larson and Foote, 1975).

Testicular size in bulls is more highly correlated to body weight than to age (Fagerlin, Carroll and Brinks, 1971). However, there is a closer relationship between

testis size and age in young bulls than in aged ones. The linear correlations between age and testis size declined from 0.85 before 6 years of age to 0.12 after that age in one study (Hahn, Foote ane Seidel, 1969). Decrease in testis size after bulls are 8 to 9 years old has also been reported (Iljinskaja, 1966).

1.3 INFLUENING FACTORS

Several factors combine to influence the development of the testis. These can be grouped broadly into four, namely nutritional, endocrine, genetic and pathological factors.

Nutrition

The influence of nutrition on the development of the testis in bulls fed on low or high planes of nutrition is more apparent before puberty after which no difference exists (Bane, 1954; Davies et al 1957; Abdel-Raouf, 1960) . In maturing bull-calves underfeeding has a marked delaying effect on the appearance of spermatozoa (Davies, Mann and Rowson, 1958). This effect appears to be due to inadequate stimulation of the gonads by the gonadotropic hormones, since injection of the latter elicits a prompt appearance of fructose and citric acid in the semen. Alterations in semen composition caused by underfeeding paralleled histological changes in the testis and accessory

male reproductive organs.

Mudra (1974) examined the effect of rearing on semen production of young bulls. The author found a highly significant correlation (r=0.60) between age at first ejaculation and nutrient consumption to 9 months of age. Underfeeding delays semen production and replenishment in Holstein bulls (Van-Demark *et al.*1964).

Endocrine

In males generally it is thought that the formation of spermatogonia from the germinal epithelium in the seminiferous tubules is stimulated by testosterone (Niswender, Nett and Akbar, 1974). Hooker (1944) reported that the androgen content of bull-calf testis was low, increasing gradually until 28 months at which time the Leydig cells began to show vacuolation. Androgen levels then showed an abrupt and very large increase. After five years, a steady decline was reported by the author.

The release of LH in Holstein bulls was found by Rawlings, Hafs and Swanson (1972) to be related to the

onset of testosterone secretion and to the onset of spermatogenesis. However, the authors postulated the mediation of factors other than LH, since variations in the latter only partially accounted for the variance in gonadal sperm numbers or testosterone levels.

Genetics

Limited evidence available indicates there are differences between breeds with regard to the pattern of testicular growth. In a comparative study involving two breeds, Coulter, Larson and Foote (1975) found that Angus bulls tended to have larger testes than Holsteins up to 3 years of age but were surpassed after that age by Holstein bulls.

It is a widely held view that certain congenital defects of the bull testis, such as cryptorchidism, testicular hypoplasia and uneven testes size may be hereditary (Ehlers, 1968; Smith, Jones and Laing, 1970; Faulkner and Carroll, 1974; Humphrey and Ladds, 1975).

High heritability estimates have been reported for testicular size in bulls (Coulter, Rounsaville and Foote, 1974). This suggests that testicular size, and hence spermatozoa production, may be improved in bulls by pedigree selection.

Pathology

Testicular growth, development, and function may be affected by various pathological factors which may be congenital, or acquired during postnatal life.

Congenital defects can be found in the testes as in all other parts of the male reproductive tract. The incidence of such malformations is however low, and their impairment of reproduction has not been established other than for a limited number of conditions (Ehlers, 1968; Smith, Jones and Laing, 1970; Faulkner and Carroll, 1974; Humphrey and Ladds, 1975). The more widely recognized of these defects are cryptorchidism, monorchidism, polyorchidism, testicular hypoplasia, uneven testes size and segmental aplasia of the mesonephric duct. Others are

ectopia of the testis and anorchidism. Two congenital cystic conditions of the bovine epididymis, paradidymis and aberrant ductules, were studied in detail by Blom and Christensen (1956, 1958 and 1960).

Pathological conditions acquired during postnatal life are more frequent than congenital defects in the reproductive tract of the bull. These include testicular degeneration, orchitis, fibrosis, calcification and testicular tumors. Frequencies of gross lesions in the testes of mature bulls from Fulani herds were reported to be 2.8%, 2.1% and 1.4% for degeneration, orchitis and hypoplasia respectively (Kumi-Diaka, 1976).

2. SEMEN QUALITY

2.1 Semen Composition

2.2 Evaluation of Semen

2.3 Factors Affecting Semen Quality

2.1 SEMEN COMPOSITION

Bull semen consists mainly of spermatozoa produced in the testes, and seminal plasma, the suspending medium produced by the accessory sex glands. The spermatozoa and the seminal plasma become mixed only at the time of ejaculation (Melrose, 1962).

The biophysical and biochemical characteristics of bovine semen have been reviewed by a number of workers (Melrose, 1962; Melrose and Laing, 1970; Mann, 1969; White, 1968, 1974).

The specific gravity of bull spermatozoa varies from 1.03 to 1.113, which values depend to some extent on the method of determination (Lavon *et al.*, 1966; Drevius and Eriksson, 1966 Drevius, 1972b). The specific gravity of seminal plasma from Israeli-Friesian bulls varies from 1.0281 to 1.0421 (Lavon *et al.*, 1966). The volume of a bull spermatozoon under strictly isotonic conditions lies between 32.0 and 33.3 μm^3, while the mean area of a non-swollen epididymal bull spermatozoon is about 144-5 μm^2 (Drevius, 1972a). The head, middle

piece and main tail piece of a bull spermatozoon are 9.15, 14.84 and 45 to 50μ long respectively, while the corresponding widths are 4.25, 0.67 and 0.51μ (Bonadonna *et al.*, 1953).

The chemical compositions of whole semen, spermatozoa and seminal plasma, as determined by different workers using various analytical techniques, and involving many breeds, have been summarized and listed in the review by Mann (1969). Most of the components, including pH and spermatozoa concentration, were subject to considerable variation.

Freshly ejaculated bovine semen has an average dry weight of 9,486 mg/100ml, a hydrogen ion concentration (pH) of 6.48 to 6.99, and a freezing point of - 0.587 C. The freezing point for seminal plasma is - 0.533 C.

Mean chloride content is 371 mg/100 ml for whole semen and 174.7mg/100ml for seminal plasma.

The major cations in semen are sodium, potassium, calcium and magnesium (Quinn, White and Wirrick, 1965). Their respective levels in whole semen are 233±12.0,

44.1±2.55 and 8.8±0.18 mg/100ml. Spermatozoa contains less sodium and calcium but more magnesium amd potassium than seminal plasma. The level of iron in seminal plasma is low (2.1 mg/100ml). Total phosphorus content of whole semen is about 82 mg/100 ml with an inorganic phosphorus content of 9 mg/100 ml, acid-soluble and lipid phosphorus being 33 and 9 mg/100 ml respectively.

The average total nitrogen content of semen is 756 mg/100 ml, of which non-protein nitrogen accounts for 48 mg/100 ml. Urea, uric cid, ammonia, creatine and adrenaline are present in whole semen at levels of 5.0 mg/100 ml or below. Creatinine level is 12.1 mg/100 ml. The nitrogenous base glycerylphosphorylcholine (GPC) occurs in high concentrations in whole semen (232 mg/100 ml) and seminal plasma (350 mg/100 ml).

Bovine semen is very rich in citric acid (720 mg/100 ml) and fructose (540 mg/100 ml) while lactic acid and inositol occur at lower levels (35 and 34.9 mg/100 ml respectively).

Thiamine, riboflavin, pantothenic acid and niacin are present in whole semen at levels below 0.6 mg/100 ml, while the amino acids alanine, aspartic acid, glutamic acid, glycine, histidine, phenylalanine and serine occur in seminal plasma at levels ranging from 0.09 to 0.35 mg/100 ml. Arginine, cystine, proline, tryptophan, tyrosine and threonine occur in trace amounts.

Phosphatases and glycosidases constitute the major enzymes of bovine seminal plasma. Spermatozoa contain lactic dehydrogenases, glutamic oxalacetic transaminase, hyaluronidase, glycerokinase and various other enzymes essential for cell metabolism.

Three previously unreported constituents of bovine semen were determined by Clarke *et al.*, (1967). These are the polyhydric alcohols D-mannitol, erythritol and glycerol, present in whole semen at levels of 17 mg/100 ml, 6.9 mg/100 ml and 2 mg/100 ml, respectively.

Seminal plasma which serves as suspending medium for spermatozoa is a complex fluid, representing the combined secretions of the epididymides, seminal vesicles

and prostate glands. Apart from facilitating the transfer of spermatozoa from the male to the female reproductive tract during coitus, it serves as a buffer and also as an energy source for the ejaculated spermatozoa. However, the functions of several constituents of seminal plasma, and the significance of the considerable variation in levels of each component are unknown. Seminal plasma is not essential to fertility (White, 1968; Shah *et al.*,1968; Faulkner *et al.*,1968).

2.2 EVALUATION OF SEMEN

Several techniques which are based on physical, morphological or biochemical criteria are ut-ilised for the assessment of semen quality. Many reviews on the subject exist (Melrose, 1962; Melrose and Laing, 1970; Foote, 1969; Ball *et al.*, 1974).

The physical tests are, the measurement of ejaculate volume, determination of spermatozoa concentration in semen by haemocytometer counts, photoelectric methods or by electronic counting (Iversen, 1964). Activity of spermatozoa may be electrically measured as changes in electrical impedance between a pair of platinum electrodes placed in the semen. Glover, Berridge and Cliffe (1974) have described a method for the calorimetric measurement of activity in semen samples. Although very subjective, the estimation of motility of spermatozoa under coverslip is perhaps the most common of the physical tests.

Morphological examination of spermatozoa for abnormalities or damage due to processing is carried

out on stained semen smears under the light microscope. Supravital staining (Dott and Foster, 1972) is used for the estimation of percentage of 'live' and 'dead' spermatozoa. Unstained samples may be examined by phase-contrast or differential-interference-contrast microscopy (Saacke and Marshall, 1968; Pursel and Johnson, 1974). Examination of ultrastructure may be by electron microscopy (Jones, 1973) or may involve freeze-etching techniques utilizing the scanning electron microscope (Friend and Rudolf, 1974).

Biochemical tests form by far the largest group of tests used in semen evaluation. The group may be sub-divided into analytical tests and metabolic tests.

Analytical tests consist mainly of the determination of various biochemical components of semen, especially the nucleic acid content of spermatozoa, as well as enzymes, lipids and cations. Metabolic tests consist of the manometric determination of oxygen consumption rates in suspensions of spermatozoa, methylene blue reduction

test, fructolysis test and a few others such as the forma-zan test for catabolic activity.

The ultimate measure of semen quality is its fertilizing capacity. The various laboratory tests enumerated above are aimed at assessing that capacity in any given semen sample. It should be noted however that there is as yet no distinct relationship established between any of these tests and fertility. Melrose (1962) has observed that the laboratory test cannot measure differences in fertility between semen samples which may occur either through the handling, dilution and insemination procedures, or through variations in the fertility of the cows inseminated.

Earlier investigators (Bishop *et al.*, 1954; Cummings, 1954) obtained significant correlations between conception rate and live/dead counts, motility rating and impedance change frequency. By contrast, Buckner *et al.*, (1954) investigated a series of simple tests considered suitable for routine use at an AI centre but obtained only low correlations between these tests and fertility.

Munro (1961), using buffered nigrosin-eosin stain, reported between-bulls difference in mean incidences of live spermatozoa but within-bull sample variations were very high. Fertility appeared to the author to be reduced in samples with live spermatozoa counts below 70 per cent. However, there were discrepancies between live spermatozoa counts and motility, while the degree of spermatozoa abnormality had no obvious influence on fertility.

A lack of significant correlation between a number of undisclosed subjective tests and fertility of bull semen was reported by Glover (1971). The only correlation of undisclosed magnitude found by the author was between the motility of spermatozoa, measured with a motility meter, and fertility (Glover, 1968 1969). The heat output of bull spermatozoa, measured by a special calorimeter, was less well correlated with fertility than was the case with motility (Glover, Berridge and Cliffe, 1973).

The authors found that the laboratory tests used were inadequate for predicting the fertility of semen. Nevertheless, it was shown that limits may be set outside which poor quality semen could be discarded.

A multiple regression of conception rate (Y) on six semen traits, viz. fructose (X1), citric acid (X2), glycerylphosphorylcholine (GPC, X3), total nitrogen (X4), total solids (X5) and the number of progressively motile spermatozoa after freezing and thawing (X6), was derived by Hirao (1976). The equation was given as $Y = 93.925 - 21.416(X1) + 10.072(X2) - 21.227(X3) + 22.970(X4) - 2.405(X5) - 8.840(X6)$. Removal of citric acid as a factor from the equation maximised the variance ratio while removal of GPC gave a minimum value.

Lack of standardization of techniques used in assessing semen quality remains a major problem. Repeatability of gross motility estimates is not very good under field conditions but better results are obtainable with diluted samples under carefully controlled conditions (Ball *et al* ., 1974). With metabolic tests,

careful standardization of procedures is required, but the possibility of interactions with other factors not recorded exists which may influence the results (Melrose, 1962). Repeatability of estimates of live spermatozoa made by the live/dead staining method is not dependable and it is difficult to duplicate results (Ball *et al* ., 1974).

In general there is a tendency for correlation of seminal characteristics and fertility to be high when samples are drawn from a large, unselected population of males and each ejaculate is used to inseminate a large number of females (Faulkner and Carroll, 1974). Particularly poor correlations are obtained when the range of values for the semen trait is small and when levels of fertility are established from natural matings. It is similarly poor when only a small number of females are inseminated.

2.3 FACTORS AFFECTING SEMEN QUALITY

The factors which affect semen quality in bulls are reviewed under the following sub-titles: Testicular size and consistency, sexual preparation and frequency of semen collection, age, nutrition and management, and season. Much of the early work has been reviewed by Melrose (1962).

Testicular size and consistency

Testicular size is closely correlated to spermatozoa output in bulls. This relationship is especially high in young bulls and at high frequency of ejaculation. Willett and Ohms (1955) reported correlations between scrotal circumference and spermatozoa production ranging from r=0.30 to r=0.46 for bulls in regular service. The correlation increased to r=0.68 when a smaller number of bulls were exhausted 8 times, followed by a single ejaculation 1,3,5 or 7 days later and then by an additional rest period of 7 days. In another study,

the correlation between scrotal circumference and spermatozoa production was 0.43 and 0.32 with bulls in routine service, but with 4 exhaustions at weekly intervals with each bull, the correlation was 0.92 with 16 young bulls and 0.53 with 6 old bulls (Willett and Ohms, 1957). In Charolais bulls, scrotal circumference at 1 and 1.5 years of age was significantly correlated (0.44 and 0.52) with six-times weekly spermatozoa output at 2 years of age, and (0.62 and 0.75) with six-times weekly spermatozoa output at 3 years of age (Almquist, Branas abd Barber, 1976). However, the authors reported that testis growth rate, based on the percentage increase in scrotal circumference between 1 and 1.5 years of age, was not significantly correlated with six-times weekly spermatozoa output at 3 years of age. The correlation increased to 0.61 when testis growth rate during the first 24 weeks after puberty was used.

Components of semen quality other than spermatozoa number have also been correlated with testis size and consistency by a few workers. Heersche (1976) reported a direct relationship between total progressively

motile spermatozoa and scrotal circumference adjusted for 140-day weight, age and breed. Significant correlations ranging from 0.59 to 0.94 have also been reported between measures of testis consistency obtained with a tonometer and various tests of semen quality in 64 Holstein bulls ranging from 17 to 150 months of age (Hahn, Foote and Cranch, 1969). Measures of testis consistency with the tonometer was suggested by the authors as a simple quantitative means of predicting semen quality and potential fertility in dairy bulls. The view has however been expressed by Almquist, Branas and Barber (1976) that except for early detection of bulls with a potential for severe testicular underdevelopment, scrotal measurements of young bulls cannot be used to predict subsequent spermatozoa output precisely. Factors other than testes size, such as genetic and environmental factors which affect total testicular spermatozoa production may also limit the value of scrotal measurement for predicting subsequent spermatozoa output.

Sexual preparation and frequency of semen collection

Proper sexual stimulation of bulls prior to semen collection improves the quality and quantity of semen obtained (Amann, 1970). This may be achieved by intensive teasing and false mounting.

Several workers have shown the importance of frequency of ejaculation on semen quality. Both the physical and chemical characteristics of ejaculated semen are altered, the severity being dependent upon the frequency of collection. In young Holstein bulls on adequate nutrition, volume and concentration of semen decreased while total number of spermatozoa per week increased with increase in ejaculation frequency from bi-weekly to six-times per week (Almquist and Amann, 1962). Ejaculate volume was significantly smaller, but total spermatozoa and total motile spermatozoa per week were significantly greater when ejaculation frequency was increased from bi-weekly to six-times per week in young Hereford and Angus bulls (Almquist and Cunningham, 1967). Differences in semen concentration were not

significant. For Charolais bulls on six-times weekly compared to bulls on single collection per week, ejaculate volume was smaller but total spermatozoa and total motile spermatozoa per week were greater (Almquist *et al.*, 1976). However, differences in spermatozoa motility and semen concentration were not significant. Ejaculation of Charolais bulls six-times weekly from puberty to 2 years of age was not deleterious to semen traits, weekly spermatozoa output or testes growth and yielded 3.3 times more motile spermatozoa per week than ejaculation once weekly.

In young Czech Pied bulls ejaculate volume, spermatozoa motility and semen concentration as well as total spermatozoa per ejaculate and fructose concentration decreased from first to final ejaculations during exhaustion tests (Louda and Smerha, 1974). By contrast, when four consecutive ejaculates were collected within one hour at weekly intervals for 4 weeks from 5 mature Holstein bulls, significant increases were found in the pH of fresh semen, and plasma concentrations of fructose and citric acid (Kirton, Hafs and Hunter, 1964).

However, concentration of total free amino nitrogen declined from first to fourth week while plasma concentrations of protein and zinc, as well as total citric acid and fructose per ejaculate were unaffected.

Age

Investigations by earlier workers showed that semen producing capacity in bulls increases with age and is also related to bodyweight and testicular size increases (Melrose, 1962). Willett and Ohms (1957) estimated that nine young bulls the first year averaged 48.5×10^6 spermatozoa per gram of testicular tissue per week; seven of these bulls averaged 63.4×10^6 a year later while the old bulls averaged 46.7×10^6. Ejaculate volume, semen concentration and total sperm per ejaculate increased in Holstein bulls from puberty (35-46 weeks) to two years of age (Almquist and Amann, 1962).

In Angus and Hereford bulls, highly significant increases in ejaculate volume and weekly total and total motile spermatozoa output, and a significant improvement in semen concentration were found by Almquist and Cunningham (1967), as the bulls aged from one to two years of age. Spermatozoa motility showed a highly significant increase during the first 20 weeks after puberty, but the authors found little change thereafter. Data were obtained on 1021 Slovakian Pied Al bulls aged 2-11 years by Fasko (1974). The highest conception rate to first insemination (55.8%) was achieved by 5-year-olds and the lowest (49.2%) by the 11-year-olds. The author further found that the largest ejaculate volume (5.55 ml) was obtained from 11-year-olds, and the smallest (4.52 ml) from 2-year-olds. A similar study by Ivankov (1975) showed that bulls aged 3-7 years had better semen quality than younger or older bulls.

Semen production in Charolais bulls between puberty and 2 years of age was studied by Almquist *et al.* (1976). Significant increases were found in semen concentration, sperm motility, weekly total spermatozoa

and total motile spermatozoa output and ejaculate volume as bulls aged. From 1-2 years of age, average weekly spermatozoa output for bulls on six-times weekly collection increased 2.4 times from 14.0×10^9 at 53 to 56 weeks of age to 33.6×10^9 at 101 to 104 weeks of age while bulls on once-weekly collection increased 2.2 times from 5.0×10^9 to 11.2×10^9 at the same ages.

Nutrition and management

Much of the early investigations, reviewed by Melrose (1962), were on the effects of feed level, and type of ration on sexual maturity, semen production and fertility. Puberty may be delayed in underfed bulls but the effects on semen volume and quality are variable. In most studies, differences in semen quality attributed to the level of feeding were not large. Variable effects of type of ration and level of feeding on libido have also been reported. Impairment of fertility could not be demonstrated in most reports.

Specific dietary deficiencies like zinc and vitamin A may impair reproductive function under experimental conditions but a significant effect on fertility under natural management conditions is yet to be demonstrated.

Semen production and the rate of sperm replenishment after partial depletive collections were determined in two groups of Holstein bulls fed on 60 or 100% of the recommended total digestible nutrient intake by Van-Demark *et al.* (1964). It was found that underfeeding from 8 weeks to 46 months of age caused the bulls to be slower in replenishing depleted supplies of sperm reserves, in addition to reducing their capacity to produce adequate semen volume and sperm numbers.

For young bulls fed *ad libitum* and those given approximately 25% less nutrients, Mudra (1974) reported that age at first ejaculate was highly significantly correlated with nutrient consumption to 9 months of age ($r=0.60$). Nutrient consumption to 14 months was also significantly correlated with the number of ejaculates to 14 months ($r=0.59$) and with the number of spermatozoa ($r=0.55$). Other semen characteristics

and semen constituents were reported by the author to be unaffected by nutritional differences.

Five groups totalling 61 Czech Pied bulls were fed; (1) hay and silage, (2) maize silage, (3) ad lib-grazing, (4) haylage, and (5) a pelleted food mixture respectively in a study by Pilat and Pindak (1974). Average daily gain for the five groups respectively were, 948, 876, 1129, 1003 and 1081g, from 6-12 months of age. Group (2) animals with the lowest average daily gain produced the best quality semen having the highest ejaculate volume, sperm motility, concentration and resistance. Group (1) animals produced semen next best in quality as judged by the last three semen traits mentioned above, followed by grous (3), (5) and (4).

Season

Seasonal differences in the ejaculate characteristics of Angoni bulls were reported by Igboeli and Rakha (1971). Semen was collected from ten mature bulls

four times per week for six weeks during the rainy, cold and hot seasons and evaluated. Results obtained by the authors showed that total sperm per ejaculate, % morphologically normal sperm, % live sperm, fructose (mg %), protein (g %) and the metabolic activity of sperm were lowest during the hot season. However, the authors did not report any fertility tests over the period of investigation.

Experiments carried out in a temperate climate indicated that fertility of semen frozen in the winter was slightly higher than semen frozen in the summer (Foote, 1972). It should be noted however that a similar investigation in which semen was collected either in spring or in fall, frozen and used for insemination during fall and spring clearly demonstrated that seasonal variation in cattle fertility was due mainly to the female (Courot *et al.*, 1968).

3. SEMEN STORAGE

3.1 Semen Extenders

3.2 Liquid Storage

3.3 Deep-frozen Storage

3.1 SEMEN EXTENDERS

The purpose of extending semen is to enable the insemination of a large number of females from a minimum number of ejaculates while maintaining normal conception rates. Conditions which should be met by any extender in providing suitable environment for spermatozoa were summarized by Salisbury and VanDemark (1961) and Foote (1972). These are; proper osmotic pressure and pH, supply of an energy source, bacterial control, protection of spermatozoa against cold shock, buffering against toxic end-products of cell metabolism, and freedom from substances harmful to the fertilization and implantation processes and the subsequent development of the zygote.

The development of bull semen extenders has been reviewed by Salisbury and VanDemark (1961), Melrose (1962), Melrose and Dawson (1970), and Foote (1974). Most extenders for liquid or frozen storage of semen have either egg yolk or milk as a basic ingredient

(Foote, 1974). Much of the early investigations on the composition of semen extenders were carried out by Russian workers, in particular Milovanov. However, little progress was made until the clear demonstration by Lardy and Phillips in 1939 of the beneficial effects of egg yolk for above-freezing, low temperature preservation of bull semen. The original diluent of the latter investigators consisted of 50% egg yolk by volume, made up with phosphate buffer. Subsequent studies, reviewed by Lanz, Pickett and Komarek (1965), revealed the beneficial effects of egg yolk to be due to the lipid components, particularly lecithin.

The use of milk as a diluent for bull semen dates from the work of Kolliker published in 1856 but which aroused little interest. Later investigations by Phillips and Lardy (1940), Underbjerg *et al* . (1942), Mihailov (1949), Jacquet (1951), and Thacker and Almquist (1951) led to the development and successful usage of heated milk diluents. The components of milk essential for semen preservation were investigated by Choong and Wales (1963). Both casein and lactose were implicated as important constituents.

Buffers commonly used in semen extenders to maintain a near neutral pH and proper osmotic pressure are citrate, phosphate, Tris and other organic buffers (Foote, 1974). The effects of diluent pH and osmotic pressure on the survival and metabolism of bull spermatozoa during liquid and frozen storage were elucidated in a number of studies (Johnson *et al.*, 1956; Cragle and Salisbury, 1959; Strinbach and Foote, 1967). There was no difference in survival of bull spermatozoa when diluents having freezing point depressions of -0.50C, -0.70C and pH of 6.2, 6.6 or 7.0 were examined for liquid storage of semen by Johnson *et al.* (1956). However, Cragle and Salisbury (1959) reported that low pH, when combined with high potassium concentrations in the diluent, was inhibitory to the metabolism of bull spermatozoa during liquid storage. In yolk-containing diluents, Martin (1963b) reported that hypotonic media were superior to hypertonic ones. A previous investigation by the same author (Martin, 1963a) revealed interactions between diluent tonicity, sugar

content and holding time of the extended semen at 5C before deep freezing. Davis *et al* . (1963) reported optimum survival of bovine spermatozoa at 5C storage in Tris-buffered egg yolk diluents having a Tris molarity of 0.2M and a pH adjusted to 6.75 with citric acid.

A systematic study was carried out by Steinbach and Foote (1967) in which bull semen was extended and deep-frozen in egg yolk-fructose diluents buffered with five concentrations of Tris (0.15, 0.20, 0.25, 0.30 and 0.35 M) adjusted to four pH levels (6.0, 6.5, 7.0 and 7.5) and containing two levels of glycerol (6.4 and 8.8%, v/v). The freezing point depressions of the extenders without glycerol ranged from -0.508 to -0.955C. The authors reported that the post-thawing recovery rate of spermatozoa was significantly affected by Tris molarity, pH and glycerol concentration within the ranges examined. There was an interaction between pH and osmotic pressure: The higher pH levels tended to be detrimental at the lower Tris concentrations while the lower pH levels showed a similar tendency at the

higher Tris concentrations. This relationship was further complicated by a significant second order interaction between Tris molarity, pH and glycerol concentration. According to the authors, the best overall result was obtained with the extender containing 0.20M Tris, 6.4% glycerol, and having a pH of 6.5 and a freezing point depression of -0.62C. Few studies have been conducted to examine the effects of osmotic presure and pH of extender on conception rates. In two field trials totalling 85,000 first inseminations, use of extenders with pH values of 6.50, 6.65 and 6.80 yielded similar results (Foote, 1963).

The need for bacterial control in semen intended for artificial insemination was recognized a long time ago. Spallanzani was reported to have observed putrefaction in toad semen and Iwanow (1917), to havd investigated various chemical compounds for controlling pathogenic bacteria in semen. Renewed interest was shown in this field following elaborations on the problem of non-specific bacterial contaminants in semen by

Salisbury *et al* .(1939). Microbial contamination of semen is practically impossible to prevent although the level can be minimised by maintaining rigid hygeinic conditions at collection (Melrose, 1962). Sulphanilamide, penicillin, streptomycin and other antibacterial agents are used to control pathogens such as *Vibtio foetus* *Brucella abortus*, *Trichomonas foetus* and *Leptospira pomona* . They are also used in particular to control commensal bacteria such as *Pseudomonas aeruginosa* and *Corynebacterium pyogenes* which cause putrefaction of semen under conditions of liquid storage. Although antibiotic levels and type may vary depending on the extender, a combination of 1000 units and 1 mg of penicillin and streptomycin respectively per milliliter of diluent is considered adequate (Melrose, 1962; Foote, 1974).

Experiments by Hurst (1951) and Page *et al.* (1968) have shown that for convenience, a large quantity of extender can be prepared, divided into relatively small portions, stored at -20C and thawed for use as required. The authors found no difference in spermatozoa survival when either freshly prepared diluents or frozen extenders were used for semen storage. Also, no effect due to length of frozen storage of extender was observed.

3.2 LIQUID STORAGE

The storage of bull semen at temperatures above freezing involve a number of factors which often interact in their effects on the survival of spermatozoa. These are diluent composition, method and rate of dilution, cooling rate, storage temperature and duration.

Most diluents for liquid storage of semen consist of egg yolk in combination with citrate, Tris or lactose and glycerol, or a heaued milk-glycerol combination (Foote, 1975). Albright *et al.*, (1960) investigated the effects of yolk-citrate, glycine, whole milk and skimmilk extenders containing glycerol on the motility of bovine spermatozoa at 37C and 5C. Sixteen semen samples were extended 1:50 in 14 extenders with 7.5% glycerol added by direct and step-procedures to give 28 different extender treatments. The samples were stored either at 37C for 12 hours or at 5C for 7 days. According to the authors, direct glycerolation generally gave better results than step-wise, as evaluated by progressive sperm motility, but

these effects were not significant by 7 days of storage at 5C. It was further reported that extenders composed solely of milk constituents performed favourably at 5C and poorly with prolonged storage at 37C. Almquist and Wickersham (1962) claimed that in the skimmilk-glycerol diluent, the beneficial effect of glycerol on the maintenance of sperm motility during storage at 5C was related to both the level and method of glycerol addition. The optimum result obtained by the authors was with 5% glycerol, when the glycerol-containing fraction was added stepwise to the partially diluted semen at 5C.

Neither the optimum cooling rate to 5C nor the optimum extension rate for liquid storage of semen has been fully elucidated. Both factors appear however not to be very critical. The tendency amongst workers is to employ a cooling time of a few hours at most, as well as high extension rates. The latter tendency is of practical significance since high extension rates enable greater use to be made of the semen of top quality bulls. Almquist and Wickersham (1962) extended

semen to provide a final concentration of 15 million motile spermatozoa per milliliter. The authors employed a cooling time to 5C of 3 hours. By contrast, Foote (1970) used a shorter cooling time of 75 minutes and a higher extension rate to give a final concentration of 8 million motile spermatozoa per milliliter. Foote and Dunn (1962) found no difference in fertility between semen samples extended to 5 million or 10 million motile spermatozoa per milliliter in the CUE extender. Similarly, the fertility of bull spermatozoa were preserved at high extension rates in a 0.20M Tris-buffered egg yolk extender containing glycerol and fructose (Foote, 1970).

For most practical purposes, chilled semen is stored at 5C. The length of storage is of critical importance and it is established that the highest conception rates are obtainable with semen used on the day of collection. However, some workers have shown that only a slight decline in fertility occurs within 30 hours after collection (Willett, 1953; Hewetson, 1955). In the majority of reports reviewed by Melrose (1962), the decline in conception rate for each day of storage up to the fourth day varied from 3.0 to 9.0%.

3.3 DEEP-FROZEN STORAGE

A major constraint to the widespread use of artificial insemination in cattle was removed three decades ago with the successful development of long term storage techniques for bull semen. polge *et al.* (1949) first reported the protection of spermatozoa by glycerol against freezing injury. By late 1950 the first calf from an insemination with frozen semen was born (Stewart, 1961). Subsequently fertility trials by various workers have shown no decline in conception rates with frozen semen stored for several years (Stewart, 1961; Melrose, 1962; Foote, 1972).

The early work on deep-frozen storage of bull semen has been reviewed (Salisbury and Van-Demark, 1961; Melrose, 1962). However, most of these investigations were empirical, involving the examination of effects of various procedural manipulations on the post-thawing motility and fertility of spermatozoa. Nevertheless, several semen processing methods with acceptable results were developed and some are in routine use.

More recently, attention has been focused by several workers on the ultrastructural and biochemical changes induced by deep freezing of spermatozoa (Osinowo, 1975). The most incisive of these studies dealt with mechanisms of freezing injury and cryoprotection of cells. An excellent review was published by Mazur (1970). It seems that further elucidation of the basic principles involved in cell survival at low temperatures is required.

Dilution, Cooling and Equilibration

Both the method of dilution and cooling time to 5C are similar for liquid and frozen storage of semen. O'Dell and Almquist (1957) claimed that stepwise addition of glycerol to semen diluted and frozen in skimmilk gave slightly better post-thawing motility than adding the glycerol all at one time. Martin and Emmens (1958) reported a significant effect of dilution rate on post-thawing motility of spermatozoa. Motility at a dilution

of 30 million spermatozoa per millilitre was significantly higher than that of 13.3 million spermatozoa per millilitre. Fertility was not affected. Martin (1965) reported that a decrease in time taken to cool diluted semen from 30 to 5C from 2 hours to 30 minutes did not affect the motility of spermatozoa. However, according to the author, there was an increase in the proportion of stained spermatozoa indicating that rapid cooling was detrimental. Iida (1966) used a cooling time to 5C of 2 to 4 hours. The optimum extension rate during cooling to 5C has not been extensively studied, nor has the optimum cooling rate to 5C been established for frozen semen (Foote, 1975).

Results obtained by early workers on the importance of equilibration time at 5C were conflicting (Melrose, 1962; Martin and Emmens, 1958; Foote, 1975). For instance, O'Dell and Almquist (1957) found that in skimmilk diluent, prefreezing glycerol equilibration periods of 30 minutes and 4 hours resulted in spermatozoa survival equal to or better than 18 hours'

equilibration. It was also found by the authors that equilibration time was not related to the level of glycerol present in the diluent. Martin (1963) claimed that an improvement in survival after freezing occured if semen was stored for up to 12 hours after dilution, but confirmed that such improvement due to equilibration was not dependent on the presence of glycerol in the diluent during the process. Martin (1965) reported that a six-hour period of storage at 5C before freezing improved revival rates. Martin and Emmens (1958) carried out a factorial test in which three factors were examined. These were dilution rate (30, 20 and 13.3 million spermatozoa/ml), sugar in the diluent (fructose or arabinose) and equilibration time (1 hour or 18 hours). Only equilibration time had a significant effect on fertility. One hour equilibration resulted in lower fertility than 18 hours (58.4 v 65.3% non-returns to service at three months). Although motility was affected by the sugar used and by dilution, equilibration time had no effect.

Freezing methods

Three main methods have been used for deep freezing bull semen. The oldest method involved freezing by dipping semen-filled glass ampules in a bath containing a mixture of dry-ice (solid carbon dioxide) and alcohol (Blackshaw, Emmens and Martin, 1957). Pellet freezing on dry-ice was first described by Nagase and Niwa (1964). This method involved freezing semen directly on dry-ice without using a packaging container. The third method involves freezing of semen in liquid nitrogen vapour either in straws or ampules (Roussel, Kellgren and Patrick 1964; Clegg, Pickett and Gibson, 1965; Rajamannan, 1966).

Freezing of ampuled semen in dry-ice-alcohol bath was compared to a liquid nitrogen freezer (Linde Model BF-1) by Bean *et al.* (1963). Both freezing methods were planned to provide a cooling rate of 1C per minute from +5 to -15C and 4C per minute from -15 to approximately -79C. The authors reported that motility of

spermatozoa frozen with the dry-ice-alcohol bath was significantly higher for all treatments than samples frozen with the liquid nitrogen freezer. By contrast, Clegg *et al.* (1965) claimed that freezing semen in a in a properly programmed automatic liquid nitrogen semen freezer was equal to the best dry-ice-alcohol freezing method. Also, motility of spermatozoa frozen in the nitrogen vapour of a large semen storage unit was equal to semen frozdn in a large automatic programmed semen freezer. According to the authors, the large variation in rate of freezing semen in ampules by the vapour method did not appear to adversely affect sperm survival.

The effects of freezing rates on sperm survival were reviewed by Martin (1962) and Iida (1966).

Storage of frozen semen

The two major factors associated with storage of deep-frozen semen are storage temperature and length of storage. Early work on the effect of storage temperature on sperm viability was reviewed by Martin (1962).

Sullivan and Mixner (1963) compared storage at -196C with -79C over 18 months. The authors reported that the mean motility, sugar utilization, and lactic acid production during 3-hour post-thawing incubation at 37C were significantly greater for semen stored at -196 than at -79C. With increasing time of storage, the mean motility and sugar utilization of the semen declined linearly, with the decline being less for semen stored at -196C. With lactic acid, significant interaction was found between storage time and storage temperature. On the basis of metabolic activity per percentage unit of motile spermatozoa, the authors concluded that a storage temperature of -196C was more beneficial than a temperature of -79C.

Bean *et al.* (1963) reported that motility values of semen extended in egg yolk-citrate and stored at -92 and -196C were approximately equal and in all cases higher than comparable samples stored at -79C. Furthermore, the authors claimed that the samples extended in milk exhibited higher motility values after three months storage when stored at -196C than at -92 or -79C.

Deep-frozen semen can be transferred from -79 to -195C or vice versa repeatedly without apparent damage (Martin, 1962). However, repeated warming and cooling above this range is detrimental to spermatozoa.

Thawing of frozen semen

The thawing temperature for deep-frozen semen appears to have an effect on viability of spermatozoa. Martin (1963) reported that 40C was superior to 0C as a thawing bath temperature. By contrast, Pickett *et al.* (1965) found no significant difference in motility between semen thawed at 40 or 1C. Although the

REFERENCES

Abdel-Raouf, M. (1960). "The postnatal development of the reproductive organs in bulls with special reference to puberty (including growth of the hypophysis and the adrenals)." *Acta Endocrinologica*, suppl. 49 (1L).

Albright, J.L., Erb, R.E. and Ehlers, M.H. (1960). "Influence of yolk citrate, yolk citrate, yolk glycine, whole-milk, and skimmilk extenders containing glycerol on motility of bovine sperm at 37 and 5C. "*J. Dairy Sci.* 43, 250-255.

Almquist, J.O. and Amann, R.P. (1962), "Effect of a high ejaculation frequency on sperm output and semen characteristics of Holstein bulls from puberty to 2 years of age. "*J. Dairy Sci.* 45: 688 (Abstract).

Almquist, J.O. and Wickersham, E.W. (1962). "Diluents for bovine semen. XII. Fertility and motility of spermatozoa in skim-milk with various levels of glycerol and methods of glycerolization." *J. Dairy Sci.* 45, 782-787.

Almquist, J.O. and Cunningham, D.C. (1967). "Reproductive capacity of beef bulls. 1. Postpuberal changes in semen production at different ejaculation frequencies, *J. Anim. Sci.* 26, 174-181.

Almquist, J.O., Branas, R.J. and Barber, K.A. (1976). "Postpuberal changes in semen production of Charolais bulls ejaculated at high frequency and the relation between testicular measurements and sperm output." *J. Anim. Sci.* 42, 670-676.

Amann, R.P. (1970). "Sperm production rates." In *The testis* Vol. 1. A.D. Johnson, W.R. Gomes and N.L. Van-Demark (eds.), New York, Academic Press.

Asdell, S. A. (1955). *"Cattle fertility and sterility."* Boston, Little, Brown and Company. Toronto.

Ashdown, R.R. and Hancock, J.L. (1974). "Functional anatomy of male reproduction. " In *Reproduction in farm animals*,ed. E.S.E. Hafez, Lea and Febiger, Philadelphia, P. 3-23.

Ball, L., Nelson, L.D., Furman, J.W. and Seidel Jr., G.E. (1974). "Semen Collection and evaluation of bulls for breeding soundness." Paper presented at the *annual meeting of the AVSSBS* Columbia, Missouri, Sept. 6-7.

Bane, A. (1954). "Studies on monozygous cattle twins. XV Sexual functions of bulls in relation to heredity, rearing intensity and somatic conditions. *Acta Agric. Scand.* 4, 95,208. (Cited by Melrose 1962.)

Bean, B.H., Pickett, B. W. and Martig, R.C. (1963). "Influence of freezing methods, extenders, and storege temperatures on motility and pH of frozen bovine semen." *J, Dairy Sci.* 46, 145-149.

Bishop, M.W., Campbell, R.C., Hancock, J.L.. and Walton, A. (1954). "Semen characteristics and fertility in the bull." *J, Dairy Sci.* 44, 227-248. (Cited by Melrose, 1962).

Blackshaw, A.W., Emmens, C.W., Martin, I. and Heyting, J. "The preparation of deep frozen semen." *A.I. Digest*, 5, 6-8.

Blom, E. and Christensen, N.O. (1956). "Examination of the genitals of slaughtered male calves as a means of elucidating the frequency of genital malformation in the bovine male. Studies on pathological conditions in the testis, epididymis and - accessory sex glands in the bull." III *Proc. IIIrd. Inter. Congr. Anim. Reprod. A.I.*, Cambridge Sect. II, p. 76-79.

Blom, E. and Christensen, M. O. (1958). "Cysts and eyst-like formations (inter alia spermiostasis) in the genitals of the bull. Studies on pathological conditions in the testis, epididymis and accessory sex glands in the bull. IV. *Arsskr. K. Vet. Landbohjsk.* P. 101-133.

Blom, E. and Christensen, N.O. (1960). "The etiology of spermiostasis in the bull. Studies on pathological conditions in the testis, epididymis and accessory sex glands in the bull. V. *Nord Vet. Med.* 12,453-470.

Bonadonna, T., Caretta, A. and Corrias, A. (1953). *Zootec. Vet.* 8,369. (Cited by Ortavant 1969).

Buckner, P.J., Willett, E.L. and Bayley, N. (q 1954). "Laboratory tests, singly and in combination, for evaluating fertility of semen and of bulls." *J. Dairy Sci.* 37, 1050-1060. (Cited by Melrose 1962).

Carmon, J.L. and Green, W.W. (1952). "Histological study of the development of the testis of the ram." *J. Anim. Sci.* II, 674-687.

Choong, C.H. and Wales, R.G. (1963). "The use of various diluents for deep-freezing bull spermatozoa." *Aust. J. Biol. Sci.* 16, 896-904.

Clark, J.B.K., Graham, E.F., Lewis, B.A. and Smith, F. (1967). "D-Mannitol, erythritol and glycerol in bovine semen." *J. Reprod. Fert. 13, 189-197.*

Clegg, E.D., Pickett, B.W. and Gibson, E.W. (1965). "Non-mechanical nitrogen vapor freezing of bull semen." *Research Report,* 5, Agric. Expt. Sta., Univ. of Connecticut, Storrs.

Coulter, G.H., Rounsaville, T.R. and Foote, R.H. (1974). "Heritability of bovine testicular measurement." *J. Anim, Sci.* 39, 204 (Abstract).

Coulter, G.H., Larson, L.L. and Foote, R.H. (1975). "Effect of age on testicular growth and consistency of Holstein and Angus bulls," *J. Anim. Sci* 41, 1383-1389.

Courot, M., Goffaux, M. and Ortavant, R. (1968). Ann. *Biol. Animale Biochim. Biophys.* 8, 209. (Cited by Thibault and Levasseur, 1974).

Cragle, R.G. and Salisbury, G.W. (1959). "Factors influencing metabolic activity of bull spermatozoa. IV. pH, osmotic pressure and the cations, sodium. potassium and calcium." *J. Dairy Sci.42* 1304.

Fagerlin, P.T., Carroll, E.J. and Brinks, J.S. (1971). "Testicular growth and semen traits in beef bulls." *J. Anim. Sci.* 33,253 (Abstract).

Fasko, J. (1974). "(Fertility, semen quality, body conformation and reasons for culling of slovakian pied bulls.) Plodmost, kvalita spermatu, zevnejsek a priciny vyrazovam byku slovenskeho strakateho skotu." *Vyzkum v Chovu Skotu* 16,33-35. (A.B.A. (1976) 44,408).

Faulkner, L.C., Hopwood, M.L. and Wiltbank, J.N. (1968). "Seminal vesiculectomy in bulls. II. Seminal characteristics and breeding trials." *J. Reprod. fert.* 16,179-182.

Faukner, L.C. and Carrol, E.J. (1974). "Reproductive Failure in males. "In, *Reproduction in farm animals,* P. 373-383. End. E.S.E. Hafez, Lea and Febiger, Philadelphia.

Foote, R.H.(1963). "Influence of pH on survival and fertility of bull sperm." *J. Dairy Sci.* 47, 807-811.

Foote, R.H. (1969). "Research techniques to study reproduc.-tive physiology in the male." In, *Procedures in animal science research p.80-110. Ameri-*can Society of Animal Science, New York.

Foote, R. H. (1970a). "Fertility of bull semen at high extension rates in Tris-buffered extenders." *J. Dairy Sci* 53, 1475-1477.

Foote, R.H. (1970b), "Influence of extender, extension rate, and glycerolating technique on fertility of frozen bull semen." *J. Dairy Sci.* 53,1478-1482.

Foote, R.H. (1972a). "Tris and other organic buffers for the conservation of semen of various species." In, *'Animal reproduction and A.I.*, p.99-105. Societa Italiana peril progresso della zootecnica.

Foote, R.H. (1972b). "keeping quality and viability of deep deep frozen spermatozoa in cattle." *Proc. VII Inter. Congr. Anim. Reprod. A.I., Munich,* p. 178-181.

Foote, R.H. (1974). "Artificial insemination." In, *Reproduction in farm animals,* 'p.409-431. ed. E.S.E. Hafez. Lea and Febiger, Philadelphia.

Foote, R.H. (1975). "Semen quality from the bull to the freezer: An assessment," *Theriogenology,* 3, 219-235.

Foote, R. H. and Dunn, H.O. (1962). "Motility and fertility of bull semen extended at high rates in yolk extender containing catalase." *J. Dairy Sci.* 45, 1237.

Foote, R.H. Gray, L.C., Young, D.C., Young, D.C. and Dunn, H.O. (1960). "Fertility of bull semen stored up to 4 days at 5C in 20% egg yolk-extenders." *J. Dairy Sci.* 43, 1330-1334.

Foote, W.D. (1974). "Cattle." In, '*Reproduction in farm Animals*' p.257-264, Ed. E.S.E. Hafez, Lea and Febiger, Philadelphia.

Friend, D.S. and Rudolf, I. (1974). "Acrosomal disruption in sperm. Freeze-fracture of altered membranes. "*J. Cell Biol.* 63, 466-479.

Gier, H.T. and Marion, G.B. (1970). "Development of the mammalian testis." In, '*The testis.*' *Vol. I.* A.D. Johnson, W.R. Gomes and N.L. VanDemark (eds.), New York, Academic Press.

Glover, F.A. (1968). "A new physical method of measuring the motility of bull spermatozoa. "*Proc. VI Inter. Congr.* Anim. Reprod. AI., Paris, P. 169.

Glover, F.A. (1969). "Motility of bull spermatozoa." *N.I.R.D. Report* (1969-70) P. 133, University of Reading.

Glover, F.A. (1971). "Motility of bull spermatozoa." *N.I.R.D. Report* (1971-1972), p. 112-113, University of Reading.

Glover, F.A., Berridge, N.J. and Cliffe, A.J. (1973). "Fertility of bull semen." *N.I.R.D. Report* (1973-74). p. 97-98, University of Reading.

Graham, E.F., Schmehl, M.K.L. and Maki-Laurila, M. (1970). "Some physical and chemical methods of evaluating semen." *Proc. 3rd. Tech. Conf. Anim. Reprod. A.I.* N.A.A.B., Feb. 1970, p. 1-6.

Hahn, J., Foote, R.H. and Cranch, E.T. (1969). "Tonometer for measuring testicular consistency of bulls to predict semen quality." *J.Anim. Sci.,* 29, 483-489.

Hahn, J., Foote, R.H. and Siedel Jr., G.E. (1969). "Testicular growth and related sperm output in dairy bulls." *J. Anim. Sci.* 29, 41-47.

Heersche, G. (1976). "Estrous synchronization and factors affecting semen quality of beef cattle." *Dissertation Abstr.* B(1976) 36 (8) 3692. (A.B.A. 44,531).

Hewettson, R. W. (1955). "Storage time as a factor limiting the fertilizing capacity of diluted bovine semen stored at 5C. "*Aust. Vet. J.* 31, 129-131. (Cited by Melrose, 1962).

Hirao, K. (1975). "A multiple regression analysis on six measurements of bovine semen characteristics for fertility." *Inter. J. Fertil.,* 20, 204-208. (A.B.A. (1976) 44, 531-532).

Hooker, C.W. (1944). 'The postnatal history and function of the interstitial cells of the testis of the bull." *Amer. J. Anat.* 74,1-26.

Howe, G.R. (1973). "Efforts to relate spermatozoan motility to fertilitizing capacity." *Inter. J. Fertil.* 18,188-190 (A.B.A. (1974) 42,.)

Humphrey, J.D. and Ladds P.W. (1975). "Pathology of the bovine testis and epididymis." *The Vet. Bulletin.* 45, 787-797.

Hurst, V. (1951). "The storage of egg yolk-sodium citrate semen diluter." *J. Dairy Sci.* 34, 490. (Cited by page *et al.*, 1968).

Igbokli, G. and Kakha, A.M. (1971). "Seasonal Changes in the ejaculate characteristics of Angoni (shorthorn Zebu) bulls." *J. Anim. Sci.*, 33, 651-654.

Iida, I. (1966). "Effect of various rapid freezing rates on survival of bovine spermatozoa. "*Bulletin of the Faculty of Agriculture, Shizuoka University,* No. 16,p. 95-98.

Iljinskaja, T.P. (1966). "Changes in the testes of bulls of different ages." *Verterinariya* 43,67 (Russian). (A.B.A. 35, 429).

Ivankov, M.F. (1975). "(Variation in semen quality in relation to age of bulls and haematological parameters). Izmenenie kachestva spermy v zavisimosti ot vozrasta bykov i gematologicheskikh pokazatelei. *Trudy.* Khar' kovskii Sel'skokhozyistvennyi Institut (1975) No. 213,95-97. (A.B.A. (1976) 44, 532).

Iversen, S. (1964). "Evaluation of the number of spermatozoa in bull semen. A comparison between electronic counting, light scattering and absorptiometry." *J. Agric. Sci.* 62, 219-223.

Iwanow, E. (1917). "Moyen de rendre le sperme infecte des mammiferes incapable de transmettre l'infection." *C.R. Soc. Biol.* (Paris), 80 765. (Cited by Melrose, 1962).

Jacquet, J. (1951). "Recherches sur quelques milieux biologiques de conservation du sperme des taureaux." *Bull Acad. Vet. Fr.* 24, 429-432. (Cited by Melrose, 1962).

Johnson, P.E., Flipse, R.J. and Almquist, J.O. (1956). "Diluters for bovine semen. VIII. The effects of alterations of some physical factors of a milk diluter on the livability of bull spermatozoa." *J. Dairy Sci.* 39 180.

Jones, R.C. (1973). "Preparation of spermatozoa for electron and light microscopy." *J. Reprod. Fert.* 33, 145-149.

Kirton, K.T., Hafs, H.D., and Hunter, A.G. (1964). "Levels of some normal constituents of bull semen during repetitive ejaculation." *J. Reprod. Fert.* 8, 157-164.

Kolliker, A. (1856). "Physiological studies on the seminal fluid. (Trans. title.) Z. Wissensch. Zool. 7, 201-272. (Cited by Salisbury and VanDemark, 1961).

Kumi-Diaka, J. (1976). "Spermatogenesis in indigenous bulls in Nigeria." *MSc thesis*, Ahmadu Bello University.

Lanz, R.N., Pickett, B.W. and Komarek, R.J. (1965). "Effect of lipid additives on pre-and post-freeze survival of bovine spermatozoa." *J. Dairy Sci.* 48, 1692-1697.

Lardy, H.A. and Phillips, P.H. (1939). "Preservation of spermatozoa. "*Am. Soc. Anim. Prod. Proc.* 32, 219-221. (Cited by Salisbury and VanDemark, 1961).

Lavon, U., Volcani, R., Amir, D. and Danon, D. (1966). "The specific gravity of bull spermatozoa and seminal plasma." *J. Reprod. Fert.* 11, 447-449.

Linford, E., Glover, F.A., Bishop, C. and Stewart, D.L. (1976). "The relationship between semen evaluation methods and fertility in the bull." *J. Reprod. Fert.* 47, 283-291.

Louda, F. and Smerha, J. (1974). "(Sperm reserves in young Czech pied bulls.) Zasoby spermatu u mladych byku ceskeho stakateho plemene." *Vyzkum v Chovu Skotu* (1974) 16 (1) 73-75. (A.B.A.(1976) 44, 409).

Macmillan, K.L. and Hafs, H.D. (1969). "Reproductive tract of Holstein bulls from birth through puberty." *J. Anim. Sci.* 28, 233-239.

Mann, T. (1969). "Physiology of semen and of the male reproductive tract." In, *Reproduction in domestic animals*, p. 277-312. Eds. H.H. Cole and P.T. Cupps. Academic Press, New York.

Martig, R.C. and Almquist, J.O. (1966). "Effect of glycerol equilibration time on fertility of bull spermatozoa frozen in homogenized milk-fructose diluent." A.I.-Digest, May 1966.

Martin, I.C.A., (1962). "Rates of cooling and storage temperatures for deep-frozen bull spermatozoa." *Australian Vet. J.* 38, 414-420.

Martin, I.C.A. (1963a). "Revival of bull spermatozoa after deep-freezing to -79C following equilibration in diluents of various tonicities." *J. Reprod. Fert.* 6, 433-439.

Martin, I.C.A. (1963b). "The influence of filuents and processing time after ejaculation on the revival of deep-frozen bull spermatozoa." *J. Agric. Sci* 61, 55-63.

Martin, I.C.A. (1965a). "Effects of rate of cooling to 5C storage at 5C, glycerol concentration, sodium chloride, fructose and glycine on the revival of deep-frozen bull spermatozoa." *J. Agric. Sci.* 64, 425-432.

Martin, I.C.A. (1965b). Influence of equilibration, freezing rate, method of dilution and diluent on the survival of deep-frozen bull spermatozoa." *Austr. J. Biol. Sci.* 18, 901-915.

Martin, I. and Emmens, C.W. (1958). "Factors affecting the fertility and other characteristics of deep-frozen bull semen." *J. Endocrin.* 17, 449-455.

Mazur, P. (1970). "Cryobiology: The freezing of biological systems." *Science*, 168, 939-949.

Melrose, D.R. (1962). "Artificial insemination in cattle." In, *The semen of animals and artificial insemination*, p. 1-181. Ed. J.P. Maule. Commonwealth Agricultural Bureaux.

Melrose, D.R. and Dawson, P.L.L. (1970). "Artificial insemination." In, *Fertility and infertility in the domestic animals*, P. 161-210. Ed. J.A. Laing. Bailliere, Tindall and Cassell, London.

Melrose, D.R. and Laing, J.A. (1970). "The characteristics of normal semen." In, *Fertility and infertility in the domestic animals*, p. 128-160. Ed. J.A. Laing. Bailliere Tindall and Cassell, London.

Mihailov, N.N. (1949). "Molocnye razbaviteli spermy zivotnyh. (Milk as a diluent for the semen of animals) *Konevodstvo*, 6, 14-16. (Cited by Melrose, 1962).

Morstin, J. and Jasiorowski, T. (1976). "(A characteristic of testes, epididymes and the semen obtained by electroejaculation as a criterion of the fertility value of young bulls and of the selection of their sires.) Charakterystyka jader, najadrzy i nasienia uzyskanego metoda elektroejakulacji, jako dryteriow oceny zdolnosci rozplodowej mlodych buhajow oraz selekcji ich ojcow." *Prace i Materialy Zootechniczne*, 10, 7-16.

Mudra, K. (1974). "(The effect of rearing on semen production of young bulls.) Sledovani odchovu a produkce spermatu mladych byku." *Vyzkum v Chovu Skotu* 16, 25-27. (A.B.A. (1976) 44, 410).

Munro, I.B. (1961). "Bovine semen characteristics and fertility." *J. Reprod. Fert.* 2, 513-515.

Nagase, H. and Niwa, T. (1964). "Deep freezing bull semen in concentrated pellet form. I. Factors affecting survival of spermatozoa." *Proc. Vth Int. Congr. Anim. Reprod. A.I., Trento, IV*, 410-415.

Niswender, G.D., Nett, T.M. and Akbar, A.M. (1974). "The hormones of reproduction." In, *Reproduction in farm animals*, p. 57-81. Ed. E.S.E. Hafez. Lea and Febiger, Philadelphia.

O'Dell, W.T. and Almquist, J.O. (1957). "Freezing bovine semen. I. Techniques for freezing bovine spermatozoa in milk diluents." *J. Dairy Sci.* 40, 1534-1541.

O'Dell, W.T., Almquist, J.O. and Amann, R.P. (1959). "Freezing bovine semen. V. Practicability of collecting and freezing a large number of successive ejaculates." *J. Dairy Sci.* 42, 1209-1215.

Ortavant, R., Courot, M. and Hochereau, M.T. (1969). "Spermatogenesis and morphology of the spermatozoon." In, *Reproduction in domestic animals*, 2nd Edition, p. 251-276. Eds. H.H. Cole and P.T. Cupps. Acad. Press. New York and London.

Osinowo, O.A. (1975) "Studies on deep freezing boar spermatozoa." *MSc. thesis*, University of Sydney.

Page, R.D., Gebauer, M.R. and Pickett, B.W. (1968). "Extending bull semen with post-frozen diluents." A.I. Digest, December, 1968.

Phillips, P.H. and Lardy, H.A. (1940). "A yolk-buffer pabulum for the preservation of bull semen". *J. Dairy Sci.* 23, 399-404.

Pickett, B.W., Hall, R.C., Lucas, J.J. and Gibson, E.W. (1965). "Investigations on thawing frozen bovine spermatozoa." *Fertility and sterility*, 16, 642-651.

Pilat, Z. and Pindak, J. (1974). "(The effect of different nutrition during rearing on semen production of young bulls) Vliv rozdilnych krmnych davek behem odchovu na produkci spermatu rocnich byku." *Vyzkum v Chovu Skotu*, 16, 30-32.

Polge, C., Smith, A.U. and Parkes, A.S. (1949). "Revival of spermatozoa after vitrification and dehydration at low temperatures." *Nature*, 164, 666.

Pursel, V.G. and Johnson, L.A. (1974). "Glutaraldehyde fixation of boar spermatozoa for acrosome evaluation." *Theriogenology*, 1, 63-68.

Quinn, P.J., White, I.G. and Wirrick, B.R. (1965). "Studies of the distribution of the major cations in semen and male accessory secretions." *J. Reprod. Fert.* 10, 379.

Rajamannan, A.H.J. (1966a). "Vapour freezing of bull semen." *A.I. Digest*, 14, Feb. 1966.

Rajamannan, A.H.J. (1966b). "A new semen freezing technique." *A.I. Digest*, 14 Aug. 1966.

Rawlings, N.C., Hafs, H.D. and Swanson, L.V. (1972). "Testicular and blood plasma androgens in Holstein bulls from birth through puberty." *J. Anim. Sci.* 34, 435-440.

Roberts, S.J. (1971). "Veterinary obstetrics and genital diseases." Published by the author. Distributed by Edwards Bros. Inc., Ann Arbor, Mich. 2nd Edition.

Roussel, J.D., Kellgren, H.C. and Patrick, T.E. (1964). "Bovine semen frozen in liquid nitrogen vapor." *J. Dairy Sci.* 47, 1403-1406.

Saacke, R.G. and Marshall, C.E. (1968). "Observations on the acrosomal cap of fixed and unfixed bovine spermatozoa." *J. Reprod. Fert.* 16, 511-514.

Salisbury, G.W., Willett, E.L. and Gunsalus, I.C. (1939). "Some problems in bull semen storage." *Amer. Soc. Anim. Prod. Proc.* 32, 210-212. (Cited by Salisbury and VanDemark, 1961).

Thacker, D.L. and Almquist, J.O. (1951). "Milk and milk-products as diluters for bovine semen." *J. Anim. Sci.* 10, 1082.

Thibault, C. and Levasseur, M. (1974). "Reproductive life cycle." In, *Reproduction in farm animals*, 3rd. Edition. Ed. E.S.E. Hafez. Lea and Febiger, Philadelphia. p. 82-100.

Underbjerg, G.K.L., Davis, H.P. and Spangler, R.E. (1942). "Effect of diluters and storage upon fecundity of bovine semen." *J. Anim. Sci.* 1, 149-154. (Cited by Melrose, 1962.)

VanDemark, N.L., Fritz, G.R. and Mauger, R.E. (1964). "Effect of energy intake on reproductive performance of dairy bulls. II. Semen production and replenishment." *J. Dairy Sci.* 47, 898.

Watson, R.H., Sapsford, C.S. and McCance, I. (1956). "The development of the testis, epididymis, and penis in the young merino rams." *Austr. J. Agric. Res.* 7, 574-590.

White, I.G. (1968). "Mammalian semen." In, *Reproduction in farm animals*, 2nd. edition, p. 39-59. Ed. E.S.E. Hafez. Lea and Febiger, Philadelphia.

White, I.G. (1974). "Mammalian semen." In, *Reproduction in farm animals*, 3rd. edition, p. 101-122. Ed. E.S.E. Hafez. Lea and Febiger, Philadelphia.

Wiggin, H.B. and Almquist, J.O. (1974). "Effect of flycerol equilibration time and thaw rate on acrosomal maintenance of bull sperm frozen in plastic straws." *J. Dairy Sci.* 57, 625 (Abstract).

Willett, E.L. (1953). "Decline in fertility of bull semen with increase in storage time as influenced by dilution rate." *J. Dairy Sci.* 36,1182-1185.

Willett, E.L. and Ohms, J.I. (1955). "Testicular size and production of spermatozoa by bulls." *J. Anim. Sci.* 14, I261 (Abstract).

Willett, E.L. and Ohms, J.I. (1957). "Measurement of testicular size and its relation to production of spermatozoa by bulls." *J. Dairy Sci.* 40, 1559-1569.

Yai, T.S. and Eaton, O.N. (1954). "Postnatal growth and histological development of reproductive organs in male goats." *Amer. J. Anat.* 95, 401-431

PART II

TESTICULAR SIZE IN WEST AFRICAN BULLS

Production Research Institute (NAPRI), Shika, were used to determine the repeatability of scrotal measurement. This group consisted of 5 Bunaji, 3 Sokoto-Gudali, 2 Friesian, and 10 crossbred bulls, ranging in age and liveweight from 13 to 58 months and 164 to 453 kg respectively. Each bull was restrained with ropes while scrotal measurements were taken in turn by three technicians using an independent recorder. Scrotal circumference was measured with a flexible canvas tape while the left and right scrotal diameters were measured with a pair of steel calipers. All measurements were taken at the point of greatest circumference of the scrotum.

The second investigation was to determine the relationship between scrotal circumference and testicular size in West African bulls and was carried out at four abattoirs located at Mokwa, Kaduna, Zaria and Kano in Nigeria. A total of 30 bulls were involved, consisting mainly of Sokoto-Gudali, Bunaji and Rahaji breeds. A few however appeared to be crosses between two or all

of these breeds but their precise ancestry could not be ascertained. Scrotal circumference was measured either prior to slaughter or immediately after. The pair of testes was excised, trimmed of the epididymides and weighed. The paired testes volume was determined by water displacement using a 2-litre glass vessel with a run-off.

In the third investigation scrotal circumference measurements were taken on a total of 145 animals consisting of 34 N'Dama, 33 Bunaji and 78 Sokoto-Gudali bulls. All the N'Dama bulls were located at the Pashola Stock Farm, Oyo State. Most of the Sokoto Gudali bulls were located at four Sokoto State Livestock Investigation and Breeding Centres (L.I.B.C.) while majority of the Bunaji bulls were located at the Shika Dairy Multiplication Centre and Kabomo LIBC, Kaduna State. The rest of the bulls were from the NAPRI herds at Shika.

All measurements were taken between November 1976 and March 1977. Only data for bulls having normal testes were analysed by computer using a statistical package (Nie *et al.*, 1975). In the third investigation, pairwise deletion of missing data was adopted to make maximum

use of available data. However, listwise deletion of missing data was used to obtain the same subpopulation of samples within each breed for the calculation of partial correlations and for regression analysis. Only the regression equations that best fit the data are presented in Table 4.

RESULTS

Results of the investigation into the repeatability of scrotal measurements between technicians are summarized in Table 1.

The scrotal circumference (SC) of bulls involved in the second investigation ranged from 21.1 to 40.0cm.

age constant, were 0.30 (P<0.05), 0.44 (P<0.06) and 0.47 (P<0.05) respectively for the three breeds. Scatter-diagrams illustrating the relationship of SC to log of age and to log of liveweight are presented in Figure 2. Regression equations derived from the data for the prediction of SC from age or liveweight of bulls are given in Table 4.

DISCUSSION

The repeatability estimate obtained in this study for scrotal circumference measurements between technicians agrees closely with an earlier estimate of r=0.98 obtained with Holstein bulls by Hahn, *et al.*, (1969). The inclusion of Bunaji, Sokoto-Gudali, Friesian, and cross-bred bulls in the present investigation makes it possible to infer that repeatability of scrotal measurement is unaffected by breed. The lower repeatability estimates obtained for scrotal measurements with a pair

of steel calipers may be partly due to the greater difficulty experienced by technicians in manouvering the calipers into position.

Previous studies with temperate cattle breeds demonstrated the close relationship between scrotal cirumference and testicular size in bulls. A correlation of r=0.94 between scrotal circumference and weight or volume of excised testes was reported by Willett and Ohms (1957). Hahn *et al.*, (1969) similarly reported a high correlation (r=0.92) between scrotal circumference and testes weight in Holstein bulls.

In the present study a highly significant correlation was found between scrotal circumference and testes weight or volume. However, the actual correlation was somewhat lower than those obtained by previous workers and only 76 per cent of the total variation in testes size was accounted for by scrotal circumference. This may be attributed to three factors, namely, variation in scrotal skin thickness, difference in testis shape, and the compounding breed effects. Considerable within and

between-breed variation in skin thickness had previously been reported for both temperate and tropical cattle breeds (Osinowo and Steinbach, 1973). Hence, it is suggested that a higher correlation between scrotal circumference and testes size may be obtained by adjusting for scrotal skin thickness.

Testicular shape varied from fairly oval in some bulls to cylindrical in others. Testicular size tends to be overestimated by scrotal circumference in oval organs but underestimated in cylindrical ones. However, the proportion of the total variance attributable to differences in testis shape in these breeds is unknown. It is probable that a breed by breed examination will yield higher correlations.

Testes weight and volume were closely related in all bulls although their ratio to one another varied by up to 5 per cent. The significance of the observed variation is not known but it may be related to variations in testicular consistency of bulls reported by Coulter *et al.* (1975).

The regression equation for estimating testes weight (Y) from scrotal circumference (X) in West African bulls was calculated for 30 slaughter-house bulls. The equation is comparable to the one calculated by Willett and Ohms (1957) for 16 bulls (Y=-776.18+37.73X), and another computed by Hahn *et al.* (1969) for 35 bulls (Y=-1298.5+50.2X). However, the equation obtained in the present study reduces the minimum value of scrotal circumference from which testes weight may be estimated, and therefore may be useful in young bulls.

The scrotal circumference of N'Dama, Bunaji and Sokoto-Gudali bulls are smaller than those reported for most temperate breeds at corresponding ages (Ball *et al.*, 1974; Coulter *et al.*, 1975; Almquist *et al.*, 1976). In view of the close relationship between scrotal circumference and spermatozoa production (Willett and Ohms, 1957; Hahn *et al.*, 1969; Almquist *et al.*, 1976) it may be expected that sperm output potential in the West African breeds will be lower than in temperate breeds, although this needs to be investigated. On the basis

of either simple correlations or partials, scrotal circumference appears to be more related to body weight than to age. Nevertheless, the high correlations obtained makes it possible to predict scrotal circumference from age or liveweight. Ball *et al.* (1974) have suggested incorporating scrotal circumference measurements for evaluating bulls for breeding soundess because it is a more accurate index of semen producing capacity. However, further research needs to be done before standards for selection can be set.

SUMMARY

A series of studies were conducted to estimate testicular size from scrotal measurement and to determine the relationships between testicular size, age and liveweight in some West African bulls.

Repeatability estimate for scrotal circumference measurements between technicians was 0.96. Scrotal circumference was highly correlated to paired testes weight (r=0.88) and volume (r=0.87). Testes weight and volume were closely related (r=0.997) but their ratio (g/cc) varied in 30 bulls from 0.962 to 1.119, with a mean and standard deviation of 1.027±0.029. The regression equation for estimating testes weight (Y) from scrotal circumference (X) was Y=-506.17+27.70X.

Significant correlations existed between scrotal circumference and age (r from 0.59 to 0.79) or liveweight (r from 0.62 to 0.83) in N'Dama, Bunaji and Sokoto-Gudali bulls. However, examination of the partial correlations showed that most of the age effects were attributable to changes in liveweight.

REFERENCES

ALMQUIST, J.O., BRANAS, R.J. and BARBER, K.A. 1976. Postpuberal changes in semen production of Charolais bulls ejaculated at high frequency and the relation between testicular measurements and sperm output. J. Anim. Sci. 42, 670-676.

AMANN, R.P. and ALMQUIST, J.O. 1962. Reproductive capacity of dairy bulls. VIII. Direct and indirect measurement of testicular sperm production. J. Dairy Sci. 45, 774-781.

BALL, L., NELSON, L.D., FURMAN, J.W. and SEIDEL, JR., G.E. 1974. Semen collection and evaluation of bulls for breeding soundness. Presented, Annual Meeting AVSSBS, Columbia, Missouri.

COULTER, G.H., LARSON, L.L. and FOOTE, R.H. 1975. Effect of age on testicular growth and consistency of Holstein and Angus bulls, J. Anim. Sci. 41, 1383-1389.

HAHN, J., FOOTE, R.H. and SEIDEL, JR., G.W. 1969. Testicular growth and related sperm output in dairy bulls. J. Anim. Sci. 29, 41-47.

NIE, H.H., HULL, C.H., JENKINS, J.G., STEINBRENNER, K. and BENT, D.H. 1975. *Statistical Package for the Social Sciences*. Second Edition. McGraw-Hill Book Company, New York etc.

OSINOWO, O.A. and STEINBACH, J. 1973. Variations in the skinfold thickness of Nigerian and imported cattle breeds. Proc. 14th. Ann. Conf., Science Association of Nigeria, p24. Abstract.

WILLETT, E.L. and OHMS, J.I. 1957. Measurement of testicular size and its relation to production of spermatozoa by bulls. J. Dairy Sci. 40, 1559-1569.

TABLE 1

Comparison of means[1] of scrotal measurements obtained by three technicians on the same 20 bulls.

Scrotal measurement (cm)	Technician			Repeatability
	A	B	C	
Scrotal circumference[2]	30.6±4.0	31.3±4.2	30.4±4.3	0.96
Left scrotal diameter[3]	4.8+1.1	5.1+1.1	5.2+1.1	0.83
Right scrotal diameter[3]	4.8±1.0	5.0±1.0	5.1±1.1	0.78

[1] ±standard deviation

[2] Measured with a tape

[3] Measured with pair of steel calipers

TABLE 2

Age and scrotal circumference in N'Dama (N), Bunaji (B) and Sokoto-Gudali (SG) bulls

Age (months)	Number of bulls			Average scrotal circumference (cm)[1]		
	N	B	SC	N	B	SG
10-15	-	3	12	-	25.4±2.3	16.2±5.7
16-20	2	3	8	20.3±4.2	25.5±0.4	20.8±4.5
21-25	18	4	12	23.7±1.9	25.4±2.6	21.0±3.9
26-30	9	2	4	24.8±1.8	26.6±4.0	23.8±4.7
31-35	2	-	6	25.2±2.7	-	25.8±3.9
36-60	2	3	8	26.6±3.1	34.3±3.1	30.1±2.6
61-85	-	-	5	-	-	34.3±1.4
> 120	-	-	2	-	-	36.8±0.4

[1] ± standard deviation

TABLE 3

Relationship between scrotal circumference, age and bodyweight[1]

Breed	Correlation between scrotal circumference and: Age	Bodyweight
NDAMA	.59	.62
BUNAJI	.79	.82
SOKOTO-GUDALI	.77	.83

[1]All Correlation coefficients highly significant (P<.001).

TABLE 4

Regression equations for predicting scrotal circumference (Y), from age (X_1) or bodyweight (X_2)

Breed	Predictor	Prediction equation	S.E.b	S.E.e
N'Dama	X_1	$Y=2.091+15.628 \log X_1$	3.622	1.935
	X_2	$Y=-26.198+22.433 \log X_2$	3.561	1.621
Bunaji	X_1	$Y=106.047-131.493 \log X_1 +53.442(\log X_1)^2$	62.797;22.638	2.983
	X_2	$Y=15.4444+0.044X_2$	0.005	1.634
Sokoto-Gudali	X_1	$Y=3.690+15.822 \log X_1$	1.937	2.468
	X_2	$Y=-41.122+28.095 \log X_2$	2.209	1.709

S.E.b.= Standard error of slope

S.E.e. = Standard error of estimate

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Figure 1.

Relationship between testicular weight and scrotal circumference in West African bulls.

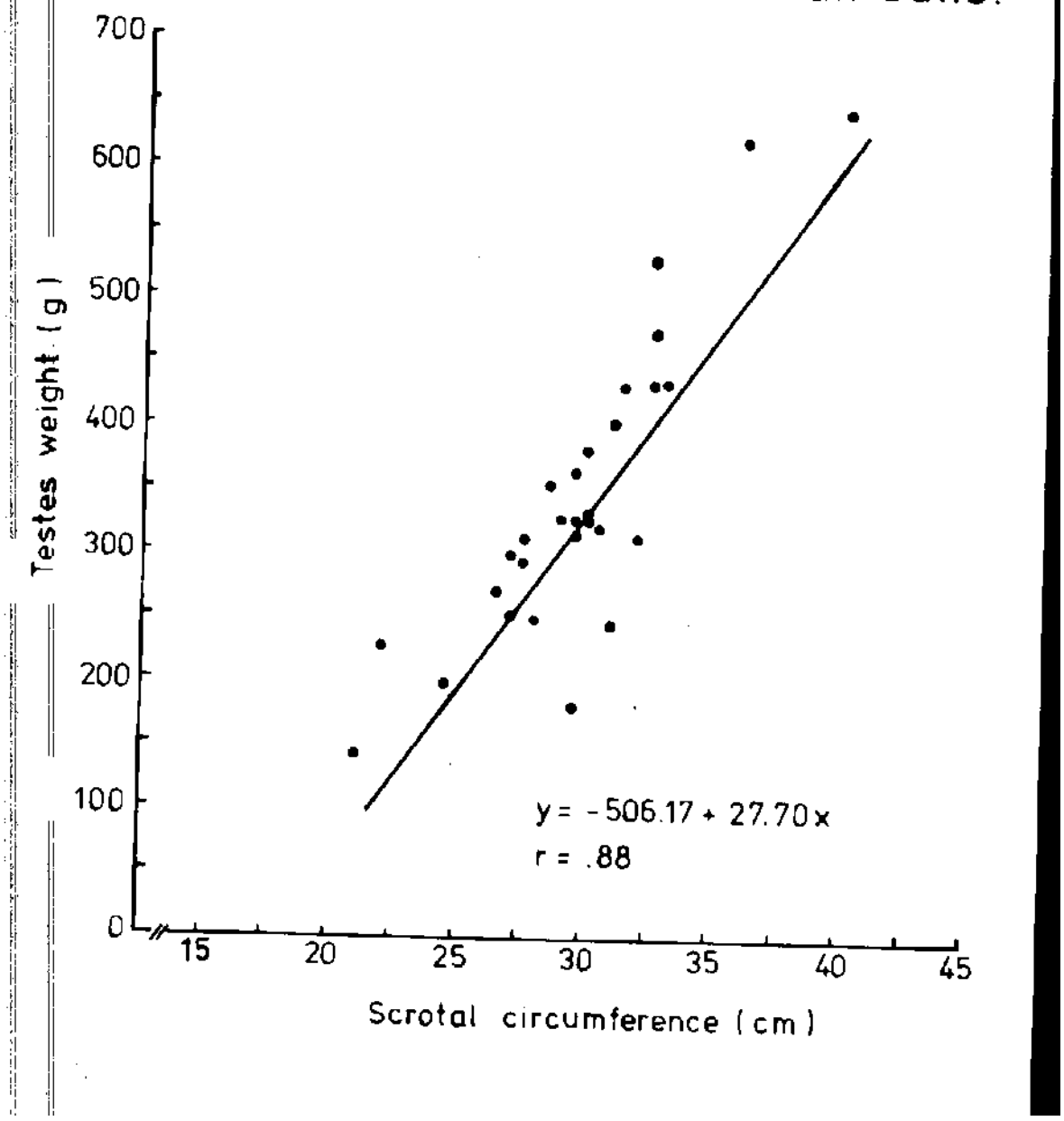
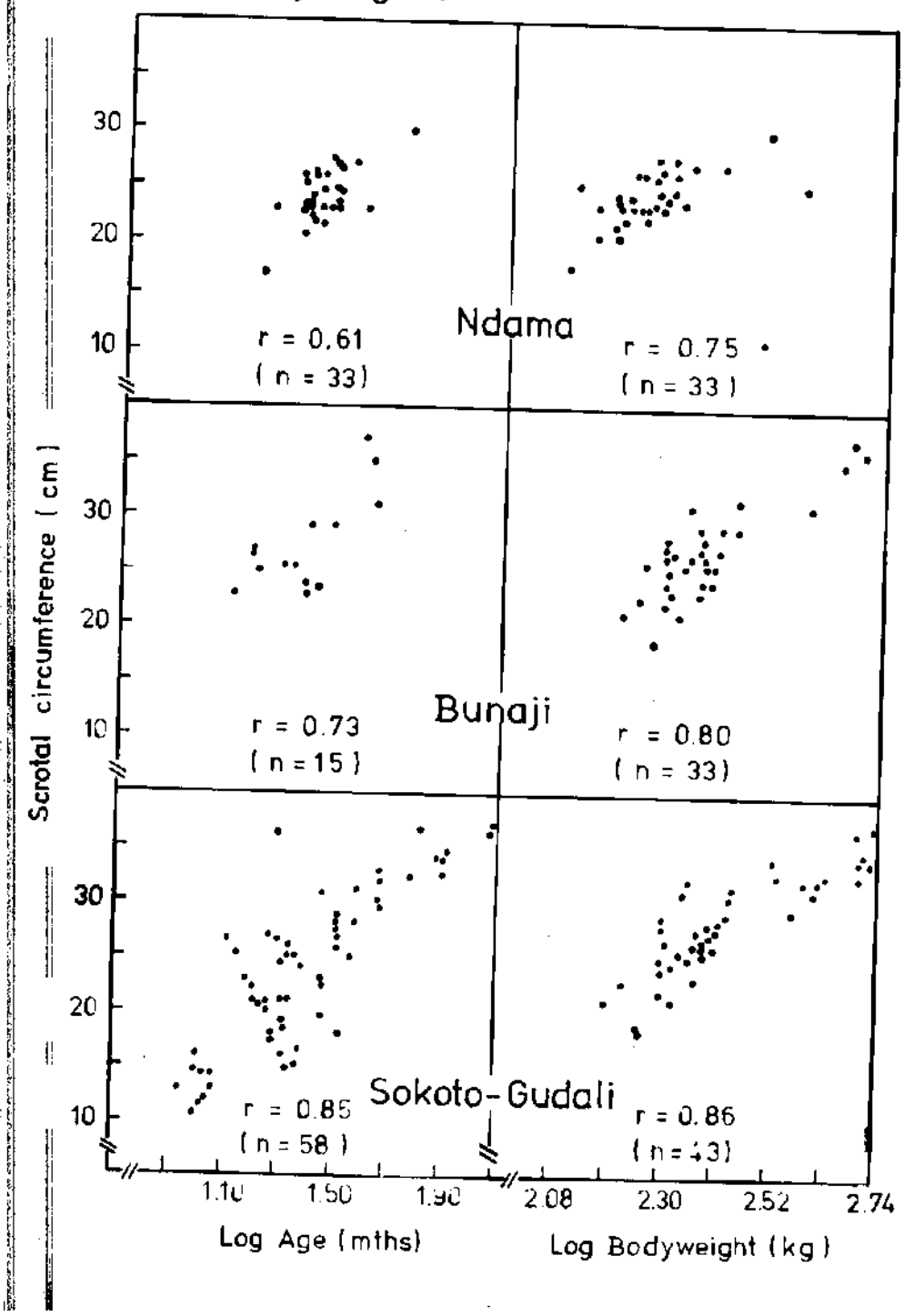


Figure 2
Scrotal circumference of bulls in relation to log (age and log (bodyweight)



PART III

GROWTH, TESTICULAR DEVELOPMENT AND SPERMATOGENESIS IN BUNAJI BULLS

INTRODUCTION

The relationship between growth, testicular development and spermatogenesis in bulls is a very important one for Artificial Insemination Centres and Progeny Test Stations and has been studied in several breeds (Willett and Ohms, 1957; Almquist and Amann, 1962; Macmillan and Hafs, 1969; Almquist *et al.*, 1976). Commencement of semen collection at the earliest possible age enhances quick determination of the genetic merit of bulls.

Few systematic investigations on growth and spermatogenesis have been carried out on West African breeds, hence the present study on Bunaji cattle.

MATERIALS AND METHODS

Twenty Bunaji bulls calves aged 3-18 months were fed hay or silage *ad libitum* plus 2.27 kg/head/day of concentrate containing 22% cottonseed, 27% groundnut meal and 51% ground guineacorn, with water and mineral salt also supplied *ad libitum*.. The bulls were weighed and

their heartgirth and scrotal circumference measured monthly for one year. Before each bull was slaughtered, the bodyweight, scrotal circumference and scrotal skinfold thickness were determined. After slaughter, the testes were excised and the epididymides were carefully removed from the organs with a scalpel. The right and left testes were weighed separately and their volumes determined by water-displacement. Thin slices of tissue for histology were removed from both testes. Each epididymis was divided into caput, corpus and cauda portions and separately weighed.

Gonadal and epididymal sperm reserves were determined by the method of Igboeli and Rakha (1971) with some modifications. After removal of the tunica albuginea with a scalpel, the testis was homogenized in 50 ml physiological saline using an 'atomix' blender operated at full speed for 2 minutes. Included in the physiological saline were the antibiotics streptomycin sulphate (1 mg/ml) and sodium penicillin G (1000 I.U./ml). The honogenate volume was measured after rinsing the blender container with 20 ml of saline and adding the effluent.

Five ml of the homogenate was transferred to a conical flask and further diluted with 80 ml of saline. The caput, corpus and cauda epididymides were minced separately in 20 ml of saline with a pair of scissors. The diluted testicular homogenates and the epididymal samples were stored overnight at 5C before the latter were filtered through gauze and the filtrate volumes were measured. One ml of the epididymal filtrate was further diluted with 2 ml of saline. The sperm/spermatid numbers in the testicular and epididymal samples were determined with a haemocytometer.

The testicular tissue slices for histology were fixed in Bouin's fluid for 6 hours, washed in several changes of 50% alcohol for another 6 hours and then stored in 70% alcohol. The tissue blocks were embedded in paraffin wax and sections 7μ thick were prepared for staining with haematoxylin and eosin. Three slides, each containing 2-4 sections, were made per bull. Seminiferous tubule diameter was calculated as the mean diameter of 30 approximately round tubules, 10 from each slide.

Analysis of data was by standard statistical procedures (Steel and Torrie, 1960).

RESULTS

The data on heartgirth, bodyweight and scrotal circumference taken monthly on the 20 bulls over the one year period of the study are summarized in Table 1. Excluded from the table are data for age-groups below 5 months and above 24 months since only two bulls were involved. Correlation coefficients between all the possible pairs of variables were highly significant ($P<0.01$) and were as follows: Age and bodyweight ($r=0.87$), age and heartgirth ($r=0.84$), age and scrotal circumference ($r=0.88$), heartgirth and scrotal circumference ($r=0.86$), bodyweight and scrotal circumference ($r=0.88$), heartgirth and bodyweight ($r=0.95$) .

The relationship found between scrotal circumference (SC) and testes weight (TW) when the bulls were slaughtered is described by the regression equation:

$$TW = -408.57+25.6(SC)$$

(r = 0.94; standard error of estimate=27.98)

Subtraction of scrotal skinfold thickness (SST) from scrotal circumference before regression on testes weight yielded the equation:

$$TW=-406.46+25.97(SC-SST)$$

(r=0.94; standard error of estimate=27.2)

Table 2 shows the ages at which the bulls were slaughtered and the respective paired testes weights, mean seminiferous tubule diameters and, gonadal and epididymal sperm reserves. The relationships between various parameters examined at the time of slaughter (ante- and post-mortem) are presented in Table 3. All the correlations in the matrix are positive and highly significant ($P<0.01$).

The relationship between gonadal sperm/spermatid numbers (GS; *in billions*) and scrotal circumference (*in centimeters*) in this study is described by the regression equation:

$$GS = -28.9 + 1.52\ (SC)$$

(r=0.69; standard error of estimate = 4.66)

Inclusion of age (A; *in months*) and bodyweight (BW; *in kilograms*) increases the coefficient of determination (r^2) from 0.48 to 0.53. The resulting multiple regression equation is:

$$GS=-28.89+0.47(A)-0.003(BW)+1.17(SC)$$

(r=0.73; standard error of estimate = 4.7)

Plates I-IV are photomicrographs showing details of the seminiferous tubules of different bulls in cross-section.

DISCUSSION

A number of inferences can be made from the results of this investigation. Bunaji bulls have smaller body size and scrotal circumference when compared to Holstein, Angus, Charolais and Hereford bulls at corresponding ages (Amman and Almquist, 1962; Hahn *et al* ., 1969; Coulter *et al* ., 1975; Elmore *et al* ., 1976). The relationships between age, body size and scrotal circumference are very close in young Bunaji bulls as evidenced by the high correlation coefficients. Between the ages of 3 to 26 months, liveweight (in kg) may be accurately predicted in this breed from heartgirth measurements (in cm). The regression equation is Y=-288.68+3.50X, which has a high correlation coefficient (r=0.95).

The relationship between scrotal circumference and paired testes weight was closer in this investigation than in the study reported in Part II (r=0.88 v r=0.94). This may be partly due to the involvement of only one breed in the present study. It appears that the variation in

scrotal skin thickness may be disregarded within each breed without much loss in accuracy of prediction, since the subtraction of scrotal skinfold thickness from scrotal circumference before regression on testes weight did not significantly improve results (r^2=0.83 v$\frac{s}{s}^2$=0.89).

In Bunaji bulls, puberty can be assumed to occur before 13 months of age, as evidenced by the presence of spermatozoa in the testes and epididymides by that age (Table 2). However, sexual maturity is not attained till about 17 months of age at which time consistently large gonadal and epididymal sperm reserves first became apparent. It would appear pointless to start routine semen collection before this age.

Scrotal circumference appears to be a good indicator of testicular development and when considered along with age and bodyweight, it can also serve as a good indicator of spermatogenesis in young Bunaji bulls.

SUMMARY

The relationship between growth, testicular development and spermatogenesis was studied in 20 young Bunaji bulls over a one year period. Highly significant correlations were obtained between age and bodyweight (r=0.87), age and heartgirth (r=0.84), age and scrotal circumference (r=0.89), heartgirth and scrotal circumference (r=0.86), bodyweight and scrotal circumference (r=0.88), and, heartgirth and bodyweight (r=0.95). The relationship between heartgirth (X) and bodyweight (Y) was described by the equation Y=-288.68+3.50X.

Scrotal circumference (SC) was highly correlated with testes weight (TW), (r=0.94); this relationship was described by the regression equation TW=-408.57+25.6 (SC).

Although spermatogenesis had commenced in Bunaji bulls by 13 months of age, consistently large gonadal and epididymal sperm reserves were not apparent until they were 17 months and above. Scrotal circumference appeared to be a good indicator of spermatogenesis in this breed, when considered along with age and bodyweight.

REFERENCES

ALMQUIST, J.O. and AMANN, R.P. 1962. Effect of a high ejaculation frequency on sperm output and semen characteristics of Holstein bulls from puberty to 2 years of age. J. Dairy Sci. 45, 699 (Abstract).

ALMQUIST, J.O., BRANAS, R.J. and BARBER, K.A. 1976. Postpuberal changes in semen production of Charolais bulls ejaculated at high frequency and the relation between testicular measurements and sperm output. J. Anim. Sci. 42, 670-676.

AMANN, R.P. and ALMQUIST, J.O. 1962. Reproductive capacity of dairy bulls. VIII. Direct and indirect measurement of testicular sperm production. J. Dairy Sci. 45, 774-781.

COULTER, G.H., LARSON, L.L. and FOOTE, R.H. 1975. Effect of age on testicular growth and consistency of Holstein and Angus bulls. J. Anim. Sci. 41, 1383-1389.

ELMORE, R.G., BIERSCHWAL, C.J. and YOUNGQUIST, R.S. 1976. Scrotal circumference measurements in 764 beef bulls. Theriogenology 6, 485-494.

HAHN, J., FOOTE, R.H. and SEIDEL, JR., G.E. 1969. Testicular growth and related sperm output in dairy bulls. J. Anim. Sci. 29, 41-47.

IGBOELI, G. and RAKHA, A.M. 1971. Gonadal and extragonadal sperm reserves of indigenous Central African bulls. J. Reprod. Fert. 25, 107.

MACMILLAN, K.L. and HAFS, H.D. 1969. Reproductive tract of Holstein bulls from birth through puberty. J. Anim. Sci. 28, 233-239.

STEEL, R.G.D. and TORRIE, J.H. 1960. *Principles and procedures of statistics*. McGraw-Hill Book Co., Inc., New York.

WILLETT, E.L. and OHMS, J.I. 1957. Measurement of testicular size and its relation to production of spermatozoa by bulls. J. Dairy Sci. 40, 1559-1569.

TABLE 1

Age-changes in bodyweight, heartgirth and scrotal circumference in Bunaji bulls.

Age (mth)	No. of animals	Bodyweight (kg)	Heartgirth (cm)	Scrotal circumference (cm)
5	3	72.0±33.9	97.3±13.3	12.7±1.0
6	4	80.8±29.2	106.0±10.4	13.0±1.3
7	6	91.2±21.8	107.8±6.1	13.6±1.8
8	7	101.0±21.3	110.9±6.2	14.0±2.0
9	10	109.8±18.7	115.3±6.7	15.5±2.2
10	11	119.5±16.7	118.3±5.4	16.5±2.1
11	12	133.3±20.4	121.7±5.7	17.6±2.5
12	16	139.8±22.1	124.7±5.0	19.2±2.0
13	15	151.7±20.2	127.2±6.0	20.1±2.1
14	15	158.1±24.4	127.5±6.7	21.0±2.3
15	15	167.7±31.9	130.1±8.4	21.9±3.0
16	15	181.0±25.8	133.5±8.1	22.8±2.5
17	13	189.5±22.6	136.5±6.7	24.2±1.8
18	13	193.2±29.1	137.2±6.4	24.4±2.2
19	9	192.6±21.9	137.7±6.3	25.1±1.9
20	8	201.0±22.9	139.6±5.4	25.5±1.5
21	7	216.0±16.4	139.9±2.4	25.4±1.6
22	4	211.5±12.1	142.0±5.0	26.4±1.0
23	4	223.3±13.9	141.8±5.4	26.8±1.7
24	3	228.0±18.7	145.3±4.0	26.4±2.3

Mean ± S.D.

TABLE 2

Testicular development and sperm reserves in Bunaji bulls.

Age (months)	Testes weight (g)	Seminiferous tubule diameter (μ)	Gonadal sperm reserve ($x10^9$)	Epididymal sperm reserve ($x10^9$)
13	167.5	194.0±24.5	3.693	0.215
14	75.0	158.6±14.3	1.488	0.010
14	105.6	146.0±13.0	0.656	0.004
16	91.5	137.5±20.4	0.184	0.015
17	225.1	232.2±18.8	11.192	6.971
17	125.7	172.0±14.8	3.369	0.204
17	276.4	222.9±16.3	8.801	4.939
18	242.1	202.9±19.1	9.767	5.063
18	232.4	220.0±17.7	8.466	2.908
19	239.5	213.4±28.4	9.251	0.900
19	318.5	217.6±17.3	13.231	7.299
19	225.0	233.8±15.7	8.050	2.345
20	195.2	195.2±20.3	4.958	0.408
21	202.0	196.0±13.9	2.202	0.116
21	189.7	230.6±17.4	7.959	5.709
22	349.0	257.8±25.2	20.453	9.025
24	172.3	187.5±13.4	3.090	1.729
24	250.6	236.7±27.5	8.652	5.455
25	347.1	244.0±29.4	11.462	9.725
27	284.2	-	24.720	6.282

Mean ± S.D.

TABLE 3

Correlation Matrix

Parameters	1	2	3	4	5	6	7	8	9
1. Age	1								
2. Bodyweight	0.67	1							
3. Scrotal circumference	0.61	0.82	1						
4. Testes weight	0.62	0.75	o.94	1					
5. Testes volume	0.62	0.76	0.93	1.00	1				
6. Epididymes weight	0.78	0.82	0.85	0.86	0.86	1			
7. Gonadal sperm reserve	0.60	0.61	0.69	0.81	0.82	0.70	1		
8. Epididymal " "	0.55	0.59	0.73	0.84	0.85	0.73	0.79	1	
9. Seminiferous tubule diameters	0.60	0.70	0.80	0.85	0.85	0.71	0.84	0.80	1

PLATE 1

Cross-section of the testis

of a 14 months old bull.

Magnification: x200

H&E stain

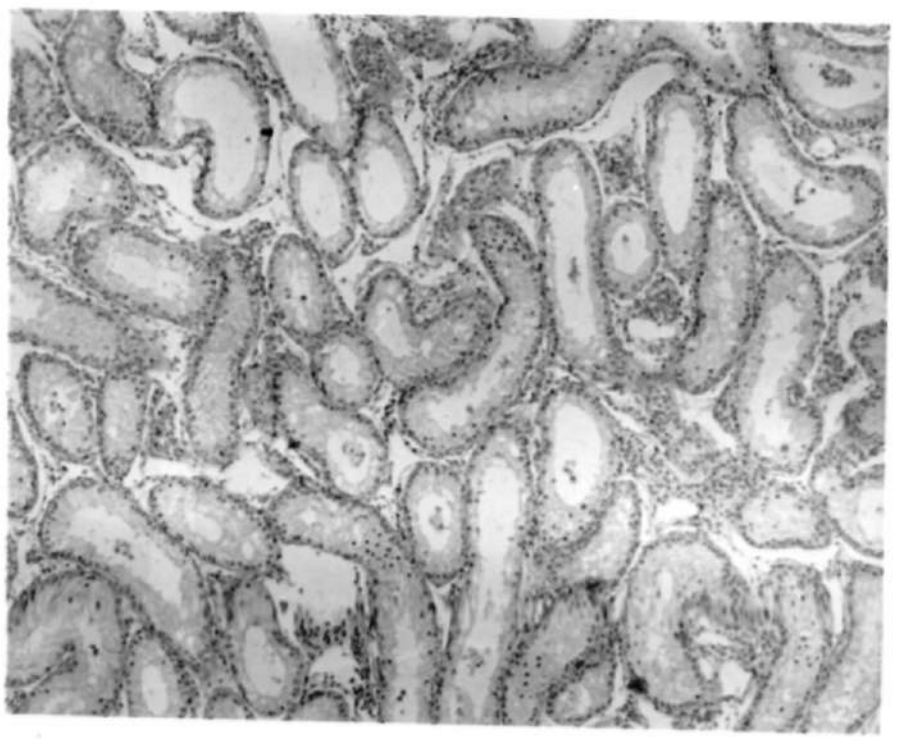
PLATE 2

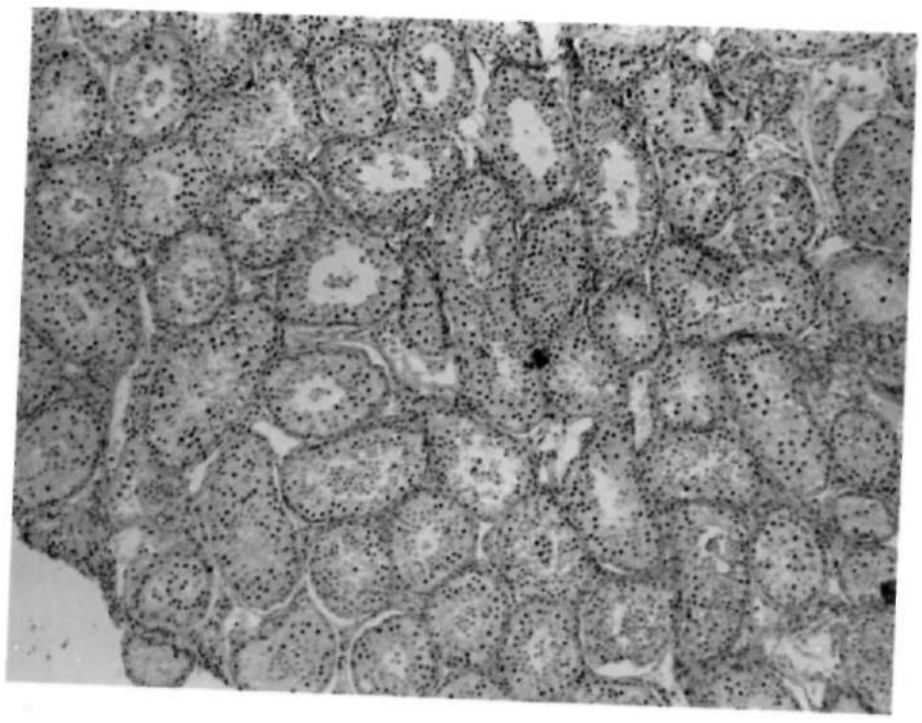
Cross-section of the testis

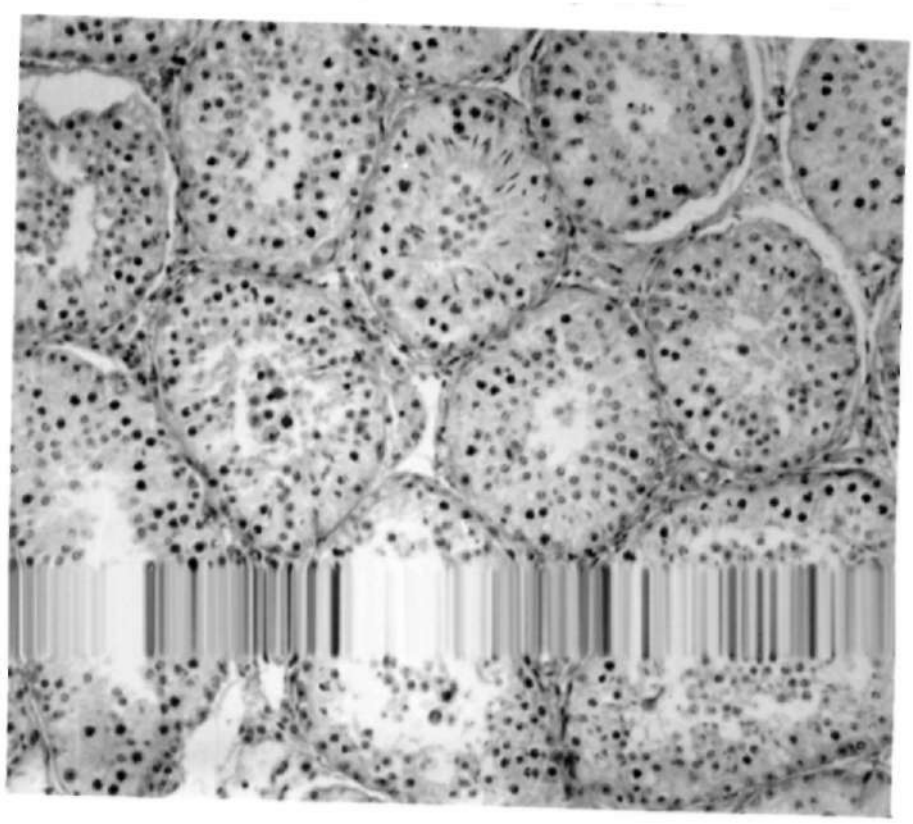
of a 24 months old bull.

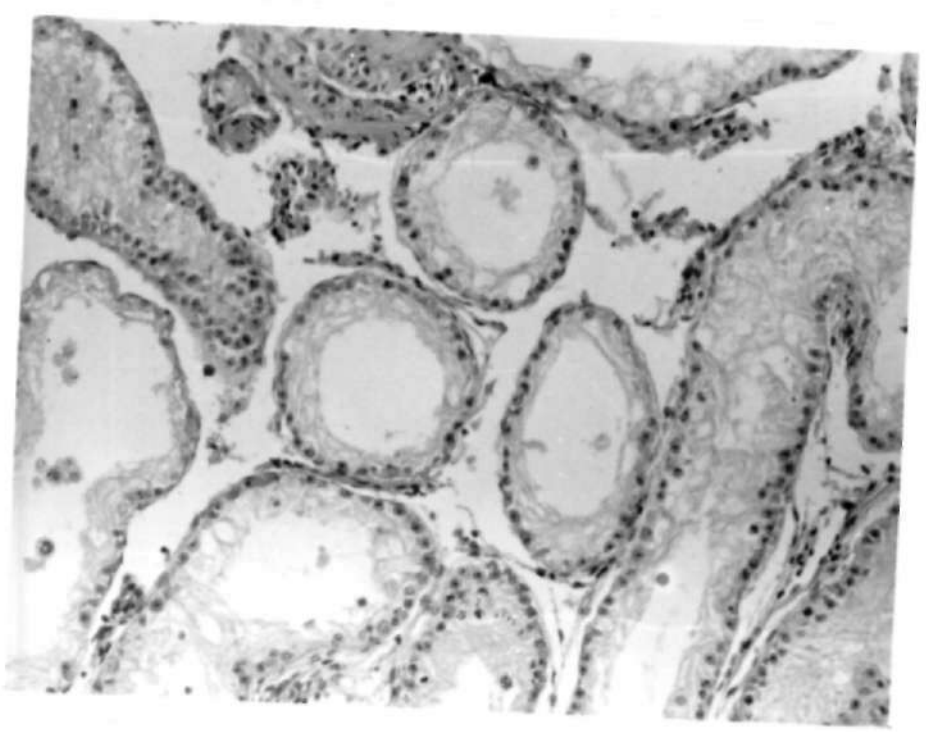
Magnification: x200

H&E stain









PART IV

SEMEN QUALITY OF SOKOTO-GUDALI AND BUNAJI BULLS

INTRODUCTION

Few systematic investigations have been conducted on the semen quality of indigenous West African bulls. Such studies are important since findings will be useful in diagnosing cases of bull infertility and in planning semen collection and storage procedures for artificial insemination.

The present investigation was carried out to determine some biophysical and biochemical characteristics of the semen of Sokoto-Gudali and Bunaji bulls normally used in the laboratory assessment of semen quality.

MATERIALS AND METHODS

The study was carried out in two parts. In the first part, the characteristics of 35 ejaculates collected from 9 Sokoto-Gudali and 2 Bunaji bulls were investigated. Parameters examined were ejaculate volume, semen concentration, sperm motility, total sperm-, and total motile sperm per ejaculate. In the second part of the

study, a more detailed investigation was conducted on the ejaculates from 10 bulls. Morphological examinations of spermatozoa were carried out. The activities of the enzymes glutamic oxaloacetic transaminase (GOT) and lactic dehydrogenase (LDH) in seminal plasma and whole semen were determined.

Semen was collected by electroejaculation in 31 cases and by artificial vagina in 4 cases. The bulls were untrained and reacted slowly to a teaser. False-mounting before semen collection was not possible on a routine basis.

Sperm motility was assessed as soon as possible after semen collection, usually within 10 to 45 minutes. A drop of semen placed on a slide was examined under coverslip at X400 magnification using a light microscope fitted with a warm stage.

Semen concentration was determined either by haemocytometer counts or by a photoelectric method. In the latter case, 0.05 ml of semen was diluted to 10 ml with 0.9% saline and the absorbance was determined in duplicate against a blank (0.9% saline) using a Beckman C-400

spectrophotometer (1 cm cuvettes, 546 nm). Actual concentration was determined from a standard curve.

Percentage of 'live' spermatozoa was determined by the method of Dott and Foster (1972). One drop of semen was added to 8 drops of eosin-nigrosin stain heated to 30C. The mixture was incubated for 5 minutes at 30C and smears made on preheated slides. The smears were cleared in acetic acid - perchloric acid mixture, washed briefly in tap-water and dried in ethanol and ether. The smears were examined under oil-immersion and the percentage of 'live' sperm was taken as the mean of duplicate counts of unstained cells per 100 spermatozoa.

Sperm morphology was determined with a phase contrast microscope using the same slides and counting technique as above. Spermatozoa with intact and undeformed head, tail and middle piece were classified as normal. Defects such as pyriform head, tapering head, micro head and giant head were classified as primary abnormalities. Swollen head, detached head, missing acrosome and coiled tails were classified as secondary abnormalities.

The enzymes LDH and GOT were assayed using Clinical Assay Kits (BDH Chemicals Ltd., Poole, England). Spermatozoa were separated from seminal plasma by centrifugation at 35,000 g for 30 minutes at 20C. For whole semen determinations, spermatozoa were first disrupted by rapid freezing in liquid nitrogen and thawing before centrifugation.

RESULTS

The results of the investigations are presented in Tables 1 and 2. In the first part of the study, semen samples collected by electroejaculation had a higher mean ejaculate volume but was less concentrated than semen collected by artificial vagina. Motility, total sperm-, and total motile sperm per ejaculate had higher averages for electroejaculated samples.

In the second part of the study involving the ejaculates from 10 bulls (Table 2), mean semen volume was 4.6 ml. Average semen concentration was 520 million spermatozoa per millilitre. Total motile spermatozoa averaged 2241 million per ejaculate.

Estimates of the mean percentages of motile, live and normal spermatozoa were 86.5, 69.0 and 76.2 respectively. The correlations between these three measures of spermatozoa viability were as follows:

% live sperm and % normal sperm, r=0.89

% live sperm and % motile sperm, r=0.78

% motile sperm and % normal sperm, r=0.66

The mean activities of LDH and GOT in whole semen were 331.1 and 82.8 milli-International Units per milliliter respectively. Whole semen contained on the average, 28% more LDH and 39% more GOT than seminal plasma.

DISCUSSION

All the parameters examined in this study were subject to considerable variation between ejaculates and between bulls. On the basis of sperm motility or morphology, the quality of most of the ejaculates examined would qualify as good to very good. However, when compared to

temperate breeds (e.g. Bishop and Hancock, 1955) the local breeds appear to have smaller ejaculates with lower sperm concentrations. This may be partly due to the limited sexual preparation before the bulls were allowed to serve the artificial vagina in this study. Another possible cause for the lower semen concentrations and volumes may be the relatively small testicular size in these breeds, as previously reported in Parts II and III of this dissertation. Testicular size is related to spermatozoa output in bulls (Willet and Ohms, 1957). Similar observations were reported for the Angoni bulls of Central Africa by Igboeli and Rakha (1971).

The ultimate proof of the viability of a spermatozoon is its ability to fertilize an ovum. In using laboratory tests to assess semen quality, it should be noted that part of the variations in the fertility of bull semen may be due to the female and other factors having nothing to do with the semen under test (Bishop and Hancock, 1955).

In the present study, different values were obtained for the mean percentages of live, motile and normal spermatozoa. It may well be that all three factors are essential for sperm viability, although this is uncertain. The incidence of primary and secondary abnormalities in the samples examined were low and certain types of abnormalities such as double head or double tail were not encountered by this investigator.

In general, it may be concluded from this study that the semen of Sokoto-Gudali and Bunaji bulls is qualitatively similar to semen of other breeds. However, semen concentration and volume appear to be lower in these breeds compared to temperate breeds.

SUMMARY

A total of 35 ejaculates were collected from Sokoto-Gudali and Bunaji bulls and subjected to physical, morphological and biochemical examination. Semen collected by electroejaculation had higher mean volume, motility and total sperm number but lower concentration than semen collected by artificial vagina.

The ejaculates from 10 bulls were examined in greater detail. Total sperm per ejaculate averaged 2.241 $x10^9$ while motility averaged 86.5%. The mean percentages of 'live' and normal sperm were 69.0 and 76.2% respectively. The incidence of sperm abnormalities was generally low. The activities of lactic dehydrogenase and glutamic oxaloacetic transaminase in seminal plasma and whole semen were determined.

REFERENCES

BISHOP, M.W.H. and HANCOCK, J.L. 1955. The evaluation of bull semen. Vet. Record 67, 363-367.

DOTT, H.M. and FOSTER, G.C. 1972. A technique for studying the morphology of mammalian spermatozoa which are eosinophilic in a differential 'live/dead' stain. J. Reprod. Fert. 29, 443-445.

IGBOELI, G. and RAKHA, A.M. 1971. Ejaculation and ejaculate characteristics of Angoni (Shorthorn zebu) bulls. J. Reprod. Fert. 26, 183-187.

WILLET, E.L. and OHMS, J.I. 1957. Measurement of testicular size and its relation to production of spermatozoa by bulls. J. Dairy Sci. 40, 1959.

TABLE I

Characteristics of ejaculates collected by artificial vagina and by electroejaculation

Characteristics	Mean	Range	n
Electroejaculation			
Volume (ml)	5.5	1.2-13.3	31
Concentration (x 10^9/ml)	0.637	0.130-1.626	29
Total spermatozoa (x10^9)	3.800	0.442-18.194	29
Motile spermatozoa (%)	87.7	60-95	31
Total motile spermatozoa (x10^9)	3.291	0.332-16.375	29
Artificial vagina			
Volume (ml)	3.6	1.5-9.0	4
Concentration (x 10^9/ml)	0.929	0.379-1.398	4
Total spermatozoa (x10^9)	2.307	1.408-3.411	4
Motile spermatozoa (%)	80.0	50-95	4
Total motile spermatozoa (x10^9)	1.948	0.704-3.240	4

ABLE 2 Semen characteristics

.l No.	Breed	Collec-tion method	Volume (cc)	Concn. ($x10^9$/ml)	Total Sperm ($x10^9$)	% Motile	% Live	% Normal	% 1° Abnor-mal	% 2° Abnor-mal	LDH (m-I.U./ml) Seminal Plasma	LDH (m-I.U./ml) Whole Semen	GOT (m-I.U./ml) Seminal Plasma	GOT (m-I.U./ml) Whole Semen
16	Bunaji	EE	3.5	0.51	1.785	95	87.0	85.5	5.0	9.5	318.7	351.7	79.2	80.7
59	S. Gu-dali	AV	1.8	0.78	1.404	50	34.0	61.0	3.0	36.0	242.7	-	66.3	-
21	"	AV	9.0	0.38	3.420	95	75.5	78.5	2.0	19.5	103.9	160.1	31.0	64.0
14	"	EE	1.2	0.55	0.660	90	55.5	76.0	4.0	20.0	226.2	-	29.7	-
13	"	EE	5.0	0.42	2.100	90	69.0	72.5	5.5	22.0	14.7	14.7	23.0	57.6
31	"	EE	8.0	0.55	4.400	95	92.5	81.0	2.5	16.5	137.0	193.1	33.6	80.7
19	"	EE	4.7	0.38	1.786	95	90.0	86.5	2.0	11.5	308.7	573.0	61.5	94.0
36	"	EE	3.3	0.42	1.386	90	52.5	63.0	2.0	35.0	549.9	761.3	49.0	80.5
17	"	EE	5.0	0.53	2.650	80	61.5	74.5	1.5	24.0	229.5	259.2	54.2	74.5
15	"	EE	4.2	0.67	2.814	85	72.5	83.5	2.5	14.0	246.0	335.2	81.0	130.3
	Mean		4.6	0.52	2.241	86.5	69.0	76.2	3.0	20.8	237.7	331.1	50.9	82.8
	Standard deviation		±2.4	±0.13	±1.101	±13.8	±18.6	±8.8	±1.4	±9.0	±144.2	±238.3	±21.1	±22.2

PART V

IN VITRO STORAGE OF INDIGENOUS BULL SEMEN

INTRODUCTION

Cattle artificial insemination practice in Nigeria has depended until very recently exclusively on imported semen of temperate breeds. However, it seems probable that an adequate AI programme will require extensive use of indigenous bull semen so as to maintain the pure bred status of most of the cattle.

Towards achieving the above objective, five factorial experiments reported in this section were undertaken to develop suitable *in vitro* techniques for storing the semen of indigenous cattle breeds. The first two experiments were on short-term storage of semen at 5C while the remaining three experiments were on long-term, deep-frozen storage.

MATERIALS AND METHODS

Semen was collected from Sokoto-Gudali bulls by artificial vagina (AV) in the first experiment and by electroejaculator (EE) in the remaining experiments. The

motility of spermatozoa was assessed under coverslip at X400 magnification and ranged from 80 to 95%. Spermatozoa concentrations were assessed by haemocytometer (Experiments 1-4) or by absorptiometry (Experiment 5). The concentrations were 1.156- and 1.318 x 10^9 spermatozoa per millilitre of semen in samples collected via AV, but ranged from 0.181- to 1.626 x 10^9 per millilitre for semen collected via EE.

The extenders used for semen dilution were the Tris-Yolk (Foote, 1970), Yolk-Citrate (Blackshaw and Salisbury, 1972), Homogenized Milk (Almquist *et al.*, 1954) and the Cornell University Extender (Foote *et al.*, 1960). The composition of these diluents are shown in the Appendix.

The designs of the factorial experiments are shown under Experimental Details and Results.

In Experiment 1, semen samples were diluted at 30C at a rate of 1:19 (semen: diluent, volume/volume), cooled to 5C in 2 hours and stored in an incubator at 5C. Portions of the semen samples were transferred into test-tubes and incubated in a water-bath at 37C for

motility assessments at the commencement of storage (day 0) and again after 1 and 4 days.

In Experiment 2, a dilution ratio of 1:20 was used. Dilution and storage temperatures were as in the first experiment. After 2 days, the semen samples were transferred to a water-bath and incubated at 37C for 30 minutes before evaluation.

In Experiment 3, semen dilution was at 30C, dilution ratio was 1:20 and cooling time to 5C was 2 hours. After cooling, the semen samples were packaged in 0.2 ml plastic straws. The straws were sealed and arranged on a small-mesh wire-gauze and lowered into a liquid nitrogen freezing tank to about 10 cm above the liquid nitrogen level. The tank was covered and the semen allowed to freeze for 30 minutes in the liquid nitrogen vapour. The straws were then transferred into liquid nitrogen for storage. After 16 days' storage the straws were thawed in a water-bath at 37C. The thawed semen samples were transferred into test-tubes and incubated at 37C for 30 minutes before motility assessment.

In Experiment 4, 1-stage dilution involved the addition of extenders containing 4% glycerol (v/v) to semen (at 30C) at a dilution ratio of 1:20. The semen samples were cooled to 5C in 2 hours and frozen with no further dilution. The 2-stage dilution involved an initial dilution of the semen at 30C with extender containing no glycerol, at a dilution ratio of 1:10. The semen samples were cooled to 5C before further dilution with cooled extender containing 8% glycerol to give a final semen dilution ratio of 1:20 and a final glycerol concentration of 4% in the diluent fraction. The semen samples were packaged in straws, frozen in liquid nitrogen vapour and stored for 5 days before evaluation. Thawing and incubation were carried out at 37C.

In Experiment 5, semen samples were diluted with Tris-Yolk diluent containing 4% glycerol at a dilution ratio of 1:10, using a 1-stage dilution at 30C. The samples were cooled to 5C in 2 hours and either packaged in straws and frozen in liquid nitrogen vapour, or frozen on dry-ice (solid carbon dioxide) by the pellet method (Nagase and Niwa, 1964). Each pellet contained 0.1 ml of semen. The frozen pellets and straws were stored in liquid nitrogen for 5 days before evaluation. Thawing of straws was carried out as previously described while the frozen pellets were thawed in dry test-tubes at 37C. Post-thawing incubation was at 37C for 2 hours while the percentages of motile spermatozoa were assessed at 0, 1 and 2 hours after thawing.

The motility data were transformed into angles in all experiments and analysed as for factorial experiments in Experiments 2, 3 and 4, and as for split-plot in Experiments 1 and 5, the sub-plots being length of storage and period of post-thawing incubation respectively. Where significant first order interaction was revealed between bulls and other factors, the interaction mean square was used to test the relevant main effect. All data presented in the results are re-transformed means.

EXPERIMENTAL DETAILS AND RESULTS

Experiment 1

This experiment was of factorial design (4x3x4) and examined the following factors:

1. Type of extender: Yolk-Citrate v Homogenized Milk v Tris-Yolk v Cornell University Extender
2. Length of storage at 5C: 0 v 1 v 4 (days)
3. Replicate: Four replicates from semen of 2 bulls.

The summary of the analysis of variance is presented in Table 1. There was no significant difference in the percentage of motile spermatozoa between replicates. The extenders differed significantly ($P<0.05$), the mean percentages of motile spermatozoa being 31.4, 45.9, 50.6 and 49.1 for Yolk-Citrate, Homogenized Milk, Tris-Yolk and Cornell University Extender respectively. Length of storage had a significant effect on spermatozoa survival ($P<0.005$), the mean percentage of motile sperm being 73.4 on day 0, 54.9 (day 1) and 9.7 (day 4). However, there was a significant interaction

between type of extender and length of storage ($P<0.005$; Table 2). This was due to the more rapid decline of sperm motility with increasing length of storage in Yolk-Citrate and Homogenized Milk diluents compared to Tris-Yolk and Cornell University Extender.

Experiment 2

The factors included in this experiment (3x4x3 factorial) were the following:

1. Type of extender: Homogenized Milk v Tris-Yolk v Yolk-Citrate
2. Glycerol concentration: 0 v 2 v 4 v 6 (%, v/v)
3. Replicate: Semen from 3 bulls.

The summary of the analysis of variance is presented in Table 3. The inclusion of glycerol in the extender did not improve survival of spermatozoa stored for 2 days at 5C. However, differences due to type of extender were highly significant ($P<0.005$). The mean percentages of surviving spermatozoa were 7.8, 62.5 and 40.5 for Homogenized Milk, Tris-Yolk and Yolk-Citrate diluents respectively. Yolk-Citrate was

highly significantly better than Homogenized Milk ($P<0.005$) while the Tris-Yolk diluent was best ($P<0.05$).

Experiment 3

This experiment (3x4x3 factorial) was on deep-frozen storage of bull semen. Factors investigated were as follows:

1. Type of extender: Homogenized Milk v Tris-Yolk v Yolk-Citrate
2. Glycerol concentration: 0 v 2 v 4 v 6 (%, v/v)
3. Replicates: Semen from 3 bulls.

The analysis of variance is summarized in Table 4. The percentage of spermatozoa surviving the freezing and thawing process was not significantly different between replicates. However, survival was highly influenced by type of extender ($P<0.005$). The mean percentages of motile spermatozoa following a 30-minute post-thawing incubation period were 6.1, 21.7 and 3.0 for Homogenized Milk, Tris-Yolk and Yolk-Citrate diluents respectively.

The glycerol concentration in the extenders significantly affected spermatozoa survival ($P<0.005$), the response being mainly linear ($P<0.005$). The percentage of motile cells for 0, 2, 4 and 6% glycerol levels were 1.0, 5.1, 19.5 and 17.0 respectively.

Experiment 4

Factors investigated in this semen freezing experiment (3x2x4 factorial) were the following:

1. Type of extender: Homogenized Milk v Tris-Yolk v Yolk-Citrate
2. Method of dilution: 1-stage dilution at 30C v 2-stage dilution at 30 and 5C
3. Replicates: Semen from 4 bulls.

The summary of the analysis of variance is presented in Table 5. There was no significant difference between replicates. The percentage of surviving spermatozoa after deep freezing and thawing was not significantly different between the 1-stage and 2-stage dilution methods (23.9 v 36.6%).

Type of extender had a significant effect on survival ($P<0.005$). The mean percentages of motile spermatozoa in Homogenized Milk, Tris-Yolk and Yolk-Citrate extenders were 20.8, 53.9 and 18.4 respectively.

Experiment 5

Factors examined in this experiment (2x3x3x4 factorial) were as follows:

1. Freezing method: Liquid nitrogen vapour-freezing of semen in straws v Pellet-freezing on dry-ice (solid carbon dioxide)
2. Type of extender: Tris-Yolk v Yolk-Citrate v Cornell University Extender
3. Post-thawing incubation: 0 v 1 v 2 (hours)
4. Replicate: Semen from 4 bulls.

The results are presented in Table 7 and the analysis of variance in Table 6. Significant differences were found between bulls ($P<0.01$). Freezing method had a highly significant effect on survival rate

(P<0.005). The mean percentage of motile spermatozoa was 17.6 for semen frozen in liquid nitrogen vapour, compared to 32.4% for semen frozen on dry-ice by the pellet method.

Tye of extender had a highly significant effect on survival (P<0.005). The mean survival were 39.9, 22.3 and 13.9% for Tris-Yolk, Yolk-Citrate and Cornell University Extender respectively. However, there was a significant interaction between freezing method and type of extender (P<0.01; Table 7). This was due to the better survival rates obtained following pellet-freezing with the Tris-Yolk and Yolk-Citrate diluents in contrast to the Cornell University Extender which gave better result with the liquid nitrogen vapour-freezing method.

Post-thawing incubation for 0, 1 and 2 hours had an increasingly adverse effect on survival rate (33.3 v 23.4 v 17.9% respectively; (P<0.005).

DISCUSSION

Results obtained in Experiments 1 and 2 for short-term *in vitro* storage of semen show the following optimal conditions for spermatozoa survival: Dilution of semen at 30C with Tris-Yolk or Cornell University Extender without glycerol and storage at 5C. Results with Yolk-Citrate and Homogenized Milk diluents were poorer and less consistent, probably due to poorer buffering capacity. Despite the heat treatment and antibiotics used for Homogenized Milk, many samples were observed as having rather high bacterial counts, which could partly account for its poor performance.

Long-term storage of semen is at present only possible through deep freezing. Of the factors examined in Experiments 3 and 4, best results were given by the Tris-Yolk extender containing 4% glycerol. A two-stage dilution also appeared better than a one-stage dilution on the basis of post-thawing motility although the difference was not statistically significant. Crabo

et al. (1970) reported that enzyme loss from spermatozoa, which is thought to be an indication of membrane damage, was higher when glycerol was added at 37C compared to addition at 5C. The latter is achieved through a two-stage dilution. In Experiment 5, freezing of semen extended with the Tris-Yolk diluent by the pellet method gave the best overall results.

Field AI trials should be planned to provide data on the fertility of semen processed using the optimal conditions for *in vitro* storage obtained in this study.

SUMMARY

Two factorial experiments were carried out on the short-term storage of semen at 5C. Factors examined were type of extender, length of storage, optimum level of glycerol in the diluent and method of dilution. The Tris-Yolk diluent and the Cornell University Extender gave good results while Yolk-Citrate and Homogenized Milk extenders gave poorer and less consistent results. Inclusion of glycerol in the extenders did not improve spermatozoa survival.

For long-term storage of semen at -196C, three factorial experiments were carried out to investigate the effects of diluent type, glycerol level, method of dilution and freezing method on spermatozoa survival. In Experiments 3 and 4, cooled extended semen was packaged in 0.2 ml plastic straws and frozen in liquid nitrogen vapour. Best results were obtained with Tris-Yolk diluent containing 4% glycerol, added as 0 and 8% in the diluent in a 2-stage dilution at 30 and 5C respectively.

REFERENCES

ALMQUIST, J.O., FLIPSE, R.J. and THACKER, D.L. 1954. **Diluters** for bovine semen. IV. Fertility of bovine spermatozoa in heated homogenized milk and skimmilk. J. Dairy Sci. 37, 1303-1307.

BLACKSHAW, A.W. and SALISBURY, G.W. 1972. The effect of storage *in vitro* on the DNA content of bull spermatozoa. Aust. J. Biol. Sci. 25, 175-184.

CRABO, B.G., BOWER, R.E., BROWN, K.I., GRAHAM, E.F. and PACE, M.M. 1970. Extracellular glutamic oxaloacetic transaminase as a measure of membrane injury in spermatozoa during treatment. In, *Current problems in fertility*. Ed. A. Ingelman-Sundberg and N.O. Lunell. Plenum Press, New York.

FOOTE, R.H. 1970. Fertility of bull semen at high extension rates in Tris-buffered extenders. J. Dairy Sci. 53, 1475-1477.

FOOTE, R.H., GRAY, L.C., YOUNG, D.C. and DUNN, H.O. 1960. Fertility of bull semen stored up to 4 days at 5C in 20% egg yolk extender. J. Dairy Sci. 43, 1330-1334.

NAGASE, H. and NIWA, T. 1964. Deep freezing bull semen in concentrated pellet form. I. Factors affecting survival of spermatozoa. Proc. Vth Int . Congr. Anim. Reprod. & A.I., Trento, IV, 410-415.

TABLE I

Summary of Analysis of Variance (Experiment 1)

SOURCE OF VARIATION		df	MS	F
Replicates	(a)	3	56.80	2.07 n.s.
Diluents	(b)	3	319.91	11.63*
Interaction:				
a x b, Error 1		9	27.50	
Length of storage	(c)	2	7098.58	418.70***
Interactions:				
a x c		6	62.53	3.69*
b x c		6	371.97	21.94***
a x b x c, Error 2		9	16.95	

* $p<0.05$

*** $p<0.005$

n.s. non-significant

TABLE 2

Effects of type of extender and length of storage on the percentage of motile spermatozoa during storage at 5C (Experiment 1).

Extender	Length of storage in days			Means
	0	1	4	
YOLK-CITRATE	70.3	37.1	1.8	31.4
HOMOGENIZED MILK	84.4	66.7	1.3	45.9
TRIS-YOLK	75.4	59.1	18.5	50.6
CORNELL UNIVERSITY EXTENDER	61.7	56.5	29.7	49.1
Overall means	73.4	54.9	9.7	

TABLE 3

Summary of Analysis of Variance (Experiment 2)

SOURCE OF VARIATION	df	MS	F
Bulls (a)	2	58.53	0.54 n.s.
Extenders (b)	2	4018.03	37.40***
: Homogenized Milk v Yolk-Citrate	1	7060.68	65.73***
: Tris-Yolk v Others	1	975.38	9.08*
Glycerol level (c)	3	219.95	2.05 n.s.
Pooled 1st order interactions	16	186.41	1.74 n.s.
2nd order interaction:			
a x b x c, Error	12	107.43	

* $p<0.05$

*** $p<0.005$

n.s. non-significant

TABLE 4

Summary of Analysis of Variance (Experiment 3)

SOURCE OF VARIATION	df	MS	F
Bulls (a)	2	26.33	0.45 n.s.
Extenders (b)	2	1035.08	17.67***
: Tris-Yolk v Yolk-Citrate	1	1908.17	32.58***
: Homogenized Milk v Others	1	162.00	2.77 n.s.
Glycerol level (c)	3	840.96	14.36***
: linear	1	2135.56	36.46***
: quadratic	1	186.78	3.19 n.s.
: cubic	1	200.56	3.42 n.s.
Pooled 1st order interactions	16	140.29	2.40 n.s.
2nd order interaction:			
a x b x c, Error	12	58.57	

*** $p < 0.005$

n.s. non-significant

TABLE 5

Summary of Analysis of Variance (Experiment 4)

SOURCE OF VARIATION		df	MS	F
Bulls	(a)	3	182.50	1.55 n.s.
Extenders	(b)	2	1182.13	10.01*
Dilution method	(c)	1	384.00	3.25 n.s.
Pooled 1st order interactions		11	651.17	5.52
2nd order interaction:				
a x b x c, Error		6	118.04	

* $p<0.05$

n.s. non-significant

TABLE

Summary of Analysis of Variance (Experiment 5)

SOURCE OF VARIATION	df	MS	F
Bulls (a)	3	567.68	10.41**
Extenders (b)	2	1838.35	33.90***
Freezing method (c)	1	1750.35	32.03***
Interactions:			
a x b	6	290.13	5.30*
a x c	3	321.98	5.90*
b x c	2	700.10	12.90**
a x b x c, Error 1	6	54.17	
Incubation period (d)	2	641.26	26.81***
: linear	1	1260.75	52.70***
: quadratic	1	21.78	0.91 n.s.
Pooled 1st order interactions	12	58.11	2.43 n.s.
Pooled 2nd order interactions	22	42.77	1.78 n.s.
3rd order interaction:			
a x b x c x d, Error 2	12	23.92	

* $p<0.05$

** $p<0.01$

*** $p<0.005$

n.s. non-significant

TABLE 7

Effects of freezing method and type of extender on the percentage of motile spermatozoa during post-thawing incubation (Experiment 5).

Freezing method	Incubation period (h)	Type of extender: Tris-Yolk	Yolk-Citrate	CUE	Means
In liquid nitrogen vapour	0	35.4	17.5	25.0	25.6
	1	24.2	15.3	12.2	17.0
	2	16.2	8.3	10.1	11.3
	Means	24.9	13.4	15.3	17.6
Pellet-freezing on dry-ice	0	67.1	38.3	20.6	41.5
	1	54.4	32.1	10.3	30.6
	2	46.1	27.7	8.1	25.5
	Means	55.9	32.6	12.6	32.4
Overall means		39.9	22.3	13.9	

Three general conclusions can be made from the results of the investigations into testicular growth and semen qualities of West African bulls, reported in this thesis:

1. Compared to temperate breeds like the Holstein, Charolais and Angus, the West African breeds had smaller body- and testicular sizes. This was probably reflected in the smaller ejaculate and lower semen concentration of the indigenous breeds.

2. Testicular development and spermatogenesis were closely related to age and bodysize.

3. Qualitatively, the semen of West African bulls was comparable to that of other cattle breeds and was *successfully stored in vitro* using established procedures.

Testicular size is better predicted from scrotal circumference using regression equations determined for each breed, since significant breed differences in this trait exist. The prediction equation obtained with Bunaji bulls

for the purpose was Y= -408.57 + 25.6X. For other breeds the equation Y= -506.17 +27.7X may be used tentatively. Analysis of scrotal size in large numbers of bulls would facilitate the detection of bulls with undersized testes.

Changes in scrotal circumference, age and bodyweight accounted for only 53 per cent of the variations in the gonadal sperm reserves of young Bunaji bulls in this investigation. Other factors such as hormones and nutrition are known to exert significant effects on spermatogenesis. While it is less certain that hormonal effects may be practically modified, emphasis should be placed on nutritional effects in future studies. It may be possible within limits to reduce age at puberty and increase sperm reserves in local bulls with better feeding.

Semen output in bulls is known to be influenced by factors such as libido, degree of sexual stimulation (e.g. through false-mounting) and frequency of ejaculation; these are apart from variations due to age, bodyweight and testicular size. It is a generally held opinion, backed by casual observation, that zebu cattle are more difficult to train for semen collection by artificial vagina than their

Bos taurus counterparts. However, while it may be true that most zebu cattle exhibit less libido within the confined space of a semen collection yard, this may not be true for bulls in the open field. Systematic investigations into animal behaviour and the management of zebu bulls for routine semen collection would be of immense value to artificial insemination centres involved with these breeds.

Finally, it is clear that many problems exist which must be solved before cattle artificial insemination becomes a practical proposition in West Africa. Perhaps the most important problem remains the absence of any effective organization charged with the promotion and control of genetic improvement of cattle in the subregion. Such an organization will encourage systematic research in reproduction, genetics and other aspects of animal production to be carried out on the West African breeds. Other problems include the absence of an efficient communications system which is of vital importance to artificial insemination practice, an unsteady electrical power supply and the absence of ready supplies of liquid nitrogen and solid carbon dioxide. However, most of these problems are certain to disappear with increasing economic development.

APPENDIX

1. Diluent Composition
2. Bouins fixation
3. Differential staining of spermatozoa

Appendix I

DILUENT COMPOSITION

	TRIS-YOLK	CUE	YOLK-CITRATE	HOMOGENIZED MILK
Tris*(g)	30.28	-	-	-
Citric acid (g)	16.75	0.87	-	-
Fructose (g)	12.50	-	-	-
Sodium bicarbonate (g)	-	2.10	-	-
Potassium chloride	-	0.40	-	-
Tri-Sodium citrate (g)	-	14.50	29.00	-
Glucose (g)	-	3.00	-	-
Glycine (g)	-	9.37	-	-
Sulphanilamide (g)	-	3.00	-	-
Homogenized milk**(cc)	-	-	-	1000
Distilled water (cc)	-	To 1000	-	-
Buffer (cc)	-	800	-	-
Egg yolk (cc)	200	200	200	-
Sodium penicillin G(IU/ml)	1000	1000	1000	1000
Streptomycin sulphate (mg/ml)	1	1	1	1
Distilled water (cc)	To 1000	-	To 1000	-

* 2-amino-2-(hydroxymethyl) propane-1,3-diol

** Heated to 92C for 10 minutes

Appendix 2

BOUINS FIXATION

Bouins fluid composition

1.	Picric acid, saturated aqueous solution:	750 ml
2.	37-40% formaldehyde :	250 ml
3.	Glacial acetic acid :	50 ml

Bouins fixation

1. Fix blocks for 4-12 hours, depending on size.

2. Wash in several changes of 50% alcohol for 4-6 hours.

3. Store in 70% alcohol.

Appendix 3

DIFFERENTIAL STAINING OF SPERMATOZOA

(DOTT and FOSTER, 1972)

Eosin-Nigrosin stain, citrate buffered.

Acetic acid/Perchloric acid mixture (1:1, v/v)

Ethanol

Ether

---

1. ADD ONE DROP OF SEMEN to eight drops of stain at 30C

2. INCUBATE FOR 5 MIN at 30C

3. MAKE SMEARS on PREHEATED slides and dry at 30C

4. SHAKE SLIDE in Acetic acid/Perchloric acid mixture for 20 SEC and WASH EXCESS OFF in running tap water (AT 37C if egg yolk diluent)

5. IMMERSE SLIDE BRIEFLY in ETHANOL and then ETHER to remove water

6. MOUNT, using DPX or EUKITT as MOUNTANT.

TESTICULAR GROWTH AND SEMEN QUALITIES OF WEST AFRICAN BULLS

By

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The studies reported in this thesis were on testicular growth and semen qualities of West African bulls. Aspects of testicular development, spermatogenesis, semen quality and semen storage were investigated.

A series of studies were conducted to estimate testicular size from scrotal measurement and to determine the relationships between testicular size, age and liveweight in some West African bulls.

Repeatability estimate for scrotal circumference measurements between technicians was 0.96. Scrotal circumference was highly correlated to paired testes weight (r=0.88) and volume (r=0.87). Testes weight and volume were closely related (r=0.997) but their ratio (g/cc) varied in 30 bulls from 0.962 to 1.119, with a mean and standard deviation of 1.027±0.029. The regression equation for estimating testes weight (Y) from scrotal circumference (X) was Y=-506.17+27.7X.

Significant correlations existed between scrotal circumference and age (r from 0.59 to 0.79) or liveweight (r from 0.62 to 0.83) in N'Dama, Bunaji and Sokoto-Gudali bulls. However, examination of the partial correlations

showed that most of the age effects were attributable to changes in liveweight.

The relationship between growth, testicular development and spermatogenesis was studied in 20 young Bunaji bulls over a one year period. Highly significant correlations were obtained between age and bodyweight (r=0.87), age and heart-girth (r=0.84), age and scrotal circumference (r=0.88), heart-girth and scrotal circumference (r=0.86), bodyweight and scrotal circumference (r=0.88), and heartgirth and bodyweight (r=0.95). The relationship between heartgirth (X) and bodyweight (Y) was described by the equation Y=-288.68+3.50X.

Scrotal circumference (SC) was highly correlated with testes weight (TW), (r=0.94); this relationship was described by the regression equation TW=-408.57 + 25.6(SC).

Although spermatogenesis had commenced in Bunaji bulls by 13 months of age, consistently large gonadal and epididymal sperm reserves were not apparent until they were 17 months and above. Scrotal circumference appeared to be a good indicator of spermatogenesis in this breed, when considered along with age and bodyweight.

A total of 35 ejaculates were collected from Sokoto-Gudali and Bunaji bulls and subjected to physical, morphological and biochemical examination. Semen collected by electroejaculation had higher mean volume, motility and total sperm number but lower concentration than semen collected by artificial vagina.

The ejaculates from 10 bulls were examined in greater detail. Total sperm per ejaculate averaged 2.241×10^9 while motility averaged 86.5%. The mean percentages of 'live' and normal sperm were 69.0 and 76.2% respectively. The incidence of sperm abnormalities was generally low. The activities of lactic dehydrogenase and glutamic oxaloacetic transaminase in seminal plasma and whole semen were determined.

Two factorial experiments were carried out on the short-term storage of semen at 5C. Factors examined were type of extender, length of storage, optimum level of glycerol in the diluent and method of dilution. The Tris-Yolk diluent and the Cornell University Extender gave good results while Yolk-Citrate and Homogenized Milk extenders gave poorer and less consistent results. Inclusion of glycerol in the extenders did not improve spermatozoa survival.

For long-term storage of semen at -196C, three factorial experiments were carried out to investigate the effects of diluent type, glycerol level, method of dilution and freezing method on spermatozoa survival. In Experiments 3 and 4 (Part V), cooled, extended semen was packaged in 0.2 ml plastic straws and frozen in liquid nitrogen vapour. Best results were obtained with Tris-Yolk diluent containing 4% glycerol, added as 0 and 8% in the diluent in a 2-stage dilution at 30 and 5C respectively.

The effect of freezing method on spermatozoa survival was examined in the fifth experiment (Part V). Semen was either frozen in liquid nitrogen vapour or on solid carbon dioxide by the pellet method. Freezing by the pellet method in the Tris-Yolk diluent gave the best results.