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SOME ENDOCRINE RESPONSE TO HORMONAL TREATMENT IN CASES OF ANESTRUM IN BUFFALO- COWS AND REPRODUCTIVE PERFORMANCE RELATIONSHIP.

(With Two Tables)

By

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استجابة بعض الغدد الصماء للعلاج ببعض الهرمونات في حالات عدم الشياح
في بعض الجاموس وعلاقته بالكفاءة التناسلية

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اجريت هذه الدراسة على عدد ١٠٠ جاموسة (٨٠ عولجت + ٢٠ ضابطه) كانت تعاني من ظاهرة عدم الشياح قسمت الحيوانات المعالجه والضابطه الى مجموعتين رئيسيتين طبقا لوجود جسم أصفر على المبيض من عدمه.. تم تقسيم كل مجموعه رئيسيه الى خمسة مجموعات فرعيه (٤ تلقت العلاج + مجموعه ضابطه) على حسب نوع العلاج المستخدم مجموعه فرعيه أولى : تم علاجها بعقار الأسترومات (٥٠٠ ميكروجرام) ، أعيدت الجرعه مره أخرى بعد ١١ يوم فى الحيوانات التى لم تستجب للعلاج بالجرعه الأولى.مجموعه فرعيه ثانيه : تم علاجها بالفيرتاجيل (٢٥ مجم).مجموعه فرعيه ثالثه : تم علاجها بالاسترومات والفيرتاجيل مجموعه فرعيه رابعه : تم علاجها بالاسترومات والبولون ٥ (٥ مجم).مجموعه فرعيه خامسه : ضابطه لم تتلقى أى علاج أخذت عينات دم قبل وبعد العلاج (عند ظهور علامات الشبق) وذلك لقياس مستوى كلا من الثيرونين الثلاثى والثيروكسين بواسطة طريقة اختبار المناعه الاشعاعيه. أظهرت نتائج التحليل لمصل الدم عن وجود زياده معنويه (٥%) فى مستوى الثيرونين الثلاثى وذلك بعد العلاج بالاسترومات + البولون ٥ معا وذلك فى حالات عدم الشياح ذات الجسم الأصفر والخاليه منه بينت نتائج التحليل لمصل الدم عن وجود زياده معنويه (٥%) فى مستوى الثيروكسين وذلك بعد العلاج بالاسترومات فى حالات عدم الشياح ذات الجسم الأصفر والخاليه منه وكذلك أوضحت نتائج التحليل لمصل الدم عن وجود زياده معنويه (٥%) وزياده معنويه جدا (١%) فى مستوى الثيروكسين وذلك بعد العلاج بكل من الاسترومات + الفيرتاجيل ،

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والأسترومات + الفولون ٥ على التوالي فى الجاموس الذى يعانى من عدم الشياح مع وجود جسم أصفر على المبيض . أوضحت نتائج علاج الجاموس الذى يعانى من عدم الشياح لوجود جسم أصفر على المبيض أن العلاج بالأسترومات + الفولون ٥ هو الأحسن (١٠٠٪ استجابة بظهور أعراض الشبق + ٨٠٪ معدل الحمل) يليه الأسترومات ثم الأسترومات + الفيرتاجيل وأخيرا الفيرتاجيل . اتضح من علاج الجاموس الذى يعانى من عدم الشياح مع عدم وجود جسم أصفر على المبيض أن العلاج بالأسترومات هو الأفضل (٩٠٪ استجابة + ٧٠٪ معدل الحمل) ، يليه الأسترومات + الفولون ٥ وأخيرا كلا من الفيرتاجيل ، والأسترومات + الفيرتاجيل لهما نفس التأثير .

SUMMARY

A total number of 100 healthy buffalo-cows suffering from anestrus were divided into two main groups based on the ovarian findings with or without palpable corpus luteum. Each main group was further subdivided into 5 subgroups. The 1st subgroup received Estrumate 500 ug (repeated once after 11 days in cases that did not respond to the first injection); the 2nd subgroup received Fertagyl (0.25 mg); the 3rd subgroup received Estrumate plus Fertagyl (repeated once after 11 days in cases that did not respond to the first injection); the 4th subgroup received Estrumate plus Folone 5 (5 mg) and the 5th subgroup act as a control and received no treatment. Blood samples were collected from all treated subgroups before and after treatment (during estrus) for estimation of serum tri-iodothyronin (T_3) and thyroxine (T_4) using radioimmunoassay. The obtained results revealed that serum, T_3 level showed a significant increase ($P < 0.05$) after Estrumate and Estrumate plus Folone treatment in anestrus buffalo-cows with and without palpable corpus luteum. Serum T_4 level showed a significant increase ($P < 0.05$) after Estrumate treatment of anestrus buffaloes with and without palpable corpus luteum. Whereas, the serum T_4 level showed a highly significant increase ($P < 0.01$) in anestrus buffalo-cows with palpable corpus luteum after treatment with Estrumate plus Folone as well as after Estrumate plus Fertagyl treatment ($P < 0.05$). For treated anestrus buffaloes with palpable corpus luteum, treatment with Estrumate plus Folone was the best as the response (appearance of estrus signs) was 100% with 80% pregnancy rate, followed by Estrumate alone, Estrumate plus Fertagyl and finally Fertagyl alone. For treated anestrus buffalo-cows without palpable corpus luteum, treatment with Estrumate alone was the best as the response was 90% with 70% pregnancy rate followed by Estrumate plus Folone and finally Fertagyl alone and Estrumate plus Fertagyl are about equal in their effects.

Keywords: Endocrine response, hormonal treatment, anestrus, buffalo-cows, reproductive performance, relationship.

INTRODUCTION

Reproduction in farm animals is closely related to the dynamic activity of the thyroid gland (SOLIMAN *et al.*, 1964 and AFIEFY *et al.*, 1970).

Thyrotrophic hormone (TSH) influences reproduction via the production of thyroxin and triiodothyronin which regulate the metabolic pools of nitrogen producing available energy necessary for the reproductive system and the developing embryo. Therefore, abnormal decrease in thyroid hormones may interfere with normal pregnancy (HAFEZ, 1980).

It was found that thyroid hormones increases the basal metabolic rate by increasing both oxygen consumption as well as the concentration of Na⁺, K⁺ and ATP in brown adipose tissue (KNCHANS and ROMSON, 1989). In this respect, KLEIN *et al.* (1984) reported that T₃ enhance the norpinephrine induced stimulation of oxygen consumption by adipose tissue. Therefore, the thyroid hormones could affect neonatal thermogenesis through the lipolytic activity of catecholamines.

Regarding the estrus cycle in buffalo-heifers, KUMAR *et al.* (1991) observed a maximum concentration of T₃ level on -3, -2, -1 and 0 days of estrus, while T₄ peak level was at the onset of estrus.

On the other hand an idea about the plasma level of thyroid hormones in infertile buffaloes is required to know the different mechanisms enhancing the reproductive processes in these infertile buffaloes. The present investigation was carried out to know the level of thyroid hormones in the infertile buffalo-cows before and after different hormonal treatments in addition to evaluate the effect of these hormonal treatments on their reproductive performance.

MATERIAL and METHODS

This work was carried out on 100 healthy buffalo-cows (4-9 Years) suffering from anestrus in Khattara farm, Sharkia province (80 treated + 20 control).

- These animals did not show any signs of heat since 3-6 months. Gynaecological examination proved the absence of any detectable pathological affections or abnormalities in their reproductive organs.

- According to the ovarian findings, the animals were classified into two main groups including cows with and without palpable corpus luteum (Table 1 and 2). Each main group was further subdivided into 5 subgroups (each subgroup included 10 cows) according to the trial of treatment.

a- The first subgroup received 2ml Estrumate* (500 ug PGF₂ α), cases that did not respond received the same dose 10-12 days after the first injection.

b-The second subgroup received 2.5ml Fertagyl alone** (0.1 mg/ml Gn-RH).

c- The Third subgroup received 2ml Estrumate plus 2.5 ml Fertagyl after 5 days (repeated once after 11 days in cases that did not respond to the first injection).

d- The fourth subgroup received 2ml Estrumate plus 25mg stilbosterol*** (Folone 5).

e- The fifth subgroup act as a control and received no treatment.

• Blood samples were collected from the jugular vein of each buffalo-cow immediately before treatment and during estrus in the responded animals. The blood was allowed to clot and centrifuged at 3000 r.p.m for 20 minutes for separation of serum. The separated serum stored at -20°C till processed further.

RESULTS

The results of the present study listed in table (1) and table (2). The

obtained results revealed a marked fluctuation in the level of serum T₃ and T₄ in buffalo-cows with or without palpable corpus luteum before and after treatments. For anestrus buffaloes with palpable corpus luteum, treatment with Estrumate plus Folone was the best (100 V 80%) response and pregnancy rate respectively followed by Estrumate alone, Estrumate plus Fertagyl and finally Fertagyl alone. For oestrus buffaloes without palpable corpus luteum, treatment with Estrumate alone was the best (90 V 70%) response and pregnancy rate respectively followed by Estrumate plus Folone and finally Fertagyl alone and Estrumate plus Fertagyl are about equal in their effects.

DISCUSSION

The present results (Table 1) showed that there was a significant increase ($p < 0.05$) in the T₃ level after Estrumate and Estrumate plus Folone therapy in buffalo-cows with and without palpable CL. On the other hand, a non significant increase in serum T₃ level was observed in buffalo-cows with and without palpable CL after Fertagyl and Estrumate plus Fertagyl.

Similar findings were recorded by IBRAHIM *et al.* (1990) who showed that, the serum T₃ content was increased significantly after injection of anestrus buffalo-cows with 25 mg

* : Coopers Animals Health Limited (England).

** : Intervet International BV. (Holland).

*** : Misr Company for Pharmaceutical Industries (Egypt).

N.B.: All drugs used were administered by i.m. route.

prostaglandin $F_2\alpha$, MELANDER *et al.* (1974) also reported an increased TSH in laboratory animals treated with $PGF_2\alpha$. Moreover, VADO-DARIA *et al.* (1978) showed a lower thyroid activity immediately after ovulation with the peak activity at the beginning of heat. The high level of T_3 during proestrus may be due to stress caused by hyperactivity as confirmed by KHURANA and MADAN (1985). Furthermore, the higher level of T_3 after hormonal treatment may be attributed to the stimulatory effect of $PGF_2\alpha$ on the secretion of gonadotrophins, steroid production by the adrenals and insulin release (JOHNSON *et al.*, 1973) and thyrotropin-like effect on the thyroid gland (MASHITER and FIELD, 1974). The obtained data (Table 1) showed a significant increase ($p<0.05$) in the T_4 level after Estrumate treatment in buffaloes with and without palpable CL, whereas, in buffaloes with palpable corpora lutea, the T_4 level showed a highly significant increase ($p<0.01$) after treatment with Estrumate plus Folone and a significant increase ($p<0.05$) after Estrumate plus Fertagyl treatment. On the other hand, the present results showed a non significant increase in the T_4 level in buffaloes with and without palpable CL. after Fertagyl treatment and in buffaloes without CL. after

Estrumate plus Folone and Estrumate plus Fertagyl treatments.

Our results were in agreement with those obtained by D'ANGELO and FISHER (1969) who observed a peak level of serum T_4 at the onset of estrus. These results were much related to the increased estrogen level at the onset of estrus, as estrogen stimulated the thyroid gland activity by direct action without the intervention of pituitary gland (KUMAR *et al.*, 1991). Estrogen might also stimulate TSH release from the pituitary gland (D'ANGELO and FISHER, 1969) causing elevation of the T_4 during estrus as observed during the present study. Moreover, IBRAHIM *et al.*, (1990) observed that, the administration of $PGF_2\alpha$ increased T_4 activity in anestrus buffaloes. In pregnant woman, SHENKMAN *et al.* (1974) recorded that, the administration of $PGF_2\alpha$ increased the concentration of T_3 and T_4 levels. The obtained results of the different trials for treatment of the buffaloes suffering from anestrus were listed in table (2). Our results indicated that, in buffalo-cows with palpable CL treated with Estrumate plus Folone exhibited the highest percentage of response (100%) with 80% conception rate, followed by Estrumate (90 V 80%), Estrumate plus Fertagyl (70 V 70%) and Fertagyl (50 V 40%), respectively.

These results are in agreement with those obtained by *INSKEEP et al.* (1975) who observed that 95% of cows came in heat after PGF₂ α plus Folone treatment.

The high response to Estrumate plus Folone in the present study, may be attributed to luteolytic action of exogenous estrogen (*MCDONALD, 1980 and WILLIAMS, 1980*), estrogen may also exert its luteolytic effect by stimulating endogenous PGF₂ α secretion (*BARTOL et al., 1980 and KNICKERBOCKER et al., 1986*). This hypothesis was supported by that obtained by *GENGENBACH et al.* (1977) who demonstrated a luteolytic interaction between estradiol and PGF₂ α in ewes independent of the uterus.

Meanwhile in the buffalo-cows without palpable CL, the treatment with Estrumate revealed the highest percentage (90%) of response and 70% conception rate followed by Estrumate plus Folone (80 V 60%), Fertagyl (60 V 50%) and Estrumate plus Fertagyl (60 V 50%), respectively. The high response to Estrumate in the present study may be attributed to the embedding lutein tissues of the CL in the ovarian

matrix that could be isdiagnosed by rectal palpation specially in the buffalo-cows that have large sized ovaries. This suggestion was supported by the findings of *EL-WISHY et al.* (1971) who stated that, the clinical evidence for the presence of CL can not be always obtained by rectal palpation, because CL in the major cases of buffaloes are either embedded in the ovarian matrix or very slightly protruded above the ovarian surface so that they could be misdiagnosed even on careful rectal examination. Moreover, this statement was strongly supported by *EISSA et al.* (1990) who stated that absence of a palpable CL in the ovaries of buffaloes should not be taken as an individual parameter indicating that the animal is not cycling at the time it is examined.

It can be concluded from this study that, Estrumate plus Folone was the best treatment used in anestrus buffalo-cows with palpable CL, while Estrumate alone was the best drug used in anestrus buffalo-cows without palpable CL, as reflected by appearance of estrus and their conception rate as well as serum T₃ and T₄ levels.

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RESPONSE TO HORMONAL TREATMENT, ANESTRUM IN BUFFALO-COW

Table (1) Mean (\pm S.E) serum values of Triiodothyronin (T_3) and tetraiodothyronin (T_4) of infertile buffalo-cows with and without palpable CL before and after hormonal treatment

Hormones used	Parameters							
	Buffalo-cows with CL				Buffalo-cows without CL			
	T_3 (ng/dl)		T_4 (ug/dl)		T_3 (ng/dl)		T_4 (ug/dl)	
	Before	After	Before	After	Before	After	Before	After
Estrumate	46.35 \pm 5.97	67.784 \pm 7.985	3.85 \pm 0.78	8.54 \pm 1.43	36.30 \pm 3.89	72.953 \pm 7.19	2.114 \pm 0.59	6.975 \pm 2.01
Fertagyl (Gn - RH)	39.837 \pm 5.79	61.755 \pm 8.834	2.85 \pm 0.78	7.31 \pm 2.017	39.237 \pm 6.44	62.398 \pm 9.37	2.732 \pm 0.123	6.773 \pm 1.97
Estrumate + Fertagyl	55.271 \pm 7.959	82.35 \pm 10.56	2.07 \pm 0.61	6.59 \pm 1.71	46.229 \pm 7.14	69.35 \pm 8.51	2.498 \pm 0.72	5.552 \pm 3.05
Estrumate + Folone	43.73 \pm 6.53	68.382 \pm 6.205	3.46 \pm 0.98	8.79 \pm 1.06	43.586 \pm 4.79	62.379 \pm 6.34	2.501 \pm 0.465	7.240 \pm 2.96

* : ($P < 0.05$)** : ($P < 0.01$) \pm : Standard error.

Table (2) Some reproductive parameters of treated anestrus buffalo-cows with and without palpable CL.

Treated groups	Buffalo-Cows with CL					Buffalo-Cows without CL				
	Treat. Cases	Resp. Cases	%	Interval Days	CR %	Treat. Cases	Resp. Cases	%	Interval days	CR %
Estrumate (PGF2 α)										
1st trial	10	6	60	4 \pm 1		10	2	20	4 \pm 1	
2nd trial	4	3	75	13.5 \pm 1.52		8	7	87.5	10.5 \pm 1.89	
Total	10	9	90		80	10	9	90		70
Fertagyl (Gn - RH)	10	5	50	12.25 \pm 3.29	40	10	6	60	13 \pm 1.26	50
Estrumate + Fertagyl 1st trial	10	3	30	4.67 \pm 0.64		10	20	20	4.5 \pm 0.5	
2nd trial	7	4	57.17	8.25 \pm 0.83		8	50	50	8.25 \pm 1.09	
Total	10	7	70		70	10	6	60		50
Estrumate + Folone	10	10	100	6.5 \pm 1.8	80	10	8	80	9.25 \pm 2.17	60
Control	10	1	10	70	10	10	1	10	55	10