ACTA UNIVERSITATIS AGRICULTURAE SUECIAE

VETERINARIA 70


# Breeding Soundness Evaluation of Zebu Bulls

## With special reference to variations in clinical parameters and sperm characteristics in sires extensively managed in the dry tropics of Costa Rica

Jorge Chacón

SWEDISH UNIVERSITY OF AGRICULTURAL SCIENCES

# Breeding Soundness Evaluation of Zebu Bulls

## With special reference to variations in clinical parameters and sperm characteristics in sires extensively managed in the dry tropics of Costa Rica

**Jorge Chacón**

Akademisk avhandling, som med tillstånd av veterinärmedicinska fakulteten vid SLU för avläggande av veterinärmedicine doktorsexamen, offentligen försvaras på engelska språket i Ettans föreläsningssal, Klinikcentrum, Uppsala, fredagen den 11 februari 2000, kl 9.15.

Av fakultetsnämnden utsedd opponent: Professor Peter Chenoweth, Department of Clinical Sciences, College of Veterinary Medicine, Kansas State University, Manhattan, USA.

## Abstract

In Costa Rica and other tropical regions, beef cattle production is based upon Zebu (*Bos indicus*) breeds extensively managed on natural pasture. The reproductive efficiency of the breeding herd is low. To what extent the sires play a major role in this problem is yet undetermined mainly due to the nature of exploitation and the lack of periodical andrological controls to determine their suitability for natural breeding.

This thesis aimed to investigate the breeding soundness of bulls extensively managed in Costa Rica, with special reference to the identification of variables associated with their final ranking as potential breeders. Particular attention was paid to the prevalence of sperm abnormalities in the ejaculate, as well as eventual seasonal variations—and their causes—in clinical parameters and the spermiogramme.

Breeding soundness evaluations (BSE) were performed under field conditions in *Bos indicus* (Bi) ($n$ = 598), *Bos taurus* (Bt) ($n$ = 252) and *Bos indicus* × *Bos taurus* bulls (Bi × Bt) ($n$ = 48) in four different climatic regions of Costa Rica. The bulls, whose number represented about 2% of the total bull population in the country, were 1–12 years of age and extensively managed. Thirty-three percent of the bulls (296/898) were classified as unsound for breeding, 9.1% owing to clinical problems (82/898) and 23.9% to unsatisfactory sperm morphology (214/898). Low scrotal circumference (SC; less than 30 cm) was the most common clinical finding in bulls more than 2 years of age. The percentage of unsound bulls with sperm abnormalities in the ejaculate, especially head defects and proximal droplets, increased as SC decreased ($P < 0.05$), being higher in bulls

having a decreased testicular consistency (TC) ($P< 0.001$) and long scrotum ($P< 0.01$). Frequencies of sperm abnormalities were also higher in bulls less than 2 years of age than in older sires ($P< 0.01$) and were highest in Bi × Bt bulls ($P< 0.001$). The prevalence of bulls unsound for breeding was lower ($P< 0.01$) in Bi sires (29%), intermediate in Bt (41%) and higher in Bi × Bt (48%).

Sperm morphological examinations in 302 Zebu bulls, aged 1.5–9 years, from the dry Pacific zone of Costa Rica, confirmed the relationship between sperm morphology and clinical andrological parameters. Bulls with a SC less than 30 cm showed a higher prevalence for proximal cytoplasmic droplets than bulls whose SC was greater than 30 cm ($P< 0.05$). Likewise, bulls with a long scrotum and decreased TC at palpation showed higher percentages of abnormal sperm heads (including nuclear abnormalities) in the ejaculate than bulls with normal scrotal length (SL) and normal TC ($P< 0.05$). Zebu bulls less than 2 years of age showed a higher percentage of missing acrosomes and proximal cytoplasmic droplets than older sires ($P< 0.05$). The abnormality most commonly seen in ejaculates from sires sound for breeding was bent tails with entrapped cytoplasmic droplets. Phase contrast microscopy of fixed wet-smears was a convenient method to assess sperm morphology on-farm since it allowed the diagnosis (based on sperm morphology) of up to 88% of unsound bulls. However, a follow-up quantification of stained smears is advisable to confirm the primary assessment.

The fine morphology of testes and cauda epididymides from 11 sexually mature Zebu bulls with normal ($n = 3$) or decreased TC ($n = 8$) at palpation was also studied. Bulls having a slight to moderate reduction in TC showed more, albeit slight, degenerative changes in the seminiferous epithelium, compared with control animals. Cellular debris present in the Sertoli cells, abnormal acrosomes and impaired chromatin condensation during spermiogenesis were also common in bulls with a decreased TC. Palpation for TC is an important component of the clinical andrological evaluation of breeding Zebu bulls.

Sexually mature Brahman bulls ($n = 25$) extensively managed, were andrologically examined once monthly for 13 months to determine whether clinical and spermatological parameters in Zebu bulls show seasonal changes under extensive rearing conditions, in the dry Pacific region of Costa Rica. Of the clinical and sperm parameters examined, only SC showed a significant seasonal variation. This variation was positively related to body condition rather than to the changes in climatic variables prevailing during the study period.

The overall results confirm the higher adaptability of Zebu bulls to the tropical environment, as well as the relationship between SL, SC and TC with testicular normality and, therefore, with a normal sperm morphology in the ejaculate. The findings also suggest that nutrition, rather than ambient temperature, is the major factor contributing to variations in the reproductive function of Zebu bulls in the tropics.

*Key words*: Zebu (*Bos indicus*) bulls, Breeding soundness evaluation (BSE), Extensive rearing conditions, Testicular consistency, Scrotum length, Testis, Cauda epididymides, Ultrastructure, Sperm morphology.

Distribution:
Swedish University of Agricultural Sciences
Department of Obstetrics and Gynaecology
Box 7039, SE-750 07 UPPSALA, Sweden

Uppsala 2000
ISSN 1401-6257
ISBN 91-576-5910-9

# Breeding Soundness Evaluation of Zebu Bulls

With special reference to variations in clinical parameters and sperm characteristics in sires extensively managed in the dry tropics of Costa Rica

Jorge Chacón
*Department of Obstetrics and gynaecology*
*Uppsala*

**Doctoral thesis**
**Swedish University of Agricultural Sciences**
**Uppsala 2000**

**Acta Universitatis Agriculturae Sueciae**
Veterinaria 70

ISSN 1401-6257
ISBN 91-576-5910-0
© 2000 Jorge Chacón, Uppsala
Tryck: SLU Service/Repro, Uppsala 2000

*Cuando naciste, me diste el mayor regalo del mundo y te convertiste en el mayor de mis ejemplos, pues jamás olvidaré la fortaleza con que luchaste por vivir desde el primer día de tu hermosa vida. Gracias por enseñarme que siempre se debe luchar hasta el final...sin desmayar.*

*Con todo mi corazón y toda mi alma, para Andrés.*

# Abstract

Chacón, J. 2000. *Breeding soundness evaluation of Zebu bulls. With special reference to variations in clinical parameters and sperm characteristics in sires extensively managed in the dry tropics of Costa Rica.* Doctoral thesis.
ISSN 1401-6257, ISBN 91-576-5910-9

In Costa Rica and other tropical regions, beef cattle production is based upon Zebu (*Bos indicus*) breeds extensively managed on natural pasture. The reproductive efficiency of the breeding herd is low. To what extent the sires play a major role in this problem is yet undetermined mainly due to the nature of exploitation and the lack of periodical andrological controls to determine their suitability for natural breeding.

This thesis aimed to investigate the breeding soundness of bulls extensively managed in Costa Rica, with special reference to the identification of variables associated with their final ranking as potential breeders. Particular attention was paid to the prevalence of sperm abnormalities in the ejaculate, as well as eventual seasonal variations—and their causes—in clinical parameters and the spermiogramme.

Breeding soundness evaluations (BSE) were performed under field conditions in *Bos indicus* (Bi) ($n$ = 598), *Bos taurus* (Bt) ($n$ = 252) and *Bos indicus* × *Bos taurus* bulls (Bi × Bt) ($n$ = 48) in four different climatic regions of Costa Rica. The bulls, whose number represented about 2% of the total bull population in the country, were 1–12 years of age and extensively managed. Thirty-three percent of the bulls (296/898) were classified as unsound for breeding, 9.1% owing to clinical problems (82/898) and 23.9% to unsatisfactory sperm morphology (214/898). Low scrotal circumference (SC; less than 30 cm) was the most common clinical finding in bulls more than 2 years of age. The percentage of unsound bulls with sperm abnormalities in the ejaculate, especially head defects and proximal droplets, increased as SC decreased ($P< 0.05$), being higher in bulls having a decreased testicular consistency (TC) ($P< 0.001$) and long scrotum ($P< 0.01$). Frequencies of sperm abnormalities were also higher in bulls less than 2 years of age than in older sires ($P< 0.01$) and were highest in Bi × Bt bulls ($P< 0.001$). The prevalence of bulls unsound for breeding was lower ($P< 0.01$) in Bi sires (29%), intermediate in Bt (41%) and higher in Bi × Bt (48%).

Sperm morphological examinations in 302 Zebu bulls, aged 1.5–9 years, from the dry Pacific zone of Costa Rica, confirmed the relationship between sperm morphology and clinical andrological parameters. Bulls with a SC less than 30 cm showed a higher prevalence for proximal cytoplasmic droplets than bulls whose SC was greater than 30 cm ($P< 0.05$). Likewise, bulls with a long scrotum and decreased TC at palpation showed higher percentages of abnormal sperm heads (including nuclear abnormalities) in the ejaculate than bulls with normal scrotal length (SL) and normal TC ($P< 0.05$). Zebu bulls less than 2 years of age showed a higher percentage of missing acrosomes and proximal cytoplasmic droplets than older sires ($P< 0.05$). The abnormality most commonly seen in ejaculates from sires sound for breeding was bent tails with entrapped cytoplasmic droplets. Phase contrast microscopy of fixed wet-smears was a convenient method to assess sperm morphology on-farm since it allowed the diagnosis (based on sperm morphology) of up to 88% of unsound bulls. However, a follow-up quantification of stained smears is advisable to confirm the primary assessment.

The fine morphology of testes and cauda epididymides from 11 sexually mature Zebu bulls with normal ($n$ = 3) or decreased TC ($n$ = 8) at palpation was also studied. Bulls having a slight to moderate reduction in TC showed more, albeit slight, degenerative changes in the seminiferous epithelium, compared with control animals. Cellular debris

present in the Sertoli cells, abnormal acrosomes and impaired chromatin condensation during spermiogenesis were also common in bulls with a decreased TC. Palpation for TC is an important component of the clinical andrological evaluation of breeding Zebu bulls.

Sexually mature Brahman bulls ($n$ = 25) extensively managed, were andrologically examined once monthly for 13 months to determine whether clinical and spermatological parameters in Zebu bulls show seasonal changes under extensive rearing conditions, in the dry Pacific region of Costa Rica. Of the clinical and sperm parameters examined, only SC showed a significant seasonal variation. This variation was positively related to body condition rather than to the changes in climatic variables prevailing during the study period.

The overall results confirm the higher adaptability of Zebu bulls to the tropical environment, as well as the relationship between SL, SC and TC with testicular normality and, therefore, with a normal sperm morphology in the ejaculate. The findings also suggest that nutrition, rather than ambient temperature, is the major factor contributing to variations in the reproductive function of Zebu bulls in the tropics.

*Key words*: Zebu (*Bos indicus*) bulls, Breeding soundness evaluation (BSE), Extensive rearing conditions, Testicular consistency, Scrotum length, Testis, Cauda epididymides, Ultrastructure, Sperm morphology.

*Author's address*: Jorge Chacón, Department of Obstetrics and Gynaecology, Faculty of Veterinary Medicine, SLU, Box 7039, S-750 07, Uppsala, Sweden. On leave from Department of Animal Reproduction (Section of Andrology), Veterinary School-UNA, P.O. Box 2556-3000, Heredia, Costa Rica.

# Contents

# Appendix

## List of original papers

The thesis is based on the following papers, which will be referred to in the text by their Roman numerals **I-IV**:

**I.** Chacón, J., Pérez, E., Müller, E., Söderquist, L. and Rodríguez-Martínez, H., 1999: Breeding soundness evaluation of extensively managed bulls in Costa Rica. Theriogenology. 52: 221-231.

**II.** Chacón, J., 1999: Assessment of sperm morphology in Zebu bulls under field conditions in the tropics. (Submitted for publication).

**III.** Chacón, J., Müller, E. and Rodríguez-Martínez, H., 1999: Morphological features of the seminiferous and cauda epididymides epithelia of breeding Zebu bulls with normal and decreased testicular consistency. J. Reprod. Dev. 45: 119-128.

**IV.** Chacón, J., Pérez, E. and Rodríguez-Martínez, H., 1999: Seasonal variations in testicular consistency, scrotal circumference and spermiogramme parameters of extensively reared *Bos indicus* bulls in the dry tropics. (Submitted for publication).

Offprints are printed with the kind permission of the journals concerned.

# Abbreviations

| | |
|---|---|
| AV | artificial vagina |
| BCS | body condition score |
| Bi | *Bos indicus* |
| Bi x Bt | *Bos indicus* × *Bos taurus* |
| BSE | breeding soundness evaluation |
| Bt | *Bos taurus* |
| DIC | differential interference contrast microscopy |
| EE | electroejaculation |
| LSM | least square mean |
| SC | scrotal circumference |
| SD | standard deviation |
| SEM | standard error of the mean |
| SL | scrotal length |
| TC | testicular consistency |
| TEM | transmission electron microscopy |

# General background

## Beef production in the Central American tropics

Cattle production in the tropics is generally based on Zebu (*Bos indicus*) breeds extensively managed on natural pasture. Beef production has been recognised as being less productive compared with more temperate areas where *Bos taurus* cattle has been used (Lamothe, 1990). Müller (1990) estimated that in Central America the yearly calving rate in beef cattle does not reach the 50% level and stressed that poor reproductive efficiency of the breeding males contributed largely to the low reproductive outcome. Other contributing factors relate to social, management, climatic, nutritional and genotype influences.

In Costa Rica, in 1991, the grasslands accounted for 29% of the country's land surface (Kaimowitz, 1996). Beef farms are distributed throughout the country, except in the Central Valley, and are mainly located in the dry north Pacific lowlands (tropical dry forest ecosystem), in which approximately 40% of the beef stock and 38% of the total number of Zebu cattle are concentrated (see map in page 27). The extensive rearing system and the low attention paid to increasing the efficiency of the breeding herd have, however, pointed out livestock production as one of the causes for the non-sustainable use of land in the tropics (Le Tacon and Laker, 1990; Murgueitio, 1990; Pérez, 1996; Ruiz, 1996).

In 1955, Costa Rica had approximately 750,000 heads of cattle on 700,000 hectares of grasslands (Kaimowitz, 1996; Pérez, 1996). With the opening of the U.S market for Central American beef cattle by the middle of the 1950s, large government subsidies were provided for cattle raising during the 1960s and 1970s (Kaimowitz, 1996). Consequently, by 1978, the cattle herd in Costa Rica had increased to 2.1 million. This expansion was supported by an increase in the area designated as pasture (2.2 millions of hectares) rather than by an increase in the efficiency of the production system (Kaimowitz, 1996; Pérez, 1996). A similar trend has been documented in all of the neighbouring Central American countries (Kaimowitz, 1996) and in Colombia (Murgueitio, 1990; Preston, 1990). By 1992, the cattle stock had decreased in Costa Rica to approximately 1.7 million heads because of an inefficient production, and an increasing demand of the growing population (approximately 3% annually). In addition, the yearly beef consumption *per capita* increased from 20.4 (between 1976–1983) to 22.9 kg/person in the period 1984–1991 (Kaimowitz, 1996). As a result, beef exports from Costa Rica have shown a declining trend year after year (26,000 tons in 1993 vs. 9,400 tons in 1999, Source: Promotora de comercio exterior de Costa Rica, 1999). If this tendency continues, the beef production system may not be able to handle the need from local consumption in the short term. Even when the causes of the problem are complex, the conditions under which cattle are managed in the tropics have significantly accounted for the low performance of the beef cattle production system.

## Breeding management of Zebu bulls in the tropics

In the tropics, breeding of beef cattle is based on natural mating by using one or several bulls per herd (McCosker et al, 1989; Rodríguez et al, 1993). In Costa Rica, natural mating accounts for approximately 95% of the cows sired in beef farms. Breeding bulls are managed under conditions of extensive rearing using continuous (i.e., the male mixed with the herd the whole year) or temporal mating seasons with one (single sire system) or several sires (multiple sire system). Data from the Department of Reproduction of the Veterinary School, UNA, Costa Rica, based on 1,100 breeding soundness evaluations (BSE) of Zebu bulls from the dry Pacific area, showed that 47.6% of the males (524 out of 1,100) were mating continuously, whereas 49.5% of them were in multiple sire systems in which mixed-age or mixed-breed groups were common (Chacón, unpublished data). In addition, around 7% of the bulls examined were older than 7 years.

The mean number of bulls per group in multiple sire systems in Costa Rica was 4.0 (range 3–11) breeding sires for an average of 101.8 (range 15–450) cows. The mean number of cows in a single sire mating system was 29.3 animals (Chacón, unpublished data). Regardless of the system used, this creates a bull/cow ratio of approximately 1:25, which is similar to that which has been reported in other tropical countries (McCosker et al, 1989). This management strategy is used by most farmers in Costa Rica since it is believed to represent the maximum breeding capacity of a sire in natural mating and because it compensates for eventual bull subfertility. However, this system has the disadvantage of favouring social dominance, especially when age is not uniform in the group, which is normally the case in the tropics (Blockey, 1979; Chenoweth, 1981; McCosker et al, 1989; Rodríguez et al, 1993; Orihuela and Galina, 1997). Dominant, older bulls are likely to win more mating opportunities than younger bulls, but they are more likely to have a higher prevalence of testicular disorders (i.e. fibrosis, calcification; Carroll et al, 1963; Rao and Bane, 1985) as well as venereal diseases (i.e. trichomoniasis; McCosker et al, 1989; Pérez et al, 1992), thus decreasing the potential fertility in the herd.

In tropical Australia, McCosker et al (1989) reported low conception rates in herds with multiple sire systems using Brahman bulls of mixed ages, where up to 82% of the calving cows were pregnant by the dominant bull. Furthermore, Rodríguez et al (1993) reported that a dominant Zebu bull would carry out 63% of the sexual activity registered in a breeding herd. Also Blockey (1979) reported similar findings for *Bos taurus* bulls. If the dominant bull performs the majority of the matings in the breeding herd, the general belief of most farmers in the tropical areas of the effectiveness of using a bull/cow ratio of 1:25 is not well-founded. In continuous mating systems, many of the cows ought to be pregnant or could also be in anestrous, thus lowering the ratio even more. Consequently, breeding the herd in a multiple sire system using bulls with mixed-ages, is not efficient and contributes to decrease the reproductive efficiency of the beef farms. In addition, the use of older bulls (i.e. > 7 years old) may also favour inbreeding, thus deteriorating the genetic perspectives of the herd.

## Physiological and reproductive characteristics of Zebu bulls

*Adaptability of Zebu bulls to tropical environments*

The term adaptability could be defined as the ability of an animal to modify and adjust its physiologic processes in response to a specific stimulus (Turner, 1980). The ability of *Bos indicus* to withstand environmental conditions prevailing in the tropics is widely recognised (Cartwright, 1955; Turner, 1980; Kumi-Diaka et al, 1981). Most of the earlier thinking was that this ability concerned only the capacity of the Zebu to resist the effects of climate. However, it is now more widely recognised that other effects characteristic of tropical areas, and with major effects in cattle productivity, such as low-quality forages, diseases, especially caused by hemoprotozoa and other parasites, are also well coped by *Bos indicus* cattle compared with *Bos taurus* (Cartwright, 1980).

Zebu bulls have a greater capacity to regulate their body temperature than *Bos taurus* bulls in a tropical environment. Studies by Carvalho et al (1995), based on a large bull population in Brazil, showed that the perimeter of the sweat glands and the number of layers in the epidermis were greater in Zebu than in native or imported Simmental bulls. Also, the activity of sweat glands (baggy-shaped) in *Bos indicus* was higher than in the exotic breeds (tubular-shaped). Consequently, the sweating ability is greater in Zebu bulls and increases more quickly in a hot environment, thus allowing the bull to loose more heat by skin evaporation than European breeds. As a result, signs of heat stress are rarely seen in *Bos indicus* bulls exposed to temperatures up to 37°C and high humidity (Da Silva and Casagrande, 1976; Carvalho et al, 1995). The smooth hair coat present in Zebu bulls has also been found to have great resistance to solar radiation (Turner, 1980; Finch, 1986; Carvalho et al, 1995).

*Bos indicus* is also recognised for its ability to better utilise low-quality forages and for its lower nutritional requirements for maintenance than *Bos taurus* (Turner, 1980; Wildeus and Entwistle, 1984). Rekwot et al (1994) suggested that Zebu bulls are able to lower their metabolic rate to basal levels during the dry season when good-quality pastures are scarce. Further, after being placed on a low nutritional plane, *Bos indicus* easily recover their reproductive potential. In regard to ectoparasites, Zebu cattle of any breed show a higher resistance to ticks and fly infestations compared with *Bos taurus* (Riek, 1962; Turner, 1980; Fordyce et al, 1996). The skin thickness and its local mechanisms of defence in Zebu animals could explain this feature.

Haematological differences between Zebu cattle and *Bos taurus* have also been reported. Turner (1980) found higher red-cell counts and haemoglobin content in Brahman bulls than in Hereford bulls in Florida, USA. Evans (1963) reported similar differences between Zebu and European breeds. This condition, together with the mechanisms of heat loss, may explain the low body temperatures and low respiration rates observed in *Bos indicus* during rest or after walking in a tropical climate (Cartwright, 1955; Turner, 1980; Finch, 1986; Carvalho et al, 1995). Finally, Turner (1980) reported that the scrotal skin in

Zebu bulls is hairless and less thick than in *Bos taurus* sires. These physiological attributes might explain the lower prevalence of testicular degeneration, presumably due to environmental heat stress in the tropics, in *Bos indicus* bulls compared with *Bos taurus* bulls (Vale-Filho et al, 1980; Kumi-Diaka et al, 1981; Wildeus and Entwistle, 1983a, 1984; Crabo, 1988; Ohashi et al, 1988).

*Puberty and sexual maturity*

Prepubertal animals frequently show mounting behaviour and erection, in relation to production of androgens by the developing Leydig cells (Wolf et al, 1965; Amann, 1983). Spermatogenesis gradually begins and puberty is attained when the male initiates substantial production of spermatozoa (Wolf et al, 1965). After puberty, both the quantity and the quality of the produced spermatozoa increase over time leading to sexual maturity, when the bull is fully apt to perform as a breeder, has normal sperm production and a characteristic low frequency of sperm abnormalities in the ejaculate. Full sexual maturity is often related to maximum fertility and varies individually (Noakes, 1988).

Even though reports regarding age of puberty attainment and sexual maturity in *Bos indicus* bulls differ according to the breed and conditions of the study, all of them agree in that *Bos indicus* bulls reach puberty at a later age than *Bos taurus* sires, even when the bulls are raised under the same conditions (Fields et al, 1979; Igboeli and Rakha, 1981; Wildeus and Entwistle, 1982; Vale-Filho et al, 1980, 1996; Silva-Mena, 1997). Oyedipe et al (1981) reported that White-Fulani and Sokoto Zebu bulls, raised under the conditions prevailing in Nigeria and supplemented with grain during the dry season, were 15 and 17 months old, respectively, at puberty. In Brahman bulls raised in Florida, USA (Morris et al, 1989) and México (Silva-Mena, 1997), age at puberty was reported to be 14 and 16 months, respectively. In the same breed, Fields et al (1979) suggested that sexual maturity might occur between 18–20 months of age when raised under the conditions in Florida, USA. Guzerat and Nellore bulls raised in tropical Venezuela reached sexual maturity at 24 months of age, with a scrotal circumference of 28–29 cm (Trocóniz et al, 1991).

Nutrition plays a significant role in the onset of puberty and attainment of sexual maturity in Zebu bulls (Igboeli and Rakha, 1981; Crabo, 1988; Vale-Filho et al, 1996). In Angoni (short-horn Zebu) bulls, kept on good pastures and with good body condition in Nigeria, puberty could be attained as early as 14 months of age (Igboeli and Rakha, 1981). A high level of protein induced early onset of puberty compared with control bulls (Rekwot et al, 1987a). In contrast, underfed young bulls show impaired body growth, delayed puberty, a retarded development of endocrine glands and of the reproductive tract, especially during the early period of rapid growth (Rekwot et al, 1994). These effects are believed to be the result of diminished gonadotrophin release by the pituitary gland (Dunn and Moss, 1992).

*Libido and mating behaviour*

The term libido is commonly used to describe the willingness and eagerness of a male animal to mount and to attempt service of a female, while mating behaviour describes the performance of the male in the period immediately before, during and after service (Blockey, 1979; Chenoweth, 1981). Several methods are used to measure libido in bulls (see Chenoweth, 1981), basically scoring the bull's sexual response during exposure to a restrained non-oestrous or oestrous cow for a certain interval. The "serving capacity test" records the number of services completed by a bull during a 40-minute period (Chenoweth, 1981). In yearling beef *Bos taurus* bulls, libido scores highly correlated ($r = 0.98$) with the proportion of heifers the bulls served during a breeding season. Moreover, the repeatability of the test obtained in two consecutive trials under corral conditions was 0.67 (Price, 1987).

However, libido and serving capacity tests have been shown to be fairly difficult to apply in Zebu bulls (Hernández et al, 1991; Piccinali et al, 1992). In contrast to *Bos taurus* bulls, Zebu bulls should be tested with unrestrained oestrous females (Price, 1987, Hernández et al, 1991; Piccinali et al, 1992). Furthermore, the sexual behaviour of Zebu bulls is very particular when exposed to a group of cows synchronised for oestrus (Galina et al, 1987; Hernández et al, 1991; Piccinali et al, 1992). First, the bull establishes dominance over the bigger cows and protects passive cows from being mounted by other cows by placing himself behind the oestrous cow or simply guarding the rear of a cow of his preference (Galina et al, 1987). Moreover, if the bull has shown interest for a particular cow, he will not attempt to mount other cows, thus limiting his availability to mount other females concomitantly in oestrus (Galina et al, 1987).

Zebu bulls have a tendency to mount only cows in mid-oestrus (Chenoweth, 1981). In Indobrasil (Orihuela et al, 1988) and Gyr (Gutiérrez et al, 1993) cows, oestrus has a short duration. Indobrasil bulls undergoing libido evaluations under grazing conditions, performed fewer services during a 30-minute period than they did in the corral during the same period just a few hours before (Hernández et al, 1991). Similar results have been reported in Gyr bulls, where almost 50% of the bulls that failed to show interest in females under corral conditions, were quite active when exposed to females on pasture (Piccinali et al, 1992). Indobrasil bulls also showed a non-constant sexual behaviour in the presence of oestrous-synchronised cows (Orihuela et al, 1988), with a higher mounting frequency at night (18:00-24:00 hours), as well as early in the morning (06:00-09:00 hours). Similar results were reported by Rodríguez et al (1993) in other Zebu breeds under tropical conditions. This particular sexual behaviour of Zebu sires may explain the lower frequency of mating reported for these bulls during libido testing (Chenoweth and Osborne, 1975; Piccinali et al, 1992). Zebu bulls also performed fewer services per cow in heat than *Bos taurus* sires (0.5 for *Bos indicus* [Hernández et al. 1991] vs. 4 mounts for *Bos taurus* [Blockey, 1979]).

Therefore, since no definite trend has been observed in *Bos indicus* bulls with respect to their performance under corral and field conditions, and since their mating ability is severely constrained in the presence of other bulls (McCosker et

al, 1989; Piccinali et al, 1992; Orihuela and Galina, 1997), testing bulls under field conditions should be done individually to reduce the hierarchy effects among sires. Due to the lower frequency of mounts reported during the first minutes of libido testing, a longer testing period for *Bos indicus* must be considered. Moreover, the observations must consider other sexual activities such as chin resting or attempts to mount if one wants to design a more accurate method to measure sexual interest in *Bos indicus*.

*Seasonal variation in the reproductive efficiency of Zebu bulls*

A male is considered to be a seasonal breeder when specific reproductive characters change in response to climatic influences during a specific season of the year. Seasonal influences on the reproductive efficiency of the bull (libido, semen quality and conception rate) are widely recognised (Erb et al, 1942; Fayemi and Adegbite, 1982; Parkinson, 1987). However, in tropical areas the male's response to seasonal influences is highly influenced by the bull's breed. In regard to libido, Hardin et al (1981) did not find any seasonal variation on libido score of young Brahman bulls, although in non-tropical areas, a depression of libido during winter has been reported in Zebu bulls as a consequence of daylight length (Chenoweth, 1991). In tropical regions, this effect is less likely to occur, since daylight length is relatively constant throughout the year.

The role of seasonal influences on the reproductive capacity of Zebu bulls in the tropics is highly controversial. Semen quality is not impaired in Zebu bulls during the summer months in Nigeria (Crabo, 1988). Similarly, Kumi-Diaka et al (1981) reported that Fulani (*Bos indicus*) bulls are not affected at all by season, in contrast to *Bos taurus* bulls, in a tropical environment. Igboeli et al (1971, 1987) and Rekwot et al (1987b) found in semen samples from Zebu bulls collected by electroejaculation (EE) and artificial vagina (AV), respectively, a decrease in the ejaculate volume during the hottest months in Nigeria. However, the same authors showed different results regarding sperm output; Rekwot et al (1987b) reported a decrease during the summer months whereas Igboeli et al (1987) could not find any seasonal influence on this parameter. In regard to sperm morphology, Wildeus and Entwistle (1984) found higher levels of missing acrosomes, proximal droplets and bent tails in bulls after slaughter during the dry season compared with the rainy season in Australia. The effect, however, was more pronounced in *Bos taurus* sires, which also had a higher prevalence of abnormal sperm heads compared with *Bos indicus* bulls. Sekoni et al (1988), found a slight decrease in the percentage of normal spermatozoa during the summer months in a population of Zebu and *Bos taurus* bulls in Nigeria, and Igboeli and Rakha (1971) found differences between seasons only for bent tails and proximal droplets, but not for other abnormalities. The levels reached during the summer period were, however, considered to be within a normal range (Igboeli and Rakha, 1971).

A major drawback of the studies done on the potential seasonal variations in reproductive capacity of Zebu bulls in the tropics has been the use of different sires, slaughtered in different seasons. In addition, the results have been based on a low number of animals, including *Bos taurus* and *Bos indicus* × *Bos taurus* bulls.

Therefore, to study the potential seasonal variations in clinical and spermiogramme parameters and to determine the causes of these variations, a homogeneous population of grass-fed Zebu bulls is needed.

## Breeding Soundness Evaluation (BSE) of Zebu bulls

The role of the bull on the reproductive efficiency of the cattle herd is well-known. Therefore, the use of basic techniques such as the breeding soundness evaluation to screen the potential reproductive capacity of bulls has proven useful in detecting males with potential low fertility (Carroll et al, 1963; Ball et al, 1983). Despite its importance as an aid to increase conception rates in the naturally mated herds, the first documented BSE of a bull in Costa Rica was done as late as in 1986 (Müller, 1991).

The BSE was here applied to sires intended for natural mating under pasture conditions. The procedure (Ball et al, 1983; McCosker et al, 1989; Chenoweth et al, 1992) involves a thorough andrological examination, including a general physical inspection, examination of the reproductive organs and a partial assessment of the spermiogramme, solely under farm conditions. After the evaluation, the bulls are judged as sound or unsound for breeding. However, in this latter group, re-evaluation is advisable because bulls may be able to recover their breeding soundness.

In Costa Rica, practitioners perform bull evaluations only sporadically and recommendations are based solely on the assessment of sperm mass activity of the collected semen. These evaluations do not include a complete clinical examination of the sire and, therefore, people consider BSE as a simple "semen examination" (Müller, personal communication). Unfortunately, many veterinarians are also still not aware of how important, yearly or at least previous to the purchase of a breeder, sire evaluations are. A similar situation has been reported in other tropical regions (McCosker et al, 1989). In addition, some traditional ranchers, who are most often the breeders of pure Zebu sires, are normally more reluctant to accept a complete BSE of their bulls, compared with those who have medium and small farms. Consequently, finding pure-bred Zebu bulls in cattle fairs in Costa Rica with severe testicular asymmetry (most probably hypoplasia) was not unusual during 1999. It is clear that in these cases, the idiosyncrasy and unawareness of Zebu breeders in the country play an important role, since they justify the presence of these bulls as breeders because they believe that "*sperm motility in the semen of a bull having testicular hypoplasia is not necessarily affected*". Other farmers hesitate to accept BSE of their bulls because they believe that rectal examination and EE can negatively affect the bulls' future sexual libido or that the examination itself can injure the bull. In consequence, the raising and breeding management of *Bos indicus* bulls in Costa Rica do not currently include the supervision of basic reproductive parameters. This has been documented in two studies in the country where a large proportion of sires showed low scrotal circumference (SC) values and were considered unsuitable for breeding (Braden, 1992; Chacón, 1997). Unfortunately, this picture

is not different from that found in other tropical areas (Chenoweth and Osborne, 1975).

Furthermore, BSE is essential when considering the introduction of exotic breeds of cattle in the tropics. In Costa Rica, particularly, the past importation of pure Simmental and Hereford bulls are examples of this problem. Those sires, performed poorly because of either severe testicular degeneration or loss of libido caused, most likely, by heat stress. Studies including the feasibility and value of evaluating sires extensively reared for breeding ability have been rarely carried out in Costa Rica. Those few screenings thus far run showed that around 70% of the bulls examined were sound for breeding (Alvarado, 1987; Müller, 1990; Braden, 1992; Arias, 1995). However, these studies did not search for the effects and interactions between season and other clinical variables on semen quality of Zebu bulls, and did not determine which variables that were associated with their breeding soundness.

*Scrotal circumference*

Scrotal circumference (SC) in the bull is an indirect but confident indicator of testicular weight and sperm production (Coulter et al, 1976; 1979; Madrid et al, 1988). It is positively correlated with the total number of Sertoli cells (Berndtson et al, 1987), as well as with semen quality, age at puberty of the offspring, and in vivo fertility in Zebu (McCosker et al, 1989; Morris et al, 1989; Trocóniz et al, 1991; Rekwot et al, 1994) and *Bos taurus* bulls (Elmore et al, 1976; Coulter and Foote, 1979; Carter et al, 1980; Toelle and Robison, 1985; Bailey et al, 1996).

Reports of breeding soundness in bulls using the system recommended by the American Society of Theriogenology, have shown a significant correlation between SC and classification of the sires after examination (Elmore et al, 1976; Barth, 1995; Chenoweth et al, 1996; Vale-Filho et al, 1996), which is not surprising in view of the score given to SC (40 points). However, even if SC was not considered as a criterion to classify bulls after BSE, sires with SC below 30 cm have more sperm abnormalities in their ejaculates and are more often classified as unsound for breeding than sires with SC above this threshold (Madrid et al, 1988). This is supported by a close relation between small testicles (below 30 cm) and a higher proportion of damaged seminiferous tubules in bulls (Veeramachaneni-Rao et al, 1986; Madrid et al, 1988).

Body weight and age are positively correlated with SC in Zebu bulls (Morris et al, 1989). Brahman sires raised under tropical conditions in Australia showed a correlation between SC, age and weight of 0.61 and 0.72, respectively (McCosker et al, 1989), whereas Trocóniz et al (1991) reported values (unadjusted) of up to 0.82 and 0.81, respectively, for Nellore bulls in Venezuela. These estimates are similar to those for beef and dairy *Bos taurus* bulls in non-tropical areas (Coulter and Foote, 1979). Breed is another important factor that accounts for the high variance seen in SC between bulls of the same age and raised under the same conditions. De Dios-Vallejo et al (1991) reported lower SC for Nellore bulls at any age compared with Indobrasil, Brahman and Gyr sires in the Mexican humid tropics, whereas Grauber et al (1990) also found a lower SC for Nellore compared

with Brahman bulls in Argentina. Trocóniz et al (1991) found a lower SC in Nellore bulls compared with Guzerat at 18 months of age (23.6 ± 0.2 and 25.6 ± 2.3 cm, respectively [mean ± SD]), although weight variations would have accounted for this difference. Supplemented Nellore bulls, for instance, had a mean SC of 32.2 cm versus 28.5 cm in control bulls at 24 months of age (Vale-Filho et al, 1996). Fordyce et al (1996) reinforced the relation between the increase in the SC and growing rate in Zebu bulls grass-fed in the tropics. Data of adjusted SC from 2,050 grass-fed Zebu bulls in tropical Costa Rica showed that Indobrasil bulls had a higher SC at any age compared with Nellore, Brahman and Gyr sires, which at 2 years of age had mean values of 34.2, 31.0, 32.2 and 31.6 cm, respectively (Chacón et al, unpublished results).

*Bos indicus* bulls have lower adjusted SC when compared with *Bos taurus* breeds at the same age (Fields et al, 1979; De Dios-Vallejo et al, 1991; Chenoweth et al, 1996). Endo et al (1978) found that Zebu breeds tend to have testes with a more elongated shape than *Bos taurus* bulls, leading to a bias in the measurements of SC. However, only a 10% prevalence of elongated testicles was found in *Bos indicus* bulls after sampling 2,050 sires in Costa Rica (Chacón et al, unpublished results). The general assumption that *Bos indicus* bulls have an inherited low SC is more likely to be the result of a poor selection among these bulls in the tropical areas, as well as their delayed sexual development when compared with *Bos taurus* breeds. In fact, low SC in Zebu sires is the most important cause for bulls being classified as unsound for breeding due to clinical deviations (Braden, 1992; Chenoweth et al, 1996). In Florida, USA, Brahman sires showed lower SC values compared with Angus and Hereford bulls at young ages. In the latter breeds, testicular growth ceased at approximately 22 months of age, whereas SC continued to increase in Brahman bulls (Fields et al, 1979; Chenoweth et al, 1996). Similar results have been found recently in Costa Rica, where comparisons between *Bos indicus* and *Bos taurus* bulls showed higher SC values in some sires from the latter, compared to Gyr, Brahman and Nellore bulls 18–36 months of age, but not older (Chacón et al, unpublished results). In addition, SC values for Holstein and Simmental bulls were lower than those for Indobrasil sires at any age and were also lower than the averages reported for these European breeds under temperate conditions (Coulter et al, 1976; 1979; Elmore et al, 1976). These findings confirm a slow growth pattern in young Zebu breeds when compared with *Bos taurus* bulls and also support the existence of an effect of the interaction between genotype and environment on SC.

Even though the benefits on the reproductive parameters of the herd, when selecting bulls by SC, and its moderate to high heritability, have been clearly demonstrated (Coulter and Foote, 1979; Bourdon and Brinks, 1986; Gipson et al, 1987; Morris et al, 1989), selection of Zebu sires for this variable in tropical Costa Rica has been rare or absent. Other tropical areas probably face a similar situation. Most research in this field has been generated in dairy or beef cattle raised in non-tropical areas and grain-fed. Consequently, there is a need for establishing adjusted standard values for SC in grass-fed *Bos indicus* bulls under tropical conditions to be able to select breeding bulls by this characteristic.

*Collection of semen in Zebu bulls under field conditions*

Semen collection in the bull can be performed by the use of an artificial vagina (AV) or electro ejaculation (EE). The AV is the most common method used in *Bos taurus* and enables evaluation of libido while semen collection is in progress (Ball et al, 1983). In contrast, the use of this method in *Bos indicus* bulls is mainly restricted to those sires kept at artificial insemination (AI) centres, since they need to be well-trained to mount a restrained cow or a teaser. Despite intensive training, many of them never succeed, since Zebu bulls are usually reluctant to mount restrained and non-oestrus cows (Chenoweth, 1981; Price, 1987, Piccinali et al, 1992). Thus, considering their sexual behaviour and their often violent temperament, which makes handling rather difficult, semen collection using an AV is difficult, if not impossible, under field conditions.

Consequently, EE seems to be the method of choice for collecting semen samples from Zebu bulls under field conditions in the tropics (León et al, 1991). Good restraint of the male in a strong chute is necessary to perform EE in Zebu bulls. The animal should be restrained so it is unable to move (see **Fig. 1**). Squeezed head chutes should be avoided when working with Zebu, since the bulls can lie down, thus compromising their safety.

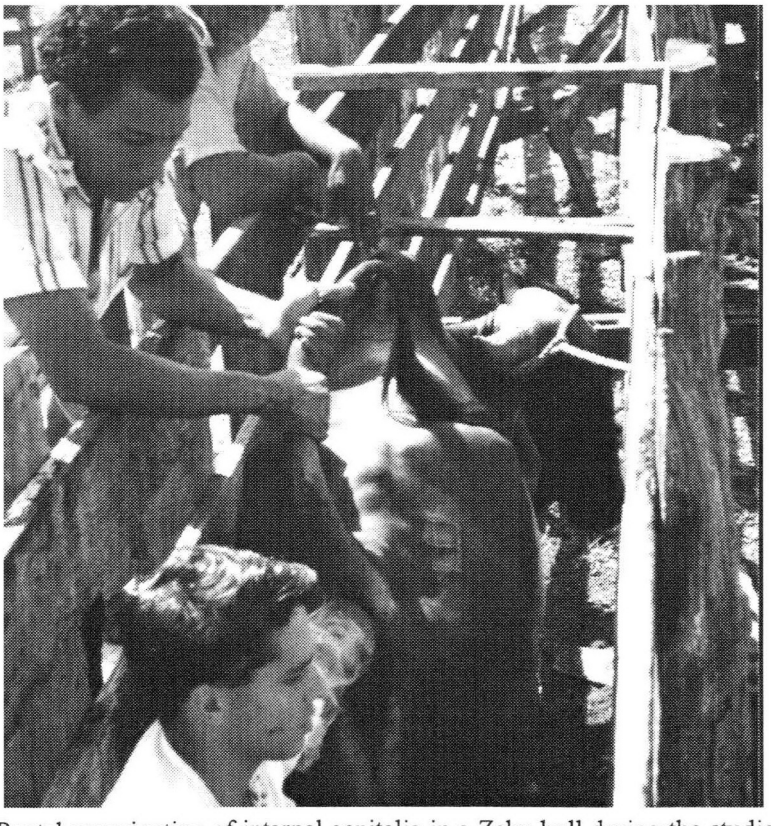

**Figure 1**: Rectal examination of internal genitalia in a Zebu bull during the studies. Note the characteristics of the chute and the simple immobilisation strategy routinely used (Photograph of the author).

The EE method is based on electrical stimulation per rectum by an electrode-probe on nervous fibres from the mesenteric and pelvic plexa as well as the lumbar (hypogastric) and sacral (intern pudendal) nerves. These nerves control the contractions of excurrent ducts, vesicular glands, and the emission process into the urethra, leading to ejaculation in the bull (Ball et al, 1983). Evacuation of the manure from the rectum and pre-stimulatory transrectal massage of the internal genitalia for at least 3 minutes facilitates EE with a minimum electrical stimulus. Even though the EE stimulus also provokes relaxation of the retractor penis muscle, ejaculation can occur into the prepuce without penile protrusion or erection (Carroll et al, 1963). Data from the Department of Reproduction, UNA in Costa Rica, based on the response to EE of 402 *Bos indicus* and 68 *Bos taurus* bulls, indicate that ejaculation without penis protrusion or erection occurred more frequently in the latter (25.0% vs. 10.7%; Chacón, unpublished results).

The pattern of stimulation and response to EE differs among Zebu bulls. Some males, usually those with wild temperament, react very violently to stimulation, whereas others do not. In the first case, previous rectal massage for a longer period of time is advisable and electrical stimulus should be applied while carefully observing the individual response. Although semen can be collected from most bulls by EE, failure rates vary largely (0.6% to 15%, Carroll et al, 1963; Chenoweth and Osborne, 1978; Chenoweth et al, 1996). A difficulty of the semen collection with EE is to determine whether failure to ejaculate is the result of poor response to the method or a pathological condition in the bull. Ejaculation failure during EE occurs in very temperamental Zebu sires or when bulls are stressed prior to EE, as when electric guns are used to handle the animals, which should be avoided. Also a quick palpation per rectum or inexperience of the operator with abrupt EE stimulation can constrain ejaculation. In these cases, a resting period of 10–15 minutes is advisable before a new attempt (Chacón, unpublished observations). Finally, practitioners should also consider that EE could cause a temporal corkscrew deviation of the penis during stimulation in some bulls (Carroll et al, 1963). Thus, this method does not allow the diagnosis of this condition in affected bulls, which instead should be diagnosed under natural mating.

Semen samples obtained by EE differ significantly from those collected by AV from the same bulls. The EE produces a high stimulus over the accessory glands (mainly seminal vesicles), thus increasing the ejaculate volume compared with samples collected by AV (Austin et al, 1959; Ball et al, 1983; León et al, 1991). Consequently, the pH, bicarbonate and fructose contents in the ejaculate are significantly higher in samples collected by EE (Carroll et al, 1963; León et al, 1991) while sperm concentration is lower (Austin et al, 1959; Ball et al, 1983; León et al, 1991). However, total sperm number per ejaculate, overall sperm motility and sperm morphology are not significantly different in semen samples from Zebu (León et al, 1991) or *Bos taurus* breeds (Austin et al, 1959; Carroll et al, 1963; Ball et al, 1983) collected alternatively with both methods.

*Semen evaluation under field conditions*

The field conditions prevailing in the tropics, together with the necessity of giving a prognosis of the potential fertility of the bull on-farm, demands a practical assessment of the spermiogramme, relating findings with the breeding management and clinical examination of the bulls. The use of EE as collection method imposes the interpretation of some spermiogramme variables, such as sperm concentration and motility, to be done cautiously.

Sperm concentration: Assessment of sperm concentration in semen samples collected from bulls during the mating season in tropical field conditions is not reliable. This unreliability is due to the huge variation of this parameter related to the frequency of ejaculation before the examination (Foster et al, 1970; Everett et al, 1978), which cannot be controlled in the field. The first ejaculates following sexual inactivity in bulls would have a normal concentration, although daily sperm output is low (Ball et al, 1983). In addition, sperm concentration is negatively correlated with ejaculate volume (Fields et al, 1979; Igboeli and Rakha, 1971), which is increased by EE. In addition, the response of bulls to EE is, as stated above, highly variable, thus also affecting the assessment of sperm concentration in the field.

Sperm motility: The significance of sperm motility upon the potential fertility of a bull examined under field conditions in the tropics should also be carefully considered. Although motility is an indicator of sperm viability and correlates with fertility of frozen semen (Januskauskas, 1999), its repeatability when assessed in natural breeding bulls is low, regardless of the method of semen collection (Almquist and Cunningham, 1966; Ball et al, 1983; Gipson et al, 1987; Wenkoff, 1988; León et al, 1991). Furthermore, the conditions under which the semen is collected may introduce urine or dust contamination, thus affecting motility estimations. In addition, motility is highly correlated with ejaculation frequency in Zebu (Igboeli and Rakha, 1971) and *Bos taurus* bulls (Almquist and Cunningham, 1966; 1967), and it could be affected according to mating activity in bulls under natural breeding. Consequently, the high variability reported for this parameter in Zebu and European breeds and the conditions prevailing at the farm where it is assessed, reduces the significance of this variable in the breeding prognosis of the bull if no other alterations are found at examination. One exception, however, may be a low sperm motility associated with a high percentage of tail defects in the semen sample as a sign of inherited conditions in the bull (Blom, 1966; Wenkoff, 1978) or epididymal pathologies (Gustafsson et al, 1972).

Sperm morphology: The relationship between sperm morphology of the semen sample and the potential fertility of the sire has been documented since the beginning of the century by Williams and Savage (1925) and later by Lagerlöf (1934). Few reports coming from the tropics concerning evaluation of methods to study sperm morphology in Zebu sires are available. Under field conditions in the

tropics, the necessity of providing a prognosis, at least a preliminary one, of the potential breeding soundness of a bull, forces the practitioner to consider the type of sperm abnormality in order to disclose whether it is inherited or acquired. Most practitioners in the tropics are still using conventional light microscopy, together with India ink or eosin-nigrosin staining, to evaluate morphology at low magnification (400×). This procedure can, however, mask many sperm defects (i.e., acrosome or nuclear abnormalities, Sprecher and Coe, 1996).

The use of phase-contrast microscopy to examine wet smears of semen fixed with buffered formol-saline or buffered glutaraldehyde, seems to be a convenient and practical method for assessing sperm morphology on-farm, since no staining is required. However, a disadvantage is the lower sensitivity in diagnosing abnormally shaped and sized sperm heads compared with carbol-fuchsin staining.

Another method for assessing sperm morphology is microscopy with differential interference-contrast optics (DIC). Coulter et al (1978) remarked on the suitability of this method for determination of some head abnormalities, especially nuclear pouches. Visintin et al (1984) reported that assessment of semen samples from Zebu bulls at AI-centres using DIC, allowed the diagnosis of 5% more acrosome abnormalities compared with when phase-contrast optics was used. Similarly, Saacke and Marshall (1968) stressed the convenience of DIC to assess acrosome morphology in bull spermatozoa. However, even when clearly advantageous, the use of DIC for routine assessment of sperm morphology is difficult to carry out under field conditions.

# Introduction to the studies

As stated above, beef cattle herds in the tropics comprise *Bos indicus* breeds, which are naturally sired and extensively reared in vast areas of grassland (McCosker et al, 1989; Chenoweth et al, 1996). These herds are also distinguished by a lack of productive and reproductive record keeping, infrequent veterinary assistance and low culling rates of animals with undesirable characteristics for breeding. In addition, the importance of the bull for its role on the efficiency of the reproductive herd, particularly in a beef production system based on extensive management, has been neglected in the tropics, even by veterinarians (McCosker et al, 1989; Müller 1990). Consequently, most bulls are selected based solely on phenotypic characteristics that are not necessarily related to their reproductive performance under natural mating conditions.

Extremely little attention has been paid to studies in the tropics of the breeding performance of *Bos indicus* bulls and the factors that interfere with their efficiency. A clear indicator of this trend is the reduced outcome of publications from this area concerning reproductive aspects in the male compared with the female and the low emphasis given to studying reproductive aspects of Zebu compared with *Bos taurus* males (Galina and Russell, 1987; Galina and Arthur, 1991). Studies concerning the breeding soundness of *Bos indicus* bulls in the tropical areas indicate a slow pattern of sexual maturity and testicular growth (Igboeli and Rakha, 1981; Kumi-Diaka et al, 1981; Oyedipe et al, 1981; Chenoweth et al, 1996; Vale-Filho et al, 1996). Thus, most young Zebu bulls failed to achieve satisfactory BSE status, compared with *Bos taurus* sires (Chenoweth et al, 1996) which might be reflected in low fertility rates, as those reported for Zebu bulls in tropical Australia (Chenoweth and Osborne, 1975; McCosker et al, 1989). The lower prevalence of testicular degeneration in Zebu breeds compared with *Bos taurus* and crossbred bulls reported in Brazil (Vale-Filho et al, 1980; Ohashi et al, 1988), suggests an interaction between genotype and environment in the tropics on the reproductive performance of the bull.

Therefore, since many gaps remain regarding the reproductive efficiency of Zebu bulls, there is an urgent need to investigate their breeding soundness and the variables associated with their final ranking after evaluation, to facilitate the diagnosis and prognosis of potentially low-fertile bulls in the breeding herd. Attention should be put on variables considered of value, as testicular consistency at clinical palpation, for their relation with the normality of the testicular tissue.

In regard to sperm morphology, few studies have been carried out to describe the frequencies of different abnormalities found in the ejaculates of extensively reared *Bos indicus* bulls and put such data in relation to clinical findings. Considering the positive relationship of sperm morphology with testicular and epididymal function and, subsequently, fertility, such studies are of utmost importance. However, there is a need for an on-farm evaluation procedure of the collected semen that does not involve laborious laboratory staining and detailed microscopy assessment. Since these methods differ in their ability to disclose

sperm details, a study where fixed wet smears and conventional stained smears (i.e., carbol-fuchsin) are compared is therefore advisable.

Additionally, the potential seasonal variations in clinical and spermiogramme variables in Zebu bulls are not fully studied. At present, the investigations performed in this area have used different bulls, usually crossbreeds, slaughtered at different periods of the year or based on a low number of sires under AI centre conditions. A well-controlled, long-term study is therefore needed.

# Aims of the study

The main objective of this thesis was to identify variables associated with the final ranking of extensively managed bulls, particularly Zebu bulls, during evaluation for breeding soundness (BSE) to facilitate a more accurate prognosis of these sires for natural breeding, under tropical conditions in Costa Rica.

The specific aims were to:

- evaluate the BSE of *Bos indicus*, *Bos taurus* and crossbred bulls located in various regions of Costa Rica, with distinct climatic conditions,

- assess the value of using phase-contrast microscopy with fixed, wet-smears for assessment of bull sperm morphology for BSE under field conditions,

- determine the frequency of sperm abnormalities found in the ejaculate of Zebu bulls according to their age, scrotal length, testicular consistency, scrotal circumference and ranking after BSE,

- assess the magnitude and nature of changes in fine morphology of the seminiferous and cauda epididymides epithelia of Zebu bulls with normal and decreased testicular consistency at clinical palpation, and

- determine whether clinical and spermatological parameters in Zebu bulls show seasonal changes under extensive rearing management in the dry tropics of Costa Rica.

# Materials and methods

## Location

In **Paper I**, the bulls were located in farms situated in four different climatic regions of tropical Costa Rica (8°00′–11°15′ N and 82°30′–86°00′ W) as defined by the National Meteorological Institute of Costa Rica, based on the yearly distribution of rainfall and ambient temperature. The regions were the North Dry Pacific, the Central Pacific, the South Pacific and the North-Caribbean (**Fig. 2**). In **Papers II-IV**, all Zebu bulls were located in the North Dry Pacific region (9°43′–11°13′ N and 84°46′– 85°57′ W).

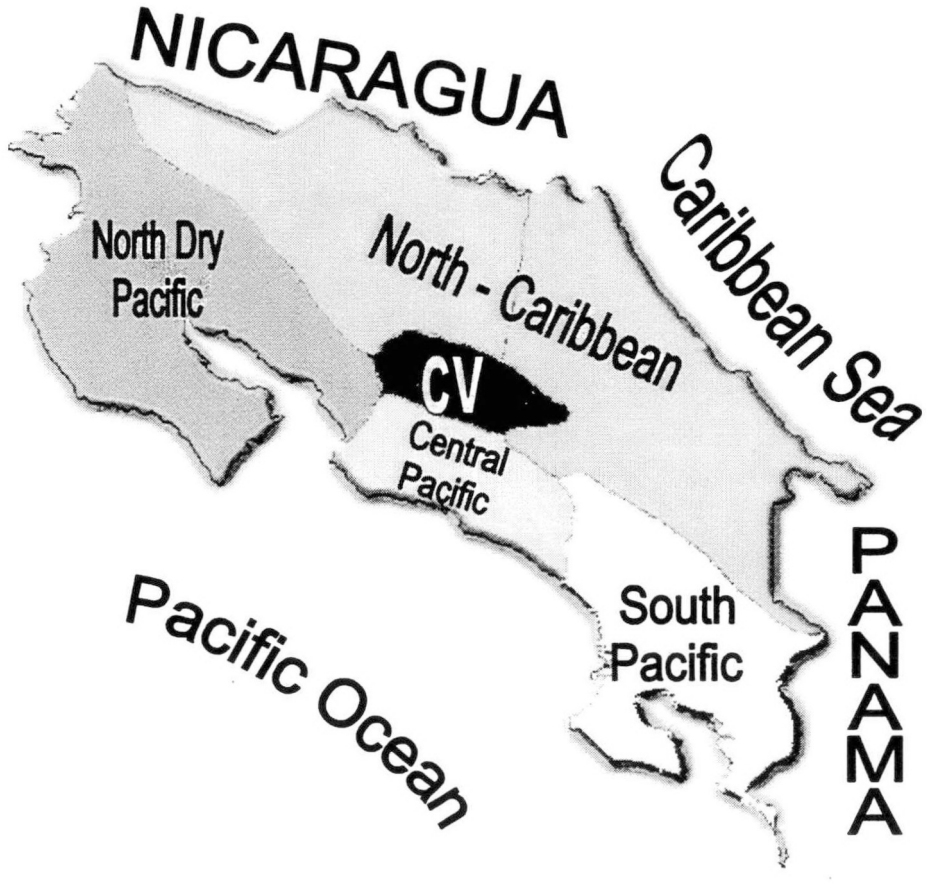


**Figure 2**: Map of Costa Rica showing the different climatic regions where the studies were carried out (**CV**: central valley, Source: National Meteorological Institute of Costa Rica).

## Climatic conditions

The climatic regions in Costa Rica (**Fig. 2**) differed from each other mainly in the distribution and amount of rainfall during the year. The yearly average rainfall for the past 20 years was 3,500 and 3,900 mm for the central and south Pacific, respectively, and 3,950 for the north Caribbean region. The monthly average humidity in these three regions ranged from 81% to 91% in the driest and rainiest months, respectively. However, daily values close to 100% usually have been reported during the most humid months (September and October). The average monthly maximum ambient temperature ranged from 29°C to 34°C, whereas the average monthly minimal temperature was never below 20°C (Source: National Meteorological Institute of Costa Rica). The north dry Pacific is the hottest and driest zone of Costa Rica with well-defined dry and rainy seasonal periods. The dry season lasts from December to April, whereas the rainy season lasts from May to November, with a maximum rainfall during June, September and October. The yearly average rainfall in the lowlands is around 1,400 mm. Maximum temperatures are reached during April, sometimes exceeding 36°C, and the monthly maximum averages are always above 31°C during the rest of the year. The relative humidity ranges between 60% and 65% during the dry period and between 80 and 85% during the rainiest months. The daylight time does not vary between the dry and rainy season (approximately 12 hours of daylight the whole year, Source: National Meteorological Institute of Costa Rica).

## Feeding management

All bulls (n= 1,236, **Papers I-IV**) grazed mixed pastures predominantly composed of *Hyparrenia rufa, Cynodon nlemfuensis, Brachiaria mutica, Panicum purpurascens,* and *Ischaemum ciliare.* Animals had free access to drinking water. *Ad libitum* supplementation with salt blocks was provided throughout the year. Additionally, molasses were supplied during the dry season in 50% of the farms (**Papers I** and **II**) and in all of the ranches involved in research for **Papers III** and **IV**. No other supplement was provided.

## Animals

The breed of the bulls was either documented or determined by their phenotypic appearance. The size of the animal sample was about 2% of the total bull population in Costa Rica (National Livestock Census, 1984). In **Paper I**, a total of 898 breeding bulls were examined with an overall mean age of 4.0 ± 1.7 years (± SD, range from 13 months to 12 years). The mean age for the *Bos indicus* bulls (*n*= 598) was 4.2 ± 1.8 years and included the following breeds (number of bulls in parenthesis): Brahman (254), Indobrasil (168), Gyr (54), Nellore (41) and Crossbred Zebu (81). The *Bos taurus* bulls (*n*= 252) had an average age of 3.7 ± 1.5 years and included: Simmental (63), Holstein (63), Brown Swiss (51), Charolais (20), Chianina (17), Jersey (14), Romosinuano (4) and crossbred *Bos taurus* (20). The *Bos indicus* × *Bos taurus* bulls (*n*= 48) were aged 3.9 ± 1.6 years and were comprised of Brahman bulls crossed either with

Simmental (21), Brown Swiss (11), Charolais (6), Chianina (6), Holstein (2) or Aberdeen Angus (2). In **Paper II**, 302 Zebu bulls, ranging from 18 months to 9 years of age, were examined (4.3 ± 2.0 years, mean ± SD). The bulls belonged to Brahman (138), Indobrasil (70), Nellore × Brahman (39), Nellore (33), Gyr (12), and Indobrasil × Brahman (10) breeds. In **Papers III-IV**, all bulls were of Brahman breed, ranging between 3 and 3,5 years (**Paper III**, n= 11) and between 3 and 6 years of age in **Paper IV** (4.5 ± 1.2, n= 25).

## Experimental design

All the bulls included in this thesis belonged to commercial beef or dual-purpose cattle farms (**Papers I-IV**). The evaluation of the bulls included a record of the breeding history and a thorough clinical inspection, including a partial spermiogramme assessment which included evaluation of sperm motility and sperm morphology of semen collected by EE. The general clinical inspection of the sires, as well as the specific examination of their reproductive organs, were performed under field conditions by the same operator (**Papers I-IV**). In **Papers I-II**, the visit was initiated at the farm by filling in a questionnaire, which included date of examination, name of the farm/owner, location, and nutritional management of the animals. A detailed description of the bull, including breed, tattoo number, and age, was made. The clinical background included previous illness history and mating system used (single or multiple sires, with restricted or continuous mating, length of breeding season, number of females per bull, mating behaviour, and dominance status). The general clinical inspection included the eyes and the muscle-skeletal system, with special emphasis placed on foot and leg conformation. The specific examination included inspection of the prepuce and penis (only when penile protrusion occurred during semen collection), palpation and examination of the scrotum and its contents, and assessment of the internal genitalia by rectal palpation. After the clinical and seminal evaluation on-farm, the bulls were diagnosed as sound or unsound for breeding, according to the standards proposed by Ball et al (1983) and Chenoweth et al (1992), and the farmer was advised to either keep, re-examine, or cull the bull (**Paper I-II**). In **Paper II**, a detailed classification of the sperm abnormalities found in ejaculates of grass-fed Zebu sires was made using either fixed wet- or stained smears. Only bulls found free of congenital or inflammatory problems in their reproductive organs were included in this survey. The frequencies of sperm abnormalities were disclosed according to age, testicular consistency (TC), scrotal circumference (SC), scrotal length (SL), or BSE-grading, and comparisons between groups were made. In **Paper III**, testicular and cauda epididymides tissue samples were collected immediately after slaughter from extensively managed Brahman bulls with normal or decreased TC at palpation. The testes were fixed by vascular perfusion and the cauda epididymides and their contents immersion-fixed with buffered glutaraldehyde solution and then submitted for routine processing for transmission electron microscopy (TEM). In **Paper IV**, clinical and spermiogramme parameters of Brahman bulls reared in the North

Dry Pacific region of Costa Rica and considered as sound for breeding after a preliminary BSE, were assessed monthly during a 13-month period.

## Methods

*Recording of clinical parameters*

Determination of body condition score (BCS, **Papers I-IV**): BCS of the bulls was scored according to the system used for Zebu cattle by the International Livestock Centre for Africa (Nicholson and Butterworth, 1986). This method uses a five-point scale with half-point increments. For analytical purposes, the BCS was categorised (**Paper I**) as low (< 3) or normal (≥ 3) or used as a continuous variable (**Paper IV**).

Classification of scrotal length (SL, **Papers I-IV**): SL was classified according to the distance between the bottom of the scrotum and the hock joint. ***Short SL***: Bulls having a scrotum clearly close to the body without signs of contraction of the cremaster muscle, normally >15 cm above the hock joint; ***Normal SL***: Sires with a scrotum that was less than 15 cm above the hock joint down to the level of the hock joint; ***Long SL***: Bulls with a scrotum below the hock joint.

Measurement of scrotal circumference (SC, **Papers I-IV**): SC was determined by the same operator during the clinical examination of the bull using a standard scrotal metal tape (Nasco®, Wisconsin, USA) placed at the widest mid-scrotal point. In **Paper I**, SC was categorised as < 30 or ≥ 30 cm, whereas in **Papers I-IV** it was used as a continuous variable.

Determination of testicular consistency (TC, **Papers I-IV**): TC was determined by palpation by the same operator and categorised as soft, normal or hard.

*Semen collection*

Semen collection (**Papers I-II** and **IV**) was performed through EE immediately after the clinical examination. The bull was well restrained in a cattle chute using two pieces of strong wood behind the rear legs for the safety of the operator during rectal palpation and to prevent back movement of the bull (**Fig. 1**). After removing the manure from the rectum with a gloved hand, the accessory glands and pelvic urethra were massaged per rectum for at least three minutes. Then, a 33-cm long electro-ejaculator probe, comprised of three longitudinal electrodes (Standard Precision Electronics®, Colorado, USA) was lubricated and fully inserted into the rectum. Immediately after, the operator performed slight and progressive increases in the electrical stimulation until semen was collected in a latex funnel with a glass tube attached to its end.

*Semen evaluation*

*Evaluation of sperm motility* (**Papers I-II and IV**): The same operator assessed the overall percentage of motile spermatozoa immediately after semen collection. The estimation was done subjectively after examining five different fields in the

wet smear of undiluted semen using a phase-contrast microscope (Zeiss®, Germany) at 400×.

*Evaluation of sperm morphology* (**Papers I-II** and **IV**): Semen samples were fixed with buffered formol-saline solution (Hancock, 1952). Sperm morphology was examined in wet-smears using phase-contrast microscopy at 1,000x (**Papers I-II** and **IV**) and in air-dried smears stained with carbol-fuchsin (Williams and Utica, 1920) using bright field light microscopy at 1,000× (**Papers II** and **IV**). Sperm morphology was assessed in the field (**Paper I-II**) and at the laboratory (**Papers II** and **IV**). The percentages of abnormal spermatozoa (abnormalities of the acrosome, abnormally shaped and sized heads, nuclear invaginations, mid-piece and tail abnormalities, and the presence and location of cytoplasmic droplets) were recorded and classified according to Lagerlöf (1934), after counting 200 (**Paper I**) and 400 (**Papers II** and **IV**) spermatozoa per smear.

## Statistical analyses

Analyses were performed using the Statistical Analysis System software package (SAS® 1988, Cary, North Carolina, USA; **Papers I, II** and **IV**). The level of statistical significance was set to $P< 0.05$. In **Paper I**, the relationship between the climatic and clinical parameters with the classification of the bulls after BSE and the spermiogramme variables studied was analysed by means of linear multiple regression (GLM procedure). These analyses included only males free from congenital or inflammatory clinical abnormalities at the time of evaluation and for which climatic data were available (701 sires). With the aim of studying the potential effect of maximum ambient temperature on the variables of interest, the temperature value utilised was the one to which the bull had been exposed a month before the evaluation. Comparisons between least-square means (LSM) for categorical variables were performed when differences were statistically significant. Age, BCS and TC were included as categorical variables in the statistical analyses. In **Paper II**, analysis of variance was performed focusing the effects of age, TC, SC, SL and BSE categories using the Bonferroni test for means comparisons (SAS®, 1988). The mean percentages of abnormal heads (size and shape) determined using either carbol-fuchsin stain or formol-saline wet smears were compared using ANOVA. In **Paper IV**, the potential variations between clinical and spermiogramme variables during the period of study were analysed using a GLM regression model for repeated measurements.

# Results

## Prevalence of unsound bulls after BSE (Paper I)

Thirty-three percent (296 out of 898) of the bulls examined in four different regions of Costa Rica were classified as unsound for breeding. Of these, 82 were classified unsound because of clinical unsoundness (9.1%, 82/898) and the remaining (23.9%, 214/898) due to deviations in sperm morphology. Regarding clinical findings, abnormalities associated with low SC (testicular asymmetry, hypoplasia, fibrosis, and atrophy) were the most common alterations found [6.8 %, $n$ = 61/898]). The prevalence of other clinical abnormalities was below 1% and, as a rule, the sires having these anomalies were culled by the farmer. The prevalence of bulls classified as unsound for breeding after BSE did not differ statistically among the climatic zones under study.

## Relationship between the climatic variables and the clinical and spermiogramme parameters, and BSE classification (Paper I)

*Clinical parameters*

No statistically significant relation was found between average rainfall or ambient temperature and BCS or TC, respectively. In contrast, a negative effect was observed between the ambient temperature, to which the bulls were exposed the month before the BSE was performed, and SC ($\beta$ = –0.28, $P< 0.01$). This relationship was independent of the breeds under study since the interaction of breeds by temperature was not statistically significant in the model studied ($P> 0.05$). In addition, bulls with a low BCS had a significantly smaller SC than bulls with a normal BCS (34.9 vs. 36.0 cm, LSM, $P< 0.01$).

*Spermiogramme parameters*

Sperm motility: No relationship was found between the climatic variables studied and sperm motility ($P> 0.05$).

Sperm morphology: A positive relationship was found between the monthly mean temperature and rainfall to which the bull was exposed the month before the evaluation and the frequencies of abnormal tails and proximal cytoplasmic droplets ($\beta$ = 1.44, $P< 0.05$ and $\beta$ = 0.015, $P< 0.01$ respectively). No statistical relationship was found between the climatic variables and the frequency of abnormal sperm heads and mid-pieces.

*Classification after BSE*

A statistically significant positive relationship ($\beta$ = 0.24, $P< 0.05$) was observed between the percentage of bulls classified as unsound for breeding and the ambient temperature.

## Effect of breed, age, body condition score, scrotal length, scrotal circumference and testicular consistency on spermiogramme parameters and BSE classification (Papers I-II)

*Sperm motility*

No relationship was found between breed, age, SL and SC and sperm motility. However, mean sperm motility was lower in bulls with a low BCS and a lower TC compared with bulls having a normal BCS or TC (56 vs. 61% and 57 vs. 61%, respectively; $P< 0.05$, **Paper I**).

*Sperm morphology*

The mean percentage of sperm-head abnormalities was higher ($P< 0.001$) in *Bos indicus* × *Bos taurus* bulls compared with both *Bos taurus* and *Bos indicus* sires (22%, 15% and 13%, respectively). No significant difference was found between *Bos indicus* and *Bos taurus* bulls in this respect (**Paper I**). Zebu sires less than 2 years old had higher frequencies of missing acrosomes and proximal cytoplasmic droplets in their ejaculates (12.1 vs. 2.4% and 23.9 vs. 3.6%, respectively; $P< 0.05$) compared with older bulls (**Paper II**). No significant differences were observed between the groups of older bulls in the percentages of sperm abnormalities (**Papers I-II**). No statistical relationship was found between BCS and sperm abnormalities in the ejaculate (**Paper I**). The frequency of sires with a long scrotum was higher in *Bos taurus* bulls (16%) compared with Zebu (12%) and crossbred bulls (11%). However, independent of the breed, bulls having a long scrotum and a soft TC had a higher percentage of abnormal sperm heads in their ejaculates compared with bulls with a normal scrotum length (19 vs. 14%; $P< 0.01$) and a normal TC at palpation (20 vs. 13%, $P< 0.001$, **Paper I**). The same results were also found in Zebu breeds (**Paper II**) with a long scrotum and a soft TC ($P< 0.05$) compared with normal bulls (32.7% vs. 12.8% and 30.7% vs. 10.3%, respectively). Neither TC nor SL affected the mean percentages of abnormal acrosomes or tails, or the presence of cytoplasmic droplets. A negative relationship ($\beta = -1.16$, $P< 0.05$) was observed between SC and the frequency of sperm head abnormalities (**Paper I**). However, Zebu bulls older than 2 years with a SC less than 30 cm did not show this pattern, albeit a higher prevalence of proximal cytoplasmic droplets was seen in their ejaculates compared with sires having SC equal to or more than 30 cm ($P< 0.05$). No differences in the other sperm abnormalities were found with regard to the SC classification (**Paper II**).

*Classification after BSE*

The percentage of bulls classified as unsound for breeding (**Paper I**) was lower for the *Zebu* sires (29%) compared with both *Bos taurus* (40%, $P< 0.01$) and *B. indicus* × *B. taurus* bulls (46%, $P< 0.01$). This trend was independent of the region studied. The prevalence of unsound bulls was also higher in sires with

a long scrotum (16.6 vs. 11.5%; P< 0.05) and a soft TC score (42.7 vs. 25.3%, P< 0.001) compared with bulls with a normal SL and normal TC. In the same way, the percentage of bulls unsound for breeding increased with decreasing SC ($\beta = -0.5$, P< 0.05).

*Sperm morphology in Zebu bulls and classification after BSE* (**Paper II**)

The knobbed sperm defect was the most common abnormality found in the acrosome of Zebu bulls ranked as unsound for breeding, (4.0 ± 7.3%, mean ± SD). There were no statistical differences in the percentage of missing acrosomes between the bulls classified as unsound and sound for breeding. In regard to the sperm head, the most common abnormalities found in Zebu bulls ranked as unsound for breeding were heads narrow at the base (11.5 ± 16.2%) and undeveloped head forms (10.7 ± 17.5%). Concerning other abnormalities, bent tails with an entrapped cytoplasmic droplet was the most common tail abnormality found in both the groups classified as unsound and sound for breeding (8.3 ± 13.3 and 3.0 ± 3.7%, respectively). In addition, this was the most common sperm abnormality found in bulls classified as sound for breeding after BSE.

*Prevalence of specific sperm abnormalities in Zebu bulls* (**Paper II**)

In general, all the specific sperm defects reported in the literature in *Bos taurus* bulls were found in the Zebu sires studied. Although the overall mean percentage for these abnormalities was less than 5%, some bulls showed high frequencies of these defects, especially tail and nuclear crater defects. The tightly coiled tail under the head was the most common defect, appearing in 27 bulls in more than 10% and one bull having 65% of its spermatozoa affected. The crater defect appeared in 21 bulls with more than 10% of affected spermatozoa and in one bull affecting 64% of the spermatozoa. The knobbed acrosome appeared in 15 of 27 bulls in a frequency of more than 10% of the spermatozoa and in one bull with 34%.

## Formol-saline vs. carbol-fuchsin staining for assessment of sperm head morphology in Zebu bulls (Paper II)

Evaluation of sperm head morphology (i.e., size and shape of heads) in smears stained with carbol-fuchsin allowed the detection of a higher percentage of heads that were narrow at the base and undeveloped, compared with wet smears examined under phase-contrast microscopy (12.0% ± 1.1 and 11.7% ± 1.3 vs. 3.2% ± 1.1 and 5.3% ± 1.3, respectively; mean ± SD, P< 0.001) in samples from the same ejaculate. No statistically significant differences were found between the methods regarding rounded, tapered, giant or double heads (P> 0.05). The use of carbol-fuchsin for determination of head size and shape, therefore, made it possible to diagnose 16 more bulls as "unsound" than did the use of phase-

contrast microscopy of formol-saline–fixed samples (136 vs. 120), which gives a sensitivity of 88% for the latter method.

## Morphology of the seminiferous and cauda epididymides epithelia in Zebu bulls with normal or decreased testicular consistency (Paper III)

Bulls having a slight to moderate reduction in TC showed more, albeit slight, degenerative changes in the seminiferous epithelium, compared with control animals. Cellular debris present in the Sertoli cells, abnormalities in the acrosome and condensation of the chromatin during spermiogenesis were also common in cases of decreased TC. In contrast, the epithelia from the cauda epididymides of these bulls did not show morphological deviations compared with controls, although the presence of intra-epithelial macrophages and of cell debris in the lumen, together with foreign cells, was more pronounced.

## Seasonal changes in clinical and spermiogramme parameters of extensively reared Zebu bulls (Paper IV)

*Body condition (BCS)*

The bulls maintained a good body condition throughout the study period. However, BCS were higher from January to May compared with other months ($P< 0.01$) and started to decrease in June. The lowest seasonal mean, which differed significantly from the January–May mean ($P< 0.01$), was registered for September–December. No statistical relation was found between BCS and any of the climatic variables studied.

*Scrotal circumference (SC) and testicular consistency (TC)*

The mean SC's were lower during the period September–February and highest during March–August ($P< 0.001$). A positive relationship was found between SC and BCS of the Zebu bulls, since every unit of increase in BCS was followed by an increment of 0.7 cm in SC ($P< 0.001$). Age was also positively associated with SC during the study period ($\beta = 0.05$, $P< 0.01$). In contrast, no relationship was found between SC and the climatic variables studied. No significant changes were observed in the TC of the Zebu bulls during the study period. Furthermore, no statistically significant relationship was found between TC and the climatic variables or between TC and the body condition of the sires.

*Sperm motility and morphology*

A significant variation was observed in the mean percentage of overall sperm motility during the study period ($P< 0.05$), which decreased from 63% in September to 47% in December. The mean sperm motility decreased a second time from 65% in April–May to 45% in July–August ($P> 0.05$). Moreover, sperm motility was higher in ejaculates obtained during the mating season (62%) than in

those collected when the sires were out of the breeding period (52%) ($P< 0.01$). No statistical relationship was found between motility and any of the climatic variables studied or between motility and BCS, SC, or TC, respectively.

No significant variations were found during the study in the percentage of abnormal acrosomes, sperm heads, mid-pieces or cytoplasmic droplets. In contrast, significant fluctuations in the frequency of bent tails with entrapped cytoplasmic droplets were observed during the study period ($P< 0.05$), with values being highest (up to 21%) during August. This was the single most common sperm abnormality found during the study. However, no relationship was observed between the frequency of this abnormality and the climatic, BCS, SC and TC variables, respectively.

# General discussion

Soundness for breeding of a bull implies a complex interaction of factors such as physical and reproductive health, libido and mating ability, semen quality and interactions among animals in the breeding herd.

Practising a BSE in bulls is very important and perhaps essential in the tropics, considering the type of beef production system prevailing in these regions. The extensive management characterised by a poor selection of bulls based on reproductive characters and the scarcity of programmes monitoring herd health and reproduction makes it difficult to discriminate low-fertile bulls based on their *in vivo* fertility performance. Therefore, it is of utmost importance to carry out an andrological evaluation of the young bulls before they are placed in the breeding herd. In addition, and since environmental conditions in the tropics (i.e., temperature, humidity, nutrition, diseases) may adversely affect the reproductive health of the bulls at any time, a BSE of the bulls used for breeding is advisable at least once a year or before the breeding season starts in farms with seasonal mating.

Among the bulls considered clinically unsound for breeding (**Paper I**), a low SC was obvious evidence of the poor selection of reproductive traits done in breeding sires (*Bos indicus*, *Bos taurus* and crosses) in the tropics. The higher prevalence of sires classified as unsound for breeding due to sperm abnormalities (**Paper I**) in bulls with low SC agrees with earlier findings in Brahman (Zebu) (McCosker et al, 1989; Morris et al, 1989; Chenoweth et al, 1996) and *Bos taurus* bulls (Coulter and Foote, 1979; Madrid et al, 1988). This was also supported by findings in **Paper II**, where a higher prevalence of proximal cytoplasmic droplets were found in Zebu bulls older than 2 years with a SC less than 30 cm. According to Madrid et al. (1988), an increased percentage of proximal droplets in young *Bos taurus* bulls with a SC less than 32 cm may be an indicator of delayed sexual maturity or might suggest an impairment in the testicular or epididymal function. An andrological follow-up of these sires and in-depth studies regarding peri-pubertal changes in SC values and spermiogramme traits of Zebu bulls are necessary to eliminate the two latter possibilities. In addition, SC in *Bos indicus* and *Bos taurus* bull is highly correlated with testicular weight, with sperm output generally increasing in sires as SC increases (Elmore et al, 1976; McCosker et al, 1989; Rekwot et al, 1994). Unfortunately, the majority of data concerning standards of SC by age in Zebu breeds come from non-tropical regions or are based in animals fed with grain supplements. Therefore, the use of these data on grass-fed Zebu bulls in tropical areas should be used with caution. The use of a minimal SC value to select *Bos indicus* bulls for breeding, together with further training courses for farmers as well as veterinarians, are required to modify the present tendency shown by breeders and farmers in the tropics to ignore the importance of measuring SC when selecting bulls for breeding purposes.

It is a general belief that climate (often mainly translated as ambient temperature) is one of the major factors accounting for impairment of spermatogenesis in the bull (Skinner and Louw, 1966). This relationship has been studied and demonstrated in *Bos taurus* breeds (Johnston and Branton, 1953;

Meyerhoeffer et al, 1986; Parkinson, 1987). However, this negative effect seems to be lower in *Bos indicus* than in *Bos taurus* bulls (Vale-Filho et al, 1980; Kumi-Diaka et al, 1981; Wildeus and Entwistle, 1983a; 1984). The negative effect observed in **Paper I** between the ambient temperature to which the bulls studied were exposed the month before the examination and the SC might suggest an impairment of testicular function during hot periods in the tropical areas. In addition, the relationship between the ambient temperature and the frequencies of cytoplasmic droplets and tail abnormalities may suggest an impairment of the epididymal function during the summer season. However, since the bulls studied were free from clinical abnormalities and ranked only according to their sperm morphology, the relationship between temperature and classification after BSE may have been the result of the increasing presence of these sperm abnormalities in the semen samples. Artificial scrotal insulation of Zebu crosses caused, after 2 weeks of insulation, an increase in the frequency of proximal cytoplasmic droplets, probably as a result of epididymal dysfunction (Wildeus and Entwistle, 1983a). Igboeli and Rakha (1971) reported a slight rise in the frequency of the same abnormality in ejaculates from Angoni (Zebu) bulls during the hottest period of the year. The mechanism behind this apparent impairment of the epididymal function related to heat stress in the bull is unclear. However, since the normal function in this organ depends on a proper supply of testicular testosterone and its conversion to dihydrotestosterone, it could be hypothesised that it either reflects a hormonal deprivation or an impaired ability of the organ to convert the hormone to its active form. This could be circumstantially supported by reports from Rhynes and Ewing (1973) and Parkinson (1987) showing that periods of heat stress in *Bos taurus* were associated with a reduction of up to 43% in the testosterone levels as well as an increase in progesterone (Parkinson, 1987). However, there is no experimental evidence yet provided that supports these assumptions in Zebu sires.

It is possible that the relationships found in **Paper I** between ambient temperature and SC values, prevalence of cytoplasmic droplets and classification at BSE were influenced by individual variations. Therefore, the effect of the climatic variables on the clinical and spermiogramme parameters of the bulls should be studied in the same individuals over time and not based on assessments of single ejaculates of different bulls evaluated at different seasons, as performed in **Paper I**. In addition, grouping different sperm abnormalities (i.e. proximal cytoplasmic droplets together with abnormal sperm tails) could have been a factor of confusion when analysing the potential relationship between ambient temperature and sperm morphology.

In **Paper I**, the lower number of Zebu bulls found unsound for breeding compared with *Bos taurus* and *Bos indicus* × *Bos taurus* sires indicates a higher capacity of the Zebu bulls to adapt under tropical conditions. This high adaptability is supported by physiological and anatomical attributes that allow them to regulate their body temperature more efficiently (Turner, 1980; Finch, 1985). In addition, Zebu cattle is also recognised for having a better utilisation of poor pastures and high resistance to ectoparasites, two conditions that are common in the tropics (Cartwright, 1955; Rekwot et al, 1994; Fordyce et al,

1996). The adaptability of *Bos indicus* sires to hot climate was also clear when looking for potential seasonal variations in clinical and spermiogramme parameters (**Paper IV**) since the ambient temperature had no effect on any of the variables studied. In contrast, nutrition under extensive management practice, as in the tropics, seemed to play a major role on the reproductive performance of Zebu sires. The fact that the SC was significantly affected by changes in the body condition score (BCS) of the Brahman bulls studied (**Paper IV**), but not by the changes in ambient temperature, strongly suggests that nutrition (i.e., grass availability) is an important factor (Kumi-Diaka and Zemjanis, 1978). In Australia, Wildeus and Entwistle (1984) reported higher percentages of sperm abnormalities during the dry season, when pastures are scarce but temperatures are lower, compared with the rainy season. The reduction observed in SC values, due to a decrease in the BCS (**Papers I and IV**) was, however, not reflected in significant changes in the sperm morphology of the bulls studied. Spermatogenesis has been considered normal at any season, as studied histologically in the testes of Zebu bulls (Kumi-Diaka et al, 1983). A reduction in the diameter of the seminiferous tubuli was, however, found during the dry season when the quantity and quality of the pastures decreased (Kumi-Diaka et al, 1983). These results indicate that a reduction in the SC related to a decrease in BCS may be reflected in a lower sperm output in grass-fed Zebu bulls, despite the fact that other parameters, such as sperm morphology and TC, are not significantly affected. Unfortunately, determinations of sperm output were not done in the bulls hereby studied, since the sires were often breeding at the moment of collection and further, their semen was collected by EE.

The increased percentages of proximal cytoplasmic droplets found in ejaculates from Zebu bulls less than 2 years of age is in agreement with the slow attainment of sexual maturity reported for *Bos indicus* managed under pasture conditions (Rekwot et al, 1987a; Silva-Mena, 1997). A higher percentage of this abnormality, similar to the findings in **Paper II** for *Bos indicus*, has been found earlier in sexually immature *Bos taurus* bulls (Madrid et al, 1988; Mortimer et al, 1991). Wildeus and Entwistle (1983b) studied the epididymal development in *Bos indicus* cross bulls and found that the caput is the last segment completing differentiation during puberty. Since it is in this region that the migration of the cytoplasmic droplet to distal regions of the mid-piece takes place (Crabo, 1965; Gustafsson et al, 1972), this finding may explain the association found between the prevalence of this abnormality and sexually immaturity in *Bos indicus*. In addition, the high prevalence of missing acrosomes found in young Zebu bulls (<2 years of age, **Paper II**) may also reflect a low sperm turnover in the epididymal cauda as a result of low mating frequency (Saacke and Marshall, 1968; Wells et al, 1971), since *Bos indicus* bulls are not placed in the breeding herd until they reach 2 or more years of age. No significant differences in sperm morphology were found between Zebu bulls older than 7 years and sires 2 or more years and less than 7 years old (**Papers I-II**). This agrees with previous findings (Rao and Bane, 1985) that senile atrophy of the germinal epithelium in bulls is not necessarily associated with deviations in sperm morphology in the ejaculate. However, since some degree of proliferation in the amount of interstitial connective tissue and tubular calcification

occurred in bulls older than 7 years, it might be assumed that senile atrophy leads to a decreased sperm production. In addition, the use of old bulls in the herd is not recommended because it increases the risk for transmission of venereal diseases such as trichomoniasis (Pérez et al, 1992), as well as inbreeding, due to the dominance of older Zebu sires (i.e., >7 years; McCosker et al, 1989).

The relationship found between the presence of a long scrotum in the sires, sperm abnormalities and classification after BSE (**Papers I-II**) has not been documented in breeding bulls. The results obtained in the present thesis indicate that spermatogenesis was severely impaired in *Bos indicus*, *Bos taurus* and crossbred bulls having a long scrotum, since abnormalities in the sperm nucleus, as well as abnormal size and shape of the sperm heads, were more frequently seen in semen samples from these bulls compared with sires with a normal scrotum. The mechanism of the impairment in testicular function associated with a pendulous scrotum is not determined. However, it is clear that bulls having this characteristic are more exposed to testicular lesions by the constant beating upon the pendulous scrotum. Waites (1970) and Riemerschmid and Quinlan (1941) reported that the long, pendulous scrotum of bulls has a decreased gradient of temperature between its inguinal region and the bottom of the scrotal sac. Since this gradient directly depends on testicular thermoregulation mechanisms (i.e., pampiniform plexus; Cook et al, 1994), the presence of a long scrotum might, in some way, affect the heat exchange by the plexus. The incidence of bulls with a pendulous scrotum was higher in *Bos taurus* bulls (**Paper I**), attributed to a permanent relaxation of the cremaster muscle in an attempt to down-regulate scrotal temperature and might be a sign of maladaptation to the tropical climate in this species. Obviously, the pathogenesis of this relationship deserves to be fully studied in the future.

A significant relationship has been reported to exist between TC measured by tonometry and semen quality in *Bos taurus* bulls (Hahn et al, 1969; Coulter and Foote, 1979; Cook et al, 1994). However, similar reports are scarce in Zebu breeds extensively reared in the tropics. The relationship found between a decreased TC and a higher frequency of sperm head abnormalities in Zebu sires (**Papers I-II**) indicates that TC might be a reliable and practically useful indicator of the integrity in the seminiferous epithelium. This was confirmed by the histology of testes from Brahman (*Bos indicus*) bulls studied in detail in **Paper III**. Bulls with a clinically decreased TC at palpation had testes whose seminiferous epithelium presented multiple intercellular hollow areas. These empty spaces, at the level where spermatocytes are usually localised, indicate that these spermatogenic stages died and were then either sloughed to the tubular lumen or (most likely) phagocyted by the Sertoli cells. The latter is supported by the increased phagocytic activity found in the Sertoli cell cytoplasm, indicated by the presence of cellular debris in phago-lysosome–like structures. Goyal (1982) and Sinowatz et al (1979) also reported an increased phagocytic activity by Sertoli cells in bulls with impaired spermatogenesis. The presence of abnormalities in the acrosome and a defective condensation of the chromatin during spermiogenesis were also more commonly found in Zebu bulls with decreased TC at palpation. Such changes have been reported in bulls with impaired spermatogenesis due to testicular degeneration

(Lagerlöf, 1934; Müller et al, 1992). The normal picture found in the cauda epididymides of bulls with decreased TC at scrotal palpation indicates that this organ has lower propensity to degenerative epithelial changes, compared with the germinal epithelium. The presence of macrophage-like cells in the epithelium of the cauda epididymides suggests that spermiophagy could be present at this level in bulls with decreased TC, as it occurs in bulls with impaired spermatogenesis (Roussel et al, 1967). In contrast with these findings, spermiophagy seems to occur more frequently in the straight tubules and rete testis than in the corpus and cauda epididymes in bulls with normal TC (Roussel et al, 1967; Crabo et al, 1971; Goyal, 1982). Finally, the higher incidence of bulls with decreased TC at palpation being classified as unsound for breeding reinforces the importance of recording this parameter during the breeding soundness evaluation of Zebu bulls under field conditions.

The relationships found between a lower overall sperm motility in bulls with a reduced BCS and decreased TC (**Paper I**) are, in opinion of the author, of little relevance. The significance of this parameter should be carefully interpreted when the assessment is done under field conditions and on samples collected by EE. Since these results (**Paper I**) were based on examinations of a single ejaculate from different animals, the high individual variation reported for this parameter in bulls (even managed under controlled conditions) could have accounted for the differences found between groups studied. This is supported by the absence of a relationship between variations in sperm motility and any climatic or clinical variable in the more homogeneous group of Brahman bulls examined at monthly intervals (**Paper IV**). The results in the latter study also suggest that sperm motility is largely influenced by management conditions in the breeding group (i.e., mating frequency). This is in agreement with findings in *Bos indicus* (Igboeli and Rakha, 1971) and *Bos taurus* bulls (Almquist and Cunningham, 1966; 1967) where sperm motility increased with a higher ejaculation frequency.

Considering the above, the assessment of sperm morphology is of utmost importance during the BSE of Zebu bulls extensively reared in the tropics. Under these conditions, evaluating a bull's fertility by the herd's conception rate may be misleading, since breeding is based on natural mating with several bulls in the breeding herd and since most farms do not keep reproductive records. In addition, the assessment of other important spermiogramme variables, such as sperm concentration and sperm motility, may provide erroneous information, since under extensive rearing management, EE is the method currently used to obtain semen samples from Zebu bulls. Since the assessment of sperm morphology is done at the farm, it is obvious that the method used to determine the different abnormalities must allow a reliable evaluation of the semen sample. The higher percentage of sperm head abnormalities (size and shape) diagnosed when using stained dry smears with carbol-fuchsin (Lagerlöf, 1934) compared with using only wet-smears of formol-saline fixed semen samples, emphasises the importance of using both methods when assessing sperm morphology (**Paper II**). Despite the usefulness of combining the methods to evaluate sperm morphology, there is a need for evaluating the breeding soundness of the Zebu

bulls *on site* and providing the farmer with an immediate diagnosis of the potential performance of the examined sire. Since it is difficult to perform microscopy using carbol-fuchsin–stained smears in the field, a useful and practical procedure is to give a preliminary evaluation of the sperm morphology using wet smears under phase-contrast microscopy on the farm, and then, at the laboratory, to do a follow-up of the stained smears of those samples which, according to the judgement of the evaluator, need to be more closely studied for a confirmation of the diagnosis.

Another important consideration when assessing sperm morphology of Zebu bulls in the field is the prognosis for the bull. This is largely influenced not only by the frequency but also by the type of sperm abnormality found in the semen sample. Evidence is provided that some sperm abnormalities can be the result of genetic influences (Blom, 1966; Barth, 1986; Kojima, 1988a, 1988b) or they can be acquired either by impairment of testicular/epididymal function (Lagerlöf, 1934; Gustafsson et al, 1974) or excessive sexual rest (i.e., missing acrosomes) (Saacke and Marshall, 1968; Wells et al, 1971). The method applied in **Paper II** summarising the abnormalities by sperm domain, as well as counting the specific defects separately, not only allows assumptions to be made about the aetiology of the abnormality (for instance, the relationship found between head abnormalities and TC or SL, respectively), but also allows for a better prognosis to be made of the potential recovery of the breeding capacity of the bull, since abnormalities with a genetic origin may not change significantly in repeated semen collections, in contrast to acquired abnormalities (Koefoed-Johnsen et al, 1980; Barth, 1986; Kojima, 1988a; 1988b). Bent sperm tails with entrapped cytoplasmic droplets was the single most common sperm abnormality found in Zebu bulls classified as sound for breeding after BSE (**Paper II**). The finding was repeated in **Paper IV**, where the frequency of the abnormality showed a fluctuating pattern, without significant relation to clinical or climatic variables. The presence and levels of this abnormality has been earlier reported in Bos Taurus (Swanson and Boyd, 1962; Barth and Oko, 1989) and Bos indicus (Igboeli and Rakha, 1971, Fayemi and Adegbite, 1982, Wildeus and Entwistle, 1984). Additionally, bent tails with an entrapped cytoplasmic droplet have been reported as the dominant tail abnormality in ejaculates from bulls affected by epididymal dysfunction (Swanson and Boyd, 1962; Gustafsson et al, 1972; 1974). The aetiology of this sperm abnormality should be studied further.

The results obtained when studying potential seasonal variations in clinical and spermiogramme variables in Zebu sires (**Paper IV**) confirm not only the high adaptability of *Bos indicus* to a tropical environment, but also that nutrition is probably the major factor influencing their reproductive performance. These findings agree with studies from Da Silva and Casagrande (1976), Kumi-Diaka et al (1983), Wildeus and Entwistle (1984) and Rekwot et al (1987b; 1994). However, it should be considered that although no clear signs of testicular degeneration were seen in Zebu bulls, as reflected in changes in the TC or deviations in sperm morphology throughout the year, the reduction in SC due to a decrease in BCS values might reflect a decreased sperm production. This is

supported by the reduction in seminiferous tubular diameter found in Zebu bulls during periods of low pasture availability (Kumi-Diaka et al, 1983). This factor needs to be considered when evaluating bulls for breeding soundness in order to improve nutritional status of the sires before the mating season on the farm or during the periods when quantity and quality of the available pastures decrease. The lowering in BCS of the Zebu bulls during and up to two months following the rainy season has not been reported previously. The general belief has been that nutritional conditions in the tropical areas are poor only during the dry season. However, the results obtained in **Paper IV** indicate that during the periods of high rainfall (i.e., September–October in Costa Rica), the nutritional status — as seen reflected by the BCS of the bulls — is also impaired. This could be explained by the increased water contents in the pastures, which also decreases the consumption and digestion by the bull (US National Research Council, 1984).

# General conclusions

- Of the bull population explored by BSE, more than 30% were classified as unsound for breeding, mainly due to low scrotal circumference and unsatisfactory sperm morphology. Zebu (*Bos indicus*) bulls were the less affected, independently of the climatic region studied.

- Phase-contrast microscopy of wet-fixed smears was a convenient method to assess sperm morphology on-farm since it allowed the diagnosis (based on sperm morphology) of up to 88% of unsound bulls. However, a follow-up of stained semen smears is advisable to confirm the primary assessment.

- Zebu bulls with a long, pendulous scrotum, low SC and decreased TC at palpation were more frequently classified as unsound for breeding and had a higher frequency of sperm head abnormalities, compared with normal sires. Zebu bulls older than 2 years and with SC values lower than 30 cm showed a higher frequency of proximal cytoplasmic droplets.

- Decreased testicular consistency at palpation in Zebu bulls was related to minor morphological alterations in the seminiferous epithelium but no obvious deviations at the cauda epididymides duct epithelium. Palpation for testicular consistency is to be considered an important component of the clinical andrological evaluation of breeding bulls.

- Of the clinical and spermatological parameters examined in mature Zebu bulls followed for 13 months, only SC showed a significant seasonal variation. This variation was positively related to BCS rather than to the climatic variables studied.

# Acknowledgements

This study was carried out as part of my postgraduate education at the Faculty of Veterinary Medicine, Swedish University of Agricultural Sciences (SLU), Uppsala, with three cooperating institutions, the Departments of Obstetrics and Gynaecology, Department of Anatomy and Histology and the Department of Clinical Chemistry. With its character of "sandwich-programme", the field studies were performed in Costa Rica, patronised by the Department of Reproduction, Section of Andrology, School of Veterinary Medicine, Universidad Nacional Autónoma (UNA), Heredia, Costa Rica which so kindly granted me study leave during the periods at SLU. The study was supported by a grant from the Swedish Agency for Research and Cooperation with Developing Countries (SAREC), Stockholm, Sweden, and the School of Veterinary Medicine, Universidad Nacional Autónoma (UNA), Costa Rica.

I express my sincere gratitude to the team of advisers for their scientific support to this thesis and for believing that, despite the somewhat adverse field conditions that characterised this project, the results would contribute to improve the knowledge of reproductive aspects of Zebu bulls in the tropics of Central America. Special thanks are due to Professor **Heriberto Rodríguez Martínez** - my main adviser- for his constant aid, orientation, international knowledge and interest shown in the development of this thesis. I also express him my gratitude for having accepted my as his student from the early stages of the SAREC project. I will forever keep in my mind his particular way to "grow water-melons in the Pacific's sand". Thanks for everything, extensive to his family.

Dr. **Lennart Söderquist**, my co-adviser, for his valuable comments to my manuscripts and his good disposition in trying to understand the different way bull management is done in the tropics. Dr. **Enrique Pérez**, my co-adviser, for the time he kindly spent with my constant questions, as well as for the help, suggestions and interesting discussions during this period. My other co-advisers, Dr. **Mats Forsberg** and Agr. Dr. **Nils Lundeheim**, for their kind interest in my work and the valuable suggestions verted.

During my study periods at SLU, I must recognise the kindness of the staff members of the Department of Obstetrics and Gynaecology. Special thanks are due to Professor **Stig Einarsson**, head of the department, for welcoming me as a postgraduate student. **Tom Jangby**, for his tremendous spirit and kindness in solving daily problems around us. The excellent technical assistance of Mr. **José Rodríguez**, Mr. **Edy Gómes**, Mrs. **Marianne** and Mr. **Hans Ekwall**, Mrs. **Karin Selin-Wretling** and Mrs. **Annika Rikberg** is deeply acknowledged. The professionalism and amazing library service from **Johnny Carlsson, Cecilia Ekström** and **Mikael Eklund** is also recognised. Thanks to all the student partners, for making a nice atmosphere at work and giving "some light" during the autumns we shared in Uppsala. It is necessary to point out the encouragement and support I received from some "pura vida" people like Drs. **Mariel Regueiro, Fernando Dutra, Elize van Lier, Jorge Gil** and family, **Raquel** and family, and to **Carlos Lamothe**. I will remember your good friendship forever.

I express my sincere gratitude to Dr. **Eduard Müller** for introducing me to the study of bull reproductive aspects in the tropics. I will always be grateful for your guidance and support. Thank you also for the enjoyed travels to the farms and the good times shared after work. I give special thanks also to Dr. **Sandra Estrada** for providing me some of the facilities of the SAREC project in Costa Rica, and to Drs. **Carlos Jiménez** and **Humberto Cedeño** former and current directors of the Veterinary School in Costa Rica for always giving support to my projects. Thanks also to Drs. **Laura Castro** and **Magaly Caballero** for assuming many of my responsibilities at the andrology section during my study periods in Uppsala.

My sincere thanks to the farmers who allowed the development of some of the surveys included in this thesis. In this respect, I would like to mention the important collaboration received from Dr. **Max Figueroa**, Mr. **Allan Rivas** and Mr. **Modesto Hernández** from the GISA group in Liberia currently Rancho San Jerónimo; Ing. **Werberth Martínez** from Rancho La Julianita in Abangaritos; Dr. **Ezio Segnini** from Finca La Trampa in Abangares; Mr. **Rodrigo "Macho" Chan** and Mr. **Carlos Bonilla** the "Masters" from Rancho Salinero in Abangares and Mr. **Lanfranco Tretti** from La ensenada in Costa de Pájaros. Thanks to all of you for always trusting me in my work and supporting the efforts of the andrology section.

Para toda una **señora** y todo un **señor**†, quienes a pesar de que no contaron con los medios para terminar siquiera su educación primaria, fueron sabios al darme disciplina, llevarme por buen camino, y además enseñarme que el estudio y los principios morales son la mejor herencia que se le puede brindar a un hijo. A esa linda señora que creyó no tendría tiempo de ver a su hijo graduarse de primaria, y a mi recordado y querido viejo, debo decirles que les admiraré por siempre su increible empuje y disposición para surgir en la vida. **Papá y Mamá**, muchísimas gracias.

Gracias a mis hermanos y hermanas por ser el constante ejemplo que todo hermano menor necesita, y a todos los que de una y otra forma me brindaron soporte mental y enviaron buenas vibras a las dos almas que me acompañan y son mi vida por siempre. Agradezco profundamente a la familia **Pastor-Poso** por la inagotable amistad y solidaridad mostrada en todo momento.

Finalmente, eternas gracias a mis "coautores de tesis", quienes a pesar de no aparecer como tales, han sido el combustible que me hizo llegar hasta acá sin desmayar. Gracias gata por tu incondicional apoyo, tu paciencia y sobre todo por haberte identificado con mis metas. Y quien no podía faltar, ese que me rayó constantemente mis separatas con sus "dibujos", que traveseaba y escribía garabatos a mis artículos en la pc cuando yo me descuidaba, y que cortaba mi ritmo de escritura a cada momento pidiendo su merecido tiempo para que jugara con él, -algo que debo aceptar disfruté- pues sacó de mi ese alto porcentaje de niño que todos guardamos. A ese "gordo bandido", debo decirle que ha sido el regalo mas grande que Dios podría darme. Gracias Andy por darme vida!

# References


Almquist, J. and D. Cunningham, 1966: Semen traits of beef bulls ejaculated frequently. J. Anim. Sci. **25**, 916.

Almquist, J. and D. Cunningham, 1967: Reproductive capacity of beef bulls. I. Postpuberal changes in semen production at different ejaculation frequencies. J. Anim. Sci. **26**, 174-181

Alvarado, E., 1987: Desarrollo de una metodología para la evaluación andrológica del toro cebuino en el Pacífico Seco de Costa Rica (Development of a methodology for breeding soundness evaluation of Zebu bulls in the dry Pacific of Costa Rica). Lic. thesis, Universidad Nacional, Heredia, Costa Rica.

Amann, R., 1983: Endocrine changes associated with onset of spermatogenesis in Holstein bulls. J. Dairy Sci. **66**, 2606-2622.

Arias, F., 1995: Evaluación andrológica de toros cebuinos en la zona Atlántica de Costa Rica (Breeding soundness evaluation of Zebu bulls in the Atlantic zone of Costa Rica). Lic. thesis, Universidad Nacional, Heredia, Costa Rica.

Austin, J., Hupp, E. and R. Murphree, 1959: Effect of method of collection on quality of bull semen. J. Anim. Sci. **18**, 1556.

Bailey, T., Monke, D., Hudson, R., Wolfe, D., Carson, R. and M. Riddell, 1996: Testicular shape and its relationship to sperm production in mature Holstein bulls. Theriogenology. **46**, 881-887.

Ball, L., Ott, R., Mortimer, R. and J. Simons, 1983: Manual for breeding soundness examination of bulls. J. Soc. Theriogenology. **12**, 32-38.

Barth, A., 1986: The knobbed acrosome defect in beef bulls. Can. Vet. J. **27**, 379-384.

Barth, A., 1995: The effect of amount of body fat and loss of fat on breeding soundness classification of beef bulls. Can. Vet. J. **36**, 758-763.

Barth, A. and R. Oko, 1989: Defects of the sperm head and tail. In: Iowa State University Press (eds.). Abnormal morphology of bovine spermatozoa. Iowa: Ames, 130-228.

Berndtson, W., Igboeli, G. and B. Pickett, 1987: Relationship of absolute numbers of Sertoli cells to testicular size and spermatogenesis in young bulls. J. Anim. Sci. **64**, 241-246.

Blockey, M., 1979: Observations on group mating of bulls at pasture. Appl. Anim. Ethol. **5**, 15-34.

Blom, E., 1966: A new sterilizing and hereditary defect (the dag defect) located in the bull sperm tail. Nature. **12**, 739-740.

Bourdon, R. and J. Brinks, 1986: Scrotal circumference in yearling Hereford bulls: Adjustement factors, heritabilities, genetic, environmental and phenotypic relationships with growth traits. J. Anim. Sci. **62**, 958-967.

Braden, S., 1992: Andrologische untersuchungen von bullen in der trockenen pazifikregion (pacífico seco) Costa Ricas unter besonderer berücksichtigung der hodendegeneration (Andrologic evaluation in bulls from the dry Pacific region of Costa Rica with emphasis in testicular degeneration). PhD. thesis, Faculty of Veterinary Medicine, Hannover, Germany.

Carroll, E., Ball, L. and J. Scott, 1963: Breeding soundness in bulls. A sumary of 10940 examinations. JAVMA. **142**, 1105-1111.

Carter, A., Wood, P. and P. Wright, 1980: Association between scrotal circumference, live weight and sperm output in cattle. J. Reprod. Sci. **59**, 447-451.

Cartwright, T., 1955: Responses of beef cattle to high ambient temperatures. J. Anim. Sci. **14**, 350-362.

Cartwright, T., 1980: Prognosis of Zebu cattle: Research and application. J. Anim. Sci. **50**, 350-362.

Carvalho, F., Lammoglia, M., Simoes, M. and R. Randel, 1995: Breed affects thermoregulation and epithelial morphology in imported and native cattle subjected to heat stress. J. Anim. Sci. **73**, 3570-3573.

Chacón, J., 1997: Evaluación andrológica en el toro: Factores de riesgo asociados a la clasificación final de los sementales (Breeding soundness evaluation in the bull: Risk factors related with the final ranking after examination of the sires). MSc. thesis, PCVET, Universidad Nacional, Heredia, Costa Rica.

Chenoweth, P., 1981. Libido and mating behavior in bulls, boars and rams. A review. Theriogenology. **16**, 155-177.

Chenoweth, P., 1991: *Bos indicus* bulls, How different are they? Proc. Soc. Theriogenology AGM. 117-122.

Chenoweth, P. and H. Osborne, 1975: Breed differences in the reproductive function of young beef bulls in central Queensland. Aust. Vet. J. **51**, 405-406.

Chenoweth, P. and H. Osborne, 1978: Breed differences in the response of young beef bulls to electro-ejaculation. Aust. Vet. J. **54**, 333-337.

Chenoweth, P., Spitzer, J. and F. Hopkins, 1992: A new breeding soundness evaluation form. Proc. Soc. Theriogenology AGM. 63-70.

Chenoweth, P., Chase, C., Thatcher, M., Wilcox, C. and R. Larsen, 1996: Breed and other effects on reproductive traits and breeding soundness categorization in young beef bulls in Florida. Theriogenology. **46**, 1159-1170.

Cook, R., Coulter, G. and J. Kastelic, 1994: The testicular vascular cone, scrotal thermoregulation, and their relationship to sperm production and seminal quality in beef bulls. Theriogenology. **41**, 653-671.

Coulter, G., Rounsaville, T. and R. Foote, 1976: Heritability of testicular size and consistency in Holstein bulls. J. Anim. Sci. **43**, 10-11.

Coulter, G., Oko, R. and J. Costerton, 1978; Incidence and ultraestructure of "crater" defect of bovine spermatozoa. Theriogenology. **9**, 165-171.

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

Crabo, B., 1965: Studies on the composition of epididymal content in bulls and boars. Acta Vet. Scan. **6**, Suppl. 5. 13-14.

Crabo, B., 1988: Sperm production by farm animals under tropical conditions. Proc. 11th Int. Cong. Anim. Reprod. and AI, Dublin, V, 238-245.

Crabo, B., Gustafsson B., Nicander, L. and A. Rao, 1971: Subnormal testicular function in a bull concealed by phagocytosis of abnormal spermatozoa in the efferent ductules. J. Reprod. Fert. **26**, 393-396.

Da Silva, R. and J. Casagrande, 1976: Influence of high environmental temperatures on some characteristics of Zebu bull semen. Proc. 8th Int. Congr. Anim. Reprod. and AI, Cracow, **II**, 939-942.

De Dios-Vallejo, O., De la Cruz, J., Falcón, J., Arriola, H., Leyva, P. and J. Santos, 1991: Efecto de la época del año y edad en el desarrollo testicular de cuatro razas cebuinas en área tropical húmeda (Effect of season and age on testicular development of four Zebu breeds in the humid tropics). Veterinaria México. **21**, 3-8.

Dunn, T. and G. Moss, 1992: Effects of nutrient deficiencies and excesses on reproductive efficiency of livestock. J. Anim. Sci. **70**, 1580-1593.

Endo, Y., Seifert, G. and H. Christensen, 1978: Differences in testicle shape and weight of different breeds of cattle at weaning. Proc. Australian Soc. Anim. Prod. **12**, 190.

Elmore, R., Bierschwal, C. and R. Youngquist, 1976: Scrotal circumference measurements in 764 beef bulls. Theriogenology. **6**, 485-494.

Erb, R., Andrews, F. and H. Hilton, 1942: Seasonal variation in semen quality of the dairy bull. J. Dairy Sci. **25**, 815-826.

Evans, J., 1963: Adaptation to subtropical environments by Zebu and british breeds of cattle in relation to erythrocyte characters. Aust. J. Agric. Res. **14**, 559-571.

Everett, R., Bean, B. and R. Foote, 1978: Sourses of variation of semen output. J. Dairy Sci. **61**, 90-95.

Fayemi, O. and O. Adegbite, 1982: Seasonal variations in sperm abnormalities in bulls in a tropical climate. Revue D'Elevage et Medicine Veterinaire Des Pays Tropicaux. **35**, 69-72.

Fields, M., Burns, W. and A. Warnick, 1979: Age, season and breed effects on testicular volume and semen traits in young beef bulls. J. Anim. Sci. **48**, 1299-1304.

Finch, V., 1985: Comparison of non-evaporative heat transfer in different cattle breeds. Aust. J. Agric. Res. **36**, 497-508.

Finch, V., 1986: Body temperature in beef cattle: Its control and relevance to production in the tropics. J. Anim. Sci. **62**, 531-542.

Fordyce, G., Howit, C., Holroyd, R., Rourke, P. and K. Entwistle, 1996: The performance of Brahman-Shorthorn and Sahiwal-Shorthorn beef cattle in the dry tropics of northern Queensland. 5: Scrotal circumference, temperament, ectoparasite resistance, and the genetics of growth and other traits in bulls. Aust. J. Exp. Agric. **36**, 9-17.

Foster, J., Almquist, J. and R. Martig, 1970. Reproductive capacity of beef bulls. IV. Changes in sexual behavior and semen characteristics among succesive ejaculations. J. Anim. Sci. **30**, 244-252.

Galina, C., Orihuela, A. and A. Duchateau, 1987: Reproductive physiology in Zebu cattle. Unique reproductive aspects that affect their performance. Vet. Clin. North Am. Food Anim. Pract. **3**, 619-632.

Galina, C. and J. Russell, 1987: Research and publishing trends in cattle reproduction in the tropics: Part 1. A global analysis. Anim. Breed. Abst. **55**, 755-749.

Galina, C. and G. Arthur, 1991: Review of cattle reproduction in the tropics. Part 6. The male. Anim. Breed. Abst. **59**, 403-412.

Gipson, T., Vogt, D., Ellersieck, M. and J. Massey, 1987: Genetic and phenotypic parameter estimates for scrotal circumference and semen traits in young beef bulls. Theriogenology. **28**, 547-555.

Goyal, H., 1982: Light microscopic and ultrastructural evidence of ephitelial phagocytosis of sperm in the rete testis and ductuli efferentes in the bull. Am. J. Vet. Res. **43**, 785-790.

Grauber, C., Acosta, A. and P. Repetto, 1990: Circunferencia escrotal en toros *Bos indicus* y derivados (Scrotal circumference in *Bos indicus* bulls and their crosses). Vet. Argentina. **7**, 466-472.

Gustafsson, B., Crabo, B. and R. Rao, 1972: Two cases of bovine epididymal dysfunction. Cornell Vet. **62**, 392-402.

Gustafsson, B., Einarsson, S., Nicander, L., Holtman, M. and O. Soolalu, 1974: Morphological, physical and chemical examination of epididymal contents and semen in a bull with epididymal dysfunction. Andrología. **6**, 321-331.

Gutiérrez, C., Galina, C. and I. Rubio, 1993: The influence of the social structure of a Zebu herd on the manifestation of signs of oestrus. World Rew. Anim. Prod. **23**, 58-70.

Hahn, J., Foote, R. and E. Cranch, 1969: Tonometer for measuring testicular consistency of bulls to predict semen quality. J. Anim. Scie. **29**, 483-489.

Hancock, J., 1952: The morphology of bull spermatozoa. Br. J. Exp. Biol. **29**, 445–453.

Hardin D., Chenoweth P., Randel R. and C. Scott, 1981: Effects of seasonal variations on seminal parameters and libido in Angus and Brahman bulls. J. Anim. Sci. **53**, 326.

Hernández, J., Galina, C., Orihuela, A. and R. Navarro, 1991: Evaluación de la líbido de toros Cebú en pruebas en corral y en potrero (Libido evaluation in Zebu bulls under pen and grassing conditions). Veterinaria México. **22**, 41-45.

Igboeli, G. and A.M. Rakha, 1971: Seasonal changes in the ejaculate characteristics of Angoni (Short horn Zebu) bulls. J. Anim. Sci. **33**, 651-654.

Igboeli, G. and A.M. Rakha, 1981: Puberty and related phenomena in Angoni (Short horn Zebu) bulls. J. Anim. Sci. **33**, 647-653.

Igboeli, G., Nwakalor, L., Orji, B. and G. Onuora, 1987: Seasonal variation in the semen characteristics of Muturu (*Bos brachyceros*) bulls. Anim. Reprod. Sci. **14**, 31-38.

Januskauskas, A., 1999: Assessment of viability and function of post-thaw spermatozoa from Swedish dairy AI bulls. Ph.D. thesis, SLU, Uppsala, Sweden.

Johnston, J. and C. Branton, 1953: Effects of seasonal climatic changes on certain physiological reactions, semen production and fertility of dairy bulls. J. Dairy Sci. **36**, 934-942.

Kaimowitz, D., 1996: Livestock and deforestation in Central America in the 1980s and 1990s: A policy perspective. Ciencias veterinarias. **Jan-Dec**, 113-161.

Koefoed-Johnsen, H., Andersen, J., Andreser, E., Blom, E. and H. Philipsen, 1980: The dag deffect of the tail of bull sperm. Studies on the inheritance and pathogenesis. Theriogenology. **14**, 43-47.

Kojima, Y., 1988a: Morphologically abnormal spermatozoa suspected inheritance: Head. Jap. J. Fertil. Steril. **33**, 186-196.

Kojima, Y., 1988b: Morphologically abnormal spermatozoa suspected inheritance: Tail. Jap. J. Fertil. Steril. **33**, 438-451.

Kumi-Diaka, J. and R. Zemjanis, 1978: Seasonal variations in spermatogenesis in bulls indigenous to Nigeria. Br. Vet. J. 134, 537-540.

Kumi-Diaka, J., Nagaratnam, V. and J. Rwuaan, 1981: Seasonal and age-related changes in semen quality and testicular morphology of bulls in a tropical environment. Vet. Rec. **3**, 13-15.

Kumi-Diaka, J., Osori, D., Njoku, C. and D. Ogwu, 1983: Quantitative estimation of spermatogenesis in bulls (*Bos indicus*) in a tropical environment of Nigeria. **6**, 215-222.

Lagerlöf, N., 1934: Morphologische untersuchungen über veränderungen im spermabild und in den hoden bei bullen mit verminderter oder aufgehobener fertilität (Research concerning morphologic changes in the semen picture and in the testicles of sterile and subfertile bulls). Acta Path. Microbiol. Scand. Suppl. **19**, 66-77.

Lamothe, C., 1990: Reproductive performance of Zebu cattle in Mexico. M.Sc. thesis, SLU, Uppsala, Sweden.

León, H., Porras, A., Galina, C. and R. Navarro-Fierro, 1991: Effect of the collection method on semen characteristics of Zebu and European type cattle in the tropics. Theriogenology. **36**, 349-355.

Le Tacon, F. and J. Laker, 1990: Deforestation in the tropics and proposals to arrest it. AMBIO. **19**, 372-378.

Madrid, N., Ott, R., Rao, D., Parret, D., Vanderwert, W. and C. Willms, 1988. Scrotal circumference, seminal characteristics and testicular lesions of yearling Angus bulls. Am. J. Vet. Res. **49**, 579-584.

McCosker, T., Turner, A., McCool, C., Post, T. and K. Bell, 1989: Brahman bull fertility in a North Australian range land herd. Theriogenology. **32**, 285-297.

Meyerhoeffer, D., Wettermann, R., Coleman, S. and M. Wells, 1986: Reproductive criteria of beef bulls during and after exposure to increased ambient temperature. J. Dairy Sci. **60**, 352-357.

Morris, D., Tyner, C., Morris, P. and R. Forgason, 1989: Correlation of scrotal circumference and age in American Brahman bulls. Theriogenology. **31**, 489-453.

Mortimer, R., Seidel, G., Amann, R., Grotelueschen, D. and S. Olson, 1991: Use of in vitro fertilization to evaluate spermatozoa with proximal droplets in young beef bulls. Theriogenology. **35**, 247.

Murgueitio, E., 1990: Intensive sustainable livestock production: An alternative to tropical deforestation. AMBIO. **19**, 397-399.

Müller, E., 1990: Andrological evaluation of bulls in the tropics. Proc. Joint IFS-SIPAR Seminar in animal reproduction. Paysandú-Uruguay. 143-150.

Müller, E., 1991: Andrological evaluation of bulls in Costa Rica. Proc. World Vet. Congress. Río de Janeiro. 120.

Müller, E., Rodríguez, H., Braden, S. and L. Edquist, 1992: Testicular ultrastructure of Zebu bulls in Costa Rica. J. Vet. Med. **39**, 382-391.

National Livestock Census, 1984: Ministry of Agriculture and Livestock, Costa Rica.

Nicholson, M. and M. Butterworth, 1986: A guide to condition scoring of Zebu cattle. International Livestock Centre for Africa (eds.). Ethiopia.

Noakes, D.E., 1988: The normal genital organs. In: Fertility and infertility in veterinary practice. (Laing, J.A.; Brinley Morgan, W.J. and Wagner, W. C., eds.), Bailliere Tindall, 4th Ed., London, 1-38.

Ohashi, O., Sousa, J., Ribeiro, H. and W. Vale, 1988: Disturbios reprodutivos em touros *B. indicus*, *B. taurus* e mesticos, criados em clima Amazonico (Reproductive disturbs in *B. Indicus*, *B. Taurus* and crosses in the Amazonas climat). Pesq. Vet. Bras. **8**, 31-35.

Orihuela, A., Galina, C. and A. Duchateau, 1988: Behavioural patterns of Zebu bulls towards cows previously synchronised with prostaglandin $F_2\alpha$ or oestrogen under corral and field conditions. Appl. Anim. Behav. Sci. **21**, 267-276.

Orihuela, A. and C. Galina, 1997: Social order measured in pasture and pen conditions and its relationship to sexual behavior in Brahman (*Bos indicus*) cows. Appl. Anim. Behav. Sci. **52**, 3-11.

Oyedipe, E., Kumi-Diaka, J. and D. Osori, 1981: Determination of onset of puberty in Zebu bulls under tropical conditions of northern Nigeria. Theriogenology. **16**, 419-431.

Parkinson, T., 1987: Seasonal variations in semen quality of bulls: Correlations with environmental temperature. Vet. Rec. **120**, 479-482.

Pérez, E., 1996: Towards a sustainable animal production. Ciencias veterinarias. **Jan-Dec**, 3-6.

Pérez, E., Conrad, P., Hird, D., Ortuño, A., Chacón, J., Bondurant, R. and J. Noordhuizen, 1992: Prevalence and risk factors for Trichomonas foetus infection in cattle in northeastern Costa Rica. Prev. Vet. Med. **14**, 155-165.

Piccinali, R., Galina, C. and R. Navarro, 1992: Behavioural patterns of Zebu bulls towards females synchronised with $PGF_2\alpha$ or oestrogens under corral and field conditions. Appl. Anim. Behav. Sci. **35**, 125-133.

Preston, T., 1990: Future strategies for livestock production in tropical third world countries. AMBIO. **19**, 390-393.

Price, E., 1987: Male sexual behaviour. Vet. Clin. North Am. Food Anim. Pract. **3**, 405-422.

Rao, A. and A. Bane, 1985: Pathological conditions of the genital organs of normal fertile bulls. Indian Vet. J. **62**, 242-246.

Rekwot, P., Oyedipe, E., Akerejola, O., Kumi-Diaka, J. and J. Umoh, 1987a: The effect of protein intake on the onset of puberty in Bunaji and Friesian × Bunaji crossbred bulls in Nigeria. Theriogenology. **28**, 427-434.

Rekwot, P., Voh, A., Oyepide, E., Opaluwa, G., Sekoni, V. and P. Dawuda, 1987b: Influence of season on characteristics of the ejaculate from bulls in an artificial insemination centre in Nigeria. Anim. Reprod. Sci. **14**, 187-194.

Rekwot, P., Oyedipe, E. and O. Ehoche, 1994: The effects of feed restriction and realimentation on the growth and reproductive function of Bokoloji bulls. Theriogenology. **42**, 287-295.

Rhynes, W. and L. Ewing, 1973: Testicular endocrine function in Hereford bulls exposed to high ambient temperature. Endocrinology. **92**, 509-515.

Riek, R., 1962: Studies on the reactions of animals to infestation with ticks. Aust. J. Agric. Res. **13**, 532-550.

Riemerschmid, G. and J. Quinlan, 1941: Further observations on the scrotal skin temperature of the bull, with some remarks on the intra-testicular temperature. Onderstepoort J. Vet. Sci. Anim. Ind. **17**, 123-140.

Rodríguez, C., Galina, C., Gutierrez, R., Navarro, R. and R. Piccinalli, 1993: Evaluación de la actividad sexual de los toros Cebú bajo condiciones de empadre múltiple con hembras sincronizadas con $PGF_2\alpha$. (Evaluation of sexual activity in Zebu bulls under condicions of multiple sire system with cows synchronised with $PGF_2\alpha$). Ciencias Veterinarias. **15**, 41-49.

Roussel, J., Stallcup, O. and C. Austin, 1967: Selective phagocytosis of spermatozoa in the epididymis of bulls, rabbits and monkeys. Fertil. Steril. **18**, 509-516.

Ruiz, M., 1996: Towards the reactivation of animal production in Latin America and the Caribbean region. Ciencias veterinarias. **Jan-Dec**, 7-16.

SAS®, 1988: User's Guide. Version 6, ed. 6.03, Cary-USA. SAS Institute Inc.

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

Sekoni, V., Kumi-Diaka, J., Saror, D., Njoku, C. and S. Olorunju, 1988: Seasonal and monthly variations in the incidence of morphological abnormalities in bovine spermatozoa in Shika, Zaria, Northern Nigeria. Anim. Reprod. Sci. **17**, 61-67.

Silva-Mena, C., 1997: Peripubertal traits of Brahman bulls in Yucatán. Theriogenology. **48**, 675-685.

Sinowatz, F., Wrobel, K., Sinowatz, S. and P. Kugler, 1979: Ultraestructural evidence for phagocytosis of spermatozoa in the bovine rete testis and testicular straight tibules. J. Reprod. Fert. **57**, 1-4.

Skinner J. and G. Louw, 1966: Heat stress and spermatogenesis in *Bos indicus* and *Bos taurus* cattle. J. Appl. Phys. **21**, 1784-1790.

Sprecher, D. and P. Coe, 1996: Differences in bull spermiogram using eosin-nigrosin stain, feulgen stain, and phase contrast microscopy methods. Theriogenology. **45**, 757-764.

Swanson, E. and L. Boyd, 1962: Factors affecting coiled-tail spermatozoa in the bull. Am. J. Vet. Res. **23**, 300-308.

Toelle, V. and W. Robison, 1985: Estimates of genetic correlations between testicular measurements and female reproductive traits in cattle. J. Anim. Sci. **60**, 89-99.

Trocóniz, J., Beltrán, J., Bastidas, H., Larreal, H. and P. Bastidas, 1991: Testicular development, body weight changes, puberty and semen traits of growing Guzerat and Nellore bulls. Theriogenology. **35**, 815-826.

Turner, J., 1980: Genetic and biological aspects of Zebu adaptability. J. Anim. Sci. **50**, 1201-1205.

U.S. National Research Council, 1984: Effect of environment on nutrient requirements of domestic animals. 6th ed., National Academic Press (eds.). Washington, USA, 44 pp.

Vale-Filho, V., Pinto, P., Megale, F., Fonseca, J. and L. Soares, 1980: Fertility of the bull in Brasil. A survey of 1088 bulls and 17945 ejaculations from *Bos taurus* and *Bos*

*indicus* and crosses under tropical conditions. Proc. 9th Int. Cong. Anim. Reprod. and AI, Madrid, **VI**, 545-548.

Vale-Filho, V., Andrade, V., Bergmann, J., Reis, S., Mendonca, R. and G. Mourao, 1996: Breeding soundness evaluation (BSE-Z) of prospective young Nellore bulls (Zebu) supplemented with pasture. Proc. 13th Int. Congr. Anim. Reprod. and AI, Sydney, **II**, 69.

Veeramachaneni-Rao, D., Ott, R., Heath, E., McEntee, K., Bolt, D. and J. Hixon, 1986: Pathophysiology of small testes in beef bulls: Relationship between scrotal circumference, histopathologic features of testes and epididymides, seminal characteristics, and endocrine profiles. Am. J. Vet. Res. **47**, 1988-1999.

Visintin, J., Barnabe, V., Barnabe, R. and W. Viana, 1984: Seminal characteristics of Zebu bulls. Proc. 10th Int. Congr. Anim. Reprod. and AI, Illinois, **II**, 69-71.

Waites, G., 1970: Temperature regulation and the testis. In: Johnson, A., Gomes, W. and V. Vandemark (eds.) The testis. New York: Academic Press, 251-279.

Wells, M., Awa, O., Jay, L. and S. Fancy, 1971: Effect of sexual rest and frequency of ejaculation on sperm acrosomal morphology. J. Dairy Sci. **54**, 526-530.

Wenkoff, M., 1978: A sperm mid-piece defect of epidymal origen in two Hereford bulls. Theriogenology. **10**, 275-281.

Wenkoff, M., 1988: The evaluation of bulls for breeding soundness. 2nd. ed. Canadian Vet. Med. Assoc.

Wildeus, S. and K. Entwistle, 1982: Pospubertal changes in gonadal and extragonadal sperm reserves in *Bos Indicus* strain bulls. Theriogenology. **17**, 654-667.

Wildeus, S. and K. Entwistle, 1983a: Spermiogram and sperm reserves in hybrid *Bos indicus* x *Bos taurus* bulls after scrotal insulation. J. Reprod. Fert. **69**, 711-716.

Wildeus, S. and K. Entwistle, 1983b: A quantitative histological study of testicular and epididymal development in *Bos indicus* cross bulls. Anim. Reprod. Sci.

Wildeus, S. and K. Entwistle, 1984: Seasonal differences in reproductive characteristics of *Bos indicus* and *Bos taurus* bulls in tropical northern Australia. Trop. Anim. Prod. **9**, 142-150.

Williams, W. and N. Utica, 1920: Technique of collecting semen for laboratory examination with a review of several diseased bulls. Cornell Vet. **10**, 87-94.

Williams, W. and A. Savage, 1925: Observations on the seminal microphatology of bulls. Cornell Vet. **15**, 353-375.

Wolf, F., Almquist, J. and E. Hale, 1965: Pubertal behaviour and pubertal characteristics of beef bulls on a high nutrition allowance. J. Anim. Sci. **24**, 761-765.

**Acta Universitatis Agriculturae Sueciae**
presents doctoral theses from the faculty of agriculture, landscape architecture and horticulture, the faculty of forestry and the faculty of veterinary medicine at the Swedish University of Agricultural Sciences. Each faculty has its own subseries, Agraria, Silvestria and Veterinaria, respectively, with separate numbering.

ISSN 1401-6257
ISBN 91-576-5910-9