



Ultrasonographic Monitoring of Uterine Involution in Postpartum Buffalo Cows

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ABSTRACT

A description of normal ultrasonographic alternations of the uterus in the postpartum (PP) in buffaloes is essential for the diagnosis of pathological conditions and improves the ability to differentiate puerperal pathology from normal physiological changes. The current study aimed to characterize the changes in uterine features assessed ultrasonographically in normal parturient Egyptian buffaloes (n=20) during the post-calving period (Until Day 60 PP). In the current data, ultrasonographic findings indicated that the uterine involution was completed at 4-6 weeks PP in normal calved Egyptian buffaloes. Moreover, calving during the spring season and female new born calves were associated with an enhanced uterine involution. Early cyclicity positively influences endometrial thickness (during early PP), dorsal and ventral uterine curvature, and uterine lumen diameter (during late PP) as compared with the late cyclic group. In conclusion, the uterine involution is ended by the 6th weeks PP in normal parturient Egyptian buffaloes. Moreover, season and calves gender implicate in the changes associated with uterine involution in buffaloes. It is extremely recommended to employ ultrasound examinations in the dairy herd to improve the reproductive efficiency of females by reducing the days open and increasing the number of milking animals.

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Introduction

Reproductive efficiency is one of the key elements of a profitable dairy animal production (LeBlanc, 2008). Long postpartum anestrus intervals and long calving intervals chiefly participate in poor reproductivity of buffalo (Singh *et al.*, 2000). Therefore, the first approach to achieve a solution for these problems should be directed toward studying the normal involution of genital organs and the time of resumption of ovarian activity during the post-calving period.

Uterine evolution during gestation is characterized by changes in uterine weight, uterine blood flow, concentration of hormones and their receptors, so it can be expected that each of these changes occurs in opposite direction during the process of PP uterine involution, but may be at a different speed (Taverne *et al.*, 2001). In dairy buffaloes, the interval from calving to clinically completed involution of the uterus varied widely and ranged from 21 to 74 days (Qureshi *et al.*, 1998). In cattle, uterine involution is usually completed in 25-35 days after calving (Jainudeen and Hafez, 2000).

In buffaloes, reproductive efficiency is compromised by factors related to climate, management, nutrition, and diseases (Perera, 2008). Climatic stress depresses ovarian cyclicity, estrus expression, and conception rates (Gendelman and Roth, 2012).

The present study aimed to characterize uterine involution monitored ultrasonographically in buffaloes reared under Egyptian conditions, and some physiological factors influence on uterine measurements.

Materials and methods

Animals

The current work was conducted at the Animal Production Farm, belongs to El-Azhar University, Mostorod, Qalyubia Governorate, Egypt, during the period from August 2014 to May 2016. Clinically normal healthy newly parturient Egyptian buffaloes (n=20) of an age of 4-9 years, 420-490 kg B.Wt. and 2-4 BCS were used in this experiment. Animals had free access to water and were allowed a daily ration comprised of 5 kg concentrate mixture (consisting of cotton seed cack, line seed cack, yellow corn, bran, molasses, lime and NaCl), and ad-lib

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green fodder and rice straw. They were suckled till the end of colostrum feeding and were milked twice daily (06.00 and 18.00 h) thereafter. The average daily milk yield was approximately 4-6 kg/day.

This study was performed in accordance with the Use and Animal Care Guidelines of Faculty of Veterinary Medicine, Benha University, Egypt.

Ultrasound examination

Uterine morphology was monitored once weekly by using transrectal ultrasonography (EICKEMEYER MAGIC 2200, Germany, 6 – 8 MHz linear array probe) from calving to 60 days PP (the end of voluntary postpartum period). The transverse diameter of the anterior 1/3 section of both uterine horns, proximal to the body of uterus, measured in the dorso-ventral direction was evaluated in all animal (Kocamuftuoglu and Vural, 2008; Kandiel et al., 2013).

The progression of uterine involution was done by evaluation of uterine horn diameter that was based on dorsal, cranial and ventral diameters as well as lumen assessment; the space made between two central hyperechoic appositional lines of luminal epithelium (Kähn, 2004).

Uterine involution was defined to be completed when both horns were easily retractable, had normal echogenicity and were completely located in the pelvic cavity (Landaeta-Hernández, et al., 2004). The uterine involution was deemed complete from the occurrence of a minimal uterine horn and uterine lumen diameters and endometrial thickness, with no change between at least two to three successive examinations

(Scully et al., 2013).

Uterine data were categorized by grouping of animals to verify the effect of season, timing of ovarian cyclicity and ovulation, days-in-milk, and new-born gender on the uterine involution. Uterine lumen of less than 3 mm in diameter and had normal echogenicity was used as the cut point value for the uterine involution to differentiate between groups (Landaeta-Hernández, et al., 2004).

To analyze the effect of season, newborn gender, ovarian cyclicity and days-in-milk on uterine measures, data were re-assigned into groups as demonstrated in Table 1.

Table 1. Animal grouping and definitions to study the effect of season, newborn gender, ovarian cyclicity and days-in-milk on uterine measures

Parameter	Animal grouping			
Season	Group I (n=8)	Group II (n=7)	Group III (n=5)	
	Buffaloes calved in Autumn		Buffaloes calved in winter	
	Buffaloes calved in spring			
Newborn gender	Group I (n=8)		Group II (n=12)	
	Buffaloes gave birth of female newborn calves		Buffaloes gave birth of male newborn calves	
Ovarian cyclicity and ovulation	Group I (Early cyclic animals) (n=6)		Group II (Late cyclic animals) (n=10)	
	Buffaloes showed ovulation in less than 30 days postpartum		Buffaloes showed ovulation in more than 30 days postpartum	
Days-in-milk	Group I (n=7)	Group II (n=4)	Group III (n=5)	Group IV (n=4)
	Animals milked less than 50 days before drying	Animals milked 50-100 days before drying	Animals milked 100-200 days before drying	Animals milked more than 200 days before drying

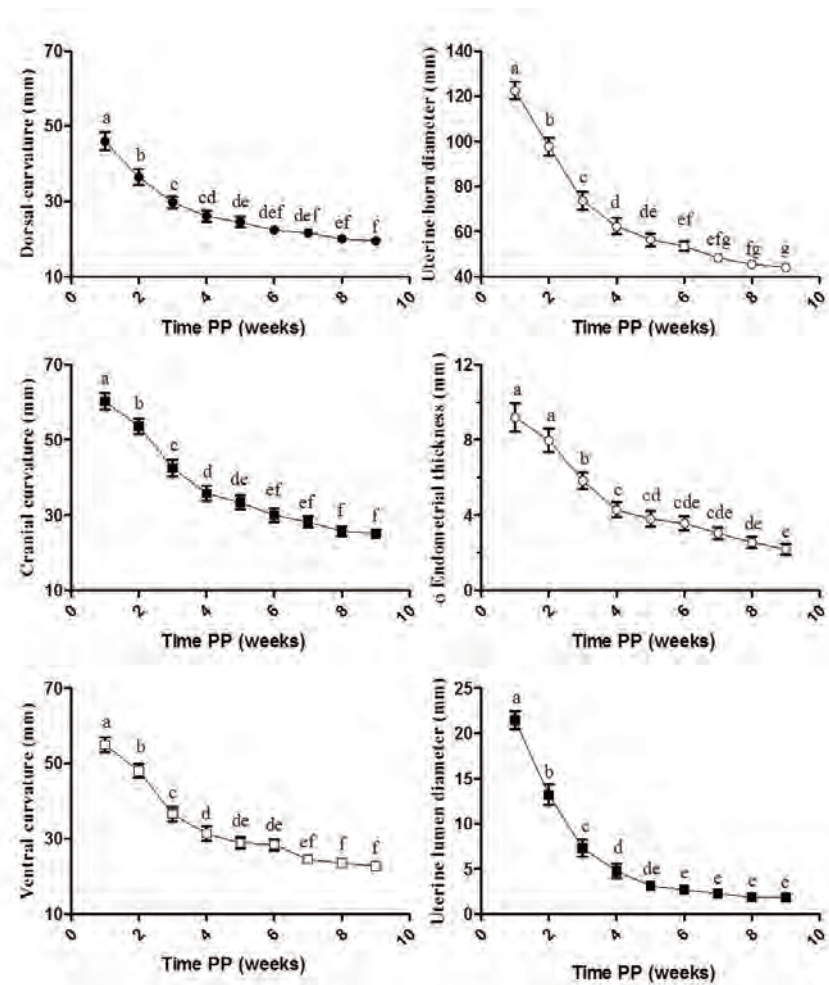


Fig. 1. Changes in uterine horn measures during postpartum period in Egyptian buffaloes. Data with different letters were significantly different at p< 0.05.

Statistical analysis

Data were computed and presented as mean±SEM. Changes in the mean uterine parameters in relation to time after calving and the differences in the means of uterine measures between animal groups were testified with one-way ANOVA using SPSS (Ver. 23) software. Dissimilarities between two groups were analyzed by independent student t-test. Means of different groups were analyzed by post-hoc Duncan multiple comparison test. P value for significance was set at < 0.05.

Results

Uterine involution characteristics in buffalo cows

Uterine horns features in this study followed algorithmic regression pattern with time postpartum (Fig. 1). However, the prompt sharp changes were noticed during the first month PP, and become steady thereafter. Statistically, the estimates of

dorsal uterine curvature and endometrium thickness indicated that the involution was accomplished by 4 weeks PP. Measures of the ventral uterine curvature, uterine lumen, and total uterine horn diameter referred to that the involution was completed by the 5th week PP. Assessed cranial uterine curvature diameter referred to that the involution was ended by the 6th week PP.

Effect of calving season, calf-gender, ovarian cyclicity and days-in-milk on uterine parameters

Season of calving, newborn gender, and estrus activity and ovulation significantly ($p < 0.05$) affected the uterine involution estimates (Fig. 2, 3 and 4, respectively). Uterus appeared involuted faster in spring, then winter and lastly in autumn. Buffaloes gave birth of female newborn calves showed a clear ($p < 0.05$) lower ventral uterine curvature diameter (at the 2nd week) and uterine lumen diameter (at the 2nd to 4th week PP) as compared with those gave birth of male newborn calves. The early cyclic group showed a decrease in dorsal uterine

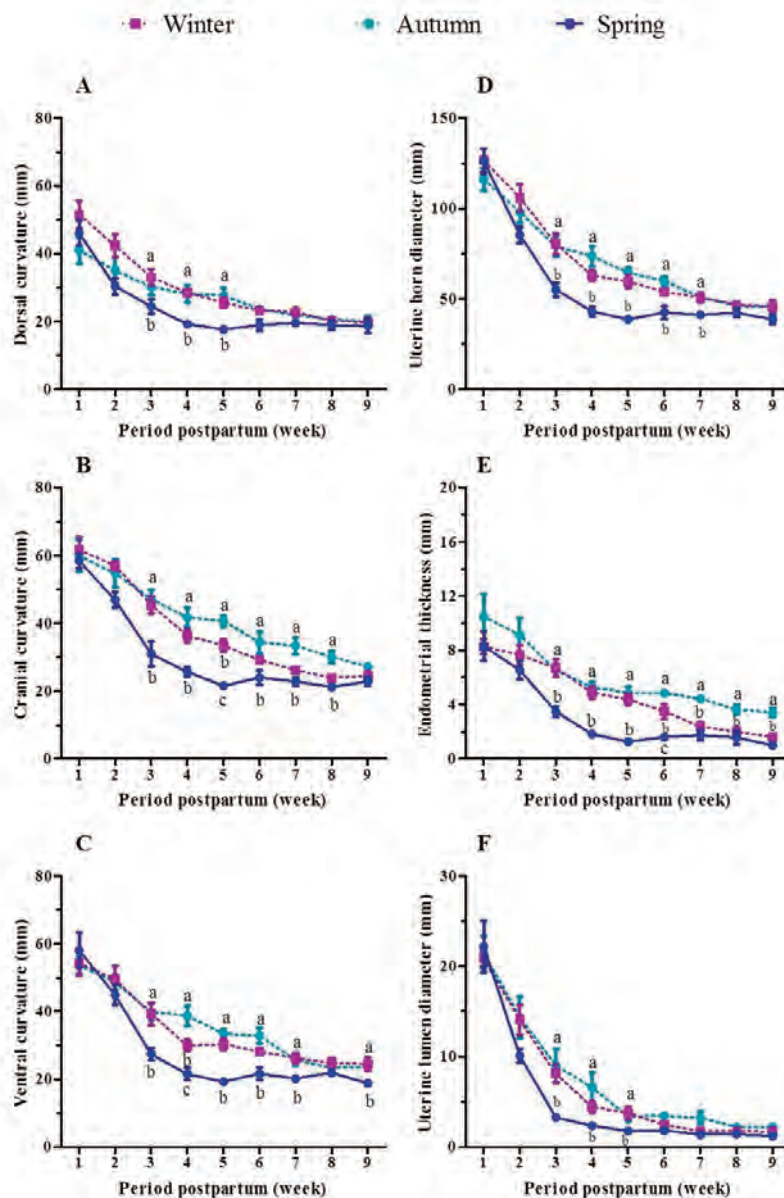


Fig. 2 Effect of calving season on postpartum uterine measures. Data with different letters were significantly different at $p < 0.05$.

curvature (from the 6th week PP), cranial uterine curvature (at the 6th week), endometrial thickness (at the 1st and 2nd week PP) and uterine lumen diameter (from the 7th week PP) as compared with the late cyclic group. Nevertheless, the effect of days-in-milk on uterine parameters was not statistically proven (Fig. 5).

Discussion

The interval from calving to clinically completed involution of the uterus in dairy buffaloes predominates the postpartum period and governs the future fertility of the animal, and this affected with many factors as the season of calving (El-Wishy 2007). The slow return of reproductive function during the puerperium period in dairy buffalos is a major limitation that affects the success of subsequent reproductive management programs, such as artificial insemination (Bell and Roberts, 2007). To maintain a calving interval of 13–14 months in buffaloes, successful breeding must take place within 85–115 days after calving (Jainudeen and Hafez, 2000). Therefore, the

uterine involution is considered a key factor that impacts the beginning of the voluntary waiting period and accordingly the time of postpartum anestrus (Opsomer et al., 2000). In the current data, ultrasonographic findings indicated that the uterine involution was completed at 4-6 weeks PP in normal parturient Egyptian buffaloes. Moreover, calving during the spring season of female new born calves improves uterine involution and enhances the buffalo's reproductive potential for the early postpartum breeding. Animals showed early cyclical activity had a faster uterine involution marked with a decrease in dorsal uterine curvature (from the 6th week PP), cranial uterine curvature (at the 6th week), endometrial thickness (at the 1st and 2nd week PP) and uterine lumen diameter (from the 7th week PP). This indicated the importance of adoption of ultrasound examination during puerperium in improving the reproductive efficiency of a dairy herd.

Involution is defined as the process by which the tubular genitalia shrink to reverse the hypertrophy that occurs as a result of pregnancy or to attain the volume, size, position, and reproductive capacity required for the next pregnancy

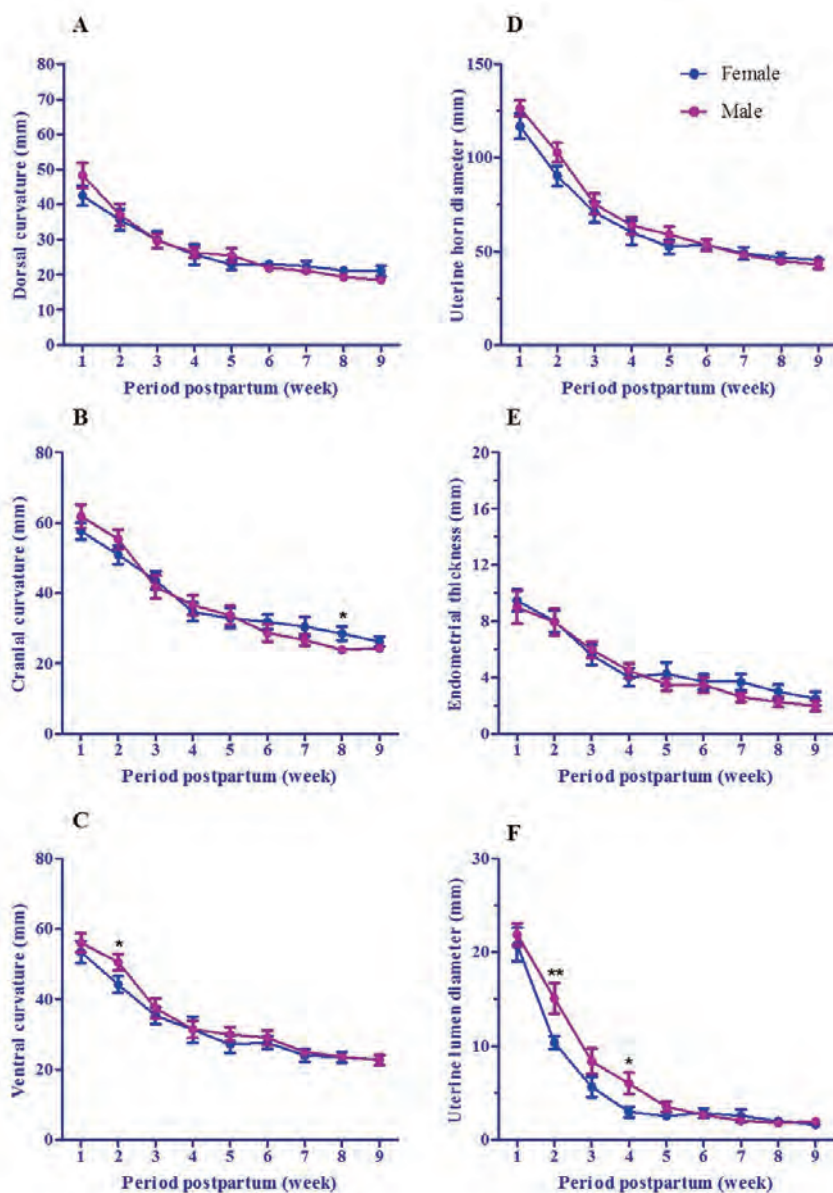


Fig. 3 Effect of newborn calf gender on postpartum uterine measures. * signified statistical differences between groups at the same time point at p< 0.05.

(Grunert and Birgel, 1998). In the current study, uterine horns parameters changed dramatically during the first month PP, and become gradual thereafter. The estimates of dorsal uterine curvature and endometrium thickness (at 4 weeks PP), ventral uterine curvature, uterine lumen and total uterine horn diameter (at 5 weeks PP) and cranial uterine curvature diameter (at 6 weeks PP) indicated that the uterus of buffaloes is completely involuted by the 6th week PP under Egyptian conditions. Alongside with our data, former studies in dairy buffaloes showed that the postpartum uterine involution interval was up to 35 days in 55% buffaloes and up to 50 days in 85% buffaloes, with a mean range was 34.30 ± 1.33 days (Qureshi et al., 1998). Chaudhry et al. (1990) showed that the meantime needed for the uterine involution to be completed was 28.37 ± 1.36 days, and in 92.68% of buffaloes and it was completed on 42 days PP. In cows, it was concluded that the uterine involution was completed at approximately Day 40 PP (Okano and Tomizuka, 1996). Also, El-Sabbagh (1993) verified that the bovine uterus returned to its pre-gravid state by about 25 days PP, though the entire involution process ended between 24 and 49 days after calving.

In the current study, the season profoundly impacted uter-

ine involution parameters. There was some evidences indicated that the season of calving may be more important than other factors affecting the resumption of postpartum uterine and ovarian activity in buffaloes (Sharma et al., 2006). These findings were in close agreement with the reports of Khan et al. (2011), and abayawansa et al. (2012), who reported that the uterine involution was complete in fewer days in the winter compared to the summer season. The obvious effect of season on uterine involution herein may be attributed to the effect of stress related to temperature fluctuation or feeding availability which increase the endometrial production of prostaglandin that augments endometrial vasoconstriction and myometrial contractions, and result in faster uterine tissue involution (Qureshi et al., 1999). Moreover, progesterone has been found to be lower after ovulation in non-breeding seasons compared to breeding seasons (Roy and Prakash, 2007). The season of calving, breed, age, the presence of uterine infection and difficult parturition may influence the time required for complete uterine involution (Bastidas, 1994). The breeding frequency (early uterine involution and ovarian cyclicity) in the buffalo was highest during winter and lowest in the summer (Shah et al., 1989).

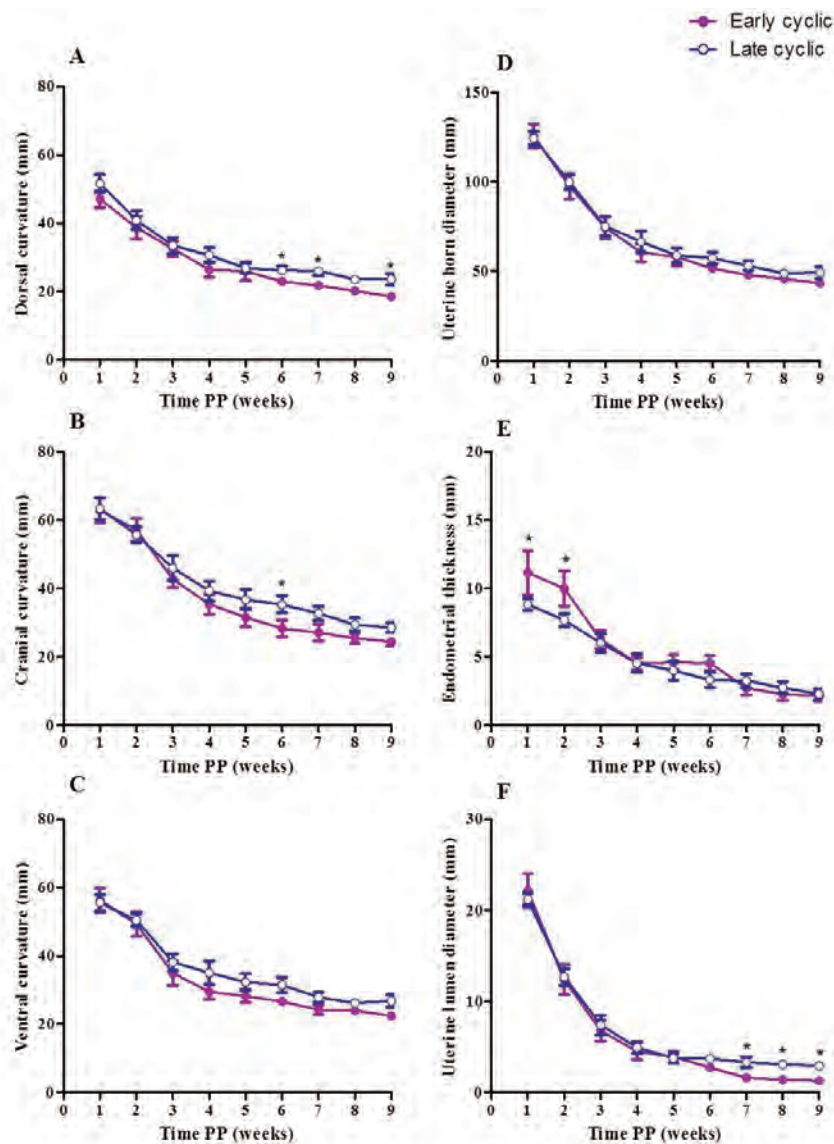


Fig. 4 Effect of ovarian cyclicity and ovulation on postpartum uterine measures. * signified statistical differences between groups at the same time point at $p < 0.05$.

In the existing study, the newborn gender had a significant effect on uterine parameters (cranial and ventral uterine curvature, and uterine lumen diameter) and consequently uterine involution. These findings agreed with former study declared that buffalo cows with female calves showed short time for uterine involution (El-Naggar, 2012). Bellows *et al.* (1982) concluded that dams nursing bull calves returned to uterine involution and to estrus more slowly than dams nursing heifer calves, as measured by day of the year when first estrus occurred. The influence of newborn gender on uterine involution could be either to the postpartum nutritional status of animals or hormonal balance required for normal uterine involution and resumption of ovarian activity (Bellows *et al.*, 1982).

Although the correlation between uterine involution and ovarian activity is not yet fully understood. The evidences show that this correlation is reciprocal and can be important for later fertility (Opsomer *et al.*, 1996). In the present study, the onset of cyclicity and ovulation marginally influenced uterine horn measures and accordingly the uterine involution. The ovarian activity is considered one of the main factors influencing the ability of the uterus to resist or eliminate bacterial in-

fections and consequently uterine involution (Jainudeen and Hafez, 2000). Stimulation of the ovarian activity, early during the post-calving period, essentially improved uterine clearance and involution in buffaloes (Kandiel *et al.*, 2013). This could be due to the influence of the follicular estradiol on the uterine endometrium and/or myometrium activity (Sheldon *et al.*, 2003). During the puerperium period, the hypothalamic-pituitary-ovarian axis releases cyclical secretions of gonadotropic and gonadal hormones which result in the first postpartum ovulation, and regular estrous cycle and, consequently help the uterus to involute quietly (Peter *et al.*, 2009). The massive postpartum release of prostaglandin F_{2α} by the endometrium largely influences the resumption of ovarian activity and uterine involution (Kindahl *et al.*, 1992).

In the current study, presented data verified that the day-in-milk has no effect on uterine involution. These findings were in accordance with former reports (El-Naggar, 2012; Hussein *et al.*, 2013). In cows, Harrison *et al.* (1990) found that there is no association between uterine involution, days to first ovulation and milk production. In the very few studies, the relationship between milk production and uterine involution has

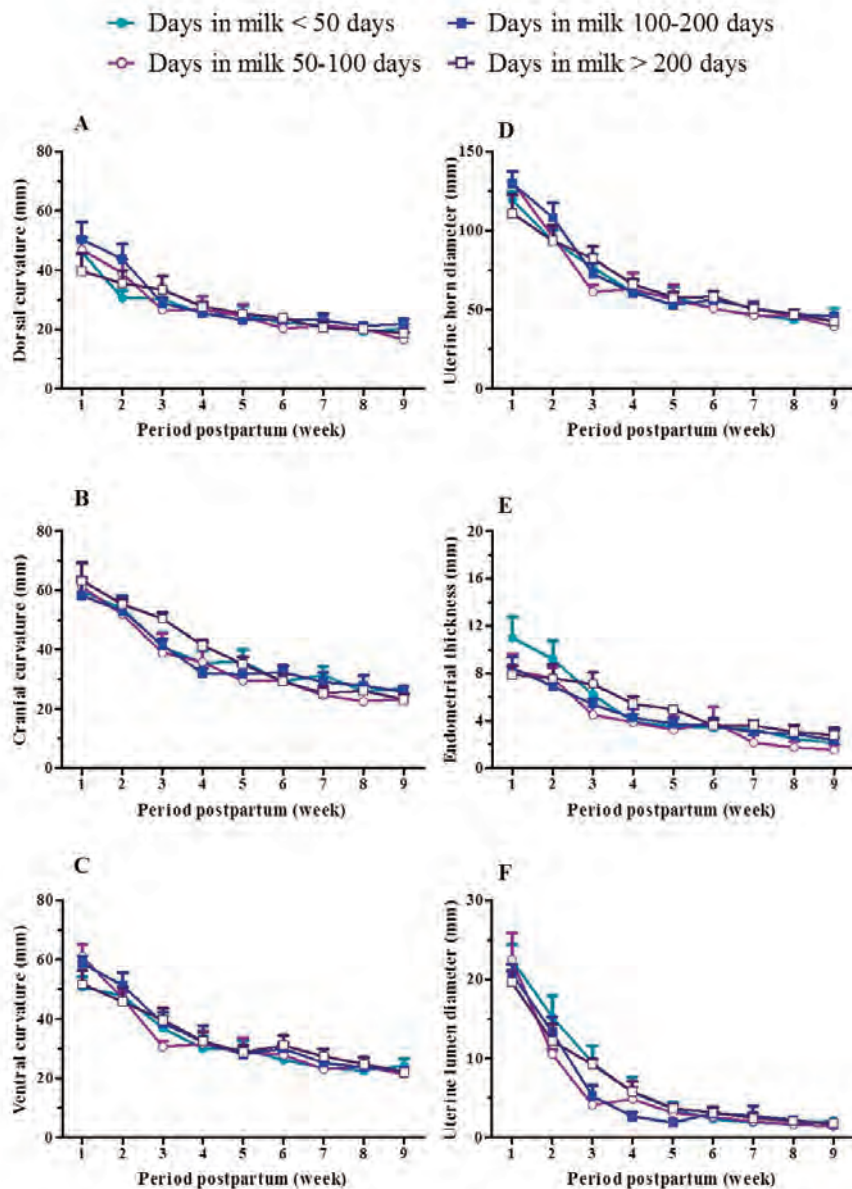


Fig. 5 Effect of days-in-milk period on postpartum uterine measures.

been verified, where a shorter PP period accompanied low milk yielding in buffaloes (El-Azab *et al.*, 1984; Bahga *et al.*, 1988). Increasing in milk yield per cow and severity of negative energy balance prompted late reproductive organs involution, reduction in estrus appearance and a decrease in pregnancy rates (Wathes *et al.*, 2007).

Conclusion

It could be concluded that the uterine involution is accomplished at 4-6 weeks PP in normal parturient Egyptian buffaloes. Moreover, calving during the spring season, female new born calves as well as early onset of cyclicity (before Day 30 PP) improve the uterine involution and enhance the buffalo's reproductive potential for the early postpartum breeding. Finally, ultrasound monitoring of genital organs during the postpartum period is of great practicality for the reproductive management of buffalo herds.

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