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Consequence of Exogenous Administration of Oxytocin on Reproductive and Productive Parameters during Postpartum Involution Period in Newly Calved Nili-Ravi Buffaloes

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ABSTRACT

Repeated use of exogenous oxytocin in newly calved buffalo has made buffalo breeder anxious about its reproductive and productive performances. Moreover, misconceptions about its harmful effect on public health are increasing day by day during postpartum period when animal has been already in stress. To know effect of oxytocin injection on cervix, uterus and milk composition during postpartum interval in Nili-Ravi buffalo, a study was conducted at Buffalo Research Institute Pattoki, Pakistan from September to October. For this purpose, 25 animals were randomly divided into three groups: group-1 (Control; n=8), group-2 (Low dose 10IU; n=8) and group-3 (High dose 30IU; n=9) while one animal excluded at milk sampling from group-3 due to unavoidable circumstances. Oxytocin was administered to each buffalo twice daily @10IU and 30IU into group-2 and group-3 respectively, whereas, saline into group-1 within one week of post calving for 48 days. Ultrasonography was performed twice a week to monitor involution changes while milk composition analysis was done once a week. Results depicted that initially, anechoic lumen filled areas with echogenic border in cervix and uterus was found but at involution, cervix and uterus became moderately hyperechoic without any fluid filled areas. Moreover, there was non-significant effect (P>0.05) of treatment on cervix, uterine body, non-gravid and gravid horn at involution among three groups when these attained non pregnant size. Regarding progesterone, it remained non-significant (P>0.05) throughout postpartum period among treatments. Furthermore, milk composition results showed that fat, protein, lactose, freezing point, SNF and solids were significantly higher (P<0.05) in group-2 and group-3 as compared to group-1 except density and pH which remained non-significant (P>0.05) in all groups. On the basis of result, it may be concluded that oxytocin had no effect on uterine involution and progesterone; however, it had some role to affect the normal composition of milk in postpartum involution interval.

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Authors' Contribution
SM and AS designed the study, MSH helped in hormone analysis. NA and MI helped in manuscript writing. MA facilitated and help in conducting research trial.

Key words
Oxytocin, Uterine involution, Progesterone, Milk composition, Nili-Ravi buffalo

INTRODUCTION

In Pakistan, buffalo is the main dairy animal and most of the milk demand is fulfilled from it. Approximately, 60.54% of milk production is contributed by buffalo (Anonymous, 2019). Buffalo has various unique characters in term of production and reproduction. It is also known as somewhat seasonal breeder but it can normally breed throughout year (Perera, 2011). As there is no production without reproduction, buffalo breeders are very anxious about its reproductive efficiency because of slow reproduction. In Pakistan, among the dairy animals, buffalo is the most popular animal as compare to cattle. In actual, there are five buffalo breeds: Nili, Ravi, Nili-Ravi, Kundi and Aza kheli (Khan *et al*., 2007) in the country. Among these, Nili-Ravi is the most leading breed of buffalo in the Punjab, Pakistan (Moioli and Borghese, 2005) and located between the belt of the two rivers of the country; Ravi and Sutlej. Nili-Ravi is considered as primarily milk breed, however, it is also being used for meat and draught purpose (Iqbal *et al*., 2007). Due to its high milk potential, it is well known as national wealth in term of living black gold reserves of the country (Bilal *et al*., 2006). Above all, Nili-Ravi buffalo is considered to be an efficient dairy animal but on the other hand, there are several infertility issues to this animal on account of which

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it has poor reproductive efficiency (Warriach *et al*., 2012).

Reproductive problems like delayed postpartum involution, prolong ovarian inactivity, silent estrous, poor conception rate and postpartum acyclicity or anestrus are also contributory to slow reproduction of these animals (Warriach *et al*., 2015). Various dairy scientists consider postpartum period an important indicator for inter calving interval which typically asses the breeding potential of animals (Snel-Oliveira *et al*., 2010). Hence, early involution of uterus to non-pregnant size and initiation of postpartum cyclicity concurrently involve improving reproductive performance of animals in term of shortening of postpartum estrous interval and simultaneously enhancing of conception chances. The conception of animal within 90-120 days of postpartum is essential factor to achieve ideal calving date within 12-14 month after last parturition (El-Wishy, 2007). Although gestation period is fixed in both species; buffalo and cow but delayed in postpartum involution results into poor reproductive efficiency. These infertility problems are due to several factors like genetics, age, nutrition, seasonality and managemental practices especially maneuvering for milk let down (Cady *et al*., 1983; Barile, 2005). For that reason, it is commonly assumed that unnatural buffalo milking practice is one of the important contributory factors to infertility and non-reproductive life of Nili-Ravi buffalo under field condition (Mustafa *et al*., 2008). Although dairy farmers use oxytocin injection regularly for milk let down yet they have some concerns about usage of oxytocin injection during postpartum period on reproductive health of animals because of their misperception about negative effects on normal uterine involution process and inter calving interval (Ijaz and Aleem, 2006). Therefore, they consider that injudicious use of exogenous oxytocin may be an important cause of poor reproductive efficiency in buffalo. Furthermore, certain studies explained the utilization of exogenous oxytocin either in combination or without it or other than oxytocin hormones like GnRH, Methergin or PGF2α to observe the effects on uterine involution in different breeds of dairy buffaloes and reported variable results regarding complete involution interval (Iqbal *et al*., 2003; Presicce *et al*., 2005; Khasatiya *et al*., 2006; Ramoun *et al*., 2006; Yindee *et al*., 2007; Snel-Oliveira *et al*., 2010; Khatri *et al*., 2013). Moreover, some studies in Nili- Ravi buffalo provided insufficient information with respect to attaining of complete involution. These studies are mostly based upon rectal palpation. In these studies neither exogenous oxytocin nor ultrasound technique are used to evaluate postpartum involution changes and interval (Usmani *et al*., 1985; Iqbal *et al*., 2003). However, a first-hand diagnostic technique like ultrasonography is commonly used now a day. It is more accurate, safe and non-invasive technique for monitoring of vibrant changes in the reproductive organs i- e cervix, uterus and ovaries during postpartum period in dairy animals (Yindee *et al*., 2007; Atanasov *et al*., 2012). Previously, one study was done in Bulgarian Murrah buffalo (Atanasov *et al*., 2012) but no single study was done by using ultrasonic technique which pin point the consequences of exogenous oxytocin on involution of cervix, body of uterus, uterine horns, caruncles in Nili-Ravi buffalo. Similarly, not a single study reported progesterone concentration in Nili-Ravi buffalo during postpartum period following exogenous oxytocin administration. To know the importance of reproductive parameters during involution period, productive parameters are also equally important to explore them following exogenous oxytocin administration in Nili-Ravi buffalo.

According to one study, it has been reported that exogenous oxytocin is used 99.66% for milk production in India (Singh and Doley, 2013). Similarly, in Italy, more than 24% heifers' buffaloes are injected with oxytocin at disturbed milking (Borghese *et al*., 2007). Likewise, in Pakistan, 25% of the dairy animals are injected with oxytocin for milk let down and among them approximately, 70 % are buffaloes (Bilal *et al*., 2008). According to one other study, 15.5 % dairy farms use oxytocin injection in Pakistan (Tariq and Younas, 2013). As buffalo is considered as hard milker and has slow milk let down on disturbed milking at the time of weaning, death or selling of calves then oxytocin injection is used as an alternative of calves to get teat stimulation and milk let down process initiation. Analogous, certain studies have also been done in buffaloes to investigate the consequences of oxytocin injection on milk composition during different stages of lactation and explained controversial results (Hanjra *et al*., 1979; Akhtar *et al*., 2012; Abbas *et al*., 2014; Shahid *et al*., 2016; Murtaza *et al*., 2017). Some have revealed positive and other have shown negative or no typical effects on milk composition. In some survey based studies in dairy cows and buffaloes, it has been reported that exogenous oxytocin may have negative or no effect on fat contents (Mustafa *et al*., 2008; Singh and Doley, 2013). Previously, one study has been conducted to know the effect of exogenous oxytocin on milk composition in first 15 days of postpartum period in Murrah buffaloes and has shown diurnal variation due to treatment effects on milk fat, lactose and chlorides but no change in protein reported (Prasad and Singh, 2001). Equally, milking and exogenous oxytocin are interlinked in dairy buffaloes and are provoking day by day. Its application during postpartum period may be another untoward effect of exogenous oxytocin on composition milk. Additionally, public apprehensions are also present about milk quality at involution period following regular use of exogenous oxytocin in newly calved buffaloes. Hence, the idea of knowing the effect of oxytocin administration on productive performance in term of milk composition during involution period is also the need of time to be investigated for public satisfaction about misconception regarding low milk quality.

Keeping in view about the researches in past, a study was designed to explore the possible effects of exogenous oxytocin administration on cervical and uterine involution and milk composition after its regular use for milk let down in newly calved Nili-Ravi buffaloes. The main objective of current study was to determine whether oxytocin has any effect on postpartum uterine involution period? This is based on the hypothesis that exogenous oxytocin administration spontaneously causes the contraction of uterus, hence, lochia may be expelled out more rapidly from the uterus into birth canal outside through vulva and rapid involution is expected parallel to non-injected animal (dos Santos *et al*., 2009).

MATERIALS AND METHODS

Selection and management of animals

For this purpose, 25 newly calved Nili-Ravi buffaloes had body condition score (BCS) ranged between 2.9 to 3.1 and age 3.5 to 8 years (mixed parity) randomly divided into three groups: group-1 (control; n=8), group-2 (low dose 10IU; n=8) and group-3 (high dose 30IU; n-=9). Uniform feeding and, watering were freely accessible to each animal in all three groups. Oxytocin was injected into group-2 and group-3 while saline into group-1 intramuscularly (IM) in the neck region of each animal twice daily at the time of morning and evening milking. The medicine (Oxytocin 50 mL vial; 10IU/mL)) was purchased from the local market, manufactured by Star Laboratory Lahore, Pakistan (Pvt).

Location

This experiment was conducted at Buffalo Research Institute Pattoki (BRI), Kasur Punjab, Pakistan which is located at latitude 31°1'0 toward North, longitude 73°50'60 toward East with an altitude of 186 m from sea level.

Blood and milk sampling

Blood samples (5mL) in yellow cap gel containing vacutainer tube were collected twice a week from each animal in all three groups for progesterone analysis during postpartum involution period and transferred to Animal Health Division for serum separation at each sampling. Milk samples were collected for milk composition analysis in 50 mL sterile falcon tubes once in a week for seven weeks from 25 animals except one from group-3 due to teat problem for some duration during postpartum involution interval. Before collection of milk samples, a few streaks of milk was discarded from each teat and samples were taken directly from four teats equally into the tube and transferred to animal health division laboratory at Buffalo Research Institute Pattoki (BRI) for milk composition analysis and kept at least two hours in refrigerator to maintained temperature between 220-25C°. For composition analysis, Ultrasonic Milk Analyzer (LM) was used except pH which was measured separately on pH meter. Before analysis, milk analyzer was washed with lactodaily wash solution and then calibrated to run the analysis. Sample holder was filled with milk and placed under input pipe of milk analyzer and then start button was entered to estimate milk composition for one minute. After one minute of analysis, result of milk composition displayed on the screen and readings were taken for statistical analysis. After every 4 to 5 five samples analysis, ultrasonic milk analyzer was washed with lactodaily, and for every week with lactoweekly.

Ultrasonography

Similarly, ultrasonography was performed twice weekly within one week of post calving for 48 days. For this purpose, Ultrasound Machine Esoate SSD 900 manufactured of USA fitted with B-mode probe having 6.0 MHz frequency, and for ultrasound images, a Video Graphic Printer Up-897MD Sony Corporation Tokyo Japan was used.

Serum separation technique and progesterone analysis

Serum was separated from blood cells after centrifugation at 2500 revolution per minute for three minutes. Separated serum was placed at -20°C in freezer and then shifted to Radio Immuno Assay (RIA) Laboratory at Nuclear Institute for Agriculture and Biology, Faisalabad-Pakistan for progesterone (P4) analysis. P4 was quantified through RIA technique by using Videogamma Rack Tacn Counter Model No. I' Acn/I' Accessorio Nucleare S.R.I. 20023 Cerro Maggiore MI Italia. Progesterone kits were purchased from Beckman Coulter, Immunotech Czech Republic Lot No. 105.

Statistical analysis

Data was analyzed through repeated measure under the one-way Analysis of Variance Technique (ANOVA) by using the SPSS 20 software as a statistical tool. Before applying the repeated measure ANOVA for cervix, uterus and progesterone parameters and one way ANOVA for milk composition, the data is fully examined with histogram and Shapiro Wilk Test. Non-normal data was further transformed into normal by Fractional Rank transformation (Templeton, 2011) for all parameters. Regression was performed to target the involution days.

The $P<0.05$ was considered significant for all analysis. Means were compared via Duncan Multiple Range Test (DMRT) (Duncan, 1955). Data was expressed as mean±SEM (Steel *et al*., 1997).

RESULTS

Cervix

From day 3 of the postpartum, cross sectional ultrasonography of the cervix and its fluid contents were viewed as curved structure with hyperechoic borders along with anechoic area and also had parallel echogenic linings in all treatment groups. Moreover, ultrasound scanning showed that anechoic fluid filled areas were decreased as involution progressed. Likewise, at the time of involution, cervix had become echogenic structure without any anechoic fluid filled cavity occupied by lumen. Ultrasonography of the cervix revealed that the reduction in the size of cervix was more in first two weeks of the postpartum period and after that went slowly till involution achieved in all treatments (Fig. 1).

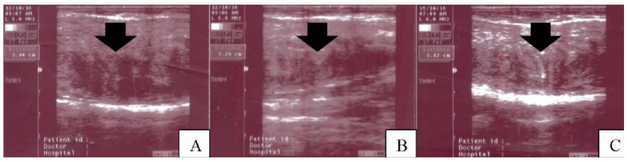

Fig. 1. Ultrasonographs of cervix among treatments: Control; A, Low dose 10IU; B, High dose 30IU; C at fifth week near complete involution. Arrow heads showing the longitudinal ultrasound images of cervix in three treatments: A, B and C with enhanced echogenicity in C part of Figure 1.

On statistical analysis, result of cervical diameter involution showed that treatment groups: 1, 2 and 3 were found significant among each other ($P<0.05$) on days: 3, 16 and 48 while the treatments performance remained non-significant ($P>0.05$) on all other days. It was also seen that the mean of group-3 (high dose 30IU) was recorded higher from the group-1 (control) and least from group-2 (low dose 10IU). Furthermore, treatment group-3 was found involuted slower significantly ($P<0.05$) as compared to group-1 but alike with group-2 on day 3. Similarly, on day 16 of postpartum involution, significant difference ($P<0.05$) was present between control and oxytocin treated groups whereas on day 48, there was no substantial difference exist between high treatment (30IU) and control while significant difference ($P<0.05$) was present with low treatment (10IU). However, throughout postpartum period, overall treatments performance remained non-significant ($P>0.05$). Involution days were countered by using ultrasonography and Regression techniques. From the slope coefficient, it could be explicate that with the advancement towards the next day, the mean value of CD decreased 0.05533, 0.05348, 0.08396 for group-1, group-2 and group-3 respectively, for every animal. Taking representative measured values by ultrasonography during postpartum involution interval, the postpartum involution values were estimated by regression equation. From the regression equation, the predicted lowest value on 48 days interval among treatments groups was 2.37 which had come from high dose 30IU. The targeted days of involutions were between 23 to 48 days. The animal was considered involuted, if the value of CD measured less than 3.25 cm from each group. The first animal was observed in involution stage on day 23 for control and low dose (10IU) while on day 27 for high dose (30IU) respectively. From the data, it was evidently noted that approximately, 65% animals got involuted between 34 to 41 days. There was small proportion (25%) of animals detected involuted before 34 days and after 41 days (10%) only. The regression suggests that all of animals which belong to group-1, group-2 and group-3 were expectedly involute on 37, 41 and 38 days one by one. However, original involution days were 43±2.81, 37±2.81 and 36±2.65 in control, low dose (10IU) and high dose (30IU) groups respectively, which were slightly inconsistent from the expected involution days. The mean cervical involution diameters in group-1, group-2 and group-3 were 2.71±0.14cm, 2.91±0.14cm and 2.65±0.14cm when measured at their analogous involution days. The original measured CD values by ultrasound showed that there was no significant ($P>0.05$) difference observed among treatment groups on involution days which justifies the regression results. Graphical representation given in Figure 2 shows the convergence of mean values of cervical diameter day by day with their respective standard error.

Body of uterus

Ultrasonic image of body of uterus showed that there were echogenic borders with hypo echoic fluid filled areas in early postpartum period. As involution progress, the body of the uterus appeared as moderate echogenic texture with some hyper echoic spots in high dose injected group (Fig. 3). Figure 4 represents the effect of oxytocin on body of uterus. The values of diameter of body of uterus are represented as mean±S.E (cm) during postpartum involution interval. The result showed that overall non-significant ($P>0.05$) difference was present among three treatment groups. However, on day 13 of postpartum, high dose 30IU was significantly different ($P<0.05$) from control and low dose 10IU. Although individual difference was presen

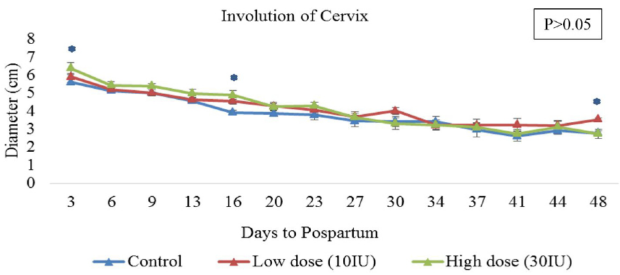

Fig. 2. Comparison of means ± S.E (cm) of cervical diameter among three treatment groups between 3 to 48 days of postpartum period in Nili-Ravi buffaloes; Means± S.E with asterisk sign on days: 3; 16 and 48 of postpartum differs among treatment groups at P < 0.05.

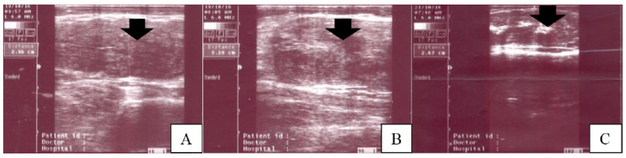

Fig. 3. Ultrasonographs of body of uterus at involution in three treatment groups: Control; A, Low dose 10IU; B, High dose 30IU; C at fifth week near complete involution. Arrow heads in A and B parts of Figure 3 indicate normal echogenicity while hyperechoic spots in C part of Figure 3.

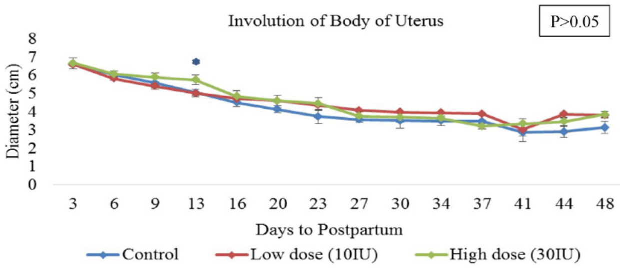

Fig. 4. Comparison of means ± S.E (cm) of the diameter of body of uterus among three treatment groups between 3 to 48 days of postpartum period in Nili-Ravi buffaloes; Means± S.E with asterisk sign on day 13 of postpartum differs among three treatment groups at P < 0.05.

t on day 44 and 48 among treatments but statistically non-significant at these two postpartum intervals while on rest of the postpartum days all the treatments remained non-significant (P>0.05). It was also observed that there was slow process of uterine regression in high dose 30IU on second week of postpartum involution period. Moreover, involution days were estimated and evaluated by using the ultra sonographic and regression techniques. From the result of regression equation, slope coefficient indicated that the mean value of diameter of body of uterus decreased 0.10089, 0.08822 and 0.12518 for group-1, group-2 and group-3 respectively, for each animal towards every next day. The expected lowest value was 2.67cm on day 48 among three treatment groups which came from group-1. The beset days of involutions were between 34 to 41 days. The animal was considered involuted, if the value of body of uterus measured less than 3.20 cm from each group. The first animal was perceived into involution stage on day 23 for group-1 and group-3 while it was day 20 for group-2 respectively. From the data, it was clearly noted that approximately, 62.5% animals got involutions between 34 to 41 days. There were 25% of animals detected in involution before 34 days and remaining 12.5% of animals resumed involution after 41 days. The regression suggested that all of animals which belonged to control, low dose (10IU) and high dose (30IU) were expectedly involute on days: 42, 43 and 42 respectively but in actual these values were existed among 41±2.00, 43±2.00 and 40±1.88 days which were closely related with expected calculated values. All of the mean values with their respective standard errors can be seen graphically in Figure 4. The original measured values of body of uterus by ultrasonography showed that there was no significant (P>0.05) difference observed in treatment groups on involution days which justifies the regression results. The mean diameters of body of uterus on the day of involution in three groups: 1, 2 and 3 were documented as 2.82±0.14, 3.02±0.14 and 3.09±0.13 respectively.

Gravid horn (GH)

On day 3 of postpartum, cross sectional image of the gravid horns represented as hypo echoic fluid filled area with echogenic walls. With the progression in involution process, the gravid horn scanned as potato chips like structures with no any fluid filled anechogenic structures found in all treatment groups. On the day of involution, gravid horns looked like moderate bright echogenic structures without any hypoechoic contents. However, it was also observed that slightly brighter echo texture of gravid horns in high dose 30IU injected group as compared to control and low dose 10IU (Figure 5). On rectal palpation and ultrasonography, it was examined that 70% pregnancies were experienced in right horn as compared to left. Overall, all three treatments: control, low dose 10IU and high dose 30IU (groups: 1, 2 and 3) remained non-significant (P>0.05) during postpartum involution interval while DMRT suggested that treatments groups: 1, 2 and 3 were found significantly different (P<0.05) among each other on day 3 of postpartum. Similarly, on day 9 of postpartum, group-3 was also significantly (P<0.05) different from group-2 but similar with group-1. However, on day 48, group 2 was significantly different (P<0.05) from group-1 and group-3. Moreover, result also showed that there was slow process of involution of gravid horn in group-3 in first two weeks of postpartum period after exogenous oxytocin administration as observed in cervix and body of uterus during postpartum involution period. The real involution days were observed on days: 41±3.02, 36±3.02 and 39±2.85 versus 43, 44 and 42 days which were calculated through regression among three treatment groups: 1, 2 and 3 respectively. Similarly, the mean values of the diameters of the gravid horns in treatment groups: 1, 2 and 3 were 3.02±0.22cm, 2.97±0.22cm and 2.97±0.21cm at their respective involution days. By considering less than 3.10 cm value as the standard for complete involution of gravid horn, the first animal was involuted on day 20 in group-1 and group-2 while in group-3, it was day 23 correspondingly. All of the animals in control were involuted between days 20 to 44. Similarly, in low dose (10IU) all animals were involuted between days 20 to 41 but this range existed between days 23 to 44 in high dose (30IU). The convergence of mean value of gravid horn towards the involution days was presented in Figure 6. The regression slope coefficient indicated that the value of pregnant horn decreased at the rate of 0.10522cm, 0.093cm and 0.13227cm per day for each treatment groups: control, low dose (10IU) and high dose (30IU), respectively. Moreover, it was observed that predicted results were somewhat closely related with the true ones.

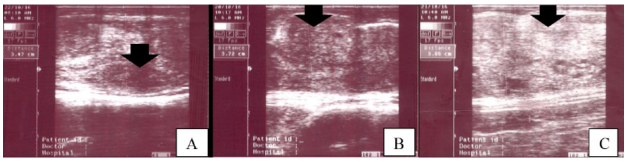

Fig. 5. Ultrasonographs of gravid horn in three treatment groups: Control; A, Low dose 10IU; B, High dose 30IU; C at fifth week near complete involution. Cross sectional ultrasound images of three treatment groups in Figure 5 showing normal echogenicity in A and B parts while enhanced echogenicity in C part.

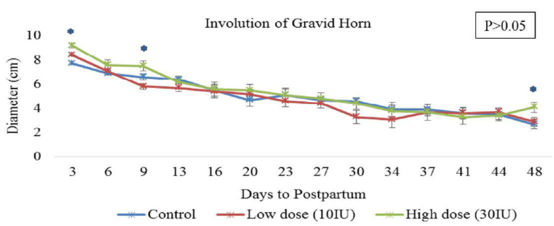

Fig. 6. Comparison of means ± S.E (cm) of gravid horn diameter among three treatment groups between 3 to 48 days of postpartum period in Nili-Ravi buffaloes. Means± S.E with asterisk sign on days: 3, 9 and 48 of postpartum differs at P < 0.05 among three treatment groups.

Non-gravid horn (NGH)

On ultrasonography, images were looked anechogenic fluid filled zones with hyper echoic borders in all three treatment groups at initial involution days. The echo texture of non-gravid horn changed as the involution approaches and became moderate echogenic potato chips like structures without any anechoic fluid filled regions (Figure 7). From the result, it was found that overall effect of treatments on non-gravid horns was found non-significant (P>0.05) throughout postpartum involution period. On pairwise comparison by utilizing the DMRT, it was found that the treatments had significant (P<0.05) effect on days: 3, 16 and 48. In addition, on day 3 of postpartum period, there was significant difference (P<0.05) among the treatment groups: 1, 2 and 3 whereas regarding about day 16 of postpartum interval, it was shown that oxytocin treated groups had significant difference (P<0.05) from control group. However, on day 48 of postpartum period, group-2 was significantly (P<0.05) different from group-3 but alike with group-1. Furthermore, it was also revealed that non-gravid horn in group-3 also attained slower progression rate of involution in first two weeks of postpartum period unlike to groups; 2 and 3. Similar trend of slow regression in group-3 was observed in cervix, cervic of uterus and pregnant horn in first two weeks of postpartum involution interval. The effect of treatments on all other days and as a whole was found non-significant (P>0.05). The lowest mean value (1.74cm) of non -gravid horn of control group-1 was observed on day 41. Similarly, the lowest mean value of low dose (10IU) group-2 and high dose group-3 was recorded on day 34 and day 41, accordingly. The value of non-gravid horn less than 3.0 cm considered the criteria for involution. From the results, the first animal in control and low dose (10IU) was involuted on day 23 while, in high dose (30IU), it was on day 27. From the results, it was noted that, all animals were got resumption of involution stage in non-pregnant horn between days 23 to 44 in group-1 and group-2, whereas, the animals that represented the group-3 involuted between days 27 to 41.

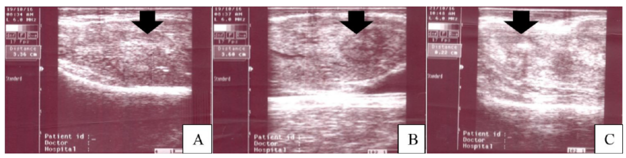

Fig. 7. Ultrasonographs of non-gravid horn in three treatment groups: Control; A, Low dose 10IU; B, High dose 30IU; C at fifth week near complete involution. Arrow head in C part of Figure 7 shows more echogenicity parallel to A and B parts.

Approximately 62.5% of involution was occurred between 34-44 days in control and low dose, while 55% in high dose respectively. Similarly, 37.5% in control, 25% in low dose exhibited involution between 23-31 days and 45% animals in high dose attained complete involution between 27-31 days. Only 12.5% involution was found in low dose after 44 days while all animals of control and high dose groups had already completed involution before 44 days. The graphical representation of mean value of non-gravid horn given in Figure 8 was also showing the interactive pattern of involution days. After running the regression analysis, it was found that the value of non-gravid horn decreased 0.10089 cm per day for control, 0.08822cm and 0.12518 for low dose (10IU) and high dose (30IU) correspondingly. The animals that were given highest dose (30IU) slightly showed more rapid involution which was closely related to regression results. The factual detected involution days were 39±2.62, 38±2.62, 37±2.50 while expected calculated involution days were 33, 39 and 36 in all the treatment groups: 1, 2 and 3 respectively. These results were somewhat associated to each other in oxytocin injected groups while some deviation from control which explicated the justification of involution facts in non-gravid horn. The mean diameters of non-pregnant horn measured at involution days in control, low dose 10IU and high dose 30IU were 2.80±0.24cm, 2.68±0.24cm and 2.94±0.23cm correspondingly (Figure 8).

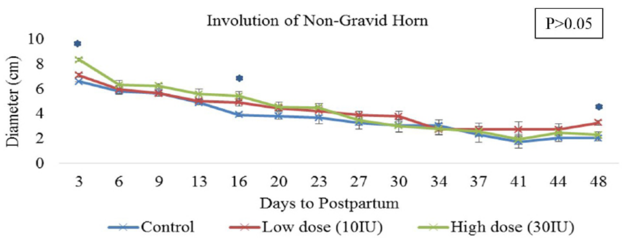

Fig. 8. Comparison of means ± S.E (cm) of non-gravid horn diameter among three treatment groups between 3 to 48 days of postpartum period in Nili-Ravi buffaloes. Means± S.E with asterisk sign on days: 3, 16 and 48 of postpartum differs among three treatments at $P < 0.05$.

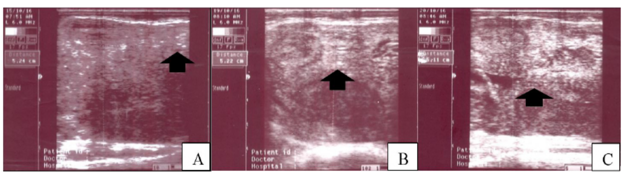

Fig. 9. Ultrasonographs of caruncles at involution in three treatment groups: Control; A, Low dose 10IU; B, High dose 30IU; C at the end of second week near complete regression. Arrow heads show caruncular points with enhanced echogenicity in C part of Figure 9.

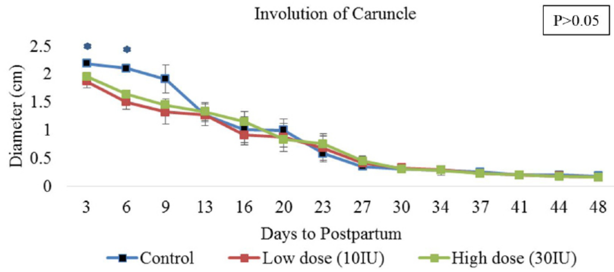

Fig. 10. Comparison of means ± S.E (cm) of caruncular diameter among three treatment groups between 3 to 48 days of postpartum period in Nili-Ravi buffaloes. Means± S.E with asterisk sign on days; 3 and 6 of postpartum differs at $P < 0.05$ among three treatment groups.

Caruncular diameter

Result showed that ultrasound scan of caruncules was imaged as bright echogenic distinctive structures in all three oxytocin administered groups on day 3 of postpartum. Further results revealed that caruncles went under almost complete regression in most of the cases within two weeks and in certain cases three weeks as shown in Figure 9. Only small pointed bright echogenic structures appeared later after involution. The mean ±S.E of caruncular diameter are shown in Figure 10. From the result, it was found that treatments had the same effect on caruncular diameter ($P>0.05$). But the DMRT showed significance ($P<0.05$) among treatments on caruncular diameter on days; 3 and 6 of postpartum interval. Result also showed that significantly ($P<0.05$) rapid regression was present between control and oxytocin treatments on day 3 of postpartum period while low dose (10IU) was significantly ($P<0.05$) different as compared to control and high dose (30 IU) on day 6 of postpartum involution period after oxytocin injection. It was known that all three treatments had non-significant ($P>0.05$) effect on caruncular diameter as a whole and on all other postpartum days. The standard of caruncular diameter was considered more or less equal to 1 cm at suggested involution as defined by other researchers (Atanasov *et al*., 2012). The standard value of caruncular diameter in control was observed on days; 16 to 20 whereas, low dose (10IU) had the standard value of caruncular diameter between days; 13 to 16. Similarly, the standard value of high dose (30IU) fell between days; 16 to 20. However, the exact caruncular measurements in the present study with respect to relative standard measurements in previously documented studies were 1.00±0.20, 1.00±0.16 and 0.99±0.15 in control, low dose (10IU) and high dose (30 IU) groups on days; 19, 20 and 19 of postpartum period respectively. Moreover, control had overall mean 0.849 cm with lowest mean 0.18 recorded on day 48 while the overall mean of injected groups; low dose (10IU) and high dose (30IU) were recorded 0.730 and 0.779 with their lowest mean 0.16 and 0.16 on day 48 respectively.

Progesterone concentration (P4)

Results of progesterone concentrations of different treatment groups revealed that non- significant (P>0.05) changes observed among all the treatment groups: 1, 2 and 3. The changes in mean ±S.E of the progesterone concentrations had been represented in Figure 11 with respect to their days. Whereas, the posthoc test DMRT showed that group-2 had significantly (p<0.05) higher progesterone concentration as compared to group-1 (control) but non-significant (P>0.05) with group-3 on day 20 of postpartum interval. Progesterone concentrations were similar in all treatments in all other days of treatment. During treatment interval, the overall mean values of progesterone were 0.368, 0.418 and 0.221 ng/mL in control, low dose (10 IU) and high dose (30IU) respectively. Furthermore, it was also observed that low progesterone concentration was detected in high dose (30IU) group but non-significantly (P>0.05) as compared to other two treatment groups; control and low dose (10IU).

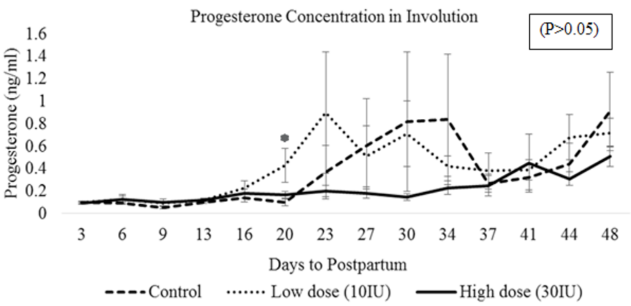

Fig. 11. Comparison of means ± S.E (cm) of P4 concentration among three treatment groups between 3 to 48 days of postpartum period in Nili-Ravi buffaloes. Means± S.E with asterisk sign on day 20 of postpartum differs among three treatments at P < 0.05.

Milk composition

Result of the milk composition during involution period showed that fat (%), SNF (%), protein (%), lactose (%), minerals (%) and freezing point were significantly (P<0.05) increased in oxytocin treatment; low dose (10IU) and high dose (30IU) parallel to control. Moreover, results also indicated that density (%) and pH were non-significant (P>0.05) among three treatments: control, low dose 10IU and high dose 30IU (Table I).

Table I. Mean±SE of milk composition parameters during involution period in Nili-Ravi buffalo.

Parameters	Control n=56	Low dose 10IU n=56	High dose 30IU n=56	P value
Fat (%)	3.81±0.20[a]	4.60±0.20 [b]	4.59±0.26[b]	0.017
SNF (%)	10.27±0.05[a]	10.55±0.05[b]	10.55±0.06[b]	0.001*
Density (m/v)	34.63±0.26[a]	35.08±0.29[a]	35.02±0.37[a]	0.533
Freezing point	0.64±0.00[a]	0.67±0.00[b]	0.67±0.00[b]	0.000*
Protein (%)	4.00±0.02[a]	4.14±0.02[b]	4.12±0.03[b]	0.001*
Lactose (%)	5.40±0.03[a]	5.55±0.03[b]	5.54±0.04[b]	0.003*
Solids (%)	0.84±0.00[a]	0.86±0.00[b]	0.86±0.00[b]	0.001*
pH	6.44±0.02[a]	6.48±0.02[a]	6.48±0.02[a]	0.219

[a,b] different lower case letters represents row wise significant difference among treatments; P<0.05 denotes significant difference among treatments.

DISCUSSION

The present study was aimed to be aware with the effect of indiscriminate use of oxytocin on cervix, uterus and progesterone concentration during postpartum period. The results revealed that overall exogenous oxytocin injection possessed no significant effect on cervix during postpartum period among treatment groups. These results were in accordance with findings of Stewart and Stevenson (1987) in Holstein cows but contradictory to Atanasov *et al.* (2012) where it was elucidated that exogenous oxytocin had rapidly decrease the size of cervix during first three weeks of postpartum involution period in Bulgarian Murrah buffaloes. In this study, the mean cervical involution days were 43±2.81, 37±2.81 and 36±2.65 in control, low dose (10IU) and high dose (30IU) groups with mean sizes 2.71±0.14cm, 2.91±0.14cm and 2.65±0.14cm, respectively, when measured at their corresponding involution days. These findings about the involution day of cervix were similar with the results in various buffalo breeds (Chauhan *et al.*, 1977; Agrawal *et al.*, 1978; Chaudhry *et al.*, 1987; Chaudhry *et al.*, 1990; Shah *et al.*, 2004; Parikh *et al.*, 2017) but either no oxytocin treatment or treated with prostaglandin F2α injection, respectively, but dissimilar with reports in in cows and buffalo (Stewart and Stevenson, 1987; Atanasov *et al.*, 2012). The mean cervical involution days in cows and buffalo after exogenous oxytocin administration were 28-34, 26, 25.6 and 28, respectively (Usmani *et al.*, 1985; Stewart and Stevenson, 1987; Atanasov *et al.*, 2012).

Regarding cervical diameter measurements, these were lower from the values illustrated in Nili-Ravi and Murrah buffaloes (Usmani *et al.*, 1985; Iqbal *et al.*, 2003; Atanasov *et al.*, 2012) which lied between 3.2 to 3.1 cm but higher than the value scanned by Parikh *et al.* (2017)

in Mehansa buffalo 1.75 to 1.64 cm at involution day nevertheless without any oxytocin treatment or some hormonal therapy PGF2α during postpartum period. Almost similar cervical diameter measurement after exogenous oxytocin administration i-e 3.0 cm to 2.8 cm on involution day as described by Stewart and Stevenson (1987) in cows. The differences in the results of cervical involution may be due to variation in breed, region, and interval to first injection used after parturition and researcher technique. In addition to these, some limited effects were also seen for short duration. There was significant difference between group-1 and group-3 but non-significant with group-2 on day 3 of postpartum period. Similarly, on day 16, there was considerably more discrepancy between control and treatment groups. The results also explained that there was slow involution in treatment groups as compared to control, hence, more regression in the size of cervical diameter of control in first two weeks after treatment application but later on no such effect was observed. This delay in the involution of cervix was suggested that it might be due to repeated usage of exogenous oxytocin which might block the action of endogenous oxytocin by changing the behaviour of oxytocin receptors and also complex hormonal interaction at hypothalamus level. At the same time, on day 48, again significant changes were observed between group-2 and groups; 1 and 3. It was assumed that enhancement of cervical activity was responsible for increased size of cervix in group-2 as compared to other two groups; 1 and 3 which was actually due to resumption of cyclicity of different animals in group-2. The increase in the size of cervical diameter was the result of estrus but not of treatment effect. It could be reflected that the increase of cervical diameter possibly due to follicular growth activity which could enhance estrogenic circulation towards cervix during postpartum involution and finally tissue swelling resulting into more average cervical diameter (Lohan *et al*., 2004).

Likewise results of cross sectional images of ultrasound related to body of uterus depicted that hypo echoic fluid filled areas with hyper echoic border initially, and on involution day, it became moderate echogenic structure with more echogenic borders without any anechogenic lumen. These sonographic finding were similar as described by Parikh *et al*. (2017) in Mehansa buffalo. However, it was found that the echo texture of cervic of uterus was slightly more echogenic in oxytocin administered group-3 as compared to group-1 and group-2. The mean diameters of body of uterus on involution day among treatment groups were 2.82±0.14, 3.02±0.14 and 3.09±0.13, respectively, while cervic of uterus attained non-pregnant size on days: 41±2.00, 43±2.00 and 40±1.88 congruently in three treated groups. By using ultrasonic tool, Ramoun *et al*. (2006) described that complete uterine involution occurred between 30.2±1.37 and 36±1.65 days in Methergin treated and control groups. Similarly, one group of researcher elucidated that complete involution occurred between 32 to 36 days with or without PGF2α treatment in buffalo (Iqbal *et al*., 2003). In the current study, the measured days at complete involution are higher as compared to studies by various dairy scientists in oxytocin injected and non-injected buffaloes (Iqbal *et al*., 2003; Ramoun *et al*., 2006). According to results in the given study, it was also seen that on days; 6 and 13 of the treatments, there were significantly slow regression in the body of uterus in oxytocin treated groups as compared to control. The results of current study are to some extent contrary to the study of Abdel-Khalek *et al*. (2013) at one point and agreeing with other point in which PGF2α and oxytocin injected cow showed rapid uterine regression in first three weeks and later on. Overall, there was no significant effect during the whole course of complete involution as compared to control. This showed that exogenous oxytocin had some negative effect on uterus to a certain degree in oxytocin treatment groups especially in group-3 at earlier days of postpartum uterine involution. Later on, these effects were minimized and perhaps due to resistant developed against the treatment. This is suggested that it might be due to competition between endogenous and exogenous oxytocin at receptors level which resisted the action of exogenous oxytocin administration. Secondly, exogenous oxytocin might affect the endogenous secretion of oxytocin at hypothalamus or pituitary level. Moreover, major part of exogenous oxytocin might be utilized for milk let down rather than exhibiting more pronounced action on myometrium of uterus in oxytocin injected groups as compared to control during milking in postpartum involution period. According to one study which has been reported by the scientists McCracken *et al*. (1984) that once oxytocin receptors are occupied, they need six hours regeneration time which may limit the exogenous oxytocin effect on uterine contraction. Previously, there were no any reports about the measurements of the uterine body diameter in Nili-Ravi buffalo. The observations of present study provided evidence that exogenous oxytocin administration had generally little or no effect (P>0.05) on gravid and non-gravid horns during postpartum involution period which is similar with finding of latest study in cows (Stephen *et al*., 2019). Likewise, ineffectiveness of treatment may be perhaps due to decrease in receptors sensivity at the time of initiation of treatment but conversely, increase between peri-partum and 24 hrs of postpartum period (Taverne *et al*., 2001). However, it was later on decreased within 48-72 hours after parturition as reported in ewe (Sheldrick and Flint, 1985). In addition, it is also identified by Del-Vecchio *et al*. (1990) that exogenous oxytocin affects differently on uterus because of dose and day of administration during postpartum involution interval. Ultrasonography of pregnant and non-pregnant horns provided supplementary information about the involution process. It was seen that both horns showed rapid regression during first two week of involution period. Later on, involution process was much slower and was approximately @ of 0.05 cm per day till complete involution was accomplished in each case of animal. These ultrasonic outcomes were similar with sonographic results in Murrah buffaloes (Lohan *et al*., 2004; Atanasov *et al*., 2012). It was also found that non-gravid horn involuted earlier as compared to pregnant horn (Usmani *et al*., 1985; Atanasov *et al*., 2012). Moreover, result shown that treatment groups: 1, 2 and 3 were significantly (P<0.05) different among each other on day 3. However, on day 9 of involution, rapid regression was seen in low dose 10IU as compared to control and high dose 30IU. This might be due to short estrous cycle experienced by some animals in low dose 10IU in which endogenous along with exogenous oxytocin had combined action on pregnant horn whereas no effect was seen in control and high dose 30IU injected groups, respectively. Nevertheless, on day 48, again significant differences were seen between low dose 10IU as compared to control and high dose 30IU treatment groups. This was due to initiation of estrous rather than exogenous oxytocin effect in different animals of the low dose 10IU group. Similarly, in non-gravid horn, a considerable (P<0.05) effect was seen on day 3 of the treatment among control, low dose 10IU and high dose 30IU. Alternatively, on day 16 again significant (P<0.05) differences were observed in control and oxytocin injected groups. These differences seem due to negative effect of oxytocin which ultimately slows the process of involution in oxytocin injected groups. Secondly, this was probably due to initiation of cyclicity in certain animals in control group-1 which naturally experienced rapid involution as compared to oxytocin treated groups-2 and 3, respectively. However, on day 48 of postpartum involution period, low dose 10IU showed noteworthy differences (P<0.05) parallel to control and high dose 30IU. This is suggested again due to initiation of cyclicity and more estrus response in certain animals in low (supraphysiological) dose 10IU group. The process of slow involution in earlier postpartum period in gravid and the non-gravid horns might be due to repeated (supraphysiological) doses of exogenous oxytocin treatments. This repeated exogenous oxytocin administration at (supraphysiological) high dose 30IU may alter the gonadotropin LH (Robinson and Evans, 1990) and perhaps indirect inhibition of estrogen hormone secretion during involution intermission which is essential for priming the uterine receptors for endogenous or exogenous oxytocin knock at myometrium. Further studies are suggested to elucidate the mechanism of slow regression of uterine horns at exogenous administration of high oxytocin doses during first two weeks of postpartum period. Moreover, a little or unavailability of oxytocin receptors after 72 hours on myometrial and endometrium cells of uterine horns can be contributory factors as earlier described in ewe (Sheldrick and Flint, 1985). In addition to these, it was also identified that non-gravid horn attained normal non pregnant size more rapidly as compared to pregnant horn during postpartum involution interval among treatment groups (Atanasov *et al*., 2012). The results of this study are one and same with the findings of Parikh *et al*., (2017) in Mehansa buffalo and Friesian cows (Abdel-Khalek *et al*., 2013) but contradictory to reports explicate in Nili-Ravi buffaloes (Iqbal *et al*., 2003). However, these researchers used PGF2α instead of exogenous oxytocin to evaluate its effect on postpartum period in buffalo. As for as involution days among three treatments groups, it was perceived that gravid and non-gravid uterine horns resumed to their normal non pregnant size on days: 41±3.02, 36±3.02, 39±2.85 and 39±2.62, 38±2.62, 37±2.50, respectively. The mean day at complete involution in all three treatments are in agreement with involution days documented by different dairy scientists in Japanese cow, Egyptian and Mehansa buffaloes, respectively, (Okano and Tomizuka, 1996; El-Wishy, 2007; Parikh *et al*., 2017) which is approximately day 40 where no any treatment or PGF2α treatment was applied. This was contrary to the findings of Atanasov *et al*. (2012) and Khatri *et al*. (2013) in Bulgarian Murrah and Kundi buffaloes, respectively, in which complete involution achieved on days; 25-34 and 34 correspondingly afterward oxytocin injection during postpartum interval. This variation in the results of involution day may be due to following possible reasons: postpartum injection day, dose regime, measuring technique and breed differences. Moreover, many researchers used other than oxytocin treatments during postpartum period to measure involution days in Nili-Ravi, Surti, Italian, Egyptian, Indian Murrah and Swamp buffaloes. In these animals, complete involution is reported on an average: 29, 31, 33, 30, 26, 26 and 29 days, respectively, which are different to present study (Usmani *et al*., 1985; Iqbal *et al*., 2003; Presicce *et al*., 2005; Khasatiya *et al*., 2006; Ramoun *et al*., 2006; Yindee *et al*., 2007; Snel-Oliveira *et al*., 2010). However, different treatments were used in these various breeds of buffaloes which were independent to one another. These consisted of PGF2α, Methergin and saline or no any treatment injected. So far diameter of uterine horns, it decreased rapidly in first two weeks and then slowly dropped until complete involution. The mean diameters of the gravid and non-gravid horns in treatment groups: 1, 2 and 3 were 3.02±0.22cm, 2.97±0.22cm and 2.97±0.21cm, and

2.80±0.24cm, 2.68±0.24cm and 2.94±0.23cm at their particular involution days, respectively. The results of average diameters of gravid and non-gravid horns of oxytocin injected groups; 2 and 3 are closely related with the findings of few researchers (Iqbal *et al*., 2003; Atanasov *et al*., 2012). In collective, there were no specific differences detected among the treatment groups during postpartum involution period on cervix, body of uterus and uterine horns after exogenous oxytocin injection. These results are similar with the findings of one research in kundi buffalo (Khatri *et al*., 2013) and contrary to work done in Bulgarian Murrah buffalo (Atanasov *et al*., 2012) after exogenous administration of oxytocin.

In the current study, it was found that all the treatments had the same effect on caruncular diameter (P>0.05). This may be perhaps due to least or unavailability of oxytocin receptors on myometrium during the treatment interval. However, it was observed that caruncular size with respect to diameter decreased rapidly as the involution progressed. This decrease in the size of caruncular diameter was found rapid and significant (P<0.05) in oxytocin treated groups as compared to control group on days 3 and 6 of postpartum. This seems oxytocin treatments might had some effect in first week of postpartum period. These results are partially one and same as described by Atanasov *et al*. (2012) in Bulgarian Murrah buffaloes. Ultrasonically, caruncles's regression appeared as hypoechic area with hyperechoic line. It was further found that completely regressed caruncles appeared as bright echogenic spots inside uterus. The caruncules on the day of involution were faintly recognizable but with careful examination. In the current study, it is suggested that caruncles acquired complete regression in similar fashion as other uterine parts and these also required 3-6 weeks for complete regression. The expected caruncular involution days at their complete regression was persisted on day 41 with their mean diameter 0.20±0.02 in all three treatment groups, respectively. The regression process in caruncles also completed parallel to cervical and uterine involution. Previously, no sonographic measurements of caruncules were made after second week of involution. In the present study, the mean caruncular diameters at complete regression in three treatment groups on the basis of relative measurements of previously reported studies were 1.00±0.20, 1.00±0.16 and 0.99±0.15 in control, low dose 10IU and high dose 30 IU groups on days; 19, 20 and 19 of postpartum period, respectively. Later on, it was difficult to differentiate between caruncular structures and under lying uterine wall. However, similar type of caruncular diameter reading; 0.80±0.60 on day 10 of postpartum involution interval was documented by Atanasov *et al*. (2012). These results are partially in consistent with finding of Agrawal *et al*. (1978) in which they had reported complete regression of caruncles occur after second week mostly and in some cases third week of postpartum involution interval but contrary to exploration of Atanasov *et al*. (2012) where they had predicted day 10 of postpartum involution period. Apparently, earlier regression was observed in oxytocin treated groups as compared to control group but all this was non- significant (P>0.05). These differences of postpartum days in caruncles regression among treatments in present and previous studies may be due to differences in response of oxytocin receptors, dose, postpartum treatment interval and breed.

As far as progesterone (P4) analysis, result showed that P4 concentration remained at the lowest level during involution period among the treatments. Only on the day 20 of postpartum interval, considerable difference (P<0.05) was present between low dose 10 IU group and other two groups; high dose 30IU and control, respectively. This difference among the treatment groups did not seem due to exogenous oxytocin administration. This perchance due to initiation of cyclicity in certain animals in group 2 which probably raised the overall level of progesterone in group-2 parallel to groups; 1 and 3 as reported in some studies in dairy animals (Shah *et al*., 2004; El-Wishy, 2007). Further, it may be suggested that the increase in the concentration of P4 in low dose 10IU may be due to dynamic interactive response of oxytocin and gonadotropins at hypothalamus pituitary axis as compared to control and high dose 30IU at the time of normal estrous cycle initiation. Moreover, results also shown that overall P4 concentration was remained non-significant (P<0.05) among the treatments. This perchance might be due to anestrous stage in most of the animals. Therefore, it is perceived that progesterone concentration maintained to a lowest level during postpartum period. Apparently, it was observed that P4 concentration was lower in high dose 30IU as compared to control and low dose 10IU groups but it was non-significant (P>0.05). Perhaps, it might be due to persistent indirect luteolytic effect of exogenous oxytocin on the regressing corpus luteum (CL) of pregnancy to influence the local triggering of PGF2α persistently during postpartum period which held responsible for complete regression of pregnancy CL actively, hence, lowered P4 concentration in high dose 30IU. Secondly, premature luteolysis is also suggested in high dose 30IU treatment as compared to control and low dose 10IU. Analogues to corpus luteum (CL), previously P4 analysis was not done after exogenous administration in postpartum involution period in buffalo. However, it was documented that P4 levels were below the 0.1ng/mL and usually at basal level at calving and normally ranged between 0.1-0.6 ng/mL (Eissa *et al*., 1995; Tiwari *et al*., 1995). This basal level of P4 was because of quiescent ovaries in most of the time during involution period but intermittent elevation was also occurred near start of cyclicity as reported by various dairy researchers (Shah *et al*., 2004).

Additionally, in the present study, milk composition is also evaluated to know the effect of exogenous oxytocin administration on milk in Nili-Ravi buffalo during postpartum involution period. In the existing study, results revealed that there was overall low fat percentage in all the three groups. The means percent values of fat measured in this study are 3.81±0.20, 4.60±0.20, 4.59±0.26 among three treatments: control, low dose 10IU and 30IU, respectively. These mean values are slightly higher in comparison to one study in Nili-Ravi buffalo (Abbas *et al*., 2014) but lower than other study (Akhtar *et al*., 2012). This overall decrease in fat value may be due to nutrition, season and early stage of lactation in which colostrum (protein) contents are more as compared to mid and late lactation intervals. However, fat percent was significantly increased ($P<0.05$) in oxytocin treated groups as compared to control. This increase of fat percent in treatment groups may be due to accelerated secretion of milk fat globules through tight junction via para-cellular pathway when oxytocin forcefully contract myoepithelial cells of alveoli in mammary glands. These results are in agreement with the findings of some studies in buffaloes at certain lactation, month and season (Abbas *et al*., 2014; Murtaza *et al*., 2017) and in cows (Stewart and Stevenson, 1987; Dymnicki *et al*., 2013) but opposite to the findings of other studies in buffaloes (Bidarimath and Aggarwal, 2007; Akhtar *et al*., 2012; Abbas *et al*., 2014; Shahid *et al*., 2016) and in cows (Allen, 1990; Nostrand *et al*., 1991; Ballou *et al*., 1993; Hameed *et al*., 2010) where either decrease or no change in fat percent values are reported. Similarly, SNF, FP, protein, lactose and solids percentages in low dose 10IU and high dose 30IU were significantly ($P<0.05$) higher as compared to control, respectively. These results reveal that oxytocin has somewhat effect on milk composition in different treatment groups. This increase was might be due to active transport of small milk molecules which were mobilized during forceful milk let down due to which concentration of all these molecules/contents increased with respect to control but similar between low and high dose injected treatment groups. The result of protein is analogue to some studies in buffalo (Bidarimath and Aggarwal, 2007; Abbas *et al*., 2014) and cows (Wheelock *et al*., 1965) while contrary to other studies in dairy buffaloes (Shahid *et al*., 2016; Murtaza *et al*., 2017) and cows (Stewart and Stevenson, 1987; Allen, 1990; Ballou *et al*., 1993; Dymnicki *et al*., 2013; Hameed *et al*., 2016) in which either decrease or no change in protein percent in oxytocin administered dairy animals's milk. The result of lactose percent in the present study is comparable to the one study in buffalo (Shahid *et al*., 2016) and cows (Penry *et al*., 2017) where increase in lactose percent was documented in certain months, and test udder with oxytocin and without oxytocin injected milk, respectively, but different to other studies in buffaloes (Shahid *et al*., 2016; Murtaza *et al*., 2017) and cows (Allen, 1990; Ballou *et al*., 1993; Werner-Misof *et al*., 2007; Hameed *et al*., 2010; Dymnicki *et al*., 2013) where decrease or unchnage lactose percent values were presented. The current results of SNF are in accordance with one study in buffalo (Abbas *et al*., 2014) but contrary to the results of other studies in buffalo (Shahid *et al*., 2016; Murtaza *et al*., 2017) and cow (Dymnicki *et al*., 2013) where SNF percent values either decreased or not changed in oxytocin test milk and control. Likewise, the result of freezing point, this is not one and same with the results of the previously conducted studies in buffalo (Murtaza *et al*., 2017) and sheep (Zamiri *et al*., 2001) in which either decreased or no change in freezing point was reported. Certainly about the result of percent solids values in current study, these are equivalent to the results of earlier studies performed in buffaloes (Abbas *et al*., 2012) and cows (Allen, 1990; Hameed *et al*., 2010) in which increase in certain solids contents were reported but different to other studies in buffaloes (Murtaza *et al*., 2017) and cows (Hameed *et al*., 2010) where decrease in certain type of solids contents had been documented. This increase in mean values of SNF, protein, lactose, freezing point and solids perhaps may be due to increase of permeability and diffusion rate in my epithelial cells tight junction and blood capillaries in oxytocin injected milk let down in comparison to normal milk let down process and as a result overall gland output increased. This was suggested in one study where increase of tiny milk molecules mobilization within the mammary tissues was reported (Lollivier and Marnet, 2005). In contrast, density and pH were alike in all three treatment groups where oxytocin injection exhibits no effect. These results are disagreeing with the previously conducted studies in buffaloes (Akhtar *et al*., 2012; Abbas *et al*., 2014; Murtaza *et al*., 2017) where either increase or decrease were noted at certain lactation stage and months. However, these results are similar with one study in sheep where no change in pH and density was reported (Zamiri *et al*., 2001). Moreover, inconsistency in the results of present and previous studies may be due to breed, species, dose, season, environment and stage of the lactation and time elapsed from calving.

CONCLUSIONS

It can be concluded that exogenous oxytocin possibly has little role on involution process, however, it may be involved to increase certain milk components in postpartum involution interval.

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Statement of conflict of interest

Authors have no potential conflict of interest.

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