EFFECTS OF TRYPANOSOMA VIVAX INFECTION ON ESTRUS SYNCHRONIZATION AND FERTILITY OF HEIFERS

BY

OBASI, ORJI LEONARD

A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL, AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN VETERINARY SURGERY AND MEDICINE (THERIOGENOLOGY).

DEPARTMENT OF VETERINARY SURGERY AND MEDICINE,
FACULTY OF VETERINARY MEDICINE.

MARCH, 1997

DECLARATION

I hereby declare that the work reported in this thesis was carried out by me in the Department of Veterinary Surgery and Medicine, under the supervision of Professor D. Ogwu, and Dr. G. Mohammed.

No part of this thesis has been accepted in any previous application for a higher degree. All sources of information are specifically acknowledged by means of references.



OBASI, ORJI LEONARD

Date 12.3.97

CERTIFICATION

This thesis entitled "Effects of Trypanosoma vivax infection on oestrus synchronization and fertility of heifers by O.L. Obasi meets the regulations governing the award of the Degree of Master of Science in Veterinary Surgery and Medicine (Theriogenology) of the Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

INTERNAL EXAMINERS

Professor D. Ogwu, DVM (ABU), M.Sc. (MISS), Ph.D (ABU)
Dept. of Vet. Surgery and Medicine
Faculty of Vet. Medicine
Ahmadu Bello University, Zaria, Nigeria.

Date: 12/3/97

Prof. D. Ogwu
Head
Dept. of Vet. Surgery and Med.
Faculty of Vet. Medicine
Ahmadu Bello University, Zaria

Date: 12/3/97

Dr. G. Mohammed, DVM (ABU), Ph.D. (Brist.)
Dept. of Vet. Surgery and Medicine
Faculty of Vet. Medicine
Ahmadu Bello University, Zaria, Nigeria

Date: 12th March 1997

Prof. J.Y. Olayemi
Dean, Postgraduate School
Ahmadu Bello University, Zaria, Nigeria.

Date: 27/3/98

DEDICATION

This thesis is unreservedly dedicated to my parents, Nze Josaiah Korie and Mrs Susan Korie.

ACKNOWLEDGEMENT

I express my heartfelt gratitude to my supervisors, Dr. G. Mohammed for his kindness and critical review of this work and to Prof. D. Ogwu, for his academic guidance and for allowing me ample opportunity to express myself. The research was funded from his EEC Trypanosomiasis Research Project.

Thanks also go to Prof. E. Okon and Dr. A. C. Okonkwo both of the University of Uyo for their encouragement and financial assistance, and to the University of Uyo for granting me study leave.

The sundry staff of the Faculty of Veterinary Medicine ABU., Zaria are complimented for providing me with a conducive environment.

I remain indebted to Drs. A. Z. Hassan, D. A. Y. Adawa, O. Okaiyeto, Femi Suleiman, L. Allams, D. Dangana, M. Raji and K. B. Kadima, who were united in making me feel at home.

I say "Thank you" to Drs. A. Sannusi, E. K. Bawa, P. I. Bekwot, J. S. Rwuaan and Prof. V. O. Sekoni and to the numerous good people who assisted me in various ways in the course of this research.

ABSTRACT

Six heifers (4 infected and 2 uninfected) were used to investigate the fertility to artificial insemination, serum progesterone profile and behavioural estrus in the Bunaji breed of cattle, experimentally infected with *Trypanosoma vivax*; and synchronized with prostaglandin F-alpha. The heifers in the infection group were artificially challenged intravenously with about 5 x 10^{i} *Trypanosoma vivax* organisms, stabilate 146 originally isolated from a Bunaji cattle in Zaria.

The six animals were given two injections of PGF$_2$alpha (Dinoprost tromethamine) at the dose rate of 25mg each on days 11 and 22 post infection, and subsequently inseminated using fresh semen at the fixed times of 72 and 96 hours after the second dose of PGF2alpha without reference to estrus.

50 and 100% of the animals in the infected group responded to the first and second PGF$_2$alpha injections, respectively while the control animals showed a 100% response to both injections. None of the animals in the infected and control groups became pregnant, although the serum progesterone levels of the control animals suggested that they were capable of becoming pregnant to a viable service. Animals in the control group also manifested a more intense behavioural estrus. The mean progesterone level in the control heifers was regular up till the end of the experiment and the basal values coincided

with the clinical manifestation of heat. The mean progesterone level in the infected group was irregular and remained at basal levels after the second dose of PGF2alpha.

The findings suggest that animals which may not become pregnant are forced to manifest heat by the strong luteolytic effect of PGF2alpha.

TABLE OF CONTENTS

		Page
	Title Page	i
	Certification	ii
	Dedication	iii
	Acknowledgement	iv
	Abstract	v
CHAPTER ONE		
	General Introduction	1
CHAPTER TWO		
2.0	Literature Review	6
2.1	Reproductive Performance of Zebu Cattle	6
2.1.1	The Cattle of Nigeria	6
2.1.2	Reproductive Efficiency of the Zebu Cattle	
2.1.3	Measurement of Reproductive Efficiency	8
2.1.4	Age of Puberty	8
2.1.5	Age at First Calving	9
2.1.6	Calving Rate	10
2.1.7	Number of Services Per Conception	10
2.1.8	Conception Rate	11
2.1.9	Calving Interval	11
2.2	Estrus and the Estrous Cycle	13
2.2.1	Heat Detection	14
2.3	Controlled Breeding	16
2.3.1	Methods of controlled Breeding Programme	18
2.3.2	Historical Development of Controlled Breeding in Cattle	18

2.3.3 Prostaglandins..... 20
2.3.4 Types of Prostaglandins..... 20
2.3.5 The Role of PGF2alpha in Farm Animal Reproduction..... 21
2.3.6 Fertility to PGF2alpha Treatment..... 23
2.3.7 Progesterone Profile and Cattle Reproduction..... 28
2.4 Artificial Insemination (AI)..... 29
2.4.1 Historical Development Of Artificial Insemination..... 30
2.4.2 Advantages of Artificial Insemination.... 30
2.4.3 Techniques For Artificial Insemination... 32
2.4.4 Time of Insemination and Optimum conception..... 32
2.4.5 Fertility to Artificial Insemination..... 34
2.4.6 Factors Affecting Conception Rate to Artificial Insemination..... 35
2.5 Bovine Trypanosomiasis in Nigeria..... 37
2.5.1 Ecology and Spread of Bovine Trypanosomiasis..... 38
2.5.2 The Pathology and Pathogenesis of Trypanosomiasis in Cattle..... 40
2.5.3 The Vector..... 43
2.5.4 Clinical Manifestation..... 44
2.5.5 Parasitaemia 45
2.5.6 Anemia in Trypanosomiasis..... 47

2.5.7 Diagnosis of Trypanosomisis..... 48
2.5.8 Chemotherapy and Chemoprophylaxis..... 48
2.5.9 Reproductive Abnormalities in Bovine Trypanosomiasis..... 52
2.5.10 Mechanism of Reproductive Dysfunction.... 57

CHAPTER THREE

3.0 Materials and Methods..... 56
3.1 Experimental Animals, Screening and Maintenance..... 56
3.2 Experimental Design..... 57
3.2.1 Experimental groups and Animal infection 57
3.2.2 Prostaglandin Administration..... 58
3.2.3 Animal Insemination..... 58
3.2.4 Blood Sampling..... 58
3.2.5 Progesterone Determination..... 59
3.2.6 Parasitological and Haematological Determination..... 61
3.2.7 Heat Detection..... 62
3.2.8 Pregnancy Diagnosis..... 62
3.2.9 Data Analysis..... 63

CHAPTER FOUR

4.0 **Results**.....64

4.1 Clinical Observation.....64

4.2 Parasitaemia.....64

4.3 Rectal Temperature.....65

4.4 Packed Cell Volume.....66

4.5 Progesterone Profile.....67

4.6 Behavioural Estrus.....68

4.7 Conception Rate.....69

CHAPTER FIVE

Discussion.....70

Conclusion 79

References.....82

List of Tables 102

List of Figures 109

Appendices 121

CHAPTER ONE

GENERAL INTRODUCTION:

Food is a basic necessity which transcends state, ethnicity and religion (Abalu *et al;* 1984). The independence and civilization of a nation depends on how much it is able to satisfy its needs - especially the need for food (Abdullahi, 1984). The prehistoric man had this natural instinct when he set out domesticating animals. Since prehistoric times, man's primary pre-occupation and aim in animal husbandry may be viewed in terms of a relentless and unremitting campaign to eliminate wastage among his stock, firstly by the control of natural disasters, and subsequently by the control of chronic wastage associated with disease and infertility (Hinks, 1987).

Even though the family *Bovidae* was not the first to be domesticated, there is ample evidence to suggest that early man preferred the meat of the *Bovidae* to that of the other animals (Morris, 1974). Cattle remains man's major source of animal protein to date. There is a nutritional necessity to include animal products in human diet. The need, therefore, to produce enough meat and meat products to satisfy the over-growing human population becomes paramount. Intensification and improvement of livestock is aimed at satisfying this need. While a lot of success has been recorded in the area of increased livestock production in the developed countries of the world, the developing countries, especially in Africa, are

[illegible] UNIVERSITY
[illegible] LIBRARY

increasingly finding it difficult to produce enough animal protein to meet their domestic needs. The grim reality, unfortunately, is that the extent of hunger and malnutrition in these countries has reached crisis proportions. This region, ironically carries more than half the worlds' cattle population (Buvanendran, 1979). These animals are unimproved and are predominantly traditionally managed and, therefore, low yielding (Pullan, 1979; Otchere, 1986). Matching production level can be achieved through improved management, breeding and disease control.

An improved cattle production enterprise must be anchored on a highly efficient cattle reproduction. Mukasa-Mugerwa (1989) had observed that the overall productivity of cattle depends on their reproductive performance. Cattle breeds indigenous to Africa have reproductive rates lower than those of the temperate regions (Knudson and Soheal, 1970; Pullan 1979; Oyedipe *et al* 1982; Otchere, 1986). Eduvie and Oyedipe (1991), while confirming the low reproductive performance of the Nigerian zebu cattle indicated the possibility for improvement if adequate management is instituted.

An indispensable management practice in cattle improvement is controlled breeding (CB). CB covers different aspects, but the most central is oestrus control (synchronized oestrus), and set-time artificial insemination (AI). As CB gains ground in Nigeria, an obvious factor that must be examined is the effect of disease on the response of cattle to the synchronizing agents. Landiver *et al* (1985) had observed that despite an increasing demand for AI. services, the resultant fertility has remained poor.

Disease incidence is more than twice higher in the tropics than temperate regions (Desai, 1989). Trypanosomiasis is the most important disease of cattle in the tropical and subtropical regions of the world. The limitation it places on livestock production and reproduction in Nigeria has ben documented (Maclennan 1970; FAO, 1975; Leeflang, 1978; Pullan, 1979; Ogwu, 1983; Sekoni, 1993, 1994).

In spite of the extensive research on trypanosomiasis, it still remains the most devastating and most complex of all cattle diseases in Africa. Due to the widespread incidence of trypanosomiasis, Africa in general produces about 70 times less animal protein per hecter than Europe (ILRAD, 1987). The behaviour of the parasite and the complexity of the epidemiology of bovine trypanosomiasis have combined to make the disease evasive to attempts at controlling it. It is,

therefore, evident that attempts at enhancing the reproduction of the zebu cattle, through the application of new technologies like estrus synchronization and set-time AI., should consider the endemicity and continued economic importance of trypanosomiasis in Nigeria. The tendency is high that cattle may be habouring pathogenic trypanosomes at the time of synchronization. It was on this basis that this study was designed with the following objectives:

1) To study the effects of *T. vivax* infection on estrus synchronization using PGF2alpha.
2) To determine the serum levels of progesterone during the estrous cycle of PGF2alpha treated heifers.
3) To determine the fertility rate to AI of heifers experimentally infected with *T. vivax* and synchronized with PGF2alpha.

Significance of the Study:

There are lots of published information on:

a) the response of the zebu cow to various synchronizing agents
b) uptimum time for insemination and
c) conception to artifical insemination.

In recent times, the deleterious effect of trypanosomasis on bovine reproduction have been highlighted by Agyemang *et al*, (1993) and Sekoni (1994). A search of the literature did not reveal any information on the effect of trypanosomiasis on synchronized estrus in the bovine. Since reproduction is the basis of any livestock production, it is necessary that a critical look be taken on how trypanosomes affect female bovine reproduction. It would also in particular be necessary to ascertain the influence of trypanosomiasis as a disease entity on estrus synchronization on which depends the commercial application of artificial insemination. Artificial insemination is presently being emphasized by the livestock subsector as an alternative means to boosting milk yield especially at the level of institutional and peri-urban farms.

CHAPTER TWO

LITERATURE REVIEW

2.1 REPRODUCTIVE PERFORMANCE OF ZEBU CATTLE

2.1.1 The Cattle of Nigeria

The national livestock census conducted for the National Livestock Project Department (FLD; 1992), estimated the entire National cattle herd at 13.9 million. The humped (zebu) cattle constitute about 90% of the total while the humpless type constitute the balance of 10%. The White Fulani (Buṇaji breed) is the most predominant and constitutes about 51% of the cattle types in Nigeria (Anon, 1981). It is widely spread in the Sudan and Guinea savannah zones. Over 90% of the entire cattle population is controlled by the pastoral Fulani who practice either nomadic pastoralism, transhumance, or agropastoralism (Ehoche, 1994). An intensive production system whereby cattle are grazed and supplemented is practiced by private ranches, institutional, and government farms.

These later farms are better placed to adopt new and improved farming practices. Unfortunately, they are less viable than the Fulani pastoral system. The number of the institutional and government farms and their stocking capacity have been on the decline since the mid 80's when they were at the peak.

The most common reasons adduced for their unviability were mismanagement, lack of dedication, lack of clear and defined goals and frudulent practices.

The Nigerian cattle is reared predominantly for meat. Dairying and traction are incidentals. Buvanendran (1979) observed that the zebu cattle is "unspecialized" in function and plays the tripartite role of a meat and milk producer as well as its uses for traction. The milk yield under traditional method of husbandry is very low, and ranges between 400-700 litres/lactation, while under research conditions the White Fulani has been found to produce about 1015 litres per lactation period of 250 days, Sokoto Gudali 1014 litres, Wadara 1290 litres, and Adamawa Gudali 1030 litres (Ehoche, 1994). Several other reports also emphasize the potentials for greater yield under optimum management (Knudson and Soheal, 1970, Oyedipe *et al.*, 1982).

2.1.2 Reproductive Efficiency of the Zebu Cattle

A high reproductive efficiency is indispensable for maximum yield from any livestock business. The breeds of cattle indigenous to Nigeria have low reproductive rates (Pullan, 1979; Otchere, 1986). These animals can, however, be exploited to play an important role in the required increases in animal protein production through improved systems of management (Eduvie and Oyedipe, 1991). To the Fulani

cattlemen, cattle rearing is a way of life, and a status symbol with more emphasis placed on the numerical strength of the cattle than on the reproductive performance. This non business oriented nomadic system of cattle production does not stand a chance of meeting the needs of a rapidly growing Nigerian population. It is therefore, necessary to strive towards increased reproductive efficiency.

2.1.3 Measurement of Reproductive Efficiency

Animal reproduction is central to livestock production. The measurement of reproductive efficiency is the key to understanding and improving animal reproduction and productivity. An efficient and reliable method for measuring reproductive efficiency is therefore very essential. A variety of indices have, over the years been employed to measure reproductive performance in cattle, viz: age at puberty; age at first calving; calving rate; conception rate; number of services per conception; and calving interval.

2.1.4 Age at Puberty

This is the time at which oestrus first occurs accompanied by ovulation (Peters and Ball, 1986). Age at puberty is influenced by the breed of the animal, nutrition, and environmental factors (Mukasa-Mugerwa, 1989). The White Fulani breed of cattle in Nigeria reaches puberty at 21.2 months. This happens to be longer than the taurine (19.5

months) and crossbreeds between *Bos taurus and Bos indicus* (20.0 months) (Knudson and Soheal, 1970). Wittbank *et al.*, (1969) reported that, on the average, the zebu reaches puberty 6-12 months later than the taurine cattle. The dairy breeds of cattle attain puberty earlier and at a lower percentage of their adult body weight than the beef breeds (Hafez, 1985).

2.1.5 Age at First Calving

This is the age at which the cow produces its first offspring. It is controlled to allow the young female cattle time to mature so as to be able to withstand the stress of parturition and lactation (Mukasa-Mugerwa, 1989). Under traditional management system age at first calving may be lower because the female running with the bull may be served at the first oestrus.

The *Bos indicus* cow in the tropical ecological zone is known for its late age at first calving. Age at fist calving reported for the White Fulani breed in Nigeria ranges from 40.4 $\pm$ 0.7 months to 60 months (Knudson and Soheal, 1970; Akpokodje and Bolarinwa, 1974; Pullan, 1979; Oyedipe *et al* 1982), with an average of about 44 months (Mukasa-Mugerwa, 1989). The age at first calving for the pure breed Freisian, 1/2 Freisian and 3/4 Freisian in Nigeria ranges from 29.0 to 31.9 months (Knudson and Soheal, 1970). Improved management shortens the age at first calving (Vaccaro, 1973; Oyedipe *et al*, 1982). It is also influenced by breed (Chlikura *et al.*,

1979), environment (Mukasa-Mugerwa, 1989), and the year and month of birth (Miranda *et al*, 1982). The effects of year of birth was found to be negative by some investigators (Sabino *et al*., 1981., Oyedipe *et al*., 1982).

2.1.6 Calving Rate

This could be defined as the number of calves born per 100 services. It is an estimate of the inseminations that resulted in the birth of live calves. The calving rate of the zebu cattle is known to be low, and like the age at first calving it is affected by the level and system of management (Mukasa-Mugerwa, 1989).

2.1.7 Number of Services per Conception

This is an index of fertility which measures the number of matings or inseminations that resulted in conception. The number of services per conception depends on the breeding system employed. It is higher under uncontrolled natural mating and low where hand mating or artificial insemination is used (Mukasa-Mugerwa, 1989). The number of services per conception in the zebu and other tropical breeds ranges from 1.4 - 2.8 (Alberro 1983; Choudhuri *et al*., 1983). Zakari (1981) reported the number of services per conception of 3.0, 1.4, and 1.6 when the Nigerian zebu cow was artificially inseminated at the early estrus, middle estrus, and post estrus, respectively.

2.1.8 Conception Rate

This refers to the number of cows that become pregnant divided by the number mated or inseminated and expressed as a percentage. It is a measure of breeding success. The traditionally managed White Fulani breed of cattle in Nigeria has been reported to have achieved a conception rate of 34.2 - 36.0% (Pullan, 1979), and 67.0% under ranch conditions (Nuru and Dennis, 1976). The native Botswana zebu breed when traditionally managed gave a conception rate of 36.2-51.9% (Reed *et al.*, 1974), and 69.0 - 82.0% under ranch conditions (Buck *et al.*, 1976).

2.1.9 Calving Interval (CI)

This is the period from one parturition to the next. It is the period from calving to conception and the gestation period. The calving interval is the most objective traditional method of assessing the reproductive success of cattle (Schultz, 1987). A calving interval of 12 months results in a maximum production efficiency. Only about 4% of Nigerian zebu cattle calve each year, 22% calve every other year, and 73% calve irregularly (Pullan, 1979). Estimates of calving interval in Zebu cattle range from 11-29 months (Oyenuga, 1967; Wheat *et al.*, 1972; Roberts and Gray, 1973; Olutogun, 1976; Oyedipe *et al., 1982*). Pullan (1979) reported an average calving interval of 27 months in a traditionally

(ANYAMBU ... UNIVERSITY)

managed White Fulani herd, while Agyemang *et al.*, (1991) reported 21 months for the N'dama under the village production system. The White Fulani breed in Niger Republic and Mali gave a mean calving interval of 19.6 ± 5.1 and 22.1 ± 6.7 months, respectively (Wagenaar, *et al.*, 1986; Wilson, 1989). Prolonged post-partum interval contributes immensely to the long calving interval of cattle in the tropical and subtropical regions of Africa (Agyemang et al., 1991).

Other reproductive indices, though less commonly employed in assessing the reproductive potential of tropical cattle, include cow efficiency, cow productivity index, most probable producing ability, expected progeny differences, and lactation index (Mukasa-Mugerwa, 1986).

The activity of the ovary is central to the reproductive status of an animal. Ovarian activity can be assessed by simple observation and rectal palpation. However, measurement of physiological parameters such as the electrical conductivity of the vaginal wall and biochemical parameters such as follicle stimulating hormone, luteinising homone and progesterone are possible.

2.2 Estrus and the Estrous Cycle

Cattle are polyestrous. The climatic effect on estrous cycle in cattle is not as pronounced as in other animals especially in the temperate zones. The reported seasonal effect on cyclicity and estrus in Nigeria (Osori, 1976) may be an indirect effect of feed deficit (Zakari, 1981). The various abnormalities in estrus manifestation include short estrus, prolonged estrus, split estrus, nymphomania, and silent estrus (Jainideen and Hafez, 1985). Zakari (1981) exhaustively documented heat manifestation in the Bunaji and Bokoloji breeds of cattle in Nigeria. Among the cardinal behavioural signs he observed included: estrus cow mounting another, estrus cow attempts standing to be mounted, sniffing, licking, nudging, butting, flehmen, pairing, urination posture, mucus discharge, and disoriented mounting.

An estrus cycle lenght range of 6-54 days, with a mean (± S.E.M) of 22.87 ± 0.70 days for the Bunaji and 6-59 days with a mean (± S.E.M) of 23.76± 0.65 days for Bokoloji cows were also observed (Zakari, 1981). Duration of estrus, which ranged from 5-12hr in both breeds, was longer in the wet than in the dry season. An average cycle lenght of 23 days has been reported for other tropical breeds (Anderson, 1944, Plasse, *et al.*, 1970; Rahka and Igboeli, 1971).

2.2.1 Heat Detection

The bull is the best detector of heat. It detects the estrus cow two days before estrus and stands in her vicinity (Zakari, 1981). The average heat detection rate by herdsmen is 55% (Hinks, 1982). The duration of estrus behaviour is controlled physiologically, the frequency of display of the various components of behaviour during this period is governed by environmental and social factors, in particular the presence or absence of another cow in estrus. (Esslemont and Bryant, 1976). Synchronized estrus is advantageous in this respect by concentrating heat and increasing the number of cows in estrus at the same time.

The various methods of detecting heat for artificial breeding include visual observation, use of marker bulls and progesterone assay (Williamson *et al.*, 1972, Mather *et al.*, 1978). Other methods include the use of Kamar Heat-mount detectors, tailpaint, pedometer, closed circuit television, and the diagnostic procedure of rectal palpation of the ovaries to determine the cyclicity of the cows. King *et al.*, (1976) reported a higher percentage of cows showing estrus by a closed circuit television and a time lapse video recorder better than observed by herdsmen. Visual observation of estrus relies exclussively on the behavioral manifestation of estrus. Whitmore (1987) divides behavioral estrus into

primary and secondary. The primary sign of estrus is standing firm while being mounted. The secondary signs of estrus include mounting of other cows, a mucus discharge, swollen vulva, hyperactivity and a limited amount of belowing. There is 90% certainty that the cow that stands to be mounted is in heat and 30% that the cow that mounts another is in heat. Standing to be mounted was successful on its own alone 79% with only 4.5% false positive (Esslemont and Bryant, 1974). Cattle bred based on primary heat signs have high pregnancy rate (60 - 75%) while those inseminated on the basis of secondary heat signs usually have lower pregnancy rates (Whitmore and Matter, 1977). Studies have shown that good heat detection (90% or more) is possible with very conscientious heat checks twice daily. The inacuracy of human observation in detecting heat has been demonstrated (Williamson *et al.*, 1972).

When fixed time insemination is carried out, cost of heat detection and decreased conception rate due to missed heat is greatly reduced. Dawuda *et al.*, (1989) reported 29.4% silent estrus, 44.1% anaestrus, 27.06% estrus behaviour in 68 post-partum Bunaji cows, as indicated by serum progesterone concentrations. The high incidence of silent estrus reported by Dawuda et al., (1989) is in agreement with previous reports by Kidder *et al.*, (1952), Rakha and Igboeli (1971), and Ozser *et al.*, (1986).

2.3 Controlled Breeding

Livestock breeders for a long time contemplated over the idea of controlled animal breeding. A break-through was made in 1948 when Christian and Casida, and also Dutt and Casida, reported the use of progesterone to control the estrus cycle of cows and ewes. Since then, research on controlled breeding and the use of synchronization by farmers continued and reached a peak in dairy cows in the USA between 1976 and 1977, and then subsequently declined (Saunders, 1980). This decrease was as a result of observed low conception rates in treated animals (10.15% over controls; Hansel and Convey, 1984).

Reliable techniques in controlled breeding were sought for many years for the numerous envisaged management advantages which have been summarized by Peters and Ball (1986) as follows:

1) Controlled breeding would facilitate the use of AI thereby allowing the greater exploitation of genetically superior sires.
2) Artificial insemination could be carried out at pre-arranged or fixed time if the control was sufficiently reliable.

3) There may be some advantages in shortening the calving to conception intervals hence the calving interval and possibly the calving season.

A drawback to controlled breeding is the high variable response between herds and between years in the same herd and also the increased labour input relative to natural mating (Peters and Ball, 1986). Capital costs of equipment may be high (Nancarrow and Radford, 1976) and may limit its usefulness and application. Gordon (1976) summed up the objective of controlled breeding as a precedure which offers the farmer means by which he can manipulate reproduction while permitting the cow to exhibit its normal fertility level.
The recipient animal of controlled breeding for best result (Schultz, 1987) should be of the following status:

1) should be disease free.
2) Heifers should be matured for normal fertility
3) Animals should be on an increasing plane of nutrition.
4) Adequate postpartum interval in cows to ensure the return of reproductive tract to normal status.
   At least an interval 45-60 days is needed.
5) Animals must be cycling
6) Animal must be non-pregnant.

2.3.1 Methods of Controlled Breeding Programme

The methods of estrous cycle control are dependent on the manipulation of the hormonal events occuring during the normal ovarian estrous cycle (Peters and Ball, 1986), with the aim of inducing a fall in peripheral progestrone level. There are two methods:

1. Shortening the lifespan of the corpus luteum (Noakes, 1986). For this, prostaglandin F2alpha or its analogs are commonly employed.
2. Administration of an exogenous source of progesterone for a number of days followed by an abrupt withdrawal (Noakes, 1986, Peters and Ball, 1986). This exerts a negative feedback effect upon the hypothalamic/pituitary axis, thereby suppressing cyclical activity. Cyclicity returns soon after they are removed. Shorter treatment periods have resulted in higher conception rates than longer treatment periods (Hansel and Convey, 1984).

2.3.2 Historical Development of Controlled Breeding in Cattle

Hansel and Beal (1979) recognised four developmental phases of controlled breeding in cattle. The first phase occured around 1960 when progestational compounds were administered through various routes. 80-90% synchrony was recorded with estrus occuring over a 4 day period begining from day 2 after

withdrawl of the progestational agent. Conception rates in the synchronized animals were lower than in the control. The second phase involved the use of progestational agents combined with either estrogens or gonadotropins. This proved to be better in terms of synchrony and conception rate than the use of progestational compounds alone. The third phase began in 1972 after the luteolytic effects of prostaglandin F2alpha-(PGF2alpha) was demonstrated. Conception rates was variable but quite promising and in some cases reported to be equal to the control. Phase four combines the use of both progesterone and PGF2alpha. Progesterone is administered via a progesterone releasing intravaginal device (PRID) inserted into the uterus and allowed to remain in place for 7 days PGF2alpha (25mg 1.m) is administered 24hr before PRID removal. Roche et al., (1981) reported conception rates equal to control in artificially inseminated Holstein heifers. PRID - PGF2alpha treatment was also reported to have given a similar conception rate compared to the control and better than twice treatment with PGF2alpha in a pre-set time - one dose artificial insemination in heifers (Smith et al., 1983).

2.3.3 **Prostaglandins**

The first published information on the smooth muscle contractile effect of prostaglandin (PG) was made almost simultaneously by Goldblatt in England and Von Euler in Sweden in 1933 and 1934, respectively. This was, however, after Kurzrok and Leib had in 1930 reported the contractile and relaxation effect of human semen on the muscles of the uterus. Later, in 1935 Von Euler found similar effect with the seminal fluid of the monkey, sheep, and goat. As a result he named the hitherto unkown active ingredient "Prostaglandin" (Szebuniewicz and McCrady, 1977). Prostaglandins are found in all tissues but with the highest concentration in human and sheep seminal plasma. Other tissues contain about 1ug/g (Meyers *et al.*, 1977)

2.3.4 **Types of Prostaglandin**

Prostaglandins are derived from arachidonic and some other related 20-Carbon essential fatty acids by cyclization and oxidation through an inactive intermediate compound known as prostanoic acid. The four major groups of prostaglandins are PGE, PGF, PGA, and PGB. PGE and PGF are known as primary prostaglandins. Prostaglandins have a very short plasma half life (about 10 mins). About 60% of an administered dose is excreted in urine, 20% in faeces and the remainder unkown (Szabuniewicz, and McCrady, 1977). Prostaglandins exist in two forms; natural and synthetic. The synthetics are more

potent and not readily metabolised. The action of prostaglandins vary in themselves and also in the different body tissues. PGE relaxes the nongravid uterus while PGF contracts it. Both have contractile effect on the gravid uterus. PGE relaxes the bronchi while PGF contracts it. Both stimulate the gastrointestinal tract.

2.3.5 **The Role of PGF2alpha in Farm Animal Reproduction.**

PGF2alpha and its synthetic analogs are drugs which have wide use especially in clinical dairy management Fetrow 1986). It is a luteolytic agent causing lysis of the corpus luteum (CL) thereby returning the female animal to heat (Cooper, 1974 Hafs et al., 1975 Seguin 1980). In this respect, it is used to regulate estrus, reducing or concentrating the effort necessary to detect estrus (Fetrow, 1986). There are four basic approaches to the use of PGF2alpha in estrus synchronization and artificial insemination viz:

1) PGF2alpha or its analogs are injected in two separate doses eleven days apart (Noakes, 1986; Schultz 1987). The presence of a mature corpus luteum is not necessary and estrus is expected 2-5 days after the second injection.

2) PGF2alpha is injected, heat is detected and the animals bred. After 11 days a second dose is injected to animals not bred. At the manifest estrus or fixed time, such animals are bred.

3) Animals with functional corpus luteum are treated. Estrus occurs 2-5 days later. this method is time consuming and the results also depend on the accuracy of rectal palpation (Peters and Ball, 1986).

4) Animals should be watched for estrus for 6 days and bred at the usual time relative to estrus. Animals not bred are injected with PGF2alpha on the 6th day and bred on the 7th and 8th day, or subsequently, relative to estrus.

After applying PGF2alpha using any of the above methods, breeding is done using one of the following options (Schultz, 1980):

a. AI on detection of estrus 2-5 days later.

b. AI at 72hr after treatment.

c. AI at 72hr and 96hr after treatment. No heat detection is required in methods(b) and (c) (Seguin *et al.*, 1986), Schultz, 1987).

The CL is responsive to PGF2alpha during the luteal phase of the estrus cycle (Day 5 or 6-17 or 18). At approximately 4-5 days before estrus (regressing CL) and 4 - 5 days after estrus (developing CL), PGF2alpha is not effective. The ovaries are refractile to prostanglandin during the follicular phase (Seguin, 1980).

Fertility of cows bred after detecting estrus following prostaglandin application is as good as, or perhaps better than, fertility on a detected estrus without prostaglandin (Cooper, 1974 Seguin, 1980;). The fertility following PGF2alpha treatment accompanied by timed AI has been reported to be variable, but often less than detected estrus.

The prostaglandins have also been found useful in the management of placental retention (Fetrow, 1986). They stimulate contraction of the muscles of the uterus and thus serve as mechanical evacuators (Patil, *et al.*, 1980; Arthur *et al.* 1982). They have however, been found to be ineffective in preventing placental retention when given later than one hour post-partum (Olson *et al.*, 1987). The incidence of retained placenta was reduced from 91 to 90% when given within 1 hr of parturition (Gross, *et al.*, 1986). PGF2alpha analogs are also used to induce parturition in cattle (Plenderleith 1978 First, 1979; Hofman *et al.*, 1979; Johnson, 1981; Diskin, 1982), and swine (First *et al.*, 1982). PGF2alpha also causes premature parturation and abortion in these animals.

2.3.6 Fertility to PGF2alpha Treatment

An obvious advantage of synchronized estrus is the opportunity it affords for a greater heat detection by concentrating the period for estrus observation (Orihuela *et al* 1983). Mounting behavior also increases if two or more cows are in estrus at

the same time (Williamson et al 1972., Esslement 1976,). Mounting behavior is very useful in heat detection. Inspite of this effective management of heat, fertility to synchronization has been variable. Voh Jr.*et al.*, (1987b), working with the Bunaji and Sokoto Gudali breeds reported a pregnancy rate of 35% in the treated group compared to 23.3% in the control in a timed dual AI (72hr and 96hr after the second PGF2alpha injection). Conception rates of 53.9% and 46.7% were respectively, recorded for the treated and the groups control. Although insemination at observed estrus resulted in pregnancy rates comparable to untreated controls, Landiver *et al.*, (1984) reported a conception rate of 18.6% in 69 zebu cattle treated with PGF2alpha on palpation of a functional corpus luteum and inseminated 8hr post detected estrus. Those 10 cows not showing estrus were inseminated 80hr after PGF2alpha injection. A control group of 57 zebu cattle inseminated 8hr post natural estrus gave a conception rate of 29.8%. The conception rate between the synchronized and non-synchronized group was significant ($P<0.05$). The 10 cows that were not detected in estrus may have exhibited silent or short estrus (Johnson and Gambo; 1979; Zakari, 1981; Dawuda et al., 1989), or were actually not in the PGF2alpha responsive phase of the cycle (Peters and Ball, 1986; Noakes, 1986).

The fertility to cloprostenol (synthetic PGF2alpha analog) induced estrus in *Bos indicus* and *Bos taurus* heifers was comparable to the naturally occuring estrus when all cattle were inseminated 12hr following the detection of estrus. (Hardin, *et al.*, 1980). Gyawu *et al* (1993) reported that the proportion of N'dama cows which manifested estrus to first and second cloprostenol injection were 85.7% and 92.9%, respectively. Logue *et al.*, (1991) compared the efficacy of two injections of luprostiol given 10 days apart, and a combination of an initial injection of norgestomet and estradiol valerate together with a norgestomet ear implant left in place for 9 days in taurine heifers. There was no significant difference between the number of animals in the two treatments which were diagnosed pregnant 33-35 days post insemination, although the implant gave a higher overall proportion of pregnancies (40%,and 51% for the respectively treatments). In an experiment designed to simulate the management practices of the migrant Fulani herdsmen, Nagaratnam et al., (1983) observed a synchronization rate of 76% and a conception rate of 59% in a herd of 17 cows made up of 12 White Fulani, 2 Sokoto Gudali, and 3 Zebu x Friesian. Synchronization was achieved with 2 injections of cloprostenol (Estrumate ICI) administered 11 days apart. The animals were inseminated twice at 72hr and 96hr using chilled semen extended in an egg-yolk citrate diluent. Adeyemo *et al.*, (1979) obtained a 100% synchronization and 61% pregnancy

rate in 3 breed of cattle (German Brown, Holstein Friesian and White Fulani) in a pioneer trial of PGF2alpha (lutalyse(R) application in the Nigerian zebu cattle. This result is comparable to the 100% synchronization reported for the taurine cattle (Stevenson *et al.*, 1984; Tanabe and Han, 1984), and in the banteng, a species of wild cattle closely related to the *Bos taurus* (Asa, 1991).

Double prostaglandin injection 11 days apart failed to induce estrus in 10-20% of treated cows. The failure was attributed to considerable variation in the duration of the luteal growth and maturation with the net result that this proportion of animals were given the second injection of PGF2alpha when the CL were not upto 5 days old (Voh Jr. *et al.*, 1987b). A preliminary trial of an inter-treatment interval of 13 days gave a response rate of 93% (Voh Jr. *et al.*, 1988a). A higher response rate was recorded during the wet (90%) than in the dry season (70%), with a mean duration of standing estrus of 19hr and 13hr respectively. About 80% of the estrus occured 43-84hr after the second PGF2alpha injection (Voh, Jr. et al., 1988a).

Even though conception to synchronized AI have been reported to be as good as those in control cows (Lauderdale et al., 1974 Hardin *et al.*, 1980;) other reports present a higher tendency of synchronized estrus leading to low pregnancy rate, attributable to poor semen quality, improper animal insemination technique (Seguin, 1981), use of acyclic animals (Turman *et al*, 1975; Cooper, 1976; Seguin 1981), and early embryonic death (Diskin and Sreeman 1980; Asa, 1991). Several authors report consistently higher response and conception rates to synchronization by heifers than cows (Larson and Ball, 1992). However, Olson (1980), reported 50% and 47% first service conception rates for control and PGF2alpha treated heifers, respectively, while first service conception rate of 62% and 41% was reported for control and PGF2alpha treated cows respectively.

PGF2alpha therapy has found good use in the management of infertility in cattle, especially infertility due to anaestrus resulting from luteal cysts (Olson, 1980; Blowey, 1981).

2.3.7 Progresterone Profile and Cattle Reproduction

Progesterone is a steroid hormone which plays an important role in the preparation and maintenance of pregnancy. The determination of progesterone concentration in milk, plasma, or serum is an excellent tool in the diagnosis of pregnancy (Bulman and Lamming, 1987) and in the determination of the general reproductive status of cattle especially when combined with clinical findings and animal records (Garcia, and Edqvist 1988).

Progesterone is synthesized from cholesterol via pregnenolone and is rapidly metabolised to pregnanediol mainly in the liver (Aufere and Hariet, 1976). The major sites of its production are the ovaries and the placenta. A small quantity is also produced by the adrenal cortex (Sorenson, 1979). The levels of progesterone in milk and plasma are positively correlated (Ginther *et al.*, 1974; Lamming and Bulman, 1976). However, the milk levels are higher than in plasma. The problem with the use of blood for progesterone assay is the reported continous enzymatic decomposition of progesterone (Vahdat *et al.*, 1981). Progesterone decomposition diminishes when serum or plasma is separated from whole blood (Garcia and Edqvist, 1988), and also when stored at lower temperature. Lower conception rate and repeat breeding have been suggested to result from inadequate progesterone levels (Casida, 1961;

Rosenberg, et al., 1977). Excessively long periods of high progesterone prior to insemination may suppress fertility. Too short a period of progesterone may also be deleterious (Lawson and Ball, 1992). In the Nigerian zebu cattle, measurement of progesterone has been used to determine estrus cycle lenght (Eduvie and Oyedipe, 1991). The range of progesterone in Bunaji cows was reported to be 0.2 ± 0.4ng/ml at oestrus to 5.3 ± 2.3ng/ml at midcycle (Eduvie and Dawuda, 1989). Variation in the concentration of progesterone is common in the zebu cows. Oyedipe *et al.*, (1988) reported a range of 1ng/ml to 9ng/ml at peak levels.

2.4 **Artificial Insemination**

Artificial insemination in cattle was introduced largely to control venereal diseases, especially bovine venereal trichomoniasis. Later, its potentials to rapidly enhance the improvement in the genetic upgrading of farm animals was realised. It is an important aspect of controlled breeding in cattle (Vaccaro. 1973).

2.4.1 Historical Background

The first recorded use of AI was in 1780 when Spallazani, an Italian physiologist, successfully inseminated a bitch by injecting fresh semen directly into the uterus. Ivanov and his co-workers in Russia successfully inseminated sheep and goat in 1930. By early 1940s, AI was of commercial application in cattle inthe USA and UK (Sorenson, 1979; Peters and Ball, 1986). The first AI in Nigeria was carried out at the defunct Livestock Improvement Centre, Vom, in 1949. The AI unit is now a prominent unit of the National Animal Production Research Institute (NAPRI). It carried out its first insemination in 1978, with locally processed chilled semen from zebu bull, (Voh Jr. 1990). Unfortunately, the benefit of field application of AI is yet to be realised in Nigeria. However, its future potential is great especially with regard to genetic improvement programmes.

2.4.2 Advantages of AI

AI is central in any attempt at cattle improvement. The main advantages of AI include the propagation of superior genes. Semen from a bull can potentially breed 500,000 cows through the use of frozen semen (Sorenson, 1979; Peters and Ball, 1986). It is the most rapid means of proving a sire's breeding ability.

The aggressivenenss of bulls make them potential dangers on farms. Safety is guranteed when cattle farmers utilize artificial insemination services. Incompatibility due to injury, size and idiosyncratic rejection of females of other breeds such as has been reported for the *Bos taurus* bulls in a zebu herd (Sorenson, 1979) are avoided. AI obviates infertility due to decreased libido and serving capacity. Zebu bulls are reported to be inferior to taurine bulls in both libido and serving capacity (Blocky, 1978; Chenoweth, 1980; Falcon, 1981).

Other advantages derived from the application of AI include genetic gain, cost effectiveness, disease control, and flexibility (Peters and Ball, 1986). It also serves as a means of sex control through separation of spermatozoa containing X and Y chromosome (Hafez, 1985), and a necessary research tool for investigating many aspects of male and female reproductive physiology (Hunter, 1982).

There are, however, some obvious disadvantages to the use of AI which Sorenson (1979) ascribed to poor techniques, controls, and management of the factors that usually are categorized as advantages. Availability of accurate breeding records necessary for good herd management imposses a great constraint.

2.4.3 Techniques for Insemination

The most commonly used insemination method is the rectocervical. This involves inserting one arm in the rectum and directing the inseminating tube through the vagina and cervix for the semen to be deposited in the anterior cervix. Clitoral message has been advocated as a stimulus to improve conception. A 10 second message of the clitoris after AI resulted in an increase of 6% conception rate over non-message in a beef herd. (Randel *et al.*, 1975). On the contrary, McDonnel *et al.*, (1977) reported a decrease of 1% conception rate in a dairy herd after 3 seconds message.

Other less commonly employed methods of insemination include the speculum (mirror) method and the vaginal method. Conception rates in both cases are confirmed to be lower than with the rectovaginal method.

2.4.4 Time of Insemination and Optimum Conception.

A lot of research has been carried out to determine the optimum insemination time for the taurine cow (Broadway, 1973; Roche, 1974). For conception to occur insemination must take place at the right time in the estrus cycle when the egg is still viable which is about 6-12hr post-ovulation (Peters and Ball, 1986). The reported optimum time for succesful AI in *Bos taurus* has varied from 12-24hr (Peters and Ball, 1986), or 12-18hr. (Sorenson, 1979), to approximately 16hr (Hafez, 1985) after standing estrus.

In Nigerian zebu cattle, a maximum conception rate of 73% occured when insemination was carried out at the middle of estrus During early estrus and post estrus insemination, conception rates of 31% and 63%, were respectively obtained. (Zakari, 1981). Estrus period ranged from 5-12hr. In a similar study, Dawuda *et al.* (1987) obtained a maximum conception rate of 70% when Bunaji cows were inseminated 12hr from standing estrus, followed closely by 65% each when inseminated 6hr and 18hr, respectively, after standing estrus. Insemination at 24hr post standing estrus reported in the same study gave a conception rate of 60%. Foot (1979) and Robbins *et al.* (1978) reported low conception rate when cows were inseminated during the first part of estrus. In general, the "Am-Pm" rule of thumb, which implies that animals detected first in estrus in the morning be bred the same afternoon and those found in the afternoon and night are bred the following morning, has given good results in all ecological zones (Sorenson, 1979; Zakari, 1981; Dawuda *et al.*, 1984).

Voh Jr. (1987a), in recognition of the difficulty of heat regulation under field conditions, advocated the insemination of cows whenever heat is observed. Such, he noted, goes "without much loss" in fertility. The problem of heat detection has been surmounted by the practice of synchronized estrus with timed AI In an experiment using the small East

African short horned zebu and Ngaunda cattle in Uganda (Rollinson, 1963), the highest conception to AI occurred when insemination was carried out between 15 and 25hr after commencement of proestrus.

2.4.5 Fertility To Artificial Insemination

First service pregnancy rates (PR) obtained in the Nigerian zebu cow ranged from 40-60% and 25-60% for insemination at spontaneous estrus, and synchronized estrus respectively (Voh Jr., 1990; Oyedipe, et al., 1980). A report on a pioneer insemination at the Livestock Improvement Centre, Vom, indicated a conception rate of 46% to using Holstein -Freisian semen. Voh Jr. *et al.*, (1987b) reported a variable conception rate (20-54%) to AI at a fixed time following synchronization with dinoprost tromathamine. Pregnancy rates of Bunaji cows varied from 56%, when inseminated at 0hr after standing heat, to 70% at 12hr after standing heat (Dawuda *et al.*, 1987).The Animals that conceived to AI were found to show higher progesterone levels than those that did not conceive (Watson and McDonald, 1984). This may suggest that the non-pregnant animals were inseminated too early for conception. Voh Jr. *et al.* (1988b) recorded 74.1% pregnancy rate to AI at natural estrus, 60.5% at observed induced estrus, 54.2% at timed insemination at 60hr. However, 45.6%, 34.9%, 40.0% pregnancy rates were obtained at preset time AI at 72, 84 and 96hr, respectively.

In the temperate breeds of cattle, a survey involving 1059 Friesian cows and heifers from 12 pedigree herds gave similar calving rates to AI and natural insemination (64.05% and 64.5%, respectively, Peters and Ball, 1986).

Cooper (1975) reported that it was unusual to record a PR of 70% or above to AI under field conditions. This observation was made in reaction to a report which though in error, confused non-return rates with PR. It was however, asserted that "this level of fertility may occur in a small population (and) under commercial circumstances a figure of 50% is more realistic" (Randel *et al.*, 1975; Hafez, 1985)

2.4.6 Factors Affecting Conception Rate To Artificial Insemination.

The major factors determining fertility in artificial breeding are:

1. The fertility of the males
2. Care with which the semen was collected, stored, and thawed before use.
3. The skill of the inseminator
4. Management of the female

The 90 and 120 day non-return rate differed significantly among inseminators, but did not differ among bulls (Busch and Furstenberg, 1984). Failure to conceive may also be due to poor insemination techniques and low bull fertility. For example, a decrement of upto 40% in conception rates was recorded when the same bulls were used in AI compared with natural service in Colombian herds. The difference was traceable to poor insemination and semen handling techniques (Zemjanis and Diegosanint, 1963).

The fertility of the semen alone could also, to a great extent, affect conception (Foot, 1979; Hafez, 1985; Sekoni, 1993). Insemination at the "proper" time of the estrus cycle is important.

Nearly all experiments have shown that more than one insemination per estrus increases conception rate. Presumably, this results from the difficulty of predicting ovulation and appropriate timing of each insemination (Hafez, 1985).

2.5 Bovine Trypanosomiasis in Nigeria

Trypanosomiasis has remained an important disease of ruminants in Nigeria, limiting the growth of the cattle industry and causing collosal economic losses. The prevalence of bovine trypanosomiasis has declined from about 80% at the early part of the 20th century to 4.3% between July 1989 and April 1991 (Anosa *et al*, 1991). There was no "remarkable difference in the prevalence among the seven ecological zones, although prevalence was consistently higher in the savannah zone. Earlier reports pointed to the endemicity of trypanosomiasis in the central and southern parts of Nigeria (Maclennan, 1963, 1970; Leeflang, 1974). Ahmed *et al*. (1994) reported 7.3% prevelance of trypanosomiasis in Damboa local government area of Borno state in a survey utilizing 151 blood samples collected from 11 sendentary herds. *T.vivax* infection accounted for 6% while *T.congolense* infection accounted for 1.3%.

2.5.1 Ecology and Spread of Bovine Trypanosomiasis

Trypanosomiasis is a disease of man and animals, caused by heamoprotozoan parasite of the genus *Trypanosoma*. With the exception of *Trypanosoma equiperdium* of equines which causes a venereal disease, all other trypanosomes have arthropod vectors (Urguhart *et al.*, 1987). Economically important typanosomes in cattle belong to two groups:

a) the monomorphic salivarian group, and
b) the pleomorphic salivarian group.

The monomorphics are represented by *Trypanosoma vivax* and T. congolense. While *T.brucei* is the only pleomorphic salivarian trypanosome of importance. These trypanosomes have a wide distribution and are transmitted cyclically by tsetse flies (Molyneux and Ashford, 1983; Urguhart *et al.*, 1987). Non-cyclical transmission is also common with *T.vivax* (Leeflang, 1978). *T.vivax* is the most pathogenic and most prevalent species of trypanosome in West Africa (Leeflang, 1978). The pleomorphic *T.brucei* group is of considerable importance because it is zoonotic, infecting humans and wildlife (Leeflang, 1974) *T. vivax* is also the most important, and most prevalent trypanosome in Nigeria (Glover, 1961; Joshua, 1986; Anosa *et al.*, 1991). In a survey which covered the seven ecological zones of Nigeria the prevalence rates of *T.*

congolense and T.brucei were 33.8 and 7.6%, respectively (Anosa et al, 1991). Infections due to unidentified species accounted for 6.4%. The infection rate in sheep and goats were mainly attributed to *T.vivax* (61.2% and 53.1%, respectively) followed by *T. congolense* (26.5% and 33.3% respectively). *T. brucei* infection in sheep and goats accounted for 16.3% and 15.6% respectively (Anosa *et al.*, 1991).

The proportion of infection due to *T.vivax and T.congolense* increases the further cattle are located from areas infested with tsetse. However, the absolute prevalence of trypanosomiasis is lower under such situations (Ford, 1964; Leeflang, 1975; Anosa *et al.*, 1991).

T.evansi, which is pathogenic to camels (Ilemobade, 1972) and *T.theileri*, a non-pathogenic stercorarian trypanosome (NITR, 1964) are only mechanically transmitted and cyclical transmission by tsetse flies has not been reported (Leeflang, 1978).

T. simiae is an important parasite of pigs causing a fulminating infection that is severly acute. However, it is non-pathogenic to cattle (Stephens, 1966).

2.5.2 The Pathology and Pathogenesis of Trypanosomiasis in Cattle

The pathology produced by experimentally induced trypanosome infection in cattle has been the object of many studies (Isoun *et al.*, 1975; Esievo *et al.*, 1982; Esievo and Saror, 1983; Nwambu, 1983; Ogwu *et al.*, 1984; Esievo *et al.*, 1984; Sekoni *et al.*, 1990a, 1990b). Reports on the response and mechanism of the natural disease is, however, scanty.

In a natural cyclical infection, tsetse flies carrying infective metacyclics inoculate them into the skin of susceptible animals. The metacyclics grow and multiply for a few days causing a localized swelling. From there they follow the lymphatics to enter the blood stream and multiply. The animals immune response reacts by producing antibodies which bind to the parasites and destroys them. However, before all the parasites can be eliminated, a population of trypanosomes bearing a new surface coat emerge and proliferate, resulting in a new wave of parasitaemia (Anon, 1986, ILRAD, 1991). This antigenic variability is responsible for the persistence of the trypanosomes and re-infection.

The production of disease by trypanosomes depend on three important factors which include the infecting trypanosome, the host, and the vector (Stephens, 1986). The host factors include age, breed, and presence or absence of stress. Stress factors such as under nutrition, intercurrent disease, pregnancy, and lactation have been found to markedly increase the susceptibility of animals to infection (Chandler, 1952; Maclennan 1970; Murray *et al.*, 1982). Undernourished West African Dwarf sheep appeared to be more susceptible to infection compared to well nourished ones (Reynolds and Ekwurike 1988).

Age is a significant factor in the susceptibility to trypanosomes in cattle. Calves resist infection more than adults (Fiennes, 1946, 1970; Welde, et al.,; 1981). Self cure is known to occur in calves (Uzoigwe, 1984). Exposure to infection at calf-hood also determines the degree of trypanotolerance in trypanotolerant breeds. Griffin and Allonby (1981) reported higher susceptibility to *T. congolense* in lambs and kids than in adult sheep and goats.

Neutropenia occurs commonly in adult cattle while lymphocytosis is relatively higher in calves than in adults (Fiennes, 1970).

The N'dama (hamitic longhorn), Muturu (dwarf shorthorn), and Keteku breeds of cattle are relatively resistant to trypanosome infection. Their ability to resist depends on infection at calfhood and constant exposure thereafter (Fiennes, 1970). Trypanotolerant breeds are able to remain healthy while carrying potentially pathogenic trypanosomes (ILRAD, 1991). The N'dama produces higher levels of detectable antibodies and control trypanosome infection better than their susceptible zebu counterpart (ILRAD,1992). The Zebu cattle, especially Bunaji, have a slight degree of resistance, and that may be the reason they are capable of surviving better than the taurine cattle in endemic areas (Leeflang, 1974). Those factors of the trypanosome known to play a role in the pathogenesis of trypanosomiasis include the strain, the size of the infecting inoculum, and geographical location of its vector and host (Maclennan, 1970). In natural infection in West Africa, *T. vivax* is of more economic importance (Unsworth, 1953; Anosa, 1985; Agu, 1983; Leeflang, 1978) while *T.congolense* is more important in East Africa (Tizard, et al., 1978; Nwambu, 1983; Stephen, 1986).

With regards to infection in Zebu bulls, T. congolense is believed to cause a more severe pathology compared to T.vivax (Sekoni 1990a & b). This is however, in disagreement with the findings of Morrison *et al* (1981) who reported that *T. congolense* is never as pathogenic as *T.vivax*.

2.5.4 Clinical Manifestation

Bovine trypanosomiasis may run an acute or chronic course. Several authors object to the description of the acute disease in endemic areas (Naylor, 1971; Stephen 1986). Stephens, (1986) described an acute disease in 17 Nigeria zebu cattle transmitted by wild *Glossina morsitans submorsitans*, in which the duration of infection ranged from 5-92 days (Average of 41.3 days). Clinical signs associated with the acute infection included rectal temperature of 40-41^{cc}, reduced appetite, and rapid pulse. Dyspnea was occassionally present. The chronic disease, which is the most common, was characterized by anaemia, starry coat, cachexia, enlarged superficial lymphnodes, nasal and ocular dischages, and abortion in pregnant animals. Although diarrhea has been suggested to be a feature of bovine trypanosomiasis, Stephen (1986) believes that it occurs secondary to other concurrent infections. However, Maikaje (1985) recorded diarrhea in Uda rams infected with *T.vivax*, although intestinal scrappings revealed the presence of *Giardia ovis*. Clinical manifestation of bovine trypanosomiasis may be influenced by the nutritional status of the animal, presence of intercurrent disease, and or stress (Murray, *et al.*, 1982; Adelekan and Adenowo,1989). The severity of disease also varies with both the species of animal and that of the infecting tyrpanosome (Leeflang, 1978; Stephens, 1986).

The outcome of bovine trypanosomiasis may be fatal, but complete or partial recovery or a chronic syndrome may occur (Stephen, 1986).

2.5.5 **Parasitaemia.**

After an infective bite by tsetse, or following artificial inoculation of trypanosomes, the parasites appear in the peripheral blood of the host later. The prepatent period and the level and duration of parasitaemia depend on both the host and trypanosome factors (Maclennan, 1970; Stephens, 1970; Desowitz, 1959). The variation in the levels of parasitaemia exhibited by different breeds of cattle may reflect differences in the nature or quality of immune responses mounted against the parasites. (Chandler, 1958; Desowitz, 1959: Murray and Morrison, 1978). The determination of the prepatent period and therefore the onset of parasitaemia, may vary with the skill of the investigator and the techniques employed (Stephens, 1986). The onset of parasitaemia depends on some host factors such as innate resistance, antibody levels, nutritional state, age, sex, intercurrent infections, and on the type of the trypanosome (Murray and Morrison, 1978).

Sekoni *et al.* (1990) reported detecting parasitaemia within 10 days in Zebu bulls inoculated with either *T. congolense* or *T.vivax*, while Esievo and Sarror (1983) reported a prepatent period of 3 to 4 days in zebu bulls aged between 12 and 24 months. The onset of parasitaemia in an experimental tsetse transmitted infection was found to be between 4 and 20 days (Luckins, 1976; Nwangela *et al.*, 1981). Four *T.vivax* infected heifers showed parasitaemia on the 4th and 5th day after intravenous inoculation (Ogwu, 1983). Intravenous challenge of Yankasa sheep with *T.vivax* (Sarror, 1980) and West African dwarf sheep with *T.congolense* (Kalu, 1983) resulted in a parasitaemia in 7-10 days. Maikaje (1985) challenged 8 Uda breed of sheep (1/v) with *T.vivax*. All showed parasitaemia within 7 days.

A typical characteristic of parasitaemia of bovine trypanosomiasis is that it is fluctuating or undulating. It rises to a peak and then quite rapidly falls to either low levels or a level at which no parasites can be found in the peripheral blood, (Doyle, 1977; Stephens, 1986,). The rise and fall of trypanosome population is due to antigenic variation (ILARD, 1991).

It is a widely held view that the degree of anemia (and therefore the extent of disease) is dependant on the level and duration of parasitaemia. On the strength of this, Stephens

(1986) queried the progressive anemia observed in some clinical cases at the time when parasitaemia could not be detected.

2.5.6 Anemia in Trypanosomiasis

Anemia is a cardinal sign of bovine trypanosomiasis (Holmes and Jennings, 1976). The type of anemia described varies from macrocytic and normochromic (Roberts and Gray, 1973), normocytic normochromic with a tendency, to macrocytic and normochromic (Saror *et al.*, 1979). It is macrocytic in acute phase but microcytic during the chronic phase (Flennes, 1954). The anemia could result from intravascular haemolysis (Losos and Ikede, 1977, Mamo and Holmes, 1975; Dargie *et al.*, 1979; Anosa and Isoun, 1980; Esievo *et al.*, 1982, 1984). Tizard *et al* (1977) found no clear evidence for intravascular haemolysis in bovine trypanosomiasis. Anemia could also be of immunologic origin (Mackenzie et al., 1978) or a result of hyperthermia. Karle (1969) reported an in-vitro study which showed that red blood cells exposed to a temperature of a few degrees above body temperature have shortened lifespan.

A practical method for anemia determination is by the measurement of haematocrit value (HCT or PCV) Sekoni *et al.* (1990) recorded a least PCV value of 22% and 11% in zebu bulls infected with *T.vivax* and *T.congolense,* respectively, over a period of 12 weeks. The mean PCV range of 28% and 38%, 17% and 38%, 31% and 38% were observed for *T.vivax, T. congolense* and control animals. A PCV of between 15% and 20% is most unlikely to result in the death of an inactive cattle. Similarly, such low PCV values in ranging animals may lead to death (Murray, 1980).

T.vivax infected cattle, maintained under normadic husbandry system, die with a PCV value of 20% (Leeflang, *et al., 1978).* A drop in PCV and other haematological values occurs within a few days following inoculation of metacyclic trypanosomes. Fiennies (1954) demonstrated a haemolysin in the blood of cattle infected with *T. congolense. T. vivax* produce a severe acute fall in PCV whereas *T. congolense* causes a more gradual fall (Sileghem *et al.*, 1994)

2.5.7 **Diagnosis of Trypanosomiasis**

The clinical signs of tyrpanosomiasis in cattle is non-pathognomonic (Stephen, 1970, 1986) and, therefore, cannot provide a confirmatory diagnosis. The demonstration of parasite is conclusive of infection. This could be achieved by microheamatocrit centrifuge technique (mHCT), thin and

thick blood smears, lymph gland smear examination (Leeflang *et al*, 1978), or by the dark ground (DG) buffycoat technique (Paris *et al*., 1982). Leeflang *et al*., (1978) demonstrated the superiority of HCT over thin and thick smears. Dark ground buffycoat technique is the most sensitive all round field test.

Laboratory animal inoculation is not commonly used in the diagnosis of trypanosomiasis. It gives a variable result depending on the species of trypanosome and the stage of infection (Robinson and Ashkar, 1972; Leeflang *et al*. 1976, 1978).

Serodiagnostic tests available for the diagnosis of animal trypanosomiasis include:

a) Complement fixation test (CFT). This test has a wide application in the detection of dourine in horses (Stephen, 1970; Luckins,. 1993).

b) Indirect flourescent antibody test (IFAT), it is both specific and sensitive in detecting antitypanosome antibodies in infected cattle (Wilson, 1969; Luckins and Mehlitz, 1978), and camel (Luckins *et al*., 1979). Ashkar and Ochilo (1972) reported antigenic cross reaction with IFAT.

Melarsomine (Cymelarsan , Melcy . Rmiio is the most recent trypanocide in 30 years (Raynard *et al.*, 1989). The long period of the field application of these trypanocides in cattle has encouraged the development of resistance. The close chemical similarities of these drugs (Williamson, 1970) has also encouraged the development of cross-resistance. Resistance to the standard recommended doses of trypanocides in Nigeria has been documented. (Jonnes-Davis, 1967; Gray and Roberts, 1971; Ilemobade, 1979). The practical implication of this resistance became doubtful if note is taken of the drastic decline in the prevalence of trypanosomiasis as reported by Anosa *et al.*, (1992). This report holds that decreased prevalence was a result of encroachment on tsetse habitat and the use of trypanocides. The joint WHO/FAO expert committee report (1976) estimated that 400,000 doses per annum of trypanocidal drugs were used in Nigeria over the period 1972- 1975. Ogwu (1983) noted the dearth of data to estimate expenditure on the therapy of trypanosomiasis but in any case concluded that the amount must be large and must constitute an important source of economic drain in the cattle industry. Drug importation is not coordinated and thus cannot be used as a basis. Drug consumption is also not adequately monitored and controlled, thus it becomes impposible to quantify the national expenditure on trypanocides.

2.5.9 Reproductive Abnormalities in Bovine Trypanosomiasis

Trypanosomiasis depresses bovine reproduction through the development of lesions in the hypothalamus, pituitary glands, gonads and the reproductive tract (Masake, 1980). Ikede and Losos (1975) reported disabling lesions of the anterior pituitary gland in bucks and bulls infected with *T.vivax*. Thyroid and adrenal gland dysfunction in the zebu cow has also been reported (Ogwu *et al.*, 1992). Endocrine organ damage is responsible for depressed blood progesterone concentration and varying degrees of abnormal estrous cycle, and in some cases, complete ceasation of estrus in animals (Ogwu, 1983; Ogwu *et al.*, 1991). Progesterone levels were elevated in *T. congolense* infection in the Boran cow (Llewelyn *et al.* 1988). Both high and low progesterone levels were reported in *T.congolense* infected goats (Luckins *et al* 1986; Llewelyn *et al.*, 1987). Abubakar (1995) did not record any significant difference between pre and post-infection estrous cycle lenght and progesterone peak values in West African dwarf ewes experimentally infected with *T.congolense*. Lorenzini *et al.* (1988) reported a deleterious effect of experimental trypanosome infection on the estrous activity of a 2 year old Boran and N'dama heifers. Deranged progestrone levels were responsible for the inability of *T.vivax* infected heifers to

conceive (Ogwu *et al.*, 1984; Fio-Ugaindiro, 1987). It was also responsible for eloganted calving inteval (Agyemang *et al.* 1995). In the ram, Mutayoba *et al.* (1994) reported a decline in both plasma luteinizing hormone (LH) and plasma testosterone following experimental infection with *T.congolense*. The decline in plasma LH was not associated with reduced sensitivity of the pituitary to gonadotropin releasing hormone (GnRH) or its ability to release LH but to a reduced GnRH stimulation of the pituitary, whereas the decline in plasma testosterone was partly caused by reduced sensitivity of Ledig cell to circulating LH.

Lesions of the reproductive organs in the cow with trypanosomiasis include ovarian cystic degeneration. (Vohradsky, 1971), quiescent ovaries (Llewelyn *et al.*, 1988), atony of both the uterus and uterine horns (Ogwu *et al.*, 1984), mononuclear cell infiltration and fibroplasia of the uterus (Ogwu and Njoku, 1991). Placental damage may be caused by phospholipases released when trypamosomes are lysed (Ogwu, 1983). Transplacental transmission of trypanosomes in zebu cows may lead to sporadic abortion, premature birth , still birth and birth of weak calves (Boyd, 1971; Ogwu and Nuru 1981; Ogwu et al., 1991). Bawa *et al.* (1991) made a similar observation on Yankasa breed of sheep in Nigeria.

Sterility and infertility have been observed in several cases of animal trypanosomiasis. Ogwu *et al.* (1984) recorded 0% conception rate in zebu heifers experimentally infected with *T.vivax* and bred by a proven bull. All the uninfected animals used in the same experiment became pregnant. The infected heifers remained anaestrus for over 5 months. Anaestrus period of 9 months and 3.5 months were recorded in heifers infected with *T.brucei* and *T.vivax* respectively. (Ige and Amodu 1975).

The enzyme neuraminidase (Esievo,1980) is known to suppress pregnancy and induce abortion in mice (Gasic and Gasic, 1970; Muller and Saothoff, 1972).

In the male, animal trypanosomiasis also produces lesions in the hypothalamus, pituitary and testes. *T.vivax* infection in rams rendered them unfit for breeding within a short period of time with increased sperm morphological abnormalities (Sekoni, 1993). Low plasma testosterone was reported in *T.congolense* infected goats (Waindi *et al.*, 1989). Testicular lesions and deteriorated semen qualities have been reported in the bovine (Isoun *et al.*, 1983; Sekoni *et al.*, 1988, 1990a, 1990b) and in the buck and ram (Isoun and Anosa, 1974; Anosa and Isoun, 1980), and in rabbit infected with *T. brucei* (Ikede and Akpavie, 1982).

2.5.10 Mechanism of Reproductive Dysfunction

Reproductive dysfuncltion is a consistent feature of both animal and human trypanosomiases (Losos, 1986). Reproductive impairement is usually manifest in chronic infections with the associated organ damage (Ogwu, 1983). The haematological and biochemical responses to acute infection may also markedly affect the animals chances of being served or conceiving and may even induce abortion (Anosa, 1988), Generally, the mechanisms through which *trypanosomes* cause *reproductive* disorders in the cow have remained indistinct. Physical damage to reproductive organs, the pituitary, and hypothalamus by trypanosomes in the embryo and the development of foetal maternal stress leading to elavated blood cortisol level have been described as possible contributing factors (Sekoni, 1994). This suggestion was based on several reports on earlier studies. Trypanosomes were detected in ovarian smears of sheep (Isoun and Anosa 1979). Abortion in ewes during mid pregnancy following T. brucei infection may be due to the stressful effect (Loke, 1982; Oyedipe and Adeyemo 1989). Immune complexes which feature in chronic trypanosomiasis may predispose to hypersensitivity and cell damage (Morrison *et al.*, 1978, 1981; Anosa and Isoun, 1980).

ABUBAKAR TAFAWA BALEWA UNIVERSITY

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Experimental Animals, Screening and Maintenance

Six (6) cycling Bunaji heifers, aged between 3 and 4 years and of a body weight range of between 196 and 219 kg, were purchased from 2 local markets in Zaria, Kaduna State, Nigeria. They were allowed to acclimatize for 5 months at the Cattle Research Unit of the Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, during which time they were regularly clinically examined. Blood samples were collected and evaluated for baseline hematological data and for the presence of parasites. The animals were also screened and treated for ectoparasites, both before and during the course of the experiment using Steladone(R) 300Ec (Chlorfenvinphos, Ciba-Geigy, Switzerland). The heifers were also treated for endoparasites using Tramaxol(R) (Albendazole, Univet, Ireland) at the dose rate of 1 bolus/33kg, body weight (7.5mg/kg body weight) on arrival at the Unit. This was repeated two weeks before the start of the experiment. Oxytetracycline HCL (Terramycin LA(R), Pfizer, Nig.) at the dose rate of 10 mg/kg body weight was used to treat a confirmed case of anaplasmosis. It was also used as a broad spectrum antibacterial prophylactic against oxytetracycline sensitive potentially complicating organisms. The heifers attained a body condition score

range of between 4-5 (Pullan, 1978) at the commencement of the experiment. The animals were allowed to graze on native pasture (6-8 hours daily) which was supplemented with digitaria hay and a concentrate mixture of cottonseed cake and maize offal (2:1). Mineral licks were occasionally provided.

3.2 Experimental Design

3.2.1 Experimental Groups and Animal Infection

On the day of infection, the heifers were randomly allocated to two groups; 2 control and 4 infected animals. Heifers in the infected group were mechanically challenged, intravenously (i/v) with $5x10^6$ *T.vivax* organisms. The trypanosomes, from stabilate 146, were originally isolated from a naturally infected White Fulani cattle in Zaria and kept at Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria. The stabilates were cryopreserved at -196^{0c} in liquid nitrogen until used. A male Red Sokoto goat was infected and the infected blood was collected when the parasitaemias of 5×10^6 trypanosomes per ml was observed. This infected blood was used to infect the 4 heifers in the infection group. The other two animals served as controls (uninfected group). The experimental period lasted 69 days from the day of infection (Day 0).

3.2.2 Prostaglandin Administration

Each of six animals received two intramuscularly injections of 25mg Dinoprost tromethamine (Lutalyse, Upjohn, Kalamozoo, USA) given 11 days apart (days 11 and 22 post-infection).

3.2.3 Animal Insemination

All the six heifers were artificially inseminated using fresh semen from a Holstein-Freisian bull, obtained from the National Animal Production Research Institute (NAPRI), Ahmadu Bello University, Zaria, at the fixed times of 72 and 96hr after the second dose of PGF_2alpha without reference to estrus.

3.2.4 Blood Sampling

Following inoculation with *T.vivax*, all infected animals were bled once daily for 4 weeks and thereafter, three times a week for 6 weeks. Control heifers were similarly bled. Daily rectal temperatures were taken between 0600 and 0900hr just prior to blood collection. The bleeding was by jugular veinpucture. Five millilitres of blood was obtained in clean sterile test tube containing ethylene diaminetetra - acetic acid (EDTA; 1mg/ml blood) as anticoagulant. Another 5ml was collected without EDTA which was allowed to clot at room temperature for 2-3hr. The serum was collected and stored at -20^{oc} until assayed for progesterone by radioimmunoassay (RIA).

3.2.5 **Progesterone Determination**

Serum progesterone concentration were qualitatively measured by Radioimmunoassay (RIA), using the FAO/IAEA no extraction solid phase progesterone assay kit. The Radioimmunoassay method depends on the competition between the progesterone in serum and the 125^{I}-labelled progesterone for a limited number of binding sites on the internal wall of the lower part of the polypropylene test tube coated with progesterone antibody. The proportion of the 125^{I}- labelled progesterone bound to the antibody is inversely related to the concentration of the progesterone present in the serum (Oschman, 1986).

Radioimmunoassay Procedure: Progesterone test tubes for the determination of total progesterone (total count tubes (TCT) and the standard curve points were provided and arranged as follows:

a) A set of two tubes not coated with progesterone antibody (TCT).
b) Another set of seven progesterone antibody coated tubes each labelled in duplicate.
c) Progesterone antibody coated tubes for each of the samples to be analyzed.

A range of standards labelled A to G (prepared for FAO/IAEA by DPC, Los Angeles) with progesterone concentrations as indicated below were used:

Standard	ng/ml
A	0
B	0.3
C	1.6
D	6.4
E	15.9
F	31.8
G	63.6

i) 100 ul of the standard "A" was pipetted into the 1st two (non-coated) tubes.

ii) 100ul of the standards "B" through "G" were also pipetted (in duplicate) into the correspondingly labelled tubes.

iii) 100ul each of the serum samples to be analyzed was pipetted into the coated tubes provided.

iv) 100ul of buffered 125^{-I} progesterone (DPC, Los Angeles, CA 90045), was added to all the tubes.

v) The tubes were arranged on a rack and vortexed for about 2 seconds and subsequently incubated at room temperature for 3 hours, after which the supernatant was decanted in all the tubes except for the non coated tubes (TCT tubes)

vi) When all residual droplets of the supernatant were completely drained the progesterone concentration of the tubes were counted (12 tubes at a time) using a computerized gamma counter (NE 1612 Turbo, Nuclear Enterprises, England UK).

3.2.6 **Parasitological and Hematological Determination**

The blood samples collected in EDTA were used for the determination of packed cell volume (PCV) and the level of parasitemia. The PCV was determined by the microhaematocrit centrifuge technique (mHCT) (Woo, 1969). About 70ul of blood in a capillary tube was centrifuged tube in a microhaemotacrit centrifuge for about 3 minutes. The microhaematocrit reader (Gelman, Hawksly Ltd., England) was used to read the PCV values expressed in percent. The same capillary tubes were placed on a clear glass slide and the buffy coat-plasma junction examined using oil immersion objectives of the compound microscope. The parasitemia scores were ranked as follows:

1) + = Occasional trypanosome seen
2) ++ = 1 - 2 typanosomes
3) +++ = 3 - 20 typanosomes
4) ++++ = > 20 trypanosome seen (per buffy coat area) (Woo, 1969).

3.2.7 **Heat Detection**

The heifers were placed on close visual observation for signs of estrus for 7 days from the day of each PGF2alpha administration. Tail painting was used to enhance estrus detection (Kerr and mcCaughey, 1984). A strip of enamel paint (18-21cm long and 5-6cm wide) was applied on the rump of each animal anteriorly 12-15cm behind the level of the tuber coxae and extended posteriorly on either side of the midline. The paint was applied with a brush first against the direction of the hair and then along the direction of the hair to obtain a smooth surface. The internal genitalia were also examined per rectum. Changes in the tubular genitalia and in ovarian structures including follicular luteal growth and ovulation were recorded.

3.2.8 **Pregnancy Diagnosis**

All the six heifers were examined for pregnancy on the 45th day after the 2nd insemination. Pregnancy diagnosis was by rectal palpation. A gloved and lubricated hand was put into the rectum and the internal reproductive organs examined for pregnancy status as described by Zemjanis (1970). A retrospective result from progesterone assay 26days after the 2nd insemination was also used to confirm pregnancy status.

3.2.9 **Data Analysis**

The data collected was subjected to statistical analysis using the students t-test. A 95% confidence level was used and where probability is equal or greater than 5% the test was considered non-significant.

CHAPTER FOUR

4.0 RESULTS

4.1 Clinical Observations

All the infected heifers developed clinical trypanosomiasis. Three animals (animal No.1,4, and 5) developed chronic trypanosomiasis. One animal (No. 2) became moribund 44 days post infection and was salvaged. Animal No.1 had mild malaise during the early stages of infection, but there was no severe loss of condition.

4.2 Parasitemia

Trypanosomes were identified in the blood of infected heifers on day 4 (animal No.1 and 5) and day 5 (animals No. 2 and 4) post infection. The mean prepatent period (+SD) was 4.5 $\pm$ 0.6. The mean weekly parasitemia was highest in week 2(2.8 $\pm$ 0.8) and lowest in week 10 (1.3$\pm$0.8) over the 10 week investigation period. The parasitemia was fluctuating and upto 4 pluses (++++) on several occasions after the infection. Some periods of aparasitemia were also recorded. One heifer in the control group (animal No. 3) became parasitaemic on day 11 post infection. It was treated with Berenil(R) (Diminazene aceturate; 7mg/kg body weight) on the same day and subsequently remained aparasitaemic upto the end of the experiment. Trypanosomes were not found in the blood of the second control heifer (animal No.6). The mean weekly parasitemia in the infected heifers differed significantly ($P<0.001$) from the control. These parameters are summarized in Table 1, Figure 1, and Appendix 1.

4.2 Rectal Temperature

The infected heifers became febrile from the 4th day of infection. Rectal temperatures were fluctuating and the highest daily mean (± SD) of 40.6±0.8 occured on day 6 post-infection while the lowest was 38.3±0.4 on day 2 post-infection. The highest individual temperature was recorded in animal No.2 (42.3°c) on day 16 post-infection. The temperature range in the infected group was 42.3 to 38.2°c with a mean of 39.2± 0.7°c while the range in the control group was 38.1 to 38.70c with a mean of 38.4±0.2°c. During the 1st, 2nd, 3rd 4th, and 8th weeks of infection, the infected group had mean rectal temperatures that differed significantly (P<0.05) from the (uninfected) control which had normalmean rectal temperatures. There were no significant differences (P>0.05) in the mean rectal temperature on the 5th, 6th, 7th, 9th, and 10th weeks between the infected and control groups, although the mean values in the infected group was consistently higher and above the normal temperature values (Tables 2a and 2b). The overall mean rectal temperature in the infected heifers differed significantly (P<0.01) over the experimental period of 10 weeks. The variation in temperature is illustrated in Figs.2a and 2b. The individual rectal temperature values for the animals is presented in Appendix II.

4.3 **Packed Cell Volume (PCV)**

The daily mean (±SD) packed cell volume in the infected and control heifers ranged between 16.6± 2.1 and 31.0± 3.4, 26.5 ± 2.1 and 31.5± 2.8 respectively. The overall mean (±SD) PCV over the experimental period of 10 weeks was 22.5±2.3 in the infected and 29.3± 0.2. in the control group. A highly significant difference ($P<0.05$) was obaserved. The weekly PCV values in the infected group was lower and differed significantly ($P<0.05$) from the values in the control group. This occured from the 5th through the 10th week post-infection (Tables 3a and 3b, Figures 3a and 3b, Appendices IIIa and IIIb). The lowest individual PCV (14%) was recorded in one animal in the infected group (animal No. 4) by the 10th week of infection. Infected animal number 2 became moribund with a PCV of 17 by the 6th week and was subsequently withdrawn from the experiment. Infected animal No.1 showed a decreasing mean weekly PCV upto week 6 post-infection 17.5% after which it subsequently appreciated rising to 21.0% by the end of the experiment. The PCV, on one occasion (day 25), rose (33.0%) higher than the mean pre-infection value. It was also higher than the PCV of the controls. The mean post-infection PCV value (±SD) for animal No.1 (23.3±4.6) was, however, significantly different ($P<0.05$) from the pre-infection value (31.3±0.6). The PCV values of the (uninfected) control remained within normal range throughout the course of the experiment.

4.4 **Progesterone profile**

The mean serum progesterone levels in the infected and control heifers from the day of infection to day 50 post-infection are presented in Table 4a and Figure 4a while the individual animal progesterone profiles in both groups are presented in Table 4b and Figure 4b(1-6). The mean progesterone level in the control heifers was regular and the basal values between these peak values coincided with the clinical manifestation of heat. The mean serum progesterone level in the infected group became irregular and remained at basal levels after the 2nd dose of PGF2alpha until the end of the experiment (Fig.4a). Both the infected and control heifers exhibited the same mean basal values of progesterone (0.1ng/ml) at the second synchronized estrus. There was a significant difference ($P<0.05$) in the peak levels of progesterone after the second dose of PGF2alpha between the infected (0.6 ± 0.7ng/ml) and control (2.5 ± 0.5ng/ml) groups. The progesterone profile of the control group fluctuated and returned to basal levels and manifested clinical heat 20 days later (Post-synchronization or post-infection day 45), while the infected group remained at basal levels without manifestation of estrus until the end of the experiment.

Infected heifers No.2 and No. 4 and control heifer Nos.3 and 6 responded to the first dose of PGF2alpha (Table 5 and Fig. 4b:(2,4,3,6). The progesterone profile of infected heifer No.5 indicated estrus at the time of first PGF2alpha administration (Fig. 4b:5). The estrus was, however, not clinically observed. Infected animal No.1 also did not respond to the first PGF2alpha administration. All the six animals responded to the second dose of PGF2alpha on day 2 (heifer Nos.5 and 6), and day 3 (heifer Nos.1,2,3,4) post-infection. Progesterone levels remained low ($\leq$0.6ng/ml) and fluctuated in infected heifer No.1,2, and 4. Animal No.5 (Fig.4b:5) showed an elevated progesterone level (1.6ng/ml) subsequent to estrus, which declined to basal levels 10 days later and 13 days after the last heat without an accompanying estrus visually recognized. The progesterone level, however, remained low ($\leq$ 0.5ng/ml) and fluctuated upto the end of sampling.

4.5 Behavioral Estrus

The enamel paint used in heat detection did not yield good result. It was irritating to the animals which resulted in self-mutilation. The remnant also soon after caked up. Homosexual mounting behaviour was also found not to be of maximum benefit as an estrus detection tool. Although allocation to treatment groups was by random sampling, it happened that the control animals were lower in the peck order and thus shied away from intense bullying. The infected heifers were lethargic and had reduced libido (Table.6).

4.6 Conception Rate

None of the animals in the infected and control groups conceived (Table 5). There was a hundred percent correlation between the results of rectal palpation on day 50 after the second insemination and the progesterone profiles for pregnancy status. In general, all the infected heifers showed a basal progesterone profile indicative of an absence of pregnancy and acyclicity, while the control heifers cycled but were also not pregnant.

CHAPTER FIVE

DISCUSSION

The one hundred percent infection rate observed in this study is in agreement with previous reports (Ogwu, 1983;Esievo and Saror, 1983; Sekoni, *et al.*,1989). This shows that the Bunaji heifers used in this study were susceptible to the trypanosome strain (stabilate 146) used for the experimental infection. The pathogenicity was variable in the infected heifers. The acute, chronic, and mild disease observed in the infected heifers may be as a result of certain factors which include the slight degree of resistance of Bunaji to trypanosomiasis Leeflang, (1974). This degree of resistance is known to vary among individuals (Fiennes, 1970; Murray and Morrison, 1978; Stephens, 1986).

The fluctuating parasitemia recorded in this study is in agreement with previous reports (Ogwu, 1983; ILRAD, 1991), while the prepatent period of 4-5 days observed in this study lies within the range reported in an experimental mechanical infection by Sekoni *et al.*(1990) and in the cow by Dargie *et al.* (1979). Esievo and Saror (1983) reported a prepatent period of 3-4 days in 16 zebu bulls. Fluctuating parasitemias observed in this study may result from the combined effects of antigenic variation (ILRAD, 1991) and the host's defense mechanism (Doyle *et al.*, 1978). In the current study,

infected animal No.2 became recumbent by week 7 was the least able to control its parasitemia and had the highest weekly mean parasitemia of 4 pluses by the second and third weeks post-infection. The parasitemia subsequently remained high (3-3.5) from week three through week six, but decreased to a weekly mean of 0.7±1.2 by week seven. Murray and Morrison (1978) and Dargie *et al.* (1979) had observed that susceptibility to African animal trypanosomiasis may be influenced by the capacity of the host to limit, reduce, or control parasitemia. This, however, contrasts with the observation in this study where animal No.1, which had a mild disease, as measured by loss in physical condition and development of clinical anaemia with a mean weekly parasitemia range of 2.0.±0 to 3.0±1, from week one through week nine, and the least parasitemia (<1) by the tenth week. Innate resistance may be responsible for variation in suceptibity within host species and possibly be acting not only through limitation of parasitemia but also via an inherent ability to withstand the destructive effect of the parasites especially on the erythrocytes. Anosa (1988) had observed that toxic metabolites released by living and dead trypanosomes may be injurious to blood cells and blood cell precursors. These may also simply accumulate in the plasma without inducing any obvious ill- effects. These toxic factors may include neuraminidase (Esievo *et al.*, 1982). Animal No.2 showed the highest mean weekly parasitemia for four consecutive weeks before it finally became recumbent by the seventh week.

Parasitemia may be a potent factor in disease production (Anosa, 1988), but there are also situations in which severe pathological changes are observed even during aparasitaemic stages of the infection (Stephen, 1986) or in chronic infections when parasites appear sporadically and in small numbers. As such, the presence of a high level of parasitemia may not be considered as the major factor responsible for the severe reaction. Animal No.1 which showed a mild disease was able to control its parasitemia, and infact showed the least parasitemia score ($<\pm$ 1) by the end of the experiment (Fig.1). However, Saror (1980) reported that the onset and peak of parasitemia is directly related to the severity of anaemia. Stephens (1986) holds the view that parasitemia and degree of anaemia do not always correlate. The results of the current study have also indicated that the heifer that had the greatest ability to withstand the infection did not,however, show a commensurate ability to control parasitemia.

One of the animals in the control group (No.3) became accidentally infected, possibly mechanically through biting flies. Mechanical transmission especially in *Trypanosoma vivax* has been documented (Leeflang, 1978). This emphasizes the need for experimentally infected animals to be restricted to fly proof housing as was recommended by Sannusi (1988).

As expected, the results of this study have shown a fluctuating rectal temperature following infection with *T.vivax*. The onset of parasitemia corresponded with hyperthermia in all the infected animals (days 4 and 5 post-infection) and in the control heifer that was accidentally infected, (day 11,post-infection). This synchrony between parasitemia and hyperthemia was not maintained through-out the course of the infections. For example, animal No.1 showed a normal rectal temperature of 38.3°C on day 11 post-infection with a parasitemia score of 1 plus (+). There was, however, no case of hyperthermia devoid of parasitemia. Pyrexia has a deliterous effect on reproduction (Ogwu *et al.*, 1984).

The degree of anaemia, as measured by PCV in this study, became significantly different ($P<0.05$) from week five post-infection, although a marked drop in PCV started from the first week of infection and immediately after the onset of pyrexia. Similar changes to those observed in the present trial have been reported in the sheep by Adenowo (1981), and in cattle by Esievo *et al.*, (1982).

Animal No.4 which showed the lowest individual PCV value (14%) thrived while animal No.2 with a higher PCV value (17%) became moribound. Low PCV, and thus clinical anaemia, may not solely be responsible for this condition. Murray (1980) reported that a PCV of between 15 and 20% is unlikely to result in the death of an inactive cattle. Similar PCV values in ranging

cattle may lead to death (Leeflang *et al.*,1978). A combination of high parasitemia and pyrexia may have been responsible for this animal's inability to thrive at the PCV of 17%, since stress due to field grazing, poor nutrition and concurrent infections were markedly reduced during the experimental period. The near steady weekly mean PCV in the infected group (Fig.3b) from week two through week four was mainly attributed to hemoconcentration recorded in animal No.1 and the near stable PCV values recorded in the other infected animals within the period. Esievo *et al.* (1982) observed a transient elevated PCV two weeks post-infection and attributed it to a compensatory increase in the production of erythrocytes by the stem cells. The blood picture of the animal that showed hemoconcentration in the present study was not investigated to ascertain the type and nature of the predominant cells.

The effectiveness of PGF2alpha in the induction of estrus in this current study is in consonance with earlier reports (Adeyemo *et al.*, 1979; Voh Jr. et al., 1994).

The estrus response following the second dose of PGF2alpha was better synchronized than that following the first dose of PGF_2X . This agrees with the observation made by Adeyemo et al. (1979) and Cooper (1978) in healthy cows. One heifer not observed to be in estrus after the first treatment had basal progesterone levels from serum samples taken before the treatments. This most likely suggests that the corpus luteum was not at the luteal phase (Seguin, 1980). This

situation was confirmed on rectal palpation of the ovaries. The first and second PGF2alpha administration were carried out on the 11th and 22nd days post-infection, respectively. PGF_2alpha was in this sense adminstered early in the course of the disease, and therefore at a time when serious reproductive organ dysfunction may not have set in. Chronic infections were noted to be more likely to result in reproductive anomalies (Ogwu, 1983; Mutayoba, 1988; Sekoni, 1994).

Mean progesterone pulse amplitude was lower in the infected than the control heifers at the time of the two PGFalpha administration. However, this difference was not statistically significant. It is most likely that the early response to trypanosome infection is associated with a gradual decline in progesterone production and during the chronic stage this decline becomes marked. The early decline may be associated with changes in hematological and biochemical parameters (Anosa, 1988), and not organ damage. Oyejide and Adeyemo (1979) reported a gradual increase in progesterone level (for 3 weeks) in sheep infected with *Trypanosoma brucei* during the 13th week of pregnancy, which then sharply declined 7 weeks later culminating in abortion. In normal healthy sheep, pregnancy is associated with increasing progesterone level which stabilizes at a peak before a sharp decline culminating in parturition (Arthur *et al.*, 1982). The mean progesterone levels at the first synchronized estrus was lower in the control (0.1ng/ml) than the infected (0.5ng/ml). This

difference was not statistically significant (P>.0.05). The mean progesterone levels in both the infected and control groups during the second synchronized estrus following which the animals were artificially inseminated, was similar (0.1ng/ml) (Fig.4). The progesterone levels in all the animals at the second estrus was within the range compatible with conception (Eduvie and Dawuda,1989). High progesterone levels at estrus may result in infertility (Adeyemo and Heath, 1980). High progesterone levels would via a negative feedback mechanism restrict the release of gonadotrophins, which subsequently inhibits follicular growth and ovulation. (Hughes and Valley, 1980).

The fluctuating but progressive decline in progesterone levels recorded in this study with respect to animal Nos.1,3,and 4 may in part be explained by impaired ovarian steroidogenesis (Oyejide and Adeyemo, 1989), and lesions in the pituitary, thyroid, and adrenal glands (Mutayoba et al.,1988; Ogaa *et al.*, 1991; Ogwu *et al.*,1992) following trypanosome infections. The only infected animal (No.5) which showed a post-estrus elevation in progesterone level (1.6ng/ml) may have manifested short estrual cycle (13 days). Subsequently, the progesterone level remained basal. Short estrous cycle is a common feature of zebu cattle (Johnson and Gambo,1979; Zakari, 1981; Dawuda *et al.*, 1989). Short estrous cycles have been observed in trypanosome infected heifers Ogwu (1983). The short estrous cycles reported in this study were not confirmed to be

accompanied by behavioral signs of heat. This was so because at this period the animals were no longer being intensively observed for heat. The serum progesterone profiles in the estrous cycles of the control heifers were similar to some previous studies in normal uninfected zebu cattle (Adeyemo and Heath, 1980; Voh Jr. *et al.*,1988). Both heifers cycled normally and returned to estrus 20days later. These progesterone profiles cast no doubts as to the animals physiological disposition to settle to an effective service.

The intensity of estrus was highest in the uninfected heifers. For example, mounting activity was not observed in 75% of the infected animals (Tables 6). The uninfected group, on several occasions, attempted mounting each other and/or the infected group. The depressed behavioral estrus exhibited by the infected heifers suggests a possible impairement of or malfunctioning of the hypophysial-gonadal axis, central to the neuro-endocrine mechanism involved in the regulation of the reproductive process.

Several factors may have contributed to the lack of conception in the control animals. These may include:-

1) Lowered semen quality and faulty semen handling prior to insemination. The fertility of the male animal is a potent factor in the success of any artificial insemination programme (Sorenson 1979; Peters and Ball, 1986), so also is the care with which the semen was collected and processed before use. Zemjanis and Diegosanint (1963) traced 40% loss in conception rate to poor insemination and semen handling techniques.

2) In spite of the better synchrony in estrus afforded by PGF2alpha treatment (Orihuela *et al.*, 1982), fertility to synchronization and artificial insemination has been variable. Conception rates ranging from 18.6 - 53.9 % have been reported (Landiver *et al.*, 1984; Voh. Jr. *et al.*, 1989)

3) Another important factor which is believed to have contributed to this failure to conceive is the small sample size. This ultimately increased the margin of error. More animals in the control group would have increased the chances for conception. The reliability of the result of this study for field application is anchored on the behavioral estrus, good physical condition and a progesterone profile recorded for the infected animals which were compatible with conception.

CONCLUSION

This study is important in several ways

1) It shows conclusively that animals which may not become pregnant are forced to manifest heat by the strong luteolytic effect of PGF2alpha. Hematological and biochemical derangement in trypanosome infection may cause the cessation of cyclic ovarian activities even before serious pathologies are produced.
2) It is also a contribution to the existing literature on the efficacy of PGF2alpha in estrus synchronization in the zebu cattle.
3) Serum progesterone levels is depressed in trypanosome infection. The progesterone level remained basal after the 2nd induced estrus. This occured at about the 30th day post infection. It might be necessary to investigate how long after infection before progesterone levels would become basal in trypanosome infected, but unsynchronized heifers. This would reveal whether PGF2alpha administration enhanced the onset of depression of progesterone level.
4) Conception is most improbable in trypanosome infected heifers. The progesterone profile clearly revealed that even if conception took place, pregnancy might not be successful since progesterone is necessary for the survival of the embryo and maintenance of pregnancy. Exogenous progesterone may also not sustain pregnancy in

the presence of trypanosomiasis. Damage to the placenta caused by phospholipases released when trypanosomes are lysed (Ogwu, 1983), and transplacental transmission of trypanosomes may lead to abortion, premature births, and birth of weak calves (Boyd, 1971, Ogwu *et al* 1991).

A lot of work has been carried out on trypanosomiasis and of recent on the effect of trypanosomiasis on animal reproduction. To achieve better results, and an appreciable understanding of the pathogenesis of the neuro-endocrine dysfunction, a hollistic approach to the study of the effect of trypanasome infection on female reproduction is recommended. The gonadotropin (luteinzing hormone), estrogen and progesterone levels should be studied in synchrony so as to determine their interaction, and account for their probable difficiencies, excesses and impotence. There is also the need to improve on the efficiency of sample handling and storage prior to analysis for progesterone. This, also, would confirm the validity in the wide variation in progesterone levels reported by Oyedipe et al, (1988) and Eduvie and Oyedipe (1991) in the zebu cattle This would enhance the use of progesterone in the diagnosis of the reproductive status across the individual animals.

Finaly, estrus synchronization, as an aspect of controlled breeding in cattle, and as a tool in genetic improvement yields maximum benefits when artificial insemination is employed. The artificial insemination operator, therefore, in order to make the benefits of estrus synchronization realizable should endeavour to be as serious as the bull.

REFERENCES

1984.

Abalu, G.I.O./ The Green Revolution Strategy in Nigeria: Major economic problems and issues. Proceedings of National seminar, Dept. of Agric. Econs. and Rural Sociology, ABU. Zaria. Sept 21-24, 1981. Pp. 77-94.

Abdullahi, A. 1984. The problems and prospects of the Green Revolution for agricultural and rural development of Nigeria. Technical and environmental perspectives. Proceedings of a National Seminar, Dept. of Agric Econs and Rural Sociology, ABU., Zaria Sept. 21-24, 1981. Pp.1-12.

Abubakar, Y.U. 1995: Effects of experimental *T.congolense* infection on the oestrous cycle of West African Dwarf ewes. Faculty of Veterinary Medicine, Ahmadu Bello University, Staff and Postgraduate Seminar

Adenewo, T.K.O. 1989. effects of experimental *Trypanosoma vivax* infection on the oestrous cycle of mature Yankasa ewes. Ph.D Thesis ABU, Zaria, Nigeria.

Adeyemo, O.; Akpokodje, U.U. and Odili, P.I. 1979. Control of estrus in *Bos indicus and Bos taurus* heifers with prostaglandin F2alpha. Theriogenology 12(5):255-262.

Adelekan, O. and Adeyemo, O. 1989. The effect of *Trypanosoma brucei* infection on sheep in mid - pregnancy: Preliminary findings. Zariya Veterinarian (1):30-32.

Adeyemo, O and Heath, E. 1980. Plasma progesterone concentration in *Bos taurus* and *Bos indicus* heifers. Theriogenology, 14:411-419.

Agu, W.E. 1983. Animal trypanosomiasis in Nigeria ACMR.Proceedings Series No 4:55-60.

Agyemang, K.; Little, D.A. Mathioli; R. Sonko; E. and Janneh, H. 1993. Effects of trypanosome infection and postpartum liveweight change on resumption of reproductive activity in N'Dama cows. Theriogenology 39(4): 985-995.

Ahmed, M.I.; Osiyemi, T.I.O and Ardo, M.B. 1994. Prevelence of bovine trypanosome infections in Damboa LGA, Borno State, Nigeria. Journal Anim. Prod. No.21(1 and 2):186-187.

Akpokodje, J.U. and Bolarinwa, R.O.A. 1974. Reproductive efficiency of White Fulani cattle of Northern Nigeria in a Sourthern Environment. J. Nig. Vet Med Ass. 3(1):6-11.

Alberro, M. 1983. Comparative performance of F1 Friesian x zebu heifers in Ethiopia. Animal Production. 37:247-252.

Anderson, J. 1944. The periodicity and duration of oestrus in zebu and grade cattle. J. Agric Sci. 34:57-67.

Anene B.M.; Chime, A.B. and Anika, S.M. 1991. The production performance of imported Friesian cattle under heavy *Trypanosoma* challenge in a rain forest zone of Nigeria. Br. Vet. J. 147:275-282.

Anon, 1981. The Green Revolution: A livestock production plan for Nigeria: Interim report. 2(1):1-114.

Anon., 1986. The Merck Veterinary Mannual 6th edition. Pp.77-79. Merck and Co. Inc, USA.

Anosa, V. O. and Isoun, T.T. 1980. Haematological studies on *Trypanosoma vivax* infection of goats and intact and splenomectomized sheep. J. Comp Pathol 90:155-168.

Anosa, V.O. 1988. Haematological and Biochemical changes in human and animal trypanosomiasis. Revue. Elev. med-vet pays trop. 4192 : 151-164.

Anosa, V.O.; Ikede, B.O.; Agbede, R.I.S.; Ajayi, S.A.; Anika, S.M.; Aning, K.G.; Kalu, A.U.; Oyejide, A.; Ogunsusi, R.A.; Nantulya, V.M. 1991. Prevalence of trypanosomiasis in Nigeria Ruminants Paper presented at the ISCTRC meeting, Yamousoukro Cote D'vorie.

Arthur, G.H. Noakes, D.E. and Pearson, H. 1982. Veterinary Reproduction and Obstetrics 5th ed. Balliere Tindall, London.

Asa, C.S 1991. Synchronization and ovulation in Banteng (Bos javanicus) with prostaglandin F2alpha.Proceedings of the Society for Theriogenology. August 16-17.

Ashkar, T and Ochilo, M. 1972. The application of the indirect flourescent antibody test to samples of dried sera from cattle in the Lambwe valley, South Nyanza, Kenya. Bull. WHO,47:769-772.

Aufere, M.B. and Harriet, B. 1976. Progesterone: an overview and recent advances. Journal of Pharmaceutical Sciences 65:783-800.

Bajyana-songa, E; Hammers-Castermance, C; Hammers, R.; Pholpark, M.;Pholpark,S.; Leidl, K.; Tanchaitrong, S.; Chaichanopoonpol, I.; Vitoorakool, C. and Thirapataskum T. 1987. The use of a card agglutination test in detection of *T. evansi* infection. A comparison with other trypanosomiasis diagnostic tests under field conditons in Thailand. Am. soc. Belg. med Trop. 67:137-148.

Bawa E.K.; Oyedipe, E.O.; Sannusi, A. and Eduvie, L.O. 1991. Comparative clinical observations between infection at second and third trimester of pregnancy. NAPRI Report. ABU. Zaria, Nigeria.

Blowey, W. 1980 Prostaglandin past, present and future. Vet.Rec. 107(18):411.

Blocky, M.A. 1978. The influence of serving capacity of bulls on herd fertility. J. Anim Sci. 46:589-595.

Buck, N.G.; Light, D;, Rutherford, A.; Miller, M.; Prachett, T.W.; Tapper, B. and Trail, J.C.M 1976. Environmental factors affecting beef cow reproductive performance in Botswana. Anim Prod. 23:357-353.

Busch, W. and Furstenberg, L. 1984. The environmental influences upon the reproductive performance in cattle. 10th International Congress on Anim. Reprod. and AI 10-14 June, Illinois, USA. Paper No 132 Pp.3.

Broadway, J.L. 1973. Optimum time for artificial insemination of the bovine species at various times of the year. M.Sc Thesis, Texas A and M, Univ College Station, Texas, USA.

Bulman, D.C. and Lamming, G.E. 1978. Milk progesterone levels in relation to conception, repeat breeding, and factors influencing cyclicity in dairy cows. J.Reprod.Fert. 54: 447-458.

Buvenandran, V. 1979. A review of results of breeding for milk production in the tropics and application of these results to Nigerian condition. NAPRI Seminar 1:259-275.

Chandler, R.L. 1952. Comparative tolerance of West African N'dama cattle to trypanosomiasis. Ani. Trop Med, Parasit 46:127-134.

Chandler, R.L. 1958. Studies on the tolerance of N'dama cattle to trypanosomiasis. J. Comp. Pathol. 68:253-260.

Casida, L.E. 1961: Present status of the repeat breeder cow problem. Journ. Dairy Science. 44:2323-2329.

Choudhuri, G.; Banerjee, G.C. and Guha, H. 1984. Studies on the breeding efficiency traits and some of the factors affecting them in the Hariana type cows. Ind. Vet. J. 61:585-590.

Chenoweth, P.J. 1980. Libido and mating behaviour in bulls, boars and rams. A review. Theriogenology. 16:155-177.

and
Chhikara, B.S.;Bolarinwa, D.S./Chopra. S.C. 1979. A study on age at first calving in Zebu cattle. Anim. Breed. Abstr. 47:5988.

Christian, R.E. and Casida, L.E. 1948. The effects of progesterone in altering the estrous cycle of the cow. Journal of Animal Science 7:540-546.

Cooper, M.J. 1974. Control of estrous cycles of heifers with a synthetic prostaglandin analog. Vet. Res. 95:200-203.

Cooper, M.J. 1976. The use of cloprostenol (Estrumate) in the controlled breeding of cattle. An assessment of European field trials. In: Proceedings Symposuim on Estrus Synchronization in Cattle. ICI Australia Limited, Melbourne.

Dawuda, P.M.; Oyedipe, E.O.; Pathiraja, N. and Voh. Jr. A.A. 1987. Serum progesterone concentrations of indigenous postpartum Nigerian zebu cows. Brit. Vet. J. 144:253-257.

Dawuda, P.M.; Eduvie, L.O.; Esievo, K.A.N and Molokwu, E.C.I. 1989. Silent oestrus manifestation in Nigeria Bunaji zebu cows. Animal Reproduction Science 21:79-85.

Desai, M., 1989. Livestock production and diseases in the tropics. In: Livestock Production and Disease in the Tropics. H. Kuil.; R.W; Palling and J.E. Hahu (Eds) Pp.158

Desowitz, R.S. 1959. Studies on immunity and host-parasite relationships. *The immunological responses of resistant and* susceptible breeds of cattle to trypanosomal challenge Ann. Trop. Med. Parasit. 53: 293 - 313.

Diskin, M.G. 1982. Induction of parturition in cows using betamethasone. Vet Rec. 110:268.

Diskin, M.G. and Sreenam, J. 1980. Fertilization and embryonic mortality rates in beef heifers after artificial insemination J. Reprod. Fert. 59:463-468.

Dutt, R.H. and Casida, L.E. 1948. Alteration of the estrual cycle in sheep by the use of progesterone and its effects upon subsequent ovulation and fertility. Endocrinology. 43:208.

Doyle J.J. 1977. Antigenic variation in the salivarian trypanosomes. In:Immunity to Blood Parasites of Animal and Man. Miller, L.H.; Pino J.A.; McKelvey Jr. J. (Eds)., Plenum Press N/Y. Pp.2763.

Eduvie, L.O. and Oyedipe, E.O., 1991. Improving the management of reproduction in indigenous cattle in the semi-arid and subhumid zones of West Africa. Proceedings of International Symposium *on Nuclear Techniques in Animal Production and* Health. IAEA/FAO Vienna, 15-19 April. Pp.317-340.

FIO-Ngaindiro, G. 1987. Development of animal industry and improvement of animal health in the Central African Republic. Rev. Sci. et Techn. 6:955-967.

First, N.L. 1979. Mechanisms controlling parturition in farm animals. In:Animal Production H. Hawk (ed). Allenheld Osmum, Montclair, N.J. Pp.15-257.

First, N.L.; Lohse, J.K. and Nara, B.S. 1982. The endocrine control of parturition. In: Control of pig reproduction. (D.J.A Cole and G.R. Foxcroft, eds). Butterworths, London Pp.311-342.

FLD (Federal Livestock Department), 1992. National Livestock Population Estimates. Section II: National Overview: Environment and Livestock In: Nigerian Livestock Resources Inventory and Management Ltd. U.K. Pp.33.

Ford, J. 1964. The geographical distribution of trypanosome infection in African cattle population. Bulletin of Epizootic Disease of Africa. 12:307-320.

Foot, R.N. 1979. Time of artificial insemination and fertility in dairy cattle. J. Dairy Sci. 62:355.

Gasic, G.J. and Gasic, J.B. 1970. Total supression of pregnancy in mice by postcoital administration of neuramidase. Pro National Acad. Sci. 67:793-798.

Garcia, M. and Edqvist, I.E. 1988. Progesterone determination and clinical examination of reproductive organs in purebred and crossbred female zebu cattle. In: On the reproductive efficiency of pure and cross bred zebu cattle in the Amazon Basin of Peru. M.Garcia (ed), Uppsala, Sweden. Pp. 1-20.

Ginther, O.L.; Nuti. L.; Wentworth, B.C. and Tyler, W.J. 1974. Progesterone concentrations in Milk and blood during pregnancy in cows. Proc. soc. Exp. Biol. Med. 146:354.

Glover, P.E. 1961. The tsetse fly problem in Northern Nigeria. Patwa News Agency, Nairobi. Kenya. Pp. 383.

Gordon, I. 1976. Controlled breeding in cattle Part 1. Hormones in the regulation of reproduction, oestrus control and set time artificial insemination. Anim Breed. Abstr. 44:265-275.

Gray, A.R. and Roberts, C.J. 1971. The cyclical transmission of strains of *T.congolense* and *T.vivax* resistant to the normal therapeutic doses of trypanocidal drugs. Parasit. 63:67-89.

Griffin, L and Allonby, E.W. 1981. The susceptibility of young sheep and goats to an experimental *T.congolense* infection. Bulletin of Animal Health and Production in Africa. 29:49-53.

Gross, T.S.; William, W.F. and Moreland, T.W.1986. Treatment lowers retained placentae. Vet Med. 45:1011-1014.

Gyawu, P.; Kabuga, J.D.; Asare, ; K.; Karikan, P.K.; Appih, P.; Kwarteng, F.A. and Awunya. P.K. 1993. Estrus response of trypanotolerant N'dama cows after treatment with prostaglandin F2alpha analogue (cloprostenol) in the humid forest zone of Ghana. Anim Rep. Sci. 31:167-173.

Hardin, D.R.; Warnick, A.C.; Wise, T.H.; Schultz, R.H. and Fields, M.J. 1980. Arficial insemination of substropical cattle following synchronization with cloprostenol(ICI, 80996). I. Fertility. Theriogenology. 14:249-258.

Hafez, E.S.E. 1985. Artificial insemination In: Reproduction in Farm Animals. 5th Edition. ESE Hafez (Ed) Lea and Febiger, Philadelphia. Pp. 481-497.

Hafs, H.D.; Mans, J.C. and Drew. B. 1975. Onset of oestrus after PGF2alpha in dairy cattle. Vet. Rec. 96:134-135.

Hansel, W. and Beal, W.E., 1979. Ovulation control in cattle. In: Animal Reprodution (H.W. Hawk ed). BARC Bettsville Symp Agric. Res. Synposiuinz, Allenheld.

Hansel, W. and Convey, E.M. 1984. Physiology of the estrus cycle. Journal Anim Sci. 57 (supl.2):404-418.

Hinks, J. 1987. Breeding Dairy Cattle. Garden City Press Ltd. Lethworth, Great Britan.

Hofman, B.J.; Schmidt, J. and Schellenberger, E. 1979. Hormonal mechanisms involved in control of parturition in the cow. In: Calving Problems and Early Viability of Calf. B. Hoffman, I.L. Mason, J. Schmidt (eds). Martinus Nig hoff Publishers. The Hague.

Holmes, P. and Jennings, F.W. 1976. The effect of treatment on the anaemia of African trypanosomiasis. In: Soulsby E.J.(ed). Pathophysiology of parasitic infection. Academic Press, New York. Pp199-210

Hunter, R.H.F. 1982. Reproduction of Farm Animals. Longman, London.

Hughes, P.E. and Varley, M.A. 1980. The basis of reproduction. Reproduction in the Pig. Butterworth and Co. London Pp.5-13.

Ilemobade, A.A. 1972. Studies on the pathogenicity and incidence of *Trypanosoma evansi* in Nigeria. 1. The incidence of *T.evansi* in camels. Proc of 13th Meeting of International Scientific Council for Trypanosomiasis Research, Lagos, 1971 pub No. 105, OAU SRC Pp.107-114.

Ilemobade, A.A. 1979. Drug sensitivity of mouse infective *T.vivax* isolates in cattle and sheep. Proc. 16th Meeting of the International Scienfific Council for Trypanosomiasis Research and Control, Younde Cameroon, Eleza Services, Nairobi, OAU/STRC Publ. No. 111 Pp.251-253.

Ige, K. and Amodu A.A. 1975. Studies on the pathogenesis of trypanosomiasis for N'dama Inter. Sc. Council for Tryps. Res. Pub No. 109:151-156.

Ikede, B.O. and Losos, G.I-1975 Pathogenesis of *Trypanosoma brucei* infection in sheep. III. Hypophysial and other endocrine lesions. J. Com. Pathol. 85:37-44 Ikede, B.O. and Akpavie-1982.

Ikede, B.O. and Akpavie, S.O. 1982. Delay inresolution of trypanosome induced genital lesions in male rabbits infected with *Trypanosoma brucei* and treated with diminazene aceturate Res. Vet.Sci. 32:374-376.

ILRAD, 1987. Trypanosomiasis.ILRAD Annual Report. Pp.35

ILRAD, 1991. Trypanosomiasis, Annual Report. Pp.31-47.

ILRAD, 1992. ILRAD 1992 Annual Report.Pp.40.

Isoun, T.T. and Anosa, V.O. 1974. The effect of experimental infection of *Trypanosoma vivax* on the reproductive organs of sheep and goats - a preliminary report. Nig. Journ. Animal Prod. 1:143-150.

Isoun, T.T., Akpokodje, J.U. and Anosa, V.O. 1975. Testicular changes in a White Fulani zebu (Bunaji) cattle experimentally infected with *T.vivax*. A preliminary report. Journ. Nig. Vet Med. Assoc. 4:107-108.

Jainideen, M.R. and Hafez, ESE 1985. Reproductive failure in females. In: Reproduction in Farm Animals, 5th edition ESE , Hafez (ed) Pp. 399 - 422.Lea and Fabiger, Philadelphia, USA.

Johnson, C.T. 1981. Induction of parturition in cattle. In: Prostaglandins in Animal Prod. L.E. Edquistand H. Kindahl (Ed). Acta. Vet Scand. Suppl. 77:521.

Jones-Davies, W.J. 1967. Berenil resistant strain of *T.vivax* in cattle. Vet.Rec. 81:567-568.

Johnson, O.A. and Gambo, A. 1979. Estrus behaviour in Bunaji (White Fulani) heifers. J. Agric.Sci. 92:689-694.

Joshua, R.A. 1986. The prevalence of trypanosomiasis in cattle in low-lying zone of the Jos-Plateau, Nigeria. Bulletin of Animal Health and Production in Africa. 34:71-74.

Kidder, H.E. Barret, G.R and Casida, L.E. 1952. A study of ovulation in six families of Holstein-Freisians. J.Dairy Sci. 35:436-444.

King, G.J.; Hurmick, J.T. and Robertson, H.A. 1976. Ovarian function and estrus in dairy cows during early lactation. J. Anim. Sci. 42:688-692.

Kundson, P.B. and Soheal, A.S. 1970. The Vom herd: A study of the performance of a mixed Friesian-Zebu herd in a tropical environment. Trop. Agric. 47:189-203.

Landiver, C.; Galina, C.S.; Dachateau, A and Navarro-Fierro, R. 1985. Fertility trial in zebu cattle after a natural or controlled estrus with prostaglandin F2alpha, comparing natural mating with artificial insemination. Theriogenology. 23(3):421-427.

Lauderdale, J.W.; Seguin, B.E.; Stellflug, J.N.; Chenault, J.R.; Thatcherm, W.W.; Vincent, C.K.; Loyancano, A.E. 1980. Fertility following PGF2alpha injection. Journ. of Anim. Sci. 38(5):694-967.

Leeflang, P. 1974. Bovine trypanosomiasis and Livestock industry in Northern Nigeria. J. Nig. Vet Med. Assoc. 3(2):71-78.

Leeflang, P. 1975. The epizootics of trypanosomiasis in Tranch cattle (Shika Agric Research Station, Northern Nigeria) at some distance from known primary foci of savannah tsetse. Proc of the 14th. International Scientific Council for Trypanosomiasis Research and Control, Dakar, Senegal. Pp.94-100.

Leeflang, P.; Ige, K. and Olatunde, D.S. 1976. Studies on *Trypanosoma vivax*: The infectivity of cyclically and mechanically transmitted ruminant infections for mice and rats. International Journal of Parasit. 6:453-456.

Leeflang, P. 1978. Bovine trypanosomiasis in Northern Nigeria. A contribution to the epidemiology, host specificity and drug sensitivity of *Trypanosoma vivax*. Utretch. Pp 9-10.

Leeflang, P.; Buys J. and Blotkamp, C.1978. Studies on *Trypanosoma* vivax. Comparison of parasitological diagnostic methods. International Journal of Parasitology. 8:15-18.

Llewelyn, C.A.; Luckins, A.G.; Munro, C.D. and Perrie, J. 1987. The effects of *T. congolense* infection on the estrus cycle of the goat. Br. Vet.. 134:423-431.

Llewelyn C.A.; Munro, C.D.; Luckins, A.G.; Jordt. T.; Murray, M.; Lorenzini, E. 1988. The effects of *T. congolense* infection on the estrous cycle of the Boran cow. Brit. Vet. Journal. 144:379-387.

Logue, D.N; Salaheddine, M. and Reuton, J.P. 1991. A comparison of two techniques for the synchronization of estrus in dairy heifers. Vet. Rec. 129(8):171-173.

Loke, Y.W. 1982. Transmission of parasites across the placentae. Advances in Parasit. 31:155-228.

Losos, G.J. 1986. Trypanosomiases. In: Infectious Tropical Disease of Domestic Animals. 1st edition. Longman Harlow. Pp.183-263.

Lorenzini, E; Scott, J.R.; Palling, T. and Jordt T. 1988. The effect of *T. Congolense*, infection on the reproductive cycle of N'dama and boran heifers. In: ILCA/ILRAD. The African Trypanotolerant Livestock Network.Pp. 168-172.

Luckins. A.G. and Mehlitz, D. 1978. Evaluation of an indirect flourescent antibody test, enzyme linked immunosorbent asay and quantification of immunolglobulins in the diagnosis of bovine trypanosomiasis. Trop. Ani. Health and Prod. 10:149-159.

Luckins A.G. Boid, R.P. Mahmoud. M.M.; Malik, K.N. and Gray, A.R. 1979. Serodiagnosis of infection with *T. evansi* in camels in the Sudan. Tropical Animal Health and Prod. 11:1-12.

Luckins, A.G.; Llewelyn, C.A.; Munro, C.D. and Murray, M. 1993. Effects of pathogenic trypanosomes on mamallian reproductive system. Nuclear and related techniques in Animal Prod. and Health. Proc. Inter. Atomic Energy Agency and FAO. Vienna, Austria. 1986. Pp.351-353.

Luckins A.G. 1976. The immune response of zebu cattle to infection with *trypanosoma congolense* and *I. vivax*. *Ann. Trop Med. Parasit.* 70:133-145.

Maclennan, K.J.R. 1963. Cattle trypanosomiasis in Northern Nigeria. The problem in field. Bull. Epizoot. Dis. Africa. 11:381-390.

Maclennan, K.J.R. and Jone-Davies, W.J. 1967. The occurence of Berenil resistant *Trypanosoma congolense* strain in North Nigeria. Vet.Rec. 80:389-390.

Maclennan. K.J.R. 1970. The epizootiology of trypanosomiasis in livestock in W. Africa. In: The Africa Trypanosomiases. H.W. Mullingan (ed). Allen and Unwin London. Pp.751-765.

Maikaje, D.B. 1985. The susceptibility of Uda sheep to experimental *Trypanosoma vivax* infection.Ph.D.Thesis ABU, Zaria.

Mamo, E. and Holmes, P.K. 1975. The erythrokinetics of zebu cattle chronically infected with *Trypanosoma congolense*. Res. Vet Sci. 18:105-106.

Masake, R.A. 1980. The pathogenesis of infection with *Trypanosoma vivax* in goats and cattle. Vet. Res. 107:551.557.

Mather, E.C.; Camper, P.M.; Vahdat, F.; Whitmore, H.L. and Gustafsson, B.G. 1978. Assessment of ovarian activity in the postpartum dairycow by use of a milk progesterone assay. Theriogen. 10:119-129.

Mackenzie, P.K.I.; Boyt W.P.; Nesham, V.W. Pirue, E. 1978. The aetiology and significance of the phagocytosis of erythrocytes and leucocytes in sheep infected with *T.congolense* (Broden, 1904). Res. Vet. Sci. 24:4-7.

McDonnel, W.E.; Edgerton L.A. Olds, D. Harned, F.D. 1977. Clitoral stimulation and non-return rate in the bovine J. Dairy Sci. Suppl 1:85 (Abstr).

Miranda, J.J.F.; de, Gasrnerio, G.G.; Fonseca, C.G. and Bergman, J.A.G. 1982. Environmental and genetic factors affecting calving interval in Nellore cows. ABA. 51:184.

Meyers, L.J.; Nicholas, H.B. and Leslite, E.M. 1977. Prostaglandins. Vet. Pharm and Therapeutics, 4th edition. The Iowa State Univ. Press. Pp.459-460.

Mohammed, G. 1991. The comparative pathogenicities of genetically defined trypanosomes of the subgenus Nannomonas, with special reference to a new species, Ph.D. Thesis, University of Bristol, U.K.

Molyneux, D.H. and Ashford, R.W. 1983. The biology of *Trypanosoma* and *Leishmania* parasites of man and domestic animals. Taylor and Francis LTD. London Pp. 71-74.

Motlick, J.; Parlock, A and Fulks, J. 1976. Journal of Reproduction and Fertility. 47:8-7.

Morris, J.G. 1974. Animal Agriculture. The biology of domestic animals and their use by man. H.H. Cole and M. Ronning (eds), Freeman and Co, USA.

Morrison, W.I.; Murray, M. and Sayer, P.D. 1978. Pathogenesis of tissue lesions in *T.brucei* infection. In: pathogenicity of trypanosomes, Losos G.J. and A. Chouinard (eds). Proc. Workshop Nairobi, Kenya, 20-23 Nov. 1978. Pp.171-177.

Morrison, W.I.; Murray, M. and McIntyre, I. 1981. Bovine trypanosomiasis. FAO/SIDA Followup Seminar on Vet. Pathology. Faculty of Vet. Med., University of Nairobi, 7th-27th Sept.

Mukasa-Mugerwa, E., 1989. A review of reproductive performance of female *Bos indicus* (zebu) cattle. ILCA Monograph No.6, Addis Ababa Ethiopia.

Muller, H.E and Saathoff, M. 1972. The pathogenic role of Trichomonas foetus neuraminidase. ZBL Bakt. Hyg. 222:275-279.

Murray, M. Morrison, W.I. 1978. Parasitaemia and lost susceptibility to African trypanosomiasis In: Logos G, and Cheuinard,A (eds) Proc. Workshop Nairobi Kenya, Nov. 20-23 Pp. 71-81.

Murray, M.; Morrison, W.I. and White-Law, D.A. 1982. Host susceptibility to African trypanosomiasis: Trypanotolerance. Adv. Parasit. 21:1-68.

Mwambu, P.M. 1983. Clinical and pathological findings in experimental *Trypanosoma congolense, Trypanosoma vivax* infection in cattle. 17th Meeting of the International Scientific Council for Trypanosomiasis Research and Control. Arusha, Tanzania. 19-24 Oct. 1981. Pp.17-224.

Mwangala, G.N.;Kovatch, R.M. and Fazil, M.A. 1981. Acute *T. vivax* infection in dairy cattle in coast province, Kenya. Tropical Animal Health and Production. 13.63-69.

Mutayoba, B.M.;Eckersall, P.D.; Jeffcoate I.A.; Gestnicks, V. and Holmes, P.H. 1994. Effects of *Trypanosoma congolense* infection in rams on the pulsatile secretion of LH and testosterone and responses to injection on GnRH. Journal Reprod. Fert. 102:425-431.

Na'isa, B.K. 1967. Follow up of a surveyon the prevalence of homiduim resistant strains of trypanosomes in cattle in North Nigeria and drug cross-resistance tests on the strains with Samorin and Berenil. Bull Epizoot.Dis. Afri. 15:231-241.

Nancarrow, C.D. and Radford, H.M. 1976. The endocrine basis of synchronization techniques. Proc. Symposium on Estrus Synchronization in Cattle, ICI LTD. Australia.

Naylor, D.C. 1971. The heamatologyand histopathology of *Trypanosoma congolense* infection in cattle. Trop. Anim. Health and Prod. 3:95-100.

Nagaratnam, V.; Sooriyamoorthy, T.; Oyedipe, E.O. and Zakari, A.Y 1983. Synchronization of estrus with cloprostenol and subsequent calving rates inartificially inseminated zebu heifers. Br. Vet. J. 139-440-4.

NITR (National Institute for Trypanosomiasis Research). 1964. The incidence of *Trypanosoma theileri* in cattle at Vom. In: Annual Report, 1964. Nigeria Institute for Trypanosomiasis Research, Kaduna, Pp.22-23.

Noakes, D. 1986. Fertility and obstetrics in cattle. Blackwell Scientific Publishers. Oxford, London.

Nuru, S. and Dennis, S.M 1976. Abortion and reproductive performance of cattle in Northern Nigeria. Trop. Anim. Hlth and Prod. 8:213-219.

Ogaar, J.S.; Kamar, K.K.; Gombe, S. and Njoku, A.R. 1991. Abortion in goats following experimental infection with *Trypanosoma congolense*. 21st Metting of the International Scientific Council for T;rypanosomiasis Research and Control. October 21-25, Yamonssonkro.

Ogwu, D. and Nuru, S. 1981. Trasplancental transmision of trypanosomes in animals and man: A review. Vet. Bull. 51:381-383.

Ogwu, D. 1983. Effects of *Trypanosoma vivax* infection on female reproduction in cattle. Ph.D Thesis, ABU Zaria.

Ogwu, D.; Njoku, C.O.; Osori. D.I.K.; Ezeokoli, C.D. and Kumi-Diaka, J. 1984. Effect of experimental *Trypanosoma vivax* on fertility of heifers. Theriogenology. 22:625-633.

Ogwu, D.and Njoku, C.O.; 1991. Genital lesions inexperimental *T. congolense* infection in heifers. Anim. Reprod. Sci. 26(1-2):1.

Ogwu, D.; Njoku, C.O. and Ogbogu, V.C. 1992. Adrenal and thyroid dysfunction in experimental *Trypanosoma congolense* infection in cattle. Vet Parasit. 42:15-26.

Olson, J.D. 1980. The use of PGF2alpha as a replacement for heat detection in dairy cattle. Proc. Soc for Therio. Fall Conf. 180-196.

Olson, J.D.; Less Ball; Oetzel, G.R and Mortimer, R.G. 1987. Retained foetal membranes. Journal of the Soc. for Therio. 14:56-63.

Olutogun, O. 1976. Reproductive performance and growth of N'dama and Keteku cattle under ranching conditions in the Guinea Savanna of Nigeria. Ph.D Thesis, University of Ibadan, Nigeria.

Orihuela, A.; Galina, C.; Escobar, J. and Requelme, E. 1983. Estrus behaviour following prostaglandin F2alpha injection in zebu cattle under continuous observation. Therio. 19 (6): 795-809.

Ooijen, C.J.P.G. 1993. Improving the diagnosis and control of trypanosomiasis and other vector-borne diseases of African livestock using immunoassay methods. IAEA-TECHDOC-707 June.

Ooijen, C.J.P.G. 1993. Improving the diagnosis and control of trypanosomiasis and other vector-borne diseases of African livestock using immunoassay methods. IAEA-TECHDOC-707 June.

Osori, D.I.K. 1976. Seasonal variations in reproductive activity of indigenous cattle of Northern Nigeria. Ph.D. Thesis, ABU, Zaria, Nigeria.

Oschmann, S. 1986. The FAO/IAEA Progesterone solid phase Radioimmunoassay Kit IAEA Viehna Pp. 1-3.

Otchere, E.O. 1986. Traditional cattle production in the subhumid zone of Nigeria. In: livestock systems research in Nigerias' subhumid zone. Von Kaufmann, R.; Chaters, S. and Blanch, R. (Eds). ILCA, Addis Ababa, 110-140.

Oyedipe, E.O.; Osori, D.I.K.; Jagun, A.C.; Adunde, B. and Eduvie, L.O. 1980. Postpartum uterine involution and resumption of ovarian activity in White Fulani (Bunaji) and Friesian x Bunaji cows as determined by rectal palpation. Nigeria J. Anim. Prod. 7(2): 112-116.

Oyedipe, E.O.; Buvanendran. V. and Eduvie L.O. 1982. Some factors affecting the reproductive performance of the White Fulani (Bunaji) cattle. Tropical Agriculture. 59: 231-234.

Oyedipe, E.O.; Voh Jr. A.A.; Pathiraja, N.; Dawuda, P.M.; Rekwot P.I.; Bawa, E.K.; Adenowo, T.K.; Opaluwa, G.I.; Ojo, E.G. and Buvanendran, V. 1986. Report of intensive cattle breeding programme for 1984 NAPRI (MISC publications).

Oyedipe, E.O.; Pathiraja, N.; Voh Jr. A.A.; Buvanendran, V. 1988. Use of plasma progesterone profiles for the assessment of reproductive functions in indigenous Nigerian zebu cattle Therio. 30: 629-641.

Oyejide, A. and Adeyemo, O. 1989. The effect of Trypanosoma brucei infection on sheep in mid-preganancy: Preliminary finding. Zariya Vet. 4: NO.1.

Oyenuga, V.A. 1967. Agriculture in Nigeria, FAO, Rome, pp 308.

Ozser, S.; Guven B,; Izgur, H. Emre Z. and Cosar, S. 1986. Some managerial factors affecting the reproductive performance of diary cows in Turkey. In: Nuclear and Related Techniques in Animal Prod. and Hlth. Proc. sysmp vienna 17-21 March, 1986 pp. 621-622.

Parris, J.; Murray, M. and McoDimba, F. 1982. A comparative evaluation of the parasitological techniques, currently available for the diagnosis of African trypanosomiases in cattle. Acta. Trop. 39: 307-316.

Robbins, R.K., Sullivan, J.J., Pace, M.M., Elliot, F.I., Bartlett, D.E. and Press, P.J. 1978. Timing of insemination of beef cattle. Therio. 10:247.

Robinson, J. and Ashkar, T.S. 1972. Trypanosomiasis in domestic livestock in the lambwe valley area and field evaluation of various diagnostic techniques. Bull WHO. 47: 727-734.

Rodes, F., Galina, C.S., Duchataeu, A. and Soto, C. 1985. Investigations into the properties of bovine semen in the Mexican tropcis. World Rev. of Anim. Prod. xxi (2).

Roche, J.F. 1974. Attempts to determine the optimal time of artifical insemination to heifers. J. Reprod. Fert. 41: 223-225.

Roche, J.F., Ireland, J. and Mawhinney, S. 1981. Control and induction of ovulation in cattle J. Fert. Reprod. (supl. 30) 211.

Rollinson, D.H.L., 1963. Reproduction habbits and fertility of indigenous cattle to artificial insemination in Uganda. J. Agric Sci. 60:279.

Rosenberg, M., Herz, Z., Davidson. M. and Folman, T. 1977. Seasonal Variation in postpartum progesterone levels and conception in primiparous and multiparous diary cows. J. Reprod. Fert. 51: 363-367.

Sabino, L., Montori, D., Maurique N., Batistini J. and Garcia E. 1981. Reproductive performance in a herd of Gir and Zebu cows in Venezuela II. Age at first calving. Mem. Ass. Lat. de. Prod. Anim. 16:120

Saunders, R.W. 1980. Prostaglandins, past, present and future. Vet Rec. 107/18:411.

Sannusi, A. 1988. Some suggested guidelines on experiments with pathogenic trypanososmes in Nigeria with reference to Trypansoma vivax Vet. Med seminar, ABU., Zaria 10th Feb.

and

Saror, D.I. Ilemobade, A.A., Nuru, S. 1979. The heamatology of N'dama and Zebu cattle experimentally infected with *Trypanosoma vivax* In: 16th ISCTRC Conference meeting, Younde, Cameroon, Nov. 1979.

Saror, D.I., Ilemobade, A.A. and Nuru, S. 1981. The haematology of N'dama and zebu cattle experimentally infected with T. vivax. In: International Scientific Council for Trypanosomiasis Research and Control, 16th meeting, Yaounde, Cameroon, 1979, OAU/STRC No. 111.

Sekoni, V.O. Saror, D.I., Njoku, C.O., Kumi Diaka, J. Opaluwa, G.I. 1990a. Comparative haematological changes following T.*vivax* and T. *Congolense* infections in the zebu bulls. Vet. Parasit. 35:11-19.

Sekoni, V.O. Kumi-Diaka, J. Saror, D.I. and Njoku, C.O. 1988. The effect of *Trypanosoma vivax* and *Trypanosoma congolense* infection on the reaction time and semen characteristics in the zebu bull. Br. Vet. J.

Sekoni, V.O., Njoku, C.O., Kumi-Diaka, J. and Saror, D.I., 1990b. Pathological changes in male genitalia of cattle infected with *Trypanosoma vivax* and *Trypanosoma congolense*. Br. Vet. J. 146:181-185.

Sekoni, V.O. 1993. Elavated sperm morphological abnormalities of Yankasa rams consequent to *Trypanosoma vivax* infection. Anim Reprod. Sci. 31:242-248.

Sekoni, V.O. 1994. Reproductive disorders caused by animal trypanosomiasis. A review. Therio. 42:557-570.

Seguin, B.E., 1980. Role of prostaglandin in bovine reproduction. Journal Anim. Vet. Med. Assoc. 176:1178-1181.

Seguin, B.E. 1981. Use of prostaglandin in cows with unobserved estrus. Acta Veterinaire Scadinavica supply. 77:343-352.

Schultz, R.H. 1987. Synchronization of ovulation/estrus. A: Prostagladin/Prostaglandin analogues and GnRH J. Soc Therio 14:104-113.

Sileghem, M., Flynn, J.N., Logan-Henfrey, L, and Ellis, J. 1994. Tumor necrosis factor production by monocytes from cattle infected with *T. vivax* and *T. congolense:* possible association with severity of anemia associated with the disease. Parasit. Immunol. 16(1)51-54.

Smith, M.F. Nix, K.J. Karaemer, D.C., Amosa, M.S. Herron, M.A.and Wiltank, J.N., 1982. Journal. Animal Sci. 54:1005.

Smith R.D., Pomerantz, A.J., Beal, W.E., Mccann, J.P., Pilbeam, T.E.and Hansel, W, 1983. Insemination of Holstein heifers at a present time after estrous cycle synchronization with progesterone and PGF2alpha.J. Anim. Sci.

Sorensen, Jr. A.M. 1979. Animal Reproduction Principles and practices McGraw-Hill Book Co. NY. Pp.292-354.

Stephens, L.E. 1966. Pig trypanosomiasis in Africa. Comm. Wealth Agric Bureaux. England. Pp.5

Stephens, L.E. 1970. Clinical manifestation of the trypanosomiasis in livestock and other domestic animals In: H.W. Mulligan (Ed). The African Trypanosomiases. George Allen and Unwin Ltd. London Pp.775-792.

Stephens, L.E. 1986. Trypanosomiasis: A veterinary perspective. Pergamon Press, Oxford, New York.

Steveson, J.S., Schmidt, M.K., Call, E.P. 1984. Stage of estrous cycle, time of insemination and seasonal effects on estrous and fertility of Holstein heifers after PGF2alpha J. Dairy Sci. 67:1798-1805.

Sullivan, J.J., Barlett, D.E., Elliot, F.I., Bronwer, J.R., Kloch, F.B. 1972. A comparison of rectovaginal, vaginal and speculum approaches for insemination of cows and heifers AI Digest 20:6-8.

Szabunie, Wicz, M. and Macrady, J.D. 1977. Histamine Serotonin, Kinins Prostaglandins and allergy - Anaphylaxis phenomena. In: Vet pharmacology and therapeutics, 4th edn Meyers, J. Nicholas H.B. Leslie E.M. (ed) Ames, IOWA.

Tanabe T.Y. and Hann, R.C. 1984. Synchronized estrus and subsequent conception in dairy heifers treated with PGF2alpha In: influence of stage of cycle at treatment. J. Anim. Sci. 55:805-811.

Tizard, I.R., Holmes, W.L., York, D.A. and Mellors, A. 1977. The generation and identification of the hemolysin of *Trypanosoma congolense*. Expt. 33:961-962.

Turman, E.J. Wettenmann, R.P. Rich., T.D., Lyons, D. Sharp, W.E and Frahm, R.R. 1975. Oklahoma Agric Exp. St. Anim. Sci. Res. Pp.176-185.

and

Urquhart, G.M. Armour, J. Duncan, J.L. Dunn, A.M./Jennings, F.W., 1987. Vet. Parasit Longman, UK. Pp. 206.

Uzoigwe, R.N. 1984. Immunity engendered through self cure in zebu calves experimentally infected with *T.vivax*. M.Sc Thesis, ABU. Zaria. Pp.29-45.

Vaccaro, L. 1973. Some aspects of theperformance of purebred and cross-bred dairy cattle in the tropics. part 1. Reproductive efficiency in the females. Anim. Breeding abst. Vol. 41.

Vivanco, W, and Gutierrez, O. 1980. Use of PGF2alpha in estrus synchronization and its effect on conception rate of dairy cows in the peruvian coast under stabilated conditions. International Congress on Animal Reproduction and AI Madrid, Spain.

Voh. A.A. (Jr.) Oyedipe, E.O. Buvanendran, V. and Kumi-diaka, J., 1987a. Estrus response of indigenous Nigerian zebu cattle after PGF2alpha treatment under conditinuous observation for two seasons. Therio. 28:77-99.

Voh, A.A. (Jr.); Oyedipe, E.O.; Pathiraja, N.; Buvanendran, V. and Kumi-Diaka, J. 1987b. Peripheral plasma levels of progesterone in Nigeria zebu cows following synchronization of estrus with prostaglandin F2alpha. Br. Vet. J. 143:254-263.

Voh, A.A. (Jr.), Ogwu, D. Oyedipe, E.O. Kumi-Diaka, J. and Buvanendran, V., 1988a Effect of intertreatment interval and of stage of the estrus cycle on estrus response of Nigerian zebu cows to prostaglandin F2alpha. Paper presented at NAPRI Seminar, Nigeria.

Voh, A.A. (Jr.); Oyedipe, E.O. Ogwu, D. 1988b. Control of estrus and AI in zebu cattle in Nigeria. Paper presented at joinc IFS/Swedish International Programme on Animal Reprod. Workshop, ILCA, Addis Ababa, Ethiopia, 17th-30th Oct. 1988.

Voh, A.A. (Jr.), 1990. A decade of practical AI service at NAPRI, (1979-1989) In: Application of Animal Reprod. and AI Techniques to Livestock Production in Nigeria, L.O. Eduvie, V.O. Sekoni, A.A. Voh, Jr. J.O. Bale, A.G. Jagun (Ed) ABU., Press, Zaria.

and

Voh, A.A. (Jr), Ogwu, D.I./Oyedipe, E.O., 1994. Determination of optimum Anie for fixed time artificial insemination of zebu cows following synchronization of oestrus with prostaglandin 2alpha. NAPRI Seminar ABU, Zaria, April 1994 15Pp.

Vohradsky, F. 1971. Clinical signs daily rate of infection, physical changes of the blood and pathomorphological changes in cattle artificially infected by **T.** *vivax*. Revue d'Elevage et de medicine veterinaire despays Tropicaux 24:251-263.

Vahdat, F, Hurtgen, J.P. Whitmore, H.L. Seguin, B.E. Johnson, S.D., 1981. Decline in assayable Progesterone in bovine plasma. Effect of time, temperature, anticoagulant and presence of bloodcell. Ani. J. Vet. Res. 42:521-522.

Wagenaar, K.T. Diallo, A. and Sayers A.R. 1986. Productivity of transhumant Fulani cattle in the inner Niger Delta of Mali. ILCA Research Report 13. ILCA Addis Ababa, Ethiopia 55Pp.

and

Waindi, E.N. Gombe, S./Odour-Okelo D. 1986. Plasma testosterone in Toggerburg goats, Achieves of Andrology 17:9-17.

and
Watson, E.E./Macdonald, B.J. 1984. Failure of conception in dairy cattle progesterone and oestradial 17B concentrations and the presence of ovarian follicles in relation to the timing of artificial insemination. Br. Vet. J. 140-398.

and
Weinde, B.T. Hockmeyer, W.T. Kontech, R.M. Bhojhal, M.S./Digs, C.L., 1981. *Trypanosoma congolense:* Natural and acquired resistance inthe bovine. Experimental Parasitology 52:219-323.

and
Wheat, J.D. Deleeuw, P.N./Kock B.A. 1972. Bunaji cattle at Shika Research Station, North Central State, Nigeria. Samaru Miscellaneous Paper No.41. ABU, Zaria, Nigeria.

and
Whitmore, H.L./Mather, E.C. 1997. Don't depend on estrous signs other than standing heat. Hoards Dairyman, 122:1287.

WHO/FAO. 1976. The African Trypanosomiasis Report of a joint WHO expert comm. and FAO expert consultation. Rome Nov. 8-12.

and
Williamson, N.B. Morris, R.S. Blood, D.C. /Cannon, C.M., 1972. A study of estrus behavious and estrus detection methods in a large commercial dairy herd 1. The relative efficiency of methods of detection. Vet. Rec. 91:50-58.

Williamson, J. 1970. Suramin complexes 1. Prophylatic activity against Ann. Trop Med. Parasitol 51:440-456.

Wilson, A.J. 1969. Value of the indirect flourescent antibody test as a serological aid to diagnosis of Glossina transmitted bovine trypanosomes. Trop Anim. Health and Prod. 1:89-93.

and
Wittbank, J.N. Kasson, C.W./Ingasils, J.E., 1969. Puberty in crossbred and straightbred beef heifers on two levels of feed. Journal Anim. Sci. 29:602-605.

Woo, P.T.K. 1969. The haematorit centrifuge technique for the detection of trypanosomes in blood. Canadian Journal of Zoo. 47. 921-923.,

Zakari, A.Y. 1981. Manifestation and detection of estrus in Bunaji and Bokoloji cows. Ph.D. thesis ABU., Zaria, Nigeria.

Zemjanis, R. and Degosanint, E. 1963. Fertility of cattle in Columbia, ABA 32:1008.

Zemjanis, R. 1970. Pregnancy examination. In: Diagnostic and therapeutic techniques in animal reproduction (2nd ed). The Williams and Wilkins Co. Paltimore, Pp.29-46.

463166

KASHIM IBRAHIM LIBRARY
RECEIVED

LIST OF TABLES

Table 1. Mean weekly parasitaemia in T. vivax infected heifers.

WEEKs POST INFECTION	EXPERIMENTAL ANIMALS NO.1	No.2	No.4	No.5
1	2.0	1.4	1.4	2.1
2	2.3	4.0	2.3	2.7
3	2.3	4.0	2.7	1.7
4	2.0	3.3	2.7	1.3
5	2.0	3.0	1.3	2.0
6	2.0	3.5	1.5	2.0
7	3.0	0.7	2.7	2.0
8	3.0	0.7	2.7	2.0
9	2.0	-	1.0	3.0
10	0.5	-	2.0	1.5

Table 2a. Mean (± SD) weekly rectal temperature (°C) in T. vivax infected and control heifers.

	Infected				Control	
Weeks Post-infection	No.1	NO.2	NO.4	NO.5	NO.3	NO.6
1	39.0	39.1	39.2	39.2	38.5	38.4
2	39.6	39.5	39.3	39.5	38.3	38.4
3	39.1	39.8	39.7	39.8	38.3	38.4
4	39.6	39.2	39.0	38.8	38.3	38.5
5	38.9	39.0	39.6	38.3	38.5	38.4
6	38.8	39.2	39.0	38.7	38.3	38.4
7	38.9	39.1	39.0	38.6	38.4	38.3
8	39.0	-	39.3	38.8	38.4	38.5
9	39.7	-	38.9	38.6	38.3	38.3
10	39.3	-	38.7	38.7	38.3	38.4

Table 2b. Mean of weekly mean (± SD) rectal temperature in T. vivax infected and control heifers.

Weeks Post-infection	Infected	Control
1	39.2±0.1	38.5±0.1
2	39.5±0.1	38.6±0.1
3	39.6±0.3	38.9±0.1
4	39.2±0.3	38.4±0.1
5	38.9±0.5	38.5±0.1
6	38.9±0.3	38.4±0.1
7	38.8±0.2	38.4±0.1
8	39.0±0.3	38.4±0.1
9	39.1±0.6	38.3±0.1
10	38.7±0.4	38.4±0.1

Table 3a. Mean weekly PCV (%) in T. vivax infected and control heifers.

Weeks	Infected				Control	
Post-infection	NO.1	NO.2	NO.4	NO.5	NO.3	NO.6
1	27.7	28.9	26.9	39.9	30.0	29.4
2	27.3	22.3	23.7	26.7	27.3	28.0
3	21.0	20.0	21.0	28.0	30.3	27.0
4	31.0	21.0	18.7	22.3	31.0	29.7
5	18.0	19.0	17.0	20.7	30.0	29.3
6	17.5	18.0	18.0	18.0	30.5	27.0
7	23.3	17.7	19.6	19.0	29.3	30.7
8	20.0	-	15.3	17.3	28.3	29.0
9	19.0	-	14.0	21.0	26.0	30.0
10	21.0	-	14.5	17.0	28.0	28.5

Table 3b. Mean of weekly means(±SD) PCV (%) of T. vivax infected and control heifers.

Weeks

Post-infection	Infected	Control
1	38.6±0.9	30.0±0.6
2	23.8±1.1	27.7±0.4
3	22.5±1.9	28.7±1.6
4	23.3±2.4	30.4±0.6
5	18.7±0.8	29.7±0.4
6	17.9±0.2	28.8±1.8
7	19.4±1.9	29.7±0.4
8	17.4±1.2	28.7±0.4
9	18.0±1.8	28.0±2.0
10	17.5±1.7	28.3±0.3

Table 4a. Mean (±SD) serum progesterone levels (ng/ml) in *T. vivax* infected (n=4) and control (n=2) heifers.

Day of ... pling	Infected	Control
0	1.6±0.7	1.6±1.8
1	1.6±0.3	1.5±1.3
2	1.5±0.9	0.9±0.4
3	1.4±0.9	0.8±0.4
4	1.6±1.1	0.6±0.1
5	2.1±1.2	0.7±0.6
8	1.6±1.6	1.4±1.6
10	2.0±1.5	2.5±2.0
11	1.3±1.3	2.7±2.3
12	1.0±0.8	1.1±0.7
13	1.0±1.2	0.4±0.5
14	0.5±0.4	0.3±0.4
15	0.5±0.6	0.1±0.1
17	0.7±0.6	0.3±0.1
18	0.8±0.9	0.8±0.4
19	1.5±0.7	1.3±0.4
22	1.7±0.5	2.0±0.4
23	0.9±0.6	1.2±0.2
24	0.5±0.5	0.2±0.1
25	0.1±0.1	0.1±0.1
26	0.1±0.1	0.2±0.3
28	0.4±0.2	0.9±0.1
30	0.6±0.7	1.4±1.0
31	0.3±0.5	2.3±1.1
33	0.3±0.5	2.3±1.1
36	0.4±0.3	2.0±0.9
38	0.1±0.1	2.5±0.5
39	0.2±0.3	1.2±0.4
40	0.2±0.3	1.2±0.9
42	0.3±0.3	0.8±0.1
43	0.1±0.1	0.8±0.8
45	0.2±0.2	0.2±0.1
46	0.2±0.1	0.4±0.4
49	0.3±0.2	0.6±0.1
50	0.2±0.1	0.4±0.2

Table 4b. Serum progesterone levels (ng/ml) in T. vivax infected and control heifers.

	Infected				Control	
Day of infection	NO.1	NO.2	NO.4	NO.5	NO.3	NO.6
0	2.3	2.2	1.1	0.9	2.8	0.6
1	1.6	2.0	1.3	1.5	2.4	0.6
2	1.0	2.8	1.0	1.2	1.2	0.6
3	0.5	2.5	1.5	1.0	1.0	0.5
4	0.7	3.3	0.8	0.9	0.4	0.7
5	0.5	3.5	2.5	2.1	0.2	1.1
8	0.1	3.4	2.5	0.4	0.3	2.5
10	0.3	2.8	3.0	-	1.1	3.9
11	0.3	2.1	2.8	0.1	1.0	4.3
12	0.8	1.0	2.1	0.1	1.6	0.7
13	1.2	0.1	2.5	0.0	0.7	0.2
14	1.0	0.3	0.3	0.2	0.5	0.0
15	1.4	0.0	0.5	0.2	0.1	0.0
17	1.5	0.6	0.3	0.3	0.2	0.3
18	2.1	0.5	0.2	0.4	1.1	0.5
19	2.3	1.3	0.7	1.5	1.5	1.0
22	2.2	2.0	1.6	1.1	1.7	2.2
23	0.5	1.7	0.9	0.5	1.0	1.3
24	0.1	1.2	0.3	0.2	0.3	0.1
25	0.0	0.1	0.2	0.0	0.0	0.1
26	0.0	0.2	0.2	0.0	0.4	0.0
28	0.1	0.6	0.4	0.3	0.8	0.9
30	0.1	0.0	0.5	1.6	0.7	2.1
31	0.0	0.0	0.0	1.0	0.9	3.3
33	0.1	0.0	0.2	1.0	1.5	3.0
36	0.5	0.2	0.2	0.8	1.3	2.6
38	0.2	0.0	0.0	0.2	2.1	2.8
39	0.5	-	0.0	0.0	1.5	0.9
40	0.6	-	0.1	0.0	1.8	0.5
42	0.5	-	0.0	0.3	0.8	0.7
43	0.2	-	0.0	0.2	1.4	0.2
45	0.3	-	0.2	0.0	0.3	0.1
46	0.3	-	0.1	0.2	0.1	0.7
49	0.2	-	0.2	0.5	0.7	0.5
50	0.2	-	0.1	0.0	0.5	0.2

Table 5. Response to $PGF_2\&$ synchronization and conception to artificial insemination in T. vivax infected and control heifers.

	Infected (n=4)		Control (n=2)	
	1st $PGF_2\&$	2nd $PGF_2\&$	1st $PGF_2\&$	2nd $PGF_2\&$
No. of animals responding to $PGF_2\&$	2 (50%)	4 (100%)	2 (100%)	2 (100%)
Conception rate*	0%		0%	

**Number of Cows pregnant divided by the number inseminated and expressed as a percentage.*

Table 6. Observations on the estrus response.

Observation	Animal					
	Infected				Control	
	No.1	No.2	No.4	No.5	No.3	No.6
Uterine tone at estrus	+	+	+	+	+	+
Homosexual mounting	+	-	-	-	+	+
Sniffing	+	-	-	+	+	+
Stringy mucus vaginal discharge during AI	+	+	+	+	+	+
Stringy mucus discharge at other times	-	-	-	-	+	-
Flehmen	-	-	-	+	-	-
Bunting/Rubbing	+	-	-	-	+	+

+ = Present
- = Absent

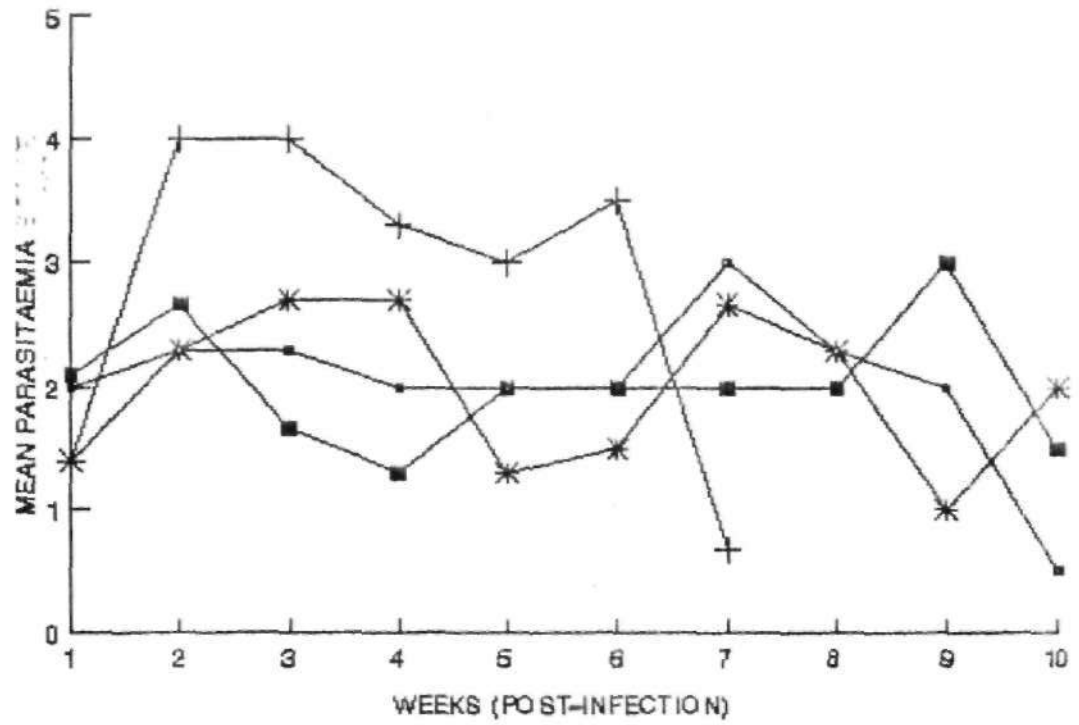


Fig 1: MEAN WEEKLY PARASITAEMIA SCORE (±S.D) T. vivax INFECTED HEIFERS

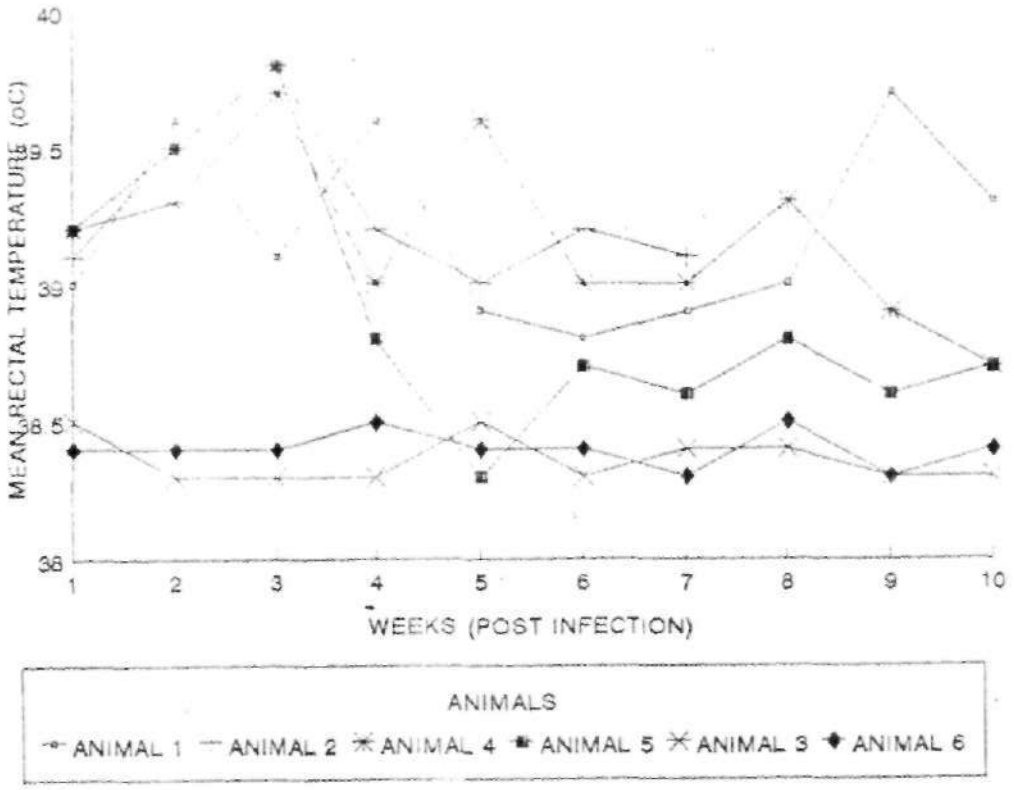


Fig 2a: MEAN WEEKLY TEMPERATUVE (oC) IN T.VIVAX INFECTED (NOS. 1,2,4 & 5) AND CONTROL (NOS. 3 & 6) HEIFERS

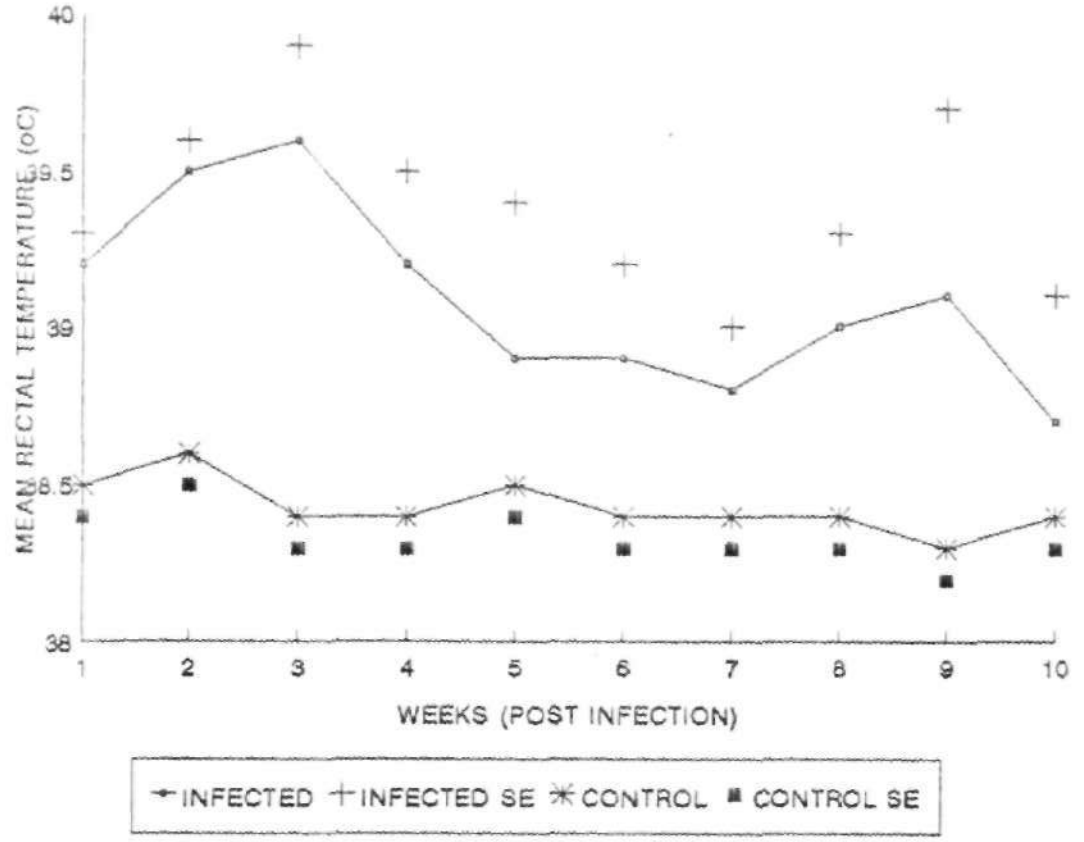


Fig. 2b: MEAN OF WEEKLY MEAN (+ SE) TEMPERATURE (oC) OF T. VIVAX INFECTED (N = 4) AND CONTROL (N = 2) HEIFERS.

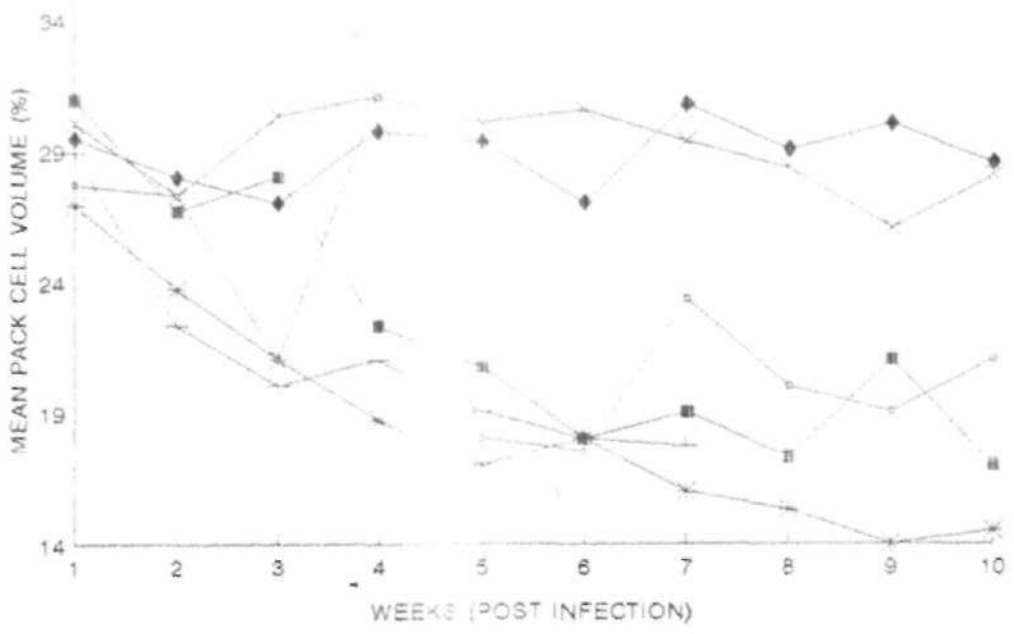


Fig 3a: MEAN WEEKLY PCV (%) IN T. VIVAX INFECTED (NOS. 1,2,4 & 5), AND CONTROL (NOS. 3 & 6) HEIFERS

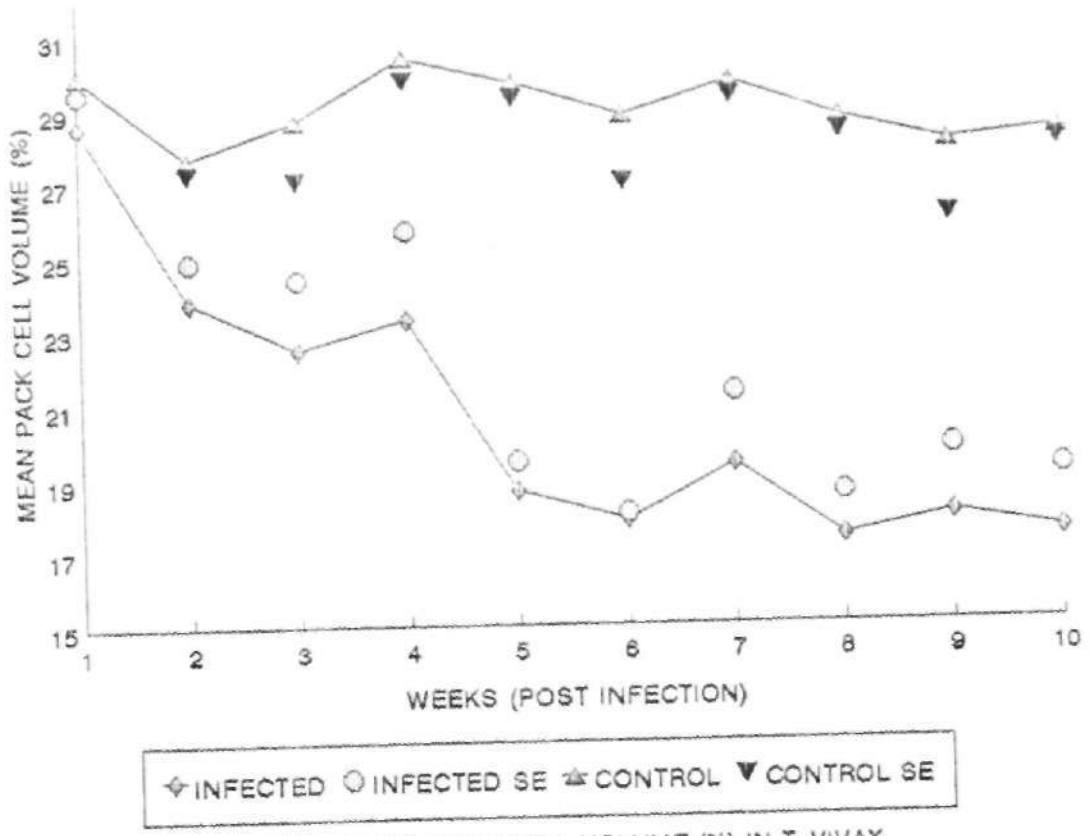


Fig. 3b: MEAN OF WEEKLY MEAN (+ SE) PACK CELL VOLUME (%) IN T. VIVAX INFECTED (N = 4) AND CONTROL (N = 2) HEIFERS.

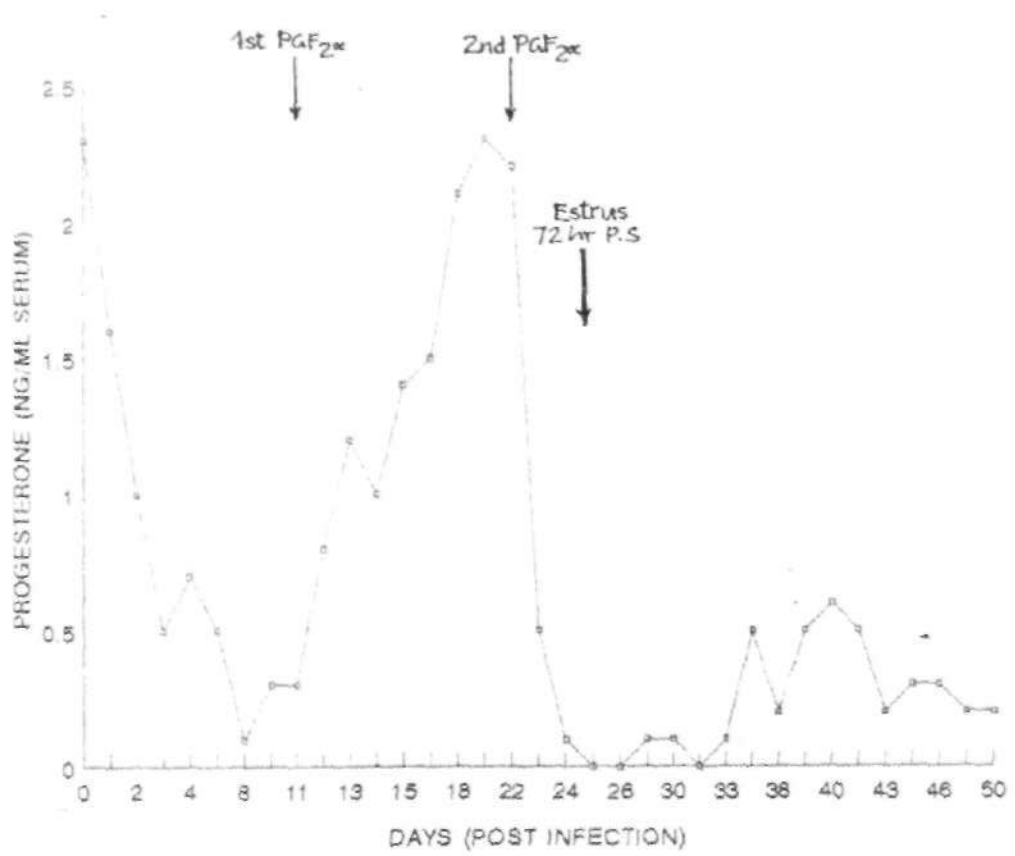


Fig. 4b: SERUM PROGESTERONE LEVELS IN T. VIVAX INFECTED HEIFER NO. 1

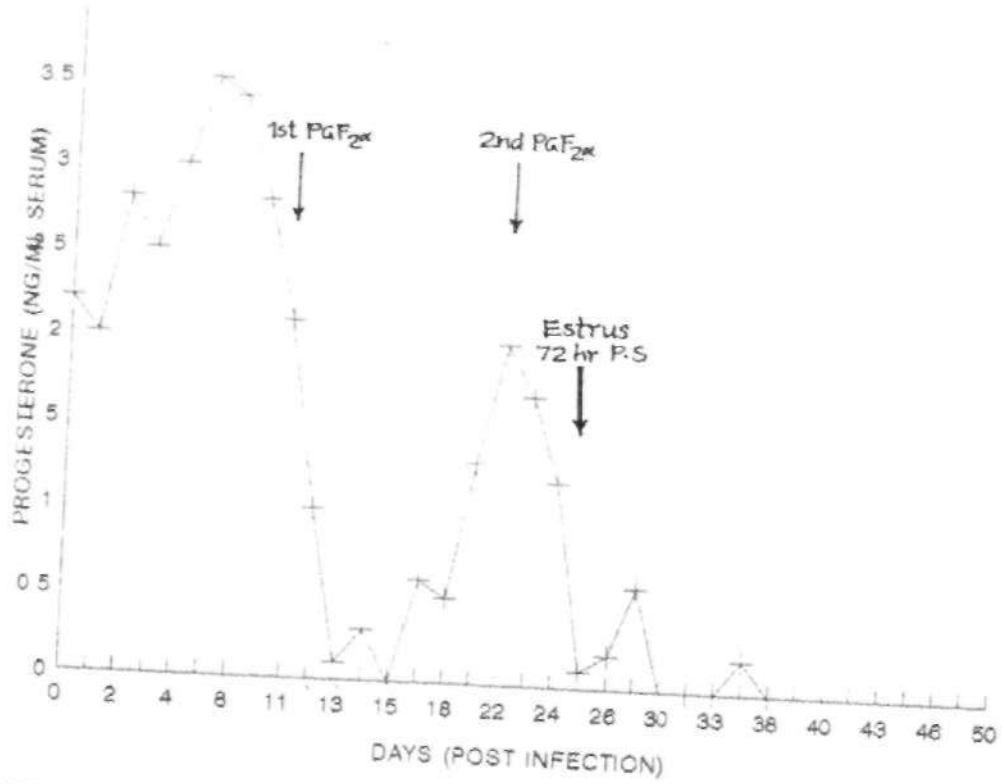


Fig. 4b: SERUM PROGESTERONE LEVELS IN T. VIVAX INFECTED HEIFER NO. 2

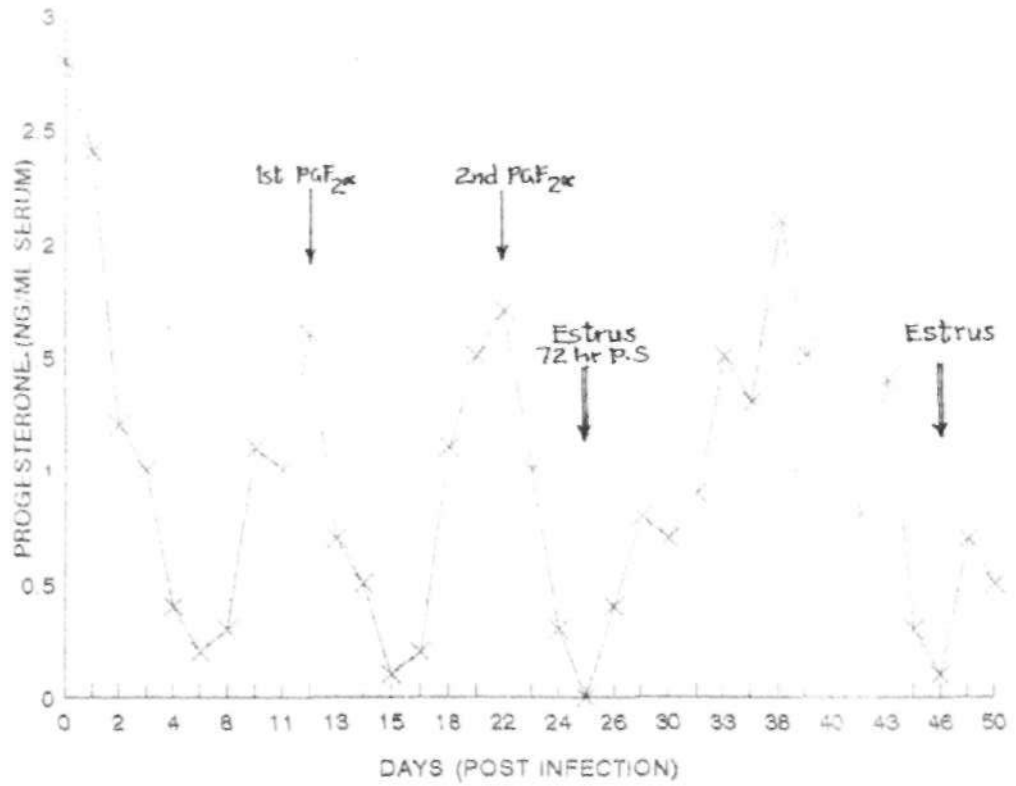


Fig. 4-b SERUM PROGESTERONE LEVELS IN CONTROL HEIFER NO.3

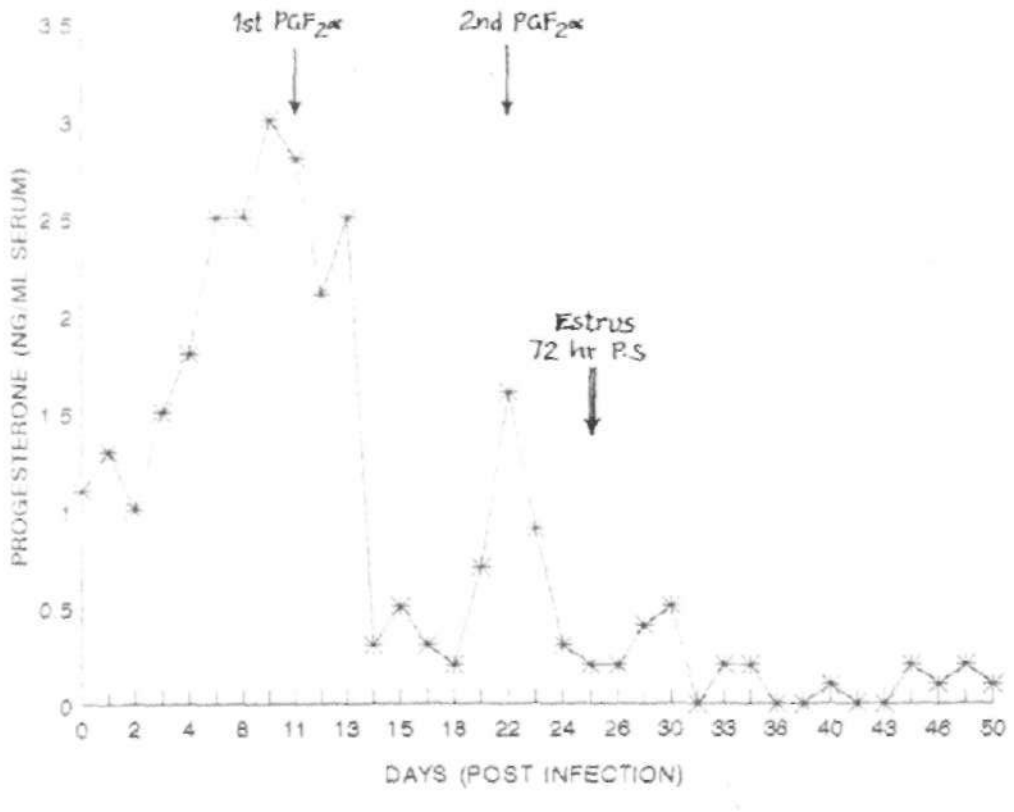


Fig. 4b) SERUM PROGESTERONE LEVELS IN T. VIVAX INFECTED HEIFER NO. 4

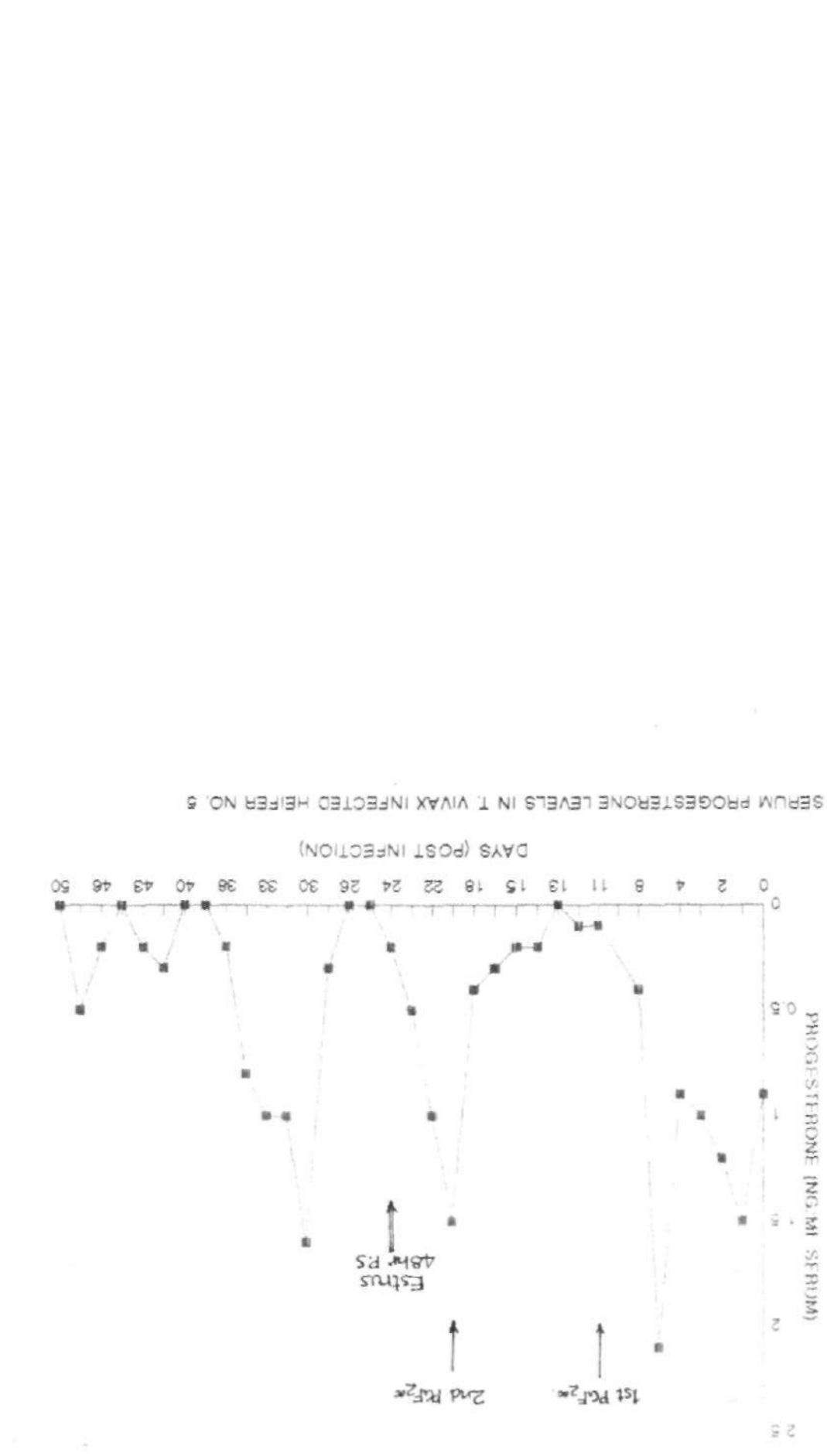


Fig. 4b. SERUM PROGESTERONE LEVELS IN T. VIVAX INFECTED HEIFER NO. 5

6

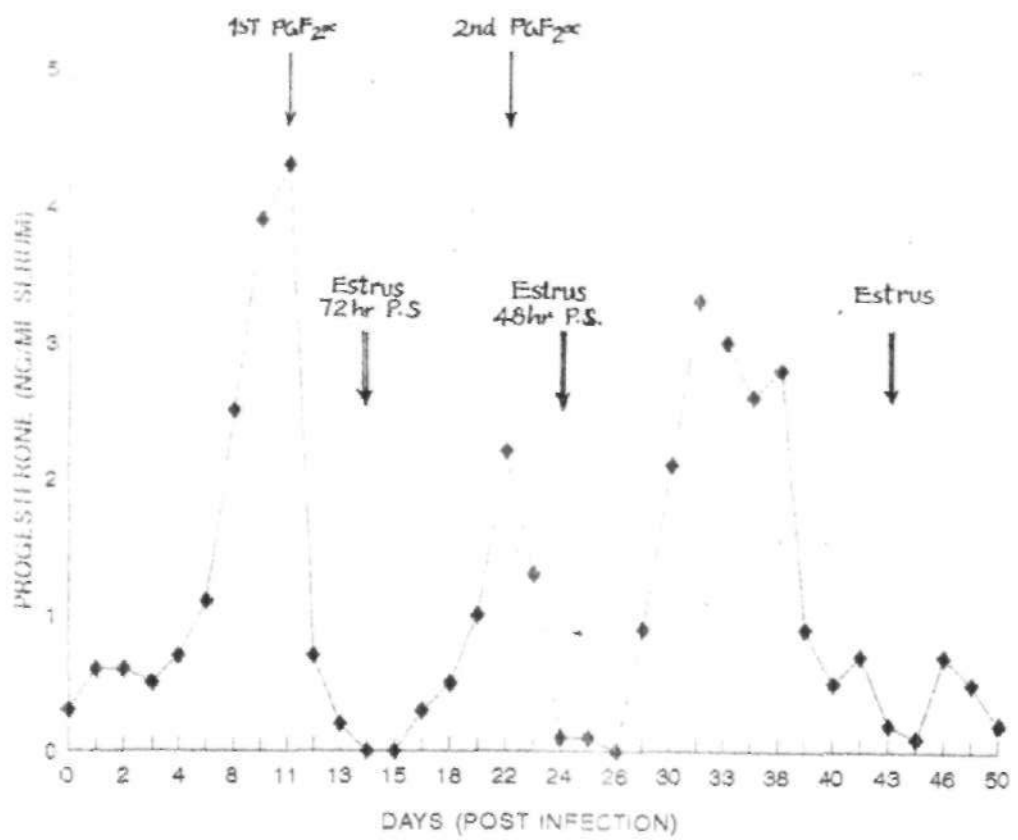


FIG. 4: SERUM PROGESTERONE LEVELS IN CONTROL HEIFERS (NO. 6)

120

APPENDICES

Appendix I. Changes in parasitemia+

	Animal					
	Infected				Control	
Day of infection	No.1	No.2	No.4	No.5	No.3	No.6
-7	*NPF	NPF	NPF	NPF	NPF	NPF
-4	NPF	NPF	NPF	NPF	NPF	NPF
0	NPF	NPF	NPF	NPF	NPF	NPF
1	NPF	NPF	NPF	NPF	NPF	NPF
2	NPF	NPF	NPF	NPF	NPF	NPF
3	NPF	NPF	NPF	NPF	NPF	NPF
4	4	NPF	NPF	4	NPF	NPF
5	4	3	2	4	NPF	NPF
6	4	3	4	4	NPF	NPF
7	2	4	4	3	NPF	NPF
9	NPF	4	1	3	NPF	NPF
11	4	4	3	2	1	NPF
13	3	4	3	3	NPF	NPF
16	2	3	3	3	NPF	NPF
18	2	2	3	NPF	NPF	NPF
20	3	4	2	2	NPF	NPF
23	2	3	4	1	NPF	NPF
25	3	4	2	1	NPF	NPF
27	1	3	2	2	NPF	NPF
30	3	3	2	3	NPF	NPF
32	2	3	2	2	NPF	NPF
34	1	3	NPF	3	NPF	NPF
38	NPF	3	NPF	3	NPF	NPF
41	4	4	3	1	NPF	NPF
44	4	3	3	2	NPF	NPF
46	2	2	2	2	NPF	NPF
48	2	-	3	2	NPF	NPF
51	4	-	NPF	NPF	NPF	NPF
53	2	-	2	3	NPF	NPF
55	1	-	2	3	NPF	NPF
58	2	-	1	3	NPF	NPF
65	NPF	-	3	1	NPF	NPF
69	1	-	1	2	NPF	NPF

*NPF = No Parasite Found.

+ = Ranking of Parasitaemia

1 = Occassional trypanosome seen per buffy coat area
2 = 1-2 " " " " " "
3 = 3-20 " " " " " "
4 = > 20 " " " " " "

Appendix II. Changes in Rectal Temperature (°C) in T. vivax infected and control heifers.

	Animal					
	Infected				Control	
Day of infection	No.1	No.2	No.4	No.5	No.3	No.6
-10	38.5	38.2	38.7	38.2	38.3	38.3
- 8	38.6	38.3	38.5	38.0	38.1	38.4
- 5	38.4	38.2	38.5	38.2	38.4	38.3
- 4	38.4	38.4	38.0	38.0	38.5	38.0
- 3	38.3	38.5	38.5	38.2	38.2	38.5
- 2	38.5	38.3	38.4	38.4	38.5	38.6
0	38.5	38.2	38.5	38.5	38.4	38.4
1	38.4	38.5	38.5	38.4	38.7	38.3
2	37.8	38.4	38.7	38.1	38.5	38.5
3	38.5	38.2	38.4	38.5	38.3	38.3
4	39.0	38.5	38.7	39.4	38.4	38.3
5	39.1	39.6	39.3	39.5	38.4	38.4
6	39.5	41.4	41.0	40.6	38.6	38.5
7	41.2	39.7	40.1	40.0	38.2	38.5
8	41.0	39.4	39.4	40.4	38.5	38.5
9	39.9	38.9	38.3	39.3	38.5	38.4
10	39.4	39.1	38.5	38.3	38.6	38.4
11	38.3	39.6	39.6	39.5	40.0	38.3
12	38.9	39.6	40.0	39.6	38.5	38.4
13	39.2	40.2	39.4	40.0	38.2	38.4
14	40.1	40.5	39.8	39.4	38.1	38.3
15	39.6	41.0	39.6	39.8	38.1	38.5
16	38.8	42.3	39.7	39.6	38.5	38.5
17	38.8	39.7	39.9	39.7	38.5	38.2
18	38.6	39.5	40.2	39.9	38.6	38.3
19	39.2	39.6	39.8	40.0	38.2	38.3
20	39.3	38.7	39.8	39.8	38.3	38.6
21	39.6	39.2	38.6	39.8	38.2	38.5
22	40.5	38.9	39.1	38.6	38.3	38.5
23	39.7	39.3	40.7	39.1	38.2	38.4
24	38.9	39.2	38.2	36.7	38.3	38.3
25	39.6	39.4	38.8	38.7	38.3	38.5
26	39.4	38.9	38.4	38.8	38.4	38.4
29	38.6	39.4	38.9	38.0	38.6	38.6
30	38.9	39.0	39.1	38.0	38.4	38.5
31	38.9	38.7	39.3	38.5	38.5	38.4
32	38.7	38.9	40.3	38.6	38.5	38.3
33	39.2	39.1	39.8	38.3	38.5	38.3
35	38.8	38.9	39.9	38.4	38.4	38.5
36	38.6	39.9	40.8	38.5	38.5	38.5
37	38.7	39.5	38.1	38.8	38.3	38.4
38	38.5	39.3	38.6	30.8	38.3	38.4
40	38.7	38.9	39.3	38.6	38.1	38.5
41	39.0	39.2	38.6	38.9	38.2	38.1
42	39.1	38.9	38.4	38.5	38.5	38.5
44	38.8	*38.8	39.4	38.4	38.4	38.3
46	38.5	-	39.8	38.5	38.5	38.4
47	39.4	-	38.4	38.7	38.2	38.4
48	38.7	-	38.6	38.6	38.6	38.2
49	38.9	-	38.6	38.8	38.4	38.5
51	38.9	-	38.8	38.9	38.3	38.4
52	39.9	-	39.5	38.6	38.3	38.6
53	38.9	-	39.1	38.5	38.5	38.3
54	38.6	-	39.8	39.1	38.3	38.5
56	38.7	-	39.4	38.9	38.4	38.6
57	40.2	-	39.2	38.7	38.4	38.5
58	40.2	-	39.6	39.5	38.1	38.3
59	39.6	-	38.3	38.5	38.3	38.3
60	39.6	-	38.5	38.9	38.2	38.1
62	38.8	-	38.9	38.7	38.5	38.3
63	39.0	-	38.8	38.5	38.6	38.4
64	39.3	-	38.8	38.4	38.3	38.5
66	39.7	-	38.6	38.8	38.1	38.7
68	38.9	-	38.6	38.7	38.4	38.5
69	39.1	-	38.9	38.7	38.5	38.4

* *Animal was treated on Day 43 at a rectal temperature of 39.1.*

Appendix IIIa. Packed cell volume (%) in T. vivax infected and control heifers.

	Animal					
	Infected				Control	
Day of infection	No.1	No.2	No.4	No.5	No.3	No.6
-7	32	30	30	29	32	27
-4	32	29	29	28	35	35
0	30	32	27	35	30	33
1	30	32	27	32	32	28
2	29	28	28	32	31	30
3	29	30	28	32	30	30
4	27	29	29	30	30	27
5	26	29	23	29	32	31
6	28	26	26	31	31	29
7	26	28	27	27	30	26
9	24	24	25	26	28	27
11	21	23	22	26	25	28
13	22	20	24	28	29	28
16	21	20	21	30	30	27
18	22	19	20	28	31	25
20	20	21	22	26	30	29
23	29	20	19	23	29	30
25	33	22	19	24	31	30
26	31	21	18	20	33	29
30	20	20	15	22	30	28
32	17	19	17	19	29	31
34	17	18	19	23	31	29
38	17	17	18	18	32	26
41	18	19	18	20	29	28
44	19	17	17	20	29	32
46	25	-	16	19	28	30
48	26	-	15	18	31	30
51	19	-	15	16	28	29
53	20	-	17	17	27	27
55	21	-	14	19	30	31
58	19	-	14	21	26	30
65	22	-	14	18	29	28
69	20	-	15	16	27	29

Appendix IIIb. **Mean (±SD) PCV (%) in T. vivax infected and control heifers.**

Day of infection	Infected	Control
0	30.3±2.4	30.0±2.8
1	31.0±3.4	31.5±2.1
2	29.3±1.9	30.5±0.7
3	29.8±1.7	30.0±0.0
4	28.8±1.3	28.5±2.1
5	26.8±2.9	31.5±0.7
6	27.8±2.4	30.0±1.4
7	27.8±0.8	28.0±2.8
9	24.8±0.8	27.5±0.7
11	23.0±2.2	26.5±2.1
13	23.5±3.4	28.5±0.7
16	23.0±4.7	28.5±2.1
18	22.3±4.0	28.0±4.2
20	22.3±2.6	29.5±0.7
23	22.8±4.5	29.5±0.7
25	24.3±6.1	30.5±0.7
27	22.5±5.8	31.0±2.8
30	19.3±2.3	29.0±1.4
32	18.0±1.2	30.0±1.4
34	19.3±2.6	30.0±1.4
38	17.5±0.6	29.0±4.2
41	18.3±0.5	28.5±0.7
44	18.3±1.5	30.5±2.1
46	20.0±4.6	29.0±1.4
48	19.7±5.6	30.5±0.7
51	16.6±2.1	28.5±0.7
53	18.0±1.7	27.0±0.0
55	18.0±3.0	30.5±0.7
58	18.0±3.6	28.0±2.8
65	19.0±4.4	28.5±0.7
69	17.0±2.7	28.0±1.4