

**CASE REPORT:-**

**VERMINOUS PNEUMONIA IN THE EGYPTIAN FREE-RANGING RED FOXES ( VULPES VUPLES ) CAUSED BY FILAROIDES HIRTHI**

**BY**

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**ABSTRACT**

*Out of 6 red foxes ( Vulpes vulpes ) captured randamly from different localities in Sinai desert, one was found to be infected with Filaroides hirthi. Grossly, the fox displayed focal pulmonary and hepatic lesions and the peritonium filled with ascitic fluid. Histopathologically, the lung granulomas revealed the occurrence of adult female worms. Verminous pneumonia was noticed around the parasite.*

*To our knowledge, the red fox is recorded as a new host for F. hirthi which herein is considered for the first time in the Middle East area.*

**INTRODUCTION**

Filaroides hirthi has been first recovered by Hirthi and Hottendorf (1973) in the beagles dogs. Then repeatedly reported by Georgi et al. (1976), Erb and Georgi (1982), Genta and Schad (1984), Rubash (1986) and Bahnemann and Baul (1994). Among other breeds, the parasite has been identified from Yorkshire terriers by Craig et al. (1978) and Pinckney et al. (1988). Also in boxers by Beveridge et al. (1983) and in charles spaniel by Andreasen and Carmichael (1992). Imported beagles to Japan and Israel were found infected with F. hirthi by Kagei et al. (1976) and Waner et al. (1991), respectively. The adult filaroides worms usually cause bronchopneumonia in canides ( Jubb et al., 1993 ) .

In Europe, F. hirthi was reported by Sacco and Genchi (1988) and Sacco et al. (1989) in Italy, Bourdeau and Ehm (1992) in France and by Carrasco et al. (1997) in Spain.

In Egypt, studies of Kamel (1994) and Abdel Aal (1995) were not concerned with pathological changes due to helminthic infections. Consequently we aimed at diagnosing of such changes that could met with in the free-ranging red fox (*Vulpes vulpes* ) .

**MATERIALS AND METHODS**

**Wild Animals**

One to two - years- old ( age determined by tooth wear ) , six clinically healthy free-ranging male red foxes ( *Vulpes vulpes* ) were captured randomly using steel traps baited with sardine from different localities in Sinai desert. Animals were submitted alive to the Departments of Wildlife and Pathology, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt.

### **Animal Handling**

Before handling, red foxes were anaesthetized with an intra-muscular injection of a combination of 30.0 mg/kg Bw ketamine hydrochloride and 3.0 mg/kg Bw xylazine hydrochloride (Kamel and Zagloul, 1997).

### **Coprological Examination**

Fresh fecal samples were obtained and examined coprologically (Soulsby, 1982).

### **Necropsy and histopathological sampling**

All foxes were killed and necropsied immediately. After death, organs (kidney, lung and liver) were collected and grossly examined for lesions or abnormalities. Specimens from kidney, lung and liver were fixed in 10% buffered formalin for 24-48 hrs, embedded in paraffins, sectioned at 5 microns and stained with Hematoxylin and Eosin (Harris, 1968) and examined microscopically. The suspected parasitic granulomas in the lungs were identified after George (1979) and Uchikawa et al. (1983).

## **RESULTS**

### **Clinical Signs**

Clinically, animals were apparently healthy except one fox was debilitated and has slight respiratory distress.

### **Coprological Results**

Coprological examination revealed absence of any internal and external parasites.

### **Pathological Findings**

#### **Gross Picture**

At necropsy, the emaciated fox displayed focal pulmonary and hepatic lesions and the peritoneum filled with ascitic fluid. The multifocal greyish brown areas were surrounded by red zones and localized in the left cranial and middle pulmonary lobes (Fig. 1). Congestion and pale emphysematous area were seen in right cranial and caudal lobes. The greyish foci were firm (hard) in consistency. Abundant frothy exudate was noticed in the bronchial tree. The kidneys were found normal.

#### **Microscopic Picture**

Histopathologically, lungs had moderate multifocal interstitial pneumonia. The alveolar tissue contained adult female nematodes (Fig.2). The female worms contained larvated eggs at different stages of development. Granulomas were not always associated with adult nematodes. Alveoli surrounding the parasite were collapsed.

The inflammatory cells were mainly eosinophils, lymphocytes and macrophages. Mucinous degeneration was seen in the epithelial cell lining of some bronchioles. Arteritis characterized by leukocytic infiltration in the tunica media and tunica intima was seen.

The liver granulomas (Fig.3) did not contain any parasitic nematode. Hepatic parenchyma showed vacuolar degeneration.

## DISCUSSION

The histopathological findings of pulmonary granulomas were to great extent similar to that given by Craig et al (1978), August et al. (1980), Carrasco et al. (1997) and others for Filaroides hirthi. We did not find any larval forms of F. hirthi in the parenchyma or in the faecal samples of the alive fox that may be due to the immaturity of the female worms or the strong immune response of foxes. Filaroides hirthi is one of the most important pulmonary nematodes in different breeds of dogs especially those used for the research and toxicological studies (Bahnemann and Bael, 1994). The parasite was considered by Wilson (1990) as an opportunistic one in the immune compromised dogs. The free-ranging red foxes ( *vulpes vulpes*) may attract infections from wild dogs and the disease then developed due to non-specific immunosuppressive factors such as nutritional stress and captivity conditions (Tizard, 1987). Large numbers of F. hirthi usually associated with severe granulomatous reactions in specifically immuno-suppressed dogs such as in canine distemper, adrenal carcinomas and prolonged corticosteroid treatment (Carrasco et al.1997). In the present case, the non-specific immuno-suppressor factors was not so severe that the granulomas contained few or just one adult parasite. Liver granulomas and kidneys were free of any parasitic forms of F. hirthi. However, August et al. (1980) found fragments of larvae in most of them. Also he found a larvae in a glomerulus of one kidney. Filaroides hirthi was reportedly the most important parasite in beagle colonies and has the potential to be so in red foxes.

Within the limits of our knowledge, red fox is considered a new host for F. hirthi. Also, with the exception of Waner et al. (1991), F. hirthi is recorded for the first time in red fox in the arid-area of the Middle East.

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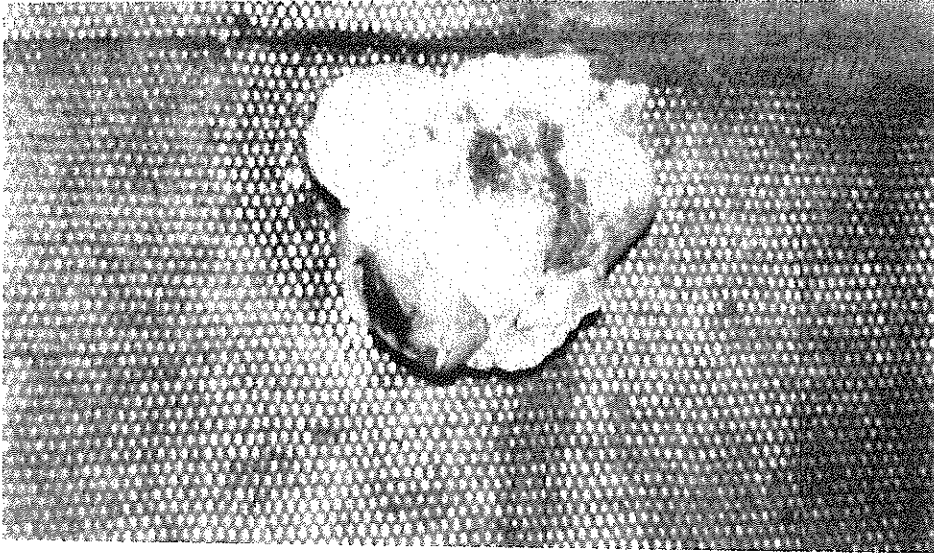
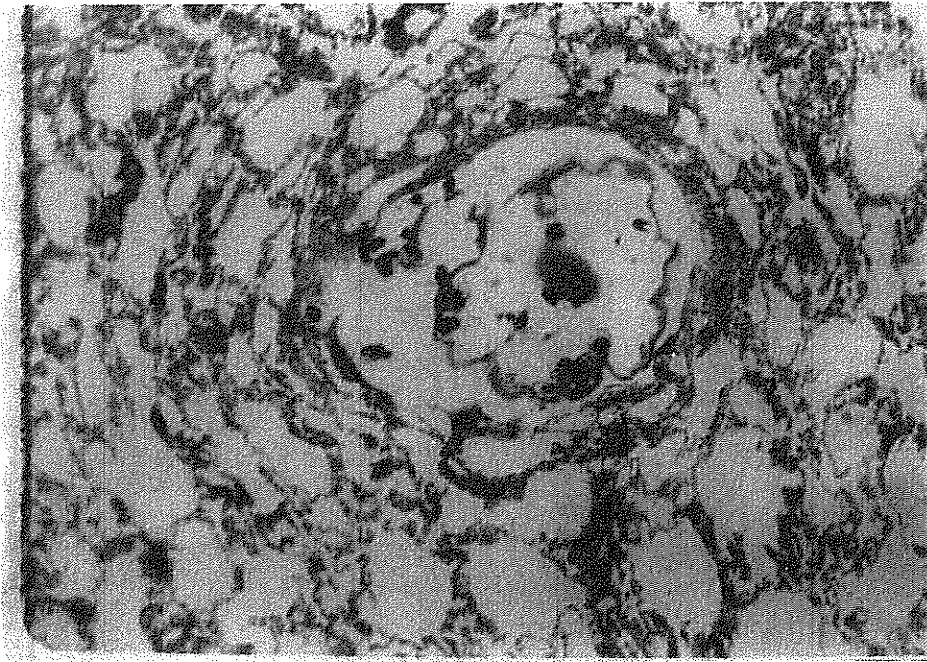


Figure 1. Lung of a red fox (*Vulpes vulpes*) showing a pale emphysematous middle lobe and a red congested cranial lob.



Figure, 2. Lung of a red fox ( *Vuples vuples* ) showing adult *Filaroides hirthei* female containing larvated eggs. H&E, X 250.

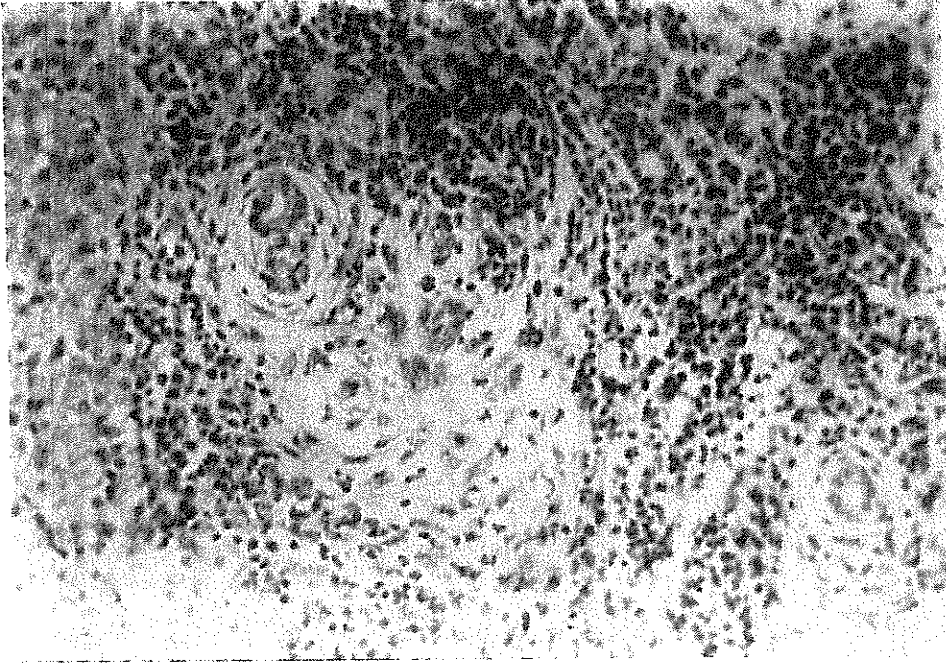


Figure 3. Liver of the red fox shows focal granulomas with vacuolar degeneration of the hepatocytes. H&E, X 400.

### الملخص العربى

فيلارويد هيرسى يسبب الالتهابات التنفسية فى الثعالب الحمراء فى مصر  
عاطف كامل و حسين عمر

بعد فحص عدد ستة ثعالب حمراء تم اصطيادها عشوائيا من صحراء سيناء وجد انها تعاني من  
اصابات تنفسية وبعمل الصفة التشريحية وجدت بعض العلامات المرضية وبالفحص النسيجية المصابة  
بالطرق الهستوباثولوجية اتضح ان سبب الصابات التنفسية هو طفيل فيلارويد هيرسى  
وتعتبر هذه هي المرة الاولى التى يسجل فيها هذا الطفيل فى منطقة الشرق الاوسط

## GNRH AGONIST ENHANCES THE EFFICACY OF PGF<sub>2</sub> $\alpha$ ANALOGUE IN ESTRUS SYNCHRONIZATION IN BUFFALOES

BY

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### ABSTRACT

*The aim of this work was to evaluate the value of GnRH in a controlled breeding program. A total of 22 buffalo-cows were examined per rectum to identify that they had palpable corpus luteum (CL). They were injected with 500  $\mu$ g cloprostenol prostaglandin F<sub>2</sub> $\alpha$  (PGF<sub>2</sub> $\alpha$ ) (Synchromate) and 48 hrs later, they were injected 200  $\mu$ g GnRH (Fertagyl Intervet). Nineteen out of twenty two buffalo cows came in estrus (86.36%) after 48 hrs. from GnRH injection and 3 out of 22 (13.64%) did not show any signs of estrus and their ovaries had no structures when examined rectally. Progesterone concentrations decreased sharply in animals that had larger CL after injection of PGF<sub>2</sub> $\alpha$  from (4.02 $\pm$ 0.89 to 0.93 $\pm$ 0.24 ng/ml). Estradiol 17 $\beta$  concentrations increased from (3.92 $\pm$ 0.4 to 5.06 $\pm$ 0.28 pg/ml) after injection of GnRH. The protocol of single dose of PGF<sub>2</sub> $\alpha$  depends on the presence of CL followed by single dose of GnRH resulted in good estrus synchronization and allowed effective management of buffalo-cows breeding without need to heat detection under field conditions.*

### INTRODUCTION

Buffaloes are unique among farm animals in Egypt, for its good palatable milk and meat. Problems of buffalo estrus detection even with a schedule of three observation for heat is inconvenient, time consuming and therefore not often implemented (Rosenberg et al., 1990), in addition to long calving interval, when estrus detection efficiency had a high correlation with calving intervals (Barr, 1974). These are the major constraints that impede genetic improvement and productivity of buffalo-cows. The use of prostaglandin F<sub>2</sub> $\alpha$  or its analogues as a tool for managing reproductive performance can overcome these problems, where the luteolytic effect of PGF<sub>2</sub> $\alpha$  in buffaloes had achieved by the great regression in CL associated with rapid decrease in progesterone level (Jainudeen, 1976; Kamonpatana et al., 1979; Bachlaus et al. 1980; Ibrahim, 1987; and Hassan, 1989)

Combination of PGF<sub>2</sub> $\alpha$  with GnRH for an effective ovulation control was studied in buffaloes, where 10.4% respond with overt estrus within 1 to 2 days after injection of GnRH, while 54.2% revealed palpable luteal structures after 7 days and 88.5% of them came into heat after PGF<sub>2</sub> $\alpha$  injection at an interval of 61.4 hrs. (Norasimha and Venkatramiah, 1991). Administration of GnRH after PGF<sub>2</sub> $\alpha$  analogue enhance the ovulation in cows by 96-97% Vs 85% for that did not received GnRH (DoValle et al., 1997). While in buffaloes, administration of two doses of PGF<sub>2</sub> and one dose of GnRH had no advantages (Jainudeen, 1976).

increasing the ability of a follicle to ovulate. Also to record the effect of this method of synchronization on the concentrations of serum progesterone and estradiol 17 $\beta$  and the manifestation of estrus for more efficient control of ovulation for successful breeding program for buffaloes.

## MATERIALS AND METHODS

### Animals:

Twenty-two non-lactating buffalo-cows aging between 4-6 years were used in this study. Animals were fed according to NRC feeding management (Ranjhan and Palhok, 1979). Five of them were kept in the experimental farm of Animal Reproductive Research Institute for hormonal study. The rest of buffalo-cows were in a private farm at Ismailia Province. All the buffaloes were detected to have a palpable CL (by rectal palpation).

### Synchronization protocol:

Animals were injected I/M with a single dose (2ml) of PGF2 $\alpha$  analogue (Synchromat B), each 1.0 ml equal to 250  $\mu$ g cloprostenol, 48 hours later, the buffalo-cows received 2.0 ml GnRH agonist (Fertagyl Intervet). All buffalo-cows were observed for signs of estrus three times daily. These signs were categorized as:

- 1- Standing to be mounted.
- 2- Clear viscous vaginal mucous.
- 3- Increased vocalization.

Or no estrus signs.

### Blood samples:

Blood samples were collected from five buffalo-cows via jugular venipuncture in plain vacutainer tubes one day before PGF2 $\alpha$  administration (day 1) and then daily till the estrus manifestations appear. Serum was separated and stored at -20 °C until used for hormonal assay.

### Hormonal assay:

Serum progesterone and Estradiol 17 $\beta$  concentrations were estimated by direct radioimmunoassay (RIA) using coat A-count kit (Diagnostic Products Corporation, DPC). This method have been characterized and verified to measure each hormone with an extremely low cross reactivity to other hormones. In Progesterone, the sensitivity of the assay was 0.015 ng/ml while the mean intraassay and interassay coefficient of variation were 3.8% and 6.9% respectively. In estradiol 17 $\beta$ , the sensitivity of the assay was 0.30 pg/ml, while the mean intraassay and interassay coefficient were 4.1% and 7.0% respectively. The correlation coefficient between observed and expected values obtained for quantitative recovery of known addition of progesterone and estradiol 17 $\beta$  were 0.9 and 0.85 respectively.

## RESULTS

A total of 19 out of 22 buffalo-cows (86.36%) were easily detected in heat after 48 hours from GnRH administration. Those animals manifested standing estrus accompanied by clear viscous vaginal mucus, increased vocalization and restlessness. The remaining 3 buffalo-cows (13.64%) did not show any signs of estrus.

The hormonal concentrations during the experimental period were shown in table (1) and Fig. (1).



Preinjection of PGF $2\alpha$ , progesterone concentration was  $4.02\pm 0.89$  ng/ml then dropped sharply to  $0.93\pm 0.24$  ng/ml within 24 hours after injection. Serum progesterone levels decreased continuously, 24 hours after the injection of GnRH where it reached its lowest concentration ( $0.20\pm 0.07$  ng/ml). Then started to increase gradually after the appearance of estrus manifestation. On the other hand, Serum estradiol  $17\beta$  increased from  $3.92\pm 0.4$  pg/ml to  $5.04\pm 0.47$  pg/ml in the same interval. Its levels increased post-injection of GnRH reaching its highest level 48 hours after injection ( $6.23\pm 0.20$  pg/ml) concurrently with the appearance of estrus manifestation. Then decreased gradually to reach a concentration of ( $3.45\pm 0.35$  pg/ml) by day eight post injection of PGF $2\alpha$  Table (I) and Fig.(I).

### DISCUSSION

Injection of GnRH agonist 48 hours after PGF $2\alpha$  injection improved the efficacy of estrus synchronization in buffalo-cows compared with standard methods using luteolytic agent only. In the present study, estrus appeared in 19 buffalo-cows out of 22 treated animals (86.36%), while Parasad et al., (1979) reported that estrus synchronization succeeded in 12 animals out of 18 treated (72.22%). Heifers injected with GnRH on day 3 followed by PGF $2\alpha$  on day 13 of estrus, non-of them came in heat but only 43.8% showed luteolysis. These clarify that GnRH treatment before PGF $2\alpha$  diminished the efficiency of its luteolytic action (Birnle et al., 1997). GnRH initiates or plays a role in initiation of a new follicular wave and ovulated a newly formed dominant follicle between 24-32 hrs. after the second injection of GnRH (Pursley et al., 1995). It is well accepted that GnRH and its agonists act on ovarian follicular development and CL function indirectly via the induced release of pituitary LH and FSH within 2 to 4 hours after injection which act indirectly by binding to their respective receptors on follicular and luteal cells (Chenault et al., 1990; Conn and Crowley, 1990; Rettmer et al., 1992; Stevenson et al., 1993 and Twagiramungu et al., 1995).

The hormonal changes for luteal and follicular phases recorded during this study are agree with the previous reports in buffaloes (Ahmed et al., 1977; Boyd and Munro, 1979 and Bachlaus et al., 1980). The progesterone levels during day of estrus ( $0.78\pm 0.15$  ng/ml) was higher in the present study than that reported by Chauhan et al., 1985. While the level of estradiol  $17\beta$  was lower and this may be attributed to the effect of GnRH on the proportion of steroidogenic luteal cells. This lead to increase in progesterone and decrease in estradiol concentrations (Macmillan et al., 1985 and Stevenson et al., 1993).

Although the level of estradiol  $17\beta$  was lower, the manifestation of estrus resulted from the present protocol were clear.

It can be concluded that, this program of single dose of PGF $2\alpha$  followed by single dose of GnRH have a good response in synchronizing estrus in buffalo-cows with a good percentage.

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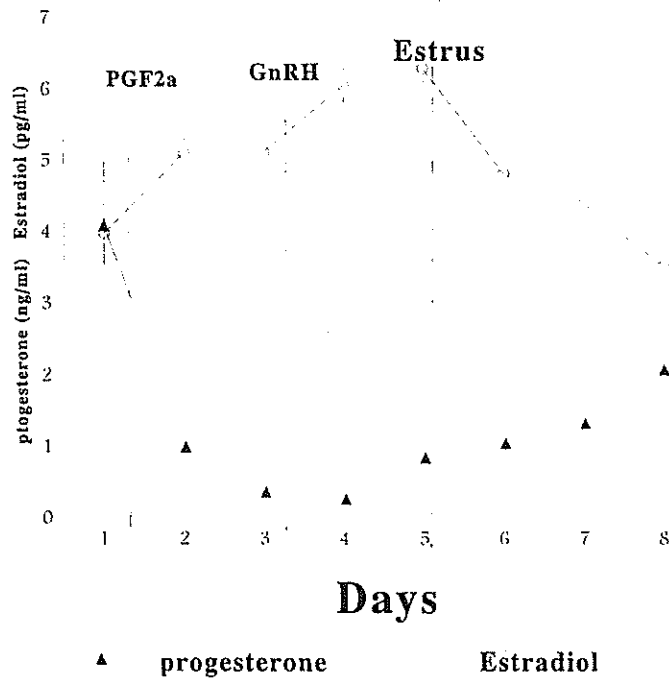
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**Table (1): Serum progesterone and estradiol 17β in synchronized buffalo-cows (n=5)**

Days of experiment	treatment	Progesterone (ng/ml) mean±S.E	Estradiol 17β (pg/ml) mean±S.E
1	Pre-PGF2 inj.	4.02±0.89	3.92±0.40
2	24 hrs.after inj.	0.93±0.24	5.04±0.47
3	Pre-GnRH inj.	0.30±0.07	5.06±0.28
4	24 hrs.after inj.	0.20±0.07	5.99±0.24
5*		0.78±0.15	6.23±0.20
6		0.98±0.23	4.75±0.09
7		1.26±0.19	4.32±0.28
8		2.01±0.25	3.45±0.35

\*day of well signs of estrus on animals

**Fig.(1): Progesterone and Estradiol 17B concentrations after prostaglandin and GnRh injection**



## الملخص العربي

أستخدام *GnRH* فى زيادة كفاءة البروستاجلاندين فى توحيد الشبق فى الجاموس

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تهدف هذه الدراسة إلى تقييم استخدام *GnRH* فى برامج التربية الحديثة. تم فحص عدد اثنان و عشرون أنثى جاموس عن طريق الجس المستقيمى للتأكد من وجود جسم أصفر على المبيض. وقد تم حقنهم ب ٥٠٠ ميكروجرام بروستاجلاندين ف٢ ثم بعد ٤٨ ساعة تم حقنهم ب ٢٠٠ ميكروجرام *GnRH* عن طريق الحقن فى العضل. وكانت الاستجابة السريعة متمثلة فى شيوع عدد ١٩ جاموسة من إجمالى ٢٢ (بنسبة ٣٦, ٨٦ %) بعد ٤٨ ساعة من الحقن. وكانت نسبة الجاموس التي لم تشع هي ٦٤, ١٣ % . وقد انخفض هرمون البروجستيرون انخفاضاً سريعاً بعد حقن البروستاجلاندين من ٠,٢ ± ٤, ٨٩± . إلى ٠,٩٣ ± ٠,٢٤ . نانوجرام/ملى وزاد تركيز هرمون الاستراديول من ٩٢, ٣ ± ٠,٤ . إلى ٠,٦ ± ٥, ٢٨ ± . بيكوجرام/ملى . وبعد حقن ال *GnRH* أنخفض مستوى البروجستيرون حتى وصل إلى ٠,٢ ± ٠,٧ . نانوجرام/ملى ٢٤ ساعة بعد الحقن , كما زاد تركيز الاستراديول إلى ٢٣, ٦ ± ٠,٢ . بيكوجرام/ملى. وكانت علامات الشبق قد ظهرت بوضوح بعد ٤٨ ساعة من حقن *GnRH* .

والخلاصة أن هذه الدراسة قد أوضحت أن برنامج الحقن على هذا المنوال قد أحدثت توحيداً للشبق فى الجاموس مما يسهل تطبيق برامج التلقيح الاصطناعي ودون النظر لاستخدام وسائل تحديد الشبق فى مختلف الأحوال الحقلية.