Postpartum Performance Of Buffaloes Treated With Gnrh To Overcome The Impact Of Placenta Retention

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Abstract: This study aimed to investigate impacts of GnRh treatment on post-partum productive and reproductive performance of buffaloes subjected to placenta retention. A number of 30 female buffaloes were used in the study among them 20 buffaloes were detected with retained placenta (RP), while 10 buffaloes were normally calved (NRP). Buffaloes with RP were divided into two groups (10 buffaloes each) where group (RPT) were injected with 10 ml GnRH at the 7th day postpartum and group (RPC) served as control group. Blood samples were collected twice weekly from each buffalo cow during late pregnancy and postpartum period for determination of progesterone (P4), estradiol 17β (EST) as well as some blood metabolites. Placental tissue samples were taken from four animals with normal and retained placenta for histological examination. Postpartum loss in live body weight was greater (P <0.01) in NRP buffaloes than animals with RP. Differences between groups in calf birth weight (CBW) were insignificant while differences between newborn males and females were highly significant (P < 0.01). Volume of fetal fluids was greater in NRP group comparing with the other groups (P < 0.01) whereas no significant differences were detected in weight of fetal membranes between groups. Time elapsed for placenta expulsion in was 4.23, 17.26 and 18.7 hr. in NRP, RPT and RPC groups, respectively. Sex of newly born calf had only a significant effect (P < 0.01) on CBW and CBW/DAM. The normal group of buffaloes (NRP) achieved the least (P < 0.01) calving interval (CI) and days open (DO) as compared with buffalo groups with RP. However, GnRH treatment had significantly (P < 0.05) reduced CI and DO for group RPT than that for group RPC by 10.41% and 28.33%, respectively. No. of services per conception declined in response to GnRH treatment (2.6) when compared with RPC group (3.5). Differences between the studied groups in milk traits (total milk yield, days in milk and daily milk yield) were highly significant (P < 0.01) not only in the current milking season but also in the previous and next milking season. Buffaloes treated with GnRH (RPT group) achieved greater milk productivity (13.27%) than RPC group. Post partum concentrations of P4 were significantly (P <0.05) greater in NRP animals than that in buffaloes with RP throughout the experimental months. GnRH treatment increased significantly (P < 0.05) postpartum EST concentrations during 5th to 8th week as compared with non-treated animals. Concentrations of all studied metabolic parameters were relatively lees in RP groups than that in non retained group (NRP). GnRH treatment had relatively ameliorated the metabolic function in treated buffaloes via increasing concentrations of blood total protein, glucose, creatine, creatinine, clacium and inorganic phosphorus. The histological sections revealed dismaturation of the RP denoted by limited number of trophblastic giant cells, decomposition and fragmentation of the placental tissue and chorionic villi concomitant with hyperplasia in the chorionic epithelial cell of the villi. [Journal of American Science 2010; 6(5):225-233]. (ISSN: 1545-1003).

Keywords: Buffaloes , retained placenta, GnRH, productive and reproductive traits.

1. Introduction

Buffaloes represent the main dairy animals raised in small or medium size holdings and play an important role in the animal agriculture eco-system in Egypt. However, buffaloes are characterized by low reproductive efficiency as they achieve longer calving intervals (El-Rigalaty, 1995). This factor compromises a major impediment for buffalo productive performance of milk and meat. Placenta retention is one of the reproductive disorders affecting profitability of buffalo production since it delays uterine involution, predisposes females to ovarian cystic degeneration and reduces fertility. El-Malky (2007) demonstrated that retained placenta (RP) was observed in 4.6% cases of buffaloes over three years of study. The higher levels of progesterone (P4) concomitant with a significant lower level of oestradiol (EST17β) at delivery leads to incidence of RP (Hashem and Amer, 2008). Several investigators pointed out some factors that predispose for RP such as pre partum metabolic disturbances (Michal *et al*. 2006) season of the year (El-Malky (2007), dystocia (Thompson *et al.,* 1983), animal age (Erb and Martin, 1980) and hormonal levels (Bous *et al.,* 1984). The retained placenta (RP) frequently results in a secondary bacterial infection and subsequently depresses fertility (Hashem and Amer, 2008), prolonged calving interval, loss of milk production (Rajala and Grohn, 1998) and higher costs of veterinary treatment.

Several attempts have been made to adopt exogenous treatment with gonadotrophic releasing hormone (GnRH) for inducing ovulation and

resumption of ovarian activity in animals-suffering from reproductive disorders (Aboul-Ela *et al*. 1985 and Barkawi and Aboul-Ela 1987). The present study aimed to investigate impacts of GnRh treatment on post-partum productive and reproductive performance of RP buffaloes. In addition, the study aimed to compare levels of some hormones and blood constituents of buffaloes during pre-partum and post-partum periods to elucidate possible association between those parameters and incidence of RP as well as to examine physiological and histological changes in intact and retained placenta taken from buffaloes.

2. Material and Methods

The experimental procedures:

This work was conducted at the Research Station of Mehallet Mousa belonging to the Animal Production Research Institute. A number of 30 female buffaloes were chosen to carry out the experimental work, among them 20 buffaloes were detected with retained placenta (RP), while 10 buffaloes were normally calved (Group NRP). As a general rule in the study, placenta was considered retained if it remained for a postpartum period longer than 12 hours (Laven and Peters, 1996). Buffaloes with RP were divided into two groups (10 buffaloes each) where group (RPT) were injected with 10 ml GnRH (Receptal™) at the 7th day postpartum and group (RPC) served as control group. During the experimental period, close observation was undertaken at the late pregnancy (one month before calving) and continued three months after delivery. Birth weight of the offspring (CBW) was recorded and the time elapsed for placenta expulsion was determined for each dam. Also, dam weights before and after parturition were recorded. Weight of completely intact (PTW) or damaged placenta from buffaloes with RP was also recorded. As regular farming system, the experimental buffaloes were included in mating groups one month after parturition, where pregnancy diagnosis through rectal palpation was adopted subsequently after two months postpartum.

Management and feeding:

Buffaloes at late pregnancy period were kept in their shed until time of delivery then they were transferred to the maternity unit. After delivery the newly born calves were kept for one week with its dam for colostrums feeding. The dam was then transferred to the milking unit. The experimental buffalo cows were housed in open sheds and subjected to regular managerial practices of the breeding stock.

Animals were fed according to their live weight and milk production, feed allowances were offered for animals as recommended by APRI (1997). The animals were fed on concentrate feed mixture (CFM) twice daily along with wheat or rice straw and clover hay, when available. Buffalo cows were hand milked twice daily and milk yield was recorded at each milking. Water was freely available in water troughs except at the milking time.

Blood sampling:

Blood samples were collected twice weekly via the jugular vein from each buffalo cow during late pregnancy and postpartum period. Blood plasma was separated after centrifugation at 3000 r.p.m. for 15 minutes, and then stored at -20 C^{o} until analysis for the different blood parameters. Using ready made kits, plasma was used for determination of total protein (TPR), albumin (ALB), Glucose (GLU), blood urea nitrogen (BUN), creatine (CRT), creatinine (CRTN), aminotransferases (AST and ALT), total cholesterol (TC), calcium (Ca) and inorganic phosphorus (PHOS). Direct radioimmunoassay technique was performed for determination of progesterone (P4) and estradiol (EST17β) in representative plasma samples. Kits of ‘‘Diagnostic Products Corporation, (DPC) Los Angles, USA’’ with ready antibody coated tubes were used according to the procedure outlined by the manufacturer.

Measurement of reproductive parameters:

One month after parturition, all animals were observed twice daily for heat detection at 8.00 a.m. and 3.00 p.m. by using a fertile bull. Mating procedure was conducted naturally after 12 hours of heat detection. The number of days open (DO) and number of services per conception (NSPC) were determined for each dam.

Histological examination:

Placental tissue samples were taken from four animals with normal and retained placenta for histological examination. Tissue samples were dehydrated by ascending graded series of ethyl alcohol 70, 80, 90, 96 and 100 %. Toluene was used as a clearing agent for 8-10 hours. The samples were impregnated in two successive baths of melted paraffin wax, then embedded in melted wax blocks. Finally the paraffin blocks were cut into thin section (5-7 micron) by rotary microtome. From each sample, 20 sections at least had been mounted and stained by haematoxylin and eusin. The slides were examined by means of light microscope

Statistical analysis:

Statistical analysis was carried out using the General Linear Model Program (GLM) of SAS (2000). Differences were subjected to Duncan’s Multiple Range Test (1955). Data concerning productive traits of different groups of buffaloes were analyzed using the model: $Y_{ijk} = \mu + T_I + Sj + e_{ijk}$

Where:
$\mathbf{Y_{ijk}}$ is the observation taken on the recorded animal ij,
μ is the overall mean,
$\mathbf{T_I}$ is the effect of group
Sj is the effect of born calf sex
$\mathbf{e_{ijk}}$ is the random error.
Data concerning blood constituents of the experimental buffaloes were analyzed using the following model:

$$Y_{ijk} = \mu + M_i + T_j + MT_{ij} + e_{ijk}$$

Where:
$\mathbf{Y_{ij}}$ is the observation taken on the experimental animal **ij**,
μ is the overall mean,
$\mathbf{M_i}$ is the effect of month of sampling,
$\mathbf{T_j}$ is the effect of group,
$\mathbf{MT_{ij}}$ is the interaction between month of sampling and group effect
$\mathbf{e_{ijk}}$ is the random error.

3. Results and Discussion

Post-partum traits of the experimental groups:

As shown in table (1), postpartum loss in LBW had the same trend of LBW being significantly ($P < 0.01$) greater in NRP buffaloes than animals with RP. Percentages of calving loss in LBW of buffaloes in proportion to the pre-partum weight were 10.30%, 11.22% and 11.16 % for NRP, RPT and RBC groups, respectively. Awara (2006) working on buffaloes found that loss in weight was 9.30 to 10.41% depending on pre-partum feed supplementation. Differences between groups in calf birth weight were insignificant while differences between newborn males and females were highly significant ($P < 0.01$). However, relative weights of calves to their dams (CBW/DAM) were significantly higher ($P <0.05$) in RP groups comparing with NRP group probably due to decline in LBW of RP group. This finding was in contrary to that obtained by Bhalaru *et al.* (1983) who indicated that percentages of RP decreased significantly, when (CBW/DAM) increased. Karen (1996) found that differences in CBW from cows with RP were insignificant. Joosten *et al.* (1988) observed that higher incidence of RP was associated with greater CBW. Bhalaru *et al.* (1985) found that dams LBW at calving and CBW were significantly affected the time elapsed until expulsion of placenta among 234 normal parturitions of buffaloes. Volume of fetal fluids reached its maximum value in NRP group comparing with the other groups ($P <0.01$) whereas no significant differences were detected in weight of fetal membranes between groups (Table 1). Time elapsed for placenta expulsion in NRP was 4.23 hr. while it was 17.26 and 18.7 hr. in RPT and RBC groups, respectively. Greater values of placental tissue weight (PTW), being 4.7 - 5.5 kg and foetal fluids (FF) 16.9- 17.4 L in NRP buffaloes were recorded by Awara (2006). Janakiraman (1981) reported that the time spent for normal placenta expulsion varied from 4.08 to 5.38 h and PTW from 3.01 to 3.40 kg denoting that the risk of RP was increased as PTW increased and that of FF volume was decreased. It seems that FF facilitates the fast expulsion of the placenta; meanwhile the detachment of placental membranes seems to be delayed with the excess in PTW. Sex of newly born calf had only a significant effect ($P < 0.01$) on CBW and CBW/DAM whereas, a significant effect ($P < 0.05$) was noticed on the dam's loss in weight and CBW due to interaction of groups and calf sex. The NRP buffaloes that calved males recorded greater ($P < 0.05$) CBW and PTW in comparison with their group mates that calved females. Meanwhile, differences in CBW/DAM were significantly ($P < 0.01$) affected by sex of the born calf or incidence of RP. El-Malky (2007) observed a significant effect of the calf sex on calf LBW in RP or NRP buffaloes.

Histological features of non retained and retained placentome :

As shown in figure (1), histological examination of the NRP revealed presence of intact basal decidua and chorionic epithelium with normal trophoblastic giant cell. The normal allantochrion was bearing simple epithelium on the side facing the allantoic cavity and the mesenchymal layer was containing blood vessels. Histological examination of RP showed decomposition and fragmentation of the placental tissue and chorionic villi. Such alteration was concomitant with hyperplasia in the chorionic epithelial cell of the villi. The number of trophblastic giant cells in RP was limited denoting placental dysmaturity while, focal areas of trophoblastic degeneration and desquamation was remarkable. The section also revealed necrosis of the lining chorionic epithelium with inflammatory cell aggregation. Hemorrhages and congestion of most blood vessels was noticeable accompanied with necrosis and calcification of the lining endothelial capillaries. Hence, thickening in the wall of blood vessels with edema in the connective tissue layer was expected. In agreement, Al-Sadi et al. (1994) observed presence of compacted degenerating deciduas, extensive necrosis and numerous clumps of bacterial colonies in placentomes of cows with RP including vascular changes (edema, thrombosis and vasculitis). The author noticed also presence of numerous binucleate cells, infiltration of polymorphonuclear cells in the connective tissue of the villi.

Table (1): Pre- and post-partum weights of buffalo dams, calf birth weight, placental tissue weight, weight of fetal fluids and time of placenta expulsion in the different experimental groups.

	Experimental groups						Level of Significance		
	NRP		RPT		RPC				
Sex of newborn calf	♂	♀	♂	♀	♂	♀	Group	Sex	Group* Sex
No. of animals	3	7	7	3	6	4			
Dam body weight (kg):									
pre-partum	607.8	572.2	490.6	494.3	482.2	511.3	**	NS	NS
post-partum	544.4	513.6	433.7	443.3	425.9	451.4	**	NS	NS
Loss in weight (kg)	63.40 a	58.60 b	56.86	51.00	56.25	59.88	**	NS	*
CBW (kg)	41.80 a	36.80 b	41.00 a	36.33 b	40.17	39.75	NS	**	*
CBW/Dam	7.68	7.22	9.51 a	8.16 b	9.45	8.83	**	**	NS
Fetal fluids (L)	16.90	17.92	12.33	10.90	12.17	11.25	**	NS	NS
Placenta tissue (kg)	4.70 a	3.88 b	3.36	3.37	4.38	3.92	**	NS	NS
Expulsion time (hr)	4.21	4.24	17.22	17.28	18.64	18.71	**	NS	NS

Reproductive performance of treated groups:

The normal group of buffaloes (NRP) achieved the least ($P < 0.01$) calving interval (CI) and days open (DO) as compared with buffalo groups with RP (Table 2). However, GnRH treatment had significantly ($P < 0.05$) reduced CI and DO for RPT buffalo group than that for group RPC by 10.41% and 28.33%, respectively. Despite, number of services/ conception (NSPC) declined in response to GnRH treatment (2.6) when compared with RPC buffaloes (3.5), the hormonal treatment failed to attain 1.3 services/ conception that recorded by the NRP group. The shorter interval from calving to 1st detected ovulation and date of conception recorded by NRP group may explain the negative impact of RP on post-partum reproductive performance. Incidence of RP causes a deleterious effect on fertility and milk productivity of cows (Jainudeen and Hafez, 1992). Swiefy (2003) found that Friesian cows with RP under Egyptian conditions had significantly ($P <0.05$) longer intervals of postpartum uterine involution, first ovulation, first estrus and days open. There are an earlier evidence that postpartum GnRH treatment shortened the interval from ovulation to peak P4 (Aboul-Ela *et al*. 1985 and Barkawi and Aboul-Ela 1987) and reduced significantly the intervals from parturition to 1st ovlulation and 1st detected oestrus (Aboul-Ela *et al*. 1985). Our finding agree with Hashem and Amer (2008) who found that GnRH treatments reduced significantly ($P <0.05$) the interval from calving to 1st insemination and NSPC, followed by a significantly ($P <0.05$) higher conception rate. However, Risco *et al* (1994) found that cows affected with RP and treated with GnRH (100 µg at d 12 postpartum) did not improve the reproductive performance.

Milk productivity of treated groups:

As shown in table (3), differences between the studied groups in milk traits (total milk yield, days in milk and daily milk yield) were highly significant ($P < 0.01$) not only in the current milking season but also in the previous and next milking season. Estimates of milking traits were greater in NRP group than the other groups with RP at the current season indicting severe negative effect of placenta retention on milk productivity of buffaloes. The decline in total milk yield of current season compared with that of the preceding season was 7.84%, 40.02% and 48.60% in the studied groups NRP, RPT and RPC, respectively. Meanwhile, the milking traits were positively maintained in the next season for buffaloes with RP. Moreover, it was noticed that buffaloes treated with GnRH (RPT group) achieved greater milk productivity (13.27%) than RPC group. These results are in agreement with those of (Paisley *et al.*,1986 and Rajala and Grohn, 1998) who reported that RP resulted in a significantly negative effect on milk yield of dairy cows for several weeks after calving. El-Malky (2007) noticed that buffaloes group with RP reduced its milk yield by 15.79% than the preceding season while NRP group increased milk productivity by 6.99% over that of the preceding season.

Table (2): Estimates of buffalo reproductive parameters in the different experimental groups.

	Treatments				
	NRP	RPT	RPC	± SE	Sign
No. of animals	10	10	10		
Calving interval (days)	411.9 c	489.9 b	541.0 a	10.96	HS
Days open	101.9 c	179.9 b	231.0 a	10.96	HS
No. services/ conception	1.3 c	2.6 b	3.5 a	0.38	HS

Interval from calving to:					
1st detected ovulation	49.9 b	85.1 a	93.6 a	6.97	HS
Conception	70.10 c	90.10 b	107.10 a	5.82	HS

Table (3): Estimates of milk productivity in the different experimental groups of buffaloes.

	Season *	Treatments			
		NRP	RPT	RPC	Significance
No. of animals		10	10	10	
Total milk yield	P	2538.6 a ± 129.97	1762.2 b ± 141.66	1815.2 b ± 114.69	HS
	C	2339.6 a ± 97.45	1056.9 b ± 106.21	933.1 b ± 85.99	HS
	N	2668.8 a ± 2668.7	1281.5 c ± 73.55	1371.3 b ± 59.55	HS
Days in milk	P	236.9 ± 9.59	227.9 ±10.46	219.9 ± 8.47	NS
	C	209.1 a ±8.47	178.7 b ± 9.23	153.2 b ±7.47	HS
	N	232.3 a ±6.72	179.2 b ± 7.33	209.1 a ±5.93	HS
Daily milk Yield	P	10.7 a ±0.49	7.8 b ±0.53	8.2 b ±0.44	HS
	C	11.2 a ±0.43	5.9 b ±0.46	6.1 b ±0.37	HS
	N	11.5 a ±0.32	7.3 b ±0.35	6.6 c ±0.28	HS

P= Previous milking season, C= Current milking season, N= Next milking season.
Means bearing different superscripts in the same raw are significantly (P <0.05) different.

Changes in levels of progesterone and estradiol hormones:

Differences between treatments in progesterone (P4) concentration were insignificant while differences between experimental months were highly significant (P<0.01) taking into account a significant interaction (P <0.01) between treatments and month of sampling. Averages of P4 concentrations were 1.97, 1.95 and 1.84 ng/ml in plasma of NRP, RPT and RPC groups, respectively. Pre-partum concentrations of P4 were almost similar between groups (3.06- 3.62 ng/ml) then it reached the minimum level at the 1st month after parturition (1.11 ng/ml) followed by a relative increase in the 2nd month (1.65 ng/ml) and a relative decrease in the 3rd month (1.46 ng/ml) for all groups. As shown in Figure (2), P4 concentration was significantly (P <0.05) greater in NRP animals than that in buffaloes with RP throughout the experimental months particularly beyond the 5th week postpartum. The diminished levels of P_4 before parturition in NRP animals was stated by several authors (El-Malky, 2007 and Amjad Ali *et al.,* (2009). It was evidenced that P_4 level was significantly higher in cows suffering from RP compared to NRP cows (Sabry *et al.* 1997 and Harendra *et al.,* 2001).

Differences in concentrations of E2 due to treatments or month of sampling were highly significant (P <0.01) with noticed interaction (P <0.01) between those effects. Averages of E2 concentrations were 60.46, 55.4 and 52.29 pg/ml in plasma of NRP, RPT and RPC groups, respectively. Pre-partum concentration of EST were almost similar among groups (103.9-109.0 pg/ml). Concentration of E2 minimized in the 1st month after parturition (22.47 pg/ml) followed by a gradual increase up to the 3rd month (52.72 pg/ml). In addition to the elevated level of E2 in NRP group over that of group RPC, the results indicated a positive effect of GnRH treatment by increasing E2 concentration significantly (P < 0.05) in the 2nd month (5th to 8th week) after parturition when compared with that of non-treated animals (Figure 1). El-Malky (2007) found that prepartum concentration of E2 was relatively higher in NRP buffaloes than that of RP group. In contrary, Harendra *et al.* (2001) did not observe difference in E2 level between RP and NRP animals. The sharp increase of E2 and decrease of P_4 just before parturition in NRP cows was reported by Gordon (1996). Such finding was evidenced also by Hashem and Amer (2008) working on cattle and Amjad Ali *et al.* (2009) working on buffaloes. Normal calving requires softening and dilation of the cervix,

particularly during LP due to the influence of relaxin and estrogen when P_4 dominance decline and uterine prostaglandin production is increasing (Taverne, 1992). On the other hand, normal expulsion of fetal membranes requires a rise in E2 before calving accompanied by a gradual and sustained fall in P_4 (El-Wardani *et al.*, 1998).

The ratio of E2/ P_4 was similar among treated groups of buffaloes. However, the respective ratio had greater (P <0.01) estimate during prepartum period in comparison with postpartum period. On the other side, that ratio was greater at the 3rd month after delivery than that at the 1st and 2nd month postpartum.

Changes in blood constituents of treated groups:

Generally, concentrations of blood proteins (TPR), glucose (GLU), blood urea nitrogen (BUN), creatine (CRT), alanine transferase (ALT), total cholesterol (TC), calcium (CA) and inorganic phosphorus (PHOS) increased significantly (P < 0.01) with advancement of month of sampling (Table 4). On the other hand, TPR, CRT, amino transferases, TC and PHOS showed higher concentrations in pre-partum month than that in postpartum months. In accordance, Some studies attributed the differences between pre-partum and post-partum periods in level of blood TPR and GLU to milk protein synthesis (Rakesh Kumar *et al.*, 2001) or increased proteins break down required for gluconeogensis (Abdul Gani *et al.*, 2003). The differences in TPR due to treatment or month of sampling and their interaction were highly significant (P <0.01). It was observed that concentrations of all studied metabolic parameters were relatively less in RP groups than that in non retained group (NRP). Malnutrition may have a significant role influencing pre-parturient hormonal balance particularly those hormones mediated in energy metabolism leading to placenta retention. In agreement with this finding, Sabry *et al.*, (1997) observed that TPR and GLU in NRP cows were greater than that in cows with RP while,. Choudhury *et al.*, (1993) did not detect appreciable change in plasma TPR of cattle or buffaloes due to RP. In contrary, Deyab (2000) working on Friesian cows noticed an increase of TPR and GLU in animals with RP. Harendra *et al.*, (2001) did not found a significant change in serum glucose in NR or RP cows. Hashem and Amer (2008) noticed that concentration of liver enzymes was significantly (P <0.05) higher, while serum GLU and TC levels were significantly lower in cows with RP compared with the NRP group.

The hypocalcaemia detected in RP buffaloes could be considered as another indicator of metabolic disorder due to malnutrition. These findings were in accordance with results reported earlier by Sabry *et al.* (1997), Deyab (2000) and Patel *et al.* (2003). However, Harendra *et al.* (2001) did not observe any significant change in concentration of CA and PHOS in blood serum of RP compared to NR cows. Hashem and Amer (2008) concluded that low serum electrolytes levels and hormonal imbalance might predispose to RP in dairy cows while, Rakesh Kumar *et al.* (2001) attributed the low CA level during late pregnancy to its diversion toward fetal skeletal formation throughout the gestation period. El-Malky (2007) noticed 26.14% and 16.94% increase in CA and PHOS content in blood of NRP than RP groups, respectively. In the current work, GnRH treatment had relatively ameliorated the metabolic function in treated buffaloes via increasing concentrations of TPR, GLU, CRT, CRTN, ALT, CA and PHOS. Hashem and Amer (2008) observed that RP cows treated with GnRH showed a higher (P<0.05) levels of GLU and total lipids, with lower liver enzyme concentrations than controls adding that protein profile returned close to control level in the treated groups.

Table (4): Least square means of concentrations of blood metabolites in the experimental buffaloes.

Blood parameter	Unit	Experimental groups			
		NRP	RPT	RPC	± SE
Total protein (TPR)	g/dl	8.48 [a]	7.35 [b]	6.94 [c]	0.04
Albumin (ALB)	g/dl	3.82 [a]	3.31 [b]	3.31 [b]	0.03
Globulin (GLOB)	g/dl	4.66 [a]	4.04 [b]	3.64 [c]	0.03
Glucose (GLU)	mg/dl	58.80 [a]	51.18 [b]	49.07 [c]	0.30
Blood urea nitrogen (BUN)	mg/dl	42.82 [a]	39.84 [b]	39.44 [b]	0.14
Creatine (CRT)	mg/dl	50.37 [a]	48.02 [a]	43.20 [b]	1.50
Creatinine (CRTN)	mg/dl	2.82 [a]	2.71 [b]	2.54 [c]	0.02
AST	(U/L)	50.53 [a]	48.17 [b]	48.04 [b]	0.20
ALT	(U/L)	21.29 [a]	21.24 [a]	20.20 [b]	0.14
Total cholesterol (TC)	mg/dl	88.53 [a]	84.32 [b]	85.67 [b]	0.52
Calcium (CA)	mg/dl	9.57 [a]	8.65 [b]	8.22 [c]	0.05
Inorganic phosphorus (PHOS)	mg/dl	5.39 [a]	5.18 [b]	4.45 [c]	0.03

Means bearing different superscripts in the same raw are significantly (P <0.05) different.

Conclusion

The current study indicated different negative impacts due to incidence of RP in buffaloes. Among those impacts, the prolonged DO and CI in addition to reduced animal milk productivity. The higher P4 with lower E2 concentrations during the week before parturition seems to be an important crucial factor predisposing to RP in cattle or buffaloes. The decreased level of E2 may be indicated as a factor enhancing RP (El-Nemer *et al.*, 2000). Prepartum hypoglycemia may act as a predictive indicator of RP risk (Markiewicz *et al*., 2001).

The current study revealed that GnRH treatment had significantly ($P < 0.05$) reduced CI and DO for group RPT than that for group RPC by 10.41% and 28.33%, respectively. Also, NSPC declined in response to GnRH treatment (2.6) when compared with RPC group (3.5). Improvement of reproductive efficiency in GnRH treated buffaloes may result from normalization of P4 level (Foote and Riek, 1999), the treatment may be helpful in hastening ovulation and establishment of a *corpus luteum.* In addition, buffaloes with RP that treated with GnRH (RPT group) achieved greater milk productivity (13.27%) than RPC group. Such response may be referring to amelioration of the metabolic activities in treated buffaloes. The role of GnRH to overcome impacts of placenta retention as well as to sustain fertility of the affected animal may be a dose dependant. Further studies are needed to focus on appropriate doses of gonadotrpins to re-maintain the indigenous hormonal balance.

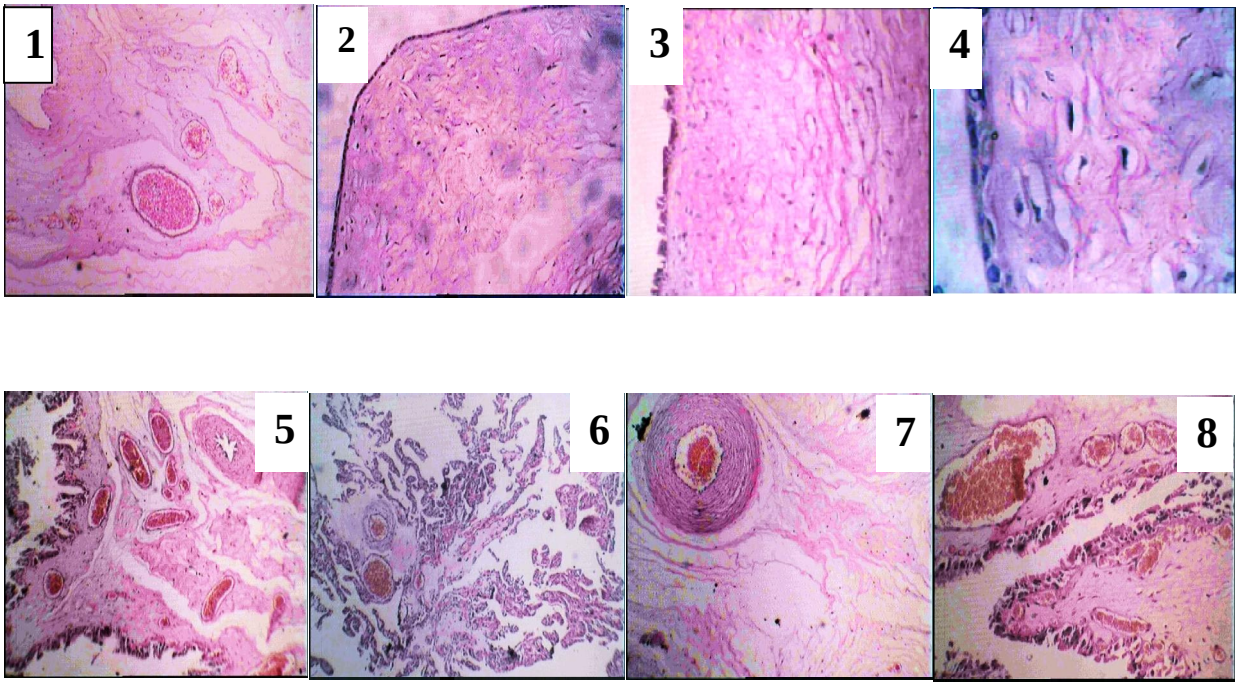

Fig. (1): Sections (H&E staining) from non retained placenta (1,2,3 and 4) of buffaloes, revealing normal allantochorion which bears simple epithelium on the side facing the allantoic cavity (1). The mesenchymal layer contains normal blood vessels (X50). The chorionic epithelium (2) contained numerous mono nucleated cells(X100) and regular arrangement of trophoblast (3)(X250). The basal decidua is intact (4) with presence of normal trophoblastic giant cell(X400). Sections through the retained placenta (5, 6, 7 and 8) showed hyperplasia in the chorionic epithelial cell of the villi (5) with congestion of most of chorionic blood vessels (X100). Decomposition and fragmentation of the placental tissue (6) and chorionic villi is abundant (X100), accompanied with thickening in the wall of blood vessels (7) with edema in the connective tissue layer (X 250). Focal areas of trophoblastic degeneration and desquamation (8) with inflammatory cell aggregation, hemorrhages and congestion of most blood vessels (X 250).

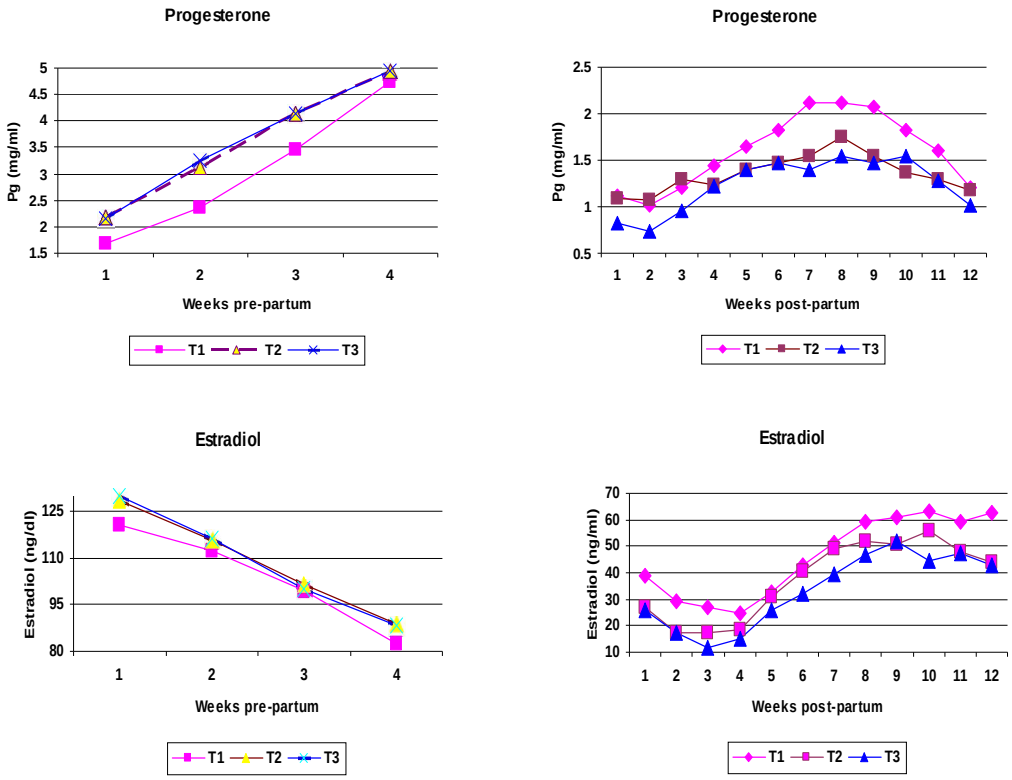

Fig. (2): Pre partum and postpartum weekly levels of progesterone and estradiol in blood of buffaloes with retained placenta (RPC), with retained placenta and treated with GnRH (RPT) and without retained placenta (NRP).

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