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Ovitone as a Novel Treatment for Ovarian Inactivity in Egyptian Buffaloes

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Abstract: Ovarian inactivity is the major constraint for proper productivity in buffaloes in Egypt and most of developing countries having buffaloes. This disorder causes great economic losses due to decreased calf crop and milk yield. The current study was carried out to monitor the current status of ovarian inactivity in Egyptian buffaloes during the last decade. Special emphases were given to some economic aspects and the evaluation of a novel mixture of phosphorus, traces, vitamins and Lasalocide, which could be used as feed additives and we refereed to it as Ovitone. Field investigations were carried out from 2004 to 2011on 2723 female buffaloes raised at Lower Egypt. Ovitone was tried for treatment of ovarian inactivity in comparison to the current commercially used hormonal preparations. Results indicated that ovarian inactivity averaged 41.53% among the studied animals, fluctuated during different years of that period, showing higher prevalence in heifers and primiparous cows. The syndrome was highly correlated with mange and coccidiosis. Ovarian inactivity predispose to national economic losses estimated as 3.296 and 3.938 million of Egyptian pounds during 2009 and 2010. It was recommended to pay more attention for this syndrome to minimize the money spent on meat and milk importation. Use of Ovitone would give the same curative effective of the commercial medicine besides its cheap price and availability.

Key words: Buffaloes · Ovarian inactivity · Lasalocide · Folligone · Economic evaluation

INTRODUCTION

Nowadays, there is evidence that the phenotypic historical decline in cow fertility has reached a nadir [1] and the condition is not the same in buffaloes, whereas ovarian inactivity prevailed and causing considerable economic losses. Reproductive dysfunction was attributed directly or indirectly to managemental, pathological and other external influences with consequent low economic benefits from breeding of such animals [2-4].

From the financial point of view, producers are not only confronted with the cost of treating their animals from a specific disorder or disease, but they often incur additional costs arising from insidious associated diseases in herdmates, together with the proven deleterious consequences for reproductive performance.

To get the proper benefit from animal breeding, a cow must have a calveing interval of 12 to 13 months and should become pregnant within ~3 months after calving. This requires normal resumption of ovarian activity before this period [5-7]. Moreover, the condition in buffaloes gets more influenced by level of nutrition, season of birth, management and season of the year [8].

The current study was carried out to throw light on the current status of ovarian inactivity syndrome in Egyptian buffaloes during the last decade, tracing the coincident disorders or diseases and to stand on the financial losses due to this syndrome. A field trial was carried out to evaluate a novel laboratory prepared mixture of phosphorus, trace elements, vitamins and Lasalocide (Ovitone) used as feed additives to treat inactive ovaries as compared with different commercial treatments. Special emphasis was given to the financial output of such novel mixture as a trial to venture forth to pursuit better reproductive performance for better profitability and animal welfare.

MATERIALS AND METHODS

A total number of 2723 female buffaloes were examined throughout 8 years (2004-2011). These animals raised in small holder farms at Lower Egypt. A full case history and owner complaint of each animal were

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recorded. The general health condition was examined and body condition score was recorded on scale of 1 (very thin) to 5 (very fat) [3]. Gynecological examinations were carried out twice for two successive weeks at least to register the reproductive status and/or disorders. Examinations were aided by ultrasonography (PiaMedical Falcs e`Saote, Netherlands) with an endorectal linear array of 8.6 M hertz. Animals which did not show estrous signs during the breeding season (September-May) and have small non-functioning ovaries were considered to suffer from ovarian inactivity. The incidence of the coincident diseases was recorded.

Sampling:

- Milk samples were collected and *in situ* investigated for the incidence of mastitis using commercial available California Mastitis test (Qmasa, Spain).
- Blood samples were collected with and without EDTA and films were prepared, stained with Giemsa stain to be examined for the presence of blood parasite.
- Fecal samples and skin scrapings were also collected and examined for internal and external parasites, respectively.

Treatment Trials: A total number of 97 buffalo cows were selected to carry out some treatment trials. These cows were selected to have an average body condition scores (3-4) and were divided into 4 groups as follow:

- The first group included 20 animals that were kept without treatment as a control group.
- The second group included 24 cows, each animal in this group was intramuscularly injected with a small dose (1000 IU) of pregnant mare serum gonadotrophin (PMSG, Folligon ®, Intervet, the Netherlands) to sensitize the ovarian activity and support folliculogenesis.
- The third group included 29 cows that were intramuscularly injected with 0.02 mg buserelin,(GnRH, Receptal®, Intervet, the Netherlands), divided into two doses with 24 hrs intervals followed by intramuscular injection of 2ml synthetic prostaglandin F2α (Estrumate®, Coopers Animal Health, Berkhamsted, UK).
- The fourth group included 24 cows that supplemented with 20g from mixture of mineral, vitamins and lasalocids® " Ovitone" in their ration for 10 successive days. This mixture was prepared in the laboratory by thorough mixing of 1kg sodium phosphate dibasic (Adwic, Egypt), 20g zinc sulphate(Modern Lab, Egypt), 6.25g copper sulphate(Fine-Chem, Egypt), 1.5 g potassium iodide (BDH, England), 300 mg sodium selenite (Adwic, Egypt), 200g vitamin AD_3E (Adwic, Egypt) and 60 g lasalocid® (Calcium ionophores, Larache, Basle, Switzerland).

Animals in all groups were checked for the occurrence of heat signs as a response for treatment. Animals came in heat were naturally mated 12 hrs after the appearance of the heat signs. Conception rate was detected 2 months later by rectal palpation.

Economic evaluation was performed before and after treatment to study the financial aspect of this problem and to stand on the prospect output of the used treatments [9].

Data were computed and statistically analyzed [10].

RESULTS

Out of the total examined animals (2723), 41.53% suffered from ovarian inactivity during the breeding season as confirmed by ultrasonography. The incidence was higher in heifers (59.23% of 341) than in cows (39.04% of 2382) (Fig. 1). Moreover, it was found that this syndrome fluctuated throughout the eight years of the study (Fig. 2).

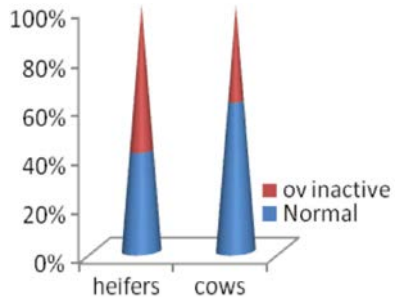

Fig. 1: Incidence of ovarian inactivity in buffalo cows and heifers

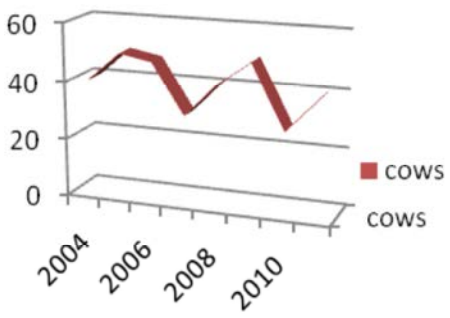

Fig. 2: Incidence of ovarian inactivity in Egyption buffaloes

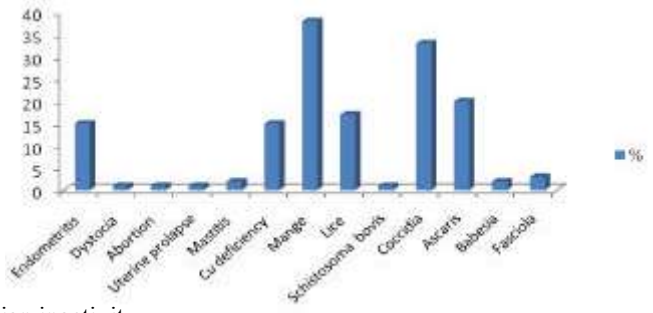

Fig. 3: Concident disease to ovarian inactivity

Table 1: financial losses due to ovarian inactivity in Egyptian buffalo cows

	Type of losses							
Breeding season	Expected buffalo population if all cows gave birth (in millions)	Ovarian inactivity %	Anticipated numerical losses due to ovarian inactivity/ million heads	Exoected buffalo population+ovarian	Financial losses due to lost calf crop in million EGP	Expected looses in annual milk yield in million tons due ov.inactivity	Financial losses due to decreased milk yield in million EGP	Total expected losses due to ovarian inactivity in million EGP
The starting nucleus	4.153	41.8	-	4.153				
After one breeding season	5.191	50	0.434	4.757	1.302	0.443	1.994	3.296
After two breeding seasons	5.795		0.519	5.276	1.557	0.529	2.381	3.938

Table 2: Comparative study between the added values due to different treatement trials for every 100 buffalo cows

parameter	PMS (Folligon®)	GnRH (Reciptal®)	Mineral mix (Ovitone)
Reponse to treatment	58.33%	62.07%	58.33%
Costs of treatment /100 cows in EGP	2250	3000	570
Total revenue in EGP	174690	186210	174690
Total net revenue	172440	183210	174120
The net revenue for every spent EGP/100 cows	64.76	61.07	305.47

Animals showed ovarian inactivity are most likely have poor body condition score (2.08 ± 0.11 varsus 2.88±0.28 for cyclic group) with higher coincidence with mange, coccidiosis, ascariasis, pediculosis, endomtritis and copper deficiency (38,33,20,17,15 and 15%, respectively) (Fig. 3).

Economic Aspect of Ovarian Inactivity: The economic evaluation of the current status of ovarian inactivity in Egyptian buffalo cows based on the losses dues to:

- Lesser progress in the expected buffalo population curve (Fig. 4).
- Financial losses due to decreased calf crop and milk yield (Table 1).

Treatment Trials: Fig 4 shows the response of the non cyclic buffalo-cows following the application of the different treatment trials. The ovarian rebound were 00.00, 79.00, 72.40 and 71.00 % among control, Folligon®, Receptal® and Ovitone groups of animals as monitored by estrous signs, 7-10 days post-treatment, respectively. Rectal palpation, 2 months later revealed conception rates of 00.00, 58.33, 62.07 and 58.33 %, respectively.

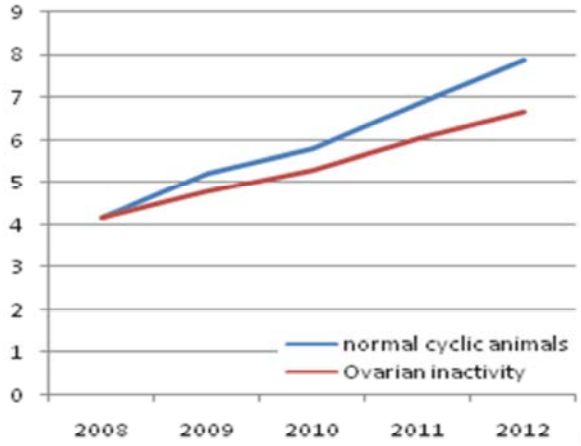

Fig. 4: Expected buffalo population growth with ovarianinactivity in million heads

The economic evaluation of different treatments used for curing ovarian inactivity in buffalo cows is shown in table 2. Ovitone gave favorable response, represented by the conception rate, as compared to the other hormonal treatments, moreover its total revenue for every spend Egyptian pound was the highest among treatments.

DISCUSSION

Reproductive efficiency is high priority in all breeding systems, yet trials were carried out to maximize the condition, especially in seasonal breeding systems as the opportunity for a buffalo cow to calve and become pregnant is time limited [11]. Consequently, one of the greatest challenges of reproductive biologists is to gain an understanding of the underlying biology of the buffalo cow that contributes to low fertility and develop strategies to improve fertility.

Poor nutrition, parasitism, inappropriate management and environmental factors are often have a significant impact on reproductive performance of a cow rather than direct genetic effects [12].

In the current study, animals having ovarian inactivity had poor body condition score if compared to normal cyclic animals and heifers and primiparous cows are more likely affected with inactive ovary whereas these animals have higher energetic demands for growth as well as lactation and may be in greater negative energy balance than multiparous cows [13].

Pulsatile secretion of luteinizing hormone (LH) in early postpartum is necessary for pre-ovulatory follicular growth, estradiol secretion and ovulation of the dominant follicle [14,15]. However, inferior body condition score coupled with severe negative energy balance during this period suppresses pulsatile LH secretion, reduces ovarian responsiveness to LH stimulation and also reduces the functional competence of the follicle characterized by reduced oestradiol production [13] and ultimately results in anestrum [15].

Other traced risk factors cited to be negatively affecting on ovarian activity are parasitic infestation, mastitis and endometritis. In this respect, the current study revealed clear correlation between parasitic infestation, especially mange and coccidiosis and ovarian inactivity and this can be attributed to the negative energy balance and poor nutritious state of the infested animals[15,16]. Also stressed animals may have low LH pulse amplitude and frequency, low estradioal level and smaller dominant follicles which can extend the interval from calving to first ovulation [17].

Recently, it has been recorded that mastitic and lame cows showed a delay in the resumption of cyclicity 7 and 17 days, respectively to the calving-conception interval [18].

Compared to their healthy herdmates, cows with clinical endometritis were 4.5 times more likely to have delayed resumption of ovarian cyclicity and 4.4 times more likely to have prolonged postpartum luteal phases [19]. Endometrial epithelial cells respond to uterine infection by altering the secretion and thereby function of prostaglandins from luteolytic (prostaglandin $F_{2\alpha}$) to luteotropic (prostaglandin E_2) action. This mechanism has been proposed to explain delayed resumption of cyclicity in infected cows [20,21].

The current supplement 'Ovitone' was formulated in response to our previous investigations [5,6]. It is considered as a supplement for phosphorus, zinc, copper, selenium, AD_3E and Iodine while Lasalocid is used as anticoccidial drug and growth promoter. It was found that supplementation with phosphorus in the used combination of mineral mixture has a prime role in the establishment of ovarian activity in the affected animals. It was reported that phosphorus has had a synergistic effect on increasing the bioavilabilty of other minerals such as Cu and Zn in basal diet and in turn increase the soluble carbohydrate in ruminal fluid and consequently increased the pituitary LH secretion [28]. AD3E is essential for cell division, protein synthesis and steroid synthesis [29]. Zn, Cu and Se are more likely improve general health, growth and fertility and this was attributed to improved synthesis/ secretion of FSH and LH [5]. Likewise Cu supplementation decreases the susceptibility to infection, growth retardation and infertility [3]. In the same time prepartum supplementation with Se greatly controlled the incidence of mastitis, ovarian cysts and anoestrum /silent oestrus during the post partum period [22].

Lasalocid is an ionophorous (transport-inducing) antibiotic produced by *Streptomyces lasaliensis* and an antiprotozoal agent used in veterinary practice as growth promoter, for the prevention of coccidiosis and cryptosporidiosis [23]. The alteration in the membrane transport of ions is the basis for the metabolic, organic and functional effects of this class of compounds. Since their pharmacologic activity is dose-related, the usefulness of these ionophores is based on selective toxicity to protozoan parasites and bacteria and margins of safety in the approved target species. It's main therapeutic application in veterinary medicine as food additives; added to animal feed at a concentration of 10-120 mg/ kg [24].

It could be interpreting that the used supplementation improves the general health condition of the affected animals. The condition is related to activation of enzymes responsible for protein synthesis and hypothalamic regulation [30] as well as increased LH pulse frequency following retaining of positive energy balance [28]. The addition of lasalocid improves the condition whereas it increases the utilization of volatile

fatty acids for energy production, modifying carbohydrate fermentation in the rumen and for its antimicrobial and anticoccidial effect [31].

Hormonal interventions are usually used to induce ovulation and estrus in farm animals by stimulating maturation of Graafian follicles, either directly as in case of PMSG or indirectly by inducing a surge in release of LH [32]. Better fertility indicated by occurrence of heat signs and pregnancy following the application of GnRH therapy was reported in 86.6 % of anestrous buffalo heifers [33] and 73.3% of anoestrous buffalo cows [34]. GnRH and $PGF_2\alpha$ were used to induce ovulation in anestrous dairy cattle and buffaloes [35]. GnRH synchronizes the development and occurrence of follicles and results in more homogenous follicular development. Also, it induces ovulation or luteinization of dominant follicle in both cyclic and non cyclic animals However, the induced ovulation in non cyclic animals stimulated luteal tissue development and function resulting in the occurrence of cyclic activity[36,37]. However, hormonal interventions are usually expensive, not frequently available in the local market and not preferred by animal breeders who are worry about its drawbacks on milk yield, therefore the current formulation could be more economic and have approximately as high curative effect as hormone therapy in the same time it is more nutritive and safe to animals,

According to the Arab Agricultural Statistic year book [38], the total buffalo population in Egypt was 4.153 million heads in 2008 out of which 207.65 thousands were slaughtered and offered in local market. In the same time, the government imported 256.75 thousand tons of red meat to meet the requirement of local market. If we anticipated that half of the buffalo population in that time were females and out of these animals 50% were in the breeding age (1.038 million heads) is considered as the starting animal colony of this study, It would give birth and increase the total Egyptian buffalo population to be 5.191 and 5.795 after the first and second breeding seasons in 2009 and 2010, respectively.

Concerning the production losses due to ovarian inactivity and according to our findings, the non cyclic buffalo cows were estimated to be 41.8 and 50% in 2008 and 2009, respectively so that these females lost the breeding season and their calf crop which is anticipated to be 0.434 and 0.519 million heads and the new population would be 4.757 and 5.276 million heads in 2009 and 2010, respectively (Table1). The financial losses due these lost calf crop was estimated as 1.302 and 1.557 million EGP (based on the price of the newly born calf is 3000EGP).

The negative effect of ovarian inactivity on the annual milk yield estimated by 0.443 and 0.529 million tons leading to financial losses was estimated as 1.994 and 2.381 million EGP in 2009 and 2010, respectively. Whereas the estimated milk production is 1.02 ton/cow/year and the price is 4.5 EGP/kg, so the total financial looses due to ovarian inactivity is 3.296 and 3.938 million EGP, respectively

Responding to the treatment it is expected to get back these lost amounts of milk yield that minimize the amount of imported milk (903.58 thousand tons) by 49.1 and 58.5% in 2009 and 2010, respectively [39,40].

Comparing the added values due to different treatment trials, it was clear that GnRH (Reciptal) treatment gave the higher added values (6.68%) if compared to Folligone and Ovitone. The total net revenue was better in Reciptal treatment if compared to both Folligon and Ovitone by 6.25 and 5.22%, respectively (based on the price of the newly born calf was 3000EGP). In spite of the apparent higher value of the total net revenue of Reciptal, the calculation of the revenue of every spent pound showed the advancement of Ovetone to reach 305.47 EGP/100 cows that equivalent to 371.69 and 400.20% for the net revenue of the spent pound /100 cows in case of Folligon and Reciptall treatments, respectively.

The official record of money spent on the imported meat in 2008 was 504 million dollars. The condition would be different if the government paid more attention to overcome this syndrome in that time so that the buffalo cows would give the expected annual calf crop, the proposed production of red meat would increase by 86.8 thousand tons in 2009 and 103.8 thousand tons in 2010 that would minimize the current shortage in food balance valued by 170.26 million dollars in 2009 and 203.61 million dollars in 2010 (based on the price of imported ton of meat costs 1961.56$) and represent about 18.5 and 22.2% of the total shortage in food balance (919 million dollars)[41]

In conclusion, Ovarian inactivity hinders the productivity of Egyptian buffalo on favorable economic level. Trials must be carried out to control this disorder using modern manegmental techniques, especially at small holder farms. Use of Ovitone provides favorable result as compared to other commercial products depending on conception rate and net economic revenue.

REFERENCES

1. Norman, H.D., J.R. Wright, S.M. Hubbard, R.H. Miller and J.L. Hutchison, 2009. Reproductive status of Holstein and Jersey cows in the United States. J. Dairy Sci., 92: 3517-3528.

2. Sharma, R.K., 2003. Reproductive problems of buffaloes and their management. Compendium of lectures delivered to the Cent. Inst. Res. Buffalo, Hisar, India, pp: 119-126.
3. Ahmed, W.A., G.M. Nabil, H.H. El-Khadrawy, E.M. Hanafi and S.I. Abdel El-Moez, 2005. Monitoring progesterone level and markers of oxidative stress in blood of buffalo-cows with impaired fertility. Proc. 2nd Intern. Conf. Vet. Res. Div., NRC, Cairo, Egypt, pp: 1-12.
4. Ahmed, W.M., 2006. Adverse conditions affecting ovarian activity in large farm animals. Proc. the 3rd Intern. Conf. Vet. Res. Div., NRC, Cairo, Egypt.
5. Beever, D.E., A. Hattan, C.K. Reynolds and S.B. Cammell, 2001. Nutrient supply to high-yielding cows. Br Soc. Anim. Sci. Occas. Publ., 26: 119-31.
6. Doepel, L., H. Lapierre and J.J. Kennelly, 2002. Peripartum performance and metabolism of dairy cows in response to prepartum energy and protein intake. J. Dairy Sci., 85: 2315-34.
7. National Research Council, 2001. Nutrient requirement of dairy cattle. 7th ed. (Revised). Washington, DC:National Academy of Science.
8. Hassan, S.G., 2005. delayed age of puberty in buffalo heifers. Proc. The 12th Intern. Soc. Anim. Hygiene, Warsaw, Poland, pp: 159-162.
9. Agricultural Statistic year book. www.aoad.org/Ass/28/index.old.htm.
10. Sndecor, G.W. and W.G. Cochran, 1980. Statistical Methods. 7th Ed., Iowa State Univ. Press, Ames, Iowa, USA.
11. Dillon, P., D.P. Berry, R.D. Evans, F. Buckley and B. Horan, 2006. Consequences of genetic selection for increased milk production in European seasonal pasture based systems of milk production. Livest. Sci., 99: 141-158.
12. LeBlanc, S., 2010. Assessing the association of the level of milk production with reproductive performance in dairy cattle. J. Reprod. Dev., 56(Suppl): S1-S7.
13. Tanaka, T., M. Arai, S. Ohtani, S. Uemura, T. Kuroiwa, S. Kim and H. Kamomae, 2008. Influence of parity on follicular dynamics and resumption of ovarian cycle in postpartum dairy cows. Anim. Reprod. Sci., 108: 134-143.
14. Diskin, M.G., D.R. Mackey, J.F. Roche and J.M. Sreenan, 2003. Effects of nutrition and metabolic status on circulating hormones and ovarian follicle development in cattle. Anim. Reprod. Sci., 78: 345-370.
15. Crowe, M.A., 2008. Resumption of ovarian cyclicity in post-partum beef and dairy cows. Reprod. Domest. Anim., 43(Suppl. 5): 20-28.
16. Butler, W.R., 2003. Energy balance relationships with follicular development, ovulation and fertility in postpartum dairy cows. Livest. Prod. Sci., 83: 211-218.
17. Ahmed, W.M., H.H. El-Khadrawy, A.R. Abd El Hameed and H.A. Amer, 2010. Applied investigations on Ovarian Inactivity in Buffalo-heifers International J. Academic Research, 2(1): 26-32.
18. De Rensis, F. and R.J. Scaramuzzi, 2003. Heat stress and seasonal effects on reproduction in the dairy cow-a review. Theriogenology, 60: 139-1151.
19. Dobson, H., S.J. Walker, M.J. Morris, J.E. Routly and R.F. Smith, 2008. Why is it getting more difficult to successfully artificially inseminate dairy cows? Animal, 2: 1104-1111.
20. Opsomer, G., Y.T. Gröhn, J. Hertl, M. Coryn, H. Deluyker and A. de Kruif, 2000. Risk factors for post partum ovarian dysfunction in high producing dairy cows in Belgium: a field study. Theriogenology, 53: 841-857.
21. Peter, A.T., P.L.A.M. Vos and D.J. Ambrose, 2009. Postpartum anestrus in dairy cattle. Theriogenology, 71: 1333-1342.
22. Sheldon, I.M., S.B. Price, J. Cronin, R.O. Gilbert and J.E. Gadsby, 2009. Mechanisms of infertility associated with clinical and subclinical endometritis in high producing dairy cattle. Reprod. Domest. Anim., 44(Suppl. 3): 1-9.
23. Kommisrud, E., O. Osteras and T. Vatn, 2005. Blood selenium associated with health and fertility in Norwegian dairy herds. Acta Veterinaria Scandinavica, 46: 229-40.
24. Hermida, J.A.C., F.F. Santos, A.M.O. López, C.A.V. Castiblanco and M.E. Ares-Mazαs, 2000. *In vitro* and *in vivo* efficacy of lasalocid for treatment of experimental cryptosporidiosis Veterinary Parasitology, 90: 265-270.
25. Novilla, M.N., 2007. Basic and Clinical Principles Chapter 83-Ionophores, pp: 1021-1041.
26. Mitani, M., T. Yananishi and Y. Miyazaki, 1975. Biochem. Biophys. Res. Commun., 66: 1231.
27. Westley, J., 1982. Polyether Antibiotics, vol. 1, Dekker, New York, chapter 6.
28. Lamming, G.E. and A.O. Darwash, 1998. The use of milk progesterone profiles to characterize components of subfertility in milked dairy cows. Anim. Reprod Sci., 52: 175-90.

29. Grimard, B., O. Hunbolt, A.A. Punter, G.P. Mialton, D. Sauvant and M. Thibier, 1995. Influence of postpartum energy restriction on energy status, plasma LH and estradiol secretion and follicular development in suckled beef cows. J. Reprod. Fert., 104: 173-179.
30. Ahmed, W.M., H.A. Sabra and A.M. Hamam, 2001. Effect of administration of vitamins AD_3E and selenium during prepartum period on the reproductive performance of native cows in Egypt. Beni-Suef Vet. Med. J., XI: 81-91.
31. Roga-France, M., T. Kosla and E. Rokicki, 1995. The evaluation of hematological indicators and blood protein in dairy cows fed a diet with mineral additives. J. Ann. Warsaw Agric. Univ., Anim. Sci., 31: 55-61.
32. Grimard, B., O. Hunbolt, A.A. Punter, G.P. Mialton, D. Sauvant and M. Thibier, 1995. Influence of postpartum energy restriction on energy status, plasma LH and estradiol secretion and follicular development in suckled beef cows. J. Reprod. Fert., 104: 173-179.
33. Rhodes, F.M., S. McDougall, C.R. Burke, G.A. Verkerk and K.I. McMillan, 2003. Treatment of cows with extended postpartum anestrus interval. J. Dairy Sci., 86: 1876-1894.
34. Metwelly, K.K., F.M. Hussein, S.A. Hattab and F.K. Khalifa, 2004. Effect of GnRH on repeat breeder buffalo cows and delayed pubertal buffalo heifers and its effect with PGE2 alpha on reproductive efficiency of postpartum buffalo cows. Assiut Vet. Med. J., 50: 362-373.
35. Heleil, B.A., I.I. El-Kon, S.A. Darwish and M.S. Fadel, 2005. Buffalo anestrum: Diagnosis and treatment. Proc 17th Annu. Cong. Egypt. Soc. Anim. Reprod. Fert., Al Menia, Egypt, pp: 229-248.
36. Abou El Ghait, H.H., 2004. Postpartum trials to improve the reproductive performance in buffaloes. M V. Sc. Thesis. Tanta Univ., Egypt.
37. Bao, G.A., P.S. Baruselli and M.F. Marques, 2003. Pattern and manipulation of follicular development in *bos indicus* cattle. Anim. Reprod. Sci., 15: 307 326.
38. Arab League-Arab Organization for Agricultural Development-Statistical Yearbook of Agricultural Statistics Editions, (29): 2011.
39. Food and Agriculture Organization-FAO.
40. Kenawy, E. and N. Solieman, 2004. Principles of Macro-economic Analysis Science Publishing House 2004.
41. Central Agency for Public Mobilization and Statistics-Information Center.