Serum progesterone profiles of zebu cattle (Bos indicus) in relationship to conception and repeat breeding after artificial insemination

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Abstract

Sixty-four zebu cows, comprised mainly of Bunaji cattle aged between 3 and 7 years old, were involved in an intensive artificial insemination programme. The study was conducted during the breeding period (May–October), and cows were inseminated with freshly prepared Friesian semen when they stood to be mounted by the vasectomised bull or herdmates. Fertility was measured by serum progesterone (P4) concentrations and pregnancy diagnosis by rectal examination post-insemination. From the records of oestrus detection and P4 profiles of cattle following breeding, 39 oestrous cycle lengths were classified into short, normal and long cycles. The mean duration for short, normal and long oestrous cycles were 15.6 ± 2.0, 21.5 ± 1.5 and 29.5 ± 2.5 days, respectively. Mean inter-oestrus intervals between the treatment groups were not different. The percentage frequency distribution was 48.7% for normal oestrous cycles. The short oestrous cycle lengths of 11–17 days were observed in 12.8% of the cases, while 38.5% of the cases of oestrus returns had long luteal phases with oestrous cycle lengths of 26–32 days.

Out of the 64 cows, 48.4% conceived to the first breeding while 18.8% conceived to the second breeding. Five and nine cows became acyclic after the first and second breeding, respectively, constituting 22.0%. Cows that displayed irregular oestrous cycles (repeat breeders) constituted 10.9%. Average number of services per conception was 1.3. Serum P4 is of practical value in monitoring ovarian activity in cattle and in the identification of acyclic and repeat breeder cows. Repeat breeder cows could be investigated by a combination of radioimmunoassay and clinical examination of the ovaries and reproductive tract. In cattle management, it is economical...
1. Introduction

In spite of all the advances in veterinary medicine, the repeat breeder remains the most economically important type of infertility in domestic animals (Vandeplassche, 1968; Ayalon, 1978). Reproductive failure or loss is one of the major factors affecting productivity of cattle herds, and is principally caused by repeat breeding (Ayalon, 1984). Almeida et al. (1984) and Maurer and Echternkamp (1985) defined a repeat breeder cow as one without morphological or clinical abnormalities that has had three or more nonfertile inseminations. The repeat breeder female is defined as nonpregnant after exposure to bulls for two breeding periods of 45–60-day duration or after four or more artificial inseminations (Ayalon, 1984). Lower fertility in repeat breeder females has been attributed to fertilization failure, endocrine dysfunction, increased embryonic mortality, and genetic and reproductive tract aberrations (Ayalon, 1984; Maurer and Echternkamp, 1985). Increased anatomical aberrations of the reproductive tract, increased anovulation, lower recovery rate of an oocyte or embryo, lower serum progesterone (P$_4$) concentration, and increased chromosomal abnormalities are possible causes for lower fertility in repeat breeder females (Ayalon, 1984).

The causes of irregularities in oestrous cycle length must be understood if herd fertility is to be improved. In tropical regions, about 65% of the intervals between services deviate from the expected (Garcia and Edqvist, 1989). In cattle, the concentration of progesterone is an index of the presence of a functional corpus luteum and reproductive status (Perara and Abeyratne, 1979). Cattle exhibiting regular oestrous cyclicity characteristically shows periodic fluctuations in progesterone concentration with a 2-week period of high levels followed by 1 week of low levels (Bulman and Wood, 1980). Concentrations of P$_4$ during the first 14 days of the oestrous cycle are similar to those occurring during the first 14 days of pregnancy (Bulman and Lammings, 1979). It is also important to note that cows which lose their embryos between days 6 and 16 after breeding would normally return to oestrus after the same interval as that of unmated cows (Ayalon, 1984). Irregular oestrous cycles with intervals shorter than 18 days or longer than 24 days are common (Macmillan and Watson, 1971; Bulman and Wood, 1980).

Mean oestrous cycle lengths were longer following breeding than normal cycle lengths in nonbred cows, suggesting the occurrence of embryonic death (Kummerfield et al., 1978). In artificially inseminated herds, the proportion of cows returning for insemination after an interval longer than a normal oestrous cycle has been used as an estimate of embryonic mortality, but the incidence of repeat insemination is also increased by inaccurate heat detection (Kummerfield et al., 1978). Early embryonic mortality has sometimes been associated with a decline in P$_4$ to baseline concentrations after a period of elevated levels lasting for more than 30 days postbreeding (Bulman and
Embryonic mortality, 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 in approximately 45 days (Committee on Reproductive Nomenclature, 1972).

Pregnancy rates lower than 30% for zebu cattle have been reported, and this poor fertility has been attributed to the inability to detect oestrus, poor signs of oestrus, and irregularity of the oestrous cycle (Hardin et al., 1980; Orihuela et al., 1983; Laudivar et al., 1985). There is also evidence that poor nutrition and management, harsh climatic conditions and seasonal effects affect fertility in zebu cattle (Zakari et al., 1981; Pathiraja et al., 1988). Embryonic mortality rates of 5.1% and 12.5% in cattle, as suggested by $P_4$ concentrations, have been reported, and that the embryos were lost between days 21 and 45 postbreeding (Rekwot et al., 1999).

There is limited information on serum $P_4$ profiles of zebu cattle in relationship to conception and repeat breeding after artificial insemination. The aim of this study is to examine postbreeding ovarian activity and oestrous cycle lengths of zebu cows.

2. Materials and methods

2.1. Location

This study was conducted using the dairy cows of the Dairy Research Programme of the National Animal Production Research Institute (NAPRI), Shika, Ahmadu Bello University, Zaria. NAPRI, Shika is located in the Northern Guinea Savanna between latitudes 11°N and 12°N, and between longitudes 7°E and 8°E at the elevation of 650 m above sea level. The climate of the area is characterised by an average annual maximum and minimum temperatures of 31.0 ± 3.2°C and 18.0 ± 3.7°C, respectively. The monthly average rainfall during the rainy season (May–October) was 148.1 ± 68.4 mm. Mean monthly relative humidity was 71.1 ± 9.7% during the study period.

2.2. Experimental cows

This study was conducted using cows from the NAPRI dairy farm, with the sole aim of generating Friesian crossbreeds using the Bunaji and Bokoloji dams. The study was conducted during the breeding period (May–October), when a greater proportion of cows are expected to be undergoing regular oestrous cycles. Cows were admitted into the study when they stood to be mounted by the vasectomised bull or herdmates (sign of standing oestrus). Cows ($n = 64$) with varying parities (1–5) aged between 3 and 7 years old were utilised. Cows were body-condition scored using a score scale of 0–5, from the most emaciated to the fattest (Pullan, 1978). Only cows with body condition scores of 2.5 and above were used.

2.3. Management of cows

The cows were grazed on free-range improved pastures and supplemented with 1–2-kg mixture of cotton seed cake and maize per cow per day. Water and mineral licks
were provided ad libitum. All cows were tagged with large plastic ear tags to enable identification of the cows from a distance during detection of oestrus. The animals were dipped once a week in 16% liquid Asuntol® (Coumaphos), containing \( O,O\text{-diethyl-O-} (3\text{-chloro-4-methyl-7-coumarinyl})\text{-phosphorothiate} \) as the active ingredient for effective control of ectoparasites, especially ticks. The dilution rate of 1 l of Asuntol® to 800 l of water was used. Before the commencement of the study, the cows were screened for blood and helminth parasites, and appropriate treatments and vaccination against certain diseases were conducted.

2.4. Detection of oestrus and insemination

As a routine activity of the Artificial Insemination Unit, animals in the Dairy Research Programme were monitored for standing oestrus and inseminated using Friesian semen. The study lasted from April to November, and a total of 64 cows were inseminated within the period. A bull with Pen-O-Block Lewish Schacht, Tallahassee, FL, 32311, harnessed with a chin–ball mating device (The Outdoors, NZ) was used to aid in the detection of oestrus. Cows were monitored for mounting activities and mucus discharge. Visual detection of oestrus by the herdsmen and inseminators in the morning and evening was also put in place to improve the reliability of detecting oestrus. Cows with standing oestrus were inseminated 12 h after the onset of oestrus using freshly prepared Friesian semen. Semen collection from a Friesian bull of known fertility was used for all the inseminations. Semen was collected by the use of an artificial vagina and extended by use of a standard buffer (2.9% w/v of trisodium citrate dehydrate), and stored as chilled semen in the refrigerator (4°C) for a maximum of 3–4 days. The insemination dose (5 ml) contained \( 600 \times 10^6 \) million spermatozoa with a mean individual motility of 90%. A single efficient inseminator conducted all the inseminations. All inseminated cows were further monitored for evidence of oestrus; and inseminations were repeated for those cows that returned to oestrus. A total of 103 inseminations were conducted during the study period.

2.5. Blood sampling

Blood samples were harvested via jugular venipuncture from the 64 cows involved in the breeding programme. Blood samples were collected on the day of oestrus (day 1), day 12 and every 5th day thereafter until the next oestrus or confirmation of pregnancy by rectal palpation, performed 45–60 days post-insemination. Blood samples were placed immediately in ice and allowed to clot at 4°C for 24 h. Serum was separated by centrifugation and stored at −20°C until concentrations of \( P_4 \) were determined by radioimmunoassay.

2.6. Radioimmunoassay

Serum \( P_4 \) concentrations were determined by solid phase \(^{125}\text{I} \) radioimmunoassay no-extraction technique. The sensitivity of the assay, defined as twice-the-standard deviation away from the zero standard, was 0.09 ng/ml. The within- and between-assay
coefficients of variation were 8.8% and 9.7%, respectively. The potencies of the samples were estimated using a linear logit–log dose response curve. A serum P₄ concentration of 1 ng/ml and above was taken to indicate the presence of functional luteal tissue (Oyedipe et al., 1986).

2.7. Statistical analysis

From the records of the standing oestrus and inseminations, a total of 39 oestrous cycles were classified as short (11–17 days), normal (18–25 days) and long (26 days and above). The proportion of the reproductive traits was determined by calculating the percentages in each category. The P₄ profiles and clinical data for each cow were individually evaluated and matched in certain cases for convenient classification into groups.

3. Results

Thirty-nine oestrous cycle lengths were classified as short (11–17 days), normal (18–25 days) and long (26 days and above, Table 1). The mean duration for short, normal and long cycles were 15.6 ± 2.0, 21.5 ± 1.5 and 29.5 ± 2.5 days, respectively. The percentage frequency distribution was 48.7% for normal cycles. The short oestrous cycle lengths of 11–17 days were observed in 12.8% of the cases, while 38.5% of the cases of oestrus return had long luteal phases with oestrous cycle lengths of 26–32 days (Table 1). Mean inter-oestrus intervals between treatment groups were not different (Table 2). The summary of reproductive performance and fertility of the cows involved in the breeding programme as monitored by P₄ concentration and rectal examination is reported (Table 2). Out of the 64 cows, 31 (48.4%) conceived to the first breeding while 12 (18.8%) conceived to the second breeding. Out of the 64 cows, five and nine cows became acyclic after the first and second breeding, respectively. Seven cows displayed irregular oestrous cycles (repeat breeders 10.9%, Table 2). A total of 103 inseminations were performed during the study period with an average number of 1.3 services per conception.

<table>
<thead>
<tr>
<th>Oestrous cycle length</th>
<th>Mean ± standard deviation</th>
<th>Number of oestrous cycles</th>
<th>Percentage frequency distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Short cycle (11–17)</td>
<td>15.6 ± 2.0</td>
<td>5</td>
<td>12.8</td>
</tr>
<tr>
<td>Normal cycle (18–25)</td>
<td>21.5 ± 1.5</td>
<td>19</td>
<td>48.7</td>
</tr>
<tr>
<td>Long luteal phase (26–32)</td>
<td>29.5 ± 2.5</td>
<td>15</td>
<td>38.5</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>39</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Table 1
Frequency distribution of oestrous cycle lengths in breeding cows
Table 2
Summary of reproductive performance of cattle monitored by progesterone concentrations and rectal palpation

<table>
<thead>
<tr>
<th>Reproductive traits</th>
<th>Number of cows</th>
<th>Number of inseminations</th>
<th>Number of cows pregnant</th>
<th>Inter-oestrus intervals (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conceived to first breeding</td>
<td>31 (48.4)</td>
<td>31</td>
<td>31</td>
<td>NA</td>
</tr>
<tr>
<td>Conceived to second breeding</td>
<td>12 (18.8)</td>
<td>24</td>
<td>12</td>
<td>21.8±4.6</td>
</tr>
<tr>
<td>Acyclicity (after first breeding)</td>
<td>5 (7.8)</td>
<td>5</td>
<td>0</td>
<td>NA</td>
</tr>
<tr>
<td>Acyclicity (after second breeding)</td>
<td>9 (14.1)</td>
<td>18</td>
<td>0</td>
<td>24.1±3.7</td>
</tr>
<tr>
<td>Repeat breeders (irregular cycles)</td>
<td>7 (10.9)</td>
<td>25</td>
<td>0</td>
<td>20.4±4.0</td>
</tr>
<tr>
<td>Total</td>
<td>64 (100.0)</td>
<td>103</td>
<td>43</td>
<td></td>
</tr>
</tbody>
</table>

Figures in parentheses are in percentages.
Average number of services per conception = 1.3.
NA = not applicable.

The $P_4$ profiles of the 64 cows are depicted (Fig. 1). Group A cows consisted of 31 cows whose $P_4$ profiles, after breeding, remained elevated throughout the sampling period indicating pregnancy as confirmed by rectal and clinical examination of the genitalia and ovaries at 45–60 days post-insemination (Fig. 1a). Group B included 12 cows that failed to get pregnant during the first breeding but conceived after the second breeding (Fig. 1b). The $P_4$ concentrations remained consistently high after the second breeding. Of these 12 cows, three subcategories were diagnosed as B₁ (seven cows), B₂ (one cow) and B₃ (four cows), which received their second inseminations on days 22 (normal cycle), 27 (long luteal phase) and 32 (luteal phase) post-insemination, respectively (Fig. 1b). Thus, cows B₁, B₂ and B₃ had oestrous cycle lengths of 21, 26 and 31 days, respectively.

Group C included nine cows which failed to conceive to both the first and second inseminations and remained anoestrous up to day 77 post-insemination (Fig. 1c). Of these nine cows, three subcategories were diagnosed as C₁ (two cows), C₂ (four cows) and C₃ (three cows), which received their second inseminations on days 22, 27 and 32 post-insemination, respectively. Thus, C₁, C₂ and C₃ cows had oestrous cycle lengths of 21, 26 and 31 days, respectively. After the second breeding, the $P_4$ concentrations remained consistently below 1.0 ng/ml, indicative of ovarian inactivity. Group D consisted of five anoestrous cows (D₁) and one cow (D₂) with irregular oestrous cycle lengths (Fig. 1d). The five anoestrous cows (D₁), immediately after oestrus and breeding, i.e. day 1, remained acyclic and anoestral throughout the experimental period. This is evident through the persistent low $P_4$ concentrations below 1 ng/ml and through subsequent confirmation by rectal and clinical examination of the genitalia and ovaries of these cows. The other cow in this group, D₂, showed three oestrous cycle lengths of 11 (short cycle), 20 (normal cycle) and 25 (normal cycle) days (Fig. 1d). This cow was bred four times (day 1, 12, 32 and 57 post-first insemination) without any conception.

Group E consisted of six repeat breeder cows with irregular oestrous cycle lengths (Fig. 1e, f and g). The $P_4$ profiles of cows E₁ and E₂ are depicted (Fig. 1e). Cow E₁ was bred on days 1, 22, 42 and 62 post-first insemination (i.e. bred four times) with no conception. The oestrous cycle lengths for cow E₁ were 21, 20 and 20 days. Cow E₂ had a short oestrous cycle length of 16 days and a normal cycle of 20 days, and then
Fig. 1. Progesterone (P₄) profiles of cows involved in a breeding programme.
remained anoestrous for the rest of the period. The \( P_4 \) profiles of cows \( E_4 \) and \( E_5 \) are depicted (Fig. 1f). Cow \( E_3 \) showed three oestrous cycle lengths of 16 (short cycle), 20 (normal cycle) and 20 (normal cycle) days. The cow was bred four times (days 1, 17, 37 and 57 post-first insemination), showing irregularities of the oestrous cycle. Cow \( E_4 \) displayed similar irregular oestrous cycle lengths, with two short oestrous cycle lengths of 16 and 15 days followed by a very long luteal phase or cycle of 30 days. Cow \( E_4 \) was bred four times without any confirmation of pregnancy by 45–60 days post-insemination. The \( P_4 \) profiles of cows \( E_4 \) and \( E_5 \) are depicted (Fig. 1g). Cow \( E_5 \) showed two oestrous cycle lengths of 26 (long luteal cycle) and 20 (normal cycle) days. This cow was bred three times (days 1, 27 and 47 post-first insemination), showing irregularities of the oestrous cycles. Cow \( E_6 \) displayed the same pattern of \( P_4 \) as that of \( E_5 \).

4. Discussion

The results of this study showed that oestrous cycle lengths were classified into short, normal and long luteal cycles, as have already been reported (Garcia and Edqvist, 1989). The incidence of 12.8% and 38.5% for short and long luteal cycles, respectively, in this study are among the major problems responsible for reductions in herd fertility and reproductive management in Africa. This corroborates the report of a high frequency of abnormal postbreeding luteal phases in a group of cows following natural service (Garcia and Edqvist, 1989). A prolonged luteal phase can reflect early embryonic mortality (Bulman and Lamming, 1978; Lamming and Bulman, 1979; Nakao et al., 1983). The reported values of 12.8% and 35.9% for short and long oestrous cycle lengths, respectively, in this study is a clear indication of deviation from the expected normal cycle. This confirms the findings of Garcia and Edqvist (1989) who reported that in tropical regions, about 65% of the oestrous intervals between services deviate from the expected. The irregular oestrous cycles with intervals shorter than 18 days or longer than 25 days in this present study corroborates the findings of some researchers (MacMillan and Watson, 1971; Bulman and Wood, 1980). In this study, the proportion of cows returning for insemination after an interval longer than a normal cycle could suggest an estimate of embryonic mortality, as has earlier been reported (Kummerfield et al., 1978). Another possibility of the repeat inseminations in this study could be caused by inaccurate detection of oestrus and faulty insemination. The reported value of 1.3 services per conception in this study appears to agree with the range of 1.7–2.1 services that has been reported by other researchers (Dawuda et al., 1988; Rekwot et al., 1999).

Group A cows conceived to first breeding as evident through the persistent elevated \( P_4 \) profiles and confirmed by rectal and clinical examination, as had earlier been reported (Oyedipe et al., 1986). Group \( B_1 \), \( B_2 \) and \( B_3 \) cows had oestrous cycle lengths of 21, 26 and 31 days respectively after the first inseminations, after which they conceived following the second inseminations as evident through the elevated \( P_4 \) profiles and confirmed by rectal examinations. A similar observation was made for group \( C_1 \), \( C_2 \) and \( C_3 \) cows; but after the second breeding, the \( P_4 \) concentrations remained consistently below 1.0 ng/ml, indicative of ovarian inactivity. The five anoestrous cows (\( D_1 \))
immediately after the first inseminations remained acyclic and anoestrus throughout the experimental period. This is evident through the persistent low P₄ concentrations below 1.0 ng/ml and was subsequently confirmed by rectal and clinical examination of the genitalia and ovaries of these cows. Cow D₂ showed one short oestrous cycle length of 11 days and two normal oestrous cycle lengths of 20 and 25 days. Cow D₂ and all the cows in group E displayed irregular oestrous cycle lengths ranging from 11 to 31 days. Our findings of 11–31 days of oestrous cycle lengths in this study corroborates the research of Ayalon (1984). The long inter-oestrus interval of cow E₄ could be indicative of long luteal phase as diagnosed by the persistent high P₄ profiles. Therefore, the subsequent decline of P₄ concentrations to baseline levels and the absence of any functional ovarian structure seem to suggest embryonic mortality, as has been reported earlier (Ayalon, 1984; Maurer and Echternkamp, 1985; Rekwot et al., 1999). Our results support the findings of Hawk et al. (1955) and Maurer and Echternkamp (1985), who have reported that most reproductive losses occurred between 16 and 34 days postbreeding.

Failure to conceive to any insemination may suggest: (a) lack of fertilization even when the cow was on oestrus, (b) fertilization took place but the embryo failed to survive or (c) the cow was not on oestrus when served (Wood, 1976). In the first case, the repeat interval is likely to be longer than the normal oestrous cycle, while in the second case, the embryo is especially vulnerable during the first days after conception, and if it should succumb during this period, a return to service will occur at the first normal cycle. In the third case, if the cow was not in oestrus at insemination, she will return at her next oestrus and, apart from an apparently unusual short first cycle, will thereafter cycle normally (Wood, 1976). Factors responsible for repeat breeder problem have, in cattle, been grouped into two main classes: (a) genetic factors, such as breed, family, in-breeding and blood groups and (b) environmental factors, such as nutrition, age, climate, infections, hormonal imbalance and uterine environment (Ayalon, 1984). Two infections generally considered to cause embryonic mortality are trichomoniasis and campylobacteriosis (Roberts, 1971). The conditions of service and the quality of semen have been shown to influence both fertilization rate and embryonic mortality (Salisbury et al., 1978). Possible reasons why a number of cows became anoestrus and repeat breeders include: faulty inseminations (inseminations when the animals were not in oestrus), delayed returns to oestrus because of endometritis and fertilization failure, and embryonic death due to heat stress.

The incidence of 12.8% and 38.5% for short and long oestrous cycle lengths respectively, in this study are greater than the values earlier reported (Ayalon, 1984; Maurer and Echternkamp, 1985). An incidence of over 10% repeat breeder cows in Sweden has been reported, and that incidence was affected by farm size, season, age and lactation (Hewett, 1968). The importance of P₄ in the survival of the early embryo and maintenance of pregnancy in cattle has been reported (Zakari et al., 1981; Oyedipe et al., 1986). Progesterone assays may, therefore, find useful application in monitoring occurrence of cyclic activities, early pregnancy and repeat breeders in cattle. Low reproductive rates, failures in oestrus detection and high frequencies of abnormal postbreeding luteal phases are among the major problems responsible for reductions in herd fertility and difficulties in reproductive management (Garcia and Edqvist, 1989). Accurate
oestrus detection is usually difficult under tropical conditions, and the incidence of repeat inseminations can be increased by inaccurate heat detection. A well-planned procedure for the detection of oestrus is essential for the success of artificial insemination programmes in tropical countries.

The findings of this research indicate that radioimmunoassay for $P_4$ in serum is of practical value in monitoring ovarian activity in dairy cattle. Serum $P_4$ profiles provide means of identifying acyclic cows and repeat breeder cows. Repeat breeder cows could be investigated by a combination of radioimmunoassay and clinical examination of the ovaries and the reproductive tract. In cattle management, it is economical and profitable to diagnose pregnancy early after insemination so that cows which fail to conceive may be rebred.

References


