Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 6 Special issue [1] 2017: 38-45

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD
Global Impact Factor 0.533
Universal Impact Factor 0.9804
NAAS Rating 4.95

FULL LENGTH ARTICLE **OPEN ACCESS**

Ultrasonographic Evaluation Of Uterine Involution In Postpartum Mehsana Buffaloes

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ABSTRACT
Eighteen pluriparous recently calved Mehsana buffaloes were randomly selected to study the effect of PGF2α on uterine involution by using real time ultrasonography consisting of trans-rectal probe of 7.5 MHz. The animals were allotted to three groups; Group-I (control), consisted of recently calved buffaloes; Group-II also included recently calved buffaloes but received intramuscular injection of Iliren 5 ml on 5th day postpartum, whereas, Group-III had animals completed 2 months postpartum without treatment. The ultrasonic evaluation of uterus and cervix was done at 2 days interval from 5th day onwards till 21st day postpartum, and subsequently followed at five days interval up to 40th day following parturition in Group-I and II animals. The ultrasonography of cervix, uterus, middle uterine artery and caruncles during the period of uterine involution depicted the images of different echogenicity. The echogenicity of involuting cervix was recorded as hyper echoic wall with anechoic lumen; similarly uterus was imaged as hyper echoic wall with anechoic lumen having hypo echoic spots. The diameter and thickness of wall of cervix reduced during the period from 5-40 days postpartum in Group-I and II, but at 40th day of postpartum it remains significantly ($p<0.05$) higher in these groups in comparison to Group-III. Similarly, the gravid and non-gravid uterus in Group-I and II reduced in diameter and thickness in its wall, however, the differences were non-significant for diameter while thickness was demarkably greater on 5th and 30th day of postpartum for the gravid horn. On the other hand, the non-gravid horn reduced in thickness at faster rate in Group-I on 5th, 7th, 9th, 11th, 13th, 15th and 17th day of postpartum. In comparison to Group-III, the uterine diameter was significantly ($p<0.05$) less and uterine thickness was greater at 40th day of postpartum in buffaloes of Group-I and II. The middle uterine artery and caruncles did not show any demarkable change from day 5 to 11 postpartum in Group-I and II except the caruncular width which remained more in buffaloes treated with Iliren. The findings envisage that the treatment of Mehsana buffaloes with Iliren advocated intramuscular at the dose rate of 25 mg on 5th day of postpartum didn't enhance the rate of uterine involution.
Kea words: Ultrasonography, Postpartum, Mehsana buffalos, Uterine Involution.

Received 18.07.2017 Revised 02.08.2017 Accepted 25.08. 2017

INTRODUCTION

The prolonged interval between two consecutive parturitions is the cause for the poor reproductive efficiency and economic losses of buffalo husbandry (Yotov *et al.,* 2016). The prerequisites for successful reproductive performance in buffaloes are rapid uterine involution and early resumption of the ovarian activity after parturition (Perera, 2011). Uterine involution occurs in a decreasing logarithmic scale with the greatest change occurring during the first few days after parturition (Noakes *et al.*, 2009). Ultrasonography is an alternative method for detection of dynamic changes in the reproductive tract after the parturition, which allows more accurate, quick and safe examination of the reproductive system (Yindee *et al.,* 2007). The method is widely used for control of uterine involution and ovarian function during the postpartum period in cattle and buffaloes (Melendez *et al.,* 2004; Kocamuftuoglu and Vural, 2008).

Various experiments for improvement of the uterine involution and resumption of the ovarian activity by hormonal treatment during the early postpartum have been performed in buffaloes (Iqbal *et al.,* 2003). An accelerated uterine involution by prostaglandin and oxytocin treatment immediately after calving was observed by Khatri *et al.,* (2013). According to Kandiel *et al.,* (2013) the inclusion of GnRH-PGF2α-GnRH

protocol on postpartum day 21 conducted to rapid uterine involution and ovarian activity resumption. Hence, this study was planned to evaluate the effect of PGF2α on uterine involution in postpartum Mehsana buffaloes.

MATERIALS AND METHODS

This study was conducted at Livestock Research Station, Sardarkrushinagar Dantiwada Agricultural University (SDAU), Sardarkrushinagar, Gujarat. A total of Eighteen recently calved Mehsana buffaloes of more than one parity with a history of normal calving were selected. All 18 were divided in three groups, each comprising of 6 animals. Group- I were untreated, Group-II given hormonal treatment (PGF2α) at 5th day postpartum and Group-III included normal non-pregnant buffaloes which had Completed more than 2 months of postpartum period with normal parturition and puperial period.

Ultrasonographic evaluation of uterus and cervix of both Group-I and Group-II was carried out from 5th day postpartum on wards till 21st day at 2 days intervals, afterwards at 25th, 30th and 40th days postpartum using 7.5 MHz linear array transrectal transducer. Group-II buffaloes were treated with 25mg of 'Tiaprost trometamol' (Inj. Iliren 5 ml, Intervet, Germany) through intramuscular route on 5th day of calving. The uterus and cervix of Group-III buffaloes were screened ultrasonographically and the measurements were compared with the ultrasonographic findings of 40 days postpartum observations of Group-I and Group-II buffaloes.

The Ultrasonography was performed using a real-time B mode ultrasound scanner (Titan, Sonosite Ltd, Hitchin, UK) equipped with a 7.5 MHz linear array transducer designed for intra-rectal placement. The transducer was positioned dorsally and parallal above the cervix as well as uterus and relevant measurements of diameter and thickness were taken. Approximately 10cm ahead from the gross bifurcation, the uterine diameter and thickness of uterine wall were measured as per the guide lines of Melendez *et al*. (2004), for both the involuting gravid and non-gravid horns. Two diameters of the previously gravid and non gravid uterine horns were evaluated. The first measurement was from serosa to serosa (A to B) to obtain the gross diameter of the uterine horn (Sheldon and Dobson, 2000). The second measurement was from mucosa to mucosa (C to D) to obtain the lumen diameter of the uterine horn (Melendez *et al*., 2004) (Plate-I). The difference between the first and the second measurements were divided to half to estimate the thickness of the uterine wall, i.e. thickness of uterine wall = AB-CD/2. The diameters of middle uterine arteries of either side were measured by keeping the transducer transverse to them (Plate-II). Caruncles located approximately at the same position of scanning for uterus were measured for its length and width (Plate-III).

Statistical Analysis:

For this mean and standard error were calculated. The 'T-test' was used to compare the differences in means between groups and Multiple Dunckon Range Test was used to compare the differences in means within groups, if any, using standard statistical procedure (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSIONS

Cervical Diameter and Thickness of Cervical Wall:

A reducing trend in the cervical diameter and wall thickness from 5th to 40th day postpartum was found in both the groups. However, the reduction in cervical diameter was faster up to 15th day postpartum in both the groups, whereas, very marginal difference in the cervical diameter was observed afterwards. A very negligible reduction in cervical diameter was seen after 25th day postpartum in both the groups of buffaloes. However, reduction in the cervical wall thickness from 5th to 40th day postpartum was significantly ($P<0.05$) faster in untreated animals as compared to treated buffaloes with PGF2α (Table-I).

Though, the buffaloes were selected randomly from the farm, the mean cervical wall thickness of the animals selected for the treatment groups was observed to be significantly lower from 5th day postpartum onwards and the difference was maintained up to the 19th day postpartum. This may be the probable reason for significantly ($p<0.05$) faster involution of cervix in treated group and may not be the effect of PGF2α. Further, from 21st day postpartum onwards, the difference between the two groups was non-significant. After 25 days postpartum, the cervix was greater than the gravid uterus. It could be concluded that PGF2α did not affect significantly on the process of cervical involution.

The present findings of both the groups of buffaloes collaborated well with Morrow *et al.* (1969) who reported that the size of the gravid horn and cervix were equal at 18 and 22 days postpartum which remained approximately of the same size for 3 to 5 days in dairy cattle. After 25 days postpartum the cervix was larger than the gravid horn. Roberts (1971) who found that prior to day 20 postpartum the diameter of cervix was smaller than the horn and after day 22-25 the cervical diameter was greater than horn diameter in cows. Sinha *et al*. (2002) found there was no significant difference with respect to reduction in diameter of cervix between Dinoprost treated and control group of crossbred cows at day 10,

20, 30, and 40 postpartum, which is in close agreement with the present findings. On contrary to the present study, Dagli (1997) reported that the prostaglandin treatment hastened involution process and completed 5 days earlier than the control group of cows.

In control and treatment group of animals, the cervical diameter and cervical wall thickness were significantly (P<0.05) higher than that of normal non-pregnant buffaloes. The sonographical observations of cervical diameter and wall thickness of cervical wall indicated that at cellular level yet the involution of cervix is not completed and it may require few more days to be at par to that of normal non-pregnant buffaloes.

Diameter and Thickness of Gravid Uterine Horn:

There was a reducing trend in the diameter and thickness of the gravid uterine horns from 5th to 40th day postpartum in both the groups. The statistical analysis revealed that from 5th day onwards up to 40th day postpartum there were non significant differences between the groups for diameter and thickness of gravid uterine horn, however, on 5th and 30th day postpartum significant (P<0.05) differences were observed for gravid uterine horn thickness. The diameters of gravid uterine horns which measured from 5th to 15th days after calving revealed a rapid decline in diameter comparison to the remain involutory period in both the groups (Table-II). The observations of the present study did not reveal any significant effect of PGF2α treatment. In control and treatment group of buffaloes, the uterine diameter and horn thickness were significantly (P<0.05) higher than the uterine diameter of normal non-pregnant buffaloes. The sonographical observations of uterine diameter and thickness of uterine wall indicated that yet the involution of uterus may require few more days to be at par to that of normal non-pregnant buffaloes.

The findings of the present study observed in both the groups of buffaloes collaborated well with Roberts (1971) who opined that between days 40-50 little change in uterine regression was occurred in cows. Melendez *et al.* (2004) studied the ultrasonographic uterine findings of gravid uterine horn of Holstein cows with acute puerperal metritis subsequently treated with 2 doses of PGF2α, at 8 day postpartum and stated that PGF2α having a positive effect on the process of uterine involution. The ultrasonographic uterine diameters and wall thickness in prostaglandin treated primiparous and multiparous cows were 49.2 ± 2.6 mm and 24.7 ± 2.2 mm, 52.3 ± 2.2 mm and 21.7 ± 1.9 mm, respectively. Jadhav (2005) studied the postpartum uterine involution by ultrasonography in Gir and Cross breed cows from the tip of horn and have stated that a reducing trend in the average diameter of gravid uterine horn was observed from second to fifth week with maximum reduction up to the end of fourth week in all the groups. Kocamuftuoglu and Vural (2008) observed that the diameter of gravid uterine horns on 20th, 30th and 40th day postpartum were 3.707 ± 0.614, 3.052 ± 0.698 & 2.716 ± 0.670 cm respectively in normal dairy cows. In contrary to present study, Iqbal *et al.* (2003), Tiwari *et al.* (2004), Hassan *et al.* (2007) in buffaloes who carried out the study by per-rectal palpation and reported that use of PGF2α hastened the uterine involution.

Diameter and Thickness of Non-Gravid Uterine Horn:

There was a reducing pattern in the diameter and thickness of the non-gravid uterine horns from 5th to 40th day postpartum in both the groups. There was no any significant difference in the diameter of non-gravid uterine horns of both the group of animals. However, reduction in the diameter of non-gravid uterine horn was faster in treated than untreated buffaloes. The reduction in the thickness of non-gravid uterine horn was significantly (P<0.05) faster in treated on 5th, 7th, 9th, 11th, 13th, 15th and 17th day postpartum (Table-III). Though, the animals were selected randomly from the farm, the measurement of wall thickness of the animals selected for the treatment groups was lower from 5th postpartum day onwards and the difference was static up to the 17th postpartum day. This may be the probable explanation for the faster reduction of thickness of non-gravid horn and may not be the effect of PGF2α. However, from 19th day postpartum onwards they differ non-significantly between the groups. This suggested that there was non-significant effect of PGF2α treatment on non-gravid uterine horn involution. In untreated and treated group, uterine diameter was significantly (P<0.05) lower than that of normal non-pregnant buffaloes, whereas, the uterine thickness of control and treatment group was significantly (P<0.05) higher than non-pregnant buffaloes. The sonographical observations of uterine diameter and thickness of uterine wall indicated that complete involution of uterus require few more days.

Jadhav (2005) studied the postpartum uterine involution by ultrasonography in Gir and Cross breed cows from the tip of horn and have stated that a reducing trend in the average diameter of non-gravid uterine horn was observed from second to fifth week with maximum reduction up to the end of fourth week in all the groups. Kocamuftuoglu and Vural (2008) recorded the diameter of previously non-gravid uterine horns on 20th, 30th and 40th day postpartum were 3.025 ± 0.650, 2.870 ± 0.742 & 2.648 ± 0.611cm, respectively in normal dairy cows.

In contrary to present study, Dagli (1997), Sheshappa *et al.* (2002) in cows, Sinha *et al*. (2002) in crossbred cows, Iqbal *et al.* (2003), Tiwari *et al.* (2004) in buffaloes, Patel (2008) in Holstein Friesian cows, Hassan *et al.* (2007) in buffaloes who carried out the study by per-rectal palpation and reported that use of PGF2α hastened the uterine involution.

Diameter of Middle Uterine Artery:

There was a reducing trend in the diameter of both right and left middle uterine artery during early postpartum in both the groups. The reduction in the diameter of middle uterine artery from 5th to 11th day postpartum was non-significant between the groups (Table-IV). The present study revealed that there was no effect of PGF2α treatment on diameter of middle uterine artery during early postpartum period.

Length and Width of Caruncles:

The reduction in length and width of caruncles from 5th to 11th day postpartum was non-significant except width of caruncles at 5th day postpartum which was significantly (P<0.05) faster in untreated than treated group of animals (Table-IV). Up to 11th day postpartum diminution in length and width of caruncles could be demonstrated. On the basis of recorded measurements of caruncles of both the group of buffaloes, there was no significant effect of PGF2α treatment on involution of caruncles.

Table:I- Ultrasonographic measurements (Mean ± SE) of cervical diameter and wall thickness(cm) in postpartum Mehsana buffaloes.

Cervical diameter (cm)				Cervical Wall Thickness(cm)			
Days of Post partum	Groups		Significance between groups (Cal.'t'Value)	Days of Post partum	Groups		Significance between groups (Cal.'t'Value)
	G-I (n=6)	G-II (n=6)			G-I (n=6)	G-II (n=6)	
5th	3.15 ± 0.19[b]	2.61 ± 0.02[a]	2.95*	**5th**	0.82 ± 0.03[b]	0.44 ± 0.01[a]	5.58*
7th	2.78 ± 0.08	2.52 ± 0.01	2.12 NS	**7th**	0.68 ± 0.02[b]	0.39 ± 0.01[a]	4.64*
9th	2.45 ± 0.02	2.46 ± 0.01	0.19 NS	**9th**	0.58 ± 0.01[b]	0.35 ± 0.01[a]	4.62*
11th	2.27 ± 0.01[a]	2.39 ± 0.01[b]	2.26*	**11th**	0.48 ± 0.02[b]	0.32 ± 0.01[a]	2.95*
13th	2.19 ± 0.01	2.31 ± 0.01	1.83 NS	**13th**	0.42 ± 0.01[b]	0.31 ± 0.01[a]	2.41*
15th	1.99 ± 0.02[a]	2.19 ± 0.02[b]	2.41*	**15th**	0.37 ± 0.01[b]	0.29 ± 0.01[a]	2.97*
17th	1.88 ± 0.01[a]	2.08 ± 0.02[b]	3.34*	**17th**	0.35 ± 0.01[b]	0.28 ± 0.01[a]	2.77*
19th	1.84 ± 0.01	1.97 ± 0.02	1.94 NS	**19th**	0.33 ± 0.01[b]	0.26 ± 0.01[a]	2.85*
21st	1.81 ± 0.01	1.88 ± 0.01	1.60 NS	**21st**	0.30 ± 0.01	0.25 ± 0.01	2.04 NS
25th	1.79 ± 0.01	1.82 ± 0.01	1.17 NS	**25th**	0.27 ± 0.01	0.24 ± 0.01	2.05 NS
30th	1.75 ± 0.01	1.78 ± 0.01	1.29 NS	**30th**	0.23 ± 0.01	0.22 ± 0.01	1.19 NS
40th	1.64 ± 0.01	1.69 ± 0.01	0.98 NS	**40th**	0.20 ± 0.01	0.19 ± 0.01	0.85 NS

- Means bearing different superscripts between the rows differ significantly (P<0.05).
- NS: Non-significant * Significance at 5%

Table:II- Ultrasonographic measurements (Mean ± SE) of Gravid Uterine horn diameter and wall thickness(cm) in postpartum Mehsana buffaloes.

Gravid Uterine Horn diameter (cm)				Gravid Uterine Wall Thickness (cm)			
Days of Post partum	Groups		Significance between groups (Cal.'t'Value)	Days of Post partum	Groups		Significance between groups (Cal.'t'Value)
	G-I (n=6)	G-II (n=6)			G-I (n=6)	G-II (n=6)	
5th	3.27 ± 0.22	3.29 ± 0.01	0.13 NS	**5th**	0.99 ± 0.02b	0.81 ± 0.01a	2.93*
7th	2.96 ± 0.05	3.00 ± 0.06	0.34 NS	**7th**	0.79 ± 0.01	0.75 ± 0.01	0.90 NS
9th	2.74 ± 0.01	2.86 ± 0.07	1.07 NS	**9th**	0.70 ± 0.01	0.69 ± 0.01	0.05 NS
11th	2.59 ± 0.04	2.61 ± 0.03	0.17 NS	**11th**	0.63 ± 0.01	0.61 ± 0.01	1.11 NS
13th	2.42 ± 0.09	2.27 ± 0.02	1.15 NS	**13th**	0.60 ± 0.01	0.58 ± 0.01	0.82 NS
15th	2.17 ± 0.03	2.10 ± 0.03	0.80 NS	**15th**	0.55 ± 0.01	0.54 ± 0.01	0.24 NS
17th	1.99 ± 0.02	1.98 ± 0.03	0.18 NS	**17th**	0.53 ± 0.01	0.48± 0.01	2.11 NS
19th	1.92 ± 0.01	1.86 ± 0.02	0.85 NS	**19th**	0.46 ± 0.01	0.45 ± 0.01	0.40 NS
21st	1.83 ± 0.01	1.79 ± 0.01	0.92 NS	**21st**	0.44 ± 0.01	0.40 ± 0.01	1.24 NS
25th	1.79 ± 0.01	1.75 ± 0.01	0.88 NS	**25th**	0.38 ± 0.01	0.33 ± 0.01	0.04 NS
30th	1.72 ± 0.01	1.66 ± 0.01	1.09 NS	**30th**	0.33 ± 0.01b	0.29 ± 0.01a	2.64*
40th	1.60 ± 0.01	1.55 ± 0.01	1.17 NS	**40th**	0.25 ± 0.01	0.23 ± 0.01	1.17 NS

- Means bearing different superscripts between the rows differ significantly (P<0.05).
- NS: Non-significant * Significance at 5%

Table:III- Ultrasonographic measurements (Mean ± SE) of Non-Gravid Uterine horn diameter and wall thickness(cm) in postpartum Mehsana buffaloes.

Non-Gravid Uterine Horn diameter (cm)				Non-Gravid Uterine Wall Thickness (cm)			
Days of Post partum	Groups		Significance between groups (Cal.'t'Value)	Days of Post partum	Groups		Significance between groups (Cal.'t'Value)
	G-I (n=6)	G-II (n=6)			G-I (n=6)	G-II (n=6)	
5th	2.33 ± 0.12	2.24 ± 0.04	0.72NS	**5th**	0.65 ± 0.02b	0.52 ± 0.03a	3.68*
7th	2.11 ± 0.12	2.10 ± 0.05	0.22 NS	**7th**	0.58 ± 0.01b	0.46 ± 0.02a	4.09*
9th	1.97 ± 0.07	1.95 ± 0.02	0.34 NS	**9th**	0.53 ± 0.01b	0.42 ± 0.02a	5.44*
11th	1.87 ± 0.04	1.89 ± 0.02	0.70 NS	**11th**	0.51 ± 0.01b	0.41 ± 0.02a	4.91*
13th	1.80 ± 0.04	1.84 ± 0.02	0.88 NS	**13th**	0.47 ± 0.01b	0.39 ± 0.02a	3.37*
15th	1.74 ± 0.04	1.74 ± 0.03	0.06 NS	**15th**	0.44 ± 0.01b	0.35 ± 0.01a	6.65*

17th	1.71 ± 0.04	1.71 ± 0.03	0.06 NS	**17th**	0.42 ± 0.01b	0.31± 0.02a	4.97*
19th	1.66 ± 0.04	1.66 ± 0.02	0.10 NS	**19th**	0.35 ± 0.02	0.30 ± 0.01	1.84 NS
21st	1.59 ± 0.04	1.62 ± 0.02	0.64 NS	**21st**	0.32 ± 0.02	0.29 ± 0.01	1.41 NS
25th	1.59 ± 0.04	1.58 ± 0.02	0.40 NS	**25th**	0.29 ± 0.02	0.27 ± 0.01	1.15 NS
30th	1.52 ± 0.03	1.52 ± 0.01	0.02 NS	**30th**	0.28 ± 0.02	0.24 ± 0.01	1.55 NS
40th	1.43 ± 0.04	1.40 ± 0.05	0.45 NS	**40th**	0.23 ± 0.02	0.21 ± 0.02	0.94 NS

- Means bearing different superscripts between the rows differ significantly (P<0.05).
- NS: Non-significant * Significance at 5%

Table:IV- Ultrasonographic measurements (Mean ± SE) length and width of caruncles (cm) in postpartum Mehsana buffaloes.

Days of Post partum	Diameter of Right Middle Uterine Artery		Significance between groups (Cal.'t'Value)	Diameter of left Middle Uterine Artery		Significance between groups (Cal.'t' Value)	Length of Caruncles		Significance between groups (Cal.'t'Value)	Width of Caruncles		Significance between groups (Cal.'t' Value)
	G-I (n=6)	G-II (n=6)		G-I (n=6)	G-II (n=6)		G-I (n=6)	G-II (n=6)		G-I (n=6)	G-II (n=6)	
5th	1.71 ± 0.08	1.93 ± 0.08	1.87 NS	1.60 ± 0.07	1.63 ± 0.06	0.31 NS	2.99 ± 0.21	3.74 ± 0.55	1.27 NS	1.18 ± 0.11a	1.76 ± 0.19b	2.74*
7th	1.57 ± 0.05	1.69 ± 0.09	1.22 NS	1.46 ± 0.06	1.57 ± 0.04	1.44 NS	2.38 ± 0.12	2.55 ± 0.17	0.83 NS	1.00 ± 0.10	1.32 ± 0.14	1.87 NS
9th	1.44 ± 0.05	1.60 ± 0.09	1.62 NS	1.37 ± 0.05	1.50 ± 0.04	2.10 NS	2.16 ± 0.11	2.76 ± 0.38	1.52 NS	1.02 ± 0.15	1.34 ± 0.14	1.57 NS
11th	1.71 ± 0.08	1.93 ± 0.08	1.87 NS	1.60 ± 0.07	1.63 ± 0.06	0.31 NS	1.91 ± 0.14	1.92 ± 0.18	0.02 NS	0.90 ± 0.10	0.93 ± 0.07	0.27 NS

- Means bearing different superscripts between the rows differ significantly (P<0.05).
- NS: Non-significant * Significance at 5%

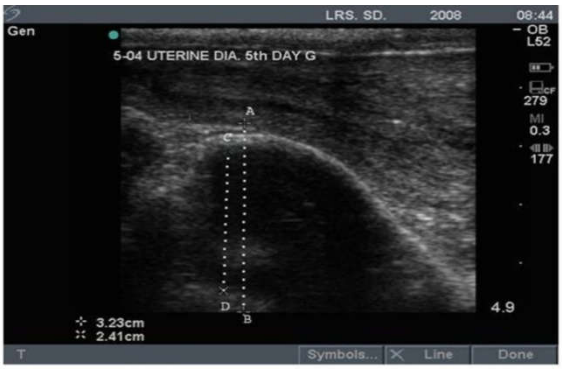

Plate-I: Ultrasonic measurements of uterus

Thickness of uterine wall = $\frac{AB - CD}{2}$

Plate-II: Ultrasonic measurements of Middle uterine artery

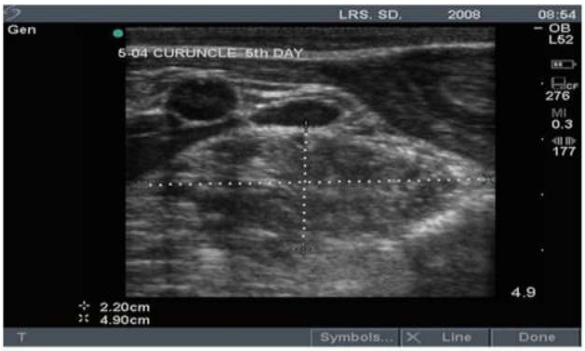

Plate-III: Ultrasonic measurements of Caruncles

CONCLUSION

The sonographic images of the uterus and cervix during the period of involution appear to be hyperechoic and hypoechoic with regard to the wall and lumen of these organs, respectively, whereas, the uterine lumen was anechoic in non-pregnant diestrus buffalo. The caruncles depict bright hyperechoic image with hypoechoic spots, while, middle uterine artery as anechoic. The $PGF_2\alpha$ treatment on day 5 postpartum did not enhance the uterine involution as the diameter and thickness of uterus and cervix remain almost similar in treatment and untreated groups of buffaloes. Though, morphologically the uterine involution was completed by 40 days, the cellular level repair was yet to be completed.

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CITATION OF THIS ARTICLE

S. S. Parikh, B. N. Suthar, T. V. Sutaria, B. D. Savaliya and R. B. Makwana.Ultrasonographic Evaluation Of Uterine Involution In Postpartum Mehsana Buffaloes. Bull. Env. Pharmacol. Life Sci., Vol 6 Special issue 1, 2017: 38-45