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Original Research Article

TRANSRECTAL ULTRASONOGRAPHY AND RECTAL PALPATION FOR JUDGING UTERINE AND CERVICAL INVOLUTIONS IN BUFFALO: A COMPARATIVE STUDY

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Abstract: Uterine and cervical involutions were judged by transrectal ultrasonography (US) versus rectal palpations (RP) in buffaloes (*n = 26*). The diameters of the pregravid uterine horn (PGUHD) and cervix (CvD) were estimated by both transrectal US and RP every three days until gross uterine or cervical involution. Also, the US-measured PGUHD and CvD were recorded on the day of gross uterine or cervical involution on the basis of RP. The combined thickness of the myometrium and perimetrium of the pregravid uterine horn (PGUHMPT) and cervical wall thickness (CvWT) were parallelly estimated with US-measuring of PGUHD and CvD. The intervals to gross uterine ($P < 0.05$) and cervical ($P < 0.01$) involutions, on the basis of RP, were shorter than those on the basis of US. Both US-measured PGUHD and PGUHMPT, on the day of involution on the basis of US, were less than their US-measured counterparts on the day of gross involution on the basis of RP. Both US-measured CvD and CvWT, on the day of cervical involution on the basis of US, were less than their US-measured counterparts on the day of involution on the basis of RP. It is concluded that US- measuring of the pregravid uterine horn diameter and the combined thickness of myometrium and perimetrium as well as CvD and CvWT, is more accurate than RP-measuring of diameters of pregravid uterine horn and cervix for assessing the involution of the reproductive tract in buffaloes.

Key words: buffalo; involution; ultrasonography; cervical; combine thickness

Introduction

Uterine involution together with the earlier resumption of postpartum ovarian activity is prerequisites for having a successful reproductive performance in buffaloes (1). Days open is shorter in cows with smaller than those with the larger diameter of the pregravid uterine horn (2). Normal cervical and uterine involutions are essential for resuming ovarian activity (3). During the involution period, a crosstalk was detected between cervix and health status of the endometrium (4) from one side and with the resumption of the ovarian cyclicity from the other side such that the larger cervical diameter (CvD) associated with endometritis can result

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in abnormal follicular selection and abnormal ovarian cyclicity (5).

Before a cow is likely to conceive, it has to undergo cervical and uterine involutions (6). For the establishment of a new pregnancy in dairy cows, the uterus must return to its normal prepartum condition (7) and the cervix should close and regain its normal size and structure quickly (8).

Although rectal palpation (RP) remains the most commonly used method for assessing uterine involution in cows, it is less accurate than transrectal ultrasonography due to its high level of subjectivity (2) and variation among individual palpators (9,10)**.**

Trans-rectal US is an alternative method for monitoring the dynamic changes in the reproductive system after calving which allows rapid, safe and accurate assessment of uterine involution and resumption of ovarian activity in cattle (10, 11)**.** It gives information about cervical and uterine sizes, endometrial thickness and uterine contents, which are variables closely related to uterine involution (12)**.** While the decrement in the diameter of the involuting uterus and cervix is expressed in ≤ 0.5 cm at least in the case of RP, it is estimated by millimeters in case of the transrectal US (13). The transrectal US is utilized not only in the follow up of puerperium but also shares in making reproductive management decision in many reproductive areas such as selection of animals to be kept in or culled from the herd, increasing the likelihood of the reproductive success and pregnancy diagnosis (14)**.** The hypothesis of the work was that the US examination would be expected to be more accurate than RP for judging uterine involution thereby help to optimize reproductive management, ensuring higher future fertility, therefore the aim of the present work was to test the efficiency of transrectal US versus RP in judging cervical and uterine involutions in buffaloes.

Material and methods

Animals and management

The study was carried out in Mahallet Mousa Buffalo Research Station affiliated to Animal Production Research Institute, Agriculture, Research Centre, Egypt during the period extending from March to August 2017. Twenty-six healthy, 5-8 years old Murrah buffaloes, were enrolled in the present study. Their body condition score averaged 3.3 ± 0.4 (on a scale; 1 = lean to 5 = fatty). They had normal parturition and spontaneous placental expulsion within the first 12 hours after parturition. The calves were isolated after they had received colostrum and the buffaloes were milked twice daily. The animals were fed on a ration that met their maintenance and milk production requirements according to the requirements of Animal Production Research Institute (APRI 1997, unpublished data). Fresh water was available *ad libitum.* The Buffaloes were kept indoors in open yards, whereas half of the area was sheltered. This study was carried out under the Animal Welfare according to the regulations of Egyptian guidelines with approval granted by the Animal Ethics Committee of the Faculty of Veterinary Medicine, Kafrelsheikh University, Kafr EL-Sheikh, Egypt.

The judgment of uterine and cervical involutions

Rectal palpation

RP of the cervix and uterus was conducted by the same examiner every three days beginning on the third day postpartum. Uterine involution was considered complete when the diameters of the two uterine horns, at their bases, became nearly symmetrical and no further changes in the diameter of the pregravid uterine horn (PGUHD) could be detected during two successive RP of the uterus [6]. The cervix was considered involuted when the CvD, at three cm cranial to external OS, became stable in two successive RP (15). The PGUHD and CvD were recorded every three days and on the day of gross uterine or cervical involution. The interval to gross uterine or cervical involution on the basis of RP was recorded.

Ultrasonography

All transrectal US examinations were conducted by digital Ultrasonic Diagnostic Imaging System Model: D-P-30Vet. 10-2012 equipped with a linear probe, 7.5 MHz. All examinations were conducted by one examiner.

Cross-sectional images of PGUH, at its base, and cervix, at three cm cranial to external OS, were obtained by the transrectal US. When the image, cross section of either uterine horn or cervix, was not spherical, the diameter was estimated by averaging two 90° dimensions (2). Uterine involution was considered complete when uterine dimensions, PGUHD and the combined thickness of the myometrium and perimetrium of the pregravid uterine horn (PGUHMPT), became nearly stable in two successive transrectal US [16].

Cervical involution was considered complete when no further reduction in CvD/cervical wall thickness (CvWT) was detected in two successive transrectal US. The US-measured PGUHD and PGUHMPT were recorded when the uterine involution was considered on the basis of RP or US. Both the CvD and CvWT were measured and recorded every three days and on the day of a gross cervical involution on the basis of RP or US.The interval to gross uterine or cervical involution on the basis of transrectal US was recorded.

The rationale for measuring PGUHMPT rather than the thickness of either the whole of the uterine wall or endometrium by transrectal-US was due to the great folding of the endometrium into the uterine lumen and presence of still enlarged caruncles, especially during the early puerperium. The US-measuring of the endometrial thickness is more presumably beneficial for the diagnosis of endometritis rather than assessing the uterine involution.

The involution rate

The involution rate of PGUH

The involution rate (cm/ 3 days) of PGUH as indicated by the rates of the reduction in the PGUHD on the basis of RP or transrectal US examination was calculated by subtracting thePGUHD recorded on the days 6, 12, 18, 24, 30, 33...etc. from those recorded on the days 3, 9, 15, 21, 27, 30…etc. respectively, each of its previous one: e.g. (PGUHD on day 3- PGUHD on day 6), and so on until complete uterine involution. Also, the involution rate of PGUH as indicated by the rate of reduction in the PGUHMPT was calculated in the same manner as in the case of calculating the rate of reduction in the PGUHD.

The involution rate of the cervix

The involution rate (cm/ 3 days) of the cervix as indicated by the rates of reduction in the CvD on the basis of RP or the transrectal US was calculated by subtracting the CvD recorded on Days 6, 12, 18, 24, 30, 36, 42…etc. from those recorded on Days 3, 9, 15, 21, 27, 33, 39...etc. respectively, each of its previous one e.g. (CvD on day 3- CvD on Day 6). Also, the involution rate of the cervix as indicated by the rates of reduction in the CvWT was calculated in the same manner as in the case of calculating the rates of reduction in the CvD.

Statistical analysis

Data were analyzed with a statistical software program (GraphPad Prism version 5.0; GraphPad Software, San Diego, CA, USA).The means±SEM of the interval to gross uterine or cervical involutions on the basis of RP were compared to those recorded on the basis of transrectal US by using t-test. Also, by using t-test, the US-measured PGUHD and PGUHMPT or US-measured CvD and CvWT on the Day that either of the pregravid uterine horn or the cervix was considered involuted on the basis of RP were compared with their counterparts measured by transrectal US on the Day of uterine or cervical involutions on the basis of transrectal US. The involution rate of PGUH as indicated by the rate of reduction in either of PGUHD or PGUHMPT was examined by repeated measures ANOVA followed by Bonferroni's multiple comparison tests. Also, the involution rate of the cervix as indicated by the rates of reduction in CvD or CvWT was examined by repeated measures ANOVA.

Results

Intervals to gross uterine and cervical involutions on the basis of RP and US

The interval to gross uterine involution as being detected by RP was shorter ($P < 0.05$) than its counterpart detected by the transrectal US. The interval to gross cervical involutions as being detected by RP was shorter ($P < 0.01$) than its counterpart detected by transrectal US (Table 1).

The US- measured PGUHD and CvD on the day of gross uterine and cervical involutions on the basis of RP and US

The US-measured PGUHD and PGUHMPT, recorded on the day of a gross uterine involution on the basis of RP, showed increases at $P < 0.01$ and $P < 0.001$ respectively compared with their counterparts recorded on the day of a gross uterine involution on the basis of transrectal US. Also, the US-measured CvD and CvWT recorded on the day of a gross cervical involution on the basis of RP showed increases ($P < 0.05$) compared with their counterparts recorded on the day of a gross cervical involution on the basis of transrectal US (Table 2).

The Involution rate of pre-gravid uterine horn

The involution rate of PGUH as being indicated by the rates of reduction in RP-measured PGUHD throughout involution period showed a decrease ($P < 0.05$) among interval: 3 to 6, 6 to 9 and 9 to 12 and no difference ($P \geq 0.05$) among intervals: 9 to 12, 12 to 15, 15 to 18, 18 to 21, 21 to 24, 24 to 27 and 27 to 30 (Fig. 1). The involution rate as being indicated by the rates of reduction in the US-measured PGUHD throughout the involution period showed a decrease ($P < 0.05$) among intervals: 3 to 6, 6 to 9,9 to 12, 12 to 15, 15 to 18, 18 to 21, 21 to 24, and no difference among intervals: 24-27, 27 to 30, 30 to 33 and 33 to 36 (Fig. 1).The involution rate of PGUH on the basis of rates of reduction in PGUHMPT showed significantly ($P < 0.05$) decrease among intervals: 3 to 6, 6 to 9, 9 to 12, 12 to 15, 15 to 18, 18 to 21, 21 to 24 and 24 to 27 days and no difference among intervals: 24 to 27, 27 to 30 and 30 to 33 respectively (Fig. 3).

The involution rate of the cervix

The involution rate of the cervix as being detected by the rates of reduction in the RP- measured CvD showed a difference ($P < 0.05$) among interval 3 to 6, 6 to 9 and 9 to 12, and no difference among intervals: 9 to 12, 12 to 15, 15 to 18, 18 to 21, 21 to 24, 24 to 27, 27 to 30 and 30 to 33**.** On the other hand, the involution rate as being detected by the rates of reduction in the US-measured CvD showed differences ($P < 0.05$) among intervals: 3 to 6, 6 to 9, 9 to 12, 12 to 15, 15 to 18, 18 to 21, 21 to 24, 24 to 27, 27 to 30, and 30 to 33 and no difference ($P \geq 0.05$) among intervals: 33 to 36, 36 to 39 and 39 to 42 (Fig. 2). The involution rate of the cervix on the basis of the rates of reduction in CvWT showed a significant decrease ($P < 0.05$) among postpartum intervals: 3 to 6, 6 to 9, 9 to 12, 12 to 15, 15 to 18, 18 to 21, 21 to 24, 24 to 27, 27 to 30, 30 to 33 and 33 to 36 and no difference ($P \geq 0.05$) between intervals from 36 to 39 and 39 to 42 days (Fig. 4).

Table 1: Intervals to gross uterine and cervical involutions detected on the basis of rectal palpation and trans-rectal ultrasonography

Parameter	Method of Judgment	
	Rectal palpation (RP)	Ultrasonography (US)
Interval to gross uterine involution	24.8 ± 0.96	29.3 ± 0.89*
Interval to gross cervical involution	30.5 ± 0.92	37.0 ± 0.10**

Means within the same row and bearing different superscripts were significantly different at * $P < 0.05$;** $P < 0.01$. The interval to gross uterine involution as being detected by RP was shorter ($P < 0.05$) than its counterpart detected by transrectal US. The interval to gross cervical involutions as being detected by RP was shorter ($P < 0.01$) than its counterpart detected by transrectal US.

Table 2: The ultrasound- measured PGUHD and CvD as well as PGUHMPT and CvWT on the days of gross uterine and cervical involutions on the basis of RP and US

Day of involution	Uterine dimensions		Cervical dimensions	
	PGUHD	PGUHMPT	CvD	CvWT
Based on RP	$2.34 \pm 0.06^{**}$	$0.64 \pm 0.02^{***}$	$2.01 \pm 0.06^{*}$	$0.52 \pm 0.02^{*}$
Based on US	2.09 ± 0.04	0.47 ± 0.01	1.72 ± 0.08	0.43 ± 0.02

Means within the same column and bearing different superscripts are significantly different at * $P < 0.05$;** $P < 0.01$; *** $P < 0.001$.

PGUHMPT means ultrasound –measured combine thickness of both myometrium and perimetrium of the pregravid uterine horn; PGUHD means the diameter of the pregravid uterine horn; RP means rectal palpation, US means ultrasonography. Both the US-measured PGUHD and PGUHMPT, recorded on the day of gross uterine involution on the basis of RP, showed increases at $P < 0.01$ and $P < 0.001$ respectively compared with their counterparts recorded on the day of gross uterine involution on the basis of transrectal US. Both the US-measured CvD and CvWT recorded on the day of gross cervical involution on the basis of RP showed increases ($P< 0.05$) compared with their counterparts recorded on the day of gross cervical involution on the basis of transrectal US.

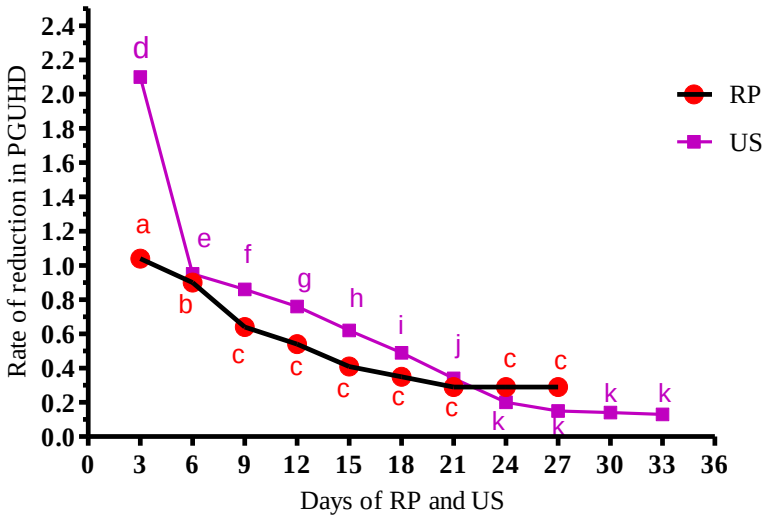

Figure 1: Rate of reduction in the PGUHD per three days interval throughout involution period on the basis of RP and US examinations in buffaloes. PGUHD = Pre-gravid uterine horn diameter; RP=Rectal palpation; US=Ultrasound examination. For rectal palpation, values carrying different letters from a - c differ at $P < 0.05$. For ultrasound examination, values carrying different letters from d – k differ at $P < 0.05$. The rate of reduction in the RP-measured PGUHD shows a decrease ($p < 0.05$) in the 3rd interval compared with either 2nd or 1st and in the 2nd compared with the 1st interval but shows no difference ($P > 0.05$) among the rest of intervals from 3rd (9-12) till 8th interval (24-27). On the other hand, the rate of reduction in the US-measured PGUHD shows a decrease ($p<0.05$) among intervals from 1st (3-6) till the 7th (21-24) but no difference are observed among intervals from 7th till 10th (30-33).

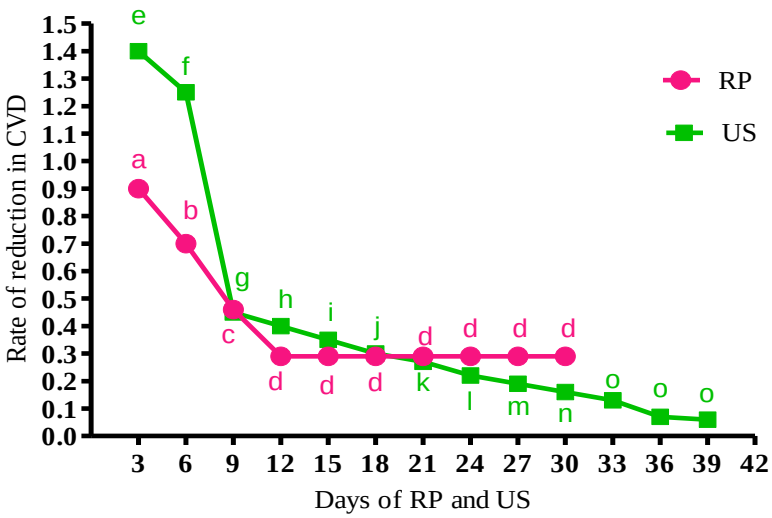

Figure 2: Rate of reduction in the CvD in buffaloes throughout the involution period on the basis of RP and US examinations. CvD = Cervical diameter; RP = Rectal palpation; US = Ultrasound examination. For rectal palpation, values carrying different letters from a - d differ at $P < 0.05$. For ultrasound examination, values carrying different letters from e – o differ at $P < 0.05$. The rate of reduction in the CvD on the basis of RP shows a decrease ($p<0.05$) among intervals: 3-6, 6-9 and 9-12 and no difference ($p \geq 0.05$) among intervals from 3rd (9-12) till 9th (27-30). On the other hand, the US-measured CvD shows among 1st (3-6) till the 10th (30-33) intervals and no difference ($p \geq 0.05$) among intervals from 10th till 13rd (39-42).

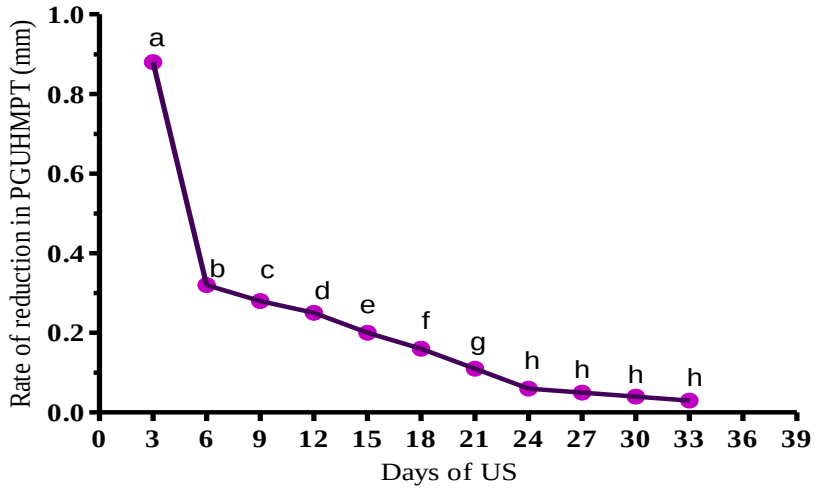

Figure 3: The rate of reduction in the PGUHMPT per 3 days throughout the involution period on the basis of US in buffaloes. US = Ultrasound examination; PGUHMPT= combine thickness of the myometrium and perimetrium. *Values carrying different letters differ at $P < 0.05$. The PGUHMPT shows a decrease ($p < 0.05$) among intervals from the 1st (3-6) till the 8th (24-27) and no difference among the intervals from 8th till 10th (30-33).

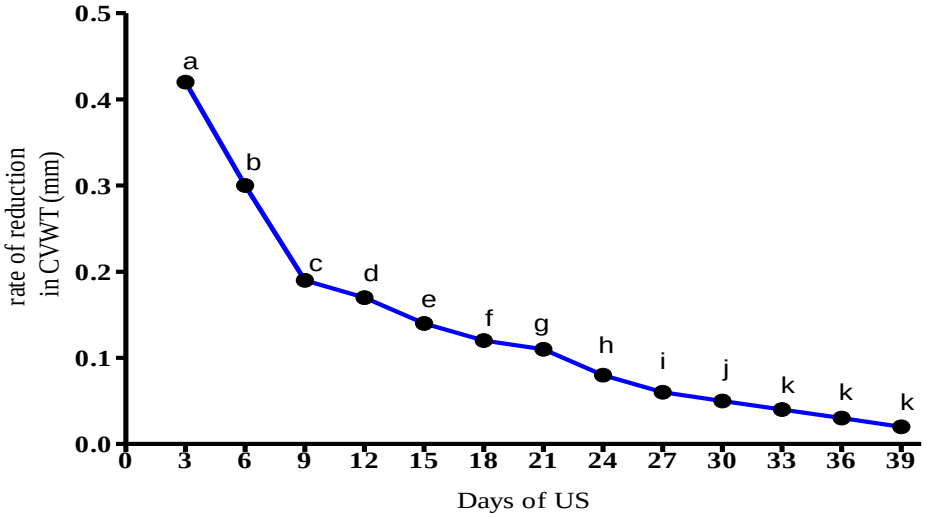

Figure 4: Rate of reduction in CvWT per 3-days throughout involution period by transrectal US in buffaloes. US = Ultrasound examination, CvWT= Cervical wall thickness. Values carrying different letters differ at $P < 0.05$. The CvWT shows a significant decrease ($P < 0.05$) among postpartum intervals: 3 - 6, 6 - 9, 9 - 12, 12 -15, 15 -18, 18 - 21, 21 - 24, 24 - 27, 27 - 30, 30 - 33 and 33 - 36 and no difference ($P \geq 0.05$) was detected between intervals from 36 - 39 and 39 - 42 days.

Discussion

The aim of the present study was to compare the efficiency of RP and the transrectal US in judging cervical and uterine involutions in buffaloes. On the basis of RP, the interval to gross uterine involution (24 days) may come in concurrence with previous studies (17, 18) in buffaloes. On the other hand, longer intervals to gross uterine involution, 24-56 days (19); 24-44 days (20) and 21-74 days (21), were recorded in buffaloes. Also, shorter intervals, 14-29 days (22) and 20 days (23) were recorded in cows on the basis of RP.

On the basis of transrectal US, the interval to gross uterine involution (30 days) in the present study, more or less, coincides with those recorded in previous studies: 30 days (24); 28 days (11); 31 days (25) and 27 days (17).

Matching the interval to gross uterine involution either on the basis of RP or the transrectal US, recorded in the present study, with their counterparts in previously mentioned studies herein revealed that while greater variations were observed in case of RP, lesser variations were noted in case of transrectal US. This observation indicates the higher accuracy of transrectal US compared with RP for judging uterine involution.

However, the wide variations between the interval to gross uterine involution on the basis of RP in the present study and intervals recorded in the previous studies might be due to the variations among the criteria used to evaluate the uterine involution and/or variations among individual palpators during measuring the dimensions of the involuting uterus (9). Also, Okano and Tomizuka. (1987) (16) attributed the inaccuracy in estimating the uterine dimension by RP to the variations in fingers width among palpators and nature of the postpartum uterus which is being relaxed and flat especially during early puerperium. However, the previously mentioned limitations, that affect the efficiency of RP in estimating uterine dimensions, are not present in case of transrectal US.

The interval to gross cervical involution on the basis of RP coincides with that (31.1) recorded byEl-Fouly et al. (1976) (26)and Usmani et al. (2001) (27) in buffaloes but was being longer than that, 25, recorded by Atansov et al. (2012) (10)in buffaloes. However, the disagreement between the current study and the study of Atansov et al. (2012) (10) may be attributed to individual variations among palpators in the two studies. The increase in the interval to gross uterine or cervical involution on the basis of transrectal US compared with RP is believed to

be expended in estimating the lesser differences (in terms of mm) in diameter of either pregravid uterine horn or cervix which would be supposed to still undergo involution on the basis of transrectal US. On the other hand, the reductions in the diameters (in terms of ≤ 0.5 cm) between successive RP were greater thus required a shorter time to reach stability.

Moreover, the US scanning of both uterus and cervix during assessing the involution gives more diagnostic criteria that cannot be accurately detected by RP such as the wall thickness of cervix and pregravid uterine horn as well as smaller amounts of intrauterine fluids (Lopez-Helguera et al. 2012) (12). Doubtless, the combining between the US- measured PGUHMPT or CvWT and corresponding PGUHD or CvD as diagnostic criteria, for assessing uterine and cervical involutions will be better than using either of them solely especially in case of assessing uterine involution.

However, US measuring of the PMGHMPT may be more accurate than measuring PMGHD because the PGUH in some cases may be compressed under linear probe while it is being positioned over it giving false results. Thus, measuring PMGHMP could be efficiently used as a complementary parameter to PMGHD for assessing uterine involution.

The benefit of assessing the involution rate of PGUH and cervix on the basis of detecting the rate of reduction in their diameters by RP or transrectal US was to check the efficiency of RP and transrectal US in determining the time when the greatest reduction in the uterine or cervical size had occurred as well as the normalcy of the involution process at any time throughout the involution period. Cengic et al. (13) reported that the speed of uterine involution could be monitored by detecting the decrease in the uterine horn diameter and its wall thickness.

The results of the present study indicated that the greatest rate of reduction in the PGUHD had occurred during the intervals 0-9 days on the basis of RP and 0-15 days on the basis of transrectal US. Afterward, the rates of reduction in the successive RPs or US examinations became relatively smaller. This indicates that the higher efficiency of US compared with RP in determining the time in which the greatest reduction in the size of PGUH had occurred. However, the pattern of reduction in US-measured PGUHD throughout involution period may come in coincidence with that described by Atansov et al. (10). Matching the involution rates between RP and transrectal US methods revealed that although there were no differences among rates of involution beyond 9th day postpartum in the case of RP, there were differences among all transrectal US examinations conducted from 3rd until 27th day except during the interval from 18th to 21st day indicating the higher efficiency of transrectal US in assessing involution rates throughout the involution period.

Matching the involution rate of the cervix as indicated by the rates of reduction in the CvD on the basis of RP with their counterparts on the basis of transrectal US revealed that although the involution rate detected by RP and the transrectal US on the Day 9 became similar, it abruptly decreased in case of RP to 0.29 cm/3 days on the 12th day and remained so until the end of involution period. On the other hand, in the case of transrectal US, it gradually decreased significantly until reaching comparable value, 0.30 cm/ 3days, on the 18th day. However, these results indicated the subjectivity of RP and higher efficiency of transrectal US in the follow up of the cervical involution.

The higher reduction rates (1.40 and 1.25 cm/3 days recorded during postpartum intervals 3 to 6 and 6 to 9 respectively) in US-measured CvD indicate that the greatest cervical involution occurs within the first postpartum week. These results partially agree with Atansov et al. (10) who found that the mean CvD decreased by more than 45% between the first and seventh days postpartum. Also, the results of the present study agree with the same authors beyond day 19 whereas CvD regressed slowly until the 25th day while non-significant differences were detected in CvD between the 25th and 34th day. However the overall pattern of the rates of reduction in the US-measured CvD may be comparable with that recorded by Pariksh et al. (1) in Buffalo who reported that the reduction in the

CvD was faster until the 15th day, marginal from the 16th to the 25th day and became negligible beyond the 25th day postpartum.

Regarding the involution rate of the PGUH on the basis of rates of reduction in PGUHMPT, it was noted that the greatest rate of reduction (0.88 cm/3 days) was recorded in the period from the 3rd to the 6th day postpartum indicating that the greatest reduction in the PGUHMPT had occurred during the first week. Afterward, it abruptly decreased to 0.32 cm between 6th and 8th day. Later on, it was regularly decreasing (at 0.04 cm) from 0.32 between 6th and 8th to 0.16 cm in the interval between 18th and 20th day. This indicates that the involution rate became slower as the time became farther from parturition. However, beyond day 21, the involution rate was similar and became nearly negligible.

Regarding the involution rate of the cervix on the basis of the rates of reduction in CvWT, it was noted that the greater rates of reduction were recorded in the intervals: 3 to 6 and 6 to 9 days. Afterward, although the differences between the rates of reduction among succeeding the transrectal US were significant, the decrease in the CvWT was slow. Beyond day 30, the rates of reduction among 3 successive transrectal US became similar and nearly negligible. This may be explained in light of the structural components of the cervix, which is mainly formed from connective tissues rather than smooth muscles as in the case of the uterus.

It is concluded that US- estimation of the diameter and the combined thickness of myometrium and perimetrium of the pregravid uterine horn as well as CvD and CvWT thickness is more accurate than RP-measured diameters of pregravid uterine horn and cervix for assessing the involution of the reproductive tract in buffaloes.

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Conflict of interest

The authors declare that they have no conflict of interest.

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