Sequential changes in ovarian follicular dynamics in *Bos indicus* heifers before and after nutritional anoestrus

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This study examined the effects of live mass on ovarian follicular dynamics in heifers before and after the onset of nutritional anoestrus. The effects of decreased live mass on secretion of progesterone, LH and FSH were also investigated. Ovarian characteristics were monitored in 16 postpubertal Brahman heifers using daily transrectal ultrasonography. After two normal oestrous cycles, 11 heifers (treatment group) were allocated to a restricted diet, until the cessation of their oestrous cycles, that resulted in a decrease in live mass of 0.8 kg day^{-1}. Heifers were then fed an *ad libitum* diet, resulting in increasing live mass until resumption of oestrous cycles. Five heifers (control group) were fed an unrestricted diet throughout the experimental period. The concentrations of progesterone in plasma were monitored twice a week and the concentrations of LH and FSH were determined on day 6 after ovulation, before initiation of dietary restriction, after a 17% decrease in live mass and after the onset of anoestrus in the treatment group. Onset of anoestrus was preceded by linear decreases in the size of ovarian follicles and corpora lutea, and in the persistence of the first dominant follicles of oestrous cycles. These changes were proportional to the decrease in live mass ($P < 0.001$). The frequency of pulses of LH or mean concentration of FSH on day 6 of the oestrous cycle were not changed after a 17% decrease in live mass, and did not increase following the onset of anoestrus, although concentrations of progesterone were < 0.2 ng ml^{-1}, suggesting that release of LH was suppressed at this time. Increasing live mass following anoestrus resulted in a linear increase in dominant follicle size and persistence, which were correlated with changing mass ($P < 0.001$); ovulation resumed when dominant follicles were $105 \pm 4\%$ of the size of initial ovulatory follicles.

Introduction

In northern Australia, as is the case in most tropical and subtropical environments, the available nutrient supply varies considerably with season of year such that during the dry season there is often an inadequate supply of forage to meet the maintenance requirements of grazing cattle. Body mass and body condition therefore undergo oscillating patterns of increase during the wet season and decrease during the dry season (Holroyd *et al.*, 1979). The severity of weight loss and decrease in condition have major effects on reproductive performance, with degree of weight loss during the preceding dry season and condition at mating correlating with conception rates in both heifers and mature cows (Doogan *et al.*, 1991; O'Rourke *et al.*, 1991).

For successful mating to occur, a sequence of coordinated endocrinological events must culminate in display of behavioural oestrus and ovulation of an ovarian follicle. Restricted dietary intake reduces the size of dominant ovarian follicles in pre- and postpubertal heifers (Murphy *et al.*, 1991; Bergfeld

et al., 1994) and delays the development of large follicles in postpartum cows (Ryan *et al.*, 1994). Decreased dietary energy intake is also associated with decreased pulsatile secretion of LH in pre- and postpubertal heifers and mature cows (Imakawa *et al.*, 1986a; Richards *et al.*, 1989; Kurz *et al.*, 1990), while extended periods of dietary restriction result in the cessation of oestrous cycles (Imakawa *et al.*, 1986b; Richards *et al.*, 1989). However, the sequential changes in ovarian follicular dynamics during such nutritional restriction events have not been reported.

The objectives of this study were (i) to characterize changes in ovarian morphology in *Bos indicus* heifers with decreasing live mass until the cessation of oestrous cycles, and to determine whether these changes were associated with changes in secretion of progesterone, LH and FSH, and (ii) to examine the pattern of follicular dynamics with increasing live mass, until the resumption of oestrous cycles.

Materials and Methods

Animals and procedures

The study was performed at James Cook University, Townsville (latitude 19°19'S; longitude 146°43'E) in the dry

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tropics of North Queensland. The climate is generally warm and humid, having a distinct hot, wet summer period (October to April) and a warm dry winter period (May to September). During the experimental period, mean monthly minimum and maximum temperatures ranged between 24°C and 31°C in February and between 15°C and 26°C in August.

A group of 16 postpubertal Brahman heifers were studied for 10 months between August and June. At the start of the study they were 18 months old, body mass was 307.0 ± 3.0 kg (mean ± SEM) and they had a condition score of 5.7 ± 0.08 (1 = emaciated and 9 = fat; Holroyd, 1978). At the start of the experimental period, all heifers were allowed sorghum hay *ad libitum* (dry matter digestibility 53%, crude protein content 56.3 g kg^{-1} and estimated dry matter metabolizable energy 8.4 MJ kg^{-1}). In addition, they had access to native pasture and an 8% urea–molasses supplement (crude protein content 349 g kg^{-1} and estimated dry matter metabolizable energy 10.7 MJ kg^{-1}). After observation of two interovulatory intervals, of mean duration 21.4 ± 0.83 days, 11 heifers (treatment group) were allocated to a restricted diet, with a daily dry matter intake of 0.5% of live mass. Heifers were fed reduced quantities of hay, with 60 g per heifer day^{-1} of a dry-mix mineral supplement, which resulted in an average daily decrease in live mass of 0.8 ± 0.05 kg until onset of anoestrus (period of decreasing live mass). Feeding of the restricted diet continued for a further 21 days and then heifers were returned to the original unrestricted diet until an interovulatory interval of ≥ 17 days was recorded (period of increasing live mass). Five heifers (control group) remained on the initial diet throughout the experimental period, which resulted in an average daily gain in live mass of 0.5 ± 0.06 kg. All animals were weighed twice a week and condition scores were recorded each week.

Ultrasonography

The ovaries of all heifers were examined daily, between 07:00 h and 08:00 h, using transrectal ultrasonography (7.5 MHz transducer, Aloka 210DX). No attempt was made to synchronize the stage of oestrous cycles during the period of the experiment. The size and position of corpora lutea and all follicles ≥ 5 mm in diameter were recorded as described by Savio *et al.* (1988). Ovulation was determined by the disappearance of a dominant follicle and subsequent formation of a corpus luteum in the same location on the ovary. The day of ovulation was defined as day 0 and the duration of oestrous cycles as the interval between two successive ovulations. Anoestrus was defined as failure of ovulation of a dominant follicle following luteolysis. The following variables were also defined: persistence of follicles or corpora lutea as the number of days when individual follicles or corpora lutea ≥ 5 mm were detected; maximum diameter as the largest diameter measured in dominant follicles or corpora lutea; growth rate (mm day^{-1}) of follicles as the maximum diameter minus the diameter on the day of detection divided by the interval in days.

Collection of blood samples

Concentrations of progesterone in plasma were monitored twice a week in jugular blood samples. Serial blood samples were collected via indwelling jugular catheters on three occasions from all heifers during the course of the experiment. During the second oestrous cycle, before initiation of dietary restriction, blood samples (10 ml) were collected every 15 min for 8 h (sampling period 1). When the treatment heifers had lost approximately 15% of their initial live mass (7.1 ± 1.8 weeks after the start of dietary restriction), blood samples were collected every 15 min for 10 h (sampling period 2). After the onset of anoestrus in treated heifers, before they were re-allocated to *ad libitum* dietary intake, samples were again collected every 15 min for 10 h (sampling period 3). On all occasions, except when treated heifers were in anoestrus, samples were collected on day 6 of the oestrous cycle, when the first dominant follicle was still increasing in size. After collection, blood samples were immediately placed on ice; plasma was separated by centrifugation at 3000 g within 1 h and then stored at − 20°C until assayed. Concentrations of LH were determined in all plasma samples and concentrations of FSH were measured in pooled hourly samples.

Radioimmunoassays

Concentrations of progesterone in plasma were determined using a modification of the Danazol method (McGinley and Casey, 1979) described by Jolly (1992). The intra- and interassay coefficients of variation (CVs) at the approximate midpoint of the standard curve (1.0 ng ml^{-1}) were 8.8% and 9.1%, respectively, and the sensitivity of the assay, as defined by the concentration at 90% of maximum binding, was 0.03 ng ml^{-1}.

Concentrations of LH in plasma were measured by double-antibody radioimmunoassay, using a modification of the method described by Niswender *et al.* (1969). Purified bovine LH (USDA-bLH-B-6, AFP-11743-B) was provided by D. Bolt (USDA Animal Hormone Program, Beltsville, MD). The antiserum used was NIDDK-anti-oLH-1 (AFP-192279), which was supplied by NIDDK, Bethesda, MD. Iodination of bLH was performed using a modification of the chloramine-T method described by Bolt and Rollins (1983). All assay reagents were prepared in 0.04 mol phosphate buffered saline l^{-1} (pH 7.2) containing 50 mg egg albumin ml^{-1} (RIAD, McNeilly *et al.*, 1976). Duplicate 100 μl aliquots of standards or samples were incubated with 50 μl of normal rabbit serum (diluted 1:250 in RIAD), 50 μl of antiserum (1:400 000 dilution in RIAD) and 50 μl of tracer (12 000 c.p.m.) for 24 h. Donkey anti-rabbit serum (50 μl; IDS, Boldon, Tyne and Wear), diluted 1:25 in RIAD, was then added and the tubes were incubated for 18 h at 4°C. After addition of 300 μl of RIAD, the tubes were centrifuged at 3000 g for 30 min at 4°C. Supernatants were decanted and the radioactivity of the pellet counted. Increasing volumes (3.125–100 μl) of plasma in three pools produced displacement of radioligand that paralleled the standard curve. Quantitative recovery of added LH was evaluated by adding 0, 0.31, 0.63, 1.25, 2.50 and 5.0 ng of USDA-bLH-B-6 to three plasma pools. There was a linear relationship between the concentrations of LH recovered and LH added ($R^2 = 0.94$). The interassay CVs for two bovine plasma pools of 0.28 and 1.82 ng ml^{-1} were 19.7 and 6.3%, respectively. The intra-assay CVs for the same plasma pools were 15.0 and 5.9%, respectively. Assay sensitivity, defined as the concentration at 90% of maximum binding, was 0.15 ng ml^{-1}.

Plasma concentrations of FSH were measured by double-antibody radioimmunoassay, using a modification of the method described by Bolt and Rollins (1983). Purified bovine FSH (USDA-bFSH-I-2, AFP-5318C) was provided by D. Bolt, USDA Animal Hormone Program. The antiserum used was NIDDK-anti-oFSH-1 (AFP-C5288113; NIDDK). Iodination of bFSH was performed using a modification of the chloramine-T method described by Bolt and Rollins (1983). Duplicate 100 μl aliquots of standards or samples were preincubated with 400 μl RIAD and 200 μl of antiserum diluted 1:50 000 with RIAD containing 1:300 normal rabbit serum, for 24 h at room temperature. Iodinated tracer (100 μl), with an activity of about 15 000 c.p.m., was added and the incubation continued for a further 48 h. Donkey anti-rabbit serum (200 μl), diluted 1:25 in RIAD, was then added with 500 μl of 6% (w/v) polyethylene glycol (6 kDa) in RIAD. The tubes were incubated for 15 min at 4°C before centrifugation at 3000 g for 20 min at 4°C. The supernatant was decanted and the tubes drained for at least 10 min before the amount of radioactivity was determined. Parallel inhibition of tracer binding by FSH in plasma samples and USDA bFSH standard was determined using two plasma pools serially diluted 1:1 to 1:32 with assay buffer. Displacement curve slopes of sample pools and assay standards were similar when compared using analysis of covariance ($P = 0.169$). Quantitative recovery of added FSH was evaluated by adding 0, 0.16, 0.32, 0.64, 1.28, 2.56 and 5.12 ng of USDA-bFSH-I-2 to 0.5 ml of each of three plasma pools. There was a linear relationship between the concentrations of FSH recovered and FSH added ($R^2 = 0.99$). The interassay CVs for two bovine plasma pools of 0.24 and 3.54 ng ml^{-1} were 10.7 and 7.1%, respectively. The intra-assay CVs for the same plasma pools were 9.0 and 5.2%, respectively. Assay sensitivity, defined as the concentration at 90% of maximum binding, was 0.12 ng ml^{-1}.

Statistical analyses

The rates of change of variables with the number of oestrous cycles during the period of decreasing live mass were examined using a linear model with an autoregressive error structure (Jennrich and Schluchter, 1986). The last oestrous cycle before the initiation of dietary restriction in treatment heifers was defined as oestrous cycle 0 and all cycles until the onset of anoestrus were included. The rates of change for treatment and control groups were estimated for each variable. The same model was used to examine the rates of change of variables with the dominant follicle number during the period of increasing live mass following anoestrus. The relationships between live mass and ovarian characteristics in treatment heifers were examined using multiple regression analysis. The mean rates of change were estimated using a random coefficients linear model (Lindsey, 1993). Results from these analyses are presented as least squares estimates together with standard errors.

The mean concentration, basal concentration, pulse frequency and pulse amplitude of LH during the three periods of serial blood collection were determined using a modified version of the algorithm developed by Merriam and Wachter (1982) and adapted for IBM-compatible personal computers (PULSAR: R. Lazarus, Department of Community Medicine,

Westmead Hospital, NSW). The 'G' parameters used were those derived empirically for LH profiles by Merriam and Wachter (1982), namely 3.8, 2.6, 1.9, 1.5 and 1.2 for G(1) to G(5), respectively. Differences in pulsatile secretion of LH and mean concentration of FSH between the different sampling periods were examined using multivariate repeated measures analysis of variance. The changes in differences between control and treated heifers from periods 1 to 2 and from periods 1 to 3 were evaluated as pre-planned contrasts.

The timing of onset of anoestrus in treated heifers was modelled using logistic regression. All explanatory variables were expressed as proportions of their values before the initiation of restriction of dietary intake. A subset of significant explanatory variables was determined using backward elimination of non-significant variables ($P > 0.05$), based on the likelihood-ratio test (Collett, 1991).

Repeated measures analysis of variance was used to compare the ovarian characteristics of oestrous cycles before dietary restriction in treated heifers with the first oestrous cycle of normal duration after the resumption of ovulation, and with oestrous cycles of control heifers occurring at a similar time.

The MIXED procedure in SAS (1992) was used for the autoregressive linear model. For all other statistical analyses, SPSS (1993) was used.

Results

Period of decreasing live mass

Linear decreases in the live mass, condition score, maximum diameter of the first dominant follicles of oestrous cycles, ovulatory follicles and corpora lutea and the persistence of the first dominant follicles were observed in the treatment group, when all oestrous cycles before the onset of anoestrus were considered ($P < 0.01$, Figs 1 and 2). During the same period in the control group, there were linear increases in the live mass, condition score ($P < 0.001$), size ($P < 0.01$) and growth rate ($P = 0.02$) of ovulatory follicles (Figs 1 and 2). There was no change in the persistence of corpora lutea or in the mean concentrations of progesterone in either treatment or control groups during this period ($P > 0.1$). The number of dominant follicles per oestrous cycle did not change over the period of decreasing live mass in treatment heifers ($P = 0.30$) or in control heifers over the same time ($P = 0.98$). Changing live mass in treated heifers was linearly associated with changes in ovarian characteristics ($P < 0.001$). For every 10 kg decrease in live mass, the maximum diameter of the first dominant follicles of oestrous cycles decreased by an estimated 0.31 ± 0.005 mm; persistence of the first dominant follicles decreased by 0.39 ± 0.018 days; ovulatory follicle size decreased by 0.31 ± 0.006 mm and the maximum diameter of corpora lutea decreased by 0.64 ± 0.018 mm. There were also linear relationships between live mass and ovarian characteristics, when expressed as proportions of values before the initiation of dietary restriction ($P < 0.001$). A 10% decrease in live mass resulted in an estimated $9.4 \pm 0.7\%$ decrease in the maximum diameter of first dominant follicles of oestrous cycles, an $8.5 \pm 0.8\%$ decrease in ovulatory follicle size and a $10.6 \pm 0.9\%$ decrease in the maximum diameter of corpora lutea.
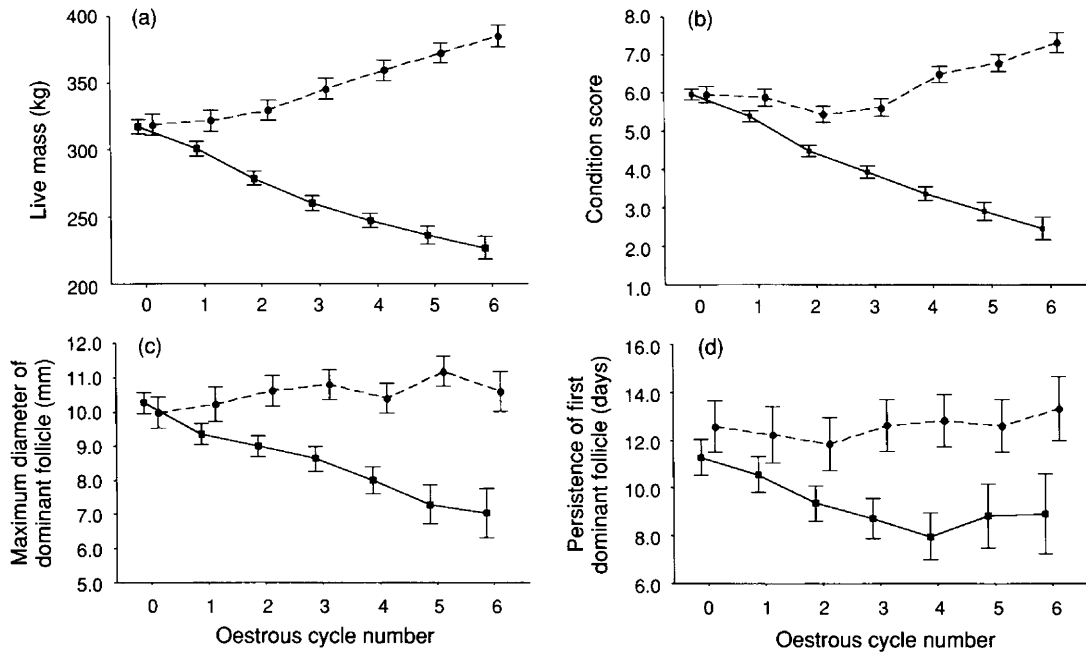
Fig. 1. Sequential changes in (a) live mass, (b) condition score, (c) maximum diameter and (d) persistence of the first dominant follicles of oestrous cycles in treated (■) heifers during the period of decreasing live mass and in control (●) heifers (least squares means ± SEM). Data are included from individual animals only until the onset of anoestrus.
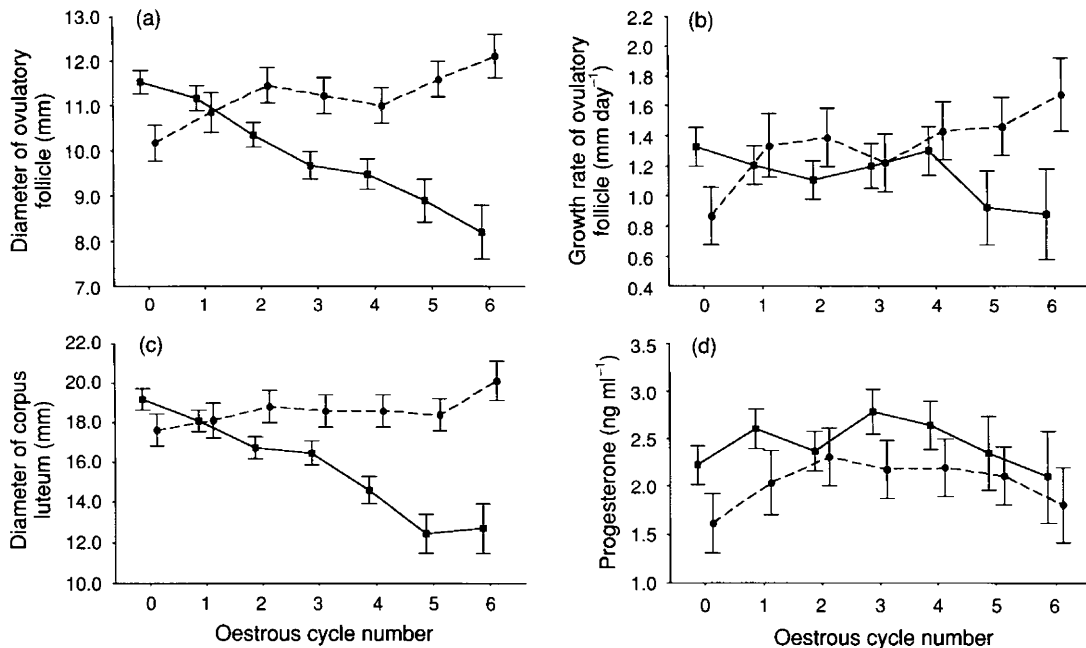

Fig. 2. Sequential changes in (a) ovulatory follicle size and (b) growth rate, (c) maximum diameter of corpora lutea and (d) mean concentration of progesterone in treated (■) heifers during the period of decreasing live mass and in control (●) heifers (least squares means ± SEM). Data are included from individual animals only until the onset of anoestrus.

A decrease in live mass of 69.8 ± 5.6 kg, or $21.8 \pm 1.6\%$ of initial mass, was required before the onset of anoestrus and occurred after 93.2 ± 8.9 days (Table 1). The diameters of final ovulatory follicles and corpora lutea before the onset of anoestrus were $83.3 \pm 3.9\%$ and $76.3 \pm 3.1\%$ of values before the initiation of dietary restriction, respectively (Table 2). The

onset of anoestrus was best predicted by live mass ($\chi^2_{(1)} = 4.37$, $P = 0.04$) and maximum diameter of corpora lutea ($\chi^2_{(1)} = 4.19$, $P = 0.04$), when expressed as proportions of initial live mass and size of corpora lutea. The percentage decreases in live mass and maximum diameter of corpora lutea to give a 50% probability of anoestrus were 21.0%

Table 1. Duration of period of decreasing live mass of *Bos indicus* heifers preceding the onset of anoestrus and period of increasing live mass to the resumption of ovulation in treated heifers, and the change in live mass during both periods and condition score at the end of each period

Parameter	Decreasing live mass		Increasing live mass	
	Mean ± SEM	Range	Mean ± SEM	Range
Duration (days)	93.2 ± 8.9	48–132	54.5 ± 9.0	8–97
Change in live mass (kg)[a]	− 69.8 ± 5.6	31–86	71.9 ± 11.1	5–117
Percentage change in live mass[b]	− 21.8 ± 1.6	10–28	22.7 ± 3.6	2–38
Condition score	3.3 ± 0.19	2.3–4.4	3.9 ± 0.3	2.5–6.0

[a]Difference in live mass between beginning and end of periods.
[b]Difference in live mass as a percentage of live mass before treatment.

Table 2. Ovarian characteristics at the onset of anoestrus in *Bos indicus* heifers expressed as percentages of values at the initiation of dietary restriction (mean ± SEM)

Parameter	Value at the onset of anoestrus (% of initial value)
Maximum diameter of first dominant follicle (mm)	8.1 ± 0.35 (79.9 ± 3.8)
Persistence of first dominant follicle (days)	8.9 ± 0.88 (80.8 ± 6.9)
Ovulatory follicle diameter (mm)	9.5 ± 0.34 (83.3 ± 3.9)
Growth rate of ovulatory follicle (mm day^{-1})	1.2 ± 0.10 (95.0 ± 11.0)
Maximum diameter of corpus luteum (mm)	14.5 ± 0.59 (76.3 ± 3.1)

(95% confidence interval = (17.7, 27.4)) and 23.0% (16.7, 37.0), respectively.

No differences were detected in the characteristics of LH secretion or in mean concentrations of FSH between groups over sampling periods 1 and 2 ($P > 0.2$, Fig. 3), although, at the time of the second sampling period treatment, heifers had lost 16.8 ± 0.8% of initial live mass, maximum diameter of first dominant follicles of oestrous cycles had decreased by 12.2 ± 3.7%, ovulatory follicle size by 11.9 ± 3.2% and maximum diameter of corpora lutea by 12.7 ± 2.6%. At the time of the third sampling period, no evidence of luteal activity was detected in treated heifers, as determined by ultrasonography or plasma concentrations of progesterone (< 0.2 ng ml^{-1}). However, no differences in the pulsatile secretion of LH or in concentrations of FSH were detected between periods 1 and 3 ($P > 0.05$, Fig. 3). All control heifers continued to exhibit oestrous cycles of normal duration (19.4 ± 0.68 days) throughout the experimental period.

Period of increasing live mass

After the initiation of feeding *ad libitum* diets to treatment heifers, subsequent to anoestrus, until resumption of ovulation, there were linear increases in live mass, maximum diameter, persistence and growth rate of dominant ovarian follicles with time ($P < 0.001$, Fig. 4). The persistence of dominant follicles during this period (Fig. 4d), in the absence of corpora lutea, was greater than the persistence of the first dominant follicle of oestrous cycles before the initiation of dietary restriction (11.4 ± 1.12 days). Changes in follicle characteristics were

linearly associated with changing live mass ($P < 0.001$), with a 10 kg increase in live mass resulting in an estimated 0.58 ± 0.02 mm increase in maximum diameter, 0.10 ± 0.001 mm day^{-1} increase in growth rate and 1.1 ± 0.05 days increase in the persistence of dominant follicles.

Ovulation resumed after the emergence of 7.9 ± 1.2 follicles or after 54 ± 9.0 days. This period ranged from 8 to 97 days, with the condition score at resumption of ovulation varying from 2.5 to 6.0 (Table 1). There was no relationship between live mass of treated heifers at the beginning of the period of dietary restriction and time to onset of anoestrus ($P = 0.20$), but the duration of the interval to resumption of oestrous cycles was inversely related to the duration of decreasing live mass before anoestrus ($R^2 = 0.39$, $P = 0.04$, Fig. 5).

The duration of the first interovulatory interval following anoestrus was on average 9.36 ± 1.43 days, and was ≤ 8 days in 8 of 11 heifers. The first ovulatory follicle was 105 ± 4% of the size before the initiation of decreasing live mass; growth rate was 118 ± 5% and a persistence was 98 ± 9% of initial values. Live mass was 101 ± 4% of mass at the start of dietary restriction. Short interovulatory intervals were followed by oestrous cycles of normal duration (19.9 ± 0.48 days) in which, when compared with oestrous cycles before the initiation of decreasing live mass, there were no differences in ovarian characteristics ($P > 0.1$).

Discussion

Decreasing live mass in *Bos indicus* heifers was associated with linear reductions in the size of ovarian follicles and corpora
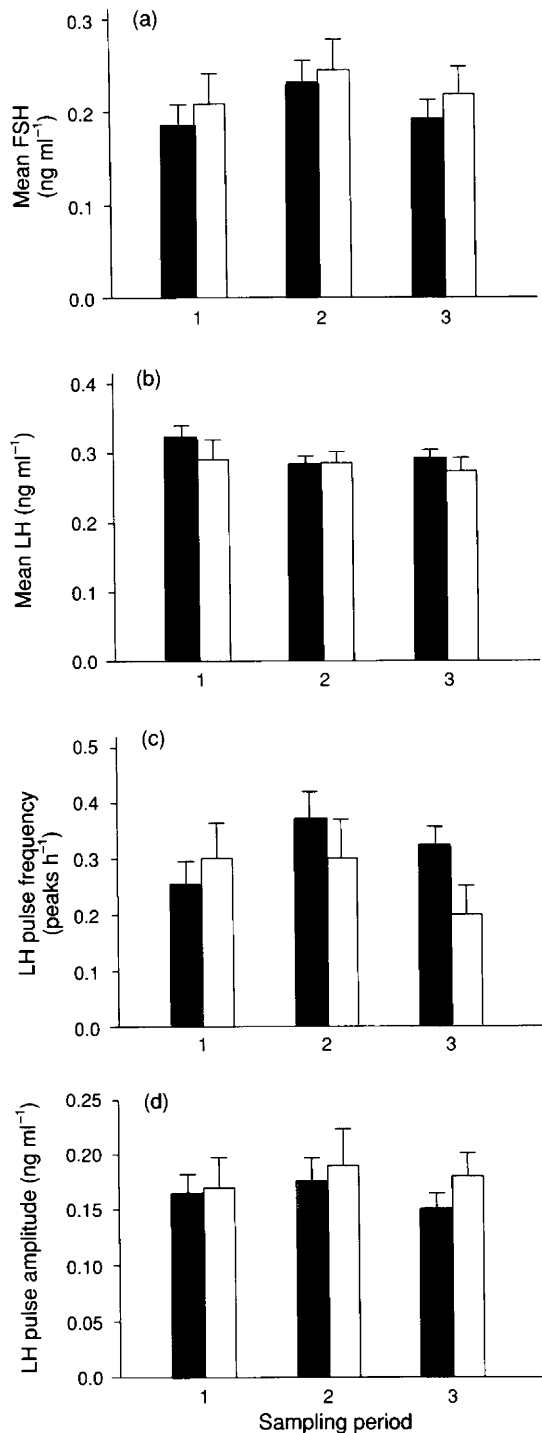
Fig. 3. Mean concentrations of (a) FSH and (b) LH, (c) LH pulse frequency and (d) pulse amplitude in treated (■) and control (□) heifers during the three periods of serial blood collection (least squares means ± SEM). Period 1 was before the start of dietary restriction; period 2 was when treated heifers had lost 17% of initial live mass; and period 3 was after the onset of anoestrus in treated heifers. For all characteristics, differences between groups from periods 1 to 2, and from periods 1 to 3 were not significant ($P > 0.05$).

lutea, and in the persistence of the first dominant follicles of oestrous cycles. These ovarian changes were proportional to the percentage decrease in live mass. In the current study, a 10 kg reduction in live mass resulted in a decrease in ovarian follicular diameter of 0.3 mm and a decrease in persistence of 0.4 days. These changes are similar to those reported by Murphy et al. (1991) for two groups of Bos taurus heifers, in which a 38 kg difference in live mass resulted in a difference of 1.4 mm in maximum diameter and a 2.1 day difference in the persistence of dominant follicles. Similar findings were reported in prepubertal hiefers, when animals fed to gain 0.3 kg day^{-1} had smaller dominant follicles, at the same age, than did heifers fed to gain 0.9 kg day^{-1} (Bergfeld et al., 1994). In spite of the decrease in persistence of dominant follicles in the present study, there was no change in number of dominant follicles per oestrous cycle with decreasing live mass, in contrast to the findings of Murphy et al. (1991). The results reported here demonstrate that decreasing live mass results in gradual changes in ovarian follicular morphology, before the onset of nutritional anoestrus.

The effect of decreasing live mass on ovarian follicular function cannot be determined directly from the results of the present study. However, the ability of dominant follicles to ovulate following luteolysis and to form functional corpora lutea despite a decrease in size of 12%, at the time of the second period of serial blood collection, suggests that granulosa cells could still respond normally to gonadotrophin stimulation. This finding is in agreement with studies on ewes (McNeilly et al., 1987), and postpartum cows (Ryan et al., 1994), in which the steroidogenic potential of follicular granulosa cells was not consistently related to body condition score when comparisons were made between follicles of equivalent status.

Although there was a significant decrease in the maximum diameter of corpora lutea with decreasing live mass, there did not appear to be any effect of decreasing mass on luteal function in the study reported here, as mean circulating concentrations of progesterone and the persistence of corpora lutea did not change with decreasing live mass. This finding is in contrast to previous reports of decreased peripheral concentrations of progesterone after reduction in live mass (Imakawa et al., 1983, 1986a), but is in agreement with other authors who found no difference in peripheral concentrations of progesterone in serum of animals losing or gaining mass (Harrison and Randel, 1986; Schrick et al., 1990; Murphy et al., 1991). An inverse relationship between level of nutrition and concentration of plasma progesterone in ewes is described by Parr et al. (1993) and attributed to differences in metabolic clearance rates, and may account for the absence of any decrease in peripheral concentrations of progesterone after reduction in live mass.

No differences were detected in the pulsatile secretion of LH and mean concentrations of FSH on day 6 after ovulation, between control and treatment groups, when treated heifers had lost 17% of live mass and the maximum diameters of follicles and corpora lutea had decreased by more than 10%. It may be that, during the luteal phase of the oestrous cycle, changes in secretion of LH and FSH cannot be detected until a greater decrease in live mass has occurred. Richards et al. (1989) found a change in frequency of LH pulses in nutritionally restricted cows only after a 24% decrease in live mass. In the study reported here, sampling performed earlier in the luteal

phase, when progesterone negative feedback on release of LH was low, or sampling during the follicular phase, may have revealed differences in pulsatile secretion of LH. In support of
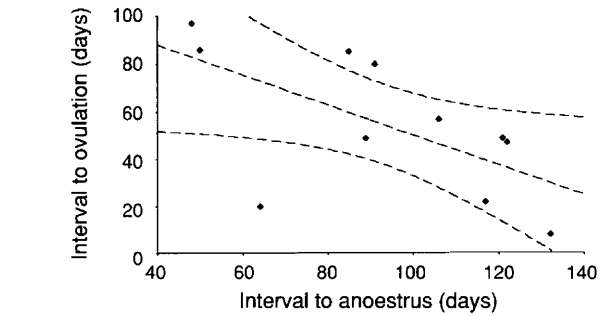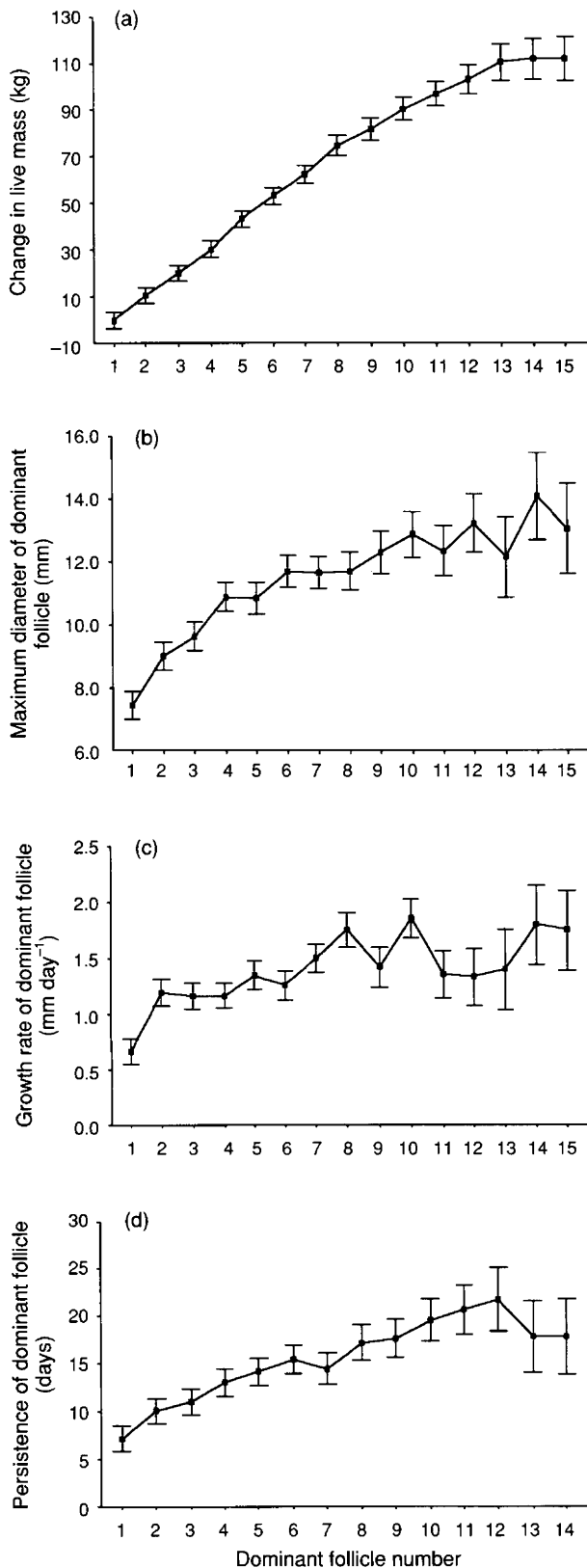

Fig. 5. Fitted linear regression, with 95% confidence intervals for the mean predicted response, of the interval to the resumption of oestrous cycles with the interval to the onset of anoestrus. Values for individual heifers are represented by different points.

this contention, secretion of LH remained low in treated heifers after the onset of anoestrus, when concentrations of progesterone were < 0.2 ng ml^{-1} and the frequency of pulses of LH would be expected to be high. This suggests that release of LH at this time was suppressed by the reduction in live mass.

Feeding unrestricted diets to heifers, following onset of anoestrus, resulted in a linear increase in ovarian follicular size and persistence with increasing live mass. Similar increased persistence of dominant follicles is reported in cattle treated with subluteal concentrations of progesterone and is associated with an increase in the frequency of LH pulses compared with control animals (Savio *et al.*, 1993; Stock and Fortune, 1993). The increase in size and persistence in the study reported here were possibly a result of gradual increases in the frequency of LH pulses, as has been observed in nutritionally anoestrous heifers and prepubertal heifers after increased feed intake (Imakawa *et al.*, 1986b; Kurz *et al.*, 1990).

Resumption of ovulation in heifers after onset of anoestrus occurred when the mean maximum diameter and growth rate of dominant follicles were similar to those observed in ovulatory follicles before initiation of dietary restriction, and when mean live mass was 101% of initial live mass. Richards *et al.* (1989) reported that resumption of oestrous cycles following nutritional anoestrus occurred at 89% of initial live mass, when body condition score had increased to 4.6 from 3.5 at the time of anoestrus, and suggested that condition score dictates the initiation of oestrous cycles. However, in the study reported here, condition score at resumption of oestrous cycles varied from 2.5 to 6.0. The concept of a critical amount of body fat being required for ovulation has been largely discounted (Bronson and Manning, 1991); instead the availability of metabolic fuels is likely to be the mechanism linking nutritional status and reproductive function (Schillo, 1992). Although reproductive competence may be correlated with percentage of

Fig. 4. Sequential changes in (a) live mass, (b) maximum diameter, (c) growth rate and (d) persistence of dominant follicles in treated heifers during the period of unrestricted dietary intake after the onset of anoestrus (least squares means ± SEM). The duration of the period displayed was 97 days and data are included for dominant follicles from individual animals only until the resumption of ovulation. The change in live mass is the cumulative increase in live mass after initiation of feeding *ad libitum*.

body fat, it is not necessarily a causal relationship, as is indicated by the considerable variation in condition scores at the onset of anoestrus and resumption of ovulation in the current study. In addition, the characteristics of the first oestrous cycles of normal duration after anoestrus were similar to those of oestrous cycles before the initiation of dietary restriction, although the mean body condition score was only 3.9, compared with 5.7 at the start of the experiment. However, no conclusions can be drawn from the current study as to the precise mechanisms responsible for the failure or resumption of ovulation after changes in live mass.

The first interovulatory interval was ≤8 days in 8 of 11 heifers, similar to that in reports of shortened first oestrous cycles after postpartum anoestrus (Copelin *et al.*, 1987), or in prepubertal heifers (Evans *et al.*, 1994). Evans *et al.* (1994) suggested that dominant follicles giving rise to short-lived corpora lutea at puberty ovulate at a later stage of development than do ovulatory follicles in mature cattle. However, in the study reported here, the first ovulatory follicle after anoestrus had a similar persistence (98%) and a greater rate of growth (118%) compared with the ovulatory follicles before the initiation of dietary restriction, indicating that follicular development was not abnormal. Early luteolysis after the first ovulation in postpartum cows is caused by premature release of prostaglandin $F_{2\alpha}$ from the uterus, rather than impaired luteal function (Copelin *et al.*, 1987). This early release is a consequence of increased concentrations of endometrial oxytocin receptors in animals with short luteal phases, resulting in increased responsiveness to oxytocin, which is responsible for stimulating release of prostaglandin $F_{2\alpha}$ (Garverick *et al.*, 1992). However, it is not known whether a similar mechanism operates following nutritional anoestrus.

The duration of the period to resumption of ovulation after onset of anoestrus was inversely related to the time of decreasing live mass required to induce anoestrus, suggesting considerable between-animal variation in sensitivity to nutritional stress. Similar differences in susceptibility to dietary induced anoestrus were reported in rats fed 50% of normal intake (Knuth and Friesen, 1983). The variation in sensitivity of *Bos indicus* heifers may represent differences in priorities for energy allocation, with more sensitive animals having a better survival strategy for the harsh environmental conditions experienced in the dry tropics.

In conclusion, onset of nutritional anoestrus was preceded by linear decreases in the size of ovarian dominant follicles and corpora lutea, which were proportional to decreases in live mass. No changes in LH or FSH secretion on day 6 after ovulation were detected in treated heifers following a 17% decrease in live mass. This was probably due to the effect of progesterone negative feedback on LH secretion at this stage of the oestrous cycle, as characteristics of LH release were not altered in treated heifers after anoestrus, in the absence of any luteal activity, suggesting that release of LH at this time was suppressed by the reduction in live mass. After re-allocation to *ad libitum* diets, linear increases in the size and persistence of dominant follicles were observed until the resumption of ovulation, which occurred when dominant follicles attained maximum diameters similar to and growth rates slightly greater than those observed in ovulatory follicles before dietary restriction was initiated. Further investiga-

tion is required into the mechanisms responsible for these morphological changes and their functional significance.

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