

SOME BIOCHEMICAL CHANGES IN SERUM OF EGYPTIAN BUFFALOES WITH SMOOTH INACTIVE OVARIES BEFORE AND AFTER TREATMENT WITH VITAMINS A, D₃, E, C AND VITAMIN E/SELENIUM

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Abstract

Twenty-four female buffaloes between 5-10 years old were used in this investigation. They were divided into two main groups according to their reproductive performance. The first group included 10 buffaloes which were normal cyclic animals and considered as control. The second group included 14 buffaloes which were anoestrus with smooth inactive ovaries. This group was further subdivided into two subgroups each of 7 buffaloes. The first subgroup was injected with vitamins AD₃EC, while, the second subgroup was injected with vitamin E/Selenium.

Serum vitamin A, β -carotene, Ca, P, total protein, protein fractions, trace elements (Zn, Cu, Fe and Co), as well as progesterone hormone and cortisol were estimated.

There were a significant increase in vitamin A and β -carotene levels in both animals treated with vitamin AD₃EC and vitamin E/Se. On the other hand, the levels of Ca and P were increased in treated groups with significant elevation of Ca/P ratio. Treatment with vitamin E/Se increased significantly the level of total protein more than treatment with vitamin AD₃EC. The albumin was increased, while, α and β globulins were not significantly changed in both treated groups. At the same time, δ globulin was significantly elevated in both treated groups. The A/G ratio fluctuated within limited range without effect of supplementation.

Concerning trace element levels, there was significant increase in serum Zn, Cu, and Co and Fe in both treated groups. The progesterone level was significantly increased in both treated animals, but its level was high in buffaloes treated with vitamin AD₃EC than the group treated with vitamin E/Se. The cortisol level increased slightly by treatment in both groups of anoestrus buffaloes. This study cleared that vitamins and selenium supplementation play a fundamental role in fertility since injection of AD₃EC and Vit. E/Selenium improved the reproductive efficiency of the anoestrus buffaloes where 85%, 71.5% of animals came in heat in both treated groups, respectively.

INTRODUCTION

Losses due to infertility and improper breeding in Egyptian cows and buffaloes have been estimated to be more than 100 million pounds annually (Osman *et al.*, 1984). Ovarian inactivity is a major cause of reproductive failure in buffaloes. The high incidence of 39.4% inactive ovaries has been reported for buffaloes in Egypt (Aggag, 1986). Ovarian inactivity can be detected at any age in buffaloes (Osman *et al.*, 1984). The basic causes of these impaired production in buffaloes are not always apparent, although improper management, poor nutrition and pathological factors could be involved (Ashturkar *et al.*, 1995).

Vitamin A, D, C and E selenium are essential nutrients for dairy animals, their deficiency may be implicated in many reproductive disturbances (Monika, 1988). The supplementation of vitamin A, D, E, niacin and β -carotene (if plasma levels are low) are considered necessary in case of reproductive failure.

The present study investigates the effect of vitamin A, D₃, E, C and selenium/vitamin E administration to buffaloes suffering from smooth inactive ovaries. Biochemical changes including immunoglobulin status of these animals before and after treatment are recorded.

MATERIALS AND METHODS

This investigation was carried out at El-Khanka farm, Kalubia province. A total of twenty-four female buffaloes 5-10 years old were used for the present study. The general health of these animals, reproductive history, rectal palpation and in some cases vaginal examinations were done. From history and clinical examination of these animals, they were classified into two head groups according to their reproductive performance.

The first group including 10 buffaloes all were normally cyclic in follicular and luteal phases of the ovarian cycle which was ensured by rectal examination, and considered as a control group.

The second group including 14 buffaloes all were suffering from smooth inactive ovaries. They were without pathological affection in the genital tract, but revealed no structures on the ovaries. Also, there was a history of postpartum anoestrus for about six months, and these were considered as the test group. That group was further subdivided into two sub-groups.

Each animal was fed daily on 5 kg. concentrate mixture, which consisted of 20% wheat bran, 65% cotton seed meal, 12% rice polish, 2% lime stone and 1% common salt. In the meantime, barseem (*Trifolium alexandrinum*) was available (25 kg/head/daily). Ample amount of fresh water was also available.

Animals of the first sub-group (7 buffaloes) were injected with 20 ml of vitamins AD₃EC/intra muscularly twice a week for three months. These were manufactured by The Egyptian Co. for Chemicals and Pharmaceuticals (ADWIA). Each ml contains: Vit. A 100.000 I.U., Vit. D₃ 10.000 I.U, Vit. E 60 mg and Vit. C 20 mg.

Animals of the second subgroup (7 buffaloes) were injected with selenium (as sodium selenite 50 mg divided into two doses with one week interval) and vitamin E (as α tocopherol 680 I.U. divided into two doses with one week interval for 3 months).

After 3 months of the study, individual blood samples were collected from control and test groups, but, in cases of the animals of the test group, blood samples were collected twice, once immediately before injection and once at the end of the study by jugular puncture into dry, clean MacCarteny tubes, then, centrifuged at 3000 r.p.m for 15 min. for serum separation. The freshly collected serum was used for the determination of vitamin A and B-carotene according to the method of Neeld and Pearson (1963); serum vitamin E determined by ferric chloride method according to Quaife (1979). Calcium was calorimetrically estimated according to Martink (1971). Also, determination of inorganic phosphorus was carried out according to Pasquinelli (1979). Serum total proteins were estimated by the method of Peters (1968). Estimation of protein fractions in serum using cellulose acetate method was carried out according to Henry *et al.* (1974).

The progesterone and cortisol hormone level in blood serum were estimated according to Ekins (1984) by radioimmunoassay using 123 Mini Gamma counter (LKB).

Serum zinc, copper, iron and cobalt were determined by using flame atomic absorption spectrophotometer (Perkin-Elmer, 2380).

The data were tabulated and statistically analyzed. Statistical analysis of the data was performed using methods of Milton and Tsokos (1983) to assess the significance of differences between means of smooth inactive buffaloes and active ones, and also, between acyclic buffaloes before treatment and after treatment with

AD₃EC and Vit. E/Selenium at each time point using t-test.

RESULTS

Table 1 illustrated serum (vitamin A, vitamin E, β -carotene, calcium, inorganic phosphorus, in addition to Ca/P ratio) in buffaloes before treatment (cases of smooth inactive ovary), after treatment with AD₃EC and after treatment with Vit. E/Se, in addition to control group.

The results revealed that there was a significant increase in serum vit A and E levels in animals treated with vitamins AD₃EC (66.02 ± 4.56 ; 215.25 ± 2.9) and those treated with vitamin E/Se (64.015 ± 3.6 ; 214.25 ± 2.5), compared to those before treatment (38.7 ± 5.6 ; 210 ± 0.7).

Regarding serum β -carotene, there was a significant elevation in serum β -carotene level in both treated groups either by vitamins AD₃EC or E/Se (53.51 ± 3.87 , 48.08 ± 6.412) compared to untreated group (30.88 ± 5.03), respectively.

On the other hand, the levels of calcium and inorganic phosphorus were increased in animals treated with both vitamins AD₃EC (10.6 ± 0.32 ; 7.5 ± 0.13) and animals treated with vitamin E/Se (10.2 ± 0.611 , 7.3 ± 0.08) if comparing with untreated group (6.21 ± 0.311 ; 5.01 ± 0.12), respectively.

The Ca/P ratio was significantly elevated in both treated groups if compared to untreated ones (1.413 ± 0.03 ; 1.397 ± 0.01 ; 1.239 ± 0.02).

Serum protein levels and their fractions before and after treatment with vitamin AD₃EC and E/Se were recorded in Table 2.

Treatment with vitamin E/Se increased significantly the levels of total protein (8.001 ± 0.2) more than treatment with AD₃EC (7.47 ± 0.36) if compared to untreated group (6.15 ± 0.267).

The albumin level was increased in treated groups (36.1 ± 0.989 , 37.21 ± 1.2) compared to untreated ones (29.95 ± 1.866). Serum globulin levels were also affected by treatment, α globulin decreased in both treated groups with vitamin AD₃EC and E/Se, respectively (7.55 ± 0.46 , 7.82 ± 0.4), while, it was (9.17 ± 0.896) in untreated group; in the meantime β -globulin increased slightly in treated groups (9 ± 0.63 ; 9.01 ± 0.89), compared to untreated ones (8.46 ± 0.925).

The δ globulin increased significantly in group treated with vitamin E/Se

Table 1. Serum levels of vitamin A, E, β -carotene, Ca and inorganic phosphorous before and after supplementation with AD₃EC and Vitamin E/Selenium in buffaloes.

Groups	Vit. A $\mu\text{g/dl}$	β -carotene $\mu\text{g/dl}$	Vit. E $\mu\text{g/dl}$	Calcium mg/dl	Inorganic phosphorous mg/dl	Ca / P ratio
Cyclic buffaloes	65.015 \pm 2.5	55.5 \pm 6.54	213.3 \pm 6.8	10.8 \pm 0.45	7.4 \pm 0.12	1.459 \pm 0.015
Acyclic buffaloes (before treatment)	38.7 \pm 5.6***	30.88 \pm 5.03**	210 \pm 0.7	621 \pm 0.311***	5.01 \pm 0.12 ***	1.239 \pm 0.2
After treatment with AD ₃ EC	66.02 \pm 4.56**	53.51 \pm 3.87**	215.25 \pm 2.9	10.6 \pm 0.32***	7.5 \pm 0.13***	1.413 \pm 0.03
After treatment with Vit. E and Selenium	64.015 \pm 3.6**	48.08 \pm 6.412*	214.25 \pm 2.5	102 \pm 0.611***	7.3 \pm 0.08***	1.397 \pm 0.01

Mean \pm S.E

* Significant at P < 0.05

** Significant at P < 0.01

*** Significant at P < 0.001

Table 2. Levels of Serum protein and seum protein fractions in buffaloes before and after supplementation with AD₃EC and Vitamin E/Selenium.

Groups	Total protein g/dl	Albumin g/L	Alpha globulin g/L	β -globulin g/L	Gamma globulin g/L	A/G
Cyclic buffaloes	7.9±0.4	37.01±0.89	8.01±0.5	9.5±0.35	24.48±0.98	0.881±0.05
Acyclic buffaloes (before treatment)	6.015±0.267**	29.95±1.566**	9.17±0.869	8.46±0.925	12.5±0.527***	0.992±0.01
After treatment with AD ₃ EC	7.47±0.3625**	36.1±0.989**	7.55±0.46	9±0.63	22.05±0.955***	0.935±0.06
After treatment with Vit. E and Selenium	8.00±0.2 **	37.21±1.12**	7.82±0.4	9.01±0.89	25.97±0.54***	0.869±0.04***

Mean ± S.E.

* Significant at P < 0.05

***Significant at P < 0.01

** Significant at P < 0.01

(25.9± 0.54) more than group treated with AD₃EC (22.05±0.955) if compared with untreated group (12.5±0.527). The A/G ratio fluctuated with limited range without effect of supplementation; its ratios were (0.935±0.06, 0.869±0.04, 0.992±0.01) in groups treated with vitamin AD₃EC, E/Se and untreated group, respectively.

Table 3 showed the effect of treatment with vitamin AD₃EC and Vit. E/Se and untreated groups serum microelements (trace-elements) Zn, Cu, Fe and Co. Significant increase in Zinc level in groups treated with both vitamin AD₃EC and E/Se (118.1±0.25, 119.5±0.38) compared to untreated group (77.9±0.1), respectively. Also, Cu and Fe levels were significantly increased in both treated groups (88.8±0.4, 89.81±0.8 and 155.52±2.03, 160.7±2.6) compared to untreated one (59.75±0.18 and 110.25±5.5), respectively.

No significant change in Cobalt level was noticed in all groups. It was (0.34±0.07, 0.31±0.02 and 0.32±0.01) in groups treated with vitamins AD₃EC and E/Se and untreated group, respectively.

Table 3. Levels of microelements in buffaloes before and after supplementation with AD₃EC and Vitamin E/Selenium.

Groups	Zn µg/dl	Cu µg/dl	Fe µg/dl	Co mg/dl
Active cyclic buffaloes	117.5±0.3	89.33±0.8	145.66±5.25	0.51±0.02
Smooth inactive buffaloes (before treatment)	77.9±0.1***	59.75±0.18***	110.25±7.5**	0.32±0.01
After treatment with AD ₃ EC	118.1±0.55**	88.8±0.4**	155.52±2.03**	0.34±0.07
After treatment with Vit. E and Selenium	119.5±0.38**	89.81±0.8**	160.7±2.6**	0.31±0.02

Mean±S.E.

* Significant at p< 0.05

** Significant at p< 0.01

*** Significant at p< 0.001

Table 4 recorded the levels of progesterone and cortisol hormones in buffaloes before and after treatment with vitamins AD₃EC and E/Se. There was a significant increase in progesterone levels in both treated groups, but, its level in AD₃EC supplemented group was higher than the group treated with vitamin E/Se (1.6±0.33; 1.521±0.34) compared to untreated group (0.123±0.21).

Table 4. Levels of progesterone and cortisol in buffaloes before and after supplementation with AD₃EC and Vitamin E/Selenium.

Groups	Progesterone µg/ml	Cortisol µg/ml
Cyclic buffaloes	2.797±0.387	0.47±0.15
Acyclic buffaloes (before treatment)	0.123±0.21***	0.230±0.018
After treatment with AD ₃ EC	1.6±0.33**	0.39±0.02***
After treatment with Vit. E and Selenium	1.521±0.34**	0.281±0.019*

Mean±S.E.

* Significant at p< 0.05

** Significant at p< 0.01

*** Significant at p< 0.001

The cortisol hormone also increased slightly in both treated groups, its level in groups treated with vitamins AD₃EC, and E/Se was (0.39±0.02; 0.281±0.019) compared to (0.230±0.01) in untreated group, respectively.

Clinically, treatment with AD₃EC, 6 buffaloes from 7 ones which suffered from smooth inactive ovaries showed oestrus, while, 5 buffaloes from 7 came in heat in the group treated with vitamin E and Selenium.

DISCUSSION

Buffaloes constituents are considered a major source of meat, milk and dairy products in Egypt. Infertility among buffaloes is quite high, and we are faced with such a challenging threat to our animal wealth. Ovarian inactivity is a considerable cause of reproductive failure in buffaloes than in cattle (Serur *et al.*, 1982). In addition, a higher percentage of buffaloes with prolonged postpartum anoestrus period indicates a major infertility problem in Egyptian buffaloes as recorded by Youssef *et al.* (1988).

The result of this study showed a significant increase in serum vitamin A and B-carotene levels (P<0.01) of animals suffering from smooth inactive ovaries after treatment with vitamin AD₃EC or Vit E/Se. In the same time, serum vitamin E was elevated in both two treated groups, but not significantly increased. This coincides with Daghash *et al.* (1993) as they reported that, blood vitamin A, E and acarotene were increased in buffalo - heifers when injected with vitamin AD₃EC twice a week for 3 months. This may be attributed to the beneficial effect of vitamin E, not on the absorption of vitamin A (Farrell, 1980), but also, on the storage of vitamin A in the

liver (Williams, 1981). on the other hand, the antioxidant effect of vitamin E on vitamin A and β -carotene have a role in protecting vitamin A and β -carotene levels (Green, 1972).

Daghash *et al.* (1993) observed a positive correlation between vitamin A and β -carotene serum levels in buffaloes treated with vitamin AD₃EC as compared with control group, and this replicates the important role of β -carotene, not only as a precursor of vitamin A, but also as it has specific action on the function of the reproductive process in female cattle.

The reason why vitamin E deficiency takes some time to affect cattle is said to be due to high vitamin E levels in the endocrine glands (Zintzen, 1976). The antioxidant effect of vitamin E on β -carotene and vitamin A establishes a further indirect link with fertility in cattle (Green, 1972). Selenium and sulphur containing amino acids cystine and methionine have effects similar to those vitamin E. It is difficult to distinguish between the effect of selenium or vitamin E alone. Kappel *et al.* (1984) found that, injection of 50 mg selenium with 200 mg vitamin E for 3 times when given approximately 21 days before calving and after artificial insemination, improved the conception rate of Simmenthal cows. Segerson *et al.* (1977) achieved optimum fertility (100%) in superovulated cows with injections of Se and vitamin E even though the overall ration was above NRC (1978) recommendations for maintenance requirements.

El-Taweel (1982) and Osman *et al.* (1984) observed that serum calcium and inorganic phosphorus levels in buffaloes with inactive ovaries were significantly decreased than those in normal cycling ones, and these concur with our findings as Ca and inorganic phosphorus were 6.21 ± 0.311 mg/dl and 5.0 ± 0.12 mg/dl in acyclic buffaloes compared to 10.8 ± 0.45 , 7.4 ± 0.12 mg/dl in cyclic ones. Ward *et al.* (1971) studied the effect of vitamin D supplementation on dairy cow fertility. He found that animal which received 300,000 IU vitamin D₃ weekly had shorter uterine involution times, more clearly marked oestrus and better fertility rates. Cohen (1962) reported that vitamin D has an estrogenic effect. Kaneko (1989) mentioned that calcium/phosphorus complex increased after administration of vitamin D in rats and children suffering from vitamin D deficiency.

In this study, the levels of serum calcium and inorganic phosphorus were significantly increased in both groups ($P < 0.001$) after treatment with AD₃EC or with vitamin E/Sel. Also, Ca/phosphorus ratio was significantly increased in both

two groups. These results agreed with Daghash *et al.* (1993) and Ashurkar *et al.* (1995). Furthermore, the addition of vitamin E eventually improved the digestibility of fat which favours the Ca absorption (Roy *et al.* 1984). Serum total Ca level was highly affected by Se where Selenium improved the absorption of calcium serum total via the intestine. Blood and Henderson (1974) mentioned that, there is a relationship between selenium and the healthy condition of intestine. In addition, Harrison and Conard (1984) recorded a correlation between Ca and Se in their absorption in intestine. The injection of AD₃EC and vitamin E/Se increased significantly the Ca/P ratio in treated animals (Table 1). The ovarian dysfunction in buffaloes was attributed to the wide range of serum Ca/P ratio (Osman *et al.*, 1984 and Hassan *et al.*, 1991).

Blood proteinogram evaluated in buffaloes with smooth inactive ovaries revealed a significant decrease in serum total protein, albumin and gamma globulin concentration as compared with those active cyclic buffaloes. After intramuscular injection of AD₃EC and vitamin E/Se in two groups of acyclic animals separately, the levels of total protein, albumin and gamma globulin concentrations in serum were elevated significantly $P < 0.01$, 0.01 and 0.001 , respectively.

Applinton *et al.* (1964) reported that vitamin A plays a role in biosynthesis of gamma globulins. Also, vitamin C is important for antibody formation as it helps in amino acid metabolism and protein synthesis (Tietz, 1986). These concur with the results obtained in the present study where the administration of AD₃EC resulted in elevation of protein level which may be due to the direct role of vitamin A in improving cell replication (Zile *et al.*, 1979), and also increase in DNA replication in most organs.

The immune response of buffalo uterus was higher during the follicular phase of estrus cycle than during the leuteal phase. The uterine leukocytes count and their phagocytic activity and total immunoglobulin content were clearly decreased in the uteri of buffaloes with bilateral smooth inactive ovaries as compared with those of active ones (Ahmed *et al.*, 1993). The administration of vitamin AD₃EC, especially vitamin A improved the leukocytic count and phagocytic activity and total immunoglobulins.

Regarding trace elements, El-Taweel (1982) mentioned that lack of trace elements (Cu and iron) in diet may lead to anestrus in buffaloes. These findings concur with our results which showed lower copper serum levels of infertile ones.

Administration of AD₃EC and Se/E significantly elevated the levels of iron and Cu at $P < 0.01$ as compared to untreated group. Results in Table 3 showed lower serum levels of trace elements Cu and Co, while, the level of iron and Zn are significantly increased in both treated groups. Khattab *et al.* (1991) and Mohamed (1993) reported that, in the anestrus buffaloes, vitamin E, Se and vitamin AD₃EC supplementation produced non-significant changes in levels of Cu and Co. Decrease in Cu level after Se administration indicated the antagonism between Se and Copper in suckling buffalo-calves. Similar findings were previously reported by Farrell (1980).

Regarding progesterone and cortisol hormone levels in buffaloes serum before and after treatment with vitamin AD₃EC and vitamin E/Se, the levels of progesterone hormones were greatly decreased ($P < 0.001$) in smooth inactive buffaloes as compared to cyclic active ones. On the other hand, there is no significant difference between cortisol hormone in cyclic and acyclic buffaloes. This agreed with Yadav *et al.* (1991) as they reported that, estimation of circulating progesterone is more useful indicator of infertility than rectal palpation. They noticed that, serum progesterone level was greatly decreased in infertile buffaloes as compared to its level in serum of fertile ones.

The progesterone levels in serum of smooth inactive ovaries animals were greatly increased after treatment either with AD₃EC ($P < 0.01$) or with vitamin E/Se ($P < 0.01$). Also, there was a significant elevation in serum cortisol level of both treated groups. This could be attributed to the role played by vitamin E effect on reproduction, as it helps in synthesis of steroid hormones as progesterone and estrogen (Jackson *et al.*, 1981). Moreover, vitamin E promotes release of FSH, ATCH and LH (Barnes and Smith, 1975). Also, vitamin C plays a role in steroidogenesis as it is present in high concentration with cholesterol in adrenal cortex (Tietz, 1986).

Out of 7 anestrus buffaloes that were supplemented with AD₃EC, 6 animals showed estrus representing 85% of treated cases, while, 5 animals came in heat in the group treated with vitamin E/Se representing 71.5% of treated cases.

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بعض التغيرات الكيميائية في مصل الجاموس الذي يعاني من خمول المبايض قبل وبعد العلاج بإستعمال السيلينيوم وفيتامين هـ أو مجموعة الفيتامينات أ، د، هـ، جـ

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^٢ كلية الطب البيطري - جامعة الزقازيق .

تمت الدراسة علي عدد ٢٤ من إناث الجاموس تتراوح أعمارها من ٥ - ١٠ سنوات
وقد قسمت هذه الحيوانات تبعا للكفاءة التناسلية الي مجموعتين.

المجموعة الأولى وعددها ١٠ حيوانات ذات خصوبة عالية ولا يوجد بها أية ظواهر
مرضية (بالفحص الإكلينيكي) وقد اعتبرت هذه مجموعة ضابطة.

المجموعة الثانية شملت ١٤ من إناث الجاموس كانت تعاني من خمول المبايض وقد
قسمت هذه المجموعة الي مجموعتين فرعيتين (أ ، ب) كل منهما بها ٧ إناث. حقنت
المجموعة (أ) بفيتامين أ د هـ ج بينما المجموعة الثانية (ب) تم حقنها بفيتامين هـ
والسيلينيوم.

تم تجميع عينات من الدم من المجموعات الثلاث ثم فصل منها المصل لقياس مستوي
العناصر الأتية : فيتامين أ ، البيتاكاروتين، فيتامين هـ ، الكالسيوم، الفوسفور الغير
عضوي، البروتين الكلي، الألبومين والجلوبيولين بالإضافة الي قياس بعض العناصر
النادرة كازنك، النحاس، الحديد والكوبلت. أيضا تم تقدير مستوي هرموني
البروجستيرون والكورتيزول.

وقد أظهرت النتائج زيادة معنوية في كل من مستوي فيتامين أ ، البيتاكاروتين
والكالسيوم والفوسفور ونسبة كل من الكالسيوم الي الفسفور في كلتا المجموعتين. كما
أوضحت الدراسة إرتفاع مستوي البروتين الكلي في المجموعة المحقونة بفيتامين هـ
والسيلينيوم عن المجموعة المعالجة بفيتامين أ د هـ ج .

أيضا أظهرت النتائج ارتفاع مستوي الألبومين وكذا المستوي المناعي لكل من
المجموعتين المعالجتين وذلك من خلال الإرتفاع المعنوي لمستوي جلوبيونات المناعة لكل
منهما. كما أوضحت الدراسة ارتفاع معنوي في مستوي العناصر النادرة ومتوي هرموني
البروجستيرون والكورتيزول في كلتا المجموعتين. وقد أسفرت هذه المعالجة لكلتا
المجموعتين عن ارتفاع الخصوبة وزيادة الكفاءة التناسلية لإناث الجاموس الذي يعاني من
خمول المبايض بنسبة ٨٥% في المجموعة (أ) ٧١.٥٢% في المجموعة (ب). وقد أوضحت
الدراسة ان إضافة الفيتامينات (أ د هـ ج) او هـ والسيلينيوم تلعب دورا أساسيا في
خصوبة إناث الجاموس.

لذا نوصي بإضافة فيتامين أ د هـ ج أو فيتامين هـ مع السيلينيوم بالنسب السابق
ذكرها في الحالات التي تعاني من خمول المبايض دون سبب مرضي واضح.