EFFECT OF HORMONAL ADMINISTRATION ON SOME TRACE ELEMENTS IN BLOOD OF INFERTILE BUFFALO - COWS

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ABSTRACT

A total number of 80 buffalo - cows (40 suffering from ovarian inactivity and 40 suffering from palpable corpora lutea ) were divided into four groups (20 cows each of both two reproductive disorders). Group A received 2 mls of oestradiol injected i.m. and repeated once after 10 - 12 days. Group B received 2 ml of Gn-RH after 5 days. Group C received 2 mls of 2.5 mg Gn-RH after 5 days. Group D received 2.5 mgs of Gn-RH injected. Estimation of serum copper, zinc, iron and manganese levels were done to all studied groups before and at the appearance of heat after treatment. Buffaloes suffering from inactive ovaries had a non significant change in the values of serum copper after hormonal treatment. A highly significant increase in serum zinc level was detected after treatment with PGF_{2a} with oestradiol and Gn-RH, respectively. Serum iron level showed a significant decrease after treatment with PGF_{2a}. A highly significant increase in serum iron level was recorded after treatment with Gn-RH (Fertagyl). A significant increase in serum manganese level was recorded after treatment with PGF_{2a}, alone in comparison with the values before treatment. Buffaloes suffering from palpable corpora lutea had a significant change in the values of serum copper after hormonal treatment. A

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very highly significant decrease in serum zinc level was recorded after treatment with PGF₂α alone. A significant decrease in serum iron level was recorded after PGF₂α treatment, this decrease became highly significant after treatment with PGF₂α with Gn-RH. Whereas, a significant increase of serum iron level was observed after treatment with Gn-HR alone. The level of serum manganese increased significantly by treatment with PGF₂α alone in comparison with the values obtained before treatment. The results of this study indicated that the use of prostaglandin F₂α and Gn-HR may be helpful in improving the reproductive efficiency in buffaloes.

INTRODUCTION

It is well accepted that nutrition plays an important role in the development and function of the reproductive organs, and faulty nutrition is by far the most likely cause of ovarian dysfunction. However, Trace elements are of light relation to reproduction and reproductive performance in animals. Morrow (1980) stated that trace elements are essential parts to maintain the optimal health condition of animals in the most critical periods of reproductive life of female animal.

Various trace elements as copper, manganese, zinc, selenium, iodine and iron can influence reproductive performance of ruminants, so reproductive failure may be induced by imbalance or deficiency of a single or combined trace elements. These elements may act as co-factor activators of enzyme or stabilize the secondary molecular structures as showed by Vallee and Wacher, (1976).

Zinc is a constituent of several enzymes systems and plays an important role in the biochemical reactions in the animal body McDonald et al. (1979). Copper present in some plasma proteins such as ceruloplasmin which oxidizes Fe⁺⁺ to Fe⁺⁺⁺, helping to transport iron across the intestinal wall and helps to mobilize iron from storage sites (Henry et al., 1974).

Furthermore, Reddy and Reddy (1988) reported that the low level of iron in serum of infertile animals may cause hypoxia or anoxia at cellular tissue level which may affect the sites on the gonads or it possibly affect the hypothalamo-ovarian axis as well as ovary itself. Finally the depressed iron level was presumed to
suppress the enzymatic reactions needed for the release of LH. Furthermore, defects in steroid synthesis which were associated with reproductive dysfunctions and irregular oestrus cycle were manifested in manganese deficiency, Hurely and Keen, (1987).

Therefore, the aim of the present investigation is to throw light on the alteration of some important trace elements (Copper, zinc, iron and manganese) in the serum of infertile buffalo cows suffering from ovarian inactivity and persistent corpus luteum, before and after treatment with different hormones to study the biochemical effect of these hormones which used in treatment of infertility in Egyptian buffaloes which may be helpful in improving the reproductive efficiency of animals.

**MATERIAL AND METHODS**

This work was carried out on 80 mature female Egyptian buffaloes in Khattara Farm belonging to the animal development and food security project Sharkia Province (40 suffering from ovarian inactivity and 40 suffering from palpable corpora lutea). The age was ranged between 4-8 years and weighed 460-560 kg and calved once or twice.

These animals were supplied daily with 4 kg. concentrates (45% cotton seed cakes, 20% wheat bran, 20% zea maize, 12% rice polish, 2% lime salts and 1% common salts) in addition to sufficient quantities of Barseem 30-50 kg. in green season or darawa with sufficient amount of rice straw in dry season. The animals were watered and libitum and were kept free in an open shelter during the day and night all over the year and naturally bred by clinically normal, sexually healthy fertile bulls.

The choosen buffalo cows did not show oestrus over a period 3-10 months after normal parturition. Complete gynaecological examination has been performed two times at 10 days intervals before the start of the experiments which revealed no pathological abnormalities of the gential organs except ovarian inactivity and presence or absence of corpus luteum on both ovaries.

According to drugs (Hormones) used in the treatment of animals were classified into 4 groups as follow:
Group A: Comprised 10 buffalo - cows with palpable corpora lutea and another 10 buffalo - cows without palpable corpora lutea treated with PGF₂-α. 500 µg (Estrumate) it is manufactured by Coopers Animal Health Limited (England), each animal in this group was given 2 mls injected i.m., and repeated once after 10 - 12 days in buffalo - cows that did not show response to the first injection. the animals come in oestrus were observed and naturally bred.

Group B: Considered of 10 buffalo - cows with palpable corpora lutea and another 10 buffalo - cows without palpable corpora lutea (smooth inactive ovaries), each animal received 2 mls PGF₂-α plus 5 mg oestradiol (Folone) injected i.m. at the same time. It is manufactured by Misr Company for pharmaceutical industries, Egypt.

Group C: Contain 10 buffalo - cows with palpable corpora lutea and another 10 buffalo - cows without palpable corpora lutea, each animal received 2 mls PGF₂-α plus 2.5 mls synthetic Gn-RH (Fertagyl) after 5 days injected i.m. It is manufactured by Intervet International B.V., Holland.

Group D: Comprised 10 matures buffalo - cows with palpable corpora lutea and another 10 buffalo - cows without palpable corpora lutea, each animal received 2.5 mls Gn-RH injected i.m.

Blood samples were taken from all animal groups from the Juglar vein immediately before treatment and at the appearance of heat after response of the animal for treatment. The sera were separated by centrifugation and were stored at -20°C until analyzed for copper, zinc, iron and manganese. Serum concentrations of copper, zinc, iron and manganese were analyzed using Atomic absorption spectrophotometer Model 2380 (PERKIN ELEMER) according to the method of Willis (1960). Statistical analysis of the obtained results were carried out using the method of Snedecor and Cochran (1967).
RESULTS

Table (1) shows mean values of serum copper, zinc, iron and manganese level of infertile buffalo cows with inactive ovaries before and after hormonal treatment. The value of serum copper level showed a non significant decrease after treatment with prostaglandine F$_2$-a alone; PGF$_2$-a with oestriadiol and PGF$_2$-a with Gn-RH respectively. However, it increased non significantly after Gn-RH treatment.

A non significant decrease in serum zinc level was recorded after treatment with PGF$_2$-a alone and PGF$_2$-a with Gn-RH. A highly significant increase in serum zinc level was recorded after treatment with PGF$_2$-a with oestriadiol and Gn-RH, respectively.

The serum iron level showed a significant decrease after treatment with PGF$_2$-a, this decrease became non significant after treatment with PGF$_2$-a with oestriadiol. A non significant increase in serum iron level was recorded after treatment by PGF$_2$-a with Gn-RH, this increase became highly significant after treatment with Gn-RH alone.

A significant increase in serum manganese level was recorded after treatment with PGF$_2$-a alone. This increase became non significant after treatment by PGF$_2$-a with oestriadiol and PGF$_2$-a with Gn-RH respectively. However manganese level showed a non significant changes after treatment with Gn-RH alone in comparison with the values obtained before treatment.

Table (2) shows mean values of serum copper, zinc, iron and manganese levels of infertile buffalo cows with palpable corpora lutea before and after hormonal treatment. A non significant decrease in serum copper level was recorded after treatment with PGF$_2$-a. However, it increased non significantly after treatment with PGF$_2$-a with oestriadiol; PGF$_2$-a with Gn-RH and Gn-RH alone, respectively.

A highly significant decrease in serum zinc level was recorded after treatment with PGF$_2$-a alone. However, it increased non significantly after treatment with PGF$_2$-a with oestriadiol; PGF$_2$-a with Gn-RH and Gn-RH alone, respectively.

A significant decrease in serum iron level was recorded after PGF$_2$-a treatment, this decrease became highly significant after treatment with PGF$_2$-a with Gn-RH. The serum iron level showed a non significant increase after treatment with PGF$_2$-a with oestriadiol. This increase became significant after treatment with Gn-RH.
(Fertagyl).

The level of serum manganese increased significantly after treatment with PGF<sub>2</sub>α. This increase became non-significant after treatment with PGF<sub>2</sub>α with oestradiol and Gn-RH respectively. However, the manganese level showed a non-significant decrease after treatment with PGF<sub>2</sub>α with Gn-RH in comparison with the values obtained before treatment.

**DISCUSSION**

The obtained data Table (1) indicated that there was non-significant decrease in serum copper concentration of infertile buffalo - cows with inactive ovaries after treatment with PGF<sub>2</sub>α alone, PGF<sub>2</sub>α with oestradiol and PGF<sub>2</sub>α with Gn-RH respectively. The obtained results were in agreement with those of Gamal (1987) who found that the injection of prostaglandin F<sub>2α</sub> at a dose rate of 60 μg/kg lower slightly the serum copper level in infertile buffalo - cows. It is evident from the present study that the serum copper level was non-significantly increased after Gn-RH treatment. The obtained data are nearly similar to the findings of Samy (1991) who found that a non-significant increase in serum copper level at the 5th and 30th days after Gn-RH (Receptal) treatment in infertile buffalo - cows with inactive ovaries. The increase in serum copper level after treatment although it is non-significant may be attributed to increase in steroid hormones as stated by Sato and Henkin (1973) and confirmed by Desai et al. (1978) who stated that the serum copper level can be utilized as an indicator of pituitary gonadotropins and gonadal steroid levels in Surti buffaloes. Moreover, progesterone could induce the synthesis of ceruloplasmin and thereby increase copper concentration.

The obtained data revealed that there was a highly significant increase in serum zinc level after treatment with PGF<sub>2</sub>α with oestradiol and Gn-RH, respectively. The highly significant increase in serum zinc level might be attributed to the direct effect of prostaglandins on serum zinc level as stated by Song and Adham (1978) as they reported that, the changes in serum zinc concentrations were secondary to the dramatic increase in prostaglandins associated with parturition as prostaglandins bound to zinc and facilitated its transport. Moreover, Gamal (1987) showed that no significant effect of PGF<sub>2</sub>α treatment was detected.
on zinc level, neither in heifers nor in cows. Similar observation were reported in Italian buffalos by Omar et al. (1973) who noticed that no significant difference in serum zinc values between age groups. Whereas, the obtained data disagrees with Samy (1991) who observed that the serum zinc level was decrease significantly after Gn-RH (Receptal) treatment. The author attributed such decrease to high serum cortisol level and the results were confirmed by the statement of Kutsky (1981) who reported that the high cortisol level decrease the serum zinc concentration.

The serum iron level showed a significant decrease after treatment with PGF<sub>2α</sub> whereas a very highly significant increase in serum iron level was recorded after treatment with Gn-RH. The increase in iron level after Gn-RH treatment was closely similar to that obtained by Samy (1991) who stated that the serum iron level showed a non significant increase at the 10th, 15th, 20th and 30th days after treatment with Gn-HR (Receptal). The increase in serum iron level may be attributed to the correlation between iron and copper and ceruloplasmin. In this respect Sirvech et al. (1974) found that ceruloplasmin has a clinical importance and possible role in iron and copper metabolism and transport. Copper acts as a co-factor causes increased ceruloplasmin (Ferroxidase) activity when ceruloplasmin activity increased, movement of stored iron from macrophages to transferrin is stimulated. The result is increased iron transport to the bone marrow with a subsequent increase in heme and ultimately in hemoglobin synthesis. Frieden (1984). Whereas, the significant decrease in serum iron level after PGF<sub>2α</sub> treatment disagrees with that reported by Gamal (1987) who observed that injection of PGF<sub>2α</sub> (Lutalyse) in different doses did not affect iron levels in all animals even those showed induced oestrus at high dose rates. This differences may be due to changes in age and or dose of the animal.

Serum manganese level was significantly increase after treatment with PGF<sub>2α</sub> alone. The increase in manganese level may be due to the oestrogen secretion Kutsky (1981) which postulated that, the oestrogen increase the serum manganese level.

It is evident from the present study that the serum copper concentration showed a non significant change after hormonal treatment in infertile buffalo - cows suffering from palpable corpora lutea Table (2). Whereas, the serum zinc level was
very highly significantly decreased after prostaglandin F₂-α treatment. The decrease of serum zinc level may be related to the altered binding affinity of zinc binding protein as stated by Pories et al. (1976). Regarding serum iron level, there was a significant decrease after treatment with PGF₂-α alone and highly significant decrease after treatment with PGF₂-α with Gn-RH. Whereas the iron level showed a significant increase after Gn-RH treatment in buffalo cows with palpable corpora lutea. Similar discussion of the obtained results was mentioned above in buffalo cows with inactive ovaries. The obtained data indicated that there was a significant increase in serum manganese level after PGF₂-α treatment. The increase in serum manganese level after treatment may be due to the involvement of manganese in the luteal activity and maximal uptake of manganese was occurred during the luteal phase when progesterone was greatest, Hidiroglou (1975).

It could be concluded that the administration of Prostaglandin F₂-α ( Estrumate ) and synthetic gonadotrophin releasing hormone ( Gn-RH ), ( Fertagyl ) may be helpful in oestrus induction and ovulation, in post partum suboestrus buffaloes, reducing intercalving period and improving their reproductive efficiency.

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Table (1): SERUM COPPER, ZINC, IRON AND MANGANESE LEVELS OF INFERTILE BUFFALO-COWS WITH INACTIVE OVARIAN BEFORE AND AFTER HORMONAL TREATMENT IN UGAL.

<table>
<thead>
<tr>
<th>Hormones Used</th>
<th>Copper (μg/dl)</th>
<th>Zinc (mg/dl)</th>
<th>Iron (μg/dl)</th>
<th>Manganese (μg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>Prostaglandin F₂-α</td>
<td>81.25±4.26</td>
<td>77.5±3.22</td>
<td>206.25±18.07</td>
<td>171.25±10.48</td>
</tr>
<tr>
<td>PGF₂-α + oestradiol</td>
<td>75.00±7.35</td>
<td>73.75±4.26</td>
<td>161.25±10.87</td>
<td>251.25±5.15**</td>
</tr>
<tr>
<td>PGF₂-α + Gn-RH</td>
<td>98.75±2.39</td>
<td>90.00±4.56</td>
<td>166.25±7.18</td>
<td>183.75±2.39</td>
</tr>
<tr>
<td>Gn-RH</td>
<td>79.75±2.05</td>
<td>90.00±2.04</td>
<td>163.75±2.39</td>
<td>182.00±3.22**</td>
</tr>
</tbody>
</table>

*: (P < 0.05)   **: (P < 0.01)   ***: (P < 0.001)

S.E. = Standard error.

Table (2): SERUM COPPER, ZINC, IRON AND MANGANESE LEVELS OF INFERTILE BUFFALO-COWS WITH PALPABLE CORPORA LUTEA BEFORE AND AFTER HORMONAL TREATMENT IN UGAL.

<table>
<thead>
<tr>
<th>Hormones Used</th>
<th>Copper (μg/dl)</th>
<th>Zinc (mg/dl)</th>
<th>Iron (μg/dl)</th>
<th>Manganese (μg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>Prostaglandin F₂-α</td>
<td>97.5±7.77</td>
<td>73.75±4.26</td>
<td>397.5±20.25</td>
<td>218.75±2.39***</td>
</tr>
<tr>
<td>PGF₂-α + oestradiol</td>
<td>68.75±3.14</td>
<td>83.75±5.15</td>
<td>148.75±2.39</td>
<td>175.0±12.07</td>
</tr>
<tr>
<td>PGF₂-α + Gn-RH</td>
<td>88.75±5.15</td>
<td>93.75±6.88</td>
<td>165.00±5.77</td>
<td>182.50±3.22</td>
</tr>
<tr>
<td>Gn-RH</td>
<td>82.50±3.22</td>
<td>87.50±3.22</td>
<td>155.0±2.04</td>
<td>156.25±3.14</td>
</tr>
</tbody>
</table>

*: (P < 0.05)   **: (P < 0.01)   ***: (P < 0.001)

Data are presented as mean ± S.E.

S.E. = Standard error.
تأثير الأعطال الهرموني على بعض عناصر الدم النادرة في ضعف الخصوبة للجاموس

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إجراء هذا البحث على 80 جاموساً (40 نرتي من خمول المبايض و40 نرتي من وجود الجسم الأصفر) وقد قسمت إلى أربعة مجموعات تعبر عن أنواع الهرمونات المستخدمة في العلاج بحيث شملت كل مجموعة على 20 جاموساً من كل من حالات الإضطرابات التناسلية. المجموعة الأولى: أعطي كل حيوان 2 ميلليتر من البروستاجلاندين F2 ألفا في الفعلي وقد تكررت الجرعة مرة بعد 10 إلى 12 يوم. المجموعة الثانية: أعطي كل حيوان 2 ميلليتر من البروستاجلاندين F2 ألفا بالإضافة إلى خمسة مليجرام من الاستروال (فولون) في الفعلي. المجموعة الثالثة: أعطي كل حيوان 2 ميلليتر من البروستاجلاندين F2 ألفا وبعد خمسة أيام تم حقن 0.2 مليلتر من ال Gn-RH (الفرتاجيل) في الفعلي. المجموعة الرابعة: أعطي كل حيوان 0.2 مليلتر من ال Gn-RH في الفعلي وجمعت عينات الدم قبل الحقن وعند ظهور الشبق بعد استجابة الحيوانات للعلاج لدراسة أثر العلاج على مستوى عناصر النحاس ، الزئبق ، الحديد والمنجنيز وقد أسفرت النتائج على الآتي:

بالنسبة للجاموس الذي عانى من خمول في المبايض كان هناك تغير غير معنوي في مستوى النحاس وزيادة غير معنوية في مستوى الزئبق وذلك بعد علاج البروستاجلاندين F2 ألفا مع
الاسترشادين وكلاً معاً Gn-RH والبروزستيجلادينين F2 آنفاً وزيدت عاليه المفعوية بعد جلامة بال Gn-RH آنفاً وتلك بالمقارنة بالعينات التي تم جمعها قبل العلاج، بالنسبة للجاموس الذي كان يعاني من وجود الجسم الأصفر أسفرت النتائج عن وجود تغير غير ملمع في مستوى عصري النحاس بعد العلاجات المختلفة كما أظهر مستوى الزنك نقصاً على المفعوية بعدي العلاج بالبروزستيجلادينين F2 آنفاً فقط، وكذلك كان هناك نقص مضوئ في مستوى الحديد بعد علاج البروزستيجلادينين F2 آنفاً وتغير هذا النقص إلى عالي المفعوى بعد العلاج بواسطة البروزستيجلادينين F2 آنفاً مع Gn-RH. فقط وأظهر مستوى المنجنيز زيدت مفعويه بعد علاج البروزستيجلادينين F2 آنفاً وذلك بالمقارنة بالعينات التي جمعت قبل العلاج. وقد استنتج من النتائج هذه الدراسة أن استخدام البروزستيجلادينين F2 آنفاً وهرمون ال الكفاءة التناسائية في الجاموس المصري.