رقم البحث (8)

STUDIES ON THE ANESTRUM IN BUFFALO IN DAKAHLIA PROVINCE

BY

F.E.Wehaish* , S.Zaabel and Y.El-Seady***

**Department of physiology ,fac.of vet.med. Mansuora university*

***Department of theriogenology ,fac.of vet.med. Mansuora university*

ABSTRACT

Infertility is the most common problem threatened promotion of livestock in Egypt .this study was performed to get in sight the causes and available attempt for solving a problem of anestrus in buffalo –cows ,under a field condition , through the applications of some available hormonal medicaments. On the other hand, to achieve an economic benefit particularly to the small farmer at Dakahlia province .

The work was conducted on 80 mature female buffalo –cows of 4-8 year of age and kept under village system in Dakahlia province and suffering from inactive ovaries (40 animals) or palpable corpora leutea{CL} (40 animals) according gynecological examination . all experimental animals showed anestrous for more than 6 months post partum and gynecological examination not revealed any pathological abnormalities in genital tract. Each group was divided into 4 subgroup that incloud 10 animals . sub group (I):- receiving prostaglandin (PGF2α) only ,sub group (II) :- receiving PGF2α plus estradiol , Subgroup (III) :- receiving PGF2α and synthetic gonadotrophin –releasing factor (Gn-RH) ans sub group (IV) receiving Gn-RH only . blood samples were collected from Jugular vein immediately before treatment and just at the appearance of heat .serum was separated and subjected to biochemical and hormonal assay.

The results revealed that ,significant decrease in serum progesterone level of animals with inactive ovaries and treated with PGF2α alone. while , progesterone level significantly decreased in animals with palpable CL and treated with PGF2α in combination with estradiol . serum estrogen levels showed significant decrease only incase of animals treated only with PGF2α . Serum calcium levels showed significant decreases in inactive ovaries

animals and treated with PGF2α in combination with Gn-RH . in-organic phosphorous levels significantly decrease in both groups treated either with PGF2α alone or in combination with Gn-RH . On the other hand , serum magnesium show significant decrease as the result of treating animals of inactive ovaries by PGF2α alone while ,significant increases were found in animals with palpable CL treating with PGF2α with estradiol .

We concluded that , administration of PGF2α in combination with estradiol is the beast treatment used in buffalo –cows with palpable CL however, PGF2α alone showed high efficiency in treatment animals suffering from inactive ovaries.

INTRODUCTION

Infertility is the most common problem threatened promotion of the livestock in Egypt. It has been recorded that more than thirty to fourty percent of the local livestock of cattle and buffaloes are suffering from infertility .If we noticed that the total animal population of cattle and buffaloes are approximately five millions **(The agriculture Ccensus, 1982**) hence the problem of infertility seems to be so big sized that it may result in an economic disaster. The total loss of infertility problem of cattle and buffaloes in Egypt constitutes about 100 million pound per year **(osman,1984)**. The target toward finding out the rapid solution for such problem comes from the fact that nearly 90 ٪ of the local buffaloes and cows are owned by the small farmer to which his actual income is mainly incorporated. Therefore, any adversible effect on the productive and reproductive performance of these livestock will be reflected badly on the living standard of the small farmer and in turn on the national economy**(El-Desouky, 1993)**.

Infertility is not a disease but a sign for the action and/ or interaction of multiple factors related mainly to hereditary, environment, management and diseases. From field investigations, under the local environmental conditions it has been emphasized that anestrum is the commonest type of infertility among the Egyptian live stocks particularly buffaloes, through the majority of animals admitted to the clinic, suffering from anestrum, appear to have normal clinical manifestation and normal feeding status especially those managed by the small farmers, yet, they generally appear to have an abnormal prolongation of anestrum which may reach in some instances to one year. Thereby, the economic value of the animal as a unit of production and reproduction will be lost. In such case, in order to

overcome the problem of anestrum, the rapid and effective approach is to find out the treatment characterized by its availability to the farmer and easy in application.

It is recommended that, to maximize profitability in dairy herd, cows produce a calf every year. This goal is seldom realized because the interval from calving to conception is prolonged by many factors including poor breeding techniques **(Graham, 1966)**,poor detection of estrus **(Barr, 1974)**, ovarian dysfunction and reproductive tract infections **(Britt, 1980**).

Early reestablishment of cyclic ovarian activity after calving is essential as well as a fewer number of services per conception, to maintain an acceptable calving to conception interval, and therefore, economic efficiency **(Benmard and Stevenson, 1986)**. Thus, treatments given to initiate normal oestrus cycles should improve reproductive performance **(Hussein, et. al., 1992)**.

Gonadotropin releasing hormone (GnRH) is a decapeptid produced by neurons in hypothalamus and secreted into capillaries into hypophyseal portal system. Within the pituitary, GnRH binds to a specific receptors and through a series of downstream steps, stimulate the release of Follicle Stimulating Hormone (FSH) and Lutinizing Hormone (LH) **(Thatcher et al., 1989)**.

Secretion of GnRH moderately stimulates follicular development through the action of FSH and stimulate ovulation of follicle and formation of corpus luteum through the action of LH **(Chenault et al., 1990).**Reestablishment of ovarian cyclicity, the interval between calving and insemination and the fertility of an insemination are reproductive outcomes to be influenced by GnRH treatment **(Beckett and Lean, 1997).**

Prostaglandin $F_{2\alpha}$ induce a premature regression of corpus luteum (luteolysis) and consequent fall in circulating progesterone concentration. By remove a negative feed back inhibition on the hypothalamus and pituitary, the fall in progesterone concentration allows a sequence of hormonal and ovarian events which stimulate estrus and ovulation **(Gordon et al., 1996)**. They added that, Prostaglandin $F_{2\alpha}$ was used to allow synchronization of oestrous cycles of group of animals, so that they could be inseminated at a fixed time with expectation of normal fertility

The present study was designed as an attempt for approaching problem of anestrum in buffalo-cows, under the field condition, by application of some hormonal medicaments

recommended to be available, cheap and to achieve an economic benefit especially to the small farmer at Dakahelia Province.

MATERIAL AND METHODS

This work was conducted on a number of buffalo cows owned to the small farmers, kept under the village system, in Dakahlia Province. 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.

Under the village condition, animals are offered to take available rations, which mainly consist 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. According to veterinary authority regulation, all animals in the village were given a circular vaccination against common infectious and contagious diseases, and circular treatment against internal and external parasites.

A real time B-and M- mode linear array ultrasound scanner (Ultra scan 50). The ULTRA SCAN* 50 a portable ultrasound scanning system that is compact, light weight and completely self contained. The device can be carried anywhere for diagnostic purposes. In addition, the scanner is provided with a transrectal linner transducer (7.5 MHZ) for endorectal scanning. Ultrasonic gel usually is used to cover short distance air interference, produce good quality images, and minimize image artifacts. A black and white video graphic printer (VP-890 MD, Soney® was used for printing the frozen image).

All experimental animals showed no estrous for more than 6 months postpartum. Complete gynaecological examination associated with ultrasonographic scanning 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 groups were then subdivided into 4 subgroups each of 10 buffalo-Cow. In addition to ultrasonographic scanning for all buffalo-cows under experiment, they receive the following treatment regime:

Subgroup I : Intramuscularly injected with 2ml. PGF2-Q..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. PGF2-0" plus 5mg oestradi-ol(Folone-5***) injected I.m. at the same time.

Subgroup III : received 2ml. PGF2-a. plus 2.5ml synthetic Gn- RH (Fertagyl"-****) after 5 days injected I.m.

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

Blood samples were collected from jugular vein immediately 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 estradiol, progesterone, calcium, inorganic phosphorus and magnesium.

Progesterone and estradiol 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 Ku-basik et. al., (1984) and Xing et. al., (1983) for progesterone and estradiol L7-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 Woot-ton (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).**

---

* Alliance Medical INC. 7800 côte-de-liesse.Japan.

** Coopers Animal Health limited (England).

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

**** intervet International B.V. (Holland).

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). In addition to rectal palpation, inactive ovary and persistence corpus luteum were also diagnosed ultrasonically. Implantation of ultrasonography in diagnosis of infertility problems at early stages is helpful and more accurate **(Griffin and Ginther, 1992)**, particulary if it is associated with sound breeding records, clinical examinations and hormonal estimations **(Douthwaite and Dobson, 2000 and Henzen et al., 2000)**. Inactive ovary in the present study (Image-1) was appeared ultrasonically as a mixed echotexture of hypo-and hyper-echoic signals. This, was agreed with the result obtained by **Metwally (2004**). Moreover, the persistent corpus luteum in the present study (Image-2) was appeared as an oval, granular and hypoechoic structure with or without cavities. These finding was agreed with the previous studies of **Edmondson et al. (1986) and Kahn and leidl (1987).** Also, in the present study pregnancy diagnosis has been demonstrated by a high resolution scanner between day 25 and 30 post mating (Image-3).This result was agreed with **Pierson and Ginther (1984).**

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 PGFa-o" was applied in combination with oestradiol in animals with palpable corpora lutea. Gonadotrophin releasing hormone (Gn-RH) had insignificant effect when was applied with PGFa-cc 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 are highly significant increase than those at the prepuberal period. The increase in serum progesterone level may be attributed to low L.H. level occured 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 FS-O" 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 PGFa-oc 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 PGF2α 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 PGF2α • In addition the rate of decrease found in case of treating animals with PGF2α. oestradiol was of insignificant- secrete estrogen to leuteal ones cant magnitude, that secrete progesterone.

Such conclusions concerning serum estradiol concentration in infertile buffalo-Cows came in accordance to those of **Hattab, (1988)** who found that the serum estrogen 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 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 $F_{2\alpha}$ 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 significant decrease in serum calcium level after Gn-RH (Receptal) treatment in infertile buffalo-Cows with inactive ovaries. Whereas, **Mikhail (1979)** recorded that the serum calcium level in buffaloes was decreased non significantly after Pro-lan adminstration. 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-cows having palpable corpora lutea. In-these animals prostaglandin-$F_{2\alpha}$ alone or prostaglandin $F_{2\alpha}$ plus oestradiol significantly ($P<0.01$) increased serum calcium level.

However Gn-RH if injected alone or with prostaglandin F2-cc 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 **Osman et. al., (1979).**

The obtained results confirmed by **Farrag (1978) and EI-Shawaf (1984)** who stated that the level of serum calcium in buffaloes and Cows during estrus 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 $F_{2\alpha}$.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 para-thormon and calcitonine may be the main cause of the changes found in calcium and inorganic phosphorus content in the blood serum. In addition prostaglandin FS-O" may be the main hormonal factor causing these changes.

It could be concluded that the administration of $PGF_{2\alpha}$ with oestradiol is the best treatment used in buffalo-Cows with palpable ovarian CL. However $PGF_{2\alpha}$ 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 recommend the physician and farmers to use these hormones which may be helpful in oestrus induction and ovulation, in postpartum suboestrus buffaloes, reducing inter calving period and improving their reproduction efficiency.

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

Parameters Hormones used	Progesterone ng/ml				Estradiol 17B (pg/ml)			
	Inactive ovary		PalpabI corpora Ituea		Inactive ovary		PalpabI corpora lutea	
	Before	After	Before	After	Before	After	Before	After
Prostaglandin pgf2α (Estrumate)	0.161 ± 006	1.512± 0.774"	0.291 ± 0.018	0.251 ± 0.030	149.± 25.81	120.97 ± 7.01'	263.9 ± 5.92	206.3 ± 46.79"
PGF2α + Oestradiol (Estrumate + Folone)	0.388 ± 0.015	0.299 ± 0.049	1.022± 0.426	0.114± 0.046"	131.1± 3.33	129.8± 2.33	132.9 ± 8.42	118.95 ± 2.33
PGF2α+Gn-RH (Estrumate + Fertagly)	0.395 ± 0.0058	0.395 ± 0.004	0.212 ± 0.028	0.045 ± 0.019	130.7± 3.36	123.3± 1.58	159.1 ± 14.38	127.0± 3.70'
Gn-RH (Fertagly)	0.33± •0.169	0.46± 0.039	0.33± 0.005	1.12± 0.079"	135.0±5 .69	145.7± 2.91	161±19 .90	115.13 2.89"

- P < 0.05 " P < 0.01

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

Hormones used	Calcium (mg/dl)				Inorganic phosphorus (mg/dl)				Magnesium (mg/dl)			
	Inactive ovary		Pa(pab) corpora iutea		Inactive ovary		Palpable corpora lutea		Inactive ovary		Palpable corpora lutea	
	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
Prostaglandin F2α Estrumate)	9.50 ± 0.06	9.68± 0.03	7.61 ± 0.26	11.58 ± 0.55"	5.66 ± 0.22	4..93± 0.32	6.63 ± 0.36	4.41 ± 0.28"	2.07 ± 0.06	1.47± 0,03"'	1.84± 0.11	2.12 ± 0.14
PGFa-a + Oestradiol Estrumate + Folone)	7.14 ± 1.14	7.25± 0.57	7.91 ± 0.25	10.54 ± 0.35"	4.32 ± 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'
PGF2α + Gn-RH (Estrumate + Fertagly)	10.22± 0.27	8.25± 0.24	11.18± 1.25	8.60 ± 0.40	5.31 ± 0.35	4.0 ± 0.33-	5.21 ± 0.31	4.35 ± 0.14-	3.68 ± 0.08	3.68 ± 0.08	3.46 ± •' 0.02	3.35 ± 0.25
Gn-RH (Fertagly)	11.15± 1.29	10.0± 1.41	10.10 ± 0.32	10.05± 1.25	4.71± 017	3.71 ± 0.18"	5.34 ± 0.25	6.11 ± 0.28	3.13 ± 0.13	3.13 ± 0.13	3.05 ± 0.08	2.88 ± 0.14

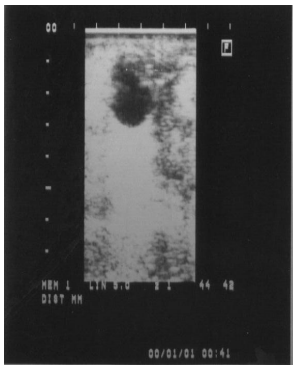

Image (1)

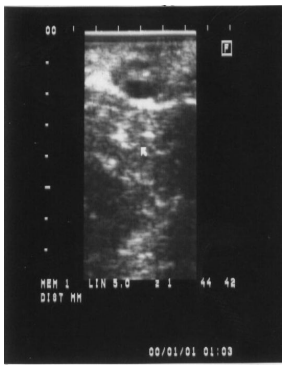

Image (2)

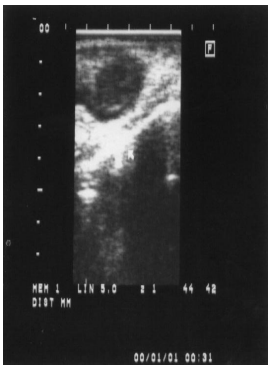

Image (3)

REFERENCES

Abd El-latifM. (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, inoragnic phosphorus, calcium and some other blood constituents in the blood of laying hens as affected by PG FS-CX 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.

Barr, (1974): Influences of estrus detection on days open in dairy herds. J. Dairy Sci., 58: 246-247.

Beckett, S. D. and Lean, I. J. (1997): Gonadotropin releasing hormone in postpartum dairy cattle: a meta-analysis of effects on reproductive performance. Anim. Reprod. Sci., 48: 93-112.

Benmard, M. and Stevenson, J. S. (1986): Gonadotropin releasing hormone and prostaglandin F2 for postpartum dairy cows: Oestrus, ovulation and fertility traits. J. Dairy Sci., 69: 800-811.

Britt, J. H. (1980): Use of gonadotropin releasing hormone and prostaglandin F2 in postpartum dairy cows. In Morrow, D.A. (ed), Current therapy of Theriogenology, W.B. Saunders Co., Philadelphia, pp. 292-296.

Chenault, J. R. ; Kratzer, D. D. ; Rezpkowski, R. A. and Goodwin, M. C. (1990): LH and FSH response of Holestein heifers to fertireline acetate, Gonadotropin and busereline. Theriogenology, 34: 81-98.

Douthwaite, R. and Dobson, H. (2000): Comparison of different methods of diagnosis of cystic ovarian disease in cattle and assessment of its treatment with progesterone releasing intra vaginal device. VET. Rec. 147 (130: 355-359.

Edmondson, A. J. ; Fissor, R. a. ; Pashon, R. L. and Bondurant, R.H. (1986): The use of ultrasonography for study of the bovine reproductive tract . I. Normal and pathological ovarian structures.

El-Desouky, A.M. (1993): Some trials for treatment of anestrum in buffaloes. M.V.Sc.Thesis, Fac. Vet. Med. Moshtohor. Zag. Univ. Benha branch.

El-Shawaf A. M. M (1984) : Some laboratory studies on infertile and sterile farm animals. Ph. D. Thesis, Vet. Med. Zag. Un

Farrag A. A. M. (1978) : Ovarian syndromes in cattle and buffaloes with special reference to certain infertility problems. Ph. D. Vet. Thesis, Assuit University.

Ganlal A. A. (1987) : Some pharmacological aspects of prosta-glandins with specical reference to their action on reproductive performance in female buffaloes. M. Vet. Sci. Thesis, Fac. Vet. Med. Cairo University.

Gordon, P. J. ; Peters, A. R. ; Ward, S. J. ; and Warren, M. J. (1996): The use of prostaglandin in combination with GnRH agonist in controlling the timing of ovulation in dairy cows. Reproduction, 24: 164-168.

Graham, E.F. (1966): Use of a dye indicator for testing, training and evaluation techniques in artificial insemination. Proc. First NAAB Tech. Conf. Ai Reprod. Pp. 57-60.

Griffin, P.G. and Ginther, O.J. (1992): Research application of ultrasonic imaging in reproductive biology. J. Anim. Sci., 70: 953-972.

Hattab S. A. (1988) : Hormonal profile during calfhood, perpuberal and puberal period and during ovarian atrophy (inactivity) in buffaloes. M.V. Sci. Thesis, Fac. Vet. Med. Alex. University.

Henzen, C.H.; Pieterse, M.; Scenczi, o. and drost, M. (2000): Relative accuracy of identification of ovarian structures in the cow by ultrasonography and palpation per rectum. Vet. Journal 159: 161-170.

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.

Hussein, F.M.; Elitus, B. E. ; Paccamonti, D. L. and Younis, M. Y. (1992): Effect of repeated injection of GnRh on reproductive parameters in postpartum anestrous dairy cows. Theriogenology, 37: 605-617.

Hussien, S A. (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.

Kahn, W. and Leidle W. (1987): Echographische Befunde an Ovaries von Stutent. Tierarztl. Umsch. 42: 257-266.

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. Saunder Comp., London.

Kamonpatana M., Van De Viet D. F. M., Koops Leenauruksa D., Negamsuriyaroj C. and Usana-kornkul S. (1979) : Oestrus control and early pregnancy diagnosis in the swamp buffalo: Comparison of EIA and RIA for plasma progesterone. Theriogynology 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 radio-immunoassay for progesterone. Clinical chemistry 30: 384.

Metwally M.A. (2004) : Some studies on postpartum ovarian rebound. M. V. Sc. Thesis, Fac. Vet. Med., Mansoura University.

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 in activity. J.Egypt. Vet. Med. Assoc. 14: 89.

Osman, (1984):

Pierson, R. A. and Ginther, O. J. (1984): Ultrasonography for detection of pregnancy and study of embryonic development in heifers. Theriogenology 22 (2): 225-233.

Pratt B. R., Butcler R. L. and Inskeep E. K (1977) : **Antiluteo-lytic effect of the conceptus and of PGF2-cx in ewes. J.Anim. Sci. 45:784.**

Rahe C. H., Owens R. E., Fleeger 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 hendran R., Sivaclel-van M. N. and Sivakanesan R. R. (1983) : Hormonal, Biochemical, physial. Behavioural and Histolo-gial hanges following PGFa-cc 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.

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 Cochron W. G. (1967) : In statistical methods 6th. Edition, Lowa State University, Press, Lowa, 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.

Some laboratory studies on infertile and sterile farm animals. Ph. D. Thesis, Vet. Med. Zagazig University.

Stacy B. D., Gemmell R. T. and Thorbum G. D. (1976) : Morphology of the corpus luteum in the Sheep during regression induced by prostaglandin F2-a. Biol. Reprod. 14:280.

Thatcher, W.W.; Drost, M. ; Savio, J.D.; Macmillan, K. L.; Entwhistle, K.W.; Scmitt, E. J. ; De la Sota, R. L.; Hanson, P.J. and Drost, M. (1989): New clinical uses of GnRh and its analogues in cattle. Theriogenology, 31: 149-164.

Theodoulou G., Mayobaulo D. S. and Mountokniakis T. H. (1977) : Calcium infusion induced hypercalcemia before and after in-domethacin adminstration in man. Hippocrates (ATHENS) 32: 85.

Willis J. B. (1960) : The determination of metals in blood serum by atomic absorption spectropho-tometery. Spector Chem. Acta 16:259.

Xing S., Sekan C. Z. and Dicz-falusy U. (1983) : Validation ofra-dio-immuno assay for estradiol 17-B by isotope dilution mass spectrometry and by a test of radi-ochemical 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.