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Ovarian morphology and estradiol-17 β concentrations in serum and follicular fluid of slaughtered zebu cattle in Ibadan, Nigeria

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ABSTRACT

Over a period of three months (January to March), ovarian morphology and estradiol-17 β concentrations in serum and follicular fluid were studied in 110 Bunaji (*Bos indicus*) cows slaughtered at Bodija abattoir, Ibadan, Nigeria, in order to determine the reproductive state and history of such cattle. Macroscopic examination of the ovaries revealed that 70 (63.6%) of the cows were undergoing active oestrous cycle. Of these, 42 (60.0%) had large ovarian follicles of greater than 3 mm diameter, while 22 (31.4%) had corpora lutea (CL). Six animals (8.6%) had corpora haemorrhagica. Of the 22 animals exhibiting CL, 12 (54.5%) had CL of pregnancy and were with calf, while 10 (45.5%) others had those of normal oestrous cycle. Corpora albicans (CA) counts showed that 90 (81.8%) of the cows had calved at least once, the mean being 4.5 calves per cow. Fifty-eight cows (52.7%) had calved 0-2 times, 34 (30.9%) 3-5 times and 18 (16.3%) 6-8 times. The mean estradiol-17 β levels in follicular fluid (1669.6 ± 9.7 pg/mL) were significantly higher than that of the serum (85.5 ± 0.8 pg/mL). The non-pregnant cyclic with corpus luteum group had the highest follicular estradiol concentration (2558.8 ± 8.9 pg/mL), while the pregnant group had the lowest (858.5 ± 6.8 pg/mL). The mean serum estradiol level among pregnant cows was 115.7 ± 1.2 pg/mL. Low levels of serum estradiol-17 β were consistently recorded among animals having active ovaries and good volume of follicular fluid, indicating that they were reproductively active and were being sold for reasons other than infertility.

Key words: ovaries, serum, estradiol-17 β , zebu cows, Nigeria

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Introduction

Sound economic livestock management demands that cattle sold for slaughter should be mainly bulls and reproductively inactive cows. Appropriate data of the reproductive state and age of any cow assigned to be slaughtered should be kept and effectively evaluated in order to avoid the slaughtering of reproductively active females.

However, information on the reproductive activity or status of cattle being sent for slaughter in Nigeria are usually unavailable because over 80% of the country's livestock population, especially cattle, sheep and goats, are in the traditional herds reared mostly by illiterate Fulani nomads more as symbol of status than as meat animals (IKEME, 1990; ADUBI and AROMOLARAN, 1998). Abattoir data studies have revealed a high incidence of foetal wastage among cattle slaughtered in Nigeria (MATHEW et al., 1982; OYEKUNLE et al., 1992; WEKHE et al., 1992; OKOLI et al., 2001). These studies may not, however, reveal the exact magnitude of the problem since they highlight mainly the prevalence of inadvertent slaughtering of pregnant animals in the country.

Ovaries harvested from slaughtered cattle have been shown to contain evidence of current and past reproductive state of such animals in the form of follicles in varying degrees of development, corpus haemorrhagicum, corpus luteum of pregnancy or estrous cycle and corpus albicans, among others (ARTHUR et al., 1982). Studies of ovarian morphology could therefore yield useful data on the reproductive conditions and history of slaughtered cattle.

Similarly, measurements of hormonal levels in different body fluids, especially blood and urine, is a highly informative tool for indirect evaluation of the functional status of an endocrine gland (BELL et al., 1992). Radioimmunoassay of steroid hormones, based on the ability of antibodies directed against a steroid determinant to react preferentially with that particular steroid, has been used successfully in the assay of protein hormones (BEG et al., 2001). Analysis of serum and follicular fluid oestrogen concentrations in slaughter cattle using such a technology could therefore yield valuable data on the functional status of their reproductive organs.

The present study was designed to investigate the current reproductive state and history of slaughter cattle in Ibadan, South-western Nigeria as revealed by ovarian morphology and estradiol-17 β levels in serum and follicular fluid.

Materials and methods

Over a period of 3 months (January to March), gross morphological studies of ovaries and radioimmunoassay of serum and follicular fluid for estradiol-17 β concentrations in 110 Bunaji cattle (*Bos indicus*) slaughtered at Bodija abattoir, Ibadan, Nigeria, were carried out in order to determine the reproductive state and history of such slaughtered cows. The

abattoir was visited once every week and pairs of ovaries from slaughtered cows were collected in properly labelled sterile glass dishes. At the same time, the morphological nature of the uteri was observed and thereafter the uteri were opened to determine if they contained any foetal material. Blood samples were collected from each of the 110 slaughter cattle in labelled sterile universal bottles by positioning bottles at the jugular vein such that about 50 mL of blood was collected as the vein was severed.

Both the ovary and blood samples were taken to the laboratory within two hours of collection. The blood was centrifuged at 2000 rpm and the serum produced decanted into labelled glass vials and kept frozen for radioimmunoassay.

The ovaries were examined for size, weighed and their dimensions (length of pole-to-pole, attached-to-free and side-to-side borders) measured using a venier caliper. The number of follicles, corpus luteum, corpus albicans, corpus haemorrhagicum and other gross observations, such as adhesions and atrophy on each ovary, were recorded. The follicles were examined for size and those measuring more than 3mm in size were aspirated for follicular fluid using a Pasteur pipette.

Altogether, ovarian follicles of 64 cows, comprising 12 pregnant and 52 non-pregnant animals were aspirated, while those from the remaining 46 were not aspirated. The follicular fluid of each follicle was then transferred into labelled sterile glass vials and frozen for radioimmunoassay.

Radioimmunoassay. Clean extraction tubes (Dyna-tech. Laboratories Inc.) (16 \times 100 mL) were arranged in a test tube rack and numbered S₁ to S₁₁₀ for serum samples, and F₁ to F₆₄ for follicular fluid samples. Each sample was assayed in duplicate to ensure accuracy. Two hundred (200) μ L of serum samples were aliquated into the tubes assigned to serum, while for follicular fluids 100 μ L were used. Radioimmunoassay was carried out according to the methods previously described by ADEYEMO et al. (1981) and ADEYEMO (1984). Results were read with the aid of a Beta Scintillation spectrometer (Packard) and the curve of percentage (%) bound standard versus the concentration of hormone was converted into a straight line graph by using the logit of % bound (of radioactivity) versus the log of the concentration of hormones. Samples were then read on this graph using a desk programmable calculator (Texas Instruments) to fit the logit and log of concentration of the standard into a regression analysis.

The analysis of variance technique was used to determine significant differences in estradiol contents in serum and follicular fluids of animals that were pregnant, non-pregnant acyclic with large follicle and non-pregnant cyclic with corpus luteum. Where significant differences were observed between the groups, means were separated using the Least Significant Difference (LSD) method (STEEL and TORRIE, 1980).

Results

The results of macroscopic examination of the ovaries revealed that of the 110 Bunaji cows (*Bos indicus*) slaughtered at Bodija abattoir, 70 (63.6%) of the animals were undergoing active oestrous cycle, while 40 (36.4%) were acyclic (Fig. 1A). Of the cyclic group 42 (60.0%) had large ovarian follicles (>3 mm in diameter), 22 (31.4%) had corpora lutea, while 6 (8.6%) had corpora haemorrhagica (Fig. 1B). Figure 1C shows that 12 (54.5%) of the corpora lutea observed were those of pregnancy, indicating that 10.9% of the cows slaughtered during the period were pregnant. The remaining 10 (45.45%) were those of normal oestrous cycle.

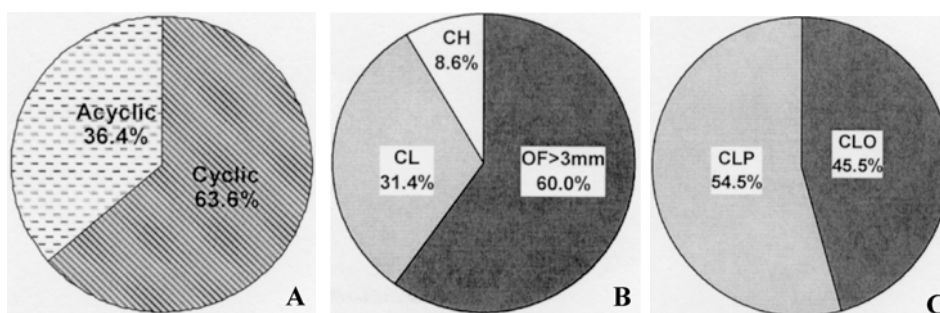


Fig. 1. Pie charts depicting different ovarian morphologic features observed in Bunaji cows slaughtered at Bodija abattoir, Ibadan, Nigeria. Cyclic = animals undergoing reproductive cycles; Acyclic = animals without signs of reproductive cycle; CL = corpora lutea; OF = ovarian follicle; CLP = corpus luteum of pregnancy; CLO = corpus luteum of normal oestrus cycle; CH = corpora haemorrhagica

Corpora albicans (CA) counts on the ovaries (Table 1) indicating the number of times the animals had calved, show that 90 (81.8%) of the cows had calved at least once. Mean CA per cow was 4.5 ± 0.7 . A total of 30 (27.3%) cows had calved only once, while 20 (18.2%) had never calved at all. On the other hand, 14.6%, 10.91%, 1.8% and 3.6% of the slaughtered cows had calved 4, 6, 7 and 8 times, respectively. On group bases, 58 (52.7%) of the animals fell under the 0-2 CA per pair of ovary group; 34 (30.9%) belonged to 3-5 CA group, while 18 (16%) were of the 6-8 CA per pair of ovary group.

Table 2 depicts the mean follicular fluid and serum concentrations of estradiol-17 β . Levels in follicular fluid (1669.6 ± 9.7 pg/mL) were significantly ($P < 0.05$) higher than serum levels (88.5 ± 0.8 pg/mL). The non-pregnant, cyclic with corpus luteum group had the highest follicular fluid concentrations of estradiol-17 β (2558.8 ± 8.9 pg/mL), while the cyclic with large ovarian follicles (>3 mm in diameter) and the pregnant group had 1591.6 ± 2.0 pg/mL and 858.5 ± 6.8 pg/mL, respectively.

Table 1. Number of corpora albicans observed on ovaries recovered from 110 Bunaji cows slaughtered at Bodija abattoir, Ibadan

Group	N ^o of CA/pair of ovary	N ^o (%) cows	N ^o (%) CA/group
0-2 CA per pair of ovary	0	20 (18.2)	58 (52.7)
	1	30 (27.3)	
	2	8 (7.3)	
3-5 CA per pair of ovary	3	8 (7.3)	34 (30.9)
	4	16 (14.6)	
	5	10 (9.1)	
6-8 CA per pair of ovary	6	12 (10.9)	18 (16.4)
	7	2 (1.8)	
	8	4 (3.6)	
Total Mean CA per cow		90 (82)	
		4.5 \pm 0.7	

CA = corpora albicans

Table 2. Mean serum and follicular fluid estradiol-17 β concentrations in Bunaji cattle in different reproductive conditions

Reproductive condition	Serum estradiol-17 β concentrations pg/mL	Follicular fluid estradiol-17 β concentrations pg/mL
Pregnant	115.7 \pm 1.2*	858.5 \pm 6.8
Non-pregnant acyclic**	84.9 \pm 0.1	
Cyclic/large ovarian follicle (>3 mm)	78.1 \pm 0.2	1591.6 \pm 2.0
Non-pregnant with corpus luteum & cyclic	78.8 \pm 0.8	2558.8 \pm 8.9

*Mean \pm standard error. **Non-pregnant acyclic cows with <3mm follicles were not tested for follicular fluid estradiol content.

Serum estradiol-17 β levels among the pregnant animals were 115.5 \pm 1.2 pg/mL, while the cyclic large ovarian follicle, (>3 mm), non-pregnant with corpus luteum and cyclic and non pregnant acyclic groups recorded 78.1 \pm 0.2 pg/mL, 78.8 \pm 0.8 pg/mL and 84.9 \pm 0.6 pg/mL, respectively.

Discussion

Data on the reproductive status of cattle sent for slaughter are important tools for sound economic livestock management. Cattle for slaughter should be mainly bulls and reproductively inactive cows. The present study showed that 63.6% of cows slaughtered at the Bodija abattoir, Ibadan, were still under reproductive activity, indicating a high level of reproductive wastage by cattle farmers in the city.

The study was carried out in the late dry season months of January to March, which has been associated with low pasture availability (AGBOOLA, 1979). Previous studies on foetal wastage in different zones of Nigeria (MATHEW et al., 1982; WEKHE et al., 1992; ABIOLA, 1996; OKOLI et al., 2001) have shown that a high incidence of foetal wastage in cattle was associated with this period of low pasture availability. The present result of 10.9% incidence of foetal wastage is in agreement with the annual 12.5% reported by OYEKUNLE et al. (1992) for nearby Ogun State, and the 14.1% reported by OKOLI et al. (2001) for Imo State. However, it was not in agreement with the 19.3% reported for the same period of the year by OKOLI et al. (2001) for Imo State.

Corpora albicans counts on pairs of ovaries of the slaughtered animals showed that this may be a better tool for highlighting the problem of reproductive wastage among animals slaughtered in Nigeria. According to ARTHUR et al. (1982) previous reproductive history in the form of fibrosed remains of corpus luteum of pregnancy (corpus albicans) persist for life in the majority of cows, and this preserves a record of the number of pregnancies undergone by each animal. Our results showed that 18% of the slaughtered cows had never calved, while 27.3% had calved only once. On group bases, 52.7% had calved only between 0 and 2 times, indicating that over half of the cows being slaughtered at Bodija abattoir during the late dry season were in the early stages of their reproductive life. These results therefore highlight the insufficiency of foetal wastage measurements as sole methods for evaluating reproductive wastage among slaughtered animals.

The fact that only 16.4% of the cows had calved 6 to 8 times is slightly in agreement with the reports of IKEME (1990) and TUKUR and UMAR (1998) that 20% of cattle slaughtered at Mina abattoir were over 10 years of age. A more detailed study using corpora albicans counts as a tool is needed in order to establish the annual figure of reproductive wastage for the area.

Results of the radioimmunoassay showed that the concentrations of estradiol-17 β were higher in follicular fluid than in serum. Levels of oestrogen in the circulation were appreciably higher during pregnancy, although it was still low compared to those of follicular fluid. Concentrations were usually high during the follicular phase of the oestrous cycle and began to decrease immediately after ovulation, remaining low during subsequent pregnancy in the animals (BELL et al., 1992). Again, the relatively low level of

circulating of estradiol-17 β obtained in most of the cows examined corresponded with the active ovaries from which good volumes of follicular fluid were aspirated.

Most previous reports of oestrogen concentrations in bovine follicular fluid and serum emanate from data obtained from *Bos taurus* (GINTHER et al., 2000; BEG et al., 2001; KOMAR et al., 2001). These reports give a value range of 300 to 500 ng/mL for follicular fluid oestrogen in *Bos taurus*, with peaks reaching up to 1000 ng/mL. Our range of 858 to 2558 pg/mL seems to suggest that *Bos indicus* have lower concentrations of follicular oestrogen. Similarly, our serum estradiol-17 β volume of 84.9 pg/mL among non-pregnant acyclic animals is also lower than values reported in the literature for *Bos tarus* (KANEKO et al., 1991; KOMAR et al., 2001). Synthesis of estradiol-17 β in bovine follicles cultured in vitro has been correlated with the number of granulose cells (TIAN et al., 1995). Again, frequent gonadotrophin-releasing hormone and LH pulses are known to lead to prolonged growth of follicles (GLENCROSS, 1987; TAFT et al., 1996) and increased follicular oestrogen synthesis (GLENCROSS, 1987; SIROIS and FORTUNE, 1990). These probably account for the lower levels of follicular steroidogenesis reported here for *Bos indicus*. This requires further investigation.

Conclusion

This study reveals that over 60% of the Bunaji cows examined among the volume slaughtered at Bodija abattoir for beef were still in their active reproductive stages and are being sold for slaughter for reasons other than reproductive inactivity. An appreciable amount of objective information on the reproductive status of a cattle population can be readily obtained by the examination of their ovaries and assay of their serum and follicular oestrogen levels at slaughter points.

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SAŽETAK

U razdoblju od 3 mjeseca (siječanj, veljača, ožujak) proučavana je morfologija jajnika i mjerena koncentracija estradiola 17 β u serumu i folikularnoj tekućini u 110 Bunaji (*Bos indicus*) krava podrijetlom iz Bodija klaonice, Ibadan, Nigerija radi određivanja reproduktivnog statusa i mogućih patoloških promjena. Makroskopski pregled jajnika je pokazao da se 70 (63,6%) goveda nalazilo u spolnom ciklusu. Ukupno 42 životinje (60%) imale su na jajniku velike folikule, veće od 3 mm u promjeru, dok ih je 22 (31,4%) imalo žuta tijela (ŽT). Šest goveda (8,6%) imalo je corpora haemorrhagica. Od 22 životinje sa ŽT, njih 12 (54,5%) imalo je graviditetno ŽT i tele u utrobi, dok ih je preostalih 10 (45,5%) imalo cikličko žuto tijelo. Pregled statusa corpora albicans (CA) na jajnicima pokazao je da se 90 (81,8%) goveda telilo najmanje jedanput, dok je prosjek bio 4,5 teladi po kravi. Od toga njih 58 (52,7%) telilo se 0 do 2 puta, 34 (30,9%) 3 do 5 puta te njih 18 (16,3%) 6 do 8 puta. Srednja vrijednost estradiola 17 β u folikularnoj tekućini ($1669,6 \pm 9,7$ pg/mL) bila je znakovito viša od serumske ($85,5 \pm 0,8$ pg/mL). Najvišu folikularnu razinu estradiola imale su negravidne cikličke životinje sa žutim tijelom ($2558,8 \pm 8,9$ pg/mL), dok je najnižu razinu imala skupina gravidnih krava ($858,8 \pm 6,8$ pg/mL). Srednja vrijednost serumskega estradiola u gravidnih je životinja iznosila $115,7 \pm 1,2$ pg/mL. Niska razina serumskega estradiola uvijek je bilježena u životinja koje su imale aktivne jajnike i dobar volumen folikularne tekućine što pokazuje da su bile reproduktivno aktivne, odnosno da razlog prodaje nije ležao u neplodnosti.

Ključne riječi: jajnici, serum, estradiol 17 β , zebu goveda, Nigerija
