

**HORMONAL PROFILE AND SOME MINERALS IN  
RELATION TO INFERTILITY IN BUFFALO-COWS**

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**Abstract**

A total numbers of 80 buffalo-Cows were equally grouped, according to their reproductive disorder, into two main groups (animals having inactive ovary and others with palpable ovarian corpora lutea). Each main group was then subdivided into four subgroups, according to the hormonal treatment applied. Animals of the first group were intramuscularly injected with 2 ml. PG F<sub>2</sub>-α repeated once after 10-12 days in case that the animals did not come in estrus. Those of the second group were injected with PG F<sub>2</sub>-α plus 5 mg. Oestradiol 17 B. Intramuscularly injection of 2ml. PG F<sub>2</sub>-α plus 2.5 ml. Gn-RH was applied to animals of the third group. While those of the fourth group were intramuscularly injected with 2ml. Gn-RH.

Serum progesterone, 17B-Oestradiol, calcium, inorganic phosphorus and magnesium levels were estimated in all experimental animals before treatment and at the appearance of heat thereafter.

Buffaloes having inactive ovary showed a significant increase in serum progesterone level after PG F<sub>2</sub>-α injection ( $P < 0.01$ ), whereas, serum oestradiol level showed a significant decrease ( $P < 0.05$ ). A significant decrease in serum calcium level ( $P < 0.05$ ) was detected after treating ani-

mals with PG F<sub>2</sub>-α plus Gn-RH. The serum inorganic phosphorus level showed a significant decrease after PGF<sub>2</sub>-α plus Gn-RH injection. This decrease was of a highly significant magnitude after treating animals with Gn-RH. A very highly significant decrease in serum magnesium level was recorded after treatment with PG F<sub>2</sub>-α alone.

Buffaloes having a palpable ovarian C. L. had a highly significant decrease in serum progesterone level after treating them with PGF<sub>2</sub>-α plus oestradiol. Whereas, serum progesterone level showed a highly significant increase in animals injected with Gn-RH. A significant decrease in serum oestradiol level was recorded after treatment with PGF<sub>2</sub>-α plus Gn-RH. This decrease was highly significant in case of treating animals with PGF<sub>2</sub>-α or Gn-RH alone. A highly significant increase in serum Calcium level was observed as a result of treating animals with either PGF<sub>2</sub>-α alone or PGF<sub>2</sub>-α plus oestradiol. A highly significant decrease in serum inorganic phosphorus level was recorded after treatment with PGF<sub>2</sub>-α. This decrease was of significant magnitude in case of treating animals with PGF<sub>2</sub>-α plus Gn-RH. A significant increase in serum magnesium level was detected after treatment with PGF<sub>2</sub>-α plus oestradiol. Results of this work indicated that applying of PGF<sub>2</sub>-α with oestradiol may be a practical therapeutic solution in case of inactive ovaries and palpable C. L. in buffaloes.

### Introduction

Minerals are essential part of the diet and properly balanced intake is necessary to maintain the optimum health condition of the animals. Various elements such as calcium, phosphorus and magnesium can influence the reproductive performance in ruminants. Delayed puberty and failure in oestrus duration are the usual symptoms of phosphorus deficiency in

heifers that usually have small and incomplete quiescent ovaries (Robert, 1971). Magnesium does not have any direct role in reproduction, but it is involved in many of the enzymatic reaction catalyzed by ATP (Aikawa, 1978). It also influences the absorption of calcium and phosphorus.

Endocrine changes during spontaneous regression of Corpora

lutea is well defined for cows and ewes. Decrease in concentrations of progesterone in plasma are accompanied by increasing luteinizing hormone (Rahe et al., 1980), prolactin (Kann and Denamur, 1974) and Oestradiol 17B (Pratt et al., 1977).

Prostaglandin F<sub>2</sub>-α (PGF<sub>2</sub>-α) is a potent naturally occurring luteolytic in sheep. Administration of PGF<sub>2</sub>-α effectively induces early regression of the corpus luteum (Stacy et al., 1976).

Abdo et al., (1983) reported a decrease in the level of Calcium without change in the level of serum inorganic phosphorus after injecting laying hens with PGF<sub>2</sub>-α. Moreover, Theodoulou et al., (1977) observed that, PGF<sub>2</sub>-α had no significant effect on serum calcium in man. Also Rajamahendran et al., (1983) reported that injection of 30 mg. PGF<sub>2</sub>-α in buffaloes did not cause any significant change in serum calcium and phosphorus during 24 hours after injection.

Gamal (1987) reported no

changes in serum Calcium, phosphorus and magnesium levels after injecting buffaloes and Cows with PGF<sub>2</sub>-α. Komonpatuna et al., (1979) reported that progesterone level was sharply declined after 24 hours in heifers and cows injected with the effective doses of PGF<sub>2</sub>-α. Moreover, Rajamahendran et al., (1983) found that, after PGF<sub>2</sub>-α treatment, serum progesterone levels decreased and minimum levels were noticed on day 2, 3, 7 and 11 after first injection. Furthermore, Gamal(1987) observed that the progesterone level was not affected by PGF<sub>2</sub>-α injection in dose at level up to 50 ug/kg, body weight. On the other hand, the luteolytic effect of PGF<sub>2</sub>-α on corpora lutea was noticed at dose levels of 60 and 70 ug/kg body weight. However, under complete luteolysis of corpora lutea in the responded animals the progesterone reached its lowest value at induced oestrus (0.03 ug/ml).

Therefore, the object of the present study was to throw light upon the biochemical effect, the physiological and reproductive responses of buffalo-Cows having in-

active ovaries or palpable corpora lutea to some hormonal treatments which usually applied to treat infertility in Egyptian buffaloes.

#### Material and Methods

This work was conducted at Khattara Farm belonging to the Animal Development and Food Security Project Sharkia province, during the Spring of 1993. A total number of 80 mature female buffalo-Cows suffering two main reproductive disorders (having inactive ovaries (40), as well as palpable corpora lutea (40)) were subjected to this study. The age of the studied animals varied between 4-8 years and calved once or twice.

Each of these animals were supplied with a uniformed ration. Composed of 4 kg. concentrats (65% cotton seed cakes, 20% wheat bran, 12% rice polish, 2% lime salts and 1% common salts), in addition to a suitable amount of Barseem (*Trifolium alexandrinum*) during the green season, as well as maize straw (darawa) and rice straw in dry season. The animals were offered water ad libitum and

were kept free in the yards all over the year.

All experimental animals showed no estrous for more than 6 months postpartum. Complete gynaecological examination has been carried out two time at 10 days intervals before the start of the experiments which revealed no pathological abnormalities of the genital organs.

Animals of each two main reproductive disorder groupes were then subdivided into 4 subgroups each of 10 buffalo-Cow, treated as follows:

**Subgroup I :** Intramuscularly injected with 2ml. PGF<sub>2</sub>-α 500 ug. (Estrumate\*) repeated once more after 10-12 days in case that animal did not show response to the first injection.

**Subgroup II :** received 2ml. PGF<sub>2</sub>-α plus 5mg oestradiol(Folone-5\*\*) injected i.m. at the same time.

**Subgroup III :** received 2ml. PGF<sub>2</sub>-α plus 2.5ml synthetic Gn-

RH (Fertagyl<sup>\*\*\*</sup>) after 5 days injected I.m.

**Subgroup IV** : received 2.5ml Gn-RH (Fertagyl) injected I.m.

\* Coopers Animal Health limited (England).

\*\* Misr Company for pharmaceutical industries (Egypt).

\*\*\* Intervet International B.V. (Holland).

Blood samples were collected from juglar vein immediatly before treatment and at the appearance of heat. The serum were separated by centrifugation at 3000 r. p. m. for 10 minutes and stored at -20°C till the time of chemical determination of oestradiol, progesterone, calcium, inorganic phosphorus and magnesium.

Progesterone and oestradiol 17-B were assayed in blood serum by the radioimmuno assay (RIA) using commercial kits provided by diagnostic products corporation, los Angeles, USA. According to Kubasik et. al., (1984) and Xing et. al., (1983) for progesterone and oestradiol 17-B assay, respectively.

Serum calcium was analyzed using plasma emission (Spectraspan V), model V-DCP (BECKMAN).

The serum inorganic phosphate was determined colorimetrically according to the method of Wootton (1982).

Serum magnesium was determined 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 and Discussion

Data concerning the effect of treatments applied on serum sexual steroidal hormones (Progesterone and oestradiol 17-B) on animals of both inactive ovary and of palpable corpora lutea are presented in table (1).

Inspection of obtained data revealed significant increase in serum progesterone level of animals

with inactive ovary ( $P < 0.01$ ) while those of palpable corpora lutea showed an insignificant decrease in the level of this hormone in their serum. However, serum progesterone level sharply and significantly decreased when  $\text{PGF}_2\text{-}\alpha$  was applied in combination with oestradiol in animals with palpable corpora lutea. Gonadotrophin releasing hormone (Gn-RH) had insignificant effect when was applied with  $\text{PGF}_2\text{-}\alpha$  on the level of serum progesterone of animals of either inactive ovary or with palpable corpora lutea. However, it sharply and significantly ( $P < 0.01$ ) increased the serum progesterone level when applied alone to animals with palpable corpora lutea only. The obtained result was in agreement with those of Zaabel (1993) who found that the progesterone level at the follicular phase of the puberal period, revealed a highly significant increase than those at the prepuberal period. The increase in serum progesterone level may be attributed to low L.H. level occurred at this phase. This possibility is advocated by the finding of Kaltenback (1980) and Schillo et al., (1982).

Serum oestradiol level in animals with inactive ovary showed no significant response to treatments applied except in case of treating animals with prostaglandine  $\text{F}_2\text{-}\alpha$ . In this case serum oestradiol level significantly decreased ( $P < 0.05$ ) as a result of this treatment. However the rate of decrease had no significant magnitude in case of applying  $\text{PGF}_2\text{-}\alpha$  with either oestradiol or with Gn-RH. On the other hand Gn-RH insignificantly increased serum estradiol level when applied alone to animals of inactive ovaries.

Response to different treatments applied was quite different in animals of palpable corpora lutea. In those animals serum estradiol 17-B level decreased as a result of treatments applied. However, the rate of decrease differed according to treatment. Generally it could be stated that  $\text{PGF}_2\text{-}\alpha$  and Gn-RH were more effective in decreasing the level of serum estradiol when applied alone than other when applying the former with oestradiol or the later with  $\text{PGF}_2\text{-}\alpha$ . In addition the rate of decrease found in case of treating animals with

PGF<sub>2</sub>-α oestradiol was of insignificant magnitude.

Such conclusions concerning serum estradiol concentration in infertile buffalo-Cows came in accordance to those of Hattab, (1988) who found that the serum oestrogen level are significantly decrease in infertile buffalo-Cows and be attributed to insufficient release of gonadotrophins necessary for initiation and regulation of the cyclic ovarian activity.

From the previously mentioned results it was found that the mode of response to various treatments applied differed according to the physiological status of the animal. However, it was more clear on serum estradiol level of animals of palpable corpora lutea. In addition serum estradiol level showed approximately a reverse response to treatment to that of serum progesterone level. This is scientifically quite logic since the site of secretion differed from hormone to another, and progesterone is secreted from the leuteal cells formed after ovulation by transforming of the granule cells that

secrete oestrogen to leuteal ones that secrete progesterone.

Data presented in table (2) show the effect of hormonal treatment applied on serum calcium, inorganic phosphorus and magnesium level in buffalo-Cows having either inactive ovary or palpable corpora lutea.

Data obtained showed that serum calcium level showed a significant (P<0.01) response when animals having inactive ovaries were treated with prostaglandin F<sub>2</sub>-α in combination with gonadotrophin releasing hormone. It decreased from 10.22 mg/dl before treatment to 8.25 mg/dl. after the previously mentioned treatment. In addition treating animals Gn-RH alone insignificantly decreased serum calcium content (from 11.15 to 10.0 mg/dl.). However no significant effects were found due to treating animals with prostaglandin either alone or in combination with oestradiol.

The obtained results are nearly similar to the finding of Samy, (1991) who found that a signifi-

cant decrease in serum calcium level after Gn-RH (Receptal) treatment in infertile buffalo-Cows with inactive ovaries. Whereas, Michail (1979) recorded that the serum calcium level in buffaloes was decreased non significantly after Pro-lan administration. The decrease in serum Calcium level at fertile heat after treatment of buffaloes may be due to high level of oestrogen as confirmed by Soliman et. al. (1964).

The mode of action of treatment applied differed in buffalo-ows having palpable corpora lutea. In these animals prostaglandin-F<sub>2</sub>- $\alpha$  alone or prostaglandin F<sub>2</sub>- $\alpha$  + plus-oestradiol significantly (P<0.01) increased serum calcium level.

However Gn-RH if injected alone or with prostaglandin F<sub>2</sub>- $\alpha$  had insignificant effect on serum calcium level in animals of the same gynaecological status. The increase of serum calcium level after treatment may be attributed to the greater mobilization of calcium ion due to the increase in metabolic activity during the oestrogenic phase of the cycle as stated by Os-

man et. al. (1979).

The obtained results confirmed by Farrag (1978) and El-Shawaf (1984) who stated that the level of serum calcium in buffaloes and Cows during oestrus period were significantly higher than during other stages of the oestrus cycle.

The effect of treatments applied on serum inorganic phosphorus differed according to the physiological status of the animals. While inorganic phosphorus significantly (P<0.01) decreased when treating animals having palpable corpora lutea with prostaglandin it is significantly decreased in those of inactive ovary treated with the same treatment. In addition prostaglandin F<sub>2</sub>- $\alpha$  had no significant effect on serum inorganic phosphorus when applied to animals of either inactive ovary or of palpable corpora lutea. However, serum inorganic phosphorus significantly (P<0.05) decreased when applied to both groups of animals in combination with Gn-RH. Finally, injecting buffalo-cows with Gn-RH significantly (P<0.01) decreased serum inorganic phosphorus. This



decrease may be attributed to the time of sampling or and nutritional status of the animals. Abd El-latif (1993).

Moreover, it is suggested that infertility as a result of phosphorus deficiency can be produced when there is excess of Calcium in the ration that interferes with phosphorus metabolism or when most of the calcium was excreted as calcium phosphate and there is insufficient phosphorus in blood, such stress condition might affect the anterior pituitary and so upset the production of gonadotrophic hormone (Hignett and Hignett, 1951).

Serum Magnesium content significantly decreased ( $P < 0.001$ ) as a result of treating animals of inactive ovary with prostaglandin alone. However, the other treatments applied did not show any effect on serum magnesium level. On the other hand, a significant ( $P < 0.05$ ) increase in serum magnesium level was found when treating animals of palpable corpora lutea with prostaglandin plus oestradiol. However, other treatments applied

showed insignificant effect in this aspects.

From the previously mentioned results it could be stated that changes in serum calcium, inorganic phosphorus and magnesium levels in response to treatment applied differed according to the gynaecological status of an animal which reflect different hormonal coordination condition related to mineral metabolism. In addition the negative correlation found between serum calcium and inorganic phosphorus may reflect the possibility to state that the effect of the hormonal treatment applied may be through its effect on the hypothalamo - hypophyseal - thyroid - parathyroid axis. The parathormon and calcitonine may be the main cause of the changes found in calcium and inorganic phosphorus content in the blood serum. In addition prostaglandin  $F_2\text{-}\alpha$  may be the main hormonal factor causing these changes.

It could be concluded that the administration of  $PGF_2\text{-}\alpha$  with oestradiol is the best treatment used in buffalo-Cows with palpable

ovarian CL. However PGF<sub>2</sub>-α alone showed high efficiency in treatment of buffalo-cows with inactive ovaries as revealed by their response, changes and return of different blood serum parameters to normal after treatment. So we rec-

ommend the physician and farmers to use these hormones which may be helpful in oestrus induction and ovulation, in postpartum suboestrus buffaloes, reducing intercalving period and improving their reproduction efficiency.

Table 1: Serum progesterone and estradiol levels (X±SE) of Inactive Buffalo-Cows in response with treatment applied.

Parameters Hormones used	Progesterone (ng/ml)				Estradiol 17β (pg/ml)			
	Inactive ovary		Palpable corpus lutea		Inactive ovary		Palpable corpus lutea	
	Before	After	Before	After	Before	After	Before	After
Prostaglandin F <sub>2</sub> -α (Estrumate)	0.181 ± 0.06	1.512 ± 0.774**	0.291 ± 0.018	0.251 ± 0.030	149.8 ± 25.81	120.97 ± 7.01*	253.9 ± 5.88	206.3 ± 46.79**
PGF <sub>2</sub> -α + Oestradiol (Estrumate + Folone)	0.388 ± 0.015	0.299 ± 0.049	1.022 ± 0.426	0.114 ± 0.046**	131.1 ± 3.35	129.8 ± 2.33	132.9 ± 8.42	118.95 ± 2.35
PGF <sub>2</sub> -α + Gn-RH (Estrumate + Fertagyl)	0.396 ± 0.0058	0.205 ± 0.004	0.212 ± 0.028	0.045 ± 0.019	130.7 ± 3.26	123.3 ± 1.58	159.1 ± 14.38	127.0 ± 3.70*
Gn-RH (Fertagyl)	0.337 ± 0.160	0.467 ± 0.039	0.033 ± 0.005	1.123 ± 0.079**	135.0 ± 3.59	145.7 ± 2.91	161.2 ± 19.90	115.13 ± 2.80**

\* P < 0.05  
\*\* P < 0.01

Table 2: Serum Calcium, inorganic phosphorus and Magnesium levels (X±SE) of Inactive Buffalo-Cows in response with treatment applied.

Parameters Hormones used	Calcium (mg/dl)				Inorganic phosphorus (mg/dl)				Magnesium (mg/dl)			
	Inactive ovary		Palpable corpus lutea		Inactive ovary		Palpable corpus lutea		Inactive ovary		Palpable corpus lutea	
	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
Prostaglandin F <sub>2</sub> -α (Estrumate)	8.50 ± 0.08	9.68 ± 0.03	7.61 ± 0.26	11.58 ± 0.58***	3.66 ± 0.25	4.93 ± 0.32	0.63 ± 0.36	4.41 ± 0.28**	2.07 ± 0.06	1.47 ± 0.03***	1.84 ± 0.18	2.12 ± 0.14
PGF <sub>2</sub> -α + Oestradiol (Estrumate + Folone)	7.14 ± 1.14	7.25 ± 0.57	7.91 ± 0.25	10.84 ± 0.36**	4.02 ± 0.29	3.61 ± 0.39	5.09 ± 0.49	5.06 ± 0.42	2.20 ± 0.18	2.05 ± 0.11	0.94 ± 0.11	1.25 ± 0.10*
PGF <sub>2</sub> -α + Gn-RH (Estrumate + Fertagyl)	10.20 ± 0.27	9.25 ± 0.24	11.18 ± 1.25	8.60 ± 0.40	5.31 ± 0.35	4.0 ± 0.33*	5.31 ± 0.31	4.55 ± 0.14*	3.68 ± 0.06	3.68 ± 0.08	3.46 ± 0.02	3.20 ± 0.25
Gn-RH (Fertagyl)	11.15 ± 1.29	10.0 ± 1.41	10.10 ± 0.32	10.05 ± 1.25	4.71 ± 0.17	3.71 ± 0.18**	5.34 ± 0.25	6.11 ± 0.25	3.13 ± 0.13	3.13 ± 0.13	3.05 ± 0.06	2.88 ± 0.14

\* P < 0.05  
\*\* P < 0.01  
\*\*\* P < 0.001

**References**

- Abd El-latif M. (1993)** : Recent trials on treatments of infertility in bovine. M.Vet.Sci. Thesis, Fac. of Vet. Med. Zagazig University.
- Abdo M. S., Salem H. A. H. and Younis M. (1983)** : Alkaline phosphatase, inorganic phosphorus, calcium and some other blood constituents in the blood of laying hens as affected by PG F<sub>2</sub>- $\alpha$  injection. J. Egypt. Vet. Med. Ass. 43:25.
- Aikawa J. K (1978)** : Biochemistry and physiology of magnesium. World Review of Nutrition and diet. 28:113.
- El-Shawaf A. M. M (1984)** : Some laboratory studies on infertile and sterile farm animals. Ph. D. Thesis, Vet. Med. Zagazig University.
- Farrag A. A. M. (1978)** : Ovarian syndromes in cattle and buffaloes with special reference to certain infertility problems. Ph. D. Vet. Thesis, Assuit University.
- Ganial A. A. (1987)** : Some pharmacological aspects of prostaglandins with special reference to their action on reproductive performance in female buffaloes. M. Vet. Sci. Thesis, Fac. Vet. Med. Cairo University.
- Hattab S. A. (1988)** : Hormonal profile during calthood, perpuberal and puberal period and during ovarian atrophy (inactivity) in buffaloes. M.V. Sci. Thesis, Fac. Vet. Med. Alex. University.
- Hignett S. L. and Hignett P. G. (1951)** : The influence of nutrition on reproductive efficiency in cattle. 1- The effect of Calcium and phosphorus intake on the fertility of Cows and heifers. Vet. Rec. 63: 603.
- Kaltenbach C. C. (1980)** : Initiation of puberty and post-partum oestrous in beef-Cattle. Current therapy of theriogenology by Morrow, D. A. (1980), W. B. Saunders Comp., London.
- Kamonpatana M., Van De Viet D. F. M., Koops Leenauruksa D., Negamsuriyaraj C. and Usanakornkul S. (1979)** : Oestrus con-

- trol and early pregnancy diagnosis in the swamp buffalo: Comparison of EIA and RIA for plasma progesterone. *Theriogenology* 11: 399.
- Kann G. and Denanlur R. (1974)** : Possible role of prolactin during the oestrous cycle and gestation in the ewe. *J. Reprod. Fertil.* 39:473.
- Kubasik N. D. (1984)** : Evaluation of a direct Solid-phase radioimmunoassay for progesterone. *Clinical chemistry* 30: 384.
- Mikhail F. A. (1979)** : Studies on ovarian inactivity in buffaloes in A. R. E. Ph. D. Vet. Thesis Cairo University.
- Osman A. M., El-Naggar M. A., Allom F. and Farrag A. A. (1979)** : Variations in some of the blood constituents of cattle during the oestrus cycle and ovarian inactivity. *J.Egypt. Vet. Med. Assoc.* 14: 89.
- Pratt B. R., Butler R. L. and Inskoop E. K (1977)** : Antiluteolytic effect of the conceptus and of PGF<sub>2</sub>- $\alpha$  in ewes. *J.Anim. Sci.* 45:784.
- Rabe C. H., Owens R. E., Fleege J. L., Newton H. J. and Harms P. G. (1980)** : Pattern of plasma luteinizing hormone in the cyclic Cow: Dependence upon period of the cycle. *Endocrinology* 107:498.
- Rajama bendran R., Sivacleivan M. N. and Sivakanesan R. R. (1983)** : Hormonal, Biochemical, physial, Behavioural and Histological changes following PGF<sub>2</sub>- $\alpha$  in Local buffaloes. Proc. of the pre-conference symposium of the 5th world conference on animal production, Japon - P. 147-152 (August, 12-13).
- Roberts S. J. (1971)** : Veterinary obstetric and genital diseases. 2nd Ed., Ithaca, New York and London.
- Samy A. H. (1991)** : Some biochemical studies on the effect of certain drugs in relation to infertility in buffaloes. Ph. D. Thesis, Fac. of Vet. Med. (Benha branch), Zagazig University.

- Schillo K. K. Diershke D. J. and Hauser E. R. (1982)** : Regulation of lutenizing hormone Secretion in prepubertal heifers: Increased threshd to negative feed back action of estradiol. *J.Anim. Sci.* 54: 325.
- Snedecor G. W. and Cochran W. G. (1967)** : In statistical methods 6th. Edition. Iowa State University, Press, Iowa, U. S. A.
- Soliman F. A., Nasr H., Zaki K. Abdo M. S. and Soliman N. K (1964)** : Metabolic effect of oestrogen with special reference to blood chemistry of Friesian Cow. 2- Effect of stilboesterol on serum electrolytes in Friesian Cow. *Indian Vet. J.* 43: 1062.
- Stacy B. D., Gemmell R. T. and Thorburn G. D. (1976)** : Morphology of the corpus luteum in the Sheep during regression induced by prostaglandin F<sub>2</sub>- $\alpha$ . *Biol. Reprod.* 14:280.
- Theodoulou G., Mayobaulo D. S. and Mountoknlakis T. H. (1977)** : Calcium infusion induced hypercalcemia before and after indomethacin administration in man. *Hippocrates (ATHENS)* 32: 85.
- Willis J. B. (1960)** : The determination of metals in blood serum by atomic absorption spectrophotometry. *Spector Chem. Acta* 16:259.
- Xing S., Sekan C. Z. and Diczfalusy U. (1983)** : Validation of radio-immuno assay for estradiol 17-B by isotope dilution mass spectrometry and by a test of radiochemical purtly. *Clinica chemica Acta.* 135: 189.
- Zaabel S. M. M. (1993)** : Some studies on puberty in buffaloes. Ph. D. Vet. Sci.Thesis, Fac. of Vet. Med. Suez canal University.