FERTILITY AND EMBRYONIC MORTALITY RATES OF ZEBU COWS FOLLOWING OESTRUS SYNCHRONIZATION AND ARTIFICIAL INSEMINATION

BY

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DECLARATION

I, Dr. A.A. Voh (Jr.), do hereby declare that the work reported in this thesis was carried out and written by me in the Department of Veterinary Surgery and Medicine, Theriogenology Section, and National Animal Production Research Institute (NAPRI), Shika, Ahmadu Bello University, Zaria under the supervision of Professor D. Ogwu and Professor E.O. Oyedipe.

It is a record of my own research work and no part of it has been presented in any previous application for a higher degree or diploma. The works of other investigators are specifically and duely acknowledged by means of references.

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CERTIFICATION

This thesis titled **"Fertility rates of Zebu cows following oestrus synchronization and artificial insemination"** by Ambrose Alikidon Voh (Jr.) meets the regulations governing the award of the degree of Doctor of Philosophy of Ahmadu Bello University and is approved forits contribution to scientific knowledge and literary presentation.

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DEDICATION

This thesis is dedicated to the late mother MRS BOGORON DEDE VOH who slept in the Lord on the 15th day of November, 1996 and my Late cousin-in-Law MRS. ELSIE MAJORITY BINAUTO who slept in the Lord on the 31st day of December, 1992 during child birth.

Jesus said to her, "I am the resurrection and the life. He who believes in me will live, even though he dies; and whoever lives and believes in me will never die. Do you believe this?" (John 11: 25 - 26)

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ABSTRACT

The experiments embodied in this thesis were carried out at the National Animal Production Research Institute (NAPR1) of Ahmadu Bello University, Shika. Zaria, Nigeria. The broad objective was to study fertility rates in artificially inseminated zebu cows following oestrus synchronization with Prostaglandin F2alpha (PGF2&) and progesterone releasing intravaginal device (PR1D). The specific objectives were:

1. To determine the effect of inter-treatment intervals and stage of the oestrous cycle on oestrus response following oestrus synchronization with P(iF2 alpha.
2. To determine the optimum fixed-time for insemination following synchronization of oestrus with PGF2 alpha.
3. To determine oestrus response and fertility rates of zebu cows following oestrus synchronization with PRU), alone and/or in combination with PGF2 alpha.
4. To determine the incidences of late embryonic mortality (LEM) and Repeal breeder syndrome (RBS) in artificially inseminated Bunaji cows following oestrous synchronization.
5. To use rectal palpation, to chronologically characterise changes in the reproductive organs and fetus that are associated with pregnancy and to use the changes in the diagnosis and ageing of pregnancy in zebu cattle.

To determine the effect of intertreatment interval and stage of the oestrous cycle on oestrus response, a total of 140 non-suckled Bunaji cows were given the first injection of prostaglandin F2 alpha regardless of the stage of the oestrous cycle. For the second injection, however, the cows were randomly alloted to 5 treatment groups of 2S cows each for intertreatment intervals of 10, 11, 12, 13 and 14 days respectively following the first

injection. The cows were visually observed for oestrus continuously tor 7 consecutive days. Standing oestrus was used as the sole sign of oestrus.

The respective oestrus response rates were 73.1. 85.5. 80.0, 92.3 and 83.9% for the 10, 11. 12, 13 and 14 days intertreatment intervals. The oestrus response rate for the 10-day intertreatment interval was significantly lower than the other groups while the oestrus response rate for the 13-day inter-treatment interval was significantly higher than the other groups.

It is concluded that 13 days intertreatment interval, a regime that places 91% of the cows in mid-dioestrus at the time of second injection, is the optimum for Bunaji cows. Also, higher oestrus response rates and tighter synchrony of oestrus was achieved when cows within the same stage of dioestrus were synchronized with PGF2 alpha.

To deterimine the optimum fixed-time for Al following double injection of PGF2 alpha given 1 1 days apart, a total of 420 Bunaji cows were randomly alloted to 6 groups of 70 cows per group as follows:

1. Controls which were inseminated approximately 12 hr..following onset of spontaneous oestrus.
2. A.I. at approximately 12 hr. following observed PGF2 alpha-induced oestrus post-second PGF2& injection.
3. Fixed-time Al 60 hr. post-second PGF2 alpha injection
4. Fixed-time Al 72 hr. post-second PGF2 alpha injection
5. Fixed-time Al 84 hr. post-second PGF2 alpha injection
6. Fixed-time Al % hr. post-second PGF2 alpha injection. The pregnancy rates, were 74.1. 60.5, 54.2. 45.6. 34.9 and 40.0% for groups I to 6 respectively. The corresponding

conception rates relative to cows detected in oestrus were 74.0, 60.0, 54.3, 45.7, 41.4 and 40.0% for groups 1 to 6 respectively.

It is concluded that following 2 consecutive injections of PGF2 alpha given 11 days apart. 60 hr. is the optimum interval from second injection to A.I. for fixed-time Al for Bunaji cows.

To determine oestrus response and fertility rates of zebu cows following oestrus synchronization with PRID, alone and/or in combination with PGF2 alpha, a total of 184 non-suckled cycling Bunaji cows were randomly alloted to 4 treatment groups of 4b cows per group as follows:

1. PRID-12: PRID only for a period of 12 days.
2. PRID-7 + PGF -6: PRID for 7 days and PGF2 alpha injection 1 day prior to PRID withdrawal i.e. on day 6 of treatment.
3. PRID-7 + PGF -7: PRID for 7 days and PGF2 alpha injection on the day of PRID withdrawal.
4. 2xPGF - 13: Double injection of PGF2 alpha 13 days apart. Blood samples were taken prior to. during and after treatment. Serum was harvested and progesterone profiles were determined by radioimmunoassay.

The respective oestrus response rates were 78.3. 76.1. 87.0 and 89.1% for Groups I to 4. while the corresponding pregnancy rates were 30.1, 41.3, 52.2 and 52.2%, the conception rates were 50.0, 54.3, 60.3 and 58.6% for Groups 1 to 4 respectively. The differences were not significant. Although individual variations in levels of progesterone were observed, the progesterone profiles were generally typical.

The results of the experiment have confirmed the effectiveness of all the tested oestrus control regimes to synchronize and control oestrus in zebu cows with acceptable fertility to A**I** at **the detected** oestrus.

To determine the incidences of late embryonic mortality (LEM) and repeat breeder syndrome (RBS), two methods were used namely:

i the delayed return rate (DRR) and

ii the serum progesterone concentration **(SPC)** method. For the **DRR** method a total of 623 post-insemination oestrous cycle lengths were used. The respective LEM and RBS rates were 27.6% and 15.3%.

For the SPC method on the other hand, a total of 15 embryos were lost out of 120 embryos between days 21 and 45 post-inseminaition giving an overall LEM of 2.5% (9.2% between days 21 and 35, 3.3% between days 35 and 45).

To identify those anatomical and physiological changes in the reproductive organs and foetus that arc associated with pregnancy and to use the changes in the diagnosis and ageing of pregnancy in Bunaji cows, a total of 23 pregnancies from synchronized oestri were rectally monitored once weekly from approximately day 28 following fertile insemination to day 14 post partum. The time of first detection as well as progressive changes in the size and position of the structures were recorded.

Based on the findings. (of the study) a table of gestation characteristics was drawn up. the salient features of which include:

1. Fetal membrane slip (FMS) was detected in all the cows between days 30 to 45 (mean 35 days).

2. Amniotic vesicle (AV) was detected in only 5 cows (22% detection rate) between days 35 to 55 (mean 45 days).

3. Fetus was detected in all the cows between days 60 to 90 (mean 75 days)

4. Cotyledons (placentomes) were detected in all the cows between days 90 to UK) (mean 95 days).

5. Fremitus of the middle uterine artery was detected in all the cows between days 70 to 100 (mean 85 days).

Thus, rectal palpation was found to be 100% accurate in the diagnosis of both pregnancy and non-pregnancy. It was concluded that it is indeed possible to positively diagnose and age pregnancy in Bunaji cows as early as 30 to 40 clays of gestation and this should be the target for clinicians engaged in herd health fertility programmes, especially in herds where Al is used.

Finally, the efficacy of PGF2 alpha or its analogue should thus be considered well proven as a luteolytic agent and that fertility following PGF2 alpha-induced luteolysis is comparable to that following spontaneous oestrus. In addition, oestrus synchronization when carefully combined with rectal palpation can serve as a powerful management tool in the improvement of reproductive performance of indigenous Nigerian zebu cows.

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LIST OF ABBREVIATIONS

1. ACC Annual Calf Crop
2. AI Artificial Insemination
3. Anon Anonymous
4. AP Age at Puberty
5. AV Amniotic vesicle
6. β Beta
7. CAP Chloroacetoxy progesterone
8. CL Corpus luteum
9. CM Centrimetres
10. CO Company
11. CR Conception Rate
12. DRR Delayed Return Rate
13. EEM Early Embryonic Mortality
14. EM Embryonic Mortality
15. E_2V Estradiol Valerate
16. FAO Food and Agricultural Organisation
17. FF Fertilization Failure
18. FGA Fluorogestone acetate
19. FMS Fetal Membrane Slip
20. FSH Follicle Stimulating Hormone
21. g grame
22. GI Generation Internal

23. GnRH Gonadotropin-releasing hormone

24. HCG Human Chorionic Gonadotropin

25. hr Hour

26. ICI Imperial Chemical Industries

27. ILCA International Livestock Centre for Africa

28. I.M. Intramuscular

29. LEM Late Embryonic Mortality

30. LH Luteinizing hormone

31. LIBC Livestock Investigation and Breeding Centre

32. Ltd Limited

33. MAP Methyl acetoxy progesterone

34. MGA Molengestrol acetate

35. Mg Milligram

36. Ml Millilitre

37. MM Millimeters

38. MMB Milk Marketing Board

39. MOET Multiple Ovulaltion and Embryo Transfer

40. MUA Middle uterine artery

41. NAPRI National Animal Production Research Institute

42. NB Normal breeders

43. NDO Non-detected Oestrus

44. NS Natural Service

45. ODB Oestradiol benzoate

46. ORR Oestrus Respond Rate

47. $PGF_2\alpha$ Prostaglandin F2 alpha

48. PMSG Pregnant Mare Serum Gonadotropin

49. PR Pregnancy Rate

50. PRID Progesterone Releasing Intravaginal Device

51. RBS Repeat Breeder Syndrome

52. RIM Resource Inventory Management

53. SC Subcutaneous

54. SMB Synchro-Mate B

55. SPC Serum Progesterone Concentration

56. UK United Kingdom

57. USA United States of America

58. % Percent

CHAPTER ONE

INTRODUCTION

One of the universally accepted practical methods of rapidly improving cattle productivity is the application of a well planned and executed artificial insemination (A.I) programme. It is also fortuitous that cattle which is the species that has the most rational scope for the application of A.I. is also the one whose semen can be most readily obtained and preserved (Arthur, 1975). Artificial insemination, however, has its own peculiar problems such as capital cost of equipment, need for detection of heat, high level management and/or husbandry and an on-site or on-call inseminator. Since these factors require considerable farm labour and resources, shortening the breeding period has long been considered necessary for field application. This necessity is even more relevant in Nigeria where in addition to the afore-mentioned constraints, the availability of good quality herbage is seasonal. This shortening or demarcation of a breeding season can be achieved by the use of oestrus synchronization techniques. Data have been published that support and confirm the luteolytic potency of prostaglandin F2 alpha (PGF2&) and its efficacy in controlling estrus in different breeds of cattle under different systems of management. Various methods utilising PGF2& or its analogues to synchronize estrus have also been described. The various methods have worked successfully in synchronizing estrus. The technique which is the simplest and most attractive is the administration of 2 intramuscular injections of PGF2& separated by 10-12 days (Cooper, 1974). This has proved to be a very effective method of synchronizing estrus in randomly cycling cattle (Lauderdale et al. 1973; Hafs et al. 1973; Roche, 1976; Voh Jr. et al. 1986). However, a small proportion of treated animals have failed to respond to the second treatment. In the case of Nigerian zebu cows this failure

has been attrib- uted to considerable variation in the length of luteal growth and maturation with the net result that these animals are given the second injection when the corpora lutea have not reached responsive stage. To take care of this category of animals, intertreatment intervals longer than the conventional 11 days used were suggested (Voh, Jr. et al. 1986). For an estrus synchronization programme to be attractive and sustained, acceptable levels of fertility have to be achieved consistently. Thus, it has been rightly said that controlled breeding will only reach peak efficiency when most cows can be inseminated on a single day without jeopardising fertility. For this to occur, sexual receptivity and ovulation must be precisely controlled (Nancarrow, 1976). However, it is known that this ideal situation is far from being realised. This has been due largely to a widespread onset of estrus over a 7-day period following PGF2& injection (Voh, Jr. 1984). Thus, there may be a need to improve the precision of synchronization if this dream is to be realised. A combined treatment of PGF2& with progesterone in the form of intravaginal devices has been reported to bring about not only tighter synchrony of oestrus but to shorten postpartum anoestrus period by inducing ovarian cyclicity in postpartum cows (Hansel and Beal, 1979; Beal, 1983).

While PGF2&-induced estrus has resulted in fertility rates comparable to untreated controls (Cooper, 1974; Lauderdale et al. 1974; King et al. 1982), insemination at predetermined time(s) has resulted in variable fertility rates (Manns et al. 1977; Burfening et al. 1978) for temperate breeds of cattle. For zebu cattle and their exotic crosses the situation has been even worse because pregnancy rates lower than 30% have been frequently reported inspite of excellent estrus response rates (Hardin et al. 1980; Landivar et al. 1985; Voh, Jr. et al. 1986). This low fertility for zebu breeds has been attributed to a number of factors notable

among which is the application of heat detection criteria and reproductive physiology data from Bos taurus cattle that do not necesarily apply to Bos indicus (Orihuela et al. 1983; Voh, Jr. et al. 1986). Randel (1984) has conclusively shown the reproductive physiology and endocrinology of zebu cows (Brahman) to be different from those of Bos taurus (Hereford). It is no wonder, therefore, that the interpolation of data from Bos taurus breeds to zebu breeds has failed to yield encouraging fertility rates. Embryonic mortality has also been suggested as a probable cause of the low fertility although this has been conjectural and needs to be investigated further (Voh, Jr. et al. 1986).

This set of facts calls for a need to generate basic reproduction data relevant to zebu cattle before large scale application of controlled breeding techniques such as estrus synchronization and A.I. are embarked upon. This need has become even more relevant and indicated now that there is a call for modification of estrus synchronization protocols used for Bos taurus breeds of cattle (Watts and Fuquay, 1985) and zebu cattle (Orihuela et al. 1983; Voh, Jr. et al. 1985) because of altered response patterns. There is, therefore, a need to provide the fertility data for Nigerian zebu cattle following synchronization of estrus with PGF2& so that appropriate estrus synchronization management system or systems can be designed which will fit particular management and husbandry situations to ensure the most profitable outcome. It is to achieve this that this study was designed; the objectives of which are to:

1. determine the optimum intertreatment interval for a double injection regime using PGF2&
2. determine the optimum time for fixed-time A.I.
3. determine the response and fertility rates of zebu cows after treatment with

progesterone-releasing intravaginal device (PRID), alone or in combination with prostaglandin F2 alpha.

4 determine the incidences of late embryonic mortality (LEM) and Repeat Breeder Syndrome (RBS) in artificially inseminated Bunaji cows following oestrus synchronization.

5. identify those physiological changes of the reproductive organs that are associated with pregnancy, when the changes occur, how they progress with gestation and to use the findings in the diagnosis and ageing of pregnancy by rectal palpation in indigenous zebu cows.

CHAPTER TWO

LITERATURE REVIEW

2.1. REPRODUCTIVE PERFORMANCE

2.1.1 Cattle Population:

Statistics on Nigerian cattle population cannot be considered reliable. It has varied from one researcher to another and has been based on estimates. The F.A.O. (Anon 1965) estimates put the cattle population at 10.86 million whereas David-West (1975) and Anon (1981) reported 9.1 million. Other sources have reported various figures: 12.3 million (FAO, 1980), 15.9 million (ILCA, 1978), 11 million (Zakari, 1986), 20 million (Lamorde, 1986 citing figures compiled by Federal Livestock Department for Pan African Rinderpest Campaign). More recently, Resource Inventory Management (R.I.M., 1991) has put cattle population in Nigeria at 14 million . Not only have these figures varied with researchers but have varied from year to year in no particular predictable fashion.

This difficulty in ascertaining the exact statistics of Nigerian cattle population has been attributed to the traditional production system (nomadism) on one hand and the methodology and sources of obtaining the figures on the other hand. One fact consensual to all these reports and on which there is no ambiguity is that over 90% of the national cattle herd resides in the hands of the nomadic Fulanis (FAO 1978). The need for an accurate census figure also has long been recognized as a prerequisite for any meaningful planning and execution of cattle improvement strategies.

2.1.2 Cattle Breeds

As would be expected that in a country the size of Nigeria, extending between latitude 4o North to 14oNorth and longitude 2 oE to 14oE and covering 923,768 square kilometers, varying in climate, vegetation, topography, and peoples, the animals, including cattle, will be as varied as the conditions under which they live, and each area will have the variations of breeds and types most suitable to it. So it is in Nigeria: the dry relatively trypanosomiasis-free almost semi-desert areas of the North are inhabited by hardy nomadic cattle especially suited to travelling long distances in search of grazing and water during long periods of drought and semi-starvation, whereas in the tsetse fly-infested south, where the cattle are more closely herded and more settled, one finds smaller, more rounded and less hardy but trypanotolerant breeds (Gates, 1952).

Apart from the clearly defined distinct breeds, there are considerable numbers of cattle which have resulted from crossbreeding. This confusion is compounded further by the custom of many Nigerian cattle owners to refer to cattle by colour names, and it is not always easy to determine what is a true breed and what is a colour variation. Thus, it is not unusual that the same breed of cattle is often referred to by different names and conversely, widely differing breeds are known by the same name in different parts of the country (Gates, 1952).

Nigerian cattle herd is predominated by the humped zebu type. Zebu cattle with a few exceptions, is a triple purpose animal specialized for neither milk nor beef but used for draught, milk and beef (Buvanendran, 1979).

The approximate percentage composition of the breeds (Anon, 1981) is as follows: Bunaji (51%), Rahaji (14%), Sokoto Gudali (11.5%), Adamawa Gudali (11.5%) and others (12 %) Breeds of cattle found in Nigeria have been described by Gates (1952) as follows:

1.1.2.1 **Bunaji (White Fulani, Yakanaji, Lyre-horned zebu)**:

The Bunaji is very similar to, and possibly a direct descendant of the Hamitic Longhorn which is believed to have mi- grated from Egypt along the north coast of Africa and down the west coast about 2,000 B.C. It is found in the Northern States of Nigeria mainly in the sandy orchard bush at 1000 to 2000 feet above sea level but also on certain high grassland plateau of volcanic origin up to 5,500 ft above sea level. The defunct provinces with the greatest population of this breed are Kano, Katsina and Bauchi, although it is also found in considerable numbers in Sokoto, Zaria, Bornu, Plateau and Adamawa.

The Bunaji are fairly large animals, averaging 51 inches high at the shoulder immediately behind the hump. An average mature bull weighs about 10 centiweights and a cow 6 centiweights with about 50-55% dressing percentage. The hump is well developed, fatty-muscular and cervico-thoracic in location. The dewlap is fairly large, particularly in the bulls.

The horns are medium to long, curving outwards and upwards soon after leaving the head. Some have an outward turn again at the tip giving the lyre shape.

The body colour is white on a black skin, black ears, eyes, muzzle, feet, horn tips and tip of tail. Variations, however, exist in all combinations of black and white on a black skin and probably through crossing with red breeds there are varieties in red and white on a whitish skin.

Bunaji is the most common breed found in Nigeria (51%), and it is owned mainly by the nomadic Fulani people. They make excellent work oxen, being of good size, strong and fairly tractable.

This breed has been studied in detail by the Agricultural Department on their farms at Samaru and Shika, and has been used for laboratory work by Veterinary Department at Vom and Kano.

2.1.2.2 Rahaji (Abori, Bodadi, Red Fulani, Red Bororo, Red Longhorn)

This breed may have originated from southern Egypt as Sanga cattle i.e a cross between the Hamitic Longhorn and the Lateral horned zebu which is now the Afrikander of South Africa. They may have migrated across Africa south of the Sahara.

It is distributed in the northern borders from Sokoto to Lake Chad in the sandy orchard bush about 2000 feet above sea level and also on the high grasslands of Bamenda in the Cameroons.

They are large framed animals averaging 55 inches behind the shoulder (some bulls being over 60 inches). The average weight of a mature bull is 11 to 12 centweights. The hump is well developed, musculo-fatty and cervico-thoracic in location. The horns are white in colour, long, curving out- wards and upwards. The body colour is reddish brown, whole coloured except for a white tip to the tail in some in- stances. The depth of colour varies from light reddish brown in some strains to dark red brown in others. They are mainly beef cattle. They are not used as work oxen being very intractable. The breed is found mainly in Niger and Tchad where it is called "Bororo"

2.1.2.3 **Sokoto Gudali (Bokoloji; the Sokoto; Short horned zebu)**

Their appearance being very like some short horned zebu breeds of India and the Sudan, it is thought probable that this type is a recent importation from India to East Africa from where it has found its way westwards across Africa. It is distributed in the north-eastern districts of the defunct Sokoto Province. It is medium to fairly large in size, averaging 51 inches high. A mature bull weighs an average 10 centiweights. It has a well developed, fatty-muscular cervico-thoracic hump. The dewlap and sheath are very pronounced. The ears are pendulous. The horns are very short in the bull growing in a lateral and somewhat upward direction. The horns are slightly longer in the cow. The head is carried very low.

It is white or cream whole coloured in the case of females. In males the colour is often light grey or cream with dark shading over the head, neck, shoulders and tail. The shading varies in intensity in different individuals, and bulls with blue grey shading are sometimes seen.

Docility is one of the main characteristics of this breed. They have been used successfully as work oxen.

2.1.2.4 **Adamawa Gudali (Adamawa, The Yola, Tattabareji)** It is thought to have originated from cross breeding of the basic Adamawa Cattle with the Bunaji and Nigerian Shorthorn (Muturu breeds). It is the introduction of Shorthorn blood which makes the Yola cattle different from the Adamawas of the French Camerouns. The breed is found predominantly in the Central Adamawa division and Muri Emirate of the defunct Adawama Province about 700 ft above sea level. It is a medium sized breed averaging about 47 inches behind the shoulder. A mature bull weighs about 8 centiweights. The hump is medium to

small; the dewlap is also small. The horns are short, curving outwards, slightly downwards and upwards. The colour is in mixtures of red, black, dun, brown, blue roan and white either in patches or speckles. It is the speckled olouration of some which gives the name Tattabareji. This breed is in the process of formation as a separate entity.

2.1.2.6 **(The Azaouak, Adar, Azawal).**

This breed is thought to have orignated from the Azaouak country north east of Nigeria and it is distributed all along the extreme northern border of Nigeria. It is a medium-to small-sized animal averaging 48 inches behind the shoulder with a mature bull weighing 8-9 centiweights. While the hump is well developed, in most cases the dewlap is not well developed. The horns are short curving outwards, upwards and forwards.

Colour: Mixtures of red, white, brown and black. The animals are docile and the head is carried low. This breed is used extensively for pack transport. The cows have good milk potentialities. The main centre of this breed is in Niger Republic.

2.1.2.6 **Wadara (Shuwa, Tur).**

The origin of this breed is un- known but they were probably brought to Nigeria by the Shuwa Arabs from Wadai and Dafur. They are distributed mainly in the Dikwa area, south and south-east of Lake Chad. The breed is also used extensively for pack transport and for riding particularly by the Shuwa Arab women.

2.1.2.7 **The Biu:**

This breed found in the hilly volcanic rock area of Biu Emirate in Borno State, may have resulted from crossbreding between the Wadara and the Nigerian shorthorn breeds with an appearance similar to very small Bunaji. It is a small compact animal averaging 43 inches in height with a small cervico-thoracic hump and small dewlap. It has short horns that curve outwards and upwards. The colour is white or white or black points or black markings.

2.1.2.8 **Kuri (Lake Chad, Kouri, Buduma, Jos Koram)**

It is suggested that these Sanga cattle originated in Southern Egypt and migrated across Africa where, in and around Lake Chad they became changed by their environment into the type of animals that they are today. It has also been suggested that these animals are Bos taurus. There is a similarity between them and the Asiatic wild Gaur and the Banteng found in India and Malaysia. They bear little or no resemblance to any other breed in Nigeria but is possible that the Kuri cattle may be African Gaur. They are mostly found in the Islands and marshes of Lake Chad.

The Kuri are large framed animals with an average height of 58 inches behind the shoulder with a mature bull weighing about 11 centiweights. The hump is mostly absent but when present it is small and cervical in location. The dewlap is very small. The horns are very large, bulbous and conical. They vary in size considerably but a 25 inch circumference and 30 inch length is about the average, although in some cases the base circumference may exceed the length. The colour is white with occasional red brown markings. The head is disproportionately large, with rounded frontal area and is carried low.

The breed has large spreading feet and move with a shambling gait. The Kuri are fond of wallowing in water. The breed is not suitable for draft work.

Other breeds include the Keteku (Borgu, Kaiama), Muturu (Nigerian Shorthorn, Dwarf shorthorn), N'dama (Fouta Longhorn, Hamitic Longhorn), Banyo, Ngaundere, Montbelliard and few imported exotic breeds.

2.1.3 Husbandry Practices

Existing systems of husbandry have been reviewed extensively by Osori (1976). There are two basic ways by which systems of management are classified. While one of the ways is based on the intensity of the operation, the other is based on such climatic factors as temperataure and rainfall.

Under the system of classification based on intensity, three husbandry systems exist:

1. Intensive system where planned grazing is supplemented with feeding of good quality concentrates, silage or hay and mineral salt lick. This system is practised only in organized government owned farms, institutional farms, and in large private-owned farms

2. Semi-nomadic system where the owners although having a permanent place of abode move out in search of grazing and water during the dry season. During the wet season the animals are grazed on fallow lands close to the place of settlement of the owners. This system has been reported to provide more cattle needs in terms of feed and water (Van Raay and De Leeuw, 1974)

3. Nomadic system: The owners do not have any permanent place of settlement but are in a state of perpetual seasonal migration; moving from north to south and south to north during the dry and wet seasons respectively. The north to south movement in the dry season is one of necessity in search of grazing and water at the risk of encountering tsetse fly challenge and trypanosomiasis. This nomadic husbandry system has resulted in over grazing of available forage while the north south seasonal migratory movement encourages disease spread.

The distinct seasonality in rainfall has resulted in inadequate year-round availability of pasture. This has further resulted in a pastoral calendar (Van Raay and de Leeuw, 1974) in the Savannah areas of Nigeria that has been divided into 5 phases viz:

1. Dabunde (December to February). This is the "harmatan" period which is characterized by low atmospheric temperature and humidity. Fodder resources become scarce and time spent grazing is longer.

2. Chudu (February to April). During this period grazing conditions have deteriorated to such a level that cattle rely almost soley on browsing. This is a critical and difficult period for cattle production. The feed scarcity is compounded by high ambient temperature resulting in drying up of most sources of livestock water.

3. Seeto (April to June). This is the stormy period with occasional rains that preceeds the rainy season. It is the most critical and difficult period in cattle production because water points are dry at this time and there is nothing on the fields for the animals to browse.

4. Dungu (June to September). This is the wet season and grazing conditions quickly improve. At the peak of the rain fall, Yuka (July to September) the herdsmen and their cattle have to move continuously to relatively drier grazing grounds. In recent years the grazing areas have shrunken considerably due to land cultivation by large scale arable farmers. This situation if left unchecked may result in a situation of "starvation in the midst of plenty" for the livestock.

5. Yamde and Nyaile (September to December). This is the hot dry post-rainy period but forage is still plentiful. However, towards the end of this period, Nyaile, decreasing forage is supplemented by crop residues from sorghum, maize, cotton, millet, rice, groundnuts and cowpeas (Powell and Bayer, 1985). Crop residue supplementation is becoming in- creasingly important such that soon after harvest the residues are gathered and stored for dry season feeding dur- ing the critical months of Chudu and Seeto (Livestock Systems Research Report, 1986).

2.1.4 Reproductive performance of zebu cows in Nigeria

The reproductive performance of a cattle population is assessed by the level of fertility or infertility of the population.

Infertility of the female is generally defined as the temporary failure to conceive, or if conception does occur, the inability to sustain pregnancy for more than the duration of one or two estrous cycles (Drost, 1981) or the inability to produce viable young within a stipulated time characteristic for each species (Jainudeen and Hafez, 1980). Infertility has therefore been rightly considered as one of the most important problems that a cattleman deals with on

a year-to-year basis (Roberts, 1971; Osburn and Kennedy, 1981).

The reproductive performance of a cattle population is best determined when the following criteria are evaluated:

a. age at puberty

b. age at first calving

c. calving cycle (gestation length plus interval between calving and conception)

d. conception rate/calving rate

e. post-partum interval and uterine involution

f. prenatal, perinatal and neonatal mortality (abortions, calf crop)

g. oestrous cycles (ovarian functions and dysfunctions; oestrous cycle lengths and duration of oestrus)

Variations in these performance indices in different breeds of indigenous cattle have been observed by many workers.

2.1.4 1 Age at puberty (AP)

An animal is said to have attained puberty when it is able to release gametes and to manifest complete sexual behavioural sequences. It is basically the result of a gradual adjustment between increasing gonadotropic activity and the ability of gonads to simultaneously assume steroidogenesis and gametogenesis.

In a study of the performance of Bunaji and Friesian x Bunaji crossbred herd in Vom, Knudsen and Sohael (1970) found the age at first successful service to be 40.2 months for the Bunaji heifers and 21.7, 20.8 and 19.5 months for 0.5 Friesian, 0.75 Friesian and

Purebred Friesian heifers respectively. Lamorde and Weinman (1972) in a survey study found the age at first estrus to be 36 months and 22.3 months for local herds and government owned farms respectively. These findings were confirmed by Nuru (1974) who in a similar survey study of six northern states (North-East, North-West, North-Central, Benue-Plateau, Kano and Kwara States) found the age at first conception to be 2.5 years in government herds and 3.5 years in local herds. Adeyemo *et al.* (1979) reported age at puberty ranging from 20 to 25 months in the hot humid zone of Ibadan. Oyedipe *et al.* (1982b) in a study of the effect of level of nutrition on the onset of puberty in Bunaji and Sokoto Gudali heifers found the mean age at puberty for heifers in the high, medium and low protein groups to be 19, 21 and 24 months respectively. From these results, it can be concluded that the age at puberty can be reduced by providing adequate nutrition in terms of quantity and quality.

2.1.4. 2 **Age at first calving (AFC)**

This is the age at which a heifer delivers a calf for the very first time. Variations in age at first calving have been reported. In one of the pioneer studies, Armour *et al.* (1961) reported mean age at first calving of 50 and 32 months for Bunaji and Friesian x zebu cows reared on the Vom Plateau. Other workers reported 42 months (Wheat and Broadhurst, 1958; Wheat *et al.* 1972), 50 months (Lamorde and Franti, 1973), 40 months (Oyedipe *et al.* 1982a), 50-60 months (Voh Jr and Otchere 1989) for Bunaji cows; 43 months for Sokoto Gudali cows (Wheat and Broadhurst, 1972) and 45 months for Wadara cows (Wheat, 1975). Thus, in general, age at first calving have ranged from 30 to 60 months for zebu cows.

2.1.4 3 **Calving interval (CI)**

Calving interval is the time interval between one delivery and the next. Calving intervals have varied: 365 days (Robertson, 1950), 438 days (Wheat and Broadhurst, 1968), 388 days (Wheat and Broadhurst, 1968), 390 days (Knudsen and Sohael, 1970), 411 days (Wheat *et al.* 1972), 365 to 568 days (Wheat, 1975), 369 days and 440 days (Oyedipe *et al.* 1980; 1982; Eduvie and Dawuda, 1986) and 24 months (Voh Jr. and Otchere, 1989). In general, calving intervals have ranged from 1 to 2 years.

In a comparative study of postpartum uterine involution and resumption of ovarian activity in Bunaji and Friesian x Bunaji crossbred cows by rectal palpation, Oyedipe et al.; (1980) found that involution of the uterus and cervix was complete by 16 to 30 days and 17 to 27 days for the crossbreds and Bunaji cows respectively. The mean interval from calving to first oestrus was 19 and 21 days for Bunaji and Friesian crossbreds respectively.

In a similar study, Eduvie (1985) reported completion of uterine involution 26 days postpartum, interval postpartum to detection of first follicle of 45 to 54 days for Bunaji and Sokoto Gudali cows. In a related study Eduvie and Dawuda (1986) reported the mean interval from calving to conception for suckled and non-suckled Buanji cows to be 232 and 73 days repectively. For postpartum Bunaji cows that calved in the rainy and dry season, Dawuda *et al.* (1988) reported mean intervals from calving to first observable oestrus of 153 and 123 days respectively.

The interval from fertile service to parturition is called gestation length. There are few avilable reports on the gestation length of indigenous Nigerian zebu cows. Egbunike and Togun (1980) studied this parameter in Ibadan, Southern Nigeria. They reported an

average gestation length of 291, 281, 281, 278 and 277 days for Brown Swiss, Jerseys, Jersey crossbreds, zebu (Bunaji) and Holstein Friesians respectively. In a more recent study involving artificially inseminated cows, Voh, Jr. *et al.* (1986) found mean gestation lengths of 281, 283 and 282 days, respectively for Bunaji, Sokoto Gudali and Friesian x zebu crossbred cows. The variations were within narrow limits indicating that this parameter is genetically determined and is only modified very slightly within species by maternal, paternal, fetal and environmental factors (Jainudeen and Hafez, 1980).

2.1.4.4. **Generation interval (GI)**

This is the average age of the two parents. Generation interval is determined by age at first calving and calving interval. For Bunaji at Birmin Kudu, Kabomo and Shika the G.I. were found to be 72, 75 and 72 months respectively (Wheat and Broadhurst, 1968; Wheat *et al.* 1972). At Bulassa and Dogondaji, generation intervals were 72 and 100 months for Sokoto Gudali (Wheat and Broadhurst, 1972) and 94 months for Wadara cows at Dalori in Borno State.

2.1.4.5 **Conception rate (CR) and/or Pregnancy rate (PR)**

Is the number of animals pregnant divided by the number inseminated and expressed as a percentage.

a. **Natural Service (NS):**

In an analysis of seasonal variations in the conception rate of two types of West African cattle, Borgu (Keteku) and N'dama, in southern Nigeria, involving 8,971 birth records,

Steinbach and Balogun (1971) reported a mean annual concep- tion rate of 72% over a 10-year period, with monthly varia- tion in CR.

Nuru and Dennis (1976) in a questionnaire survey study on abortion and reproductive performance of cattle in the Northern States reported pregnancy rates ranging from 24 to 36% in local Fulani herds and about 59% in government Livestock Investigation and Breeding Centres (LIBCs). Oyedipe et al. (1982b) showed the effect of level of nutrition on conception in zebu heifers when they reported CRs of 59%, 28% and 17% CRs for the high, medium and low protein groups respectively at the end of a 90-day breeding period. In another study that showed the effect of suckling on postpartum cow, Eduvie and Dawuda (1986) reported 21% and 73% for suckled and non-suckled cows respectively at 60 to 90 days postpartum, with an overall PR of 49%. Voh Jr. and Otchere (1989) in a study of the reprodutive performance of zebu cattle under traditional agropastoral management in northern Nigeria reported a pregnancy rate of 42%.

These studies have shown some of the factors that could affect conception and/or pregnancy rate in a cattle herd. Notable among these are nutrition, season and suckling.

2.1.4.6 **Annual calf crop (ACC)**

Percentage calf crop, defined as the number of calves under 1 year of age divided by the number of females of breeding age and expressed as a percentage, has been reported for indigenous Nigerian cattle. In a survey study involving 17,158 nomadic cattle Lamorde and Weinman (1972) reported ACC ranging from 27 to 55%. In another separate survey study involving 8,970 breeding cows, Nuru and Dennis (1976) reported ACC ranging from 34

to 55% for nomadic Fulani herds and 67% for government-owned LIBCs. Earlier studies by Johnson (1974) using the records of 74 Bunaji cows at Shika Research Station (now NAPRI) and Sohael (1974) in Vom reported ACC of 62% and 100% respectively. Furthermore, the survey conducted by Lamorde and Franti (1973) found that about 17% of cows calved once a year, 12% calved every other year and 71% calved irregularly.

In a more recent study Voh. Jr. and Otchere (1989) re- ported an annual calving rate of 55% for agropastoral herds in northern guinea savanna of Nigeria.

2.1.4.7 **Estrous Cycle**

This is the cyclical interval from the end of one heat period to the beginning of the next. The estrous cycle is divided into four stages viz:

a. proestrus

b. estrus

c. metestrus

d. diestrus

There are a few available reports on the estrous cycle of indigenous Nigerian zebu animals. In a pioneer classical comparative study of estrous cycle in Ibadan over a 15-month period with Bunaji, German Brown and Holstein-Friesian heifers, Adeyemo *et al.* (1979) reported average estrous cycle lengths of 21.4, 21 and 20 days for Bunaji, German Brown and Holstein-Friesian heifers respectively. Generally, the estrous cycle length for the Bunaji ranged from 16 to 29 days with about 95% of the estrous cycle lengths between 18 to 24 days. Duration of estrus in Bunaji heifers ranged from 7 to 30 hours with a mean of 14.6 hr. The

largest proportion of estrus periods (48%) lasted 9-14 hours with most estrus onsets commencing in the morning hours of 0500 to 1200 hours. They also reported that ovulation occurred 15 hours on the average after the end of estrus or 31 hours from the onset of estrus. Although 100% of the estrus periods that occurred were detected, a 17% incidence of subestrus was reported. They however, did not find seasonal effects and the heifers cycled all the year round without showing anestrus, an efficiency which they attributed to adequate nutrition of the animals.

Working in Shika, Johnson and Gambo (1979) reported mean estrous cycle length of 22 days and with most estrus onsets between 0600 and 1200 hours.

Out of a total of 703 estrous cycles studied, Zakari (1981a) reported a mean cycle length of 23 days and mean estrus duration of 6 hr and 11 hr in the dry and wet seasons respectively with 54% of the onsets occurring between 0200 and 1000 hours. He reported seasonality of estrus and anestrus. There have been reports on the occurrence and incidence of silent heat (Richards, 1946; Dawuda *et al.* 1986), anestrus (Richards, 1946; Osori, 1976; Zakari, 1981; Voh, Jr. 1986), split estrus (Voh, Jr. 1986), seasonality in estrous cycle (Osori, 1976; Zakari, 1981).

Richards (1946) and Adeyemo *et al.* (1979) reported the incidence of silent heat to range from 10 to 17%. Thus, both the mean and modal oestrous cycle length were about 21 days for Nigerian zebu cows.

2.1.5 The reproductive pattern

Seasonal variation in the fertility of cattle have frequently been reported (Thibault *et al.* 1966) but the comparison, for instance, the non-return rates reveals a considerable amount of disagreement among various authors. Most of the studies reported previously have been carried out on dairy cattle kept in the temperate climates where management conditions, such as feeding, housing and the breeding season, vary with economic priorities, and where most dairy cattle are, to a large extent, protected from the natural ecological conditions. Thus, seasonal effects may have been obscured by those of management. This argument is supported by reports on zebu cattle which were kept under less intensive management conditions indicating more pronounced seasonal variations in cattle fertility (Wilson, 1946; Steinbach and Balogun, 1971; Zakari, 1981; Voh, Jr. et al 1982; Butterworth, 1983). This indicates that good management obscures seasonal effects which are related to quantity and quality of available nutrition.

Seasonal sexual rhythms are believed to be less pronounced in areas of lower altitudes, the tropics (Marshall 1942) since the variations in temperature and daylight are less pronounced, although Anderson (1948) postulated that in Kenya, at very low altitudes, increased temperatures and sun- light are associated with increased sexual function in cattle.

In an analysis of 8,971 birth records of Keteku and N'dama cattle over a 10-year period in the seasonal-equatorial climate of Southern Nigeria, Steinbach and Balogun (1971) reported a mean annual conception rate of 72%. The monthly conception rate varied significantly throughout the year with the highest number of conceptions occurring in April (7.3%) and the lowest in October (4.4%). Photoperiodism was reported to have contributed

significantly to the total monthly variation. Also Wilson (1946) observed seasonal variations in the incidence of the calving rate of zebu cattle in Nyasaland. He attributed the high conception rates between October and November to the prevailing hot and dry conditions.

In a similar study with unimproved cattle in Swaziland Butterworth (1983) reported a unimodal distribution of calving pattern with peak calving occurring during the period October to December. This was correlated with rainfall 10 months earlier, thus conception coincided with a high nutritional plane.

In a study of the reproductive aspects of sedentary cows in central Mali, Wilson (1985) also reported a unimodal distribution of calving pattern with peak calving between April and June, with a very highly significant correlation between these births and rainfall 9 and 10 months previously. He concluded that nutritional stress was the main cause of low reproductive rates and of the seasonal pattern of births.

Similarly, in a study of Kenana cattle in Sudan, Saeed *et al.* (1987) reported the distribution of 3,988 births over a 27-year period. They also reported a unimodal distribution of calving with peak calving taking place in December and January and least between July and September.

Investigating the productivity of transhumant Fulani cattle in the inner Niger delta of Mali, Wagnenaar et al. (1986) reported a pronounced calving peak in the early rainy season (June, July) which was related to improved body condition during the latter part of the previous rainy season. A lesser peak was also observed in the cold dry season (January and February) which they attributed to intake of high-quality regrowth or grass availability in the northern delta. These findings reinforce findings earlier reported by Coulomb (1972)

and Diallo (1978) for Fulani cattle in Sudan and Pullan (1979) and Otchere (1983) who found similar sea- sonal patterns for the Bunaji in Nigeria.

Otchere (1986) reported bimodally distributed calvings and conceptions with peak conceptions occurring between No- vember and February with another but smaller peak between June and August for Bunaji cattle in Southern Kaduna.

In a more recent report (Voh Jr. and Otchere, 1988) reported a unimodal distribution of calving with peak calving taking place between December to March corresponding with conceptions having taken place in the dry months of March to June in Giwa, Zaria, which is in the northern guinea savanna ecological zone.

The effect of season of breeding on conception rates is not limited to natural service situations alone. Similar seasonal effects have been reported even in artificial insemination programmes, with higher pregnancy rates when animals are inseminated in the wet season than in the dry season (Zakari, 1981; Voh, Jr. et al. 1984). This seasonality was attributed to differences in nutritional planes during different seasons.

2.1.6 Factors affecting reproductive performance

Among the major constraints that impede the productivity and reproductive efficiency of zebu cattle, long age at first calving and long calving intervals are of paramount importance. A number of factors are known to affect this reproductive traits. Broadly these include genetic (Armour *et al.* 1961) and environmental factors. Prominent among the environmental factors are the climate (Knudsen and Sohael, 1970; Steinbach and Balogun, 1971), season (Steinbach and Balogun, 1971; Voh, Jr. *et al.* 1984), management (Voh, Jr.

et al. 1984) nutrition (Oyedipe *et al.* 1982, Voh, Jr. and Otchere 1989), selection (Buvanendran, 1979), suckling (Eduvie, 1985; Eduvie and Dawuda, 1986, Voh, Jr. and Otchere 1989, diseases (Nuru and Dennis, 1976) and breed (Wheat *et al.* 1972). These factors affect the fertility index of the herd.

2.1.7 Reproductive performance of zebu cattle in Tropical countries

A lot of reports concerning zebu cattle in other tropical countries have shown similarity between them and their Nigerian counterparts.

Various studies on zebu cattle have been conducted in other tropical countries (Mahadevan and Marples, 1961; Mahadevan et al. 1962; Mahadevan and Hutchison, 1964; Butterworth, 1983; Wilson, 1985; Amble *et al.* 1958a,b; Alim, 1962; Rahka *et al.* 1971; and Garcia, 1988). Most of the reports show that mean age at first calving, calving interval, length of dry period, length of lactation and lactation milk yield to be around 40 months, 420 days, 150 days, 230 days and 200 gallons respectively.

The same factors that affect Nigerian zebu cattle affect zebu cattle in other areas of the tropics, such as breed and environmental factors (Mahadevan and Marples, 1961; Buck and Light, 1982) and season (Butterworth, 1983; Wilson, 1985, Garcia, 1988).

These reports reflect the influence of genetic and environmental and an interplay of these factors on the reproductive performance of zebu cattle in general and Nigerian zebu in particular.

2.2 TECHNIQUES FOR CONTROLLED BREEDING

Some publications, Hansel and Beal (1979), Britt and Roche (1980), Roche (1977 and 1979), Roche *et al.* (1981) and Voh, Jr. (1984) have reviewed techniques used in the control of estrus in cattle.

Historically, knowledge in estrous cycle control has been developed in four distinct phases characterized successively by the use of:

i. synthetic progestational agents

ii synthetic progestational agents combined with estrogen or gonadotropins

iii prostaglandin F2 alpha

iv prostagland F2 alpha combined with progestational agents

The bovine estrous cycle is controlled mainly by the secretion of progesterone from the corpus luteum (Edqvist and Kindahl, 1980). Thus, effective synchronization of estrus necessitates control of the life span of the CL either by causing precocious regression of the existing CL or by delaying the periovulatory endocrine events until the corpora lutea have regressed. This is because progesterone secretion from the transitory CL dominates the major part of the cycle, the luteal phase. In the non-pregnant animal, the CL regresses rapidly between day 16 and 17, progesterone levels fall and estrogen, FSH and LH levels rise resulting in estrus and ovulation within 2 to 4 days of regression of the CL (Roche, 1976).

Practical methods of estrous cycle control in cattle are based on either (a) preventing ovulation (Roche, 1975, 1978) or (b) by causing regression of the CL with luteolytic substances (Kindahl and Edqvist, 1980; Voh, 1987). Thus, this review will discuss the present status of estrous regulation in cattle with particular reference to these two methods.

2.2.1 Control of estrus using progesterone

Since the first report in 1960 (Hansel and Malven, 1960), synthetic compounds having progestational activity, such as 6-methyl-17-acetoxyprogesterone (MAP), chlormadionone acetate (CAP), melengestrol acetate (MGA), fluorogestone acetate (FGA), an 17&-acetoxy-11B-methyl,19-nor-preg-4-ene-20-dione (norgestomet) have been adminis- tered by a variety of routes including oral administration, whether in the feed or in the drinking water (Wiltbank and Kasson, 1968), as subcutaneous implants (Wishart and Young, 1974; Thimonier *et al.* 1975), intravaginal sponge pessaries (Sreenan, 1975), intravaginal silastic coils (PRID) (Roche, 1976) or by intramuscular injection (Higgins *et al.* 1986). Some of these routes of administration have their limitations. For instance, oral administration is not practical under pastoral conditions and it is difficult to ensure constant intake of the drug in each animal under group feeding. Intravaginal administration of progestagens especially in pessary form has low retention rates especially in cows.

Length of progesterone administration

The length of time of administration of progesterone has been reported to affect both response and fertility rates (Roche, *et al.* 1981).

i. **Long-term administration (18-21 days)**

The classical method of controlling the corpus luteum has been to administer progesterone for the period of an estrous cycle i.e 18-21 days. Roche (1974b) found that progesterone given for 18-21 days effectively synchronized estrus but the conception rates for animals inseminated at the controlled estrus were significantly lower than for con- trols.

Many other experiments in which progestagens were used whether orally, by injection or by implants have given similar poor results (Hansel and Beal, 1979).

ii **Medium-term administration (12-14 days)**

It has been shown that there is an interaction between the requirement for estrogen and the length of progesterone treatment (Roche, 1978). When treatment period is extended to 14 days, estrogen is not required to synchronize estrus. However, in field trials using dairy cows, fertility following 14 days of progesterone treatment was significantly lower than in control cows or in cows which had had a 12-day progesterone treatment with estrogen given at the start of treatment. However, other works have shown no reduction in fertility following one or two fixed-time insemination after 12-or 14-day treatment periods (Ellicott *et al.* 1977).

iii **Short-term progesterone treatment (7-9 days)**

Using progesterone or progestagens to synchronize estrus in cattle, the apparent key to obtaining normal fertility to matings at the synchronized estrus is to limit the period of administration of the progesterone to between 9 and 12 days as first described by Roche (1974a). However, the results of a series of experiments conducted by Roche (1978) indicated that a luteolytic agent is required at the start of treatment when a 9-12 day treatment period is being used. The luteolytic agent initially used was 5mg oestradiol valerate (Wiltbank and Kasson, 1968) injected at the start of treatment. Even when oestradiol benzoate was used at the start of a 12-day treatment, the onset of estrus was delayed in 10-20% of treated animals (Roche, 1974a). Animals with progesterone concentrations greater than 2ng/ml had a delayed onset of estrus when compared to animals with lower levels (Roche and Gosling, 1977). In

a further research to determine whether the delay in onset of estrus after coil removal was due to poor control of luteal function or to variation in follicular growth, Roche (1982) concluded that the major reason for the variation was poor control of luteal function. The stage of the estrous cycle at the start of a 12-day progesterone treatment affects the subsequent response. Animals treated between Day 0 and 5 of the estrous cycle are most likely to have delayed estrus (Roche, 1974a). However, Woods *et al.* (1967) reported that progesterone shortened the estrous cycle in animals treated between Days 0 and 3. Ginther (1970) showed that progesterone was less effective in shortening the oestrous cycle as the interval between injection and the previous oestrus increased and no effect occurred when progesterone was given after Day 3. Oestrogens can shorten the estrous cycle (Wiltbank, 1966) and oestradiol or oestradiol-progesterone combinations can function as a luteolytic complex at the start of a 9-12 day progesterone treatment.

The need to use progesterone in the luteolytic complex is determined by the speed of the initial rise in progesterone. It is important to get the concentration of progesterone close to mid-luteal phase concentrations (3-6ng/ml) as soon as possible to prevent the oestrogen inducing ovulation in animals in the follicular phase (Roche, 1974) and to produce a maximum luteolytic effect in animals that have recently ovulated (Ginther, 1970). Therefore when progestagen implants are used extra progesterone is required at the start of treatment (Roche, 1974b) but with silastic coil the initial release rate is sufficient (Roche and Gosling, 1977). If a treatment period of less than 12 days is used, the onset of estrus is more variable. Significantly more heifers and dairy cows were observed in estrus within 3 days of coil removal after a 12-day treatment period compared to a 9-day treatment period (Roche, 1978).

Fertility in animals bred at estrus after 9 or 12 days of treatment was similar to that obtained in control cows.

2.2.2 Control of estrus using progestational agents plus estrogens or gonadotropins

In order to take advantage of the higher fertility associated with a 9-or 12-day treatment the poor estrus noted by Roche (1974a) had to be overcome. This was achieved by combining a luteolytic agent with a short-term treatment with a progestational agent. This followed the demonstration by Wiltbank *et al.* (1961) that a single injection of 5mg of estradiol valerate (E2V) was capable of regressing corpora lutea in heifers treated on days 2 to 3 or 7 to 8 of the cycle. When the luteolytic effect of E2V was combined with a 9-day feeding programme of a progestational compound (Wiltbank and Kasson, 1968), fertility equal to untreated controls was obtained in the treated animals. Ninety-five percent (95%) of the treated heifers were observed in estrus, and 52% of both the treated and control heifers became pregnant after a single service. Later, Roche (1978) conducted a series of 4 experiments to determine the effect of length of treatment, stage of cycle at start of experiment and administration of estradiol benzoate or progesterone coils on estrus re- sponse and fertility.

The results of this series of experiments indicated that when the progesterone coil is being used to synchronize estrus, estrogen must be administered at the start of treat- ment when a 9-or 12-day treatment period is used. Fertility in treated cows is similar to that in control cows. The method of administration of estrogen can be simplified by attaching a gelatin capsule containing the hormone to the inside of the coil, thus obviating the necessity to inject the animals. Based on the luteolytic effects of E2V and data suggesting that

injections of large amounts of progesterone early in the estrous cycle shortened the life span of the CL (Woods, *et al*,1967) a method of synchronization was developed. This consisted of single injections of 3mg of norgestomet and 6mg of E2V given at the same time a 6mg norgestomet implant was inserted. The implants were left in place for 9 days. This treatment resulted in excellent synchronization of estrus in cycling animals and conception rates similar to those of untreated control animals (Spitzer et al. 1976). The same treatment was claimed to induce precocious puberty in prepubertal heifers (Gonzalez-Padilla *et al*. 1975) and induce estrus in anestrous lactating beef cows (Zaied et al. 1976). Hormonal asynchrony has been advanced as the commonest cause of lowered conception rates reported in some of the studies. The most frequently noted kinds of asynchrony were premature or greatly delayed LH peaks with respect to the onset of estrus. Animals with "normal" hormone profiles associated with the synchronized estrus had conception rates of 60%. A "normal" profile was described as low plasma progesterone levels on the day following progestagen withdrawal, a peak in plasma LH occurring not earlier than four hours before and not later than two hours after the onset estrus and peak plasma estrogen levels at or slightly before the onset of estrus (Coulson *et al*, 1979; Harrison *et al, 1985; Voh, Jr et al 1987b)*.

Treatments with combinations of gonadotropins and progestational agents in cattle have met with little success. When attempts were made to control ovulation through combined injections of human chorionic gonadotropin (HCG) and progesterone implants (Roche, 1974b) or orally active progestational compounds (Roche and Crowley, 1973), concep- tion rates of the treated animals were well below those of untreated controls. Injections of

pregnant mare serum gonadotropin (PMSG) into heifers 15 days after administration of crystalline progesterone in a starch suspension resulted in only one of eight heifers becoming pregnant (Hansel and Beal, 1979).

2.2.3 Control of estrus using Prostaglandin F2 alpha and its analogs

The first reports of the luteolytic effects of PGF2& in the cow appeared in 1972 (Hansel and Schechter, 1972; Liehr *et al.* 1972; Louis *et al.* 1972 and Rowson *et al.* 1972). These reports all indicated that exogenous PGF2& was luteolytic when given between days 5 and 16 of the estrous cycle and that estrus and ovulation followed injection of a luteolytic dose by 3 to 4 days. A number of stud- ies on the use of PGF2& or its analogs (particularly the 16-aryloxy-prostaglandins, such as ICI 80996) have since been reported (Lauderdale, 1972; Lauderdale *et al.* 1974, Cooper, 1974, 1976; Edqvist *et al.* 1979; Roche and Prendiville 1979; Hardin *et al.* 1980a,b; Macmillan and Day, 1982; Orihuela *et al.* 1983; Voh, Jr. 1984; Voh, Jr. *et al.* 1987c). Dose rates used by the above authors were 25 to 30mg or 500ug of PGF2& by injection or intrauterine infusion of 1 to 4mg in divided doses 24 hours apart or a single infusion of 5mg. Louis *et al.* (1974) showed that a 5mg infusion of PGF2& into either the ipsilateral or contralateral horn of the uterus resulted in dramatic luteolysis, followed by increased estrogen secretion and an ovulatory surge of luteinising hormone (LH) coincidental with the onset of estrus and ovulation. No residual influence of PGF2& was detected on estrous cycles subsequent to PGF2& treatment.

Lauderdale *et al.* (1974) injected heifers with 50mg PGF2& and inseminated them either at synchronized estrus or at fixed times approximately 72 and 90 hours after PGF2& treatment. The distribution of estrus following treatment was over 7 days with

49% occurring on the third day. Pregnancy rates of cows inseminated twice at fixed times were 53.3%, 52.2% and 55.8% respectively.

Administering PGF2& either as a subcutaneous injection (8 or 10mg) or intrauterine infusion (dose 5mg), Donaldson (1977) successfully synchronized estrus in beef cattle. Synchronization of estrus was distributed over 7 days with the majority of cows being in estrus on the third (42.3%) and fourth (22.4%) days. The route of administration of PGF2& (injection or infusion) did not affect estrus synchronization nor the fertility of cows inseminated at either the synchronized or subsequent estrus. Pregnancy rates at the synchronized estrus were higher when cows were inseminated after detection of estrus than when they were inseminated without reference to estrus at a fixed time 3 days after PGF2& treatment.

Roche and Prendiville (1979) carried out trials on 1,184 dairy cows which had calved at least six weeks before treatment and 255 heifers to determine the effectiveness of cloprostenol to control estrus. In trial 1 following two injections of cloprostenol given 12 days apart, there was no difference in calving rate following A.I. either at 72 and 96 hr after treatment or at detected estrus compared to control cows bred at estrus. In trial 2, treated cows were injected once after 5 to 7 days of estrus detection and A.I. The calving rate following A.I. either at 72 and 96 hr after cloprostenol or at a detected estrus was similar to that in control cows bred at estrus. In trial 3, cows were bred at a detected estrus after the first cloprostenol injection. Twelve days after this injection, cows not bred were given a second injection and bred 72 and 96 hr later. The calving rate in treated cows bred at estrus after the injection was similar to calving rate in controls. However, calving rate in

cows given a second injection and bred 72 and 96 hr later was significantly lower. Similar results were obtained in heifers, except that calving rate in trial 3 after the second cloprostenol injection was not reduced.

Cooper (1974) controlled the estrous cycle of heifers with a synthetic prostaglandin analogue, cloprostenol (ICI 80,996). He found it to be a highly potent luteolytic agent, effective in a single intramuscular dose of 500ug, provided that it is administered after day 4 or 5 of the estrous cycle and before the corpus luteum begins to regress spontaneously on day 16 or 17. Two such injections, given 10 to 12 days apart to heifers exhibiting regular but random cycles, would be expected to synchronize estrus periods in all of them. The animals were in estrus between 48 and 72 hours after the second injection. Ovulation occurred normally at the syn- chronized heat; fertility to A.I. was also normal, and no side-effects were noted.

Results of these experiments and many others show that a lot of research work has been carried out in the area of syn- chronization of estrus with prostaglandins. This is partly because the response to PGF2& is influenced by such factors as season, breed and stage of the estrous cycle at treatment, lactational status, brand of PGF2& used and so on indicating clearly that estrous cycle control with PGF2& and its analogues can not be transposed but have to be established for each situation separately.

2.2.4 Control of estrus using PGF2& combined with progestational agents

The most recent efforts in estrous cycle synchronization have involved the use of intramuscular injections of PGF2& in combination with a progesterone-releasing intravaginal device (Roche 1976; Hansel and Beal, 1979) or intramuscular injection of progesterone

(Higgins et al. 1986), or progestagen implants (SC 21009, Searl) (Chupin *et al.* 1977).

In a comparative study, Thimonier *et al.* (1975) tried several methods for synchronization of estrus in groups of cyclic cows or heifers. A progestagen treatment for a period equal to that of the luteal phase resulted in a high degree of synchronzation with 90% of treated females in estrus within a 48-hr period. Artificial insemination without detection of estrus was feasible but fertility was generally low (34%).

A short progestagen treatment (9 to 10 days) in combination with a 5mg estradiol valerate injection on the first day resulted in only 68% females in estrus within 5 days after end of treatment and 58% during a 48-hr period. Insemination without detection of estrus was prohibited by the high proportion of females not synchronized, but fertility to A.I. at induced estrus was higher, 56%, than after the long progestagen treatment.

Two intramuscular injections of 500ug of PGF2& analogue (ICI 80,996) at an interval of 10 days or SC implant of 12mg of SC 21009 (progestagen) for 10 days plus an intramuscular injection of the PG analogue at implant removal gave a similar degree of synchronization with 70% of treated females in estrus within a 48-hr period.

With the progestagen-prostaglandin analogue combination, the degree of synchronization was increased. The interval from the end of treatment to onset of estrus was short (48hr) when the level of endogenous P4 was less than 0.5ng/ml at the end of the progestagen treatment. This can be obtained in all treated females with a prostaglandin analogue injection 48-hr before SC 21009 implant removal. Thus, 92% of treated females were in estrus during a 48-hr period. Artificial insemination can be done without detection of estrus. They concluded that a reduction in the length of progestagen treatment could obviate side effects

of progestagens. A minimum 7-day progestagen treatment should be sufficient with the prostaglandin injection being given two days before the end of this period.

Roche (1976) compared the fertility in cows after treatment with a prostaglandin analogue with or without progesterone. Two injections each of 500ug cloprostenol (ICI 80,996), a synthetic analogue of $PGF_{2\alpha}$, 11 or 12 days apart or a pre-treatment for 7 days with progesterone from an intravaginal silastic coil and one injection of 500ug cloprostenol were both effective in synchronizing estrus in heifers or nursing beef cows. After two inseminations at 72 and 96 hr after the end of treatment, the calving rate for cows observed in estrus after treatments or for cows that had estrous-like mucus after progesterone plus cloprostenol treatment did not differ from that of control cows, but was significantly lower for cows diagnosed as ready for insemination by the characteristics of mucus after the two injections of cloprostenol. Treated cows diagnosed per rectum as having inactive ovaries had significantly lower calving rate than those diagnosed as having active ovaries at the start of treatment. Significantly more treated than control cows were inseminated and became pregnant in the first 15 days of the treatment period, but the overall level of reproductive efficiency was low.

Variable fertility results have been reported following oestrus synchronization with prostaglandin F2 alpha with and without simultaneous use of progestagen implants (Chupin *et al.* 1977). Synchronization of oestrus was tighter following combined treatments than after prostaglandins alone (78.7% in oestrus in 48 hours vs 60.2%) with the best result when prostaglandin F2 alpha was injected 2 days prior to implant withdrawal. They concluded that treatment with a 7-day norgestomet implant plus a single injection of PGF2& 24 hr

before or at implant withdrawal appears to be a practical technique for synchronizing estrus in cycling heifers without affecting conception. Similarly, Beal (1983) reported that suckling post-partum cows treated for estrous synchronization exhib- ited estrus and became pregnant earlier in the mating period than untreated cows.

In another report, Higgins *et al.* (1986) conducted two trials to evaluate treatments combining progesterone and PGF2& on estrus response, pregnancy and calving rate in heifers and found that: 1) control (T1), 2) 25mg PGF2& on Day 0 (T2), 3) 150mg P4 im in corn oil on Day-24 plus PGF2& (T3) and 4) 150mg P4 on Day-5 plus PGF2& (T4). Trial 2 had T2 and T4 only. Heifers were artificially inseminated 8 to 16 hr after detection of estrus for 10 and 5 days in trials 1 and 2, respectively. They concluded that progesterone pretreatment combined with PGF2& appeared to enhance estrus synchronization without adversely affecting either pregnancy or calving rates. They, however, admitted the need for future research to determine optimal timing of appointment breeding following PGF2&. If such trials, they contended, can demonstrate a uniform, repeatable synchronization response and desired fertility following P4 injection plus PGF2& treatment, then a more convenient method of progesterone pretreatment than those involving subcutaneous implants or intravaginal devices may be possible. Thus, this volume of data has clearly demonstrated that progestagens employed in combination treatments to date have included norgestomet in subcutaneous implants, or progesterone-releasing intravaginal devices (or intramuscular injections of progesterone) for 5 to 9 days followed by a single PGF2& injection before, at, or after withdrawal of progestagen results in increased proportions of animals exhibiting estrus, improved synchronization, and reduced intervals to onset of estrus when compared with a

single or double PGF2& treatment. Furthermore, intramuscular injec- tions of progesterone prior to administration of PGF2& are a plausible alternative to the other routes of progesterone administration. The i.m route of administration has been based upon the demonstration by Ray *et al.* (1961) that single dosages of P4 (400 to 500mg) administered i.m on Day 8 or 16 of the estrous cycle in beef heifers prolonged cycle length for up to 12 days, but this treatment reduced ova viability. Exogenous P4 can either prolong or shorten the estrous cycle length depending upon stage of the cycle at administration.

2.2.5 Other techniques for controlled breeding

Methods other than the use of prostaglandin F2& and progestagens that have been used at one time or the other to control and/or synchronize oestrus have been reviewed (Voh Jr. 1984) and include:

2.2.5.1 Gonadotropin-releasing hormone (Gn-RH)

Gn-RH injection of cattle induces release of both LH and FSH (Schams *et al.* 1974; Foster *et al.* 1980b). Attempts to induce ovulation in post-partum cows have been made by single intramuscular injections of 100-500ug Gn-RH with variable results (Schams *et al.* 1973; Lamming and Bulman 1976). Administration of the above dose levels of Gn-RH, will result in LH release of preovulatory surge magnitude depending on the responsiveness of the pituitary. Ovarian follicles requre a two-to three-day period of rising plasma LH concentrations in order to mature fully prior to ovulation. A preovulatory LH surge will therefore induce ovulation only if a follicle at the appropriate stage of development is

already present. Alternatively, the induced LH release might result in premature luteinization of an unovulated follicle and transient secretion of progesterone sufficient to initiate ovarian cycles.

Some methods of administration have been tried. Webb *et al.* (1977) gave 2x500ug Gn-RH at 10 days interval while Lofstedt *et al.* (1981) infused 15ug/h Gn-RH intravenously for 12 hr into postpartum cows. Peters (1981) on the other hand used frequent intravenous injections of 10ug Gn-RH. The results obtained from these studies have been variable and inconclusive.

2.2.5.2. **Gonadotrophins (Pregnant Mare Serum onadotropin-PMSG and Human Chorionic Gonadotropin-HCG):**

Several workers have attempted to induce ovulation and ovarian cycles in cows using exogenous gonadotrophins PMSG and HCG (Roberts, 1971). Results have been variable since some animals do not respond, some ovulate and then revert to anoestrus; and in addition PMSG induces multiple ovulations in a proportion of cases depending on the dosage used. These problems preclude the use of this method for practical purposes.

2.2.5.3 **Estrogens:**

It has been known for some time that injections of doses of estradiol may induce oestrus in anoestrus cows. However, the use of estrogens for this purpose has not been particularly successful since anovulatory oestrus often occurs (Arthur, 1964). Exogenous estradiol is able to elicit a preovulatory-type LH surge release (possitive feedback mechanism) during the early postpartum period although ovulation and ovarian cycles do not consistently follow (Roche and Gosling, 1977; Radford *et al.* 1979).

Furthermore, estrogen causes premature luteal regression if it is injected from day 2 to day 12 of the oestrous cycle. However, Loy *et al.* (1960) reported decrease in weight of CL without an alteration of progesterone between days 1 and 13 of the oestrous cycle. Results have been variable, being affected by type of estrogen and the stage of the oestrous cycle at time of administration. The high incidence of cystic ovaries, anovulatory estrus and very poor fertility rates at the induced estrus makes this method impracticable.

2.2.5.4 **Uterine irrigation with irritants**:

Uterine infusions with such irritants as iodine have been known to affect oestrous cycle length. Infusions performed early, late and middle of the oestrous cycle, respectively, shortened, lengthened and did not affect the oestrous cycle. The mechanism of shortening the cycle has been associated with the release of PGF2& (Nakahara *et al.* 1971; Kindahl *et al.* 1977).

2.2.5.5 **Manual removal or enucleation of the CL**:

This has been used as a luteolytic technique (Casida, 1964). Enucleation at days 7 to 17 of the oestrous cycle resulted in estrus within 2-6 days in most normal cows. The obvious practical shortcomings of this technique which include difficulty in diagnosing per rectum, development of cystic ovaries post-estrus and oophoritis, have precluded the routine use of the technique (Zimbelman, 1964; Hansel, 1965; Donaldson *et al.* 1970; Zemjanis, 1970).

2.2.5.6 **Use of electrical impulses:**

The method involves the application of sinusoidal impulses to the hypothalamus or thalamus. Estrus response and pregnancy rates of 87% and 84% respectively were obtained when 60 Romanian Brown and crossbred cows were synchronized using this method (Stan, 1980).

2.3 PROSTAGLANDIN F2 ALPHA IN ANIMAL REPRODUCTION

2.3.1 Endocrinology of cows treated with PGF2&:

Prostaglandin F2& and its synthetic analogues have been available commercially and have been extensively used for estrus synchronization in cattle (Voh Jr., 1984; Peters, 1986). The sequence and timing of endocrinological changes following exogenous administration of PGF2& are similar to those following the endogenous release of PGF2& during the luteal phase of the oestrous cycle as well as those following manual enucleation of the corpus luteum (Dobson *et al.* 1977; Schams and Karg, 1982). After injection of PGF2& (single or double injection) the corpus luteum shows rapid morphological regression associated with significant precipitous fall in plasma progesterone concentration within 6-24 hr after treatment with basal values being attained within 24-48 hr. These changes have been observed for both Bos taurus and Bos indicus females (Dobson *et al.* 1975a; Coulson *et al.* 1979; Schams and Karg, 1982; Harrison *et al.* 1985; Jimenez *et al.* 1985; Voh Jr. *et al.* 1987b). Following PGF2& injection there is a dramatic increase in plasma prolactin and oxytocin concentrations within 5 to 30 minutes after (Schams and Karg, 1982). How PGF2& causes the release of prolactin and oxytocin is not fully understood but may be either by inhibiting the release of dopamine, which is the inhibitory hypothalamic hormone for

prolactin (prolactin inhibitory factor PIF) or by stimulating a release of the postulated prolactin releasing factor. The neuroendocrine regulation of oxytocin is even more unclear (Schams and Karg, 1982) although there is some evidence of dopaminergic link in the control of oxytocin release (Schams and Karg, 1982 citing Fluckiger and Del Pozo, 1980).

Following the administration of PGF2& there is a small increase in LH about 6 hr after treatment with a preovulatory surge occurring about 60 to 100 hr after the first injection and 48 to 62 hr after the second injection which is then followed by ovulation (Coulson *et al.* 1979). The increase in LH is concurrent with the decrease of progesterone concentrations (Dobson *et al.* 1975a; Coulson *et al.*; 1979; Schams and Karg, 1982).

There is usually no clear change in FSH concentrations either before or after PGF2& injection (Schams and Karg, 1982). Estradiol concentration in large follicles remains elevated until 24 hr, then declined steadily until 72 hr post injection (Harrison *et al.*, 1985). Dobson *et al.* (1975a) reported a small peak of estradiol -17B 4 to 6 days after induced ovulation, similar to what has been reported for cattle after natural ovulation (Dobson *et al.* 1973; Glencross *et al.* 1973). The origin of estradiol has been suggested to be from the growing follicles present at the time which may progress and ovulate rather than regress. This may account for the observation that the induction of oestrus is more rapid and precise following the second than first injection of PGF2&.

Luteal levels of progesterone were attained by days 5 to 6 for zebu cattle reaching maximum mean levels by day 12 to 13 (Jimenez *et al.* 1985; Voh Jr. *et al.* 1987b).

In studies that compared different PGF2& analogues (Schams and Karg 1982) and Bos taurus and Bos indicus females (Jimenez *et al.* 1985), there were no differences between the different analogues and the pattern of progesterone. Nevertheless, significant difference was established between breeds (Indobrazil vs Brown Swiss) in respect to progesterone levels before and after PGF2& injection (Jimenez et al. 1985).

2.3.2 Comparative reproductive endocrinology of Bos indicus and Bos taurus:

Although the endocrine regulation of reproductive functions in zebu cattle has not been sufficiently documented there are a few available reports that have compared the reproductive endocrinology of Bos indicus with that of Bos taurus breeds. Bos indicus females have been reported to have a shorter period of standing estrus than do Bos taurus females (Anderson, 1944; Plasse *et al.* 1970). Estrogen has been shown to be the primary stimulus for estrus and for the preovulatory LH surge (Randel, 1984). Ovariectomized Bos indicus females are less responsive to exogenous estradiol than are ovariectomized Bos taurus females. Ovariectomized Bos indicus females failed to accept heterosexual mount at any dose level of estradiol - 17B and showed lower homosexual activity than Bos taurus and Bos taurus x Bos indicus females (Rhodes and Randel, 1978). Duration of estrus was equally shorter in ovariectomized Bos indicus females than in ovariectomized Bos taurus. Furthermore, Bos indicus females required a longer time from injection of estradiol - 17B to estrus than did the Bos taurus or Bos taurus x Bos indicus females (Randel, 1984).

The endogenous rise in serum estrogens has been found to occur 24 hr, 18 hr and 8 hr before estrus in Bos indicus, Bos indicus x Bos taurus and Bos taurus females respectively (Randel, 1980). The possibility exists that follicular growth patterns and estrogen secretion

or metabolism patterns are somewhat different in Bos indicus females. Behavioural pattern in estrus expression certainly differ between Bos indicus and Bos taurus females (Randel, 1984).

Timing of physiological events leading to ovulation have shown that Bos indicus females had the preovulatory LH surge earlier in relation to estrus and ovulated earlier after the onset of estrus than did Bos taurus or Bos indicus x Bos taurus females (Randel, 1976). Furthermore, the data by Randel (1976) indicate that the Bos indicus female has a smaller preovulatory LH surge, which occurs earlier in relation to the onset of behavioural estrus compared with Bos taurus or Bos taurus x Bos indicus females.

Corpora lutea have been reported to be more difficult to detect per rectum (Plasse *et al.* 1968), weigh less (Irvin *et al.* 1978), are smaller, contain less progesterone in both heifers (Irvin *et al.* 1978) and cows (Hansen *et al.* 1982) in Bos indicus females than in Bos taurus females. These reports along with those of Randel (1974), Jimenez et al. (1985), have indicated that serum levels of progesterone were lower in Bos indicus females compared with Bos taurus and Bos taurus x Bos indicus females.

2.3.3 Physiologic aspects of PGF2& in reproduction

The ability to measure PGF2& metabolite in peripheral circulation made it possible for the role of PGF2& to have been elucidated during physiological and pathological conditions such as oestrous cycles, pregnancy, parturition and postpartum. To date all physiologic, therapeutic and practical applications of PGF2& in cattle are based on its ability to in- duce luteolysis, which means therefore that the presence of an active CL is required in each case that PGF2& is applied.

2.3.3.1 **Prostaglandin during the Estrous Cycle:**

A common reproductive pattern in large domestic species (bovine, ovine, porcine and equine) concerns the way in which non-pregnant animals terminate luteal activity to develop new waves of follicle(s) and thus return to a potentially fertile state. PGF2& is released from the uterus in a pulsatile manner during luteolysis in the cow (Kindahl *et al.* 1976). Initially it was difficult to evaluate if PGF2& caused luteolysis or if decreasing progesterone levels stimulated the PGF2& release (Kindahl and Edqvist, 1980). It has since been confirmed that PGF2& initiates luteolysis and is released as long as progesterone levels are elevated above a low and critical level (Kindahl *et al.* 1976).

PGF2& is released from the uterus in a pulsatile manner during luteolysis in the cow, at approximately 14 days after ovulation. It is conveyed to the ovary or CL by local utero-ovarian vein to the ovarian artery via a counter current exchange mechanism (Hansel, 1975; McCracken, 1971) or by a systematic blood vascular route. The patterns of release of PGF2& in the cow occur in well-defined surges of about 6 hr duration, with luteolysis requiring about 36 hr (Kindahl *et al.* 1976, 1979). Occasional time lapses have been reported to occur between synthesis and release resulting in partial recovery of luteal function (Kindahl et al. 1977). The result of this is a longer duration of the oestrous cycle for about 12-24 hr (Stabenfeldt, 1980).

The mechanisms involved in initiating PGF2& release at the end of the luteal phase are not precisely understood. But in the light of present knowledge it is safe to assume that PGF2& synthesis and release is triggered by estrogens secreted from the ovary and, once started, is maintained by both estrogens and progesterone or progesterone alone and finally

only progesterone level being crucial for termination of PGF2& release (Kindahl *et al.* 1976, 1979, 1980).

2.3.3.2 **Prostaglandin levels in pregnant animals**

One essential feature of the establishment of pregnancy in the large domestic species, as well as many others, involves the prolongation of the luteal function beyong the approximately 2-week duration that occurs in non-pregnant animals. The basic mechanism appears to involve suppression of the release of luteolytic amounts of PGF2& into the blood vascular system (Kindahl *et al.* 1976). The patterns of PGF2& release observed immediately prepartum suggest that PGF2& has an essential role in the delivery process. Fairclough *et al.* (1975) and Edqvist *et al.* (1978) found marked increase in PGF2& in peripheral blood (through the metabolite) or utero-ovarian blood (through PGF2&) just prior to parturition respectively. The first marked increase in PGF2& occurred 48 hr before parturition, with progesterone concentrations markedly decreased by 24 hr before parturition (Stabenfeldt *et al.* 1970; Edqvist *et al.* 1978). The PGF2& concentrations continued to increase after luteal regression. This probably has a role in fetal presentataion by increasing myometrial contractility. Thus, the release of PGF2& is the key hormonal event in terminating the luteal function in the pregnant cow.

The control mechanism for PGF2& synthesis at the expected time of parturition in the cow is not fully known. One possible factor which might trigger the PGF2& release is the rise in estrogen levels (Challis *et al.* 1972).

2.3.3.3 **Prostaglandin during the Postpartum period**

Prostaglandin F2& is released for a considerable time period, 10-20 days, after parturition in the cow (Edqvist *et al.* 1981). A similar sustained release of PGF2& has also been reported following dexamethasone-induced premature deliveries (Lindell *et al.* 1977) but differed following PGF2&-induced abortion (Lindell *et al.* 1980/81). Based on these and other findings, Edqvist *et al.* (1981) reported that PGF2& release pattern following fetal expulsion seems to be related to the stage of pregnancy at which delivery occurred. Animals induced to abort before about 80 days of gestation do not have a pronounced postpartum PGF2& release pattern. After this stage of pregnancy the pattern is similar to that seen in cows calving at the expected time, although in these animals the release is more massive and of longer duration. The release might reflect the degree of endometrial damage and/or repair. The duration and possibly the magnitude of the release seems to be related to the time for completion of uterine involution with uterine involution being most rapid in animals with greater PGF2& concentrations. Animals showing clinical signs of postpartum uterine disorder or that have had retained fetal membranes have a prolonged release of PGF2& and also require comparatively longer time periods for completing uterine involution. The first postpartum ovulation seems to occur irrespective of the PGF2& release profile, although when progesterone levels for the first time are elevated the PGF2& level is close to baseline. The postpartum PGF2& release may influence the incidence of short luteal phases, with higher incidence if first ovulation occurs relatively early postpartum.

In a study to establish the relationship between interval from calving to first ovulation and length of oestrous cycle, Lindell *et al.* (1982) observed that short cycles are much more frequent among early ovulating than in late ovulating cows. In cows that ovulated within 15 days after calving there were no obvious differences in PGF2& levels 10, 15 and 20 days after calving between cows with short (9-12 days) or normal (17-24 days) cycle lengths.

2.3.4 Assay methods for Prostaglandins

In recent years considerable efforts have been devoted to find methods for quantitative studies of PGF2& and related compounds, which are sensitive and specific enough to study the amounts of these compounds in physiological and pathological situations (Kindahl, 1981).

The three most used methods today are bioassay (Moncada *et al.* 1978), gas liquid chromatography combined with mass spectrometry (Green et al. 1978) and radioimmunoassay (Kindahl, 1981). None of these three methods can be used uncritically. Bioassay is a very suitable method when measuring unidentified sustances, although absolute specificity can never be achieved. Furthermore, the method can not detect biologically inactive substances, which in many cases might be good parameters for quantitation of PGF2& biosynthesis and release.

Gas-liquid chromatography on the other hand is a very exact chemical method which can be used for both active and inactive substances and the results are very reliable especially when combined with mass spectrometry. It is considered as a reference method with both sensitivity and specificity being very high. The drawbacks of the method, however, are its low sample capacity and rather high costs.

Radioimmunoassay techniques could be used against all kinds of prostaglandins. Its major problem is its low specificity for some antisera, although a high enough specificity can be achieved. Its sensitivity and high sample capcity at moderate costs have rendered RIA the most employed method today (Kindahl, 1981).

2.3.5 Practical Uses of Prostaglandin F2&

To date, all therapeutic and practical applications of PGF2& in cattle are based on induction of luteolysis. Therefore, the presence of an active CL is required in each case (Seguin, 1980). Prostaglandin F2& has been used in cattle in the following practical situations.

i cows and heifers with unobserved estrus (Seguin et al. 1978; Seguin, 1980; 1981)

ii a tool in the management of cattle (Cooper, 1976; 1981)

iii cattle breeding (Lauderdale, 1981)

iv induction of parturition (Zerobin *et al.* 1973; Johnson, 1981; Edqvist *et al.* 1981)

v treatment of ovarian luteal cysts (Kesler et al. 1978; Lauderdale *et al.* 1978; Gunzler and Schallenberger. 1981).

vi induction of abortion or termination of unwanted pregnancies (Copeland *et al.* 1978; Schultz and Copeland,1981).

vii treatment of postpartum infections (Gustafsson et al. 1976; Ott and Gustafsson, 1981).

viii treatment of mummified fetus (Talbot and Hafs, 1974; Jackson and Cooper 1977; Voh, Jr. 1991 unpublished data)

2.4. FERTILITY RATES IN CATTLE

Because of the very important role that reproduction plays in the entire economy of a cattle farm (Louca and Legates, 1968; Esslemont, 1974), it is essential to trace reproductive failure to its source as rapidly as possible. Fertility of a herd is best determined when three criteria are adopted: (a) pregnancy rate after the first insemination; (b) the number of inseminations per conception; and (c) the interval between parturition and conception (De Kruif, 1978). As a rule, these criteria and the corresponding normal values are as follows for a cattle population:

i. pregnancy rates of 80% after first insemination,

ii. a number of 1.3 inseminations per conception

iii. an average interval of 85 days between calving and conception (De Kruif, 1978).

Based on all these considerations fertility has been defined simply as the ability of a cow to give birth to a live calf at approximately 12-monthly intervals (Noakes, 1986).

2.4.1 Fertility rates following natural service (NS):

Apparently there are no available controlled studies on N.S. in Nigerian cattle whereby it would be possible to determine pregnancy rate after first insemination and the number of services per conception. Rather, bulls are allowed to run with the cows for a specified or unspecified period of time. To make a distinction between the two situations, Chenworth and Lennon (1986) defined gross pregnancy rate as pregnancy rate for the entire breeding season (3 to 4 cycles) and net pregnancy rate to first cycle breeding as number of cows pregnant at the first oestrous cycle as a percentage of the number pregnant from matings at all cycles.

In a study to assess the effect of level of nutrition on the onset of puberty and conception rates of zebu heifers, Oyedipe *et al.* (1982b) using two bulls in a herd of 60 heifers reported 60-day pregnancy rates of 11.1%, 11.1% and 0.0% for heifers on high, medium and low protein groups respectively. The corresponding 90-day pregnancy rates were 58.8%, 27.8% and 16.7% while the 120-day pregnancy rates on the other hand were 58.8%, 33.3% and 16.7% respectively.

Using post-partum cows, Eduvie and Dawuda (1986) reported pregnancy rates at 60-90 days post-partum for suckled and non-suckled cows to be 21.1% and 72.7% in a herd of 41 Bunaji cows that had an active bull running with them.

According to other results published over the years for other zebu breeds of cattle in other parts of Africa, pregnancy rates following natural service have ranged from about 60-90% (Wilson, 1946; Plasse *et al.* 1970; 1979; Steinbach and Balogun, 1971; Bartha, 1975; Marincowitz, 1978).

In a study of the reproductive performance of a small herd of 35 Holstein-Friesians heifers in Ghana, Kabuga and Alhassan (1981) reported conception rate of 56.2% and 61.2% for cows bred 3 and 4 times respectively. The number of services per conception ranged from 1 to 13 with a mean of 3.4 services per conception.

Studies elsewhere in the world have reported pregnancy rates ranging from 60 to 100% (Husdjur, 1971; Alpan and Aritan, 1971; Beljaeva, 1971; Vianna and Jondet, 1977). Studies comparing pregnancy and/or non-return rates following artificial insemination and natural service (O'Farrel, 1977; Williamson *et al.* 1978) reported first service conception rates and 60 to 90-day non-return rates of 58.2% and 69.3% respectively. For

all herd - years studied, they reported conception rates to AI and NS of 57.5 and 58.0% respectively. Similar results were also reported by O'Farrel (1977).

The number of services per conception have varied. Vianna and Jondet (1977) reported the number of services per conception for imported Charolais cows, Charolais cows born locally in Brazil, 5/8 Charolais x3/8 zebu and 5/8 zebu x x3/8 Charolais to be 3.2, 3.5, 1.3 and 1.4 respectively.

Saeed *et al.* (1987) reported mean number of services per conception of 1.21 in a study of 1.229 cases. Earlier studies by Mohamed *et al.* (1985) indicated 1.41 services per conception which later increased after a four-year period to 1.84 services per conception in Sudan.

In a study to assess the effect of single-sire and multiple-sire natural mating on pregnancy rate, Lunstra (1987) found no difference in pregnancy rate among heifers mated by the same bull regardless of whether they had 1 or 3 services. For multiple-sire matings, however, pregnancy rate was 11% higher (74%) than the rate for single sire services.

2.4.2 **Fertility rates following conventional A.I.**

Data on fertility rates following naturally occurring or spontaneous estrus for Nigerian zebu breeds are scanty since the report on the first documented A.I. that was carried out in August 1949 (Anon, 1954). In this pioneer report, 41 heifers were inseminated using liquid semen collected from Bunaji bulls. First service conception rate was 52% while conception rate to second and third inseminations were 64% and 66% respectively. The present progress of crossbreeding indigenous zebu cows using Friesian did not begin until 1952 using

air-transported chilled semen from England. A total of 63 animals were inseminated. First, second and third service conception rates were 51.1%, 37.7% and 25.0% giving an overall conception rate of 46.0% (Armour and Sainsbury, 1956).

Zakari (1981) conducted studies to determine fertility of inseminations relative to service periods. Conception rates for cows inseminated at early, mid and late estrus were 31%, 73% and 63% respectively with the corresponding number of services per conception of 3.0, 1.4 and 1.6 with an overall services per conception of 1.8. In a more definitive study to determine the optimum time for A.I., Dawuda et al. (1987) reported conception rates to AI at 0, 6, 12, 18 and 24 hr from onset of standing estrus to be 55%, 65%, 70%, 65% and 60% respectively.

In a study to assess the effect of management and of season on pregnancy rates to A.I. in Nigerian zebu cows, Voh Jr. et al. (1984b) reported an overall pregnancy rate of 50.4%. Seasonal and management effects were confirmed. The overall number of services per conception were 1.8.

These results are generally in consonance with those obtained for zebu cattle elsewhere in the tropics. Rollinson (1963) reported 54.3 to 65.4% conception with the highest percentage of animals conceiving when inseminated between 12 and 15 hr after onset of estrus which was in agreement with Anderson's (1948) recommendations that inseminations should be performed at 18 hr after onset of estrus.

Garcia (1988) investigated breeding data from 1,543 inseminations in the Amazon basin of Peru, performed on 763 purebred and crossbred zebu cattle. Least square mean overall conception rates for zebu, undefined crossbreds and European x zebu crossbred were

35.7%, 41.8% and 50.7% respectively. Corresponding number of services per conception were 2.6, 2.3 and 2.1 whereas conception rates at first service were 36.4%, 41.8% and 52.0%. Cow breed did not significantly influence conception rate. Farm effect, however proved to be the most important factor influencing conception rate.

2.4.3 Fertility rates following PGF2& injection

There is a large volume of clinical data on the fertility of cattle following synchronization of estrus with PGF2&.

In a study that compared fertility rates between control heifers inseminated at detected spontaneous estrus and heifers synchronized with PGF2& and inseminated at detected estrus, Adeyemo *et al.* (1979) reported higher first service conception rate for treated (61%) than control (45%) heifers. Nagaratnam *et al.* (1983) reported 59% conception rate folowing double injection of PGF2& 11 days apart followed by dual fixed-time insemination at 72 and 96 hr after the second injection using chilled semen. Pregnancy rates comparable with and sometimes superior to untreated controls have also been reported for zebu cows (Vujosevic *et al.* 1980; Voh, Jr. et al. 1987, 1988) following estrus synchronization with PGF2&.

Although inseminations at observed PGF2&-induced estrus have resulted in conception rates comparable to untreated controls (Cooper, 1974; Lauderdale *et al.* 1974; King *et al.* 1982), inseminations at predetermined time(s) have resulted in variable conception rates (Manns *et al.* 1977; Burfening *et al.* 1978) for temperate breeds of cattle. For zebu cattle and their crosses, the situation has even been worse because conception rates lower than 30% have frequently been reported (Vivanco and Delgado, 1980; Landivar *et al.* 1985; Voh Jr *et al.* 1987a,b; 1988).

Anon (1980) selected data to show examples of "low" and "high" pregnancy rates for individual herds following estrus synchronization with PGF2&. The PGF2&-treated cows had about 60% pregnancy rate on the synchronized estrus and over 80% pregnancy rate by day 28 of the breeding season. Pregnancy rate of the control cattle was close to the theoretical line (5% in estrus per day and 75% conception rate) indicating that cattle were estrous cycling and conception was good. The cattle in these cases were on a rising plane of nutrition, the working facilities were adequate, estrus detection was good, and the inseminator's proficiency was excellent.

In contrast, 24% to 40% first service conception rate was reported for the "low" pregnancy rate category. The poor conception rate was due primarily to a low conception rate prior to synchronization, errors in estrus detection and poor timing of insemination. These data reinforce the fact that high pregnancy rates can be achieved if cattle are estrous cycling, healthy, if semen of high fertility is inseminated properly, if estrus is detected accurately, and if physical facilities allow cattle handling without detrimental effects to the animals (Anon, 1980; Cooper, 1981).

This large volume of data has indicated very clearly that there can no longer be any doubts about the efficacy of PGF2& as a luteolytic agent in cattle given either as single or double injections. It should also now be considered well proven that the estrus and ovulation following PGF2&-induced luteolysis is fertile, and infact as fertile as a spontaneous heat (Cooper, 1974, 1981; Lauderdale *et al.* 1974; Leaver *et al.* 1975; and Rowson, 1975). Furthermore, the two injection regime earlier described by Cooper (1974) and Hafs (1976) have been successfully followed by insemination at a pre-determined time or times

without reference to the observation of estrus.

2.4.4 Factors affecting fertility rates:

There are many factors which may influence the fertility of a cattle population. Some of these factors have been extensively reviewed by De Kruif (1978) and Shanon (1978). These factors have been responsible for the failure of reproduction to follow an optimum course. The many factors which may influence fertility rates in cattle have been broadly classified as (1) the bull; (2) methods of insemination; (3) the cow; (4) the conditions of herd management and (5) chance.

2.4.4.1 The bull

When natural service is employed, then it regularly happens that the bull is the cause of the failure of cows to become pregnant (Ayalon, 1964; Roberts, 1971). However, when A.I. is employed a high selection rate of bulls is used and the role of the bull in infertility is considerably minimized. This notwithstanding, considerable differences in fertility between the remaining bulls still exists (Roche *et al.* 1978) and a difference of about 15% with regard to conception rate after first service between the best and poorest bull is a common phenomenon with bulls whose semen does not lend itself readily to freezing showing even poorer fertility results (Mann, 1976). Other factors relating to the bull have been adequately reviewed by Shannon (1978).

2.4.4.2 **Method of insemination**

A satisfactory method of insemination is essential for obtaining high fertility rates (Roberts, 1971; 1974). There are marked differences not only between inseminators, which often ranges from 5 to 10% (Milk Marketing Board, 1985; De Kruif, 1978; Roche et al. 1978, Garcia, 1988), but also between sites of semen deposition (Hunter, 1984; Voh Jr. *et al.* 1987a) as well as the sperm numbers.

2.4.4.3. **The cow**

The cow plays a very important role in the determination of fertility rates of a cattle herd. The age of the cow has been reported to have a marked effect on the fertility rate after the first insemination (Boyd and Reed, 1961; De Kruif, 1978). De Kruif (1978) observed a difference of 5% pregnancy rate between primiparous and secundiparous cows as well as establishing a relationship between age and pregnancy rate. Other female-related factors affecting fertility rates include reproductive diseases like campylobacteriosis (Roberts, 1971; Bawa *et al.* 1987), brucellosis (Nuru, 1974; Bale, 1991), trichomoniasis (Winter, 1972), trypanosomiasis (Ogwu *et al.* 1984), dermatophilosis (Ogwu *et al.* 1981) and individual reproductive pathological abnormalities (De Kruif, 1978; Ogwu *et al.* 1981, 1984).

2.4.4.4. **Herd management**

Conditions of herd management that affect fertility in- clude size of herd (Hewett, 1968; Ayalon et al. 1971), housing system (Willems, 1971), choice of bull (De Kruif, 1978), hygiene at time of calving (Roberts 1971), detection of estrus (Esslemont and Ellis,

1974; Pelissier, 1970), time of insemination (Trimberger and Davis, 1943; Robbins *et al.* 1978; Dawuda *et al.* 1989), nutrition (Lammond, 1970; Oyedipe *et al.* 1982b; Voh Jr. *et al.* 1984b), milk yield (Hewett, 1968), selection (Foote, 1970) and culling of cows (Van Dieten, 1968).

2.5 EMBRYONIC MORTALITY IN CATTLE

Embryonic mortality (EM), strictly interpreted, should refer to fertility losses during the embryonic period, that is the period extending from conception to completion of the stage of differentiation, which in the cow, occurs at approximately 45 days (Committee on Reproductive Nomenclature, 1972). Reproductive failure is one of the major factors affecting output in cattle herds. While it is generally accepted that fertilization rates after natural or artificial insemination are normally close to 90%, calving rates to a single insemination are closer to 50%. Fertilization rates of 90-100% have been reported following the use of bulls of proven fertility but embryo survival rates are considerably lower (Boyd *et al.* 1969; Henficks *et al.* 1971; Sreenan and Mulvehill, 1975; Roche *et al.* 1977; Mawhinney and Roche, 1978; Diskin and Sreenan, 1980).

The most important factor in the reduction of the net calf crop is the failure of the female to become pregnant or calf. Factors affecting net calf crop have been summarised to include fertilization failure (FF), fetal deaths during gestation, perinatal calf death and calf deaths between birth and weaning (Bellows *et al.* 1979). Maurer and Chenault (1983) and Humblot *et al.* (1988) have reported that about 60% of the reduction in calf crop could be attributed to fertilization failure and embryonic mortality.

The foregoing literature have one thing in common, and that is their agreement that embryonic death comprises the largest proportion of prenatal loss in cattle and it is of significant economic importance in cattle enterprises. For this reason a lot of research efforts have rightly been directed at this phenomenon. There are several reports to show that the major portion of embryonic losses occurs well before day 15 post-insemination (Ayalon *et al.* 1968; Boyd et al. 1969; Ayalon, 1972, 1978; Diskin and Sreenan, 1980) and therefore the embryo dies too early to prevent the secretion of PGF2& which causes regression of the CL and such cows therefore return to estrus after the same interval as unmated females. For this reason EM has been divided into two: early embryonic mortality (EEM) to refer to embryonic losses occuring before day 16 and late embryonic mortality (LEM) to refer to embryonic losses occurring after day 16 post-insemination.

2.5.1 Methods of study

Under experimental conditions, EM has been studied by a number of methods notable among which are:

2.5.1.1. Breeding records

It is widely considered that an increase in the interval between service and return to estrus beyond the usual range of 17 to 25 days reflects EM (Erb and Holtz, 1958). This is supported by observations such as those by Marion et al. (1950) who demonstrated that mating with a vasectomized bull had no significant effects on the subsequent time of return to estrus, and those by Boyd (1973) who found a significant difference in intervals between

periods of oestrus before and after first inseminations: 90% of pre-insemination cycles were of normal length, as compared with only 44% of post-insemination cycles. The differences between non-return rates up to 3 months and actual pregnancy diagnosis is of course, well known, and delayed returns due to unobserved estrus is a common problem in cattle herds. These facts highlight the merit of this method. Despite this, however, there are strong objections to using increased intervals between insemination and return to estrus, as the chief evidence for EM for at least 3 reasons namely:

i. Cows which had not been in estrus may have been inseminated. Reports by Appleyard and Cook (1976), Hoffman *et al.* (1976) and Shemesh *et al.* (1968) have shown that up to 20% of cows presented for insemination are not in estrus.

ii Post-service inflammation or infection is associated with the persistence of the corpus luteum and delayed return to estrus (Ginther, 1968).

iii The method does not take cognisance of EM occurring be- fore day 16 which represents the major portion of EM.

EM rates by this method have ranged from 10% to 30% (Wijeratne, 1973; Wood, 1976; Kummerfeld *et al.* 1978).

2.5.1.2 **Sustained milk or plasma progesterone concentrations**

Analysis of milk or plasma progesterone concentrations has become a possible and routinely used means of measuring oestrous cycle length directly, and prolonged cycles from these measurements have been used to estimate EM rates. The principle behind this method is based on the fact that while levels of progesterone in plasma or milk at 21 to 24 days

post-insemination declines and returns to cyclic patterns following non-fertile inseminations, the luteal phase levels are sustained following fertile inseminations during this period and up to end of gestation (Kumerfeld *et al.* 1978).

This method has been used by several workers to estimate EM (Pope and Hodgson-Jones, 1975; Kummerfeld *et al.* 1978; Bulman, 1979; Humblot *et al.* 1988) with EM rates ranging from 7 to 30%. This method is also bequeathed with shortcomings which include:

i it does not overcome problems associated with estrus during pregnancy

ii post-service endometritis or infection associated with persistence of corpus luteum and sustained progesterone concentration

iii does not detect embryonic deaths occurring earlier than day 16 post-insemination.

It has been suggested (Ayalon, 1981) that when progesterone evaluation on the day of insemination is included the accuracy of this method will be increased.

2.5.1.3 **Planned slaughter and embryo recovery after insemination.**

This approach has yielded the most reliable and accurate information about the nature of fertility losses (Ayalon, 1981) especially when it is supplemented by biochemical studies. The most important merit of this method is its ability to detect embryonic deaths occurring before day 16 post-insemination. In recent years, the problem of EM is being delineated more clearly and pertinent information has become available concerning the critical period of EM and some of the concomitant biochemical changes that occur. For instance, while some of these reports demonstrate the existence and extent of EM, few of them have gone a step further to indicate the time at which it occurs. Ayalon (1972) has shown that most losses occur

before Day 15 after breeding and that for repeat breeder cows (Ayalon, 1972) Day 7 is the critical day on which embryonic death occurs, whereas the greatest proportion of losses in normal cows occurred before day 16. These results have been confirmed by Maurer and Chenault (1983) who reported that about 70% of EM occurs between day 8 and day 16 of gestation with the remaining 30% occurring between day 16 and 42 of gestation in both nonparous and parous beef cows.

Embryonic mortality rates from these studies have ranged from 10% to 40% for normal cows and from 15% to 70% for repeat breeder cows (Bearden *et al.* 1956; Hawk *et al.* 1955; Ayalon, 1969; Boyd *et al.* 1969; Diskin and Sreenan, 1980; Maurer and Chenault, 1983; Gustafsson and Larsson, 1985).

Using slaughter after reciprocal transfer of embryos, Gustafsson and Larsson (1985) found this technique as a useful model not only to study EM but for evaluating mother-conceptus relationships.

2.5.2 Aetiological and influencing factors

Aetiological factors have been classified mainly into two: a) genetic factors and (b) environmental factors.

Genetic factors causing embryonic mortality have included breed (McFeely and Rajakoski, 1968), family (Mares *et al.* 1961; Menge *et al.* 1962), in-breeding (Casida, 1961; Conneally *et al.* 1963, and blood groups (Ashton and Fallon, 1962).

Some evidence for the possible involvement of a particular bull in late embryonic mortality (between 30-80 days post-insemination) has been reported (Bulman, 1979).

An interesting theoretical approach to the question of genetic involvement in embryonic mortality has been presented by Bishop (1964) who suggested that the genetic factors involved in EM are not necessarily inherited by the parents but rather arise in each parent generation and that some are likely to arise in definitive gametes. He contended that a considerable part of EM is unavoidable and should be regarded as normal and as a means of eliminating unfit genotypes at low biological cost. Environmental factors causing EM on the other hand have included nutrition (Hill *et al.* 1970; Francos *et al.* 1977), age (Ball, 1978), climate (Ingrahan *et al.* 1974), infection (Adler, 1959; Bawa *et al.* 1987), conditions at service (Barret, 1948), semen quality (Bearden *et al.* 1956), hormonal imbalance (Erb *et al.* 1976; Maurer and Echternkamp, 1982), and uterine environment (Almeida *et al.* 1984a,b; Gustaffson and Larsson, 1985).

2.6 MANAGEMENT TECHNIQUES FOR IMPROVING REPRODUCTIVE EFFICIENCY

Reproductive efficiency in cattle is said to be achieved when a cow gives birth to a live calf at approximately 12-month intervals. For this to happen in actual practice means that a cattle population must have a pregnancy rate of 80% to first insemination, 1.3 inseminations per conception and an average of 85 days between calving and conception (De Kruif, 1978). The true situation on the field is undoubtedly a far cry from this optimum situation because it is common place to find in a normal population of cows that the average calving rate to each insemination is 55% with 45% failing to calve (Noakes, 1986) because:

i 10-15% of ovulated oocytes are not fertilized

ii 15-20% of fertilized oocytes or early embryos die before day 16 of the estrous cycle

iii 10% of late embryos die between days 16 and 42

iv 50% of fetal death occurs after 42 days

The essentiality of improved reproductive efficiency for increased productivity of livestock, particularly in developing countries where poor reproduction is caused by factors associated with management, nutrition, climate and disease, needs no elaborate argument. This is made even more urgent in view of increasing human populations which puts a lot of pressure on animal protein supplies. Thus, any management technique that can bring about reproductive efficiency, no matter how small, should be considered a noteworthy venture. In cattle, attempts have been made in the past and are still currently on, to manipulate various phases of the reproductive cycle in order to bring about the much-desired improvement in reproductive efficiency (Oyedipe and Voh Jr. 1990). Some of the techniques in use today include A.I., estrus synchronization, embryo transfer and fertility control.

2.6.1 Effective animal management:

This includes good nutrition and strategic feeding, proper housing, and effective animal health husbandry practices.

2.6.2 Semen collection and evaluation

The mature sperm cell is a terminal cell, the end product of a complex developmental process, and it cannot undergo further cell division or differentiation. The ideal method of evaluating the fertility of a breeding male, other than its ability to produce pregnancy, is by the evaluation of its semen. The standards for a species can be established and any deviations from these "normal standards" can be recognized and correlated with fertility (Hafez, 1980).

2.6.3 Artificial insemination (AI)

AI is the most important single technique ever devised for the genetic improvement and disease control of animals. This is possible because a few highly selected males produce enough spermatozoa to inseminate millions of females annually, whereas only relatively few progeny pre-selected female can be produced per year even by embryo transfer. AI has many advantages and is practically essential in conjunction with oestrus synchronization programmes. Recent advances are opening up horizon for sex control through separation of spermatozoa containing X- and Y- chromosomes (Hafez, 1980). It is becoming increasingly possible to carry out preselection and complete separation of X- and Y-sperm before artificial insemination. Sex of the offspring can thus be predetermined (Krzyzaniak and Hafez, 1987).

2.6.4 Synchronization and induction of oestrus and ovulation

Oestrus synchronization is a technique whereby a group of randomly cycling females are made to come into oestrus at about the same time so that they can ovulate and be bred at about the same time. Similarly young females that are closely approaching puberty can be stimulated to cycle. Also, older females that are experiencing lactational or seasonal anestrus can sometimes be stimulated to resume oestrous cyclicity (Beal, 1983; Britt, 1987).

2.6.5 Early pregnancy diagnosis:

Knowing whether an animal is pregnant or not is of considerable importance. In general, an early diagnosis of pregnancy is required soon after mating or AI for an early identification of non-pregnant animals so that production time lost as a result of infertility may be reduced by appropriate treatment or culling (Jainudeen and Hafez, 1980).

2.6.6 Multiple ovulation and embryo transfer, in-vitro fertilization and genetic engineering

Multiple ovulation and embryo transfer (MOET) is a technique by which a female is made to produce more ova than normal (super ovulation) and the ova are fertilized, recovered from a valuable cow (donor) and transferred into the reproductive tract of a less valuable cow (recipient) which serves as a surrogate mother in which gestation is complete. Thus, just as the reproductive rate of superior males has been maximised through AI, the reproductive rate of superior females is currently being maximised through MOET (Wagner, 1987; Hafez, 1987; Del Campo, 1990).

2.6.7 In-situ and ex-situ preservation and cryopreservation of gametes and embryos

This is the ability to preserve gametes and embryos inside or outside the animal at ambient temperature or at cold temperatures (Hafez, 1987). This will further improve reproductive efficiency.

2.6.8 Herd health fertility programme

This is the set of activities and procedures that are carried out to attain optimum reproductive efficiency in a herd. The programme has to be designed to fit each type of animal husbandry and the individual herd concerned (Zemjanis, 1970). Implementation of the programme requires:

(a) accurate permanent recording of all relevant data;

(b) early identification of infertile animals;

(c) proficiency in diagnostic skills for early pregnancy diagnosis, diagnosis of genital abnormalities and functional ovarian structures.

CHAPTER THREE

EFFECT OF INTER-TREATMENT INTERVAL AND STAGE OF THE OESTROUS CYCLE ON OESTRUS RESPONSE OF NIGERIAN ZEBU COWS TO PROSTAGLANDIN F2 ALPHA INJECTION

3.0 INTRODUCTION

Prostaglandin F2 alpha and its synthetic analogues are luteolytic substances that bring about morphological egression of the corpus luteum that is associated with significant precipitous fall in plasma progesterone concentration within 6 to 24 hours following its exogenous orendogenous administration (Dobson et al. 1975).

One limitation of prostaglandin F2 alpha (PGF2&) is its inability to cause luteolysis in cattle when injected before day 5 (day 0 = day of estrus) of the estrous cycle (Lauderdale, 1972; Liehr et al. 1972; Louis et al. 1974).

To circumvent this limitation, numerous methods for the control of estrus and ovulation in cattle have been developed as reviewed by Hansel and Beal (1979) and Roche (1979).

One of the more promising methods reported has been the 10 to 12 days double injection technique (Cooper, 1974; Lauderdale et al. 1974; Voh Jr. et al. 1987a). Yet one of the short-comings of this technique has been its inability to induce estrus in about 10 to 20% of treated cows (Voh Jr. et al. 1987b). This failure has been attributed to considerable variation in the length of luteal growth and maturation especially for zebu cows, with the net result that these animals are given the second injection when the corpora lutea have not reached responsive stage (Voh Jr. et al. 1987b).

Thus, an effect of the stage of the estrous cycle at the time of treatment has been reported by several researchers with higher percentage of heifers or cows showing

synchronized estrus with each advancing stage of the estrous cycle (King et al. 1982; Tanabe and Hann, 1984; Watts and Fuquay, 1985). Furthermore, the day of the estrous cycle at treatment also could affect conception rates since in some studies (Manns et al., 1976; Hafs et al., 1975; Tanabe and Hann, 1984) insemination at fixed time after PGF2& injection did not lower conception rates, but in others it did (Burfening et al., 1978; Hardin et al., 1980a; King et al., 1982; Watts and Fuquay, 1985).

The objectives of this study therefore were to determine the optimum intertreatment interval for PGF2& double-injection technique, and the effect of the stage of the estrous cycle on the estrus response of zebu cows.

3.1 MATERIALS AND METHODS

3.1.1 Experimental animals:

Two experiments were conducted at NAPRI and in the rainy season. A total of 140 Bunaji (White Fulani), mostly di and tri parous cows weighing between 250-380 kg with an average body condition score of 3.5 (Pullan, 1978) and ranging in age from 4 to 8 years were used. The animals were individually identified by means of large numbered plastic eartags. The cows were grazed daily from 0700 to 1800 hours. At the end of the grazing period they were kept in fenced paddocks with improved sown pastures but without supplementary feeding.

During the heat observation period the animals remained in the fenced paddocks continuously and were not taken out for grazing but grazed the sown pastures in the paddocks.

3.1.2 Pre-experimental stage:

During a pre-experimental period of 3 months the cows were visually observed for estrus from 0700 to 1800 hours daily. The cows were palpated rectally once a month to make sure the genital tracts were normal and that the ovaries had structures indicative of estrous activity. Only cows cycling normally as evidenced by regular estrous cycles or ovarian structures were used.

3.1.3 Experiment 1:

A total of 70 Bunaji cows were used. All the cows received an intramuscular injection of 25 mg PGF2& (Lutalyse, Upjohn Co, Kalamazoo, Michigan). For the second injection the cows were randomly assigned to 5 treatment groups of 14 cows each for inter-treatment intervals of 10,11,12,13 and 14 days. The cows were then allowed a rest period of 2 months to "de-synchronize" the estrous cycle. The experiment was then repeated such that each treatment group had a total of 28 cows. Using the day of estrus onset following the first injection as day 0, the proportions of animals that were in early, middle and late diestrus at the time of second injection were computed retrospectively.

3.1.4 Experiment 2

After estrus was observed following the injection of PGF2&, a total of 140 Bunaji cows were given a second injection of PGF2& either during early diestrus (day 5-8), middle diestrus (day 9-12) or late diestrus (day 13-16). This classification was used because we had reported that progesterone does not rise early after estrus (Oyedipe et al.

1986; Voh Jr. et al. 1987b). The respective numbers of cows allocated to each stage of diestrus were 44, 51 and 45 for early, middle and late diestrus respectively. Estrus was day 0.

3.1.5 Estrus detection

Following each treatment the cows were observed for estrus continuously for 7 days as reported earlier (Voh Jr. et al. 1987a). While secondary signs of estrus such as clear mucus discharge, trailing and mounting other cows, reddening and swelling of the vulva, frequent adoption of urination posture, isolation from herd mates (Zakari 1981; Voh Jr. 1984a) were noted, standing to be mounted by other cows (homosexual mounting) was taken as the primary and sole determinant of estrus. Estrus periods occuring within 1 to 7 days post-injection were classified as synchronized, although earlier investigators (Lauderdale, 1972; Hafs et al. 1975) restricted the range to 2 to 5 days.

3.1.6 Data analysis

The data were analysed using chisquare techniques (Steel and Torrie, 1960).

3.2 RESULTS

3.2.1 Experiment 1

Out of 28 cows or observations per treatment group, estrus was detected in 20 (71.7%), 24 (85.8%), 23 (82.7%), 26 (92.9%) and 23 (82.7%) for the 10, 11, 12, 13 and 14 day inter-treatment interval groups respectively with an average of 83.2% overall response

rate. The response rate for the 10-day group was significantly lower ($P<0.05$) than those of the other groups (Table 3:1). Estrus onsets were generally later as longer the inter-treatment interval increased (Table 3:2).

While 10 days between PGF2& treatments placed 95% and 5% of cows in early (day 5-8) and middle (day 9-12) diestrus at the time of second injection, the corresponding percentages for the 11 days inter-treatment interval were 72% and 28% respectively. However, for the 12 and 13 days intertreatment intervals 21% and 79% for 12 days, and 9% and 91% for 13 days were in early and middle diestrus respectively. For the 14-day group 6%, 83% and 11% were placed in early, middle and late diestrus, respectively at the time of second injection (Table 3.3).

3.2.2 Experiment 2

Overall the intramuscular (i.m) injection of 25 mg of PGF2& in 140 cows induced estrus in 135 cows (91.3%) within 144-hour period (range 24 to 168 hr) post-injection. The estrus response rate progressively, but non-significantly increased with each advancement of the stage of the estrous cycle at which PGF2& was injected: 86.4%, 93.2% and 93.3%) for early (days 5-8), middle (days 9-12) and late (days 13-16) diestrus groups respectively (Table 3:4).

The comparative distribution of estrus onsets as affected by the stage of estrous cycle when PGF2& was injected is shown in the histogram (Figure 3.1). In general, induction of estrus was earliest among cows injected during early diestrus, latest among cows injected during late diestrus and intermediate among cows injected during middle diestrus. Over the

24 hours interval from 24-48 hr after PGF2& injection, only 3.6% of treated cows in middle diestrus group exhibited estrus. Over the 24 hr interval from 48 to 72 hr post-injection the comparative percentages of estrus onsets varied markedly: 44.7%, 20.0% and 19.0% for the early, middle and late diestrus groups respectively. Over the 24 hr interval from 72 hr to 96 hr post-injection, percentages were 21.1, 41.8 and 54.8 for cows injected during early, middle and late diestrus respectively (Table 3:5).

Estrus occurred within a 96-hr period (48 to 144 hr post-injection) for cows injected in early diestrus but extended over 144 hr and 120 hr periods for cows injected in middle and late diestrus respectively. By 144, 168 and 168 hr post-injection, estrus induction was complete for cows injected in early, middle and late diestrus respectively.

3.3 DISCUSSION

3.3.1 Experiment 1:

The estrus response rates found in this study are in consonance with what we had reported previously (Voh Jr. et al. 1987a,b,c) for Nigerian zebu cows. The significantly lower response rate recorded for the 10 days inter-treatment interval coupled with the wide distribution of onsets of estrus over a 7 day post-injection period indicates that 10 days between two injections of PGF2& cannot be optimal for Nigerian zebu cows. This lower response rate for the 10-day interval is not surprising because we had reported earlier (Voh Jr et al. 1987b) using the 11-day double injection regime that a proportion of cows was given the second injection when the corpora lutea had not reached responsive stage. This was attributed to slow luteal maturation (Oyedipe et al.1986, Voh Jr. et al. 1987b). It

would seem logical, therefore that inter-treatment intervals shorter than 11 days will aggravate the lowered response situation.

Furthermore, on the basis of the high estrus response rate (92.9%) and the compactness of estrus onsets (78.3%) of responding cows having onsets between days 2 and 4 following the second PGF2& 13 days between 2 consecutive i.m. injections of PGF2& seem to be the optimum inter-treatment interval for Bunaji cows.

The rather earlier peak onset of estrus observed for the 11 days group may partially explain the low conception rates frequently reported for zebu cows (Hardin et al., 1980; Landivar et al., 1985) following fixed time inseminations at 72 or more hours after injection of PGF2&. The net result is that a high proportion of cows will be inseminated after they are out of heat. This finding further supports earlier suggestions by Orihuela et al. (1983) and Voh Jr. et al. (1987a) that zebu cows be inseminated 60 hr following the 11-day double-injection regime.

In the current study the 11-day inte-rtreatment interval placed 72% of cows in early diestrus at the time of second injection. This is in consonance with the findings of Hansel and Beal (1979). Similarly, the 76.9% response rate following the first injection is in agreement with the theoretical 75% that would be expected in a group of randomly but normally cycling cows with an average estrous cycle length of 20 days (Voh Jr. 1984b).

The results of the two experiment are in close agreement in certain aspects. For example, the estrus response rate (85.8 vs 86.4%) and the peak of onsets of estrus (on day 2 for the 11-day group) (Experiment 1) and the early diestrus groups (Experiment 2) are very identical. These findings are not surprising in view of the fact that the 11 days regime

places 72% of cows in early diestrus at the time of second injection. The same argument is adduceable to the day 3 estrus onset peak for the 12, 13 and 14 days groups (Experiment 1) and the middle diestrus group (Experiment 2) since these 3 inter-treatment intervals, respectively placed 79%, 91% and 83% of cows in middle diestrus at the time of second PGF2& injection.

The practical implication of these findings is that the optimum interval from PGF2& injection to single insemination at a predermined time may vary depending upon whether 10 and 11 days apart are used on the one hand or 12, 13 and 14 days on the other hand. This is particularly so since the degree of synchrony within 24 hour intervals is of great importance from the point of view of fixed-time inseminations.

3.3.2 Experiment 2

In the present study, the estrus response rates of 86.4, 93.2 and 93.3% are in consonance with the findings of 86, 90 and 98% reported by Tanabe and Hann (1984) for heifers injected at 7, 11 and 15 days of diestrus respectively. These findings however, are at variance with those of Watts and Fuquay (1985) who reported significantly differing response rates of 43, 83.6 and 100% among dairy heifers injected with PGF2& during early, middle and late diestrus respectively.

For the responding cows the interval from injection to onset of estrus was shorter for the early than the middle and late diestrus groups. This is in agreement with what has been reported by many workers (Johnson, 1978; Jackson et al 1979; King et al 1982; Tanabe and Hann, 1984; Watts and Fuquay, 1985). All these workers reported that early diestrus

cows and/or heifers showed estrus about 12 to 24 hours earlier than did middle or late diestrus animals. This earlier onset of estrus for cows treated at early diestrus may account for much of the reduced fertility when the 11-days-apart double injection regime is coupled with timed insemination at 72 hr after treatment.

Compactness of the onsets of estrus is an important factor when considering fixed-time AI. Too wide distribution of onsets, even if the estrus response rate is high is clearly unsuitable for fixed-time AI. On the basis of this, cows injected during middle and late diestrus seem to show slight superiority over the early diestrus group.

Although it may be conjectural, fertility may be improved by any one of two simple steps, either 1) to use conventional two injections 11 days apart coupled with inseminations earlier than the conventional 72 and 96 hours (Voh Jr. et al. 1987a) or 2) to use two injections 13 days apart coupled with the conventional insemination at 72 and 96 hours post injection. Additional research on fertility is however, needed to elucidate and confirm these contentions.

Table 3:1 Oestrus response rates of Bunaji cows following treatment with PGF2 alpha at various inter-treatment intervals

Parameter	Intertreatment interval (days)					
	10	11	12	13	14	Total
No treated	28	28	28	28	28	140
No in oestrus	20	24	23	26	23	116
% in oestrus	71.7[a]	86.8[b]	82.7[b]	92.9[c]	82.7[b]	83.2

a,b,c = data with different superscript within the same row differ significantly ($P < 0.05$).

Table 3:2 Interval from end of treatment to onset of oestrus following treatment of Bunaji cows with PGF2 alpha at various inter-treatment intervals

No. inoestrus on day:	Intertreatment interval (days)					
	10	11	12	13	14	Total
1	1	1	2	2	2	8
2	9	10	5	4	6	34
3	5	5	7	13	8	38
4	3	4	4	4	4	18
5	1	2	3	1	0	7
6	0	2	2	2	2	8
7	1	0	1	0	1	3
No. in oestrus	20	24	23	26	23	116
No. treated	28	28	28	28	28	140

Table 3:3 The effect of inter-treatment interval on the proportion of cows given the second injection at various stages of diestrus expressed as percentages

Stage of diestrus	Intertreatment interval (days)				
	10	11	12	13	14
Early diestrus (day 5-8)	95	72	21	9	6
Middle diestrus (day 9-12)	5	28	79	91	83
Late diestrus (day 13-16)	0	0	0	0	11

Table 3:4 The effect of stage of diestrus at time of PGF2 alpha treatment on oestrus response in Bunaji cows

Parameter	Stage of diestrus			
	Early (day 5-8)	Middle (day 9-12)	Late (day 13-16)	Total
No treated	44	51	45	140
No in oestrus	38	47	42	127
% in oestrus	86.4	92.1	93.3	91.0

Table 3:5 Interval from end of treatment to onset of oestrus following treatment of Bunaji cows at various stages of diestrus

No. in oestrus on day*	Stage of diestrus Early (day 5-8)	Middle (day 9-12)	Late (day 13-16)	Total
1	0(0.0)	2(3.6)	0(0.0)	2(1.5)
2	17(44.7)	9(20.0)	8(19.0)	36(26.7)
3	8(21.1)	21(41.8)	23(54.8)	54(40.0)
4	5(13.2)	8(18.2)	5(11.9)	20(14.8)
5	3(9.0)	2(5.5)	5(11.9)	11(8.2)
6	5(13.2)	3(7.3)	1(2.4)	10(7.4)
7	0(0.0)	2(3.6)	0(0.0)	2(1.5)
No treated	44	51	45	140
No in oestrus	38(86.4)	47(92.1)	42(93.3)	127(91.0)

*Percent in parentheses.

CHAPTER FOUR

DETERMINATION OF OPTIMUM TIME FOR FIXED-TIME ARTIFICIAL INSEMINATION OF ZEBU COWS FOLLOWING SYNCHRONIZATION OF OESTRUS WITH PROSTAGLANDIN F2 ALPHA

4.0 INTRODUCTION

Accurate detection of oestrus and the correct timing of insemination are two prerequisites for the successful use of artificial insemination (A.I.) (Appleyard and Cook 1976). Both attributes are difficult to attain. To reduce the problems associated with these, much research emphasis has been placed on hormonal regulation of the oestrous cycle so that the time of oestrus, and hence ovulation, can be predicted.

Controlled breeding using PGF2& has been particularly attractive and promising in alleviating these problems. Several workers have presented evidence that timed A.I. (from 72 to 96 hrs) following PGF2& treatment of Bos taurus cattle in the temperate zones resulted in acceptable first service pregnancy rates comparable to controls (Lauderdale et al., 1974; Cooper, 1974; Hafs *et al.*, 1975; Christie and Medcalf, 1976).

Hitherto applications of PGF2& controlled breeding protocols have been based on extrapolation of data from Bos taurus breeds to Bos indicus which may not be necessarily be applicable since pregnancy rates lower than 30% have been frequently reported (Hardin *et al.*, 1980a; Landiver *et al.*, 1985; Voh Jr. *et al.*, 1987a). In a controlled preliminary fertility trial (Voh Jr. *et al.*, 1987a) unacceptably low pregnancy rates to inseminations at conventional fixed-times following double injection of PGF2& 11 days apart have been reported. Reports by Orihuela *et al* (1983), Voh Jr. et al., (1984) and Voh Jr. *et al.* (1987a) highlighted the need for further investigations to determine the optimum time(s)

for predetermined A.I. in zebu cows following PGF2& treatament. These suggestions became necessary in view of oestrus response data (Orihuela *et al.*, 1983; Voh Jr. 1984b; Voh Jr. *et al.* 1987b) and fertility data (Voh. Jr. *et al.*, 1987a) following treatment of zebu cows with PGF2& and artificial insemination.

The current study was therefore undertaken to determine the optimum fixed-time for AI following synchronization of oestrus with PGF2& in zebu cows.

4.1 MATERIALS AND METHODS

4.1.1 Location and season

The study was carried out at the A.I. Unit of National Animal Production Research Institute, Shika, Nigeria. The study was executed during the rainy season between mid-August and end of September (Voh Jr. *et al.*, 1987) for three consecutive years (1986, 1987 and 1988).

4.1.2 Experimental animals

Four hundred and twenty (420) non-lactating cycling Bunaji (zebu) cows aged between 5 and 9 years were used for the study. The animals were grazed on improved pastures between 0700 and 1800 hr following which they were herded into fenced paddocks sown with improved pasture where they remained overnight. The animals did not get any supplementaray feed. Water and mineral salt lick were, however, provided ad libitum. The cows were eartagged with large plastic eartags (Allflex, New Zealand) to make identification from a distance easy.

4.1.3 Pre-experimental period

This lasted 3 months during which time the ovaries and tubular genitalia of the cows were regularly palpated per rectum to determine oestrous activity. In addition, the animals were visually observed for oestrus. Only cows that were found to be cycling normally were preselected for the experiment. Furthermore, the body condition of the animals was scored as reported by Pullan (1978). Only cows with a body condition score equal to or greater than 3 at the commencement of the experiment were used. The cows were sprayed against ticks once a week as was the routine.

4.1.4 Experimental procedures

A total of 420 cows, balanced for parity, were assigned randomly to one of six experimental groups of 70 cows per group (Table 4.1). All cows except those in the control group were each given two single intramuscular injections of prostaglandin F2 alpha (Lutalyse, Upjohn, Michigan) 11 days apart.

Although it was not necessary for cows in Treatments 3 to, all the cows were visually observed for oestrus from 0700 to 1800 hr daily. Standing to be mounted by other cows (homosexual mounting) was the only criterion used to consider an animal as having been in oestrus.

Thereafter, the cows were artificially inseminated as follows:

4.1.5 Treatment 1 (controls):

AI at approximately 12 hr after the onset of spontaneous oestrus detected during a 45-day breeding period. Treatment 2: AI at approximately 12 hr after detected oestrus within 7 days after 2nd PGF2& injection. Treatment 3: Fixed-time AI at 60 hr after 2nd PGF2&

injection Treatment 4: Fixed-time AI at 72 hr after 2nd PGF2& injection Treatment 5: Fixed-time AI at 84 hr after 2nd PGF2& injection Treatment 6: Fixed-time AI at 96 hr after 2nd PGF2& injection.

For the PGF2& - treated cows only those that came into oestrus within 7 days after PGF2& injection were considered to have responded.

A single inseminator (AAV) carried out all the inseminations using the recto-cervical method. Deep frozen semen from a single Friesian bull packaged in French mini straws and stored in liquid nitrogen was used for AI.

Pregnancy was determined by rectal palpation at approximately 60 days following AI. First service pregnancy rate was calculated as the number of cows pregnant divided by the number assigned to the group and expressed as a percentage. First service conception rate on the other hand was calculated as the number of cows pregnant divided by the number detected in oestrus and expressed as a percentage. Statistical analysis: Chi square test was used to determine if treatments differed statistically.

4.2 RESULTS

The oestrus response and fertility rates are summarised in Table 4.2. The percentage of cows in oestrus (within 45 days for controls and within 7 days after second PGF2& injection for the remaining groups) was 92.9, 95.7, 92.9, 91.4, 92.9 and 95.7% for treatments 1 to 6 respectively. The differences were not significant.

First service pregnancy rates were 68.6, 57.1, 54.3, 45.7, 41.4 and 40.0% for treatments 1 to 6 respectively. The corresponding first service conception rates were 74.0, 60.0, 54.3, 45.7, 41.4 and 40.0% for treatments 1 to 6 respectively. Differences

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in fertility among some of the groups were statistically significant ($P<0.05$) and non-significant among others (Table 4.2). The conception rate of the controls inseminated at detected spontaneous oestrus (74%) was superior to the conception rates of all the other groups ($P<0.05$). The CR of cows inseminated at detected synchronized oestrus (60%) was similar to AI at 60 hr fixed-time (54.3%) but superior to fixed-time AI at 72 hr (45.7%), 84 hr (41.4%) and 96 hr 40% ($P<0.05$); the differences among these last 3 groups were however, not significant. Thus, the results showed a progressive decline in fertility rate with advancing interval from PGF2 alpha injection to AI.

All animals diagnosed pregnant, except 4 that aborted, carried their pregnancies to term and calved successfully without any veterinary attention. Two of the abortion cases turned out to be due to Campylobacteriosis (Bawa et al. 1987). Thus, there was an abortion rate of 1.9% in the herd. The infertility and abortion incidence of one of the trials has been published previously (Bawa *et al* 1987).

4.3 DISCUSSION

The effectiveness of PGF2& in inducing oestrus in the current study is in consonance with earlier reports that during the rainy season 90% oestrus response rate can be achieved when zebu cows are injected with PGF2& (Voh Jr *et al* 1987c. The study has shown that good oestrus response is attainable if care and time are taken to select cycling animals for PGF2& oestrus synchronization regimes. It is noteworthy that this good response was achieved without supplementaray feeding. Hardin *et al.* (1980a) showed that timed AI suppressed oestrus in Brahman-crossbred heifers. This suppressing effect was not evident in the present study since the oestrus response rate was very good.

There was no difference in the final number of cows inseminated in the control or treated groups. The treated cows were however bred within 7 days of the breeding season thereby helping to bring forward the average date of conception in the herd.

The important point to note when using PGF2& to allow fixed-time AI is to ensure that cows have a functional corpus luteum at the time of PGF2& injection. Cyclic cows can be selected for treatment with PGF2& based on rectal palpation of ovaries and oestrus detection prior to injection. Any method that ensures that only cyclic cows are treated will undoubtedly improve the effectiveness of PGF2&-based synchronization systems.

The herdsmen were already conversant with oestrus signs and also detection of oestrus was already being efficiently carried out before the commencement of the study. This would in part explain the high oestrus response rate and agreed with what has been reported by Roche (1977) and Voh et al. (1987b).

Generally, the fertility rates recorded in the current study were acceptable and superior to earlier reports (Voh Jr. *et al.* 1984b; 1987a, 1987c) and reports by others who worked with zebu cattle (Hardin *et al.* 1980a; Landiver *et al.* 1985). This could be attributed to perfection of techniques over the years.

Lauderdale *et al.* (1974), Roche (1979), Macmillan and Day (1982) and Graves *et al.* (1985) have reported comparable fertility rates between controls inseminated at detected spontaneous oestrus and cows inseminated at detected synchronized oestrus.

Fixed time insemination at 60 hr after PGF2& treatment gave conception rate similar to AI at detected synchronized oestrus and superior to AI at other fixed times (72, 84 and 96 hr post-treatment). This is the most notable and significant finding in the current study. This is however, at variance with reports by Roche (1976) where in heifers, a single insemination on a fixed time basis at 48 or 60 hr after treatment resulted in lower fertility whereas insemination at 72 or at 80 has been reported to give normal fertility (Lamming *et al* 1975; Roche, 1977). We had reported earlier that at 60 hr after treatment 53% of treated cows had shown oestrus (Voh Jr. *et al.* 1987b), a finding that would in part justify the good fertility to a single insemination at 60 hr after treatment.

Orihuela *et al* (1983) and Voh Jr. *et al.* (1987a, 1989a) had hypothesised that fixed time AI earlier than the conventional 72 hr might result in better fertility. The current study has confirmed the hypothesis. This may be a turning point and a solution to the problems hitherto encountered in the use of double injection of PGF2& to

synchronized oestrus in zebu cattle. However, fixed time AI at 60 hr has to be tested more extensively under field conditions before adoption on a large scale.

The problem that can be foreseen with this double injection regime is the cost of the drug in Nigeria. Any system that will reduce the cost of the drug will certainly be a welcome development. But it must be remembered that cost should not be the only consideration in choosing a synchronization regime. Other benefits like convenience and ease of planning need to be considered as well.

The practical implication of these findings is that for zebu cows treated with PGF twice 11 days apart, single fixed time insemination should be carried out 60 hr after the second injection. A second insemination may be carried out 24 hr following the first, that is double fixed-time AI at 60 and 84 hr. To do this it is recommended that the injections be given in the evening so that the animals will eventually be inseminated in the morning since the times from PGF injection to the onset of oestrus, LH peak or ovulation are not affected by the time of the day PGF was given (Nkutuhe and Manns, 1985). This suggestion has become necessary so that inseminations do not stretch into the darkness of the evening especially when a large number of animals is involved.

Table 4.1: Experimental design showing assignment of animals and treatments

Treatment	PGF2 alpha	Breeding Protocol	No: -- Assigned
1	None	At spontaneous oestrus detected within 45 days	70
2	25mg twice 11 days apart	At detected oestrus within 7 days after PGF2&	70
3	25mg twice 11 days apart	At approximate 60 hr after 2nd PGF2&	70
4	25mg twice 11 days part	At approximately 72hr after 2nd PGF2&	70
5	25mg twice 11 days apart	At approximately 84hr after 2nd PGF2&	70
6	25mg twice 11 days apart	At approximately 96hr after 2nd PGF2&	70
Total			420

Table 4.2: A comparison of fertility following PGF2 alpha injection and A.I.

Insemination Group	Numbers				Rates		
	Assi-gned	Oes-trus	Inse minated	Preg-nant	Oes-trus	Preg-nant	Concep-tion
1. AI at detected natural oestrus	70	65	65	48	92.9	68.6[a]	74.0[a]
2. AI at detected synchronized oestrus	70	67	67	40	95.7	57.1[b]	60.0[b]
3. AI at approx 60 hr after $PGF_2\alpha$	70	65	70	38	92.9	54.3[b]	54.3[b]
4. AI at approx 72 hr after $PGF_2\alpha$	70	64	70	32	91.4	45.7[c]	45.7[c]
5. AI at approx 84hr afater PGF2&	70	65	70	29	92.9	41.4[c]	41.4[c]
6. AI at approx 96 hr after PGF2&	70	67	70	28	95.7	40.0[c]	40.0[c]
Total	420	393	412	215	93.6	51.2	52.1

a,b,c: data within the same column with different superscript differ significantly ($P < 0.05$).

CHAPTER FIVE

OESTRUS RESPONSE AND FERTILITY RATES OF ZEBU COWS AFTER TREATMENT WITH PROGESTERONE-RELEASING INTRAVAGINAL DEVICE (PRID), ALONE OR IN COMBINATION WITH PROSTAGLANDIN F2 ALPHA (PGF2&)

5.0 INTRODUCTION

Based on the available knowledge of the main endocrine and ovarian events of the bovine oestrous cycle, there are two logical approaches to the control of the oestrous cycle viz: (1) to apply an artificial corpus luteum or source of progesterone for a specific period of time and (2) to control the lifespan of the natural corpus luteum, the origin of progesterone.

Long-, medium - and short - term treatments with progesterone and progestational agents have been used to synchronize oestrus in cattle (Roche 1974; Wishart and Young, 1974; Sprott *et al.*, 1984).

Although oestrus has been successfully synchronized, first-service fertility after treatment has been low for long and medium-term treatments, which has been compounded by a high incidence of early embryonic mortality and abnormalities of the ovum (Roche 1974; 1975; Chupin *et al.*, 1975; Heersche et al., 1979; Sprott *et al.* 1984; Wilson *et al.*, 1986). Shorter progestagen treatments have resulted in tighter synchrony as well as higher fertility rates than long-term and medium-term treatments. But adequate control of endogenous CL is required in short - term treatments. This is achieved by the incorporation of luteolytic agents like oestradiol. Doubts have however, been expressed about the luteolytic potency of oestradiol in that if luteolysis does occur at all, it is a very gradual process. Thus, PGF2&

is sometimes injected towards the end of progestagen treatment (a day before or on the day of withdrawal of progestagen (Smith *et al.*, 1984).

Thus, a combination of short-term administration of progestagen and single injection of PGF2& not only overcomes the limitations of the two methods used singly but has been reported to shorten the postpartum anoestrus period by inducing ovarian activity in anoestrus postpartum cows approaching their first ovulation (Beal, 1983). The objective of this study therefore, was to determine the oestrus response and fertility rates of zebu cows treated with PRID, alone or in combination with PGF2&.

5.1 MATERIALS AND METHODS

5.1.1 Pre-treatment cyclicity assessment

All cows were assessed for ovarian cyclicity by rectal palpation performed at weekly intervals for 4 consecutive weeks before being included in the trial. Only animals with palpable ovarian structures-corpus luteum or follicle, in at least 2 examinations were used for the study.

5.1.2 Treatment groups.

A total of 184 dry (non-suckled) randomly cycling Bunaji cows were randomly alloted to 4 treatment groups of 46 cows per group as follows:

5.1.2.1 Group 1 (PRID-12)

Progesterone releasing intravaginal device (PRID) in the form of silastic coils were inserted using a speculum. The PRID was removed 12 days after insertion. A gelatine capsule containing 10mg oestradiol benzoate (ODB) was attached to each device.

5.1.2.2 Group 2 (PRID-7 + PGF2&-6)

PRID was inserted and left in place for 7 days and prostaglandin F2 alpha (PGF2&) was injected intramuscularly 1 day prior to PRID withdrawal i.e on day 6.

5.1.2.3 Group 3 (PRID-7+PGF2&-7)

PRID was inserted and left in place for 7 days and PGF2& was administered on the day of PRID withdrawal i.e on day 7.

5.1.2.4 Group 4 (2 x PGF2& -13)

Two injections of PGF2& 13 days apart.

5.1.3 Blood Sampling

Six cows each were randomly selected from each treatment group to study and characterise progesterone profile prior to, during and after treatment. Blood samples were collected via jugular vein puncture. Two samples were collected 3 days apart, prior to commencement of treatments and another sample on the day of treatment. Thereafter the cows were bled daily until they came into oestrus after which further blood samples were collected every other day for 30 days. The samples were centrifuged a day after collection, serum harvested and stored at -20oC until assayed by radioimmunoassay for progesterone.

5.1.4 Assay Method

Serum progesterone levels were determined using solid-phase iodinated gamma counter (Voh Jr., 1984).

5.1.5 Oestrus detection and insemination:

The cows were kept in paddocks and at the end of the treatment period they were observed continuously for 7 days for behavioural oestrus (Voh, Jr. et al., 1987c).

Standing-to-be-mounted was the only criterion used to judge a cow to be in oestrus. Cows in oestrus were artificially inseminated about 12 hours after the onset of oestrus using deep frozen semen from a proven bull.

5.1.6 Pregnancy diagnosis

Pregnancy status was determined by rectal palpation at 40 to 60 days after insemination.

5.1.7 Data analysis

Comparisons of oestrus response, intervals from treatment to the first post-treatment oestrus, pregnancy and conception rates were made. The data were analysed using chisquare techniques (Steel and Torrie, 1960).

5.1.8 PRID and its insertion

PRID is a registered trade mark of Abbot Laboratories Ltd, USA. The device consists of a stainless steel spiral (30cm x 3.2cm x 3 mm when uncoiled, Roche 1976) covered with an inert silicon elastomer with a gelatine capsule attached. Each device contains 1.55g of progesterone which is uniformly distributed throughout the elastomer, a capsule containing 10mg oestradiol benzoate (ODB) and inert silicon rubber. The progesterone and oestradiol benzoate are released rapidly and absorbed through the vaginal wall resulting in serum progesterone being maintained at luteal levels during treatment period. The ODB is converted to 17B oestradiol which assists in the premature regression or in the prevention of the formation of the corpus luteum. Following proper disinfection procedures of the rod and speculum, the speculum was lubricated with a centrimide cream. The device was loaded into the bevelled end of the disinfected speculum, with the string clear of the opening. The rod was then inserted into the loaded speculum.

The vulva was cleaned immediately prior to insertion using a suitable paper wipe. Holding the speculum with one hand, the vulva lips were spread apart with the other hand and the loaded speculum was gently inserted into the vagina. The slot of the speculum should be in the 9 o'clock position for ease of insertion. Still holding the speculum pointed slightly upwards the speculum was turned so that the slot was in the 12 o'clock position after which it was gently pushed into the interior vaginal area until it reached the cervix. The device was then expelled into the vagina by gently retracting the speculum over the rod, whilst holding the rod stationary. The speculum and rod were then withdrawn leaving the string extended from the vulva. The string was gently pulled to straighten. The speculum and rod were disinfected and re-used. The device was withdrawn by gently pulling on the exposed string at the end of the treatment period.

5.1.9 PGF2& Used:

Cloprostenol (Estrumate, Coopers, formerly ICI, UK), was the PGF2& that was used. It was given at a dose rate of 500 ug in 2ml per cow, regardless of age and weight, by deep intramuscular injection in the gluteal region.

5.2 RESULTS

The data on oestrus response, interval from treatment to oestrus, PRID retention and fertility rates are summarised in Table 5.1 - 5.5. All treatments effectively synchronized oestrus as indicated by the following parameters that were assessed.

5.2.1 Interval from end of treatment to onset of oestrus and tightness of synchrony:

The interval from end of treatment to onset of oestrus was shorter in all PRID-based treatments than in the PGF2&-only treatment. In all the PRID-based treatments (Groups 1-3), 45 to 55% of all oestrus onsets were on day 2 with 80% of onsets within

days 2 and 3 whereas in the PGF2&-only treatment (Group 4) about 50% of all oestrus onsets were on day 3 with 70% of onsets within days 3 and 4. The PRID-only treatment (Group 1) had the tightest synchrony of oestrus with all oestrus onsets within days 2 to 4 (Figure 5.1 and Table 5.2).

5.2.2 Oestrus response rate (ORR):

Eighty-three percent (83%) of all the treated cows were observed in oestrus within 144 hr after end of treatment. The respective oestrus response rates were 78.3%, 76.1%, 87.0% and 89.1% for Groups 1 to 4. The ORR for Groups 3 and 4 were superior to the ORRs for Groups 1 and 2 although the differences were not significant ($P>0.05$; $X2 = 1.4$) (Table 5.1).

5.2.3. PRID retention rate (PRR)

The overall PRR (95%), was high and acceptable for all the PRID-based treatment groups. The PRRs for groups 1,2 and 3 were 93.5%, 93.5% and 97.8% respectively indicating very high and acceptable retention rates for the individual treatment groups. Two of the cows that lost their PRIDS came into oestrus within the same period of study and were inseminated (Table 5.3).

5.2.4. Fertility rates:

The pregnancy rates (PR) were 39.1%, 41.3%, 52.2% and 52.2% for groups 1 to 4 respectively with an overall PR of 46.2%. The corresponding conception rates (CRs) were 50.0%, 54.3%, 60.0% and 58.6% for groups 1 to 4. The overall CR for all the treatment groups put together was 56.0%. Fertility rates differed across the treatment groups. The highest pregnancy and conception rates were obtained in groups 3 and 4

although the differences between the groups were not significant ($P > 0.05$). ($X2$ for PR = 3.29; $X2$ for CR = 0.93) (Table 5.4).

The 2 cows that lost their PRID but came into oestrus and were inseminated both became pregnant.

5.2.5 Vaginal discharge following PRID withdrawal:

Muco-purulent and sometimes pungent smelling vaginal discharge was observed in 15 cows at the time of PRID with drawal in all the PRID-based treatment groups with 9, 3 and 3 cases for groups 1, 2 and 3 respectively. The discharge cleared spontaneously without any treatment after PRID withdrawal. Ten (10) out of the 15 animals were observed in oestrus and inseminated. Six of the ten inseminated animals became pregnant indicating that the vaginal discharge had no explicit detrimental effect on both ORR and fertility rate.

5.2.6 Progesterone profiles during treatment

Based on progesterone concentrations, 6 cows each were at the luteal and follicular phases of the oestrous cycle at the commencement of treatments as summarised in Table 5.5. The mean ($\pm$ standard error) daily serum levels of progesterone during the treatment for the various groups are presented in Figure 5.2. Standard errors ranged from 0.2 to 1.0 ng/ml and generally were proportional to the means.

It can be seen from the figure that PRID increased blood levels of progesterone within 24 hours of insertion and maintained luteal levels sufficient to inhibit oestrus for the duration for which the coil was intravaginal. The levels of progesterone during the period for which PRID was in place were similar irrespective of the treatment group and consisted of luteal or high levels the day after insertion which declined very steadily to the end of the treatment for PRID-based treatment groups. The decline in levels of progesterone

following PRID withdrawal was very transient and complete, dropping to basal levels within 24 hours of withdrawal. For the 2 x PGF2&-13 group the progesterone profiles were typical: for cows in the luteal phase, and all the cows were in the luteal phase at the time of second injection, the progesterone levels were high on the day of PGF2& injection, which declined to basal levels within 24 to 48 hours following treatment and was then followed by oestrus.

Individual variations in levels of progesterone were observed across treatment groups but the variations appeared to be more noticeable in the PRID-only group suggesting that as the period of PRID treatment increased the ability of PRID to effectively maintain luteal-phase levels of progesterone faltered slightly.

5.3 DISCUSSION

The results of the experiment have demonstrated the effectiveness of all the 4 tested regimes to synchronize oestrus and ovulation in Bunaji cows. These results agree with those reported following the use of similar systems in Bos taurus (Roche 1976; Heersche *et al.*, 1979; Smith *et al.*, 1980, 1984), zebu and crossbred cows (Lokhande *et al.*, 1983, 1984).

5.3.1 Interval to onset of oestrus

The onset of oestrus was earlier and more synchronous in PRID - based treatments than in the PGF only treatment. Other researchers have observed similar improved synchrony of oestrus when system in which PGF or its analogue was administered a day before PRID withdrawal were compared with those using PGF2 alpha at the time of progesterone withdrawal or alone (Thimonier *et al.*, 1975; Chupin *et al.*, 1977; and Smith *et al.*, 1984). The practical implication of this is from the point of view of predetermined insemination where different times may have to be used especially for the PRID - based treatments and the PGF - only treatment.

Smith *et al.* (1984) explained the improved synchrony of oestrus in the PRID - 7 + PGF2& - 6 group to be probably related to the ability of PRID to maintain plasma progesterone concentrations of 3.5 to 4ng/ml in the absence of a corpus luteum. Hansel and Beal (1979) speculated that the improved synchrony in heifers that received PGF one day before PRID removal might be due to the following: (a) a decrease in the variability of the rate at which progesterone concentrations declined after PGF (b) priming effects of PGF before progesterone withdrawal on follicle growth and/or oestrogen secretion or (c) a combination of these and/or other un identified factors. On

the other hand. Roche (1981) gave poor control of luteal function as the major reason for variation in onset of oestrus in cattle following the 12-day PRID treatment.

Roche *et al.*, (1981) reported that if a treatment period of less than 12 days is used, the onset of oestrus is more variable although fertilities following AI at detected oestrus have been similar. Our current studies further confirm this statement.

Based on the excellent synchrony of oestrus and acceptable conception rates after the treatments, it is hoped that the treatments would be precise enough to give acceptable fertility rates when combined with a single predetermined insemination at the appropriate time.

5.3.2 Oestrus response rates:

Oestrus response in terms of the number of cows observed in oestrus within 144 hr after end of treatments was very good (83%) and did not differ between groups. This is in consonnance with results reported by others (Roche 1976; Heersche *et al.*, 1979 and Smith *et al.*, 1984). The 2 x PGF-1B treatment showed higher ORR than the other treatments and further confirmed our earlier reports (Voh, Jr. et al., 1987c).

5.3.3 PRID retention rate:

The PRID retention rate (95%) was excellent and in agreement with other reports. Roche (1976, 1978) reported retention rate of 95 to 97%. This current study also showed that cows which lose their PRID are capable of showing fertile oestrus depending on the stage of the treatment at which the PRID was lost.

5.3.4 Fertility rates:

The results of this study like those before it (Voh Jr. et al. 1987a,d. 1989) have demonstrated that fertility is good if a high proportion of cows are inseminated at observed oestrus. This fact is responsible for the difference observed between pregnancy rate and

conception rate. Normal and acceptable fertility has been reported in cattle following AI at a synchronized oestrus using PRID alone or in combination with PGF (Roche, 1976, 1978; Heersche *et al.*, 1979; Smith et al., 1984). Poor fertility rates have also been reported by other workers (Thimonier *et al.*, 1975; Lokhande *et al.*, 1983, 1984).

The results of many previous experiments showed variability in fertility rates due to known and unknown factors. These emphasize the need for users of oestrus synchronization methodologies to know that variable results in terms of oestrus response and fertility rates are to be espected even under the most controlled conditions thereby necessitating caution. It is the responsibility of the clinician to design the treatment regime best suited to the farm and to ensure the most profitable outcome.

5.3.5 Vaginal discharge following PRID withdrawal:

The results of this study have indicated that there should be no cause for alarm if this discharge is seen. Its presence however, attracts flies and may be unsightly.

5.3.6 Progesterone profiles during treatment:

The progeterone profiles during treatment have been typical and in consonnance with other reports (Roche and Gosling 1977, Roche *et al.*, 1981; Voh *et al.*, 1987b).

The need to use progesterone in the luteolytic complex is determined by the speed of the initial rise in progesterone. It is important to get the concentration of progesterone close to mid-luteal phase concentrations (3-6ng/ml) as soon as possible to prevent oestrogen and luteinizing hormone (LH) from inducing ovulation in animals in the follicular phase (Roche *et al.*, 1981) and to produce a maximum luteolytic effect in animals that have recently ovulated (Ginther, 1970)6. The initial release rate of in progesterone following PRID insertion in the current study was sufficient to effect

this as opposed to the situation whereby extra progesterone is required at the start of treatment when progestagen implants are used. Thus, the method of administeration of progestagens will have an effect on the pathern of response.

Clearly defining the optimum concentration of progesterone in blood during a synchronization treatment has become apparent since it has been demonstrated that progesterone has a negative feedback effect on basal LH levels in the cow (Roche *et al.*, 1981). Therefore, a concentration of progesterone in blood sufficient to block oestrus may not suppress the LH and FSH concentrations to basal values found during the luteal phase of the oestrous cycle. The optimum concentration of progesterone required to control oestrus may be that which maintains plasma LH at concentrations found during the luteal phase of the oestrous cycle, rather than the lower concentrations of progesterone required to block oestrus during treatment (Roche et al., 1981). Because of the interrelationships between ovarian steroids, pituitary gonadotrophins and follicular growth, which currently have not been clearly defined, Roche *et al.* (1981) suggested that presently available delivery systems be refined in order to maintain higher levels of progesterone in blood during the second half of a 12- or 14-day treatment period. It is also apparent that the shorter the duration of treatment, the higher the concentrations of progesterone in blood during treatment (Figure 2). Therefore a 7-day treatment period has the advantage of maintaining blood levels of progesterone at normal luteal-phase levels during the treatment period.

The level of progesterone over the last 3 days of treatment have been reported to be significantly related to the interval from removal of coils to onset of oestrus with longer interval to oestrus in animals with higher levels of progesterone indicating incomplete inhibition of CL function and suggesting that in such situations both endogenous and zexogenous

progesterone were being measured (Roche and Gosling 1977). Since higher levels of progesterone were observed in the 7-day than the 12-day treatments, this may account for the longer interval to oestrus onset observed in the 7-day treatments.

From the foregoing it can be seen that the level of progesterone at the commencement, during and at the end of the treatment is highly related to the type of response following oestrus synchronization with progestagens thereby highlighting the need for further refined investigations into perioestrual endocrinological events to fully elucidate and understand PRID-based oestrus synchronization systems.

The results of the experiment have demonstrated or confirmed the effectiveness of all the tested oestrus control regimes to synchronize and control ovulation in Bunaji cows and therefore can be employed.

Although not statistically different, oestrus response, pregnancy and conception rates tended to be higher for the PRID -7+PGF-7 and 2xPGF-13 treatments than for the other treatments.8

PRID has the potential to provide farmers with a simple technique for effective synchronization of oestrus in zebu cows when used either alone or in combination with PGF2&. This potential will be markedly appreciated if it is tested and confirmed that PRID-based oestrus synchronization systems are capable of inducing and controlling a fertile ovulation in both oestrus and anoestrus circumstances. If this claim can be verified in Nigerian zebu cows then PRID-based treatments will be preferred over PGF2& treatments which are ineffective in inducing oestrus in postpartum anoestrus cows.

Table 5.1: Oestrus response rates of Bunaji cows following treatment with PRID alone or in combination with PGF2&

TREATMENT GROUP	PARAMETER		
	No Treated	No In Oestrus	Oestrus %
PRID-12 only	46	36	78.3
PRID-7+PGF2&-6	46	35	76.1
PRID-7+PGF2&-7	46	40	87.0
2XPGF2&-13	46	41	89.1
TOTAL	184	152	82.6

Table 5.2: Interval from end of treatment to onset of Oestrus

PARAMETER	TREATMENT GROUP				
No of cows in oestrus	PRID-12 only	PRID-7+	PRID-7+ PGF2α-6	2XPGF2α-13 PGF2α-7	TOTAL*
Days from end of treatment to onset of oestrus					
1	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
2	21(58.3)	16(45.7)	18(45.0)	4(9.8)	59(38.8)
3	11(30.6)	12(34.3)	11(27.5)	20(48.6)	54(35.5)
4	4(11.1)	5(14.3)	5(12.5)	9(22.0)	23(15.1)
5	0(0.0)	2(5.7)	4(10.0)	4(9.8)	10(6.6)
6	0(0.0)	0(0.0)	2(5.0)	4(9.8)	(4.0)
TOTAL	36(78.3)	35(76.1)	40(87.6)	41(89.1)	152(82.6)

* Percent in parentheses

Table 5.3: PRID retention rate in zebu cows

TREATMENT GROUP	PARAMETER No.Inserted	No.Lost	No. Retained
PRID-12 only	46	3(6.3)	43(93.5)
PRID-7+PGF2α-6	46	3(6.5)	43(93.5)
PRID-7+PGF2α-7	46	1(2.2)	45(97.8)
TOTAL	138	7(5.0)	131(95.0)

Percent in parentheses

Table 5.4: Fertility rates of Bunaji cows following various oestrus synchronization regimes

TREATMENT GROUP	PARAMETER Number Treated	in heat	pregnant	pregnancy rate	Conception rate
PRID-12 only	46	36	18	39.1	50.0
PRID-7+PGF2α-6	46	35	19	41.3	54.3
PRID-7+PGF2α-7	46	40	24	52.2	60.0
2XPGF2α-13	46	41	24	52.2	58.6
TOTAL	184	152	85	46.2	56.0

PR (Pregnancy rate) = Number of cows pregnant divided by the number assigned to the group and expressed as a percentage

Conception rate = Number of cows pregnant divided by the number inseminated and expressed as a percentage.

Data did not differ between groups ($P > 0.05$).

Table 5.5: Distribution of animals by phases of the oestrous cycle at commencement of treatments as determined by progesterone concentrations.

TREATMENT GROUP	PHASE OF THE OESTROUS CYCLE			
	LUTEAL	FOLLICULAR	NO. IN OESTRUS	NO. STUDIED
Group 1 (PRID only -12 only)	2	4	6	6
Group 2 (PRID 7+PGF-6)	3	3	5	6
Group 3 (PRID -7+PGF-7)	3	3	5	6
Group 4 (2xPGFα13)	4	2	5	6
Total	12	12	21	24

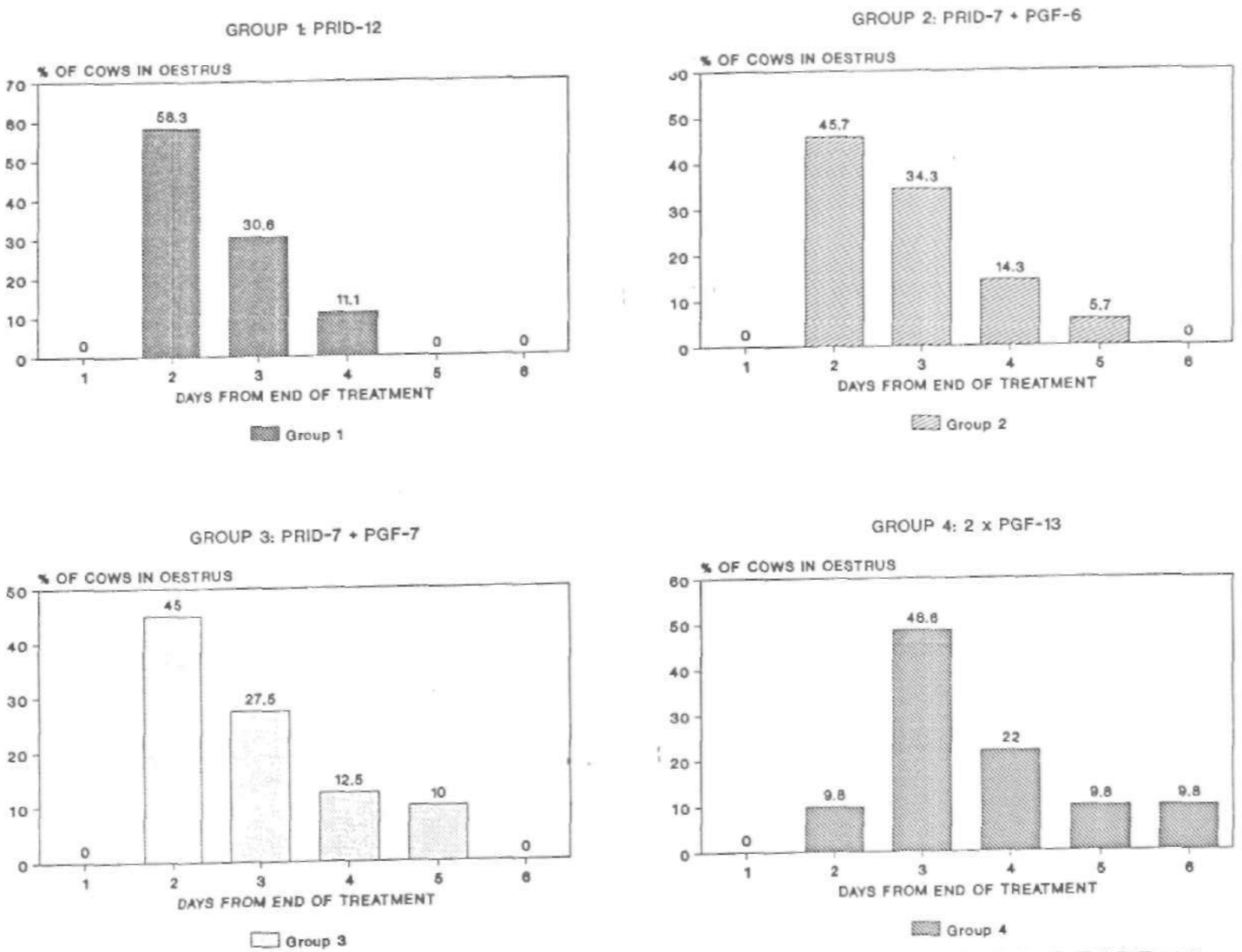

FIG.5.1. INTERVALS FROM END OF TREATMENT TO ONSET OF OESTRUS

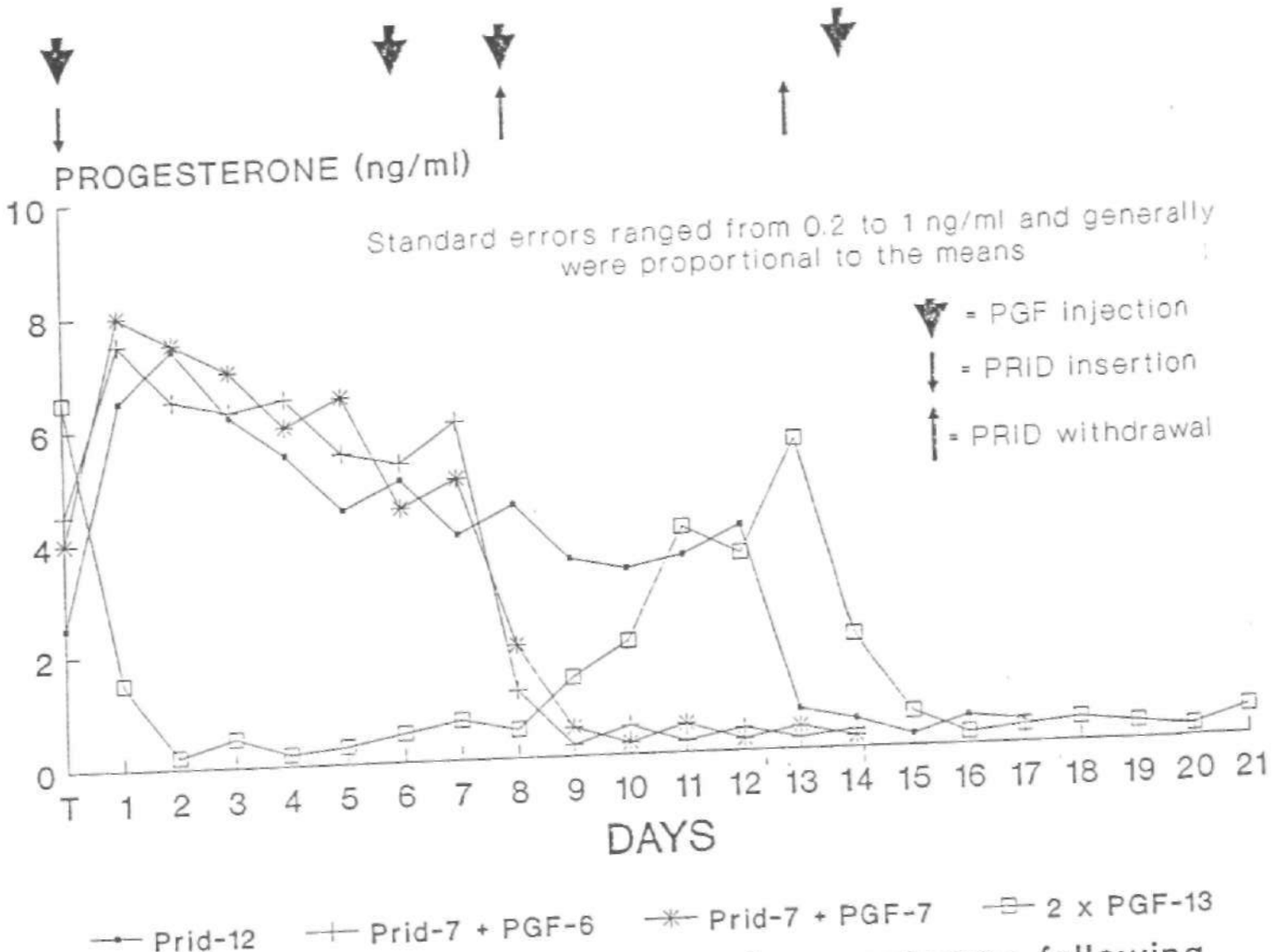

Figure 2. Mean daily serum levels of progesterone following various oestrus synchronization regimens

CHAPTER SIX

DETERMINATION OF INCIDENCES OF LATE EMBRYONIC MORTALITY (LEM) AND REPEAT BREEDER SYNDROME (RBS)IN ARTIFICIALLY INSEMINATED BUNAJI COWS FOLLOWING OESTRUS SYNCHRONIZATION

6.0 INTRODUCTION

Embryonic mortality (EM) strictly interpreted, should refer to fertility losses during the embryonic period, i.e the period extending from conception to completion of the stage of differentiation which, in the cow occurs at approximaately 45 days (Committee on Reproductive Nomenclature 1972). However, many authors have included under this term, fertilization, as well as death after fertilization (Kummerfeld et al 1978; Ayalon 1981).

A repeat breeder (RB) on the other hand is defined here as a cow without morphological or clinical abnormalities which have had four or more non-fertile inseminations (Almeida et al 1984; Maurer and Echternkamp, 1985).

Reproductive failure is one of the major factors effecting output or productivity in cattle herds. Embryonic mortality, and by extension repeat breeding, is the major cause of reproductive failure or loss in cattle (Ayalon 1981). Infertility and embryonic mortality have been reported to account for 30-40% reduction in reproductive efficiency in cattle. Maurer and Chenault (1973) have shown,in parous females, that EM (32%) accounted for 100% of the reduction in reproductive efficiency, whereas in non-parous females, infertility (17%) and EM (17%) equally contributed to the losses that occured by Day 18 of gestation with the largest proportion of reproductive failure occurring by Day 7 or 8 of gestation (Roche et al.

1981; Maurer and Echternkamp, 1982). The critical time for early EM was found to be Day 7 post-insemination i.e soon after the embryo enters the uterus, when the morula is developing into a blastocyst(Ayalon 1973). Linares (1981) found a very high incidence of abnormal embryos in RB heifers 7 days after AI which explained the high EM rate in these animals.

It has been established that the bovine embryo has to be in the uterus on Day 16 inorder to prevent luteolysis and subsequent oestrus (Betteridge et al 1980). In RBs, which return to oestrus at a normal interval after AI, it would seem likely that embryonic death had occurred before Day 16 or that embryonic signal is not delivered by the embryo or received by the mother."

In a study of reciprocal transfer of embryos followed by planned slaughter, Gustafsson and Larsson (1985) published results that suggested that the uterine environment of RB heifers is suboptimal for the support of normal embryonic development. In a survey of the incidence of the RB cow in Sweden, Hewett (1968) found an incidence of over 10% which increased with farm size, season and age. While EM rates up to 35 days for first service heifers and RB heifers ranged from 10 to 30%, it ranged from 40 to 70% in RB cows.

The above data highlights the importance of EM and RB syndrome as serious causes of production losses. Lower fertility in RB females has been attributed to fertilization failure, increased EM, genetic and reproductive tract aberrations and hormonal asynchrony. Maurer and Echternkamp (1982) have reported that administering of PGF2& intravenously rather than intramuscularly may cause the demise of CL sooner and thereby produce a uterine environment which allows more embryos to develop normally. In view

of the importance of EM and RB syndrome especially in an AI programme and the lack of information on same for Nigerian zebu cows, the current study was therefore undertaken to delineate more clearly the existence and extent of EM and RBs in an AI programme in Nigeria.

6.1 MATERIALS AND METHODS

6.1.1 Pre-experimental study

Prior to the commencement of the study, the cows were observed for oestrus for a pre-experimental period of 3 months without inseminations to determine the oestrous cycle lengths in cows not inseminated to eventually compare these with oestrous cycle lengths of cows that were inseminated and later found not to be pregnant.

The study which was conducted over a period of 4 years (1986 to 1989), consisted of synchronization of oestrus with PGF2& followed by appropriate insemination schedules intended to produce high level of fertility, for both synchronized and control non-synchronized cows. After AI the animals were observed for oestrus and reinseminated over a period of time.

In addition blood samples were collected via jugular vein puncture 21, 35 and 45 days after AI from which serum was harvested and stored at -20oC until assayed for progesterone by radioimmunoassay. Pregnancy was confirmed by rectal palpation at 45-60 days post AI.

6.1.2 Estimation of embryonic mortality

Embryonic mortality was estimated by both the delayed return rate (DRR)and serum progesterone concentration (SPC) methodsas described by Wood (1976) and Kummerfeld et al

(1978). For the progesterone concentration method a high level of progesterone followed by a sudden decline at the next sampling was assumed to indicate that pregnancy had been initiated and terminated.

For the delayed return rate method, oestrous cycle lengths were categorised as follows:

1. normal: 17-24 days;
2. genuine short cycle: 8-9 days;
3. luteal inseminations: less than 8 days or 11 to 16 days;
4. non-detected cases/silent oestrus: 37-45 days;
5. embryonic loss2: 25 to 36 or greater than 45 days. EM rate was calculated as the number of lost pregnancies divided by the total number of pregnancies initiated and expressed as a percentage.

6.1.3 Calculation of LEM and RB rates

RB

This was calculated as the number of cows which have had four or more unsuccessful inseminations divided by the total number of cows inseminated and expressed as a percentage.

LEM

This was calculated as the number of lost pregnancies divided by the total number of pregnancies initiated and expressed as a percentage.

6.2 RESULTS

6.2.1 Delayed return rate method

A total of 288 cows were used to conduct the study over a 4-year period spanning 1986 to 1989 during which a total of 623 oestrous cycle lengths following non-fertile inseminations was recorded. The year of study did not influence the results so the data were pooled. The determinations of the incidence of Repeat Breeder syndrome was carried out during 2 years (Year 1 and 3) involving a total of 170 cows. Of this number, 26 were found to RBs representing an incidence rate of 15.3% (Table 1).

The distribution of oestrous cycle lengths for non-inseminated, inseminated and repeat breeder cows is summarised in Table 2. Non-inseminated cows had a significantly higher proportion of cows with normal oestrous lengths than inseminated cows which in turn had higher but non-significant oestrous cycle lengths than repeat breeder cows.

The delayed return estimate of embryonic mortality rate for inseminated cows and repeat breeder cows were 21.4% and 33.7% respectively giving an overall embryonic mortality rate of 27.6%. The proportion of cows with genuinely short oestrous cycles (8-9 days) were 5%, 3.7% and 1.8% for non-inseminated, inseminated and RB cows respectively whereas the corresponding rates of non-detected oestrus (NDO) or silent oestrus (37-45 days) were 6.0%, 8.2% and 7.2%. Furthermore, the rates for luteal phase inseminations were 2.7% and 2.5% for inseminated and RB cows respectively giving an overall luteal insemination rate of 2.6%. Non-inseminated cows had a rate of 2.5% which is unrealistic since the animals were not inseminated. It however highlights the sources of inevitable errors inherent in the delayed return rate method.

6.2.2 Serum progesterone concentration method

Serum progesterone data was obtained from 200 samples to accurately monitor 120 cows that became pregnant by day 21 after successful insemination. Fifteen of these embryos were lost between day 21 and 45 observation period giving an overall late embryonic mortality rate of 12.5%. Eleven of the embryos were lost between days 21 and 35 while 4 were lost between days 35 and 45 representing 9.2% and 3.3% of LEM rates respectively.

6.3 DISCUSSION

Oestrous cycle lengths for repeat breeders in the current study were within the normal range (17 to 24 days) for normal Bunaji cows and is in agreement with previous studies by Ayalon (1978) and Maurer and Echternkamp (1985) who demonstrated that Bos taurus RB cows have normal oestrous cycles and fertilization rates.

The incidence rate of RB of 15.3% is also in consonance with those reported by Pelissier (1970) and Ayalon (1984) which ranged from 10.2% to 18% but is at variance with Maurer and Echternkamp (1985) who reported low rates of 1.0% and 1.7%.

The 27.6% and 12.5% embryonic mortality rates in this study as estimated by delayed return and progesterone concentration methods respectively are both within the range reported by several workers using the same methods (Ayalon, 1969; Kummerfeld et al. 1978; Gestafsson and Larsson, 1985).

Delayed return to oestrus and progesterone concentration methods, both being methods used in this study, have short comings arising from at least 4 reasons.

1. cows which had not been in oestrus may have been inseminated. Estimations of progesterone levels in blood (Appleyard and Cook, 1976) and milk (Hoffman et al. 1976) have shown that up to 20% of cows presented for AI are probably not in oestrus.
2. post-insemination endometritis or infection associated with persistence of the CL with delayed returns to oestrus (Ginther, 1968).
3. failure to observe oestrus due to anoestrus or suboestrus (Ayalon, 1981).
4. the major portion of embryonic losses occurs well before Day 15 after service (Ayalon et al. 1968; Boyd et al 1969; Maurer and Chenault, 1983; Maurer and Echternkamp, 1985) and therefore the embryo dies too early and such cows will therefore return to oestrus after the same interval as unmated animals, despite having been pregnant.

While the first two reasons will result in an over-estimation of EM, the last 2 reasons will result inunder-estimation. Inclusion of progesterone evaluation on the day of insemination has been suggested as a way of increasing the accuracy of the method.

In addition to the delayed return to oestrus and progesterone concentration method, planned slaughter after breeding has been employed to study EM and RB syndrome (Graden et al 1968; Boyd et al 1969; Maurer and Chenault, 1983; Gestafsson and Larsson, 1985). Althoguh planned slaughter method is expensive and impractical under field conditions, it has however, yielded the most reliable information about the nature of EM (Ayalon, 1981).

It is widely considered that an increase in the interval between service and return to oestrus beyond the usual range of 17-24 days reflects EM (Erb and Holtz, 1958). This is supported by observations, such as those of Marion (1950), that mating with a vasectomized bull had no significant effects on the subsequent time of return to oestrus; and those of Boyd (1973) who found a significant difference in intervals between periods of oestrus before and after first inseminations with 90% of pre-insemination cycles being of normal length, as compared with only 43.5% of post-insemination cycles. The results of the current study (85% vs 64% or 54% for RB) further supports these previous findings.

Besides having normal oestrous cycles and fertilization rates, the incidence of abnormal embryos in RB cows was not significantly different from NB cows up to and including day 6 post-breeding. On day 7 the differences became significant (Ayalon 1972; 1978). Ayalon (1978) also reported significant changes in ionic contents of uterine flushings. These results along with those of Newcomb and Rowson (1980) and Almeida et al 1984a,b) have confirmed the importance of uterine ionic and protein contents on the normal morula blastocyst development. Some of the notable causes and/or factors that may influence EM and RB are gentics (Conneally et al. 1963), nutrition (Franzos et al 1979), age (Ball, 1978), climate (Ingraham et al 1974), infection (Alder 1959; Bawa et al.,1988), semen quality (Kidder et al. 1954), hormonal imbalance (Randel et al., 1971; Erb et al. 1976) and uterine environment (Newcomb and Rowson, 1980; Almeida et al. 1984) Since embryonic mortality occuring between day 6 and day 16 can not be estimated by the methods used in the present study coupled with the fact that most embryonic losses occur during this period it is imperative to make the distinction between early embryonic mortality (EEM) and late

embryonic moratlity (LEM). In effect what has been investigated in the present study is LEM. Consequently if the two are investigated the embryonic mortality rate will be a lot higher than what has been reported. This will further show the importance of EM as a major cause of reproductive failure in indigenous Nigeria cattle.

In conclusion, while recognizing the fact that the most reliable methods for studying EM and RB entail planned slaughter, laparoscopic and systematic endocrinological approaches, the present study has no doubt confirmed the existence and to some level the extent of EM and RB syndrome in artificially inseminated Bunaji cows. More detailed work is needed to delineate more clearly the extent and economic importance of EM and RB syndrome.

Table 6.1 Experimental design by year of study

YEAR	No. of cows used	No. of oestrous cycles studied	No. of repeat breeders
1	86	150	11
2	56	108	N.I.
3	84	212	15
4	62	143	N.I
Total	**288**	**623**	**26(15.3)**

*** N.I = Not investigated**

Table 6.2 Embryonic mortality rate estimated by delayed return method and comparative distribution of pre-insemination and post-insemination oestrous cycle lengths for normal and repeat breeder cows.

		COW GROUP		
Cycle length category (days)	Interpretation	Non-inseminated	Inseminated	Repeat Breeder
8-9	Genuinely short cycle	10(5)	23(3.7)	3(1.8)
<8 or 10-16	Luteal insemination	5(2.5)	17(2.7)	4(2.5)
17-24	Normal cycle	170(85)	399(64.0)	91(54.8)
25-36*	Embryonic loss	3(1.5)	133(21.4)	56(33.7)
37-45	Non-detected oestrus or silent oestrus	12(6.0)	51(8.2)	12(7.2)
Total		**200**	**623**	**166**

% in parentheses
* category representing embryonic loss

Table 6.3 Embryonic mortality estimated by serum progesterone concentrations in Bunaji cows.

Status of pregnancy	No. of cows
Maintained	105
Lost between 21 and 35 days	11 (9.2)
Lost between 35 and 45 days	4 (3.3)
Total lost pregnancies	15
Total pregnancies initiated	120
Estimated embryonic mortality rate (%)	12.5%

CHAPTER SEVEN

THE USE OF RECTAL PALPATION IN THE DIAGNOSIS AND AGEING OF PREGNANCY IN INDIGENOUS NIGERIAN ZEBU CATTLE

7.0 INTRODUCTION

The economic value of an early pregnany diagnosis in the cow is quite clear. Reports have shown that 15-25% of all bred cows and heifers that have not returned in oestrus, and are therefore assumed to be pregnant, actually are non-pregnant. When it is recognized that every missed oestrus means a loss of approximately 21 days' production, then the significance of timely detection of pregnancy, and particularly non-pregnancy is extremely important (Zemjanis, 1970). When one is dealing with cattle, the ultimate goal is an average of 12 months calving interval for the herd. Every anagement practice which contributes to attaining this goal is well worth consideration.

Estimation of the stage of pregnancy (ageing) has manifold values. It is frequently requested by clients, especially when animals without known breeding history are purchased. It is important in herds with incomplete or lost breeding records. There are also animals which might return to oestrus and be serviced as many as four times and be pregnant from the first service. It is most valuable in herds in which pasture breeding is practised and close supervision is impossible.

The ideal pregnancy test would be one that is inexpensive and highly accurate, that would be conducted at the farm, utilizing milk, blood, urine or other easily obtained specimens, that would detect pregnancy before the first oestrus after mating. No such test is available yet today (Anon 1980). Clinical and laboratory methods are available for the

diagnosis of pregnancy. The choice of method depends on the species, stage of estation, cost, accuracy and speed of diagnosis. The progesterone assay test is conducted on milk or blood samples taken 21 to 24 days post-breeding (Voh Jr. et al., 1987; Dawuda et al., 1989). It has to be conducted in a laboratory with highly sophisticated and expensive equipment which are not easily available to most developing countries. The use of recently developed beside-cow progesterone tests like Bovitest Dipstick are overcoming this constraint. The accuracy of this method is 65 to 90% for positive diagnosis and 95 to 100% for negative diagnosis. Therefore rectal examination remains the only practical means and method of choice for the diagnosis of pregnancy in cows that is currently available to cattle men today (Jainudeen and Hafef, 1980). The accuracy of this method can be as high as 100% (Noakes, 1986) depening on the stage of gestation and experience of the palpator.

There is a dearth of clinical data on rectal findings in the diagnosis of pregnancy, and particularly the ageing of pregnancy in zebu cows. Current pregnancy diagnosis and ageing work are based on extrapolation of rectal findings from Bos taurus breeds which may not necessarily be application to Bos indicus because of breed differences. The objective of the current study therefore was to identify those physiological changes of the reproductive organs that are associated with pregnancy, when the changes occur, how they progress with gestation and to use the findings in diagnosing and ageing pregnancy in indigenous zebu cows.

7.1 MATERIALS AND METHODS

7.1.1 Breeding

A total of 45 cycling Bunaji cows aged between 5 to 9 years were synchronized using prostaglandin F2 alpha and progesterone releasing intravaginal device (PRID) (Voh Jr. et al., 1988). Thirty-eight (38) of the synchronized cows were inseminated at detected oestrus. Of the 38 inseminated cows, 25 became pregnant. Two of the cows were soon dropped from the study because of difficulty to carry out a detailed rectal examination at the early stages of pregnancy. Therefore, 23 cows were eventually used for the study.

The cows were rectally palpated once a week, on the same day of the week as much as possible, from approximately day 30 following fertile insemination to day 14 after calving (to determine when after calving fremitus disappears).

The technique consisted of insertion of a lubricated gloved hand into the rectum of the cow, evacuation of feaces and then using the cervix as the landmark structure, manual exploration of the genitalia with retraction where necessary, for detection of physiological changes of the genital organs associated with pregnancy (Zemjanis 1970). Most of the diagnosis was based on the uterus and its contents. Both horns were examined for asymmetry, the four positive signs of pregnancy (fetal membrane slip, amniotic vesicle, cotyledons and fetus) and other clinically detectable signs of pregnancy. Time of first detection of the structures were noted. Similarly, progressive changes in size and position of the structures were also noted.

7.1.2 STRUCTURES PALPATED

7.1.2.1 CERVIX

The cervix was used mainly as a landmark for locating other structures. It was recognized as a firm, cylindrical or semi-conical and nodular structure (Zemjanis 1970) lying on the midline of the pelvic floor. Except for the bony structures, the cervix was the firmest structure in the pelvic cavity.

7.1.2.2. UTERUS

Most of the diagnosis was based on the uterus and its contents. The body of the uterus and both uterine horns were examined for size, position, tone or resilience and contents. The contents examined included:

a. **Chorioallantoic membrane ("Fetal Membrane Slip" - FMS)**

This was detected by gently grasping the uterine wall between the thumb and fore finger and lifting slightly and letting it slip between the fingers. The membrane slips or "drops" first before the upper fold of the wall of the uterus. Detection of the FMS is greatly facilitated if examined for in the widest portion of the horn, the wall of which is thinner (Zemjanis, 1970; Anon 1980).

b. **Amniotic vesicle (AV):**

The amnion containing the conceptus and the amniotic fluid forms the AV (Zemjanis 1970). This is usually detected by palpating the uterine horns with the thumb and forefinger by starting near the apex of the uterine horn and applying gentle pressure as the thumb and forefinger are moved back toward the cervix (Anon, 1980).

The AV is attached to the chorioallantois by a relatively long stalk thereby permiting the vesicle to float relatively free within the allantoic fluid (Zemjanis 1970).

c. **Cotyledons (fusion of both cotyledons and caruncles):**

They are recognized as circumscribed prominences palpated through the wall of the uterus.

d. ***Conceptus or* fetus:**

When the conceptus reaches a certain age during gestation, it can be detected by palpation through the wall of the uterus. This time coincides with the period when the amniotic vesicle begins to lose its turgidity (Zemjanis, 1970). Initially the fetus could only be bumped by rubbing the hand over the outer curvature of the uterine horn. Eventually it was possible for it to be felt and grasped directly.

7.1.2.3 OVARY (CORPUS LUTEUM)

The ovaries are found a few centimetres lateral to the midline within the pelvic cavity on the same level or slightly below the level of the pelvic brim. They can be located by grasping the anterior edge of the broad ligament during retraction. Another reliable method is palpation of the bony arch formed in the pelvic inlet by the pubic and iliac bones and then making grasping attempts in an area slightly medially, anteriorly and ventrally (Zemjanis, 1970). The palpable functional structure of the ovary at this stage is the corpus luteum.

7.1.2.4 MIDDLE UTERINE ARTERY (MUA) (Fremitus, Buzz, Pulse of pregnancy):

This pulse is felt in the middle uterine artery which branches from the internal iliac and

transcends to the uterus via the broad ligament near the forward edge of the pelvis. It passes laterally, posteriorly and ventrally along the medial edge of the iliac wings for a few centimeters and then leaves the pelvix wall to descend ventrally.

7.1.3 CALVING DATA:

The date of calving for each cow, as well as its weight at calving, sex and birthweight of the calf were recorded. From these data the gestation lengths, being the interval from fertile insemination to calving, were computed.

7.2 RESULTS

7.2.1 CERVIX

From day 30 to day 80 of gestation the cervic was pelvic and on the pelvic floor. As from day 80 onwards, due to weight increases of the uterine horns and body there was traction of the cervix resulting in its being tipped over the pelvic brim and its eventual fixation in a relatively inflexible position. There was also gradual increase in the size of the cervix with advancing stages of gestation.

7.2.2 UTERUS

a. **Asymmetry:** This was brought about due to increase in size of the uterus. Asymmetry was detectable in all the 23 animals from day 28 to 43 with over 80% being detectable prior to day 40.

b. **Position:** Because of the gradual increase in weight, there was a change in the position of the uterus. Movement of the uterus to the pelvic brim at 40 to 50 days was the first

noticed change in position. This was soon followed by the uterus tilting abdominal which commenced at about 70 days of gestation and was abdominal in all cows at 100 days. At about 140 to 150 days the uterus had reached the abdominal floor (Table 7.1).

c. **Dorsal bulge**: A dorsal bulge in the body of the uterus was detected in all the cows between day 43 and 58 of gestation. The dorsal bulge was due to pressure created by the uterine contents at this thin point of the body of the uterus.

d. **Fetal Membrane Slip (FMS)**: FMS was palpated in all cows. It was detectable as from the 30th to 45th day of gestation in the pregnant horn just slightly anterior to the bi furcation. At about the 50th to 65th day of gestation the membrane could be slipped in the contralateral horn i.e membranes could be slipped in either horn. In 17 cases (75%), asymmetery or distention of the uterus preceeded detection of FMS although in 25% of the cases the two events occurred simultaneously (Table 7.2).

e. **Amniotic vesicle (AV)**: This was detected in only 5 of the 23 cows. It was detected between day 36 and day 56 of gestation as a turgid bean-shapped structure floating very freely in the allantoic fluid. This detection rate of 22% was rather low.

f. **Ballooning**: This was a marked distention of the uterine horn to about the size of a boxing glove filled with fluid. It was detected in all 23 cows between days 54 and 77 of gestation.

g. **Fetus or conceptus**: This was detected in all the 23 cows. The fetus was first detected between day 60 and day 90 of gestati6on as an "amorphous" structure with indistinct anatomical parts. However, between days 90 and 120 the fetus was clearly palpable with distinguishable anatomical parts.

Between days 90 and 100 the fetus was abdominal but within reach. Fetal descent commenced at about 90 days and between day 140 and 190 of gestation the fetus was out of reach in 20 of the 23 animals and could only be palpated by ballotment. In the 3 remaining animals, the fetus never really got out of reach during the entire gestation period. Between days 70 and 90 retraction of the uterus was no longer possible.

Ascent of the fetus commenced between day 190 and day 200 and by day 240 the fetus was at the pelvic brim. By day 250 fetal head and forelimbs were intra pelvic making further examination difficult to impossible. Movement of the fetus was detectable at this time as well (Table 7.3).

h. **Cotyledons:** Cotyledons were detected in all 23 cows studied. They first became demonstrable between day 90 and 100 as peanut-sized structures. By 130 to 140 days they were prominent and distinctly palpable. From their time of first appearance they remained palpable throughout the remaining period of gestation although between days 180 and 240 a lot of effort was needed to palpate them and in some cases palpation was infact not possible. This coincided with the time of ascent of the fetus. The cotyledons also increased in size steadily with advancing gestation.

i. **Uterine tone:** Between days 30 to 60 the tonus of the uterine wall was high (Score: 3); moderate between days 70 to 90 (Score: 2) and slight by day 100. By day 130 the uterine wall was thickened, and tight and difficult to palpate.

7.2.3. OVARY:

Corpus luteum: Was detectable in all the 23 cows and it was always present on the ovary ipsilateral to the pregnant horn. During the early stages of gestation (day 30 to 50)

the corpus luteum was clearly palpable and protruding above the surface of the ovary in all the animals while infact 12 of them (about 50%) had a very good crown within the same period. By day 60 however the corpus luteum had become imbedded in the ovary proper in 19 (about 80%) of the cows. By day 120 the ovaries had tilted abdominal but were still palpable. Between days 150 and 170 ovaries could only be palpated with difficulty. By day 180 ovaries were no longer palpable up to the end of gestation (Table 7.4).

7.2.4 MIDDLE UTERINE ARTERY (MUA)

During the early stages of pregnancy (day 30 to 40) there was slight to no palpable pulsation of the MUA. Between day 40 to 60 the pulsation of the MUA was slight to moderate whereas between day 70 to 100 there was distinctly strong pulsation and slight fremitus. Between day 110 and 130 fremitus became detectable and was moderately so in all the cows. It became distinctly detectable between days 140 and 150. Fremitus was detectable on both sides by day 120 to 140.

The middle uterine artery showed steady increase in size so that between day 160 to 190 to the end of gestation it had attained a stage when it could be readily picked up through the rectal wall and moved about freely.

From the time it first became detectable, fremitus persisted throughout gestation. However, by 10 days postpartum fremitus had disappeared in all the animals (Table 6.6).

7.2.5 PREGNANCY SIDE

While 16 (64%) of the pregnancies occurred on the right side, 9 (36%) were on the left

(pregnancy side was determined in all the 25 pregnant cows before 2 of the cows were dropped from the study). The difference was statistically significant ($P < 0.05$).

7.2.6 CALVING DATA

All 25 animals calved following a mean gestation length of 285.8 days (range 278 to 292 days). There was one case of stillbirth and one calf with congenital deformity of the right forelimb. Of a total of 25 calves born, 15 (60%) were females while 10 (40%) were males. There was no case of abortion, resorption, maceration or dystocia. Birthweight of calves ranged from 19kg to 37kg. 16 of the calves (64%) had birthweight between 24 and 29kg while 7 (28%) of the calves weighed between 30 and 40kg. The weight of the dams at calving ranaged from 300kg to 400kg.

7.2.7 GENERAL OBSERVATIONS

There were very marked and distinct structural changes between day 40 and 54 of gestation such that it was strikingly obvious to diagnose pregnancy by the structures palpable at this stage of gestation. Table 7.6 compares gestational chanages in Bunaji cows with those reported for *Bos taurus* breeds.

7.3 DISCUSSION

In herds where artificial insemination (AI) is used it is generally recommended that the first palpation should be carried out between 35 and 42 days after insemination so that cows that are not pregnant can be observed more carefully during the next few days when they should be returning to oestrus. From the results of the current study it is indeed possible to positively diagnose pregnancy in Bunaji cows as early as 30 to 40 days of gestation using the

fetal membrane slip (FMS) and the amniotic vesicle (AV) as positive signs of pregnancy. This should infact be the target for clinicians engaged in herd health fertility programmes. It should however be remembered that individual variations exist.

It is, noteworthy that the FMS was a more accessible sign than the AV since it was detected in all pregnant cows (100%) as opposed to only 22% detection rate for the AV. This may however, vary among palpators depending on experience and proficiency. The low AV detection rate could partly be due to the volume of amniotic fluid within which the AV floats and partly to the conscious efforts of the palpators to avoid rough and prolonged manipulation. It is important that the palpator should not insist on finding the AV "at all costs" as this might result in frustration, but most importantly it might cause rupture of the AV.

In all cases where the FMS and AV were detected, the animals carried their pregnancies to term and had normal deliveries thereby confirming the FMS and AV as positive signs of pregnancy. Furthermore, the study has shown that frequent (weekly) rectal palpation has no visible detrimental effect on pregnancy development as long as it is carefully carried out. In addition the 100% accuracy rate for both positive and negative diagnoses reported in the current study is in agreement with what has been reported by Noakes (1986) although this would however depend on the stage of gestation and experience of the palpator (Zemjanis 1970; Arthur, 1975).

Although 25 animals were diagnosed pregnant at the inception of the study 23, of them were eventually used indicating that situations do arise whereby it is possible to diagnose pregnancy but difficult to carry out detailed examination which is a prerequisite for ageing

of pregnancy.

The presence of non-positive or ancillary signs of pregnancy is not conclusive evidence of pregnancy. They however have their value in confirming or refuting a diagnosis or differential diagnosis for pregnancy. For instance the pres once of a mid-to late-gestational fetus without an accompanying fremitus of the middle uterine artery will be suggestive of a mumnified fetus (Zemjanis 1970, Arthur 1975, Noakes 1986).

The use of rectal palation in cattle in Nigeria has been a subject of several scientific publications. This is because of the versatility and practical application of the technique for diagnostic and therapeutic purposes. Some of the very notable studies by Osori (1976), Oyedipe et al (1980), Kumi-Diaka et al (1980), Ogwu et al (1982), Eduvie and Dawuda (1986), Pathiraja et al (1986) and Voh (Jr) and Otchere (1989) clarify this point. The current study has however gone a step further to characterise, chronologically, the events and changes associated with gestation in Bunaji cattle. This will broaden the scope of use of this versatile reproductive tool by serving as an aid in training veterinary students as well as a refresher material for practicing veterinarians or clinicians involved in herd health fertility programmes.

When compared with reports for Bos taurus breeds of cattle (Zemjanis 1970; Arthur 1975 and Anon 1980; Table 6.7) the data of the current study highlights certain interesting findings. Notable among these findings is the fact that in Bunaji cows the uterus and fetus become abdominal much later in gestation than for Bos taurus. Similarly the uterus is still retractable much later in gestation for Bunaji cows. These breed differences have been confirmed in practice by one of the authors (AAV) working with Holstein and Bunaji herds in Vom, Nigeria. Furthermore, we found in the present study that in some cows the fetus

never really got out of reach. This would however depend to some extent on the height of the palpator. We had earlier reported (Voh, Jr. et al 1987) that in more than 80% of non-pregnant Bunaji cows the genitalia was intrapelvic as opposed to what has been reported for Bos taurus breeds where the genitalia would be abdominal for most non-pregnant cows and even pubertal heifers thereby making retraction a necessary step in rectal 2palpation in Bos taurus breeds. This situation in Bunaji cows has clinical benefits in that it makes retraction unnecessary during these early stages of pregnancy, and by ex-tension increases the chances of accuracy.

From the results and experience acquired from the present study it is much easier and more reliable or accurate to diagnose and age pregnancy during the early and mid-gestational periods because there are more distinct positional and anatomical changes occuring within shorter time spans than during late gestational periods. This observation has also been made by Arthur (1975) for Bos taurus.

From the results of this study it is noteworthy that while it requires the establishment of one of the four positive signs of pregnancy to conclusively declare an animal pregnant, it may require the establishment and characterisation of two or more structures or changes to be able to age pregnancy. In other words it is easier to diagnose than age pregnancy.

Sixty-four percent (64%) of all pregnancies occurred on the right side indicating that the right ovary is reproductively more active than the left. This is in agreement with earlier findings by Voh (Jr) and Dawuda (1985-unpublished results) where they found 60% of palpable

ovarian structures occurring on the right ovary in 1150 cows and heifers rectally examined in Borno State of Nigeria in 1985.

The corpus luteum was detectable in all pregnant cows and it was always present on the ovary ipsilateral to the pregnant horn. Similar findings have been reported by Arthur (1975) and Anon (1980).

The gestation length recorded in this study is also in agreement with earlier reports for Bunaji cows carrying Friesian x Bunaji crossbred fetuses (Voh Jr. et al 1987c).

From the results of this study it is concluded that it is indeed possible to positively diagnose and age pregnancy in Bunaji cows as early as 30 to 40 days following insemination and this should be the target for clinicians engaged in herd health fertility programmes especially in herds where AI is used. The fetal membrane slip was found to be a more reliable and readily accessible sign than the amniotic vesicle at this stage. Furthermore, it is much easier and more accurate to diagnose and age pregnancy during the early-and mid-gestational periods. Of the four positive signs of pregnancy (FMS, AV, fetus and Cotyledons) the palpation of the AV was the one that presented the most difficulty.

Table 7.1: Changes in uterine position at different stages of gestation.

Stage of gestation (days)	Position
30-40	Intrapelvic
40-80	Pelvic brim
60-100	Tilting abdominal
70-100	Retraction no longer posibible
80-120	Abdominal
140-150	Abdominal floor

Table 7.2. Changes in Fetal Membrane Slip (FMS) with stages of gestation.

Stage of gestation (days)	Findings
30-45	Ipsilateral side
50-65	Contralateral side

Table 7.3. Fetal positional changes at different stages of gestation

Stage of gestation (days)	Findings
60-90	Fetus detectable as an amorphous or indistinguishable mass
90-120	Distinguishable anatomical parts
90-100	Descent begins
140-190	Fetus out of reach (abdominal floor)
190-200	Ascent begins
240	Fetus at pelvic brim
250-term	Fetal heal and fore limbs intrapelvic

Table 7.4: Changes in ovaries with stages of gestation

Stage of gestation (days)	Findings
30-50	Corpus luteum protruding above ovarian surface
60	Ovaries tilted abdominal but palpable
120	Ovaries tilted abdominal but palpable
150-170	Ovaries palpable but with a lot of difficulty
180	Ovaries no longer palpable

Table 7.5: Summary of major rectal findings in the diagnosis and ageing of pregnancy in Bunaji cows

FINDINGS	Stage of gestation (days) Mean	Range	Detection No.	rate %
Asymmetry	35	28-43	23	100
Fetal membrane slip (FMS)*	35	30-45	23	100
Amniotic vesicles (AV)*	45	36-56	5	22
Fetus*	75	60-90	23	100
Ballooning of uterus	65	54-77	23	100
Dorsal bulge	50	43-58	23	100
Ovary not palpable	180	160-200	23	100
Cotyledons*	95	90-100	23	100
Fremitus of MUA	85	70-100	23	100
High uterine tone	45	30-60	23	100

*Positive sign of pregnancy.

Table 7.6: Comparing gestational changes in Bunaji cows with those reported for some *Bos taurus* breeds

FINDINGS	BREED			
	Bunaji	***Bos taurus***		
		1	2	3
Uterus				
asymmetry	28-40	35		35-40
intra pelvic	30-40			
pelvic brim	30-50	-	-	45-50
tilting abdominal	70	75	-	60
abdominal	100			90
abdominal floor	140-150	130-140		150
retraction impossible	70-90	60-70		
dorsal bulging	40-50			30-50
ballooning	50-75			
Fetal membrane slip (FMS):				
ipsilateral	30-45	30-33	35-50	35-40
contralateral	50-65			45-50
Amniotic vesicle (AV):				
detectable	35-55	28-31	28	30-50
Fetus:				
amorphous	60-90	50		60
distinguishable	90-120		120-160	120
descent	90-120	75		

out of reach	140-190	130-140		170
ascent	190-200	210		
pelvic brim(ascent)	220-240			
head & forelimbs				
intrapelvic	250-term			
Cotyledons (Placentomes):				
detectable	90-100	65-70	110-120	120
Uterine tone score:				
3 (high)	30-60	30-50		
2 (moderate)	70-90			
1 (slight)	100			
0 (thick & difficult)	130			
Corpus luteum (Ovary):				
protruding	30-50			
imbedded	60			
not palpable	180		150	120
Fremitus:				
slight	70-100	75-80	86	120
moderate	110-130			
strong	140-150			
both sides	120-140			
disappearance	10 days postpartum			

1 = Zemjanis (1970); 2 = Arthur (1975); 3 = Anon (1980).

CHAPTER EIGHT

GENERAL DISCUSSION

8.0 INTRODUCTION

The broad objective of this Chapter of the Thesis is to discuss how the 3 reproduction management tools employed in these studies can be effectively employed to bring about improved cattle reproduction, and by extension, improved cattle productivity, in Nigeria, based on the results of the studies embodied in the Thesis. The 3 reproduction management tools are:

i artificial insemination

ii oestrus synchronization

iii rectal palpation

Highlights of the results of the studies contained in the thesis can be summarised as follows:

8.0.1 The stage of diestrus at the time of second $PGF_{2\alpha}$ injection as well as the interval between two consecutive intramuscular injections of $PGF_{2\alpha}$, both affected oestrus response rate and the tightness of synchrony. Thirteen (13) days between 2 consecutive intramuscular injections of $PGF_{2\alpha}$ gave the best response.

8.0.2 Following 2 consecutive intramuscular injections of $PGF_{2\alpha}$ 11 days apart, 60 hours after the second injection is the optimum time for fixed-time insemination.

8.0.3 Progesterone-releasing intravaginal device (PRID) used alone for 12 days or in combination with $PGF_{2\alpha}$ administered a day before or on the day of PRID withdrawal (7 days) is as effective in synchronizing oestrus as 2 injections of $PGF_{2\alpha}$ administered 13 days apart with

acceptable pregnancy rate to AI at detected oestrus.

8.0.4 About 13% reproductive or fertilization losses by way of late embryonic mortality (LEM) may be inevitable following oestrus synchronization and AI and therefore may have to be tolerated and accommodated. Similarly, a 15% incidence of Repeat Breeder cow syndrome (RBS) may have to be tolerated and accommodated.

8.0.5 It is indeed possible to positively diagnose and age pregnancy by rectal palpation as early as 30 to 40 days following artificial insemination in Bunaji cattle.

8.1 PRACTICAL SIGNIFICANCE AND RECOMMENDATIONS

One of the practical significances of the results is that in a situation whereby artificial insemination will be carried out at detected oestrus following double injection of $PGF_{2\alpha}$, the animal should be injected 13 days apart. Because of the tighness of synchrony, oestrus detection should be intensified between days 2 and 4 following the second injection i.e. a 48-hour intensive oestrus detection, and this will detect 80% of all responding animals. This contrasts with the other inter-treatment intervals where the spread of oestrus onset is over a 5-to 6-day oestrus detection period.

However, in a situation whereby fixed-time insemination has to be carried out, following double injection of $PGF_{2\alpha}$, the animals should be injected 11 days apart to be followed by fixed-time insemination at 60 hours following the second injection. A second fixed-time insemination 24 hours following the first may improve the pregnancy rate of the double injection-fixed-time insemination protocol. The cost of the semen should be considered vis-a-vis the improvement in the pregnancy rate of the double insemination over the single insemination.

The current set of studies has offered a range of options of oestrus synchronization protocols and/or synchronizing agents to choose from. They are:

8.1.1 Synchronizing agents: These are:

a Prostagalandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and

b Progesterone releasing intravaginal device (PRID) to choose from. Both synchronizing agents are equally effective provided they are used in non-suckled and cycling cows.

8.2.2 Synchronization protocols to choose from are:

i Double injection of $PGF_{2\alpha}$ 11 days apart accompanied by fixed-time AI at 60 hours post second injection.

ii Double injection of $PGF_{2\alpha}$ 13 days apart accompanied by AI at detected oestrus over a 48 hour oestrus detection period (i.e. 2 to 4 days following second injection of $PGF_{2\alpha}$

iii PRID insertion alone for 12 days to be followed by AI at detected oestrus

iv A combination of PRID for 7 days and injection of $PGF_{2\alpha}$ on the day of PRID withdrawal to be followed by AI at detected oestrus.

Regardless of which oestrus synchronizing agent or oestrus synchronization protocol or artificial insemination protocol is used, a pregnancy diagnosis using rectal palpation should be carried out at 30 to 40 days post-insemination to confirm pregnancy. This will cut down on the number of days open for cows that fail to conceive to the first insemination.

Having carefully considered the results of the present study the following operational modality is recommended for oestrus synchronization and AI in distant places. For places that

are not far, Appendix 1 provides a range of oestrus synchronization management systems to choose from. Furthermore, Appendices 2 and 3 provide oestrus synchronization highlights and commercially available oestrus synchronizing agents respectively, to choose from.

8.2 RECOMMENDED OPERATIONAL MODALITY FOR OESTRUS SYNCHRONIZATION

It has been recommended that cattle oestrus synchronization be restricted to the rainy season as much as possible in the northern parts of Nigeria due to seasonality in the availability of pastures which in turn results in seasonality in oestrus cyclicity (Voh *et al* 1986a). In view of the current high costs of synchronizing agents, semen and labour, an oestrus synchronization management system that allows the minimum number of handlings of animals single treatment and single insemination without jeopardising good oestrus response and fertility rates will be a favourite system for recommendation to farmers. The system can be operated as follows for farms that are distant from the AI service and which are embarking upon the programme for the first time.

8.2.1 First Visit:

Select non-pregnant oestrous cycling cows in good body condition (≥ 3) (Pullan, 1978) by rectal palpation in good body condition (≥ 3) (Pullan, 1978). Remove breeding bulls, if any, from the herd. Collect relevant samples for reproductive diseases screening tests in order to ascertain the herd health fertility.

8.2.2 Second Visit:

This visit should be undertaken one month after the first visit. Re-examine all the pre-selected cows in order to identify and remove any pregnant animals not so diagnosed during the first visit because the pregnancy was too early to be detected by rectal palpation. Synchronize and breed the cows at detected oestrus over a 5-day AI period.

8.2.3 Third Visit

This visit should be undertaken 30 to 40 days after the last insemination. The purpose of this 3rd visit is to carry out pregnancy diagnosis by rectal palpaation and to assess the success or otherwise of the programme.

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