J.Animal and Poultry Prod., Mansoura Univ., Vol.8(8): 261 - 268, 2017

Effect of Combined Phosphorus Preparation (Tonophosphan) on Ovarian Activity of Egyptian Buffaloes.

Abo-Farw,M. A.; E. F. Elmaghraby; W. M. Nagy; O.M. Elmalky and M. A. Aboul-Omran.
Animal Production Research Institute, Agricultural Research Center, Giza, Egypt.
Corresponding e-mail: abofarwmohamed@yahoo.com

ABSTRACT

The aims of this study was to evaluate the effect of tonophosphan (TPH), as a phosphorous compound, on resumption of estrus and ovulatory activity of anestrous Egyptian buffalo heifers and anestrous post-partum buffalo cows, in relation with blood phosphorus level. Total of 24 anestrus animals with smooth ovaries and serum progesterone of <1 ng/ml up to 90 days post-partum (12 buffalo cows, weighing 470-530 kg, aging 5-7 years and between 3-4 parities. as well as 12 buffalo heifers, weighing 390-420 kg and 2.5-3 years old) were used in this study. In each of heifer and cow groups (n=12), animals were randomly divided into two sub-groups (treated and control, 6 in each). Animals in treatment group (6 heifers and 6 cows) were injected twice at 7 day-interval with 4 ml/100 kg of TPH, while those in control group (6 heifers and 6 cows) were administrated with 4 ml distilled water/100 kg at the same time of TPH treatment. Estrous activity was detected twice daily to detect estrous signs. Blood samples were collected from all animals of each group on days 0, 3, 6, 10, 14, 18, 22, 26, 30, 34 and 38 of treatment. Results revealed that the estrus rate in treated animals was higher ($P<0.05$) in cows (83.33%) than in heifers (66.67%), while the control animals showed no estrous signs. Intensity, duration and interval from treatment to estrus were nearly similar in both heifers and cows. Concentration of serum P4 was higher ($P<0.05$) in cows than in heifers only on days 3 and 6 of treatment, while it was higher ($P\geq0.05$) in cows than in heifers on other sampling days. Concentration of P4 was higher ($P<0.05$) in treated groups than in control on all sampling days, except during the 1st and six days of treatment. Concentration of P4 was less than 1 ng/ml in control groups on all sampling treatment days, while P4 concentration level was ≥1 ng/ml after 10 days of treatment in treated groups. Concentration of Ca on most sampling days of treatment and P concentration on all sampling days were significantly ($P<0.05$-$P<0.001$) higher in heifers than in cows, while Ca:P ratio showed an opposite trend on all sampling days. In conclusion, Based on the foregoing results, twice tonophodphan injection of true anestrous buffalo heifers and cows at a week interval at a level of 4 ml/100 kg LBW has impact on resumption of estrous activity and achieving conception, being more effective for buffalo cows than for buffalo heifers.

Keywords: Buffalo cows, heifers, true anestrus, tonophosphan, progesterone, conception rate.

INTRODUCTION

Several international organizations have emphasized the potentiality of the buffaloes in the economy of a number of developing countries, due to its ability to produce and reproduce under the harsh environmental conditions as compared to the dairy cattle (Marai and Habeeb, 2010). In many countries, buffaloes are an important source of milk and meat and considered one of the most important domestic ruminants (Hinkoveski, 1990).

Poor reproductive performance in dairy buffaloes in term of anestrus cases is a problem that reduces reproduction due to long days open and consequently long calving interval (Bakr *et al.,* 2015) prolongation of the service period (Bailey *et al.,* 1999) and increases economic losses to dairy industry. Anestrus in cattle and buffaloes is generally caused by ovarian dysfunction, silent ovulation and missing heat (Das and Khan, 2010; Sah and Nakao 2010) and generally observed in large number of buffaloes leading to their culling or slaughtering every year. Buffaloes showing anestrus was 51%-66% during the first 90 days after calving, and 31%-42% for more than 150 days post-partum in Egypt (El-Wishy, 2007), 58.4% of cases were true anestrus and 33.3% of cases were silent ovulation (Ahmad and Noakes 2009), and 60% of cases were true anestrus and 33% of cases were silent ovulation in Nepal (Sah and Nakao, 2010). Anestrus is a multi-faceted problem, but inadequate nutrition, particularly dietary insufficiency of some minerals greatly contribute to anestrus (kumar *et al.,* 2014). Also, the negative energy balance is an important factor causing inactive ovaries in high-yielding dairy cows (Ziling Fan, *et al.,* 2017).

During the last few years, several studies have been attempted to treat the prolonged postpartum anestrum in cows by using hormonal (Edwell *et al.,* 2004), and non-hormonal substances as well as ovarian and uterine massage (Edwell *et al.,* 2004). Also, several methods of estrus and ovulation induction in buffaloes were carried out using hormones (Baruselli, 2001; De Rensis and Lopez-Gatius, 2007). Management and nutritional factors that may influence the response of anestrus buffaloes after treatment have not been well described (Ahmad and Noakes, 2009).

It is well established that minerals play an intermediate role in the action of hormones and enzymes at cellular level which ultimately affect the reproductive performance of female (Bearden *et al.,* 2004). Some minerals, like calcium (Ca), phosphorus (P), copper (Cu), zinc (Zn) and manganese (Mn), greatly contribute to anestrus (Terzano *et al.,* 2012). Deficiency of these elements is associated with subnormal fertility and anestrous conditions in cows (Campbell *et al.,* 1999). Mineral deficiencies and imbalances are often cited as causes of poor reproduction. It is clear that adequate amounts of minerals must be provided, but little is known about the effects of marginal deficiencies and imbalances. The same is true of excessive intakes of minerals which may indeed be harmful. Producers should avoid overfeeding minerals. If a little bit is enough, twice as much will not be better and may in fact cause problems (Elrod and Butler 1993). It is suggested that delay in post-partum ovarian activity in ruminants is related to lower level of minerals in blood (Koley and Biswas 2004).

Phosphorus is an essential element required in the high energy metabolism and the deficiency of which can prove detrimental to pituitary ovarian axis thereby causing anestrous. It is responsible for rigidity of bones and many metabolic processes as well as it is essential in the structural of phospholipids, phosphoproteins, ATP, enzymes and chemical components of the cells. It has been reported to have some known functions in the body than other mineral elements. In the anestrous cows, lower inorganic P values were observed in comparison to normal cycling ones. Approximately 31% of anestrous cows had plasma inorganic P below critical value of 4 mg/dl, hence could be declared as P deficient (Swenson, 1998). Moderate deficiency and the prime signs of P deficiency may lead to repeat breeding condition and poor conception rate reduced fertility and delayed conceptions and this can be overcome with proper P supplementation (Sathish Kumar, 2003).

In buffaloes, serum inorganic P level was significantly lower in anestrus than in cycling Surti buffalo heifers (Sarvaiya and Pathak, 1992) and in repeat breeder Egyptian buffaloes than in normal animals (Fayez *et al.,* 1992). Effect of tonophosphan, as a main source of P, in induction of estrus and conception in Murrah buffaloes (Bhandari *et al.,* 1975) and in anestrous crossbred cows (Bhaskar *et al.* (2014) was reported. In a crossbred dairy Cattle, Kumar *et al.* (2014) concluded that supplementation of acid inorganic phosphorus can be successfully used for the therapeutic management of post-parturient haemoglbinuria due to hypophosphtemia as observed by Sujatha Turkar (2013).

Therefore, the present study was conducted to evaluate the effect of tonophosphan (Sodium 4-dimethylamino-2 methylphosphonate) on resumption of estrus and ovulatory activity of Egyptian buffalo heifers and post-partum anestrous buffalo cows in relation with blood phosphorus level.

MATERIALS AND METHODS

This study was conducted at Animal Production Experimental Station, Mehallet Moussa village, Kaferelsheikh Governorate, belonging to Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture during the period from December, 2016 to May, 2017.

Animals:

Total of 24 experimental animals (12 anestrus Egyptian buffalo cows, weighing 470-530 kg and 5-7 years of age and 3-4 parities as well as 12 anestrus Egyptian buffalo heifers with 390- 420 kg LBW and 2.5-3 years old) during ≥90 days post-partum were used in this study. Aciclicity of all experimental animals was indicated by smooth ovaries (Rectal palpation, 2-3 times, at 10 day intervals (Noakes *et al.,* 2001) and serum progesterone of <1 ng/ml (Hoffman *et al.,* 1973).

All buffalo cows had a history of normal calving, completed uterine involution, lack of endometritis and without clinical illness signs. The examination of reproductive tract of experimental heifers and cows by rectal palpation and ultrasound examination revealed that the genital tract of all animals free from any pathological diseases and disorders, but did not show any visible signs of estrus up to day 90 of parturition.

Treatment and experimental design:

In each of heifer and cow group (n=12), animals were randomly divided into two sub-groups (treated and control, 6 in each). Animals in treatment group (6 heifers and 6 cows) were injected twice at 7 day-interval with 4 ml/100 kg of tonophosphan, while those in control group (6 heifers and 6 cows) were administrated with 4 ml distilled water/100 kg at the same time of tonophosphan treatment.

Tonophosphan (Inter Vet Egypt for Animal Health, Egypt) is a combined phosphorus preparation (20% Toldimfos). Each 100 ml injection solution contains 20 g toldimfos sodium anhydrous, 4 mg cobalt chloride, 9.2 mg ammonium molybdat, 33.3 mg sodium selenite, 110 mg zinc sulfate, 77 mg manganese sulfate, 500 mg nicotinic acid, and 600 mg phenyl ethyl alcohol (antimicrobial agent).

Feeding and management systems:

Animals were fed on concentrate feed mixture (CFM), fresh berseem (*Trifolium alexandrinum*) and rice straw (RS) during the experimental period. Animals were kept under the regular systems of feeding and management adopted by Animal Production Research Institute. Fresh water was available all times. Buffalo cows and heifers were housed in semi-open sheds. Chemical composition and mineral contents of feedstuffs (on dry matter basis) of CFM, FB and RS are shown in Table (1). Chemical composition (DM, CP, EE, ash, and NFE) was performed on representative samples from all experimental feedstuffs using AOAC methods (1980), while P and Ca contents were determined according to Cresser and Parsons (1979).

Table 1. Chemical composition and mineral contents of different feedstuffs of the experimental diets.

Feedstuff	CFM	FB	RS
DM%	90.74	19.8	91.33
Chemical analysis (%)			
OM	90.74	88.87	83.53
CP	17.30	16.28	3.53
CF	8.32	22.24	37.03
EE	3.11	4.15	1.35
NFE	62.01	46.20	41.62
Ash	9.26	11.13	16.47
Ca	0.545	0.925	0.023
P	0.488	0.0252	0.013

Recommended requirements of cattle (% of DM) for Ca and P are 0.43-0.60 and 0.26-0.40%, respectively, according to NRC (1989).

Detection of estrus, insemination and pregnancy diagnosis:

Estrous activity of all responded animals in the experimental groups was detected every morning and evening by personal visual observation (twice daily, 6 a.m. & 6 p.m.) for at least 30 minutes to detect signs of the estrus by a teaser buffalo bull. Intensity of estrous signs was recorded according to Zicarelli (1997). Buffalo heifers and cows in heat were naturally inseminated by fertile buffalo bull.

Pregnancy was diagnosed of each inseminated animal on day 25 post-insemination using ultrasound examination (Digital ultrasonic diagnostic imaging

System, Model Dp-30 Vet. 50/60 HZ, SHENZHEN, MINDRAY BIO-MEDICAL.ELECTRONICS, CO. LTD), 7.5 MHz Linear array transducer and Depth 4.3. Pregnancy was indicated by rectal palpation of non-returned animals on day 45-50 post-insemination, and then pregnancy rate and interval from treatment to conception were calculated.

Blood sampling:

Blood samples were collected by jugular vein puncture from all animals of each group on days 0, 3, 6, 10, 14, 18, 22, 26, 30, 34 and 38 of treatment initiation. Blood samples were collected in sterilized glass tubes and kept at room temperature. Within an hour after collection, samples were centrifuged at 3000 rpm for 15 min, and then serum was collected and transferred into sterilized vials. All serum samples were stored at -20°C till determination of progesterone (P4) concentration, and inorganic phosphors and calcium contents in blood serum.

Direct Radioimmunoassay technique (RIA) was performed for determination of serum P4 concentration using ready antibody coated tubes kit (Diagnosis Systems Laboratories Texas, USA) according to the procedure outlined by the manufacturer.

Calcium ion produces with methylthymol blue, in an alkaline medium, a blue color to the intensity of which is in proportion to the calcium concentration. The presence of hydroxyl 8-quinoline eliminate the interference due to the magnesium ions (Gindler and King, 1972). Inorganic phosphorus present in serum as phosphate forms a phosphomolybdate complex with molybdic acide. The complex is reduced by slannous chloride to a blue color which can be measured calorimetrically. Formic acid used as proleinsolubilizer and glycerol as stabilizer for assay system (El-Merzabani *et al.,* 1977).

Statistical analysis:

Statistical analysis of the obtained data was performed using general linear model of SAS (2000) as a factorial design (2 animal parities x 2 treatments) to study the effect of animal parity (cows and heifers), tonophosphan treatment (control and treated) or their interaction). The significant differences were set at P<0.05) using Duncan's Multiple Range Test (Duncan, 1955).

RESULTS AND DISCUSSION

Ovarian activity and conception rate of responded animals:

Results revealed estrus incidence only in treated heifers and cows showing significantly (P<0.05) higher estrus rate in cows than in heifers (83.33 vs. 66.67%). However, mean and range of the interval from treatment to estrus incidence as well as intensity and duration of estrus were nearly similar in both heifers and cows (Table 2).

It is of interest to note that all control buffalo cows exhibited anestrous activity during the experimental period, being with smooth ovaries. Ultrasonoghraphy examination throughout the interval from treatment up to onset of estrus in 4 out of 6 heifers and in 5 out of 6 cows revealed that all responded animals showed developing follicles and CLs on the right side and only small follicles on the left one. However, two heifers and one of non-responded animals showed small follicles on the right ovary and smooth left one.

These results indicated that tonophosphan administration as a therapeutic management of true anestrous buffaloes had impact on resumption of estrous activity, being better for buffalo cows than for buffalo heifers, the interval from treatment to estrous incidence and estrous duration were shorter in heifers than in cows.

Table 2. Effect of treatment on estrous activity and conception of responded buffalo heifers and cows.

Item	Buffalo heifers	Buffalo cows	Sign.
Treated animals (n)	6	6	-
Animals responded to estrus (n)	4	5	-
Estrus rate	66.66	83.33	*
Mean interval from treatment to 1st estrus (day)	24.75±1.931	27.40±1.961	NS
Range of interval from treatment to 1st estrus (day)	21-29	22-31	-
Intensity of estrus (%)	62.50±6.535	63.33±7.728	-
Duration of estrus (h)	16.25±2.594	20.40±2.336	-
Insemination rate	66.66	83.33	*
Number of conceived animals	3	4	-
Conception rate(1)	50	66.67	-
Conception rate(2)	75	80	-

NS: Not significant. * Significant at P<0.05). (1): Based on total treated animals. (2): Based on inseminated animals.

Differentiation between true anestrus and sub estrus is particularly important in buffaloes because of their weak estrus signs (El Wishy, 2007). In our study, only animals with true anestrus exhibited estrus and conceived post-insemination following tonophosphan administration. This finding was proved by Parmar *et al.* (2012), who induced estrous response (50%) estrus induction interval (27.14±3.74 d) and conception rate (71.4%) following treatment of anestrous buffaloes with tonophosphan plus Vitacept treatment. Estrous response presented in our study on buffalo cows (83.3%) was nearly similar to that obtained by the later authors, but was higher than that reported by Karhe (2012), who found that estrus response in cows treated with Toldimfos Sodium 20%+Vitamin A+ Lugol'siodine was 66.66%, but was nearly similar to that of buffalo heifers. In the same line, Butani *et al.* (2010) reported relatively higher estrus induction response (82.08%) and lower conception rate (69.10%) in anestrus buffaloes treated with Tono-Prepaline plus intrauterine betadine therapy twice at weekly interval. Also, Sirmour *et al.* (2006) reported the estrus induction response of 83.33% with estrus induction interval of 16.60±2.7 d and 100% conception rate in crossbred heifers treated with tonophasphan. Finally, Dabas *et al.* (1987) recorded estrus induction response and conception rate as 50 and 80% within 30 d following Tonophosphan-Prepaline therapy for 2 weeks. Almost similar interval for induction of estrus in postpartum anoestrous cows with the treatment combination of vitamin A and tonophosphan was reported in buffalo cows (22.30 days) by Kumar *et al.* (1986) and in Sahiwal cows (21.00±6.43 days) by Mathur *et al.* (2005). However, Singh *et al.* (2006) recorded estrus induction response and conception rate of 47 and 72%, respectively, following 30 to 50 days of mineral supplements of anestrus buffaloes.

Plasma progesterone profile:

Concentration of serum P4 was significantly ($P<0.05$) higher in cows than in heifers only on days 3 and 6 of treatment. Although P4 concentration was higher in cows than in heifers on other sampling days, the differences were not significant ($P\geq0.05$). It is worthy noting that , Table 3).

Table 3. Progesterone concentration (ng/ml) in blood serum of buffalo heifers and cows on different days of treatment.

Day of treatment	Buffalo heifers	Buffalo cows	±SEM
0(1)	0.350	0.400	0.037NS
3	0.442	0.558	0.042*
6(2)	0.550	0.725	0.064*
10	0.658	0.867	0.142NS
14	0.942	0.950	0.136NS
18	0.992	1.108	0.128NS
22	0.550	0.950	0.173NS
26	0.792	0.867	0.133NS
30	0.792	0.767	0.186NS
34	1.158	1.250	0.203NS
38	1.642	1.608	0.314NS

NS: Not significant. * Significant at P<0.05. 1st dose of tonophosphan. (2): 2nd dose of tonophosphan

Blood P4 estimation reflects the presence and functional status of CL and P4 level is responsible for stimulation of cyclicity and follicular development. Based on the obtained results concerning P4 profile, the present study indicated that ovarian activity was higher in cows than in heifers. This may be due to that hypothalamic-pituitary-gonado axis had more activity in cows than in heifers. In this respect, Jainudeen *et al.* (1984) observed that young buffaloes in their first lactation had inactive ovaries for a long time and showed extended period of postpartum acyclicity. Most primiparous buffaloes had inactive ovaries and lost body weight during lactation.

As affected by tonophosphan treatment, P4 concentration was significantly ($P<0.05$) higher in treated than in control group on all sampling days, except during the 1st and six days of treatment. It is of interest to note that P4 concentration was less than 1 ng/ml in control group on all sampling treatment days, while P4 concentration level was ≥1 ng/ml after 10 days of treatment in treated group (Table 4).

This may suggest that the response for elevating P4 level in treated group followed the 1st and 2nd dose of tonophosphan by 10 and 4 days, respectively. Also, such group differences indicated a positive effect of phosphorus treatment on ciclicity of animals (heifers and cows) post-treatment.

Effect of interaction between treatment and parity on P4 level on each sampling day was not significant, reflecting nearly similar P4 level in treated and control animals during treatment period (0, 3 and 6 d) and higher P4 level in treated than in control heifers or cows, especially post-2nd dose of tonophosphan (Fig. 1). This finding in both heifers and cows indicated a positive effect of phosphorus treatment on increasing P4 level in blood of treated animals.

Table 4. Effect of tonophosphan treatment on progesterone concentration (ng/ml) in blood serum of control and treated animals on different days of treatment.

Day of treatment	Control group	Treated group	±SEM
0(1)	0.408	0.342	0.037NS
3	0.475	0.525	0.042NS
6(2)	0.575	0.700	0.064NS
10	0.325	1.200	0.142***
14	0.308	1.583	0.136***
18	0.408	1.692	0.128***
22	0.392	1.108	0.173**
26	0.450	1.208	0.133***
30	0.442	1.117	0.186*
34	0.475	1.933	0.203***
38	0.583	2.667	0.314***

NS: Not significant. * Significant at P<0.05. ** Significant at P<0.01. * Significant at P<0.001. 1st dose of tonophosphan. (2): 2nd dose of tonophosphan**

When P4 level was compared among responded (conceived and non-conceived animals), non-responded and control animals either heifers or cows, it was observed similar trend of change in P4 level in both heifers and cows during treatment and post-treatment period. Also, responded-conceived animals showed the highest P4 level on all sampling days of post-treatment period in cows and on most sampling days in heifers, followed by responded-not conceived animals (heifers and cows), while control animals showed the lowest P4 level, being ≤0.5 ng/ml on all sampling days of treatment and post-treatment period (Fig. 2). These observations indicated impact of tonophosphan treatment on resumption of ovarian activity (estrus and ovulation) in responded-conceived animals and only on estrous activity of responded-not conceived animals. On the other hand, non-responded and control animals were characterized as acyclic animals according to their P4 profile during treatment and post-treatment period.

Parmar (2013) suggested that estimation of the plasma P4 levels is helpful tool to detect the current reproductive/cyclical status of the animals and to diagnose early pregnancy with reasonable accuracy in freshly bred buffaloes. In our study, increasing P4 level in responded-conceived animals (heifers and cows) at all sampling times in comparing with responded-not conceived may be attributed to that high level of serum P4 during heat period improve conception rate in buffalo-cows (Bakr et al., 2015). Also, Parmar (2013) found that plasma P4 level was significantly ($P<0.01$) higher on day 21 post-AI in pregnant than in non-pregnant buffaloes (5.20±0.00 vs. 1.58±0.67 ng/ml). In accordance with the present P4 profile, Butani *et al.* (2011) reported that the serum P4 concentration was significantly ($P<0.01$) higher in sub estrus buffaloes (3.36±0.50 ng/ml) as compared to that in anestrus (1.24±0.21 ng/ml).

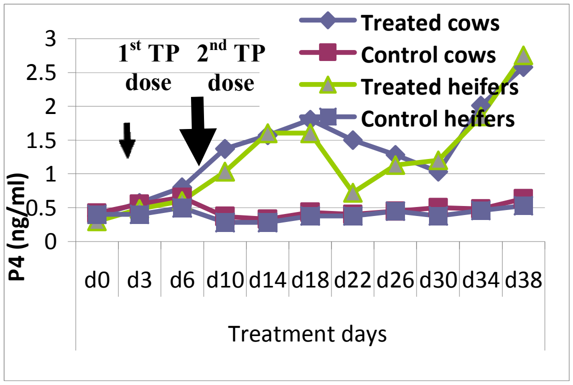

Fig. 1. Change in progesterone concentration (ng/ml) in blood serum of treated and control buffalo cows and heifers during and post-treatment period.

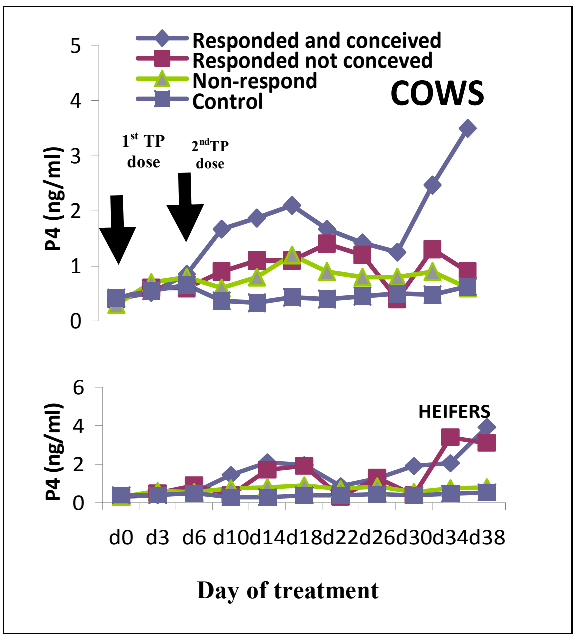

Fig. 2. Change in progesterone concentration (ng/ml) in blood serum of treated and control buffalo cows and heifers during and post-treatment period.

Calcium and phosphorus:

The obtained results revealed that Ca concentration on most sampling days of treatment and P concentration on all sampling days were significantly (P<0.05-P<0.001) higher in heifers than in cows, while Ca:P ratio showed an opposite trend on all sampling days (Table 5). However, Ca concentration on sampling days post-2nd dose of treatment and P concentration on sampling days post-1st dose of treatment were significantly (P<0.05-P<0.001) higher in treated heifers than in control animals, but Ca:P ratio was nearly similar in both treated and control animals (Table 6).

It is of interest to note that the effect of interaction between tonophosphan treatment and animal parity on Ca concentration in blood serum was not significant, reflecting higher Ca concentration in heifers than in cows as well as in treated than in control animals on all sampling days (unshown data). On the other hand, the effect of interaction between tonophosphan treatment and animal parity on P concentration in blood serum was significant (P<0.001), in particular, on sampling days post-treatment. This effect reflected marked reduction in P concentration in control cows as compared to treated cows, while nearly similarity in P concentration in control and treated heifers on all post-treatment days (Fig. 3). Such trend may suggest that tonophosphan treatment resulted in more pronounced increase in P concentration in blood of cows rather than in heifers.

NRC (2001) has revised the recommendation for dairy cattle from 0.3 to 0.4%. Increasing the concentration of dietary P above requirement (more than 0.38-0.40%) to 0.6% had no effect on estrus, service and conception (Lammoglia *et al*, 1997; Wang *et al*, 2014). Level of serum inorganic P ranged 4.32 to 7.12 mg/100 ml with a mean of 6.31 mg/100 ml in Mehsani buffaloes (Pande *et al.,* 1978), and 5.369, 3.261 and 2.030 mg/100 ml during follicular phase, luteal phase and postpartum anestrus period in rural buffaloes (Umesh *et al.,* 1995). However, levels of Ca and P were 12.33 and 7.69 v. 0.23 mg/dl in conceived and 12.15 and 6.92 mg/dl in anestrus Nili-Ravi buffalo-heifers (Paul *et al.* (2000).

Table 5. Concentration of calcium (Ca) and phosphorus (P), and Ca:P ratio in blood serum of buffalo heifers and cows on different days of treatment period.

Day of treats.	Calcium (mg/dl)			Phosphor (mg/dl)			Ca: P ratio	
	Heifers	Cows	±SEM.	Heifers	Cows	±SEM	Heifers	Cows
0(1)	8.39	7.51	0.197**	3.03	2.49	0.093***	2.77	3.01
3	8.84	7.98	0.144***	3.62	2.84	0.097***	2.44	2.80
6(2)	8.86	8.21	0.142**	3.99	3.12	0.082***	2.22	2.63
10	9.66	8.63	0.150***	4.42	3.30	0.092***	2.18	2.61
14	9.44	8.59	0.178**	4.38	3.47	0.102***	2.15	2.47
18	9.34	8.66	0.189*	4.49	3.59	0.142***	2.00	2.41
22	9.46	8.81	0.199*	4.47	3.62	0.130***	2.12	2.43
26	9.45	8.98	0.223NS	4.59	3.80	0.092***	2.06	2.36
30	9.60	9.12	0.239NS	4.81	3.72	0.128***	1.99	2.45
34	9.24	8.97	0.212NS	4.82	3.87	0.128***	1.99	2.32
38	9.58	8.93	0.180*	4.77	3.68	0.136***	2.01	2.43

NS: Not significant. * Significant at P<0.05. ** Significant at P<0.01. * Significant at P<0.001. 1st dose of tonophosphan. (2): 2nd dose of tonophosphan**

Table 6. Effect of tonophosphan treatment on calcium (Ca) and phosphorus (P) concentration (mg/100ml) as well as Ca:P ratio in blood serum of heifers and cows on different days of treatment period.

Day of treats	Calcium (mg/dl)			Phosphor (mg/dl)			Ca: P ratio	
	Control	Treated	±SEM	Control	Treated	±SEM	Control	Treated
0(1)	7.96	7.94	0.197NS	2.76	2.77	0.093 NS	2.88	2.87
3	8.36	8.47	0.144 NS	2.99	3.47	0.097**	2.79	2.44
6(2)	8.30	8.77	0.142*	3.36	3.75	0.082**	2.47	2.34
10	8.72	9.56	0.150***	3.48	4.25	0.092***	2.50	2.25
14	8.21	9.82	0.178***	3.47	4.38	0.102***	2.36	2.24
18	7.98	10.02	0.189***	3.56	4.52	0.142***	2.24	2.22
22	8.21	10.06	0.199***	3.45	4.63	0.130***	2.38	2.29
26	8.21	10.22	0.223***	3.63	4.76	0.092***	2.26	2.15
30	8.22	10.50	0.239***	3.78	4.75	0.128***	2.17	2.21
34	8.20	10.012	0.212***	3.95	4.73	0.128***	2.07	2.11
38	8.47	10.02	0.180***	3.81	4.65	0.136***	2.22	2.15

NS: Not significant. * Significant at P<0.05. ** Significant at P<0.01. * Significant at P<0.001. 1st dose of tonophosphan. (2): 2nd dose of tonophosphan**

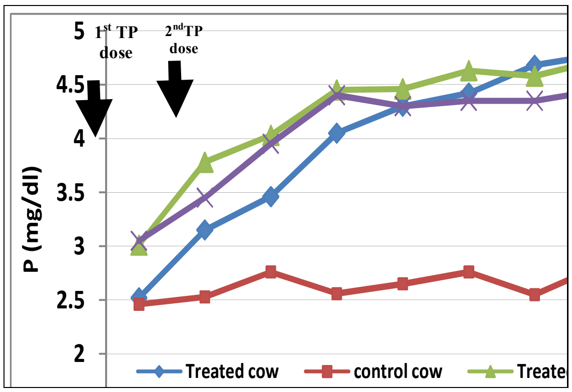

Fig. 3. Change in P concentration in blood serum of treated or control heifers and cows on different days of treatment.

Similar increasing pattern in P level after treatment was also reported by Shah *et al.* (2003). In accordance with the obtained results, it was reported that higher mean plasma P level was documented in cycling animals than that of anestrus animals in Murrah buffaloes (Lodhi *et al.,* 1998) and in Nili-Ravi buffalo-heifers (Paul *et al.,* 2000). Also, Roberts (1971) stated that the Ca deficiency may not cause reproductive failure in cattle. Significantly higher (P<0.01) concentration of P was recorded in normal cyclic buffaloes compared to anestrous buffaloes. Also, Sarvaiya and Pathak (1992) reported that serum inorganic phosphorus level was significantly lower in anestrus than in cycling Surti buffalo heifers.

The Ca: P ratio, alteration may affect ovarian function through its blocking action on pituitary gland (Sathish Kumar, 2003). Lottammer *et al.* (1974) observed that ratio of Ca:P in normal cyclic animals was 2.15:1 compared to 4.59:1 in the anestrous animals. It has been reported that absorption of Ca and P was better from a diet having a Ca:P ratio of 2:1 than one in which the ratio was 1:1. Although higher Ca:P ratios have been reported to be associated with infertility (Hignett, 1959), many others have also supported the similar views (Luca *et al.*, 1976). The present study indicated nearly Ca: P ratio in treated and control animals, which contrasted findings of Umesh *et al.* (1995) and Jani *et al.* (1995), who observed a disturbed Ca:P ratio in cyclic and noncyclic animals.

In general, the deficiency of P was reported to cause reduction in fertility rate and ovarian activity, irregular estrous cycles, increased occurrence of cystic ovaries, delayed sexual maturity and low conception rates (Martinez *et al*, 2012), appeared to play a role in the disturbance of estrus cycle (Shrivastava *et al.,* 1981) and contributes to nonfunctional ovaries (Schweigert and Zucker (1988) causing disturbances in pituitary-ovarian axis (Das *et al.,* 2002).

Correlation coefficients:

Data in Table (7) indicated significantly (P<0.01) strong and positive correlations of P level with all parameters studies, being the highest between P level and estrus response(r=0.672),followed by P4 concentration (r= 0.629) and conception rate (r = 0.577).

Table 7. Correlation coefficients of phosphorus level with different parameters studied.

Item	P	CR	ER
Overall correlation:			
CR	0.577**	-	-
ER	0.672**	0.828**	-
P4	0.629**	0.569**	0.584**
Buffalo heifers:			
CR	0.527**	-	-
ER	0.644**	0.816**	-
P4	0.595**	0.511**	0.575**
Buffalo cows:			
CR	0.649**	-	-
ER	0.724**	0.837**	-
P4	0.683**	0.619**	0.589**

**** Correlation is significant at the 0.01 level (2-tailed).**

It is worthy noting that correlation coefficients of P level with each parameter studied was higher in cows than in heifers. This indicated importance of supplementing cows with P more than heifers.

CONCLUSION

Based on the foregoing results, twice tonophodphan injection of true anestrous buffalo heifers and cows at a week interval at a level of 4 ml/100 kg LBW has impact on resumption of estrous activity and achieving conception, being more effective for buffalo cows than for buffalo heifers.

REFERENCES

Ahmad N. N. and Noakes D., 2009.Reproduction in the buffalo. In Noakes DE, Parkinson TJ, England GCW (eds) Veterinary Reproduction and Obstetrics, 9 ed., Edinburgh: Saunders Elsevier, 824-835.

AOAC methods (1980). Association of Official Analytical Chemists (AOAC) INTERNATIONAL.

Bailey T.L., Dascanio, J. and Murphy J., 1999. Analyzing reproductive records to improve dairy herd production. Veterinary Medicine 94, 269-276.

Bakr M. M., Noseir, M.B. Gamal A. and Amrawi,G., 2015 . Effect of exogenous progesterone in treatment of ovarian inactivity in the Egyptian dairy parturient buffalo-cows. /Alexandria Journal of Veterinary Sciences, 47: 191-200.

Baruselli P. S., 2001. Control of follicular development applied to reproduction biotechnologies in buffalo. pp. 128–146. In: Proceedings of the I Congress Nazionalesull'allevamentodel Bufalo, Book of the Congress.

Bearden H. J., Fuquay J. W. and Willard S. T. 2004. Applied animal reproduction, sixth edition. Pearson Prentice Hall, Upper Saddle River, New Jersey, NY, U.S.A.

Bhandari R. M., Kaikini, A. S. and Kad, M. S.,1975. A report on the use of tonophosphan (Hoechst) in Gynacologicaldisordes, Indian Veterinary Journal, 52:76.

Bhaskar Bora P., Perumal K. K., Bonia and R. K. Biswas. 2014. Effect of Non-hormonal Treatments on Postpartum True Anoestrus Crossbred Dairy Cows. International Journal of Bio-resource and Stress Management 2014, 5(2):255-261.

Butani M.G., Dhami A.J., Kumar Rajesh, Savaliya F.P., Shah R.G., Kelledar, A. K.P. Patel and Kavani, F.S. 2010. Influence of different therapies on fertility and serum progesterone, protein and cholesterol profile in conceiving and non-conceiving anoestrus buffaloes. *GAU Res. J.*, 35(2): 140-145.

Butani M.G., Dhami A.J. and Rajesh Kumar 2011. Comparative blood profile of progesterone, metabolites and minerals in anoestrus,sub estrus,repeat breeding and normal cyclic buffaloes. Indian J. Field Vets.,7(2):20-24.

Campbell C.S., Wood R., Kelly M. 1999. Social Capital and Health. Health Education Authority, London, UK.

Cresser M.S and J. W. Parsons. 1979. Sulfuric-perchloric acid digestion of plant material for the determination of nitrogen, phosphorus, potassium, calcium, and magnesium, Anal. Chem. Acta 109:431-436.

Dabas Y.P.S., S.P. Singh and O.P. Saxena 1987. Serum concentration of certain minerals in anoestrus cows and buffaloes. Indian J. Anim. Reprod.,8(2):98-101.

Das G. K. and Khan F. A., 2010. Summer anestrus in buffalo— a review. Reprod. Domest. Anim. 45: e483–e494.

Das S., Basu S., Sahoo A.K., Sarkar A.K. and Pal R.N., 2002. Comparative study of certain serum macro- and micro-minerals in anoestrous and normal cycling crossbred cows. Indian Journal of Animal Health 41(2), 99-102.

Duncan D.B., 1955. Multiple range and multiple F test. Biometrics, 11: 1-42.

Edwell S.M., Slawomir Z. and Tomasz J., 2004. Comparative study on the efficacy of hormonal and non-hormonal treatment methods in ovarian function affected dairy cows. Bulletin Veterinary Institute of Pulawy. 48, 265-267.

EI-Wishy A.B. 2007. The post partum buffalo. II. Acyclicity and anestrus. Review. Anim. Reprod. Sci., 97: 201-215.

El-Merzabani M. M., El- Aaser, A.A. and Zakhary, N.I. 1977. J. Clin. Chem. Clin. Biochem. 15;

Elrod C.C and Butler W.R., 1993. Reduction of fertility and alteration of uterine pH in heifers fed excess ruminally degradable protein, Journal of Animal Science, 71, 694-701.

Fayez I.; Marai M.; El-Darawany A.A. and Nasr A.S. 1992. Typical repeat breeding and its improvement in buffaloes. Beitrage Zur Trois chem Land wirt schaft and Veterinar medizin. 30(3): 305-314.

Gindler M. King J. D. 1972. Am. J. Clin. Path. 58, 376.

Hignett S.L. 1959.Some nutritional and other interacting factors which may influence the fertility of cattle. Vet. Rec., 71: 247.

Hinkoveski T. 1990. Buffalo breeding in Bulgaria . Animal Science Papers and Reports (Warsaw). 6:95-98.

Hoffman H, Kyrein H.J and Ender M.L., 1973: An efficient procedure for the determination of progesterone by radioimmunoassay applied to bovine peripheral plasma. Hormone Res, 4: 302-310.6.

Jainudeen M. R.; Sharifuddin, W.; Yap, K. C. and Abu-Bakr, D. 1984. Post-partum anoestrus in the suckled Swamp buffalo. Res. Coord. Mty. "Use of Nuclear Techniques to improve Domestic buffalo production in Asia". FAO/IAEA Division of Isotopes, Manila, the Phillippines.

Jani R.G., B.R. Prajapati and M.R. Dave. 1995. Hematological and biochemical changes in normal fertile and infertile Surti buffaloes. Indian J. Anim. Sci., 65: 536-539.

Karhe D.R. 2012. Induction of oestrus using hormonal and no hormonal drugs in postpartum crossbred cows during low breeding season. Master of Veterinary Science. DOI: 10.5455/ajvs.250583.

Koley S. and Biswas P. 2004. Effect of mineral supplementation on the performance of anestrous cows. Indian J. Anim. Nutr. 21(4): 268 – 270.

Kumar S., Sharma, M.C., Dwivedi, S.K., 1986. Calcium, phosphorus and serum electrolyte changes in anoestrus and repeat breeding cows and heifers. Cherion 17: 9-12.

Kumar P.R, Sri Balaji N. and Uma Rani R. 2014. Anoestrus in cattle and buffaloes: Indian Perspective. Advances in Animal and Veterinary Sciences, 2(3), 124-138.

Lammoglia M.A, Willard S.T, Hallford D.M and Randel R.D, 1997.Effects of dietary fat on follicular development and circulating concentrations of lipids, insulin, progesterone, estradiol-17b, 13, 14-dihydro-15-keto-prostaglandin F2a, and growth hormone in estrous cyclic Brahman cows, Journal of Animal Science, 75, 1591–1600.

Lodhi L.A.; Qureshi, Z.I.; Kan, A. and Hayat, S. 1998. Comparative study of blood glucose, total proteins, calcium and phosphorus in cycling, non-cycling, repeat breeding and endometritis buffaloes. Pakistan J. Biol. Sci., 1(2): 66-68.

Lottammer K.K., L. Ahleswede, H. Meyer, Schulz and N. Hoffman. 1974. Cited from Sikka, P. Indian J. Dairy Sci.,45: 159-167.

Luca L.J., De. J.H. Silva, R.J. Grimoldi and E.G. Capaul. 1976. Fertility in cattle and the practical application of some blood values, p. 972-974. In Proceedings of 20th World Veterinary Congress, Greece.

Marai I. F. M., Habeeb, A. A. M. 2010. Buffalo's biological functions as affected by heat stress. Livest.Sci. 127: 89–109.

Martinez N., Risco, C. A. , Lima, F. S., Bisinotto, R. S., Greco, L. F., Ribeiro, E. S., Maunsell, F., Galvao, K. and Santos, J. E. 2012. Evaluation of peripartal calcium status, energetic profile, and neutrophil function in dairy cows at low or high risk of developing uterine disease. J. Dairy Sci., Vol. 95, pp. 7158-7172 .

Mathur A.K., Srivatava, S., Tyagi, S. and Mandal, D.K. 2005.Effect of vitamin A and mineral administration on the induction of estrus in Firewall and Sahiwal heifers. Indian J. Anim. Reprod., 26 (1): 60-61.

Noakes David E. and Parkinson T.J., 2001.Arthur's Veterinary Reproduction and Obstetrics. W.B.SAUNDERS, A Harcourt Health Sciences Company.LONDON.8th.ed .415 – 446.

NRC ,2001. Nutrient requirements of dairy cattle (7th Rev.ed.) National Academy Press Washington.DC.

Pande M. B., Desai H. B. and Shukla P. C. 1978. Studies on blood hematology and serum mineral constituents of Mehsani buffaloes. GUJVET, 9(1-2): 43-47.

Parmar B.N. 2013. Augmenting reproductive efficiency of infertile buffaloes using controlled breeding techniques in tribal areas. M.V.Sc. Thesis, submitted to An and Agricultural University, Anand, Gujarat, India.

Parmar K.H., Shah R.G., Tank P.H. and Dhami A.J. 2012. Effect of hormonal and non-hormonal treatment on reproductive efficiency and plasma progesterone, biochemical and macro-minerals profile in postpartum anoestrus Surti buffaloes. Indian J. Field Vets., 8(2): 48-54.

Paul S. S., Chawla D. S. and Lall D. 2000. Serum mineral profile and its relationship with reproductive disorders in Nili-Ravi buffaloes. Indian J. Anim. Nut., 17(4):324-327.

Rensis De., F. and Lopez-Gatius, F., 2007. Protocols for synchronizingestrus and ovulation in buffalo (Bubalusbubalis): areview. Theriogenology 67: 209–216.

Roberts S.J. 1971.Veterinary Obstetrics, 2nd ed. College Book Store, 1701, NaiSarak, Delhi.

Sah S. K. and Nakao T., 2010. A clinical study of anestrous buffalo Esin Southern Nepal. J. Reprod. Dev. 56: 208–211.

Sarvaiya N.P. and Pathak M. M. 1992. Profile of Progesterone, 17-β oestradiol, triiodothyronine and blood biochemical parameters in Surti buffaloes heifers. Buffalo J., 8(1): 23-30.

SAS, 2000. The Statistical Analysis System. Version 9, SAS Institute Inc., Cary, North Carolina, USA.

Sathish Kumar 2003. Management of infertility due to mineral deficiency in dairy animals. In: Proceedings of ICAR summer school on "Advance diagnostic techniques and therapeutic approaches to metabolic and deficiency diseases in dairy animals" held at IVRI, Izatnagar, UP (15th July to 4th Aug.) pp. 128-137 .

Schweigert F.J., Zucker H.,1988. Concentratio of vitamin A , beta-carotene and vitamin E in individual bovine follicles of different quality. Journal of Reproduction and Fertility, 82, 575-579.

Shah A.R., Singh A.P., Kunj V., Akhtar M.H., Roy G.P. and Singh, C., 2003. Effect of mineral supplement in anoestrous buffalo cows. Indian Veterinary Journal 80, 696-697.

Shrivastava A.K., Kharche K.G. and Jain S.K. 1981. Effect of medicaments on blood levels of glucose and inorganic phosphorus in anoestrus buffaloes during non-breeding season. Indian J. Anim. Reprod., 1: 23.

Singh A.P., Shah R.S., Singh R.B., Akhtar M.H. and Roy G.P.,Singh C. and Kunj,V.2006. Response of mineral mixture, prajana and GnRH on serum biochemical constituents and conception rate in anoestrus buffalo.*Indian J. Anim.Reprod.*, 27(1):51-54.

Sirmour S., Nema S.P., Singh B.K. and Shukla S.P. 2006. Induction of estrus in delayed pubertal crossbred heifer. Indian J. Anim.Reprod., 27(1):55-58.

Sujatha T, Ranjan R, Singh N. D. and Uppal S K 2013. "Nutritional Hemoglobin urea in a
She goat", Ind. Vet. J., Vol. 90, pp. 124-125.

Swenson C.K. 1998. Influence of mineral supplementation on blood serum and liver mineral concentrations in first calf beef heifers Ph.D. Dissertation. NM State Univ., Las Cruces.

Terzano G.M. et al. 2012. Overview on reproductive endocrine aspects in buffalo. Journal of buffalo Sciences, 1, 126-138.

Umesh K.R., V. Sudhir R. Chandra A.S.S. Rao E.E. Reddy G.V.N. Reddy and C.C. Reddy.1995. Studies on certain blood biochemicalconstituents of rural buffaloes during cyclicand post-partum anestrus periods. Indian Vet.J., 72: 469-471.

Wang C., Liu, Z. Wang, D. Liu, J. Liu, H. and Wu, Z. 2014. Effect of dietary phosphorus content on milk production and phosphorus excretion in dairy cows. J. Anim. Sci. Biotechnology., Vol. 5, pp. 23.

Zicarelli L. 1997. Super ovulatory response in buffaloes bred in Italy. Third Course on Biotechnology of Reproduction in Buffaloes, Caserta, Italy., 167-188.

Ziling Fan, Shi Shu, ChuChuXu, Changsheng Li, Xinhuan Xiao, Cheng Xia, Gang Wang, Hongyou Zhang, Chuang Xu and Wei Yang 2017. Investigation of negative energy balance and postpartum anoestrus in an intensive dairy farm from the Chinese province of Heilongjiang. ACTA VET. BRNO, 86: 59–65.

تأثير مستحضر مركبات الفوسفور (التونوفوسفان) على النشاط المبيضي للجاموس المصري

محمد عبد الفتاح ابوفرو ، عماد فوزى المغربي ، وائل محمد ناجى ، اسامة مصطفى المالكي و ماجد احمد ابو العمران

معهد بحوث الإنتاج الحيواني ،مركز البحوث الزراعية ،الدقي ، مصر.

تهدف الدراسة الى تقييم تأثير استخدام التونوفوسفان كمصدر للفوسفور على استعادة النشاط المبيضي والشبقي لعجلات الجاموس واناث الجاموس بعد الولادة والتي لم يظهر عليهم علامات شياع وعلاقته بمستوى الفوسفور بالدم.أجريت هذه الدراسة على عدد ٢٤ من الحيوانات التي لم يظهر عليها شياع وذات مبايض خاملة ومستوى البروجسترون اقل من ١ نانوجرام /مل خلال ال ٩٠ يوم بعد الولادة (عدد ١٢ من أبقار الجاموس اوزانهم ٤٧٠-٥٣٠ كيلو جرام واعمارهم ٥-7 سنه و مآبين ٣-٤ مواسم حليب و أيضا عدد ١٢ عجلة اوزانهم ٣٩٠-٤٢٠ كيلو جرام واعمارهم ٢.٥-٣ سنه).كان عدد الحيوانات في كل مجموعة من مجموعة العجلات واناث الجاموس هو ١٢ وقسمت الحيوانات لتحت مجموعتين (معامل وكونترول بكل مجموعة عدد ٦ حيوانات).الحيوانات في المجموعة المعاملة (٦عجلات و ٦ من اناث الجاموس) كان يتم حقنها بالتونوفوسفان ٤ مل /١٠٠ كيلوجرام وزن حي مرتين بفاصل ٧ ايام , بينما الحيوانات بمجموعة الكونترول تم حقنها بماء مقطر ٤مل /١٠٠ كيلو جرام وزن حي في نفس ميعاد حقن مجموعة الحيوانات المعاملة بالتونوفوسفان. لكشف علامات الشياع كان يتم مراقبتها مرتين يوميا.تم اخذ عينات الدم من كل مجاميع الحيوانات عند اليوم ٠-٣-٦-١٠-١٤-١٨-٢٢-٢٦-٣٠-٣٤-٣٨ من المعاملة.وقد تم الحصول على النتائج التالية : - لوحظ ان معدل ظهور الشياع في الحيوانات المعاملة كان اعلى معنويا(P<0.05) في أبقار الجاموس ٨٣.٣% عنه في العجلات ٦٦.٦٧% , بينما الحيوانات الغير معاملة لم تظهر علامات شياع. كما لوحظ ان قوة وفترة الشياع والفترة من بداية المعاملة حتى ظهور شياع كانت تقريبا متشابهة في كل من عجلات واناث الجاموس.- زاد تركيز هرمون البروجيستيرون في سيرم الدم معنويا (P<0.05) في اناث الجاموس عن العجلات فقط عند اليوم ٣،٦ من المعاملة , بينما كان الزيادة غير معنوية (P≥0.05) في اناث الجاموس عن العجلات في جميع الايام الاخرى من اخذ العينات.كان تركيز البروجيستيرون أيضا أعلى معنويا (P<0.05) في المجموعات المعاملة عن المجموعات الغير معاملة في جميع ايام اخذ العينات, باستثناء المدة من اليوم الاول الى اليوم السادس من المعاملة. كان تركيز البروجيستيرون كان اقل من ١ نانوجرام/مل في مجموعات الكونترول في جميع أيام أخذ العينات خلال المعاملة , بينما كان اكبر من او يساوى ١ نانوجرام/مل بعد ١٠ ايام من المعاملة في المجموعات المعاملة فقط.- لوحظ ان تركيز الكالسيوم في معظم ايام اخذ العينات خلال التجربة وتركيز الفوسفور ايضا في جميع أيام أخذ العينات خلال التجربة أعلى معنويا (P<0.05-P<0.001) في العجلات عن اناث الجاموس, بينما النسبة بين الكالسيوم والفوسفور أظهرت اتجاها عكسيا في جميع أيام أخذ العينات.نستخلص من هذه الدراسة ان الحقن بالتونوفوسفان مرتين بفاصل اسبوع بين الجرعتين وبمعدل ٤مل/١٠٠كجم من وزن الجسم لعجلات واناث الجاموس التي تعانى من عدم الشياع و خمول بالمبايض كان له تأثير إيجابي على استعادة النشاط الشبقي والمبيضي وحدوث الحمل لها و كان التأثير اكثر فاعلية في اناث الجاموس عن عجلات الجاموس.