



# Improving Reproductive and Productive Efficiency of Barki Sheep by using GnRH and Selenium

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## ABSTRACT

The present study was conducted to investigate the effects of hormonal treatment (GnRH) and/or selenium (Se) supplementation on improving the reproductive and the productive efficiency of Barki ewes. Sixty-two Barki ewes were assigned into four groups. G1 group (15 ewes) served as a control group only fed CFM, without Se supplementation or GnRH injection, G2 (16 ewes) was estrus-synchronized with double injections of PGF<sub>2</sub>α 11 days apart and intramuscularly injected with 2 ml of GnRH at day 11 and were fed CFM without Se, G3 (15 ewes) received double injections of PGF<sub>2</sub>α 11 days apart and were supplemented with Se, while G4 was (16 ewes) estrus-synchronized with double injections of PGF<sub>2</sub>α 11 days apart and intramuscularly injected with 2 ml of GnRH at day 11 and supplemented with Se. Reproductive parameters, milk yield and composition as well as animal weights were recorded. Progesterone hormone concentration was also measured. The result indicated that conception and lambing rates were higher ( $P < 0.05$ ) in G4 (93.75%) as compared to G1 (80 %) while G2 and G3 recorded 87.5 and 86.66%, respectively. The numbers of lambs born alive and weaned were higher in all treated groups than the control group. Mortality rate from birth to weaning had increased in the control group than treated ones. Milk yield, milk fat and protein had increased insignificantly in Se groups (G3 and G4). The data of lambs birth weight and average daily gain showed significant increases in G3 and G4, while the weaning weight had not been affected with values being similar. GnRH administration increased plasma progesterone concentration compared with the controls. In conclusion, GnRH administration and Se supplementation improved reproductive parameters and milk yield and composition as well as their lambs' weights, probably through its beneficial effect on embryo survival by enhancing luteal function.

**Key words:** Reproductive efficiency, Barki sheep, Selenium, GnRH.

## INTRODUCTION

The efficiency of production in sheep depends heavily on the reproductive performance of females. The incidence of twin births in Barki ewes is low and considerable economic advantages would be accrued from the making available of effective methods, concerning the increase of the reproductive rate in this breed.

Selenium deficiency plays a role in numerous economically important livestock diseases, problems that include impaired fertility, abortion, retained placenta and neonatal weakness (McDowell et al., 1996). Administration of Se improves daily weight gain of lambs and reproductive performance in ewes (Gabryszuk and Klewicz, 2002). The organic selenium from selenomethionine (Se-Met) or Se - enriched yeast is an ideal additive because animals absorb and retain it more than inorganic selenium (Ortman and Pehrson, 1997).

Hormonal treatment to control ovulation and reproduction is a requirement for successful breeding and increasing the number of pregnant females as well (Motlomelo et al., 2002; Branimira et al., 2017), resulting in a short breeding period and more uniform newborn crop (Husein and Kridli 2003). Synchronization of estrus is a valuable management tool that has been successfully employed to enhance reproductive performance, particularly in ruminants (Kusina et al., 2000 and Ambrose et al., 2014). Some studies have successfully used Gonadotropin releasing hormone (GnRH) treatment in combination with progestagens, gonadotropins and prostaglandin (Husein and Kridli, 2003; Reyna et al.,

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2007; Beilby et al., 2009; Hashem et al., 2015). GnRH agonist treatment at the day of mating causes a surge of LH to be released, resulting in an improved luteal function and luteinization of developing follicles (Beck et al., 1996), which may stimulate conceptus growth (Khan et al., 2007). GnRH has been used to induce ovulation and to shorten the time to initiate estrus in conjunction with progesterone (Jabbour and Evans, 1991) or PGF<sub>2</sub>α (Naqvi and Gulyani, 1998).

Therefore, the objective of the present study was to attempt to improve the reproductive and the productive performance efficiency by injection of GnRH or/and added organic selenium to Barki ewes under the arid conditions of the North Western Coast of Egypt.

## MATERIALS AND METHODS

This study was carried out during the period from June 2015 to February 2016 at the Animal Production Unit in the Sustainable Development Center for Matrouh Resources, Matrouh Governorate, belongs to the Desert Research Center in the North Western Coast of Egypt. The purpose of the present study was to evaluate the effectiveness of GnRH and or organic Selenium for improvement reproductive and productive performance of Barki ewes.

### Ethical approval

This experiment was performed according to all ethics and animal rights (Desert Research Center). As much as this work had considering all rules and regulations in conformity with the European Union Directive for the protection of experimental animals (2010/63/EU).

### Animals and Management

Sixty two adult Barki ewes (3-5 years old with an average body weight of 41.2 ± 2.13 kg) were assigned randomly into four groups (G1 and G3 N=15 ewes, while G2 and G4 had N=16 ewes). Group 1 (G1) served as control and was not treated with hormonal treatment and was fed CFM without Se. Groups 2, 3 and 4 estrus-synchronized with double injections of prostaglandin F<sub>2</sub>α (Estrumate, Coopers Animal Health LTD, Berkhamsted-England). Each ml of Estrumate contained 250 mg Cloprostenol Acetate), 11 days apart. On day 11, both G2 and G4 were intramuscularly injected with 2 ml of GnRH (Receptal, Intervet International, B.V. Manufactured in the European Union). Groups G3 and G4 were fed diets supplemented with Se, while G2 was fed a diet without Se.

All groups were fed a concentrate feed mixture (Table 1) and berseem (*Trifolium alexandrinum*) hay to cover their requirements through the different physiological stages according to Kearn (1982). Animals were housed separately in shaded pens and were clinically healthy and free from internal and external parasites. Fresh water was available to all groups daily.

**Table 1.** Ingredients percentage and proximate chemical analysis of experimental ration (on 100% DM basis)

Ingredients	Diet	Chemical composition (%)	Diet
Yellow corn	50	Dry Matter (DM)	91.1
Wheat bran	20.5	Crude Protein (CP)	14.5
Cotton seed meal	20	Crude Fiber (CF)	7.4
Soya bean	6	Ether extract (EE)	3.1
Limestone	1.5	Nitrogen free extract (NFE)	71.8
Mineral mixture	1	Ash	3.0
Sodium chloride	1	--	--

Se (ALKOSEL) added 100 g/ton for G2 and G3.

ALKOSEL is a natural product made from the wall of yeast *Saccharmyces* and contains 2000-2400 ppm of selenium in the form of selenomethionine (98% organic selenium). 100 g ALKOSEL was mixed with 2 kg feed to obtain a homogenous mixture of ALKOSEL, which was then added to a 1 ton diet and mixed to obtain homogeneity.

Hand mating started after the second injection of PGF<sub>2</sub>α within 5 days duration, while animals in control group were left for 34 days during the mating season (equal to 2 estrous cycles). Four fertile rams were allowed to rotate among different ewe groups to avoid a ram/group confounding effect.

Milk samples (50 ml) were taken biweekly from ewes within the respective groups during the 12 weeks lactation period, in plastic bags and kept under -20 °C for further analysis. Milk yield was determined biweekly from lambing up to the 12 weeks lactation period, through the complete hand milking of the udder after having fasted the lambs for 12 hours, for two consecutive days once at night and the next at morning to cover 24 hours. The chemical composition of milk in terms of fat, protein, lactose, total solids and solids and not fat was determined using milk scan (Bentley-Belgium).

Blood samples (5 ml) from all groups were withdrawn from the jugular vein into EDTA tubes. The samples were collected from each ewe that had mated at that day, days 1, 3, 5, 7, 9, 11, 13, 15, and 17 then monthly until parturition. Blood samples were centrifuged at 3000 rpm for 20 minutes for the separation of serum and were kept at -20 °C until further analysis. Progesterone hormone was quantified by ELISA method using a BIOS kit provided by Chemux BioScience Corporation, 385 Oyster Point Blvd Suite 5-6, South San Francisco, CA 94080, USA. The standard curve ranged between 0-50 ng/ml. The sensitivity of the curve was 0.2 ng/ml.

Birth and weaning weights at day 90 after parturition and average daily gain were recorded for lambs born to ewes of this study.

### Statistical procedure

Data of the reproductive traits were analyzed using "all or non traits" according to [Snedecor and Chocran \(1980\)](#), while milk yield and composition, lambs weight and P<sub>4</sub> concentrations were analyzed using General Linear Model Procedure ([SAS, 2004](#)). The design was one way analysis. The model was as follows:

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where,  $Y_{ij}$  = any observation of  $j^{\text{th}}$  animal within  $i^{\text{th}}$  treatment;

$\mu$  = overall mean;

$T_i$  = effect of  $i^{\text{th}}$  treatment ( $i = 1-4$ );

$e_{ij}$  = experimental error.

Duncan Multiple Range Test ([Duncan, 1955](#)) was used to test the level of significance among means ( $P < 0.05$ ).

## RESULTS AND DISCUSSION

### Reproductive parameters

In this study, GnRH and/or Se improved all reproductive traits and had reduced mortality rate compared to none treated animals. Reproductive parameters (rates of conception, lambing, weaning and mortality) have been presented in Table 2. Ewes of the control group (G1) had recorded the lowest ( $P < 0.05$ ) conception and lambing rates (80%) while G4 had recorded the highest rate (93.75%). G2 and G3 recorded 87.5 and 86.66%, respectively. These results indicated that hormonal treatment and dietary Se supplementation have a positive effect on conception and lambing rates. The improvement of conception and lambing rates in treated groups might be related to the enhanced follicular development and ovulation ([Titi et al., 2008](#)).

Weaning rate showed the same trend of conception and lambing rates. On the other hand, mortality rate from birth to weaning in lambs born to those ewes that had received Se was lower than their counterparts that had no Se supplementation (7.69 and 6.25% in groups 3 and 4 vs. 16.66 and 13.33% in groups 1 and 2). These results are in agreement with those reported by [Pilarczyk et al. \(2004\)](#), who observed that lamb mortality in the experimental group that had received sodium selenite was lower than the control group (9.2% vs. 12.1%). Several studies reported better conception rate with synchronization protocols based on GnRH and PGF<sub>2</sub> $\alpha$  than those based on PGF<sub>2</sub> $\alpha$  ([Stevenson and Pully, 2012](#); [Youssefi et al., 2013](#); [Branimira et al., 2015](#)). Moreover, [Ahmadi and Mirzaei \(2016\)](#) found that twinning rate of GnRH treated ewes was significantly higher than untreated ewes, while [Cinar et al. \(2017\)](#) found that estrus synchronization protocols including PGF<sub>2</sub> $\alpha$  and GnRH hadn't significantly affected conception, kidding and twinning rates in hair goats. [Finch and Turner \(1996\)](#) showed that the diet inclusion of Se in concentrate above of requirement level was associated with improvements in animal performance and immune function.

No still births and abortion cases were found. The number of lambs born alive and weaned was higher in G4 followed by G2 and G3, while the control group had the lowest value (Table 2). This is in agreement with the previous findings of [Cam et al. \(2002\)](#), [Khan et al. \(2007\)](#) and [Lashari and Tasawar \(2007\)](#) who observed a positive effect of GnRH administration on the day of mating on embryo survival in sheep and cows. Also, the results of this study provided evidence that GnRH causes ovulation and the formation of accessory CLs. A higher number of CLs were observed in slaughter ewes (at day 25 of pregnancy) which were given 2 ml of GnRH at the day of mating after 2 doses of PGF<sub>2</sub> $\alpha$  ([Lashari and Tasawar, 2010](#)).

[Titi et al. \(2010\)](#) studied the oestrus synchronization in Awassi ewes and Damascus does using progestagen sponges and eCG (S), GnRH, and PGF<sub>2</sub> $\alpha$  (GP) and GnRH, progestagen and PGF<sub>2</sub> $\alpha$  (GSP). They found that the greatest lambing rate ( $P < 0.05$ ) in ewes was shown in the GSP group compared with the control (no treated) and S groups, while GP group was intermediate. However, similar kidding rates were observed among treatments in Damascus does. [Husein and Kridli \(2003\)](#) reported that the luteal tissue that forms as a result of the GnRH administration is responsive to PGF<sub>2</sub> $\alpha$  and is capable of undergoing luteolysis.

**Table 2.** Reproductive performance of the different experimental groups

Items	G1	G2	G3	G4	P Value
No. of ewes joined	15	16	15	16	
No. of ewes conceived	12	14	13	15	
Conception rate (%)	80 <sup>b</sup>	87.5 <sup>ab</sup>	86.66 <sup>ab</sup>	93.75 <sup>a</sup>	0.053
No. of ewes aborted	0	0	0	0	
No. of ewes barren	3	2	2	1	
No. of still births	0	0	0	0	
No. of ewes lambed	12	14	13	15	
Lambing rate (%)	80 <sup>b</sup>	87.5 <sup>ab</sup>	86.66 <sup>ab</sup>	93.75 <sup>a</sup>	0.053
No. of lamb born alive	12	15	13	16	
No. of lamb weaned	10	13	12	15	
Weaning rate (%)	83.33 <sup>b</sup>	86.66 <sup>ab</sup>	92.30 <sup>a</sup>	93.75 <sup>a</sup>	0.058
No. of mortal lambs	2	2	1	1	
Lamb mortality (%)	16.66 <sup>a</sup>	13.33 <sup>a</sup>	7.69 <sup>b</sup>	6.25 <sup>b</sup>	0.043
Litter size %	100	107.14	100	106.66	0.143

G1; control group, G2; PGF<sub>2α</sub> and GnRH without Se, G3; PGF<sub>2α</sub> and Se, G4; PGF<sub>2α</sub>, GnRH and Se. Conception rate = number of ewes conceived / number of ewes mated x100. Lambing rate = number of ewes lambed / number of ewes mated. Weaning rate = number of lambs weaned / number of lambs born. Litter size = number of lambs born / number of ewe lambed. Mortality (%) = lambs born alive - lambs weaned / lambs born alive x100.

On the other side, *Koyuncu and Yerlikaya (2007)* reported that Se and Se plus vitamin E had significant positive effects on incidence of oestrus, fertility and prolificacy in ewes, which supported our results. Positive effects of Se on fertility were also observed by *Koyuncu et al. (2006)*. Se supplementation enhances the level of Se and may indirectly improve livestock performance (*Sobiech and Kuleta, 2002*), possibly by strengthening the immunity of the animals (*Milad et al., 2001*). Moreover, Se supplementation during early and mid-pregnancy reduced the time taken for their lambs to stand and improves the immune status of the lambs, resulting in lower prenatal mortality and higher growth rates to weaning (*Munoz et al., 2009*). In addition, considered that increasing twinning in ewes in response to Se supplementation could often be attributed to the increased live weight and hence to ovulation rate (*Hemingawy, 2003*).

*Basini and Tamanini (2000)* demonstrated that Se may modulate ovarian granulosa cells proliferation and estradiol 17β synthesis in vitro, affecting ovulation and the number of live embryos. The results of reproductive parameters of this study are in agreement with *Cook and Green (2007)*, who reported that the incidence of retained placental, abortion, early embryonic death and ovarian cysts increase in Se deficient in cows and that Se supplementation overcomes some forms of infertility in ewes. Moreover, the inclusion of Se in the maternal diet improves the embryo viability and growth of the progeny (*Pappas et al., 2008*).

Whereas, *Gabryszuk and Kiewiec (2002)* found that Se plus vitamin E did not increase the reproductive performance in younger ewes. Moreover, parental Se supplementation of pregnant ewes between 15-35 days after mating results in a reduced embryonic survival rate (*Niekerk et al., 1996*).

### Milk yield and composition

Ewes fed diets supplemented with Se had a milk yield (G3 and G4) higher than the other two groups (G1 and G2) with differences being insignificant (*Table 3*), which indicated that hormonal treatments did not affect milk yield. These results are in agreement with those reported by *Pauselli et al. (2004)* who found that milk yield was higher in animals treated with vitamin E and Se but animals treated with vitamin E only seemed to have no effect on milk production or mammary health which confirm the role of Se and vitamin E in the host's defense against mastitis. On the other hand, ewes fed diets supplemented with Se showed the highest ( $P < 0.05$ ) milk fat percentage (4.70 and 4.23%) for G3 and G4, respectively, compared to G2 (3.45%), while the milk fat of the control group was near to those of Se groups (4.17%). Results of milk protein and total solids had the same trend found in milk fat (*Table 3*). No significant differences were observed in milk lactose and solids not fat.

*Othmane et al. (2002)* reported an increase of milk yield with higher litter size, while milk contents decreased or were not affected. Moreover, litter size had significantly affected milk and protein yield, while no significant effect was found for fat yield and milk contents in East Friesian sheep (*Horstic, 2001*). Better rumen parameters for organic Se supplemented to diets that may improve productive efficiency in farm animals (*Arzola et al., 2008*).

*Tufarelli and laudadi (2011)* concluded that dietary supplementation with Se had led to an increase in milk production as well as milk fat and protein of dairy Jonica goats which confirmed our results. *Lacetera et al. (1999)*

studied the effect of injecting 5 ml of selenium on day 30<sup>th</sup> before lambing (BL) and 2.5 ml on day 30 before lambing and 2.5 ml at lambing (BLL) on immune function and milk production of Sardinian ewes. They found that ewes belonging to BL and BLL groups and the number of their offspring's were significantly higher ( $P < 0.01$ ) glutathione peroxidase activity of erythrocytes (GSH<sub>px</sub>-E), moreover, milk yield increased ( $P < 0.05$ ) in two treated groups as compared to none treated group. The positive correlation between GSH<sub>px</sub>-E and milk production was reported for dairy goats (Atroshi et al., 1985) and cows (Lacetera et al., 1996). For dairy cows, Niki et al., (1991) supposed that the well-known protective role of GSH<sub>px</sub> on membrane integrity might represent at least one of the mechanisms through which Se can increase milk production. Wang et al. (2009) concluded that Se supplementation of cows ration in the form of Se yeast positively influenced milk production through the positive influence of Se yeast on fermentation in the rumen, which in turn resulted in the enhanced digestibility of nutrients contained in rations.

**Table 3.** Effect of treatments on milk yield and composition of Barki ewes during experimental period

Item	G1	G2	G3	G4	SEM	P Value
Milk yield (ml)	497	469	545	525	91.9	0.798
Milk composition						
Fat (%)	4.17 <sup>ab</sup>	3.45 <sup>b</sup>	4.70 <sup>a</sup>	4.23 <sup>ab</sup>	0.245	0.026
Protein (%)	5.34 <sup>b</sup>	5.47 <sup>b</sup>	5.62 <sup>ab</sup>	5.93 <sup>a</sup>	0.137	0.053
Lactose (%)	5.01	4.90	4.94	5.09	0.112	0.067
Total solids (%)	15.88 <sup>ab</sup>	15.20 <sup>b</sup>	16.48 <sup>a</sup>	15.72 <sup>ab</sup>	0.287	0.054
Solids not fat (%)	11.56	11.74	11.78	11.54	0.206	0.085

G1; control group, G2; PGF<sub>2</sub>α and GnRH without Se, G3; PGF<sub>2</sub>α and Se, G4; PGF<sub>2</sub>α, GnRH and Se

### Lambs growth

Effect of Se supplementation and/or hormonal treatment on lambs birth weight (BW), weaning weight (WW) and average daily gain (ADG) are shown in Table 4. The main finding was that BW and ADG had increased ( $P < 0.05$ ) in animals treated with Se supplementation (G3 and G4), while WW had insignificant increase in Se groups (15.64 and 15.68 Kg for G3 and G4 vs. 14.80 and 14.72 Kg for G1 and G2, respectively). Data obtained in this study are similar to those reported by Gabryszuk and Klewicz (2002) who found that Se given to ewes increased the average daily weight gain of their lambs from birth to 28 days of age. Similar results were obtained by Langlands et al. (1991), who observed higher weight gains in lambs born to ewes that had received Se-enriched feed than in lambs born to untreated ones.

Moreover, Koyuncu and Yerlikaya (2007) found that Se or Se plus vitamin E led to an increase in BW, WW and ADG as compared to control group. These positive responses are variable depending on species, physiological state and chemical form of Se (Rooke et al., 2004). The differences between lamb weights may be related to milk production. The amount of milk per lamb is indispensable in first weeks of life, because it is the main nutrient source for proper growth, development and health (Godfrey et al., 1997).

**Table 4.** Productive performance of Barki lambs of the different experimental groups

Performance	Experimental groups				SEM	P Value
	G1	G2	G3	G4		
Birth weight, kg	3.28 <sup>ab</sup>	3.05 <sup>b</sup>	3.63 <sup>a</sup>	3.64 <sup>a</sup>	0.089	0.040
Weaning weight, kg	14.80	14.72	15.68	15.64	0.197	0.156
Average daily gain, g	122 <sup>b</sup>	130 <sup>ab</sup>	140 <sup>a</sup>	131 <sup>a</sup>	18	0.051

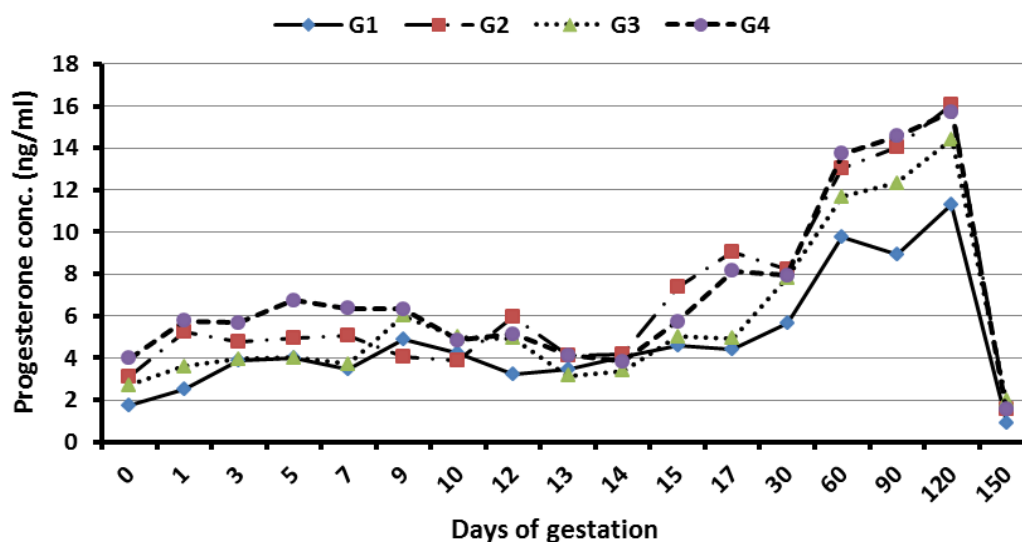
G1; control group, G2; PGF<sub>2</sub>α and GnRH without Se, G3; PGF<sub>2</sub>α and Se, G4; PGF<sub>2</sub>α, GnRH and Se

### Progesterone profile

Plasma progesterone concentration of Barki ewes in control and other treated groups are presented in Fig. 1. Progesterone profile during estrus cycle and pregnancy period was found to have followed the normal pattern. Mean plasma progesterone concentration increased significantly with advancing pregnancy in all groups from day 2 after mating due to the presence of active CL. Mean plasma progesterone concentrations of ewes treated with GnRH (G2 and G4) tended to be insignificantly higher as compared to control group. Plasma progesterone levels exhibiting peak at the 120<sup>th</sup> day of pregnancy and will gradually decline thereafter till parturition. Abd-Elaziz et al. (2004) noted that progesterone level increased during pregnancy, reached its highest level during days between 130-140 and then declined



during the last 10 days before parturition. A similar result of progesterone profiling has been observed by Lashari and Tasawar (2010).



**Figure 1.** Progesterone profile of the experimental groups during pregnancy period in Barki ewes. (G1: control group; G2: PGF<sub>2</sub>α and GnRH without Se; G3: PGF<sub>2</sub>α and Se; G4: PGF<sub>2</sub>α, GnRH and Se).

The increase in progesterone concentrations after GnRH injection suggests that GnRH through LH release might be able to provide luteotrophic stimulation to CL, which could explain the increase in CL weight observed in the ewes given this treatment (Farin et al., 1988). This luteotrophic stimulation might be related to a form of conversion of small luteal cells to large luteal cells, which then secrete higher concentrations of progesterone hormone (Farin et al., 1988).

## CONCLUSION

The results of the present study showed that GnRH administration and Se supplementation had improved the reproductive and the productive efficiency of Barki ewes as well as their lambs. We believe that these results are useful and good benefits would be accrued from the availability of such methods to improve the reproductive efficiency of the Barki sheep.

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### Competing interests

The authors declare that they have no conflict of interest with respect to the research, authorship, and/or publications of this article. The authors declare that they have no competing interests.

### Author's contribution

Dr. Bahaa Farrag designed the experiment, collected data; Dr. Ahmed Sobhy El-Hawy helped in statistical analysis, tabulation of experimental data and article writing; while, Dr. Moharram Fouad El-Bassiony helped in laboratory analyses, manuscript writing, commenting and approval. All authors have read and approved the final manuscript.

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