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Serum Biochemical Changes in Relation to Some Nutritional and Hormonal Managements for Treatment of Inactive Ovaries in Buffaloes

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ABSTRACT

Inactive ovaries may be considered as the major reproductive problem in buffaloes. This study aimed to evaluate the efficacy of hormonal and nutritional-dependent protocols to alleviate ovarian inactivity in buffaloes. In this study, thirty buffalo cows affected with ovarian inactivity were used; they were divided randomly into three groups (10/each). Group-I: were administrated with propylene glycol in addition to trace element mixture (containing zinc and selenium), Group-II: received the same previous treatment of Group-I with G-P-G protocol (GnRH-PGF-2α- GnRH), Group-III: received only the previously mentioned hormonal treatment. The results revealed that, all the treatment protocols has succeed to stimulate the ovarian activity and follicular growth which was accompanied by a significant increase in estrogen and progesterone hormone, but, only the first two protocols have a favorable effect on metabolic-related hormones (T3, T4), serum glucose concentration and mitigation of the present oxidative stress state. All of the treatment protocols have increased the concentration of insulin-like growth factor-1. Finally, it could be concluded that combination of both of nutritional and hormonal treatment has been proved to have the best plausible effect for relieving and cure of ovarian inactivity in buffaloes.

1-INTRODUCTION:

Anestrous can be defined as a reproductive cycle dysfunction which is marked by absence of clear detectable estrous due to paucity of its signs expression or its cessation (Kumar et al. 2014). Furthermore, anestrous can be classified into four main types, Type-I which is characterized by growth of follicles to reach pre deviation stage only, Type-II which is marked by atresia of dominant follicle after deviation and grow-up, Type-II which is characterized by growth of dominant follicle up to ovulatory size, but, it fail to ovulate, and finally Type-IV which is marked by normal ovulation and formation of corpus lutetium which persist for an abnormal prolonged period and last beyond the proper time of regression (Peter et al. 2009). The postpartum period representing a crucial role in bovine reproduction performance, as the prolonged postpartum period or postpartum infertility may lead to massive economical losses in dairy manufacture depending on buffaloes (Kalasariya et al. 2017). The proper nutritional management in form of supplementation of the animals with optimum levels of protein, energy, minerals (as calcium, phosphorus and iodine) and trace elements such as zinc, copper and manganese along with administration of some anti-oxidant like selenium, and vitamin-E have been proved to be highly beneficial in improvement of infertility problems in dairy cows and buffaloes (El-Amrawi, 1990; Mavi et al. 2006; Zeedan et al. 2010; Dhami et al. 2015; Khan et al. 2015; Kalasariya et al. 2017). Extensively, protein deficiency would be one of the prominent factors in occurrence of postpartum infertility (Roberts, 1979). Also, the ovarian activity is the most affected organ by minerals imbalance which may retard the proper ovarian function (Haq et al., 1999). So, restoration of buffalo cyclycity may be achieved through providing of the animals with a balanced ration during pre-partum and post-partum periods ((Baldi et al., 2000; Anita et al., 2003; Selvaraju et al., 2009). Concerning energy deficiency, propylene glycol is used extensively in ruminant diet as an energy additive, but it is used mainly due to its anti-ketogenic and glycogenic effect (Kristensen and Raun, 2007). In parallel, the

treatment of bovine infertility using hormonal therapy as GnRH may induce estrous sufficiently, but, with a poor conception rate (Giri and Yadav, 2001). In recent years, more attention was focused on finding the optimum protocols to enhance buffalo's infertility, so, our study aimed to the effect of propylene glycol, minerals and exogenous GnRH-dependent protocols on the treatment of ovarian inactivity in buffaloes.

2-MATERIAL AND METHODS:

2.1. Animals

This study was carried out during June-August 2019, thirty buffalo-cows weighting about 500-700, from a private dairy farm in Alexandria governorate, Egypt were chosen to complete this study. The farm has a proper program for prevention of infectious diseases. The nutritional program depended on supplementation of buffalo-cows with green fodder, in addition to 30% corn +15% bran. They were suffering from anestrous for about 3 month; they were free from any current pathological condition as they were examined clinically and carefully to confirm this.Ultrasonographical examination revealed no follicles or corpora lutea on both of the ovaries.

2.2. Experimental protocol

The animals were divided randomly into three groups (10/each), Group-I: each cow received 200 ml of propylene glycol 99.9 % (BASF, Germany) which were mixed with concentrate for 7 consecutive days along with subcutaneous injection of minerals mixture (Mineral Fort®, Jurox, Australia) (containing zinc 40mg/ml and selenium 5mg/ml) at a dose of 10 ml 3 times during the experimental period with 3 days interval. Group-II: received the same protocol of Group-I with a hormonal G-P-G protocol in form of injection of 2.5ml Receptal ® (10µg GnRH) (Intervet, USA) (I/M) on the first day of the treatment, followed by 2 ml of Estrumate® (500µg cloprostenol) (Vet Pharma, Germany) (I/M) on 7th day of the treatment, similar dose of Receptal ® was injected on 9th day. Group-III: received the same hormonal protocol of Group-II only. The detection of the ovarian response using ultrasonography (real time, B-mode, 7.5MHz, Sonoscope®, China) to monitor the ovarian response and the follicular sizes categories according to Harun-or-Rashid et al., (2020) was performed at time of starting of the treatment protocols and continued daily up to 24 and 48 after finishing the treatment protocols.

2.3. Blood sampling

It was performed before the starting of the treatment protocols and again on 10th day post-treatment. The blood was drained from left jugular vein in plain blood collection tubes and left for 30 minutes for complete clotting. The coagulated blood samples were centrifuged at 3000 r.p.m for 10 minutes to separate serum. Serum aliquots were kept at -20° C till the analysis time.

2.4. Serum biochemical analysis

Determination of estradiol (E2) and progesterone (Pg): serum level of steroidal reproductive hormones (E2 and Pg) was determined using highly specific ELISA Kits (Fine Test®, China).

Determination of T3 and T4 level: serum level of thyroid hormones (T3 and T4) was detected using highly sensitive ELISA kits (Cusabio Biotech®, China).

Detection of serum insulin-like growth factor-1(IGF-1): the level of IGF-1 in serum was detected using specialized species sensitive ELISA kits (Cusabio Biotech®, China).

Determination of serum glucose level: the concentration of glucose in serum samples was detected calorimetrically using commercially available kits (Biomed diagnostic®, Egypt).

Determination of serum concentration of zinc and selenium: zinc and selenium level in serum was determined using atomic absorption spectrophotometer (ContrAA® 800 F, Analytik Jena, Germany).

Determination of serum MDA and GSH level: the level of MDA and GSH in serum was detected using specialized kits (Biodianostic®, Egypt).

2.5. Statistical analysis:

The obtained data was analyzed using independent samples *t*-test, one way ANOVA test was used to obtain the difference between mean values of the different groups and the results were confirmed using one way ANOVA test Post Hoc Tukey test by the aid of the SPSS statistical package v22.0 for Windows (IBM, Armonk, NY, USA).

3-RESULTS

3.1. Hormonal changes: as shown in table (1), after application of the treatment protocols, serum level of estrogen hormone (E2) and progesterone (pg) recorded a significant elevation in all of the groups. Concerning thyroid hormones, both of serum concentration of T3 and T4 were increased significantly in Group-I and Group-II only upon application of the treatment protocols. On the other

hand, the level of IGF-1 recorded a significant elevation in all of the treated groups.

3.2. The change in some nutritional parameters: the concentration of serum glucose, zinc and selenium showed a significant increase in Group-I and Group-II, while they did not record any significant change in Group-III after the treatment protocols application. (Table: 2).

3.3. The changes in oxidative stress biomarkers: only the treatment with the first two protocols has an alleviation effect on the present state of oxidative stress as the level of GSH was significantly increased and the level of MDA was significantly decreased in Group-I and Group-II, without any significant changes in Group-III (Table: 2).

3.4. The change in the follicular size: at the end of the treatment protocols time, the total follicular size recorded more significant change in Group-II and Group-III when compared to Group-I, but, there was not any significant difference in total follicular size between Group-II and Group-III (Table: 3). The number of large sized follicles and their total sizes, 24 and 48 hours from the end of the treatment did not reveal any significant changes between all of the treated groups (Tabl:4).

Table (1): Hormonal changes upon application of the treatment protocols in different groups:

Parameter		Study Groups					
		Group-I		Group-II		Group-III	
		pre	post	pre	post	pre	post
E2 (Pg/ml)	mean± SD	6.34±0.77	14.12±10.09	6.81±0.66	29.4±6.07	6.55±1.20	23.88±7.81
	t	2.439		12.071		6.922	
	p	0.037*		0.000*		0.000*	
Pg (ng/ml)	mean± SD	0.79±0.26	2.39±0.917	0.29±0.061	1.73±0.360	0.39±0.11	1.63±0.4080
	t	6.114		12.271		9.949	
	p	0.000*		0.000*		0.000*	
T3 (ng/dl)	mean± SD	167.2±13.8	184±10.92	169.1±14.1	182.6±11.4	171±16.3	174.9±14.8
	t	3.573		3.070		0.546	
	p	0.006*		0.013*		0.598	
T4 (µg/dl)	mean± SD	3.13±0..7288	4.14±0..64	3.03±0.7056	4.02±0.7584	3.37±0.6601	3.44±0.6415
	t	3.262		1.4578		0.692	
	p	0.010*		0.001*		0.506	
IGF-1 (ng/ml)	mean± SD	16.08±2.32	18.69±3.16	15.8±2.67	18.86±2.78	13.23±2.84	15.7±2.53
	t	2.727		7.229		5.959	
	p	0.023*		0.000*		0.000*	

Pre: means the values before protocols application.Post: means the values after protocols application. *: significant change.

Table (2): The changes in some nutritional parameters and oxidative stress biomarkers upon application of the treatment protocols in different groups

Parameter		Study Groups					
		Group-I		Group-II		Group-III	
		pre	post	pre	post	pre	post
Glucose (mg/dl)	mean± SD	49.40±5.06	55.50±4.03	50.50±4.72	59.20±5.32	55.10±4.86	52.20±5.63
	t	6.515		6.483		-1.981	
	p	0.000*		0.000*		0.079	
Zinc (µg/dl)	mean± SD	162.58±7.5420	179.31±9.5953	159.59±8.1168	171.870±11.5418	161.57±8.64	163.08±9.6786
	t	4.029		2.113		0.397	
	p	0.003*		0.064*		0.701	
Selenium (µg/ml)	mean± SD	0.0716±0.0067	0.1408±0.034	0.0708±0.0082	0.1318±0.0376	0.0743±0.0083	0.0707±0.0064
	t	6.056		6.086		-0.943	
	p	0.000*		0.000*		0.370	
GSH (µmol/l)	mean± SD	2.64±1.0448	5.51±0.7310	3.01±1.1445	6.08±0.8677	2.850±1.1158	2.6±1.1146
	t	7.19		9.322		-0.633	
	p	0.000*		0.000*		0.543	
MDA (µmol/l)	mean± SD	5.49±1.2556	2.4±0.7196	6.08±1.5754	2.55±0.7906	5.62±1.6363	5.78±1.5483
	t	-7.628		-5.942		0.229	
	p	0.000*		0.000*		0.824	

Pre: means the values before protocols application.Post: means the values after protocols application. *: significant change.

Table (3): The changes in the ultrasonographically detected follicular sizes between the treated groups

Parameter's	Group-I	Group-II	Group-III	ANOVA tukey way
Total Follicular size	6.16±4.18229	13.383±2.23895	10.8360±2.60781	
F ANOVA		13.739		
p		0.000*		
Small size(mm)	2.9±0.0	2.23±0.03	2.06±0.0	A. A.0 .000*
Medium size(mm)	4.4286 ±1.09043	9.8±0.0	8.16±1.21301	B. B.0 .007*
Large size(mm)	13.85±0 .63640	13.7811±1.96379	12.62±1.34551	C. 0.181

A: comparing between Group-I and Group-II, B: comparing between Group-I with Group-III, C: comparing between group-II and group-III.*: significant change.

Table (4): The changes in the development of ultrasonographically detected large sized follicles after 24 and 48 hours from the end of the treatment between the treated groups

Parameter		Group-I	Group-II	Group-III	ANOVA tukey way
Large follicles within 24 from the end of the treatment protocols.	**number**	0	6	4	**Within groups** A. 0.999 B. 0.656 C. 0.419
	Size (mm)	0±0.0	13.555±2.3343	12.03±1.23218	
Large follicles within 48 from the end of the treatment protocols.	**number**	2	3	2	
	Size (mm)	13.85±0.63640	14.2333±1.15902	13.8±0.56569	
Within groups	**F**	0.930			
	P	0.418			
Within times	**T**	-1.294			
	P	0.215			

A: comparing between Group-I and Group-II, B: comparing between Group-I with Group-III, C: comparing between group-II and group-III.*: significant change

4-DISCUSSION:

The major restriction in obtaining the perfect reproductive efficiency in buffalo may be the high incidence of ovarian inactivity (El-Wishy, 2007). In recent years, many attempts have been applied to induce and keep regular cyclicity in anestrum-affected buffaloes by the aid of hormonal and non-hormonal treatment protocols (Nasr et al.,1983; Pant et al. 2002; Singh, 2003). Concerning our results, the increase in blood estrogen level (E2) in all of the treated groups might be a consequence of the induction of ovarian activity and follicular growth (which was detected ultrasonographically) which secrete estrogen hormone from granulose cells as proplyne glycol tend to decrease the level of non-esterified fatty acids and beta-hidroxybutyrate (BHBA) (Nielsen and Ingvartsen, 2004; Chiofalo et al., 2005), the increased BHBA concentrations may impair GnRH secretion (Beam and Butler, 1999). Also, GnRH and its synthetic analogues have been reported to have a favorable effect on induction of ovarian activity (Saini and Lohan, 2003) as the use of GnRH-PG-GnRH injections (as in Group-II) enhance follicular development and control corpus lutetium (CL) regression (Baruselli et al., 1994).On the other hand, trace elements as selenium could reduce the incidence of anoestrus (Harrison et al.,1984), also, Dutta et al. (2001) reported that low zinc concentration was associated with deficient steroid hormones concentration and anestrum in heifer as it may affect FSH and LH release (Ahmed et al., 2010), also, it was reported that zinc supplementation may improve the anestrous condition in buffalo (Khan et al., 2015). The net results of the above mentioned beneficial effects of the contents of the treatment protocol may be the reasons for the ovarian response and follicular development. The increase in progesterone level in the blood of all of the treated groups may be due starting of progesterone production from preovulatory follicle (Smith et al., 1994) as anestrous animals usually record a significant decrease in progesterone level due to absence of LH surge (Terzano et al., 2012). The increase in blood thyroid hormones level (T3 and T4) may be a result of supplementation of zinc and selenium in both of Group-I and Group-II as both of these trace elements has been reported to increase thyroid activity (Mahmoodianfard et al., 2015), the increase in thyroid hormones may stimulate the ovarian function through its direct action with FSH on differentiation of granulose cell (Saleh et al., 2011). In the same way, supplementation of zinc and selenium may influence the release of IGF-1(Lefebvre et al., 1998; Alehagen et al., 2017). In the same manner, the increase in IGF-1 in Group-III may be due to the increased estrogen level, as estrogen was previously proved to stimulate the production of IGF-1 (Murphy et al., 1987). The role of IGF-1 in reproduction may be summarized in its synergistic effect with FSH (Adashi et al, 1985) on granulose cell through granulosa cell receptors (Gates et al, 1987). Serum glucose was increased in both of group-I and Group-II which may be a result of addition of propylene glycol to the ration as it can increase blood glucose level (Nielsen and Ingvartsen, 2004; Chiofalo et al., 2005). This significant increase in serum glucose level may inhibit the blocked hypothalamic-hypophyseal-ovarian axis signal communication which may lead to nutritional anestrous condition (El-Amrawi, 1990; Kumar et al., 2015). On the other hand, oxidative stress may disturb the occurrence of efficient reproduction through its effect on folliculogenesis and steroidogenesis (Agarwal et al., 2005; Al-Gubory et al., 2010), the treatment protocols applied to group-I and Group-II have efficiently enhanced the level of GSH and decreased the elevated level of MDA, and this may be due to administration of selenium which is considered as one of strong antioxidant (Sathya et al., 2007).

5-CONCLUSION

Finally, it could be concluded that all of the treatment protocols have alleviated the anestrous condition and stimulated the ovarian activity, but, regarding the follicular size and enhanced the biochemical parameters, we can consider that the treatment protocol which was applied to group-II (combination of hormonal and nutritional treatment) was the best treatment protocol when compared to the other treatment protocols.

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