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# Husbandry Factors and the Resumption of Luteal Activity in Open and Zero-Grazed Dairy Cows in Urban and Peri-Urban Kampala, Uganda

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### Contents

The study investigated the influence of selected husbandry factors on interval to resumption of post-partum cyclicity among dairy cows in urban and peri-urban Kampala. A prospective study of 85 day post-partum period of 59 dairy cows in open (n = 38) and zero grazing (n = 21) systems was conducted on 24 farms. Cows of parity 1-6 were recruited starting 15-30 days post-partum. Progesterone (P4) content in milk taken at 10-12 day intervals was analysed using ELISA. The cow P4 profiles were classified into 'normal' (< 56 days), 'delayed' (> 56 days), 'ceased' or 'prolonged' (if started < 56 days but with abnormal P4 displays) resumption of luteal activity and tested for association with husbandry and cow factors. Of the 59 cows, luteal activity in 81.4% resumed normally and in 18.6%, delayed. Only 23.7% maintained regular luteal activity, while the others had ceased (10.2%), prolonged (37.3%) or unclear luteal activity (20.3%). There were no differences between open and zero-grazed cows. Milk production was higher (p < 0.05) in zero than open grazing, in urban than peri-urban and in cows fed on brew waste (p < 0.001) compared with mill products and banana peels. Results suggest that luteal activity resumes normally in a majority of cows, although only a minority experienced continued normal cyclicity once ovulation had occurred, in the two farming systems irrespective of feed supplements or water, and that supplementing with brew waste is beneficial for milk production.

### Introduction

Kampala, the capital of Uganda, is a fast growing city with an annual population growth rate of 5.6% (UBOS 2002). This rapidly growing urban population is challenged by food security, especially animal sourced foods. A beneficial relationship has been established between peri-urban farming and household food security (Maxwell 1994). Smallholder dairying has been reported as one approach which may transform dairy farmers from subsistence to commercial engagement (Cole et al. 2008; Garcia et al. 2008; Sebastian et al. 2008). To achieve good productivity in these smallholder dairy systems, reproductive performance of the cows is essential but reliable data about the Kampala peri-urban dairy cows and their productivity is scarce.

Reproductive performance is globally recognized as a fundamental requirement for profitable dairy farming (Galton et al. 1977; Lucy 2001). Poor reproductive efficiency has been reported as an important factor preventing peri-urban dairy farmers, in intensive production systems in Uganda, from getting good profits from their labour input (Mubiru 2007). Gillah et al. (2012) reported that delayed resumption of post-partum ovulations is responsible for increased calving to conception intervals among cross-bred cows in the East African region. Several studies (Butler 2000; Hultgren et al. 2004; Roche 2006; McDougall et al. 2011; Hudson et al. 2012; McArt et al. 2012) reported, respectively, that poor housing, malnutrition, reproductive ill-health and poor udder health affect the normal recovery of ovarian activity and reproductive efficiency of dairy cows to rebreed during or soon after the transition period.

Commencement of post-partum luteal activity as indicated by milk progesterone (P4) profile (Lamming and Bulmann 1976; Pollott and Coffey 2007) has been adopted as a key physiological event to monitor reproductive performance in dairy herds (Opsomer et al. 2000; Berglund 2008) and a convenient objective fertility index for selection of dairy cows (Royal et al. 2002; Petersson et al. 2006b, 2007). Further examination of milk progesterone profiles enabled their categorization into 'normal', 'delayed', 'prolonged' or 'pathological' (Opsomer et al. 2000; Royal et al. 2000; Petersson et al. 2006a; Garmo et al. 2008, 2009) the characterization about which has been reported for European dairy cows (Friggens et al. 2008).

The objective of the study was to investigate if selected husbandry factors influenced the resumption of luteal activity of zero and open grazing dairy cows in peri-urban Kampala using a milk progesterone monitoring tool.

### **Materials and Methods**

### Study location and design

In a longitudinal study, the monitoring of purposively selected cross-bred dairy cows in zero and open grazing farming systems was conducted within a maximum radius of 25 km of Kampala (32°33′56E:0°18′56N), the capital city of Uganda.

### Study cows

Cross-bred dairy cows ( $\leq 75\%$  Bos taurus X Bos indicus); Friesian (n = 53) and Guernsey and Jersey (n = 6) of parities one to six, calving starting from December 2011, were recruited at between 15 and 30 days post-partum. At recruitment, the following farm data: type of production system, water source and availability, feed resources, the breeding system and the farm geographical position (GPS) were recorded. In addition, cow data on calving history including assisted/

Place where the work was carried out: Urban and peri-urban Kampala, Uganda.

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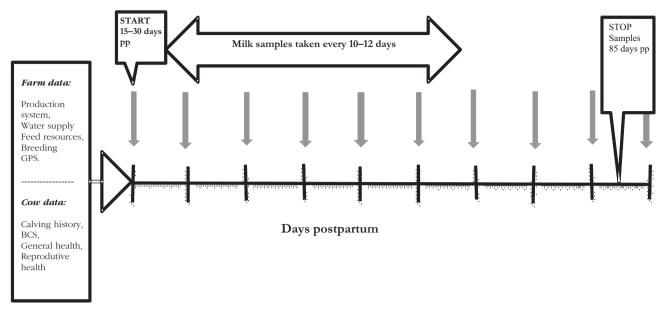


Fig. 1. Sampling schedule for monitoring husbandry factors influencing commencement of luteal activity in urban/peri-urban Kampala, Uganda

unassisted calving, retained afterbirth, abnormal discharges after calving, or stillbirth were collected (Fig. 1). All clinically healthy cows had functioning quarters and were hand-milked twice a day at intervals of 10–14 h. The reported milk yield/cow was the maximum daily yield (litres) on record during the study period.

### Cow husbandry

The cows (n = 59) on 24 farms with 1–13 cows per farm were either open grazed (n = 38; 64%) on paddocked pastures or zero-grazed cows (n = 21; 36%) in shaded cubicles or loose housing allowing minimal exercise. The latter received fresh chopped elephant grass (*Pinnestum puperium*) daily as fodder in individual feed troughs. All cows in the study received deworming once every 3 months, and ecto-parasite (tick) control once a week. Cows in either system received supplementary feeding: beer brew waste; banana peels and other crop residues; mill products (e.g. maize bran or wheat bran); mixed maize silage, brew waste plus molasses; and commercial dairy meal concentrate. In addition, the water resources on each farm and their relative accessibility to cows during the study period were recorded.

### Milk sampling

Milk samples were collected every 10–12 days from onset of the study (Fig. 1). From each cow, mid-stream bulk milk was collected from all quarters into a test tube and transported to the laboratory in ice-cooled box within 4 h of collection. Approximately 10–15 ml of the milk sample was preserved by one potassium dichromate tablet (Lactab Mk III<sup>®</sup>, Thomson and Capper Ltd, Cheshire, UK) and stored at 4°C for 1 week before P4 analysis.

### Determination of milk progesterone

Refrigerated milk samples were allowed to stabilize at room temperature (approximately 20°C) for 20 min before vortexing (Scientific Industries Inc., Bohemia, NY, USA) for homogenization. Milk P4 levels were determined by a commercial enzyme-linked immunosorbent assay (ELISA) (Ridgeway 'M' kit<sup>®</sup>, Ridgeway Science, Gloucester, UK) according to manufacturer's instructions. The concentration of P4 (ng/ml) was calculated from the standard curve ( $r^2 = 0.98-1$ ) by evaluating the optical density (OD) at 590 nm.

# Determination of intervals to post-partum ovarian activity from milk P4 profiles

The pattern of P4 levels was used to categorize four luteal activity profiles: 'normal', 'delayed', 'prolonged' and 'cessation' with the time of the lowest value of P4 prior to when content was > 2 ng/ml indicative of the beginning of luteal activity, as described for cows in European dairy production systems according to Petersson et al. (2006a). 'Normal' luteal activity was defined as P4 rise starting before d 56 post-partum followed by regular activity; 'delayed' luteal activity as P4 rise starting after d 56 post-partum. The 'prolonged' luteal activity was defined as P4 rise normally before d 56 post-partum, but P4 content remaining high for at least 20 days. The 'cessation' profile was indicated as starting P4 rise normally before d 56 post-partum but interrupted with low P4 for 14 or more days. Milk P4 onset and display outside the categories described were classified as 'un-clear' or 'ill-defined' luteal activity (Opsomer et al. 2000).

### Statistical analysis

Data were analysed using sAs version 9.3 (SAS Institute Inc., Cary, NC, USA). Data on different variables were compared between zero and open grazing farms, feed supplement resources and relative availability of water supply using Fisher's exact test and chi-square tests. The differences between means were tested using analyses of variance (ANOVA). For the tests, a value of p < 0.05 was considered significant, and p-values are only presented for statistically significant results.

Due to few observations, uneven number of cows per farm and covariance between variables, multivariable models were unsuitable and only univariable analyses were performed.

### Results

Of 59 dairy cows, the milk P4 profiles of 48 cows indicated early resumption of luteal activity: that is, less than 56 days after calving, irrespective of their calving histories as a group, except for cows with assisted calving history (OR = 4.9, 95% CI = 1.2-20.4, p = 0.03) (Table 1). Different P4 profiles were seen among cows in both types of farms (Table 2) and under the different feed supplementation (Table 3). Water was available ad libitum or half the time for 52 of 59 dairy cows under the different feed supplementation except for cows receiving banana or other crop residues diets where supply was irregular (Table 4). Although the resumption of luteal activity ranged between 15 and beyond 85 days among the dairy cows across different supplementary feeding resources and water accessibility (Table 5), the differences were not significant. Cows in zero grazing production system had a higher milk yield (p < 0.05) than cows reared in open grazing production (Table 6). Milk production was higher (p < 0.001)where brew waste was singly used as a supplement compared with where mill products were used as supplements in both farming systems. There was no significant difference in milk production between the different regimes of water access.

### Discussion

In the smallholder dairy production system of Kampala, a high proportion (81.4%) of the cows had their first ovulation and luteal activity early, before 56 days postpartum. However, most cows failed to maintain regular cyclic activity; only successfully expressed in 23.7% of the cows. Ovulation in the remaining cows occurred later or luteal activity was characterized by abnormal P4 profiles such as cycle cessation or got prolonged. The range of the time of resumption of luteal activity was wide (from 15 to beyond 85 days), but with the relatively few numbers of cows studied, it was not possible to distinguish significant differences across different production systems and feeding regimes. A

Table 1. Relationship between calving history and resumption of luteal activity in dairy cows of urban and peri-urban Kampala

Resumption of luteal activity after calving	Normal calving	Assisted Calving <sup>a</sup>	Retained placenta	Still birth	Total	
Normal resumption ovarian activity (< 56 days)	24	7	16	1	48	
Delayed resumption of ovarian activity (> 56 days)	5	5	1	0	11	
TOTAL	29	12	17	1	59	

<sup>a</sup>Assisted calving; (OR = 4.9; 95%CI = 1.2-2.04; p = 0.03).

previous study (Nassuna-Musoke 2001) conducted on small holder dairy farming systems of Kampala reported that the proportions of cows that commenced ovarian activity between days 21 and 60 post-partum was higher under zero grazing (43%) than under open grazing (14%). The study reported the mean interval to overt post-partum oestrus as 126 days for both around Kampala, and that fewer zero grazing cows (10% vs 32%) had initiated and manifested oestrus by 60 days post-partum than under open grazing conditions because of climatic heat stress. Lobago et al. (2007) in Ethiopia also reported that luteal activity commenced in 32.6% of suckled cross-bred dairy cows by 52 days post-partum while in the remaining cows (67.4%) activity was delayed. Under pastoral conditions in Tanzania, Matiko et al. (2008) reported that postpartum luteal activity was delayed up to 167 days after calving; prolonged luteal activity was not reported as a category in this study. The studies on cross-bred cows agree with our findings that early post-partum

Table 2. Number of dairy cows for normal and abnormal progesterone (P4) profiles under zero and open grazing dairy farming system of urban and peri-urban Kampala

	Farming systems						
Luteal activity, P4 profiles within 56 days post-partum	Zero grazing	Open grazing	Total				
Normal cycle P4 profile followed by regular cycles <sup>a</sup>	5	9	14				
Prolonged cycle*P4 profile <sup>b</sup>	4	18	22*				
Cessation cycle P4 profile <sup>c</sup>	3	3	6				
Delayed cycle P4 profile <sup>d</sup>	1	4	5				
Un-clear P4 profile <sup>e</sup>	8	4	12				
TOTAL	21	38	59				

\*Possible pregnant cows from bull mating included.

<sup>a</sup>P4 rise and content after start to end of study period (day 56) post-partum was repeatable every 18–24 days.

<sup>b</sup>P4 rise and content remained below 2 ng/ml beyond day 56 post-partum.

 $^{\rm c}P4$  rise and content at or above 2 ng/ml started any time before day 56, remained high for more than 20 days.

<sup>d</sup>P4 rise and content above 2 ng/ml commenced before day 56 then stopped, declined for 14 or more days.

<sup>e</sup>P4 rise and content pattern does not belong to any of the profile above.

Table 3. Relationship between type of supplementary feeds and resumption of post-partum luteal activity in dairy cows of urban and peri-urban Kampala

	Supplementary feed resource categories							
Progesterone (P4) profile categories	Feed 1	Feed 2	Feed 3	Feed 4	Feed 5	Total		
Normal cycle	0	8	4	2	0	14		
Prolonged cycle* P4 profile	1	10	2	9	0	22		
Cessation cycle P4 profile	3	2	0	1	0	6		
Delayed cycle P4 profile	0	3	1	1	0	5		
Un-clear P4 profile	3	4	4	0	1	12		
TOTAL	7	27	11	13	1	59		

Feed 1 = Banana peels + other crop residues; Feed 2 = Mill products: Feed 3 = Brew waste; Feed 4 = Brew waste + Wheat bran & Molasses; Feed 5 = Dairy meal concentrate.

\*Possible pregnant cows of bull mating are included in this group.

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Table 4. Relationship between water access and availability on farm and supplementary feeding for dairy cows of urban and peri-urban Kampala

Frequency of water availability on farm units	Supplementary feed resource categories							
	Feed 1	Feed 2	Feed 3	Feed 4	Feed 5	Total		
All time available water supply	0	17	10	13	1	41		
Half time available water supply	0	10	1	0	0	11		
Irregularly available water supply	7	0	0	0	0	7		
TOTAL	7	27	11	13	1	59		

Feed 1 = Banana peels + other crop residues; Feed 2 = Mill products: Feed 3 = Brew waste; Feed 4 = Brew waste + Wheat bran & Molasses; Feed 5 = Dairy meal concentrate.

commencement of luteal activity with continued regular cyclicity is possible in at least 20% of the cross-bred dairy cows under tropical conditions. The studies further highlight husbandry conditions such as zero, open or pastoral grazing, heat stress or suckling and the genotype of dairy cows as factors among others which might influence the interval to commencement of postpartum luteal activity.

Under temperate climate conditions, approximately 50% of cows of Holstein Friesian and other European breeds successfully resumed ovarian cyclicity within 45–60 days post-partum (Opsomer et al. 1998; Royal et al. 2002; Petersson et al. 2007; Garmo et al. 2009). This means that, disregarding husbandry factors, cross-bred dairy cows in the Kampala urban and peri-urban farming systems commence their first luteal activity by 56 days such as cows in European husbandry, and the associated categories of abnormal progesterone profiles are similar but occurring at higher frequencies. As the study indicated no intrinsic cow factor limitation to breeding

dairy cows before 85 days, truly cyclic cows should be identified and timely mated to achieve a one-year calving interval, thereby improving the reproductive and milk production capacity of smallholder dairy herds around Kampala. The study also observed that a higher than normal 'unclear' progesterone profiles were expressed under zero than open grazing, which may be attributed to husbandry differences or other factors yet to be established. This observation concurs with Mee (2012) whose review suggests that luteal and oestrous activity in pasture based, in this case open grazing, management seems better than under zero grazing system because of fewer reproductive problems in the former.

Calving assistance was frequently provided (Table 1) and interventions in 21% of the parturitions increased the risk of delayed luteal activity. On the contrary, 50% of the cows in the current study resumed post-partum luteal activity early despite abnormal calving histories. The 30% cows experiencing manual removal of retained placenta posed a risk for uterine damages and infections in the genital tract, which is known to impair reproductive performance (Sandals et al. 1979 and Roche 2006). Too early or unnecessary intervention both in calving assistance and in placenta removal will, according to Fourichon et al. (2000) and Gröhn and Rajala-Schultz (2000), interfere with post-partum luteal activity. Moreover, Sandals et al. (1979); Halpern et al. (1985); and Kaneko et al. (1997) indicated that poor uterine health status could contribute to suboptimal expression of ovarian activity through interference with endocrine function of granulosa cells (Price et al. 2013).

Although not part of this study, dietary deficiencies of trace minerals such as selenium, common in pastures in the lake side region of Uganda (Ndyanabo 1992) could also manifest as retained placenta in this study.

The study also demonstrated that more cows in the open grazing than in zero grazing systems expressed

Table 5. Average intervals from calving to resumption of luteal activity and milk production from different feed resources and access to farm water supplies in urban and peri-urban Kampala

		Supplementary feed resources*				Farm water availability			
Model variables	Feed 1	Feed 2	Feed 3	Feed 4	All time	Half time	Irregular		
Interval to resumed luteal activity (days) Milk production (litres)	34.4 10.4 <sup>a</sup>	36 13	39.7 19.2 <sup>bc</sup>	32.8 9.7 <sup>d</sup>	37.3 14	31.7 12.3	34.4 10.4		

Feed 1 = Banana peels + other crop residues; Feed 2 = Mill products; Feed 3 = Brew waste; Feed 4 = Brew waste/Wheat bran/Molasses.

<sup>a-d</sup>Means within a row with different superscripts differ (p < 0.001).

\*One farm on dairy meal concentrate excluded from analysis.

Table 6. Uni-variable model estimates indicative of intervals from calving to resumption of luteal activity under zero and open grazing systems in urban or peri-urban Kampala

		Dairy cow farming systems				Relative location to Kampala			
	Zero	Zero grazing		Open grazing		Urban		Peri-urban	
Model variables	Mean	Range	Mean	Range	Mean	Range	Mean	Range	
Interval (days) to resumed luteal activity Milk production (litres)	38.3 16.1 <sup>a</sup>	15–65 (5–35)	34.8 11.7 <sup>b</sup>	(18–91) (4–22)	37.8 15 <sup>c</sup>	(15–91) (5–35)	33.9 11.1 <sup>d</sup>	(18–65) (4–22)	

<sup>ab</sup>Means in a row with different superscript differ p < 0.05).

<sup>cd</sup>Means in a row with different superscript differ (p < 0.01).

prolonged luteal activity which could be attributed to some cows that initiated cyclicity early, got bull mated and become pregnant. Pregnancy status was however not determined, indicating that more cows in the open grazing system probably should have been favourably classified with 'normal' P4 profile. Early post-partum ovulation is generally associated with improved uterine health and fertility, but for a few individuals, it may be a risk factor for prolonged luteal activity because of incapacity of the endometrium to synthesize prostaglandins for luteolysis, due to subclinical or clinical uterine ill-health (Ranasinghe et al. 2011; Kafi et al. 2012). The described weakness for these variables reflects the difficulties that may arise while performing field studies in developing countries, but still there is a relevance of such studies for the improvement of animal production.

Approximately 80 percentage variance in cow fertility is due to environmental factors, of which 50 per cent could be nutrition (Lane et al. 2013). The study indicated that supplementation diet significantly influenced milk production. Cows on brew waste supplement produced more milk than cows on banana-crop residue, mill products or molasses-brew waste mixed diets. Basic tropical forages, deficient in crude protein have known nutrient inadequacy for dairy cows (Wilson 2009; Souza et al. 2010), unless supplemented. Accordingly, findings by Aregheore (2000) and Sampio et al. (2010) indicate that of all supplement feeds for cows in Kampala, brew waste contained a higher crude protein and was hence the better choice for milk production and cow welfare for post-partum resumption of cyclicity than crop residues, mill products or molasses.

This study also shows that dairy cows receiving a diet supplemented with banana peels and other crop residues produced less milk than others which confirms that these convenient feed supplements (Lee-Smith 2008; Nambi-Kasozi 2008; Katongole et al. 2011, 2012; Hansson 2012) are not sufficient for optimal milk yields. Noted however, is that all farms feeding banana peels and crop residues in this study irregularly supplied water to their milking cows, in contrast to other farms which provided water half or all the time. It must be remembered that the analyses did neither take into account confounding nor random effects. The limited production has been attributed to the low nutritive value of banana peels and crop residues (Aregheore 2000) despite their palatability attribute (Emaga et al.

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2011) is decimated by their low dry matter, crude protein content and tannin content that bind away dietary protein.

### Conclusions

The study established that 81.4% of studied cows in urban and peri-urban Kampala resumed post-partum luteal activity early; good enough for farmers' breeding goal of one calf per cow per year, but only 23.7% maintained normal cyclicity optimal for breeding. Both the husbandry, such as feed supplements, and cow factors, such as reproductive health, are critical for performance and hence requiring attention in this livelihood. The milk production potential from crossbred dairy cows in Kampala was high under zero grazing if brew waste was used as supplement. With improved management around and at calving, the cows have potential to recover their sexual functions and be mated early to increase production efficiency and lifetime milk production.

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### **Conflict of interest**

All authors declare that there are no financial and personal relationships with other people or organisations that could inappropriately bias or influence their work.

#### Author contributions

BMK, MGN-M, DOO, UM and RB, conceived and designed the study. BMK, MGN-M, DOO, TN and RB conducted the on farm field study. BMK, MGN-M, DOO, JL, UM and RB analysed and interpreted the data, drafted and revised and made important intellectual content to the manuscript. All authors read and approved the final version of the manuscript for publication.

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