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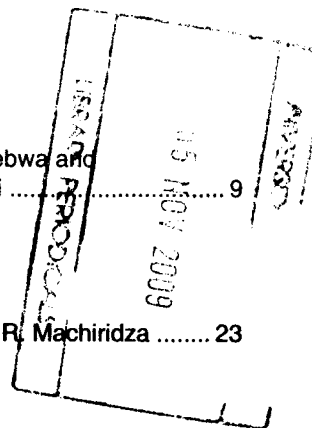
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# Reproductive performance of Tuli beef heifers mated as yearlings and synchronised for oestrus with gonadotropin releasing hormone and prostaglandin $F_{2\alpha}$

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**The objective of this study was to compare the reproductive performance of yearling heifers with that of two year old heifers and to determine whether oestrus synchronisation with gonadotropin releasing hormone (GnRH) and prostaglandin  $F_{2\alpha}$  ( $PGF_{2\alpha}$ ) could improve fertility in yearling beef heifers. The experiment was conducted at Grasslands Research Station, Marondera, Zimbabwe from October 1997 to July 1998. Supplementary feeding of 52 Tuli yearling heifers, seven to nine months old, was initiated at the beginning of the experiment. This was done to ensure that the heifers would have attained a target breeding weight of 260 to 300 kg (60 to 65 percent of the mature cow body weight) by 13 to 15 months of age. The yearling heifers and 20 two year old heifers were observed for signs of oestrus starting two months prior to the onset of breeding. At 13 to 15 months of age the yearling heifers weighed on average  $265 \pm 15$  kg. The heifers were randomly divided into two equal groups, and one group was synchronised for oestrus with GnRH (2 ml Receptal® per injection) and  $PGF_{2\alpha}$  (1 ml Prosolvin® per injection) given six days later. All heifers then ran with three Tuli bulls over a 60 day breeding period. Blood samples were taken for plasma progesterone concentration determination. The pre-breeding mean interval between oestruses for yearling heifers was longer ( $21.9 \pm 1.6$  d vs  $17.7 \pm 1.0$  d;  $P < 0.05$ ) than for two-year old heifers. Pregnancy rate for yearling heifers was lower (33.3 percent vs 90.0 percent;  $P < 0.001$ ) than that for two year old heifers, but there was no difference ( $P > 0.05$ ) between synchronised and non-synchronised heifers. The results indicated that yearling Tuli heifers showed normal oestrous activity but had lower conception rates than the two year old heifers. In addition, the results showed that reproductive performance of the yearling heifers was not improved by synchronising oestrus with GnRH and  $PGF_{2\alpha}$  and that there was little benefit in breeding Tuli heifers below 280 kg  $F_{2\alpha}$  body weight.**

**Keywords:** Heifers, Tuli, reproductive performance, oestrus synchronisation.

## Introduction

Breeding beef heifers at 13 to 15 months (yearling heifers) of age may alleviate the current shortage of beef cattle in Zimbabwe. The devastating drought of 1991/1992 reduced cattle numbers due to high mortality and heavy voluntary culling of cattle by farmers. Breeding females were also culled due to the scarcity of adequate grazing and water. Since that drought, the cattle population has not completely recovered and the whole of the Southern African region is short of slaughter cattle (Hasluck, 1997). The slow recovery may be attributed to a number of factors, among them, is the generally low reproductive performance of beef cows.

Relative to developed countries, Zimbabwean beef cows first calve at an advanced age of three to four years and have low conception and calving rates (Cundiff *et al.*, 1996; Hamudikuwanda, 1997). In Zimbabwe, beef heifers are bred at 27 months of age or later (Cattle Producers Association, 1989) which results in heifers taking a long time to join the breeding herd. The latter reduces lifetime productivity of the cows. According to Ruckebusch *et al.*, (1991), cattle breeds can attain puberty at six to 18 months of age. The Tuli, a Zimbabwean beef breed, was reported to attain puberty by 12 months of age in the United States of America (Cundiff *et al.*, 1996). Similarly, European *Bos taurus* beef breeds such as the Hereford, Shorthorn, Aberdeen-Angus, and Angus, and their crosses have been successfully bred at 13 to 15 months of age resulting in increased calf production per cow in Australia, New Zealand and the USA (Morris, 1981). However, little information is available locally on the reproductive performance of the Tuli, and the consequences of breeding the heifers at 13 to 15 months of age have not been investigated. As an indigenous breed that is adapted to the tropical climate, successful early breeding of Tuli heifers may accelerate the rebuilding and recovery of the national herd. Therefore, the objective of this study was, to assess the reproductive performance of Tuli heifers bred as yearlings and to test the possibility of inducing ovarian activity and enhancing fertility by synchronising oestrus in Tuli yearling heifers using exogenous gonadotropin releasing hormone (GnRH) and exogenous prostaglandin  $F_{2\alpha}$  ( $PGF_{2\alpha}$ ).

## Materials and Methods

### Site

The study was conducted at Grasslands Research Station, Marondera, Zimbabwe. The Station is located at 31° 30'E longitude and 18° 11' S latitude, at an altitude of 1 600 m. Grasslands Research Station receives a mean annual rainfall of 900 mm which falls mainly from October to April. The mean daily minimum and maximum temperatures are 11.5°C in winter and 23.1°C in summer, respectively. The vegetation is mainly grassland savannah with predominantly *Hyparrhenia* grass species and *Brachystegia* tree species.

### *Animal management*

Fifty two yearling heifers (13 to 15 months of age) and 20 two year old Tuli heifers were used in the study. All the heifers were grazed on natural pasture throughout the study period at a stocking rate of one livestock unit (LU) per four hectares (1 LU/4 ha). In this study, one livestock unit was equivalent to 500 kg body weight. The heifers were rotated through four paddocks, once every week. They had access to water all the time and a salt lick of monocalcium phosphate (1:1).

### *Supplementation*

Prior to the start of the study, 52 six month old heifers were selected from the beef herd at Grasslands Research Station. The heifers were group fed on a supplementary feed that provided 18 percent CP and 12 MJ ME/kg DM at a rate of two kg/heifer/day until they were 13 to 15 months old. During the feeding period, the heifers were separated from the two year old heifers. The objective of giving supplementary feed was to ensure that the heifers attained a minimum body weight of 260 kg at the time of breeding. It is generally recommended that beef heifers be bred when they have attained at least 65 percent of mature weight for successful conception. In our case, 260 kg was approximately 65 percent of the mature body weight of the Tuli cows at Grasslands Research Station. The heifers continued to receive the supplementary feed up to the end of breeding.

### *Oestrus detection*

Both groups of yearling heifers and the two year old heifers were monitored for occurrence of oestrus starting two months before breeding commenced and throughout the breeding period. Oestrus detection was by visual observation done twice per day between 0600 and 0800 hours and 1700 and 1900 hours.

### *Oestrus synchronisation and breeding*

At the start of the breeding period, the yearling heifers were randomly allocated to two groups. One group was synchronised for oestrus using Receptal® (Hoechst Veterinar, Unterschleißheim b. München, Germany), a GnRH analogue and Prosolvin® (Internet International BV, Boxmeer, Holland), an analogue of PGF<sub>2α</sub> using the procedure described by Twagiramungu *et al.*, (1992). Each heifer was given an intramuscular injection of two ml (12.5 µg) of Receptal followed by one ml (500 µg) of Prosolvin six days later. The second group of yearling heifers and the two year old heifers were not synchronised for oestrus. Immediately after the synchronised heifers received their Prosolvin injection, all heifers in the three groups were allowed to run with three Tuli bulls for a period of 60 days. Pregnancy diagnosis was performed by rectal palpation six weeks after the removal of the bulls.

### *Blood sampling*

Blood samples were collected from all heifers that were observed exhibiting oestrus signs during the breeding period. Samples were collected on days zero

(day of oestrus detection), 10 and 24 after the first signs of heat. The samples (five to six ml) were collected by jugular venipuncture into test tubes cooled to 4°C and containing 100 µl EDTA anticoagulant (10 percent w/v). The test tubes were kept in chilled water at 4°C before centrifugation at 1 000 x g for 10 minutes at 4°C. Plasma was aspirated into small vials and stored at -20°C until analysed for progesterone concentration.

#### *Hormone analysis*

Plasma progesterone concentration in the samples was analysed by solid-phase radioimmunoassay using a commercial kit (Coat-a-Count®, Diagnostic Products Corporation, Los Angeles, CA, USA). The intra- and inter-assay coefficients of variation were four percent and 15 percent, respectively. Heifers that had plasma progesterone concentrations of at least 1 ng/ml on day 10 were considered to have ovulated (Byerley *et al.*, 1987). At day 24, plasma progesterone concentration of at least 2 ng/ml was used as an indicator of possible pregnancy.

#### *Measurements*

A number of measurements were taken. First, all heifers were weighed once every two weeks throughout the experimental period. Second, the number of oestruses per heifer and interval between oestruses during the two month period before breeding were determined. In addition, the number of heifers in each group that were observed in oestrus within five days of the beginning of breeding and the interval from onset of breeding were recorded. Oestruses for synchronised yearling heifers and non-synchronised yearling heifers and the number of heifers in each group observed in oestrus during the entire breeding season were also determined.

#### *Statistical analysis*

Out of the 52 yearling heifers, 32 (15 non-synchronised and 17 synchronised heifers) reached the targeted minimum breeding weight by the end of the breeding season. Data from heifers that did not reach the target breeding weight were omitted from the statistical analysis, except for the comparison of the proportion observed in oestrus and mean interval between oestruses for oestruses observed during the two-month period prior to breeding. The effect of age on pre-breeding mean interval between oestruses and mean number of oestruses per heifer and the effect of oestrus synchronisation on mean interval from the start of breeding to oestrus were compared using the Student's t-test statistic (PROC TTEST; SAS, 1994). Effect of age and synchronisation on the number of heifers observed in oestrus during the first five days of breeding and during the entire breeding season, and pregnancy rate were compared using the Chi-square test statistic (PROC FREQ; SAS, 1994). The effect of age and oestrus synchronisation on plasma progesterone concentration was analysed using the PROC MIXED procedure of SAS (1994) for repeated measurements (Littell *et al.*, 1998). The following model for repeated measurements was used:

$$Y_{ijk} = \mu + T_i + H(T)_{ij} + D_k + (T \times D)_{ik} + E_{ijk}$$

where:

$Y_{ijk}$  = progesterone concentration on day "k" on heifer "j" in treatment group "i"

$\mu$  = overall mean

$T_i$  = fixed effect of treatment "i" (age group or synchronisation treatment)

$H(T)_{ij}$  = random effect of heifer "j" nested within treatment "i" (age group or synchronisation treatment)

$D_k$  = fixed effect of sampling day "k" (the repeated measure)

$(T \times D)_{ik}$  = fixed interaction effect of treatment "i" (age group or synchronisation treatment) with sampling day "k"

$E_{ijk}$  = random residual error on day "k" of sampling on heifer "j" in treatment "i".

## Results

The results of oestrus detection for the two month period prior to the start of breeding are shown in Table 1. There was no difference ( $P > 0.05$ ) in the mean number of oestruses per heifer between yearling heifers and the two year old heifers. However, mean interval between oestruses was longer ( $P < 0.01$ ) for yearling heifers than for two year old heifers. Mean body weight ( $\pm$  SE) of yearling heifers at the end of the breeding season was  $265.9 \pm 14.9$  kg. This body weight was lower ( $P < 0.001$ ) than that of two year old heifers of  $330.0 \pm 31.3$  kg.

**Table 1: Pre-breeding mean number ( $\pm$  SE) of observed oestruses per heifer and mean interval ( $\pm$  SE) between oestruses (days) for yearling heifers and two year old heifers.**

Parameter	Age group	
	Yearling heifers	Two year old heifers
Number of heifers	52	20
Mean oestruses per heifer	$2.0 \pm 0.2$	$1.9 \pm 0.2$
Mean interval between oestruses	$21.9 \pm 1.6^a$	$17.7 \pm 1.0^b$

<sup>a,b</sup>Values in each row with different superscripts differ ( $P < 0.05$ ).

### Effect of age

The proportion of heifers observed in oestrus during the breeding period, proportion that ovulated based on plasma progesterone concentrations and pregnancy rates for the two-year old heifers and non-synchronised yearling heifers are in Table 2.

**Table 2: Proportion (percent) of heifers observed in oestrus within five days of breeding and during the 60 day breeding period, proportion that ovulated, and pregnancy rate for non-synchronised yearling heifers (non-synch) and two year old heifers.**

Parameter	Heifer age group		Significance
	Non-synch yearlings	2 year olds	
Number of heifers	15	20	
Proportion observed in oestrus within five days of bulling (%)	40.0(6) <sup>a</sup>	15.0(3) <sup>1</sup>	NS
Proportion observed in oestrus during bulling period (%)	66.7 (10) <sup>a</sup>	65.0 (13)	NS
Proportion that ovulated	33.3 (5) <sup>b</sup>	45.0 (9)	NS
Pregnancy rate (percent)	33.3 (5) <sup>c</sup>	90.0 (18)	***

<sup>1</sup>NS = no significant difference between the yearlings and two year old heifers ( $P > 0.05$ ).

<sup>a</sup>Figure in parenthesis is the number of heifers observed in oestrus.

<sup>b</sup>Figure in parenthesis is the number of heifers that ovulated.

<sup>c</sup>Figure in parenthesis is the number of heifers that were pregnant.

\*\*\*Difference between the yearlings and two year old heifers was significant ( $P < 0.001$ )

There was no difference ( $P > 0.05$ ) between the proportion of non-synchronised yearling heifers and two year old heifers observed in oestrus during the breeding period. However, there was a tendency ( $P = 0.09$ ) for more yearling heifers than two year old heifers to come into oestrus within five days of breeding. The effect of age on pregnancy rate was highly significant ( $P < 0.001$ ) and more two year old heifers conceived than yearling heifers (Table 2). All the non-synchronised heifers that ovulated were also confirmed pregnant by rectal palpation and subsequent calving.

Least squares means of plasma progesterone concentrations for the non-synchronised yearling heifers and two year old heifers for the three sampling days are shown in Table 3. There was no interaction between age and blood sampling day on plasma progesterone concentration in non-synchronised yearling heifers and two year old heifers that ovulated. Day of blood sampling affected ( $P < 0.01$ ) plasma progesterone concentration, but the effect of age was not significant ( $P > 0.05$ ). Plasma progesterone concentration on day 24 was higher ( $P < 0.01$ ) than on day zero but marginally higher ( $P < 0.06$ ) than on day 10. There was a tendency ( $P < 0.1$ ) for plasma progesterone concentration on day 10 to be higher than day zero.



**Table 3: Comparison of mean plasma progesterone concentration (ng/ml) on three sampling days after oestrus (zero, 10 and 24 plays) for non-synchronised yearling heifers (non-synch.) and two year old heifers that ovulated.**

Age group	N	Day of Sampling		
		0	10	24
Non-synch.	5	0.26 <sup>a</sup> ± 1.57	3.37 <sup>ab</sup> ± 1.67	4.94 <sup>b</sup> ± 1.57
2 year old	9	0.98 <sup>a</sup> ± 0.98	2.32 <sup>ab</sup> ± 0.98	4.98 <sup>b</sup> ± 0.98
Mean	14	0.62 <sup>a</sup> ± 0.93	2.85 <sup>ab</sup> ± 0.97	4.95 <sup>b</sup> ± 0.93

<sup>ab</sup>Means in the same row with different superscripts differ (P<0.05).

**Table 4: Heifers observed in oestrus (percent) within five days of bulling, mean interval (± SE) from the start of breeding to oestrus, heifers observed in oestrus (percent) during the breeding period, percentage that ovulated, and pregnancy rate, for synchronised (synch. yearlings) and non-synchronised yearling heifers (non-synch. yearlings).**

Parameter	Treatment group		Significance Level
	Synch. yearlings	Non-synch. yearlings	
Number of heifers	17	15	
Percentage observed in oestrus			
Within 5 days of onset of breeding	64.7 (11) <sup>a</sup>	40.0 (6)	1NS
Mean interval from onset of breeding to oestrus, (days)	4.1 ± 1.3	6.3 ± 2.2	NS
Percentage observed in oestrus during breeding period	88.2 (15) <sup>a</sup>	66.7 (10)	NS
Percentage that ovulated	35.3 (6) <sup>b</sup>	33.3 (5)	NS
Pregnancy rate (%)	35.3 (6) <sup>c</sup>	33.3 (5)	NS

<sup>1</sup>NS = no significant difference between synchronised and non-synchronised heifers (P>0.05)

<sup>a</sup>Figure in parenthesis is the number of heifers observed in oestrus.

<sup>b</sup>Figure in parenthesis is the number of heifers that ovulated.

<sup>c</sup>Figure in parenthesis is the number of heifers that were pregnant.

### *Effect of synchronisation*

The number of heifers observed in oestrus, mean interval to oestrus during the breeding season and pregnancy rate for synchronised yearling heifers and non-synchronised yearling heifers are shown in Table 4. The plasma progesterone concentrations for the heifers that ovulated are shown in Table 5.

There was no significant difference (P > 0.05) between the number of synchronised and non-synchronised yearling heifers observed in oestrus within five days of the

onset of breeding. Similarly, the mean interval from the onset of breeding to oestrus, number of heifers observed in oestrus during the breeding season, number of heifers that ovulated and pregnancy rate were not affected ( $P>0.05$ ) by the synchronisation treatment.

**Table 5: Comparison of plasma progesterone concentration (ng/ml) on three sampling days (zero, 10 and 24 days after breeding) for synchronised yearling heifers (synch.) and non-synchronised yearling heifers (non-synch.) that ovulated.**

Treatment group	n	Day of blood sampling		
		0	10	24
Synch.	6	0.02 <sup>a</sup> ± 0.80	3.17 <sup>b</sup> ± 0.80	2.84 <sup>b</sup> ± 0.88
Non-synch.	5	0.26 <sup>b</sup> ± 0.88	3.10 <sup>b</sup> ± 0.98	4.94 <sup>b</sup> ± 0.88
Mean		0.14 <sup>a</sup> ± 0.60	3.13 <sup>b</sup> ± 0.64	3.89 <sup>b</sup> ± 0.62

<sup>ab</sup>Means in the same row with different superscripts differ ( $P<0.05$ ).

There was no interaction between the day of blood sampling and the synchronisation treatment on plasma progesterone concentration in the heifers. Synchronisation of oestrus did not affect ( $P>0.05$ ) plasma progesterone concentration but the effect of day of blood sampling was significant ( $P<0.001$ ). On both days 10 and 24, plasma progesterone concentrations were higher ( $P<0.01$ ) than on day zero.

## Discussion

The observed mean intervals between oestruses for both yearlings and two year old heifers were within the expected 17 to 25 days range for cattle (Peters and Ball, 1995). The similarity in the mean number of oestruses of the yearling heifers with that of the two year old heifers suggests that the yearling heifers had established a normal pattern of oestrus occurrence as observed in the two year old heifers.

In this study, the presence of bulls apparently induced oestrus in yearling heifers. Induction of oestrus by the presence of bulls has been reported in *post partum* cows (Fernandez *et al.*, 1996) and prepubertal heifers (Izard and Vandenbergh, 1982). Failure of the presence of the bulls to continue to stimulate oestrus in the yearling heifers suggests that the heifers had become refractory to the continual presence of the bulls. Refractoriness to bull stimulation was apparent in the two year old heifers that had previously been kept in a paddock adjacent to one with mature bulls. Fike *et al.*, (1996) reported induction of ovarian activity in *post partum* anoestrus beef cows by bulls that were separated by a fence-line.

The low pregnancy rate in the yearling heifers could be a result of the high incidence of anovulation among the heifers observed in oestrus. The proportion of the non-synchronised heifers that showed anovulatory oestrus during the breeding period (33 percent) agrees with other reports (Nelsen *et al.*, 1985; Rutter and Randel,

1986; Byerley *et al.*, 1987). Byerley *et al.* (1987) found non-pubertal or anovulatory oestrus in 25 percent of heifers and other reports quote values ranging from 13 to 63 percent (Nelsen *et al.*, 1985; Rutter and Randel, 1986). The cause of oestrus without ovulation is not known but in this study, it seemed to be linked to the induction of behavioural oestrus by the presence of bulls. The physiological basis of anovulatory oestrus could be inadequate gonadotropin support (Rutter and Randel, 1986). The hypothalamic-hypophyseal-ovarian systems of the yearling heifers may not have matured enough to secrete sufficient LH that would induce ovulation.

Most of the yearling heifers were observed in oestrus prior to breeding, and ovarian palpations done before breeding showed that all of the yearling heifers used in this study had corpora lutea and developing follicles, suggesting that they were cycling. Mukasa-Mugerwa (1989) reported that after the first ovulation, a number of oestrous periods may occur before the start of persistent ovulatory cycles. The *corpora lutea* that were palpated before the start of breeding in the present study could have been the *corpora lutea* of the first pubertal ovulation. The period from first oestrus to regular ovulation may be as long as 67 to 103 days (Mukasa-Mugerwa, 1989). Nelsen *et al.* (1985) noted that heifers that show non-pubertal oestrus have long intervals of up to 89 days to the next oestrus.

The non-synchronised yearling heifers (33 percent) that were never observed in oestrus during the entire breeding period and also failed to conceive may not have reached puberty. Gregory *et al.*, (1979) found that, on average, only 45 percent of crossbred Brahman and Sahiwal heifers (with Hereford and Angus dams) had reached puberty by 13 months of age. Although 35 percent of the two year old heifers were not observed in oestrus during the breeding season, most of them conceived. These heifers could have exhibited silent or short oestruces, which were missed during the daily observations.

Pregnancy rates for the yearling heifers obtained in the present study do not agree with previous observations as reported by Patterson *et al.* (1992). According to Patterson and co-workers, breeding yearling heifers when they have reached a minimum breeding weight, that is at least 65 percent of the mature weight of the breed, results in conception rates similar to those of heifers bred at two years of age. Cundiff *et al.* (1996) reported a very high pregnancy rate of 90 percent with yearling crossbred Tuli heifers (Tuli sires on Hereford and Angus dams) in studies conducted in the USA. This is similar to the pregnancy rate reported for the two year old heifers in the present study.

The yearling heifers used in the study by Cundiff *et al.* (1996) had a mean body weight of 300 kg at 12 months of age compared to the mean weight at the end of the breeding season of 265 kg for the yearling heifers used in this study. This suggests that the targeted minimum breeding weight for Tuli heifers required to obtain pregnancy rates comparable to those for two year old heifers could be higher than the estimate used in this study. Attainment of puberty is closely related to body weight (Morris, 1981). At the low targeted breeding weight used in this study, it is possible that some of the heifers had not yet reached puberty.

Reproductive performance of heifers bred as yearlings may vary with breed (Patterson *et al.*, 1992). In a study of Mashona yearling heifers, Tiffin (1975) reported a low pregnancy rate (25 percent). Higher pregnancy rates have been obtained after breeding of yearling heifers of Hereford, Shorthorn, Aberdeen-Angus and Angus breeds, and their crosses that were bred in Australia, New Zealand and the USA (Morris, 1981). In addition, the ability to conceive after puberty tends to improve with age (Mukasa-Mugerwa, 1989). The mean age at the end of the breeding period was 18 months for yearling heifers, compared to 30 months for the two year old heifers in the present study. The pregnancy rate of yearling heifers was not improved by synchronisation with GnRH and PGF<sub>2 $\alpha$</sub> . It is possible that induction of oestrus by bulls masked the effects of the synchronisation treatment.

## Conclusion

Yearling heifers appeared to have normal patterns of oestrus occurrence prior to breeding, but had lower pregnancy rates compared to the two-year old heifers. The targeted breeding body-weight used in this study could have been lower than the average breeding weight for the breed. Lack of ovulation among the yearling heifers was the cause of the low pregnancy rate in the yearling heifers. Synchronisation of oestrus with GnRH and PGF<sub>2 $\alpha$</sub>  did not improve pregnancy rate of the yearling heifers.

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