Indian Journal of Animal Sciences **82** (8): 859–862, August 2012

Seasonal variations in uterine involution in buffalo following normal puerperium

W D ABAYAWANSA[1], S PRABHAKAR[2], A K SINGH[3] and P S BRAR[4]

Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab 141 004 India

Received: 25 August 2010; Accepted: 27 April 2012

Key words: Buffalo, Season, Uterine body, Horn

The buffalo is considered a seasonal breeder and its reproductive performance is poor (Sharma *et al.* 2006). 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. However, gaps still exist for further clarification of basic reproductive activities during postpartum period in buffalo and deserve further investigation on this aspect using ultrasonographic assessment of reproductive organs (El-Wishy 2007). Seasonal variations in the relationship between postpartum reproductive parameters and climatic factors has been inadequately studied. Therefore, the present study was planned with the objective to study the effect of seasons on uterine involution in Murrah buffalo following normal puerperium.

Experimental animals, location, study period and general management: Healthy, lactating pleuriparous Murrah buffaloes (29), in their third to fifth parity and calved during December 2007 to May 2008 were selected at 2 organized dairy farms. Based on the season of calving, the animals were classified into 2 groups: animals calved during December 2007 to February 2008 were categorized as winter calved buffaloes (WCB; n=19); and those during April through May 2008 as summer calved buffaloes (SCB; n = 10). All the animals were maintained under standard managemental and hygienic conditions. They were fed *ad lib* with seasonal green fodder, wheat straw and concentrate mixture.

Per-rectal examination and trans-rectal ultrasonography: Per-rectal examination by palpation was commenced on day 7 postpartum and continued once a week until day 63 postpartum in both studies. Location of reproductive tract (anterior to cervix) was recorded.

Present address: [1]Animal Breeding Division (abayawd @yahoo.com), Department of Animal Production and Health, Gatambe, Peradeniya, Srilanka.

[2]Professor (sp1962 @yahoo.com); [3]Assistant Professor (assengar2001@yahoo.co.in); [4]Professor (parkashbrar @gmail.com), Department of Veterinary Gynaecology and Obstetrics, College of Veterinary Science.

Immediately after per-rectal examination of genitalia, a real time B-mode diagnostic ultrasound scanner equipped with a 7.5 MH linear array transducer was used to perform ultrasonography of the uterus. Diameter of uterine body and uterine horns (just anterior to the external bifurcation of the uterus) as well as echotexture of the uterine lumen were measured with the in-built-millimeter scale after freezing the ultrasound image and recorded.

Statistical analysis: The differences between WCB and SCB for location of uterus were tested using 2×2 analysis of variance (ANOVA) with significant interactions at 5% level. The significant interactions between WCB and SCB were tested using Duncan's multiple range test. Differences in mean (± SEM) diameter of uterine body and horns in WCB and SCB was subjected (Students 't' test) to the methods described previously (Zar 2008).

Location of the reproductive tract: The variation in the location of the anterior reproductive tract during early postpartum period is presented in Table 1 for winter and summer calving buffalo, respectively. One of the criteria used for the judgment of complete postpartum uterine involution in cattle and buffalo by per-rectal examination was the return of the uterus to a normal location in the pelvic or near pelvic

Table 1. Location of the anterior part of the reproductive tract of summer and winter calved buffaloes during first 35 days postpartum

Location	Group	Postpartum period (days)				
		7	14	21	28	35
AC	SCB	5	—	—	—	—
	WCB	19	12	4	1	—
AC/PC	SCB	2	—	—	—	—
	WCB	—	2	3	1	1
PC	SCB	3	10	10	10	10
	WCB	—	5	12	17	18

Data are presented as number of animals; AC, Abdominal cavity; PC, pelvic cavity; AC/PC, the most anterior part of the anterior tract in the abdominal cavity and the posterior part of the anterior tract in the pelvic cavity region (Chaudhry *et al.* 1987). At day 7 postpartum, the reproductive tract was located in the abdominal cavity in all WCB than in SCB (70%). However, on day 14, merely 36% of WCB had their reproductive tracts in or near the pelvic cavity as compared to SCB (100%). Thus, the average time taken by the uterus to reach pelvic cavity was significantly ($P<0.05$) more in WCB than in their summer counterparts (21.78 ± 6.30 days *vs* 11.9 ± 3.38 days). Ribeiro (2003) reported that at day 2–3 postpartum, the uterus was a large flabby sac lying in the abdominal cavity, at day 7, horns were cranial and ventral to the pelvic brim and the uterus returned to pelvic cavity by day 25 postpartum in buffalo. Summer stress further increased the endometrial production of prostaglandin which augmented endometrial vasoconstriction and myometrial contractions that might result in faster uterine tissue involution (Qureshi *et al.* 1999).

Variation in diameter of uterine body during first 63 days postpartum: Variation in the weekly diameter of uterine body with time during first 63 days postpartum has been shown in Table 2. In both the groups, the diameter of uterine body declined sharply during first 21 days and slowly during the next 14 days. There was no significant difference in the diameter of uterine body between 2 groups from day 7 to day 14 postpartum. However, from day 21 to day 49 postpartum, the diameter of uterine body was significantly ($P < 0.05$) less in SCB as compared to WCB. It was observed that the involution of the postpartum uterus in buffalo commenced soon after the fetal membranes were expelled (Campo *et al.* 2002). A marked decrease in uterine size during the early phase of involution is associated with concomitant discharge of uterine lochia, frequent peristaltic contractions of myometrium and reduction in the size of muscle fibers and endometrial edema (Usmani *et al.* 2001). Furthermore, increased plasma concentrations of acute phase proteins produced by hepatocytes in response to inflammation after parturition also reflect early uterine involution (Williams *et al.* 2007).

Table 2. Weekly averages of the diameter of uterine body in winter and summer calving buffalo during first 63 days postpartum (mean ± SEM)

Postpartum period (days)	Diameter of uterine body in winter calving buffalo (mm)	Diameter of uterine body in summer calving buffalo (mm)	Statistical significance (P value)
7	92.19 ± 2.65	86.37 ± 1.69	0.07
14	55.67 ± 2.67	58.10 ± 3.33	0.58
21	44.02 ± 1.98	34.14 ± 1.36	0.001
28	42.18 ± 2.07	35.19 ± 1.94	0.03
35	36.02 ± 1.36	26.98 ± 1.93	0.001
42	37.08 ± 2.46	29.33 ± 1.59	0.02
49	36.14 ± 1.70	27.38 ± 1.82	0.002
56	31.13 ± 1.93	26.45 ± 2.03	0.11
63	34.90 ± 2.71	27.43 ± 1.91	0.04

Variation in diameter of uterine horns during first 63 days postpartum: Based on ultrasonographic measurement, weekly average diameters of both the gravid and non-gravid uterine horns of winter and summer calving buffalo during first 63 days postpartum have been given in Table 3. In both groups, there was a rapid decline in diameter of gravid and non-gravid horns from day 7- to day 14-postpartum. Many previously reported values for the first week of postpartum in buffalo were higher to those of the present study (Usmani *et al.* 2001). This could be due to individual animal variation. However, the observations of the current study for the first week postpartum based on trans-rectal ultrasonography are in agreement with the reports by Lohan *et al.* (2004). The decline in diameter of both gravid and non-gravid horns was slow from day 14 to day 35 postpartum in the two sets of animals. From day 21–35 postpartum, the average diameter of gravid horn was significantly ($P < 0.05$) large in WCB than in SCB. Thermal stress acts as powerful stimulus for release of prostaglandin that decreases the number of days to involute the uterus (Prakash *et al.* 2005). The sensitivity of the species to increased environmental temperature plays an important role in delaying the time taken for involution of uterus (Barile 2005).

Table 3. Weekly variation in the mean diameter of gravid and non-gravid horns in winter and summer calving buffalo during first 63 days postpartum (mean ± SEM)

Postpartum	Gravid horn diameter (mm)		Significance	Non-gravid horn diameter (mm)		Significance
period (days)	WCB	SCB	(P value)	WCB	SCB	(P value)
7	95.99 ± 0.53	93.89 ± 1.13	0.07	87.87 ± 0.87	86.94 ± 1.22	0.54
14	48.07 ± 2.22	43.04 ± 2.36	0.16	40.11 ± 1.63	39.93 ± 1.91	0.95
21	38.16 ± 1.45	30.67 ± 2.42	0.01	34.36 ± 1.24	29.90 ± 1.94	0.05
28	33.81 ± 1.47	27.43 ± 1.93	0.02	31.34 ± 1.67	26.64 ± 2.01	0.09
35	28.72 ± 1.10	24.78 ± 1.42	0.04	27.34 ± 1.14	23.71 ± 1.39	0.06
42	26.56 ± 0.96	24.46 ± 1.40	0.22	25.46 ± 1.01	24.10 ± 1.46	0.44
49	24.98 ± 0.94	24.20 ± 1.55	0.65	24.33 ± 0.95	23.68 ± 1.58	0.71
56	23.37 ± 0.92	21.82 ± 0.80	0.28	22.76 ± 0.94	21.43 ± 0.88	0.36
63	23.27 ± 0.97	22.81 ± 0.83	0.76	22.96 ± 0.93	21.93 ± 0.86	0.48

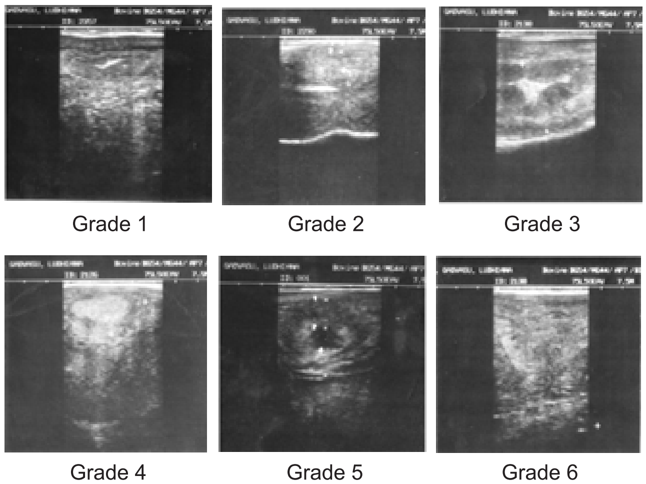

Fig. 1. Representative ultrasonographic changes for different grades of uterine lumen composition in postpartum buffalo.

Seasonal variation in the composition of uterine lumen during first 63 days postpartum: Ultrasonographic images of the uterine lumen showed that there was a high variation in the composition of the uterine lumen in both groups of buffaloes during early postpartum period that could be due to variation in the nature and quantity of fluid (blood, mucus, secretions, pus, etc.) and tissue debris or cells in the uterine environment as well as the immunity status of individual animal. Based on variations, the present study categorized the composition of uterine lumen in postpartum buffalo into 6 grades as detected by ultrasonography during first 63 days postpartum (Singh 2006), grade 1, fluid as central thin white line, grade 2, fluid as central thin black line/lines and increased echogenicity (mixture of dark and grey shades), grade 3 fluid as few echogenic spots within anechoic fluid (scattered few white spots in black lumen), grade 4, fluid as increased grey scale, grade 5, fluid as anechoic (black) and grade 6, fluid as increased echogenicity (mixture of white, black and grey shades; Fig. 1). At day 7 postpartum, both in winter and summer calving buffalo, the majority of animals had uterine ultrasonographic images with white, black and grey mixed in varying degrees. However, this appearance of uterine lumen in SCB continuously declined throughout the study period than in WCB. Theoretically, the ultrasound image of the lumen of a completely involuted uterus should be devoid of hyperechoic shades. However, the present study pointed out that even though the anterior reproductive tract reached the pelvic cavity early, size of uterine horns decreased and the postpartum ovarian activity resumed, majority of both winter and summer calving buffalo still had different ultrasonographic images with hyperechoic shades till day 63 postpartum. Kasimanickam *et al.* (2004) reported that grades with more hyperechoic spots were detrimental to the survival of sperm and embryos. This could be a possible reason for poor success rate of AI and long interval from calving to conception in buffalo as many previous studies reported. Therefore, the association between different uterine environments detected by trans-rectal ultrasonography and the conception rate in buffalo needs to be essentially evaluated.

It can thus be concluded that although, season of calving had no significant effect on the initial involution of uterus reduction in uterine body diameter was significantly affected after day 21 postpartum in summer and winter calving buffaloes. Further, composition of uterine lumen was highly variable and most of the animals in both the seasons had hyperechoic luminal contents in the uterine lumen at day 63 postpartum indicating incomplete uterine involution. Therefore, it is recommended to go for early therapeutic intervention for early involution of uterus and hence early onset of ovarian activity.

SUMMARY

The present study was conducted on healthy, lactating pleuriparous Murrah buffaloes (29), in their third to fifth parity and calved during December 2007 to May 2008. The animals were selected at 2 organized dairy farms (Mattewara and Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana). On the basis of season of calving they were divided into 2 groups, viz. winter calving buffaloes and summer calving buffaloes. A rapid reduction of uterine body was observed during first 21 days postpartum in both winter and summer calving buffalo. After day 21 postpartum, reduction in uterine body diameter was significantly slower in winter calving buffalo. The diameters of uterine horns rapidly declined during first 14 days postpartum and slowly declined from day 21 to day 35 postpartum in both groups of buffaloes. Ultrasonographic images of contents of postpartum uterine lumen were highly variable within and between animals during the study period. Based on echotexture of the content of uterine lumen 6 grades were identified. Even though uterine tracts reached the pelvic cavity early, the majority of animals in both the groups still had hyperechoic materials with varying amounts at day 63 postpartum indicating incomplete uterine involution. It can thus be concluded that although, season of calving had no significant effect on the initial involution of uterus, reduction in uterine body diameter was significantly affected after day 21 postpartum in both the groups and needs further evaluation with respect to conception.

REFERENCES

Barile V L. 2005. Improving reproductive efficiency in female buffaloes. *Livestock Production Science* **92**: 183–94.

Campo E, Alonso J C, Hincapie J J, Garcia F O and Fernandez O. 2002. Seasonal influence on uterine involution and postpartum ovarian activity in river buffaloes. *Bubalus Bubalis* **8**: 59–63.

Chaudhry M A, Ahmad M and Khan N U. 1987. Postpartum involution of the cervix and uterus in Nili-Ravi buffaloes. *Buffalo Journal* **3**: 87–92.

El-Wishy A B. 2007. Review. The postpartum buffalo 1.

Endocrinological changes and uterine involution. *Animal Reproduction Science* **97**: 201-15.

Kasimanickam R, Duffield T E, Foster R A, Gartley C L, Leslie K E, Walton J S and Johnson W H. 2004. Endometrial cytology and ultrasonography for the detection of subclinical endometritis in postpartum dairy cows. *Theriogenology* **62**: 9–23.

Lohan I S, Malik R K and Kaker M L. 2004. Uterine involution and ovarian follicular growth during early postpartum period of Murrah buffaloes (*Bubalus bubalis*). *Asian-Australian Journal of Animal Science* **17**: 313–16.

Prakash B S, Sarkar M, Vijay Paul, Mishra D P, Mishra A and Meyer H H D. 2005. Postpartum endocrinology and prospects for fertility improvement in the lactating riverine buffalo (*Bubalus bubalis*) and yak (*Poephagus grunniens* L.). *Livestock Production Science* **98**: 13–23.

Qureshi M S, Safi G M, Dhanani J and Kaka I. 1999. Reproductive performance of dairy buffaloes in the northern hilly areas of Pakistan. *Buffalo Journal* **15**: 391–96.

Ribeiro H F L, Vale W G, Andrade V J and Marques A P. 2003. Environmental effect on the ovarian postpartum activity in the buffaloes raised in low Amazon region, Brazil. *Buffalo Journal* **19**: 311–21.

Sharma R K, Singh I and Singh J K. 2006. A comparative analysis of estrus induction/synchronization protocols in buffaloes, role of follicular dynamics. *Proceedings of 22nd Annual convention of ISSAR and National Symposium*. pp. 48–60. Mhow.

Singh J. 2006. 'Studies on the reliability of sonographic examination of buffalo genitalia for determining the incidence of reproductive abnormalities and changes in the ovarian structures across estrus cycle.' M.V.Sc. thesis, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana.

Usmani R H, Ahmad N and Parveen S. 2001. Effect of subclinical uterine infection on uterine involution estrus activity and fertility in postpartum buffaloes. *Theriogenology* **55**: 563–71.

Williams E J, Fischer D P, Noakes D E, England G C W, Rycroft A, Dobson H and Sheldon I M. 2007. The relationship between uterine pathogen growth density and ovarian function in the postpartum dairy cow. *Theriogenology* **68**: 549–59.

Zar J H. 2008. *Biostatistical Analysis*. 4th edn. Dorling Kindersley Pvt. Ltd. Pearson education. Delhi, India.