The effect of restricted suckling on LH and ovarian steroids after induced ovulation in *Bos taurus* and *Bos indicus* cows

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A total of 37 first-calf cows and their calves, comprising 19 Drakensbergers and 18 Hereford × Simmentalers, were subjected to either normal or once-daily suckling for 15 days, commencing at either day 35 or 60 post-partum in a 2 × 2 × 2 factorial experiment. All cows received common hormone therapy during the normal or restricted suckling periods. A Norgestomet ear implant was inserted in conjunction with an oestrogen injection on the first day, and removed on the tenth day of the variable suckling periods. The cows were injected with gonadotropin releasing hormone (GnRh) 30 h after the removal of the ear implants, and inseminated 18 and 42 h after the GnRh injection. There was a tendency for a greater ($P \geqslant 0,05$) proportion of the cows suckled once daily to ovulate than those suckled normally, and this effect was most marked in the Drakensberger cows treated between days 60 and 75 post-partum. In the Drakensbergers, restricted suckling tended to increase the proportion of cows which conceived to the fixed-time inseminations, and to decrease the incidence of 'short' oestrous cycles following GnRh. Tonic luteinizing hormone (LH) levels, the release of LH in response to GnRh and oestrogen secretion were not affected by any of the variables studied.

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'n Totaal van 37 eerstekalf-koeie en hulle kalwers, wat uit 19 Drakensbergers en 18 Hereford × Simmentalers bestaan het, is onderwerp aan óf normale soging óf soging een keer per dag in 'n 2 × 2 × 2-faktoriale proefontwerp. Die twee sogingsbehandelings het óf op dag 35 óf dag 60 postpartum 'n aanvang geneem en het 15 dae geduur. Alle koeie het standaard-hormoonterapie ontvang gedurende die periodes van normale of beperkte soging. 'n Norgestomet-oorinplanting is tesame met 'n estrogeeninspuiting op die eerste dag toegedien en op die tiende dag van die normale of beperkte sogingsperiodes verwyder. Die koeie is 30 uur na die verwydering van die oorinplanting met gonadotropien-vrystellingshormoon (GnVh) ingespuit, en 18 en 42 uur daarna geinsemineer. Meer koeie het geneig om tydens beperkte soging te ovuleer as tydens normale soging. Hierdie effek was veral merkbaar in die Drakensbergerkoeie wat behandel is tussen dae 60 en 75 post-partum. Beperkte soging het in die Drakensbergers gelei tot 'n vermeerdering in die persentasie besette koeie asook 'n vermindering in die voorkoms van 'kort' siklusse na GnVh-toediening. Basale luteïniserende hormoon-(LH)-konsentrasies, die afskeiding van LH na die GnVh-inspuiting en estrogeensekresie is nie deur behandeling beïnvloed nie.

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Introduction

Reducing the interval between calving and reconception is a priority for efficient reproductive performance in beef herds. The duration of the post-partum anoestrous period is influenced by a number of factors, including lactation (Laster, Glimp & Gregory, 1973). The stimulus of suckling by the calf, and not the process of lactation *per se*, is important in beef cows (Wiltbank & Cook, 1958; Wetteman, Turman, Wyatt & Totusek, 1978). Measures such as early weaning (Smith & Vintcent, 1972), temporary weaning (Hollness, Hopley & Hale, 1978) and restricted suckling (Randel, 1981) have been found to hasten the re-initiation of oestrous cycles after calving. The physiological mechanisms whereby suckling depresses ovarian function are not clear, and results relating to the influence of suckling on hormone secretion are somewhat inconsistent. Echternkamp (1978), Radford, Nancarrow & Mattner (1978) and Forrest, Rhodes & Randel (1980) showed that suckling depresses tonic LH secretion, although Chang, Gimenez & Henricks (1981) and Williams, Kotwica, Slanger, Olsen, Tilton & Johnson (1982) were unable to demonstrate such an effect. Suckling also depressed LH release in response to gonadotropin releasing hormone (GnRh) in certain studies (Carter, Dierschke, Rutledge & Hauser, 1980; Troxel, Kesler, Noble & Carlin, 1980) but not in others (Echternkamp, 1978). The object of this study was therefore to determine the effect of suckling on LH, oestrogen and progesterone secretion and on ovarian function at different stages of the post-partum period. *Bos taurus* and *Bos indicus* cows were used in this experiment, since evidence exists to indicate that the problem of lactation anoestrus is greater, and overall reproductive ability lower in *Bos indicus* than in European breeds of cattle (Coetzer, Mentz, Vermeulen & Coetzee, 1975; Adeyemo & Heath, 1980).

Procedure

Animals

A total of 37 first-calf cows and their calves, comprising 19 Drakensbergers *(Bos indicus)* and 18 Hereford × Simmentaler (British crosses) were used in the experiment. When calving commenced on 11 August the cows were approximately three years old. Calving continued for 45 days. Once the cows had calved they were placed in feeding pens and fed a ration consisting of *ad lib* maize silage, approximately 3 kg *Eragrostis curvula* hay and 0,3 kg high protein concentrate per cow per day.

Treatments

The experiment incorporated a 2 × 2 × 2 factorial design

and involved a comparison of the reproductive performance of Drakensberger and British-cross cows subjected to either normal or once-daily suckling during two stages of the post-partum period, *viz* days 35 – 50 and 60 – 75. Following calving each breed type was divided into four groups, each balanced for cow and calf masses and date of calving. The four groups were randomly allocated to four treatments (Table 1).

Table 1 Outlay of experimental treatments employed

Breed	Days post-partum during which suckling intensity was varied	Suckling intensity	*n*	Treatment
British-cross	35 – 50	Normal	5	1
		Restricted	5	2
	60 – 75	Normal	4	3
		Restricted	4	4
Drakensberger	35 – 50	Normal	5	5
		Restricted	5	6
	60 – 75	Normal	4	7
		Restricted	5	8

In view of the well established need for a preovulatory increase of blood progesterone levels in anoestrous cows (Donaldson, Basset & Thorburn, 1970; Yuthasastrakasol, Palmer & Howland, 1975), all cows in the experiment were subjected to progesterone and oestrogen therapy, followed by an injection of GnRh, as standard treatment during the 15-day restricted or normal suckling periods. A Norgestomet ear implant (Intervet S.A.) was inserted in conjunction with an I.M. injection of 3 mg Norgestomet and 6 mg oestradial valerate on the first day, and removed on the tenth day of the normal or restricted suckling periods. On day 11 of these periods (30 h after the removal of the ear implants) the cows' ability to release LH and to ovulate was challenged further by injecting them (I.M.) with 500 μg GnRh (Abbott). At 18 and 42 h after the GnRh injection (48 and 72 h after implant removal) fixed-time inseminations were performed on the cows.

Cows subjected to restricted suckling were allowed access to their calves between 08h00 and 08h30 each morning during the 15-day period of restricted suckling. After suckling, the cows were kept in feeding pens approximately 400 m from the calves. The latter were housed in partly enclosed pens with free access to water, a concentrate mixture (80 % maize meal, 20 % lucerne meal) and *Eragrostis curvula* hay.

Measurements obtained

All cows were joined with masculinized teaser cows between 06h00 and 07h00, and again between 17h00 and 18h00 each day from day 20 post-partum until five days after the second fixed-time insemination was performed. The cows were rectally palpated for the presence of *corpora lutea* immediately prior to the insertion of the ear implants, and again approximately one week after the injection of GnRh. Conception rates were established from subsequent calving records.

Cows and calves were weighed at fortnightly intervals throughout the experiment. The body condition of each cow was assessed (thin = 1, fat = 5) immediately prior to the insertion of the ear implants by the scoring system described by Van Niekerk & Louw (1982).

The influence of treatment on tonic LH secretion was investigated by bleeding the cows at intervals of 15 min for a period of 2 h immediately prior to the insertion of the ear implants. This schedule was repeated five days after implant insertion, and 2 h after implant removal. LH was also measured in samples obtained at intervals of 30 min for a total period of 9 h after the GnRh injection. Indwelling polyethylene catheters (Clay Adams) were inserted into the jugular vein immediately prior to the sequential collection of samples described above. The cows were restrained in feeding stanchions during the periods of frequent blood collection.

Blood samples were obtained *via* venipuncture from all cows once daily for five days prior to, and at six-hourly intervals for 18 h prior to the GnRh injection for subsequent analysis of total oestrogens. Progesterone concentrations were determined in samples obtained at intervals of three days for a period of 20 days after the GnRh injection.

Blood samples were collected into heparinized syringes, centrifuged within 30 min of collection and stored at −15°C pending analysis.

Hormone determinations

LH was measured in plasma according to the radioimmunoassay described by Niswender, Reichert, Midgley & Nalbandov (1969) and validated by Lishman (1972) in this laboratory. When samples collected to measure tonic LH secretion were analysed, the assay was modified slightly to obtain greater sensitivity. The initial dilution of anti-LH serum was changed from 1:100 000 to 1:160 000, and the incubation of antiserum with standards and unknown plasma samples prior to the addition of labelled tracer was increased from 24 to 48 h. The inter- and intra- assay coefficients of variation for LH were 12,3 and 4,3 %, respectively. Progesterone and oestrogen were measured according to the methods described by Butcher, Collins & Fugo (1974). Recovery of labelled progesterone added to plasma varied from 92,8 to 96,3 %, and the within- and between- assay coefficients of variation were 8,8 and 12,7 %, respectively. Total oestrogens were determined, since oestradiol-17β obtained after column chromotography was undetectable. Levels were corrected for recovery of tritiated oestrogen, which varied from 72,9 to 80,0 %. The within- and between- assay coefficients of variation were 14,2 and 18,6 %, respectively.

Statistical analysis

Analyses of variance were used to test the effect of treatment on the different parameters of hormone secretion. An analysis of covariance was applied to test whether tonic LH levels before, differed from those measured during the normal or restricted suckling periods, and the Chi-squared test to determine whether treatment affected ovulatory responses and conception rates. Regression analyses were used to study the relationship between the different parameters measured.

Results

Ovulatory response of treated cows

None of the cows had exhibited oestrus or had palpable *corpora lutea* prior to the insertion of the progesterone ear implants at the commencement of the normal or restricted suckling periods. The ovulatory response of the cows (Table 2) was gauged by rectal palpation of the ovaries approximately one week after the GnRh injection. In addition, the pattern of progesterone secretion during the 21 days which followed the administration of GnRh was used to indicate ovulations. It is evident from Table 2 that there was a tendency for a greater ($P \geqslant 0{,}05$) proportion of the cows suckled once daily to

Table 2 Number of cows which ovulated, conceived and underwent 'short' cycles in response to GnRh

Breed	Days post-partum treated	Suckling intensity	*n*	No. of cows which ovulated in response to GnRh	No. of cows which conceived to fixed-time inseminations	No. of cows which underwent 'short cycles'*
British-cross	35 – 50	Normal	5	2 (40 %)	1 (20%)	0
		Restricted	5	3 (60 %)	0	0
	60 – 75	Normal	4	3 (75 %)	1 (25%)	1 (25%)
		Restricted	4	3 (75 %)	2 (50%)	1 (25%)
Drakensberger	35 – 50	Normal	5	3 (60 %)	0	2 (40%)
		Restricted	5	4 (80 %)	2 (40%)	1 (20%)
	60 – 75	Normal	4	2 (50 %)	1 (25%)	1 (25 %)
		Restricted	5	5 (100%)	3 (60%)	0

* Progesterone levels elevated above 0,5 ng/ml for only six days after GnRh administration in these cows

ovulate in response to GnRh than those suckled normally, and this effect was most marked in the Drakensberger cows treated between days 60 and 75 post-partum. A greater proportion of the Drakensbergers conceived when suckled once daily than when suckled normally (Table 2). This effect was more marked in the cows treated between days 60 and 75 post-partum than for cows treated earlier. None of the cows which ovulated displayed overt oestrus within three days of removing the ear implants. A varying proportion (0 – 40 %) of the cows allocated to each treatment underwent 'short cycles' (cows which ovulated, but where progesterone levels were subsequently higher than 0,5 ng/ml for only six days). Progesterone levels exceded 0,5ng/ml for at least 12 days in cows which displayed 'normal cycles' (Figure 1). The incidence of 'short cycles' was not affected by treatment in the British-cross cows, but 50 % less 'short cycles' occurred in the Drakensberger cows suckled once daily than in those suckled normally (Table 2).

Hormone levels

Concentrations of oestrogen in samples obtained on a daily basis for five days prior to the removal of the ear implants and then on a six-hourly basis between the removal of the implants and the GnRh injection fluctuated considerably from day to day in all the cows. Changes in the levels of oestrogen over the aforementioned periods did not follow any particular pattern, and consequently the area under the curve obtained by plotting oestrogen levels against time in each individual cow was calculated to obtain an estimate of the total quantity of oestrogen secreted. The mean area under the oestrogen curve and the mean peak oestrogen level were not affected by treatment. However, maximum oestrogen levels tended to be higher in cows suckled once daily than in those suckled normally (Table 3).

Tonic LH levels fluctuated at relatively low levels during each of the two-hour periods during which samples were obtained at intervals of 15 min. Mean tonic LH levels prior to the commencement of the normal and restricted suckling periods were not affected by breed of cow and stage post-partum. Furthermore, the once-daily suckling regime did not

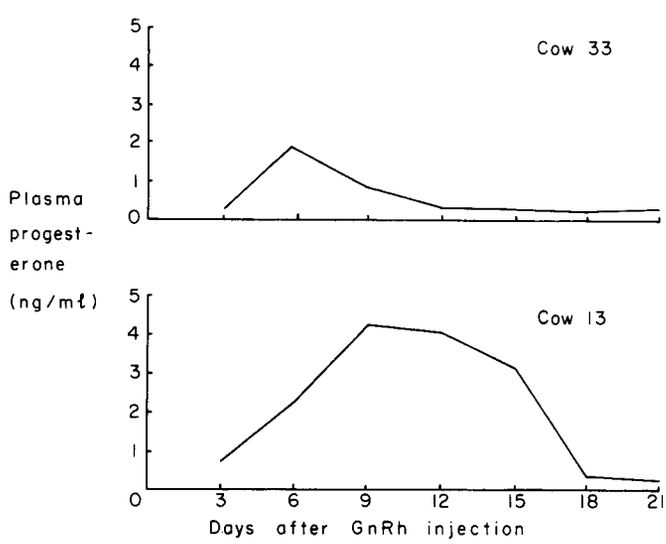

Figure 1 The pattern of progesterone secretion following GnRh in Cow 33 which displayed a 'short' cycle and cow 13 which displayed a 'normal' cycle.

Table 3 Mean area under oestrogen curve and maximum oestrogen level measured

Breed	Days post-partum treated	Suckling intensity	*n*	Mean area under oestrogen curve (mm^2)	Mean maximum oestrogen level measured over sampling period (pg/ml)
British-cross	35 – 50	Normal	5	1227,9 ± 133,4	18,8 ± 1,42
		Restricted	5	1345,2 ± 509,2	25,4 ± 10,8
	60 – 75	Normal	4	1205,9 ± 37,1	8,8 ± 3,4
		Restricted	4	1402,5 ± 441,9	45,7 ± 33,3
Drakensberger	35 – 50	Normal	5	1053,5 ± 275,3	17,0 ± 1,9
		Restricted	5	805,5 ± 113,5	23,6 ± 11,1
	60 – 75	Normal	4	740,3 ± 90,7	11,9 ± 1,7
		Restricted	5	2323,7 ± 1242,3	64,4 ± 37,3

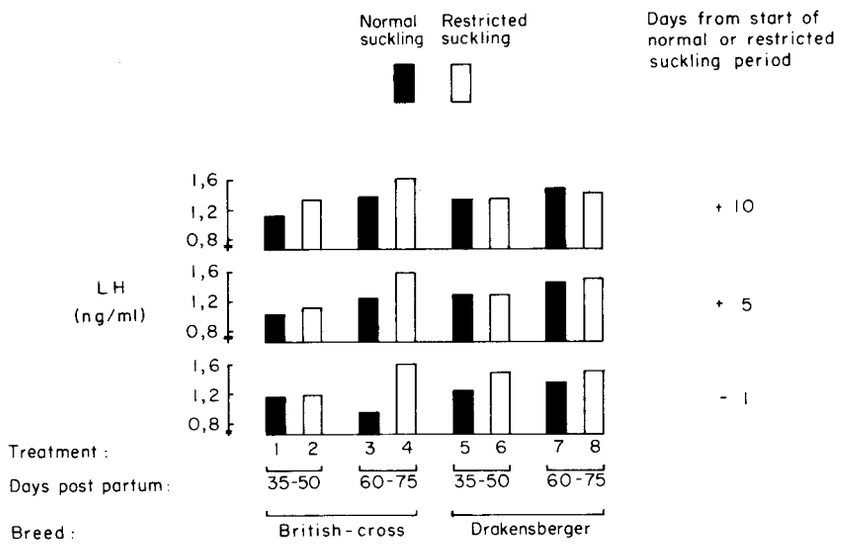

Figure 2 Mean tonic LH levels prior to and during the periods of normal or restricted suckling.

Table 4 Mean area under curve, maximum level and duration of LH surge

Breed	Days post-partum treated	Suckling intensity	*n*	Mean area under LH curve (mm^2)	Mean maximum LH level reached (ng/ml)	Mean duration of LH surge (h)*
British-cross	35 – 50	Normal	5	225,9 ± 40,7	101,0 ± 17,6	6,9 ± 0,6
		Restricted	5	202,4 ± 27,5	88,7 ± 12,0	6,2 ± 0,3
	60 – 75	Normal	4	124,6 ± 20,3	60,7 ± 6,7	6,5 ± 0,0
		Restricted	4	185,1 ± 47,0	76,9 ± 19,2	6,6 ± 0,4
Drakensberger	35 – 50	Normal	5	197,5 ± 19,7	88,8 ± 12,9	6,9 ± 0,4
		Restricted	5	173,7 ± 77,7	78,0 ± 11,0	6,1 ± 0,5
	60 – 75	Normal	4	193,6 ± 41,5	88,9 ± 17,5	7,3 ± 0,4
		Restricted	5	163,8 ± 37,1	72,2 ± 16,7	5,7 ± 1,0

* Levels > 10ng/ml.

influence tonic LH levels, since there were no significant differences between mean levels measured before and during the restricted suckling periods (Figure 2).

An LH surge was measured in all cows following the injection of GnRh. Treatment did not influence the area under the LH curve, the maximum LH level and the duration of the LH surge (Table 4). Data relating to LH release in response to GnRh in the Drakensberger and British-cross breeds were subsequently pooled in order to increase the number of cows at each of the two post-partum stages and suckling intensities employed. Mean pooled LH levels are diagrammatically illustrated in Figure 3, from which it is evident that LH surges tended to be smaller in cows suckled once daily than those suckled normally between days 35 and 50 post-partum. Between days 60 and 75 the cows suckled normally and once daily, secreted similar quantities of LH.

There were no significant correlations between the secretory patterns of the different hormones studied, the body-mass changes in the cows and their ovulatory response.

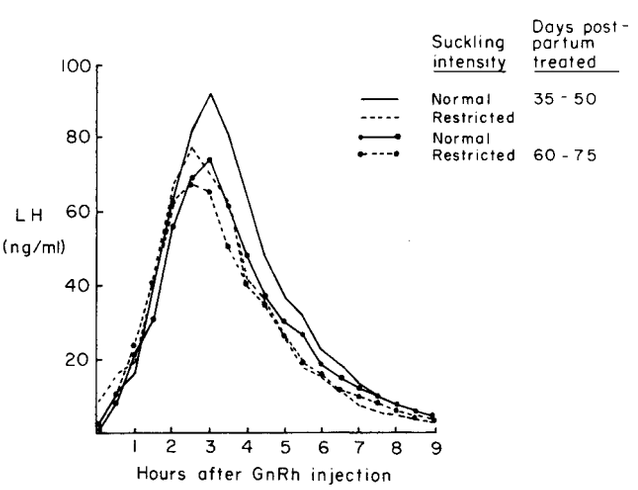

Figure 3 Mean LH levels subsequent to GnRh injection (levels obtained in two breeds pooled)

Body-mass changes

Mean masses of the Drakensberger cows within five days of calving (461,5 ± 10,2 kg) did not differ from those of the British-cross cows (456,2 ± 9,9 kg). All cows used in this study underwent mass losses ranging from 3,2 to 30,9 kg between parturition and the onset of the normal or restricted suckling periods, but these were not affected by treatment or breed. Mean condition scores in cows treated between days 60 and 75 post-partum (2,89 ± 0,08) tended to be lower than those in cows treated between days 35 and 50 (3,13 ± 0,1). Mean growth rates in the calves between birth and 90 days were not affected by the suckling intensity employed.

Discussion

In the present study the ovulatory response of the cows to the injection of GnRh was not significantly influenced by the breed

of cow, the stage post-partum or the suckling intensity employed. However, there was a tendency for a greater proportion of the cows suckled once daily to ovulate in response to GnRh than those suckled normally, and this effect was more marked in the Drakensberger than in the British-cross cows (Table 2). It could be postulated that this improved ovulatory response may have been due to relatively higher pituitary LH levels, which in turn would be expected to result in the release of relatively larger quantities of LH into the bloodstream following GnRh injection in the cows suckled once daily than in those suckled normally. However, the suckling intensity employed did not influence the quantity of LH released following GnRh injection, in fact cows suckled normally tended to release more LH than those suckled once daily at the earlier post-partum stage (Figure 3). This finding is at variance with that of Carter *et al.* (1980) and Troxel *et al.* (1980), who showed that suckling depresses LH release in response to GnRh in beef cows. Our results are however in agreement with those of Echternkamp (1978), who showed that the GnRh- induced LH release was not influenced by suckling intensity. The discrepancies described above may be due to differences in the stages post-partum during which the suckling intensities were varied, the degree of suckling stimuli imposed, the genetic constitution of the cows and the numbers of cattle used in the different studies.

The magnitude of the increase in ovulatory response owing to restricted suckling tended to be greater in the Drakensberger than in the British-cross cows (Table 2). This finding lends support to the contention that reproductive ability in *Bos indicus* cows differs inherently from that in *Bos taurus* females. For example, *Bos indicus* cows display a greater proportion of ovulations without oestrus than European breeds (Van der Westhuysen, 1972), and Randel & Moseley (1977) found that peak LH levels at the time of oestrus are significantly lower in Brahman than in Hereford heifers. Griffin & Randel (1977) found that *Bos indicus* cows release less LH in response to GnRh than *Bos taurus* cows, a finding which was not substantiated in the present study (Table 4).

The once-daily suckling regime used in the present study tended to reduce the incidence of 'short' luteal phase cycles in the Drakensberger, but not in the British-cross cows (Table 2). Carter *et al.* (1980) and Reeves & Gaskins (1981) found a greater incidence of 'short' cycles in non-suckled cows or cows nursed once daily than in normally suckled *Bos taurus* cows injected with GnRh. However, in these investigations GnRh was administered at an earlier stage post-partum than in the present study, and this could account for the different results obtained.

Tonic LH levels were not affected by breed of cow, stage post-partum and suckling intensity employed (Figure 2). In a number of other studies suckling has been shown to cause varying levels of depression on tonic LH concentrations (Carruthers, Convey, Kesner, Hafs & Cheng, 1980; Carruthers & Hafs, 1980; Forrest *et al.*, 1980). In the present study the mean tonic LH level measured over all the treatments was 1,32 ± 0,04 ng/ml which is similar to levels reported during the post-partum period by Ingalls, Convey & Hafs (1973), Edgerton & Hafs (1973) and Forrest *et al.* (1980). It should be noted though that in the present study LH concentrations in the majority of samples were measurable at, or just above, the lower limit of sensitivity for the LH assay. It is thus possible that the assay was not sensitive enough, or the number of animals used was too small to detect differences in tonic LH secretion owing to treatment. The finding that LH levels prior to and after the insertion of the progesterone ear implants did not differ supports this conclusion, since Chang *et al.* (1981) and Ireland & Roche (1982) have shown that tonic LH levels are suppressed by progesterone therapy.

It was anticipated that oestrogen levels measured in the present study would provide an indication of follicular development following the withdrawal of the progesterone ear implants, and the effect thereon of treatment. However, oestrogen concentrations varied considerably within and between individual cows (Table 3), and it is evident that a larger number of animals than was used in this experiment are necessary to measure the effect of treatment on oestrogen secretion. Such studies appear warranted, since Bellin, Hinshelwood, Robinson, Ax & Hauser (1982) found that fewer and smaller follicles, which contained smaller quantities of oestrogen, occurred in suckled than in non-suckled beef cows.

The number of cows which conceived to the fixed-time inseminations, relative to the number which ovulated over all treatments was generally dissapointing (Table 2). The stress of frequent handling imposed on the cows may have contributed to this phenomenon. However, the beneficial effect of restricted suckling on the ovulatory response obtained in this and other trials (Randel, 1981) indicate that it may hold potential as a means of increasing reproductive rates in beef herds. The use of restricted suckling techniques as a means of reducing post-partum anoestrous periods in first calvers, late season calvers and *Bos indicus* females clearly warrants attention under practical farming conditions.

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