Response of *Fasciola* free and infected buffaloes to CIDR OvSynch treatment during summer season with emphasis on sex hormone and biochemical changes

Hammam A.M. [1]; Rabab M. El Khateeb[2]; Hany A. Amer[1]; Sanaa K.A. Abou-El-Dobal[2] ; Khalied H. El Shahat[3] and Scott W. [4]

[1]Animal Reproduction and A.I. Department, National Research Centre, Dokki, Giza, Egypt
[2]Parasitology and Animal Diseases Department, National Research Centre, Dokki, Giza, Egypt
[3]Department of Obstetrics, Gynecology and A.I., Faculty of Veterinary Medicine, Cairo University, Egypt
[4]Animal Science Depart. College of Agriculture and Life Sciences, North Carolina State University, USA.
hammam56@yahoo.com

Abstract: Improvement of buffalo fertility during summer season was our goal. One hundred and sixty three buffalo-cows were examined for parasitic infection using coprological and serological methods. All animals were subjected to gynecological examination, through rectal palpation and using ultra sonic examination to detect the ovarian and genital tract condition. Thirty one non-pregnant buffalo-cows (18 healthy and 13 infected) were selected for treatment with CIDR OvSynch protocol. Blood samples were collected from animals before, during and after treatments. Serum samples were assayed for estradiol and progesterone using RIA technique. GPT,GOT, ALP, total and direct bilirubin, T. protein and glucose were measured . The percentage of infected buffaloes in the herd was 25.77% (42/163 animal), the prevalence of *Fasciola Spp.* infection among buffaloes was 6.75%. In *Fasciola* infected buffaloes, estradiol levels were decreased and progesterone concentration was increased significantly ($p<0.05$). Treatment with CIDR OvSynch protocol, elevated significantly both estradiol and progesterone levels in infected animals than healthy one, elevated direct bilirubin and total protein and decreased significantly ALP and glucose in infected animals. CIDR OvSynch regimen increased the pregnancy rate in both healthy (55.6%) and infected (30.8%) buffaloes. It is concluded that infection with *Fasciola* had adverse effects on some sex hormone and liver enzymes imbalance and animal fertility represented in decreasing response to synchronizing agents and lowering pregnancy rate.Treatment with CIDR OvSynch protocol improved buffalo fertility and resumed ovarian activity of buffaloes during summer season.

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1. Introduction:

Estrous synchronization and A.I. are tools that enhance reproductive management in cattle and buffaloes and allows for more cows to become pregnant early in the breeding season. Moreover estrous synchronization improves uniformity of a calf crop **(Dziuk and Bellows, 1983).** Recently, it is important that effective estrous synchronization protocols are developed in order to increase the use of A.I. In addition, estrous synchronization protocols should be designed to reduce time and labor inputs by limiting cattle handlings and reducing or eliminating estrus detection **(Larson *et al.,* 2006).** The application of Al is made difficult in buffaloes undergoing spontaneous estrus and ovulation due to the relatively low expression of estrous behavior, variable duration of estrus from 4 to 64 h, and difficulty in predicting the time of ovulation **(Ohashi, 1994; Seren *et al.,* 1995).** Moreover**,** there is one reason for variable responses between cattle and buffalo to estrous synchronization protocols could be that buffalo cows have a higher degree of variability in the interval from the pre ovulatory LH surge to ovulation than bovine cows in both naturally and hormonally induced ovulations **(Barkawi *et al.,* 1993**; **De Rensis and Lopez, 2007)**. One of the most prominent reasons for decreasing fertility rate in buffaloes is the heat stress and parasitic infestation. Buffaloes such are suffering from parasitic infestation which causes high economic losses. The economic losses consisted of costs of anthelmintics, drenches, labor, and losses in production due to mortality, reduction in meat, milk and wool production, reduction in growth rate, fertility and draught power **(Mendes *et al.,* 2008).** *Fasciola Spp.* could affect the reproductive performance of farm animals through impaired growth rate of young stocks, increased puberty age of heifers and prolonged estrus intervals in mature animals **(Ahmed *et al.,* 2006).** It was found that

58.4% of repeat breeder cows were seropositive to *F. hepatica* **(Simsek *et al.*, 2007).**

The main purpose of this study was to improve the reproductive performance of buffalo-cows during summer season and resume ovarian cyclicity through administration of synchronizing hormones (CIDR plus OvSynch protocol)in healthy and Fasciola infected buffaloes and to study the changes in some sex hormones and some biochemical parameters of animal sera.

2. Material and Methods

1-Animals:

The study was carried on 163 buffalo-cows maintained at private sectors and farms , Beni Suef Governorate, Upper Egypt. The experimental animals included heifers (1.8 -2 years), primiparous and multiparous buffalo-cows (3-8 years old) and were reared under a correct management system including feeding, housing, recording system and veterinary medical care. The study was carried out during summer season (June-September).

2-Experimental Design:

All animals were examined for parasitic infection via fecal and serological examination. Further selection of control and infected animals had been performed. Then, animals were subjected to gynecological examination, through rectal palpation and using ultrasonic examination to detect the ovarian and genital tract condition. Only non-pregnant buffalo-cows were used for stimulation of ovarian activity and5 synchronization of estrus using CIDR plus OvSynch protocol.

Detection of ovarian activity and pregnancy in buffalo-cows:

All animals were subjected to gynecological examination through rectal palpation and using ultrasonic examination(An endorectal linear array 6-8MHz transducer -Scanner 240, Pie Medical, the Netherlands), to detect the ovarian and genital tract condition.

Synchronization of estrus:

Thirty one non-pregnant buffalo-cows were used for estrus synchronization using CIDR-OvSynch protocol (GPG) according to **Bicalho *et al.* (2007).**

Summary of the experimental procedure is shown in Diagram (1): At day (0) buffaloes examined clinically per rectum, blood sampling and injected intramuscularly with 2 ml Estrumate® (PGF2α, synthetic prostaglandin), each ml contains 263 µg cloprostenol sodium BP-vet.- equivalent to 250 µg cloprostenol (Schering Plough, Essex Animal Health, Germany); at day 7, CIDR®(EAZI-BREEDTM, contain progesterone, 1.38 grams per EAZI-BREED CIDR cattle insert, Pharmacia & Upjohn Company Kalamazoo, Michigan 49001, USA) was inserted in the vagina(remained in the vagina for 10 days) and injected in the same day with 2 ml Receptal®(gonadotropin releasing hormone –GnRH-, each ml contains 0.0042 mg buserelin acetate equivalent to 0.004 mg buserelin, 10mg benzyl alcohol (Intervet International B.V. Boxmeer, Holland) ; at day 17 CIDR was removed, with injection of second dose of Estrumate® ($PGF_2α$), and at day 19, 2.5 ml Receptal® (GnRH) was injected I.M., followed by A.I. after 24 hours post GnRH injection.

Pregnancy was diagnosed by rectal palpation at 45- 60 days or 25 days by sonar, post A.I. for the inseminated buffaloes.

Blood sampling at days 0, 2, 7, 10, 11, 14, 16, 19, 21, 28, 35 and 45 from treatment initiation for measuring some hormonal (Estradiol and Progesterone) and some biochemical parameters such as GPT, GOT, ALP, total and direct bilirubin, total protein and glucose.

Diagnosis of the parasite:

a- Coprological diagnosis: Faecal samples were collected and examined for parasites by both Fluke finder technique **(Welch *et al.*, 1987)** and Concentration flotation technique **(Soulsby, 1982)**.

b- Serological diagnosis: Blood samples were obtained and sera were separated and kept under -20^{0}C until used for assay. The Excretory/Secretory (ES) antigen products were prepared according to **River-Marrero *et al.* (1988).** Then, the protein content of different antigenic extracts was measured using modified Lowry's method **(Lowry *et al.*, 1951)**. Finally, the Enzyme Linked Immunosorbent Assay (ELISA) was carried out as described by **(Oldham, 1983).**

Hormonal Assay:

Blood samples were collected from all buffalo-cows (Fasciola infected and free) before, during and post treatments (up to 45 days). Sera were separated and used for hormonal tests. The concentrations of Estradiol, Coat-A-Count Estradiol ($PITKE_2$-8), and Progesterone, Coat-A-Count Progesterone (PITKPG-7), were determined by Radio-Immunoassay kits obtained from Siemens Medical Solutions Diagnosis, USA according to **Batzer (1980)** and **Bauman (1981)**, respectively and read by γ-Counter.

Biochemical Tests:

The concentrations of serum GPT(AST) & GOT(ALT), Bilirubin (Total & Direct), Protein (Biuret Method), and Glucose were determined according to **Reitman and Frankel (1957), Walter and Gerade (1970), Gornal *et al.* (1949) & Trinder (1969)** respectively by colorimetric methods using reagent kits purchased from Biodiagnostic Co., Giza, Egypt and measured by spectrophotometer.

The concentration of Alkaline Phosphatase was measured using reagent kit obtained from VitroScient Co., Hannover, Germany according to **Belfield and Goldberg (1971)**.

Statistical analysis:

Data of different buffalo groups were analyzed for the means and standard deviations. Significance of the results was evaluated using Independent sample t-test, Analysis of variance (ANOVA) and Duncan using **Statistical Package for Social Science (SPSS)** computer programs **(2002).**

3. Results

Parasitological examination

A-Coprological examination of all buffalo-cows in the farm:

Fecal samples were collected from 163 female buffaloes having two different age groups, heifers (n=31) and multiparous buffaloes (n=132), and examined for internal parasites. The obtained results revealed that the total number of infected buffaloes in the herd was 42 animals (25.77%). As shown in table (1), the prevalence of parasitic infection was 6.75%, 4.29%, 4.91% and 9.82% for *Fasciola*, *Giardia, Cryptosporidia* and *Eimeria Spp.,* respectively.

The percentage of infection was 22.58% in younger animals (heifers) while, it was 26.5% in multiparous animals. In heifers, the percentage of infection was 6.45% and 16.13% for *Fasciola* and *Eimeria Spp.,* respectively. On the other hand, the percentage of infection in multiparous animals was 6.82%, 5.3%, 6.06% and 8.33% for *Fasciola, Giardia, Cryptosporidia* and *Eimeria Spp.,* respectively (Table 1).

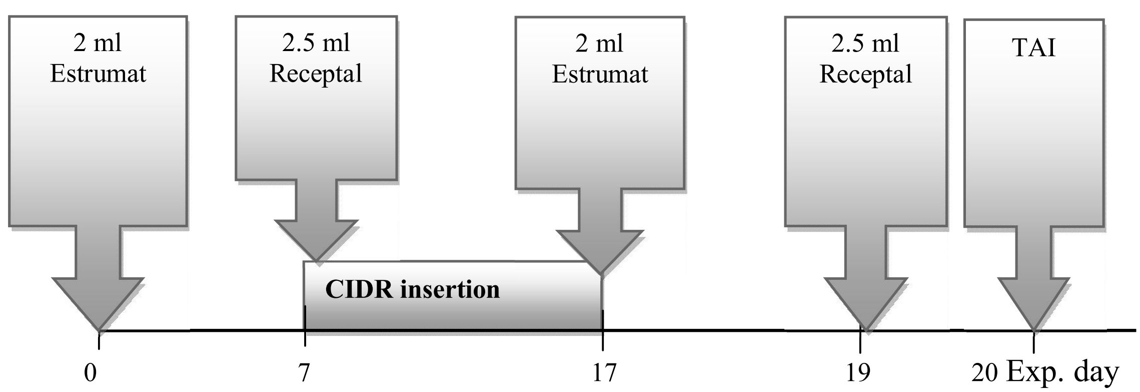


Diagram (1): CIDR OvSynch protocol in buffaloes

Table (1): Coprological examination of all buffalo-cows in the farm

Parasite	**Infected Buffaloes**		
	Heifers	**Multiparous**	**Total**
Fasciola Spp.	2 (6.45%)	9 (6.82%)	11(6.75%)
Giardia Spp.	0	7 (5.3%)	7 (4.29%)
Cryptosporidium Spp.	0	8 (6.06%)	8 (4.91%)
Eimeria Spp.	5 (16.13%)	11 (8.33%)	16 (9.82%)
Total No. of infected animals	7	35	42
Total No. of examined animals	31	132	163
Percentage of infection	22.58%	26.5%	25.77%

Table (2): Comparison between fecal examination and ELISA technique for the detection of *Fasciola* infection

Animals	Fecal examination	ELISA technique
Total number of examined animals	163	163
Number of *Fasciola* infected animals	11	23
Percentage of infection	6.75 %	14.11%

B-Percentage of Fasciola infection using two different diagnostic methods:

As shown in table (2), the total number of *Fasciola* infected buffalo-cows examined by fecal analysis was 11 animals (6.75%) whereas; the incidence of infection had increased to 23 animals (14.11%) using ELISA method.

Hormonal patterns:

Effect of OvSynch plus CIDR protocol on Estradiol (E_2) (pg/ml) and Progesterone (P4) (ng/ml) levels in healthy and *Fasciola* infected buffalo-cows serum:

In the present study, the experiments were carried out during summer season (heat stress season). All animals were randomly assigned for treatment with CIDR OvSynch protocol. In this regimen, buffalo-cows were treated with prostaglandin $F_2\alpha$ ($PGF_2\alpha$) at day (0), 7 days later CIDR(progesterone releasing device) was inserted and remained in the vagina for 10 days. GnRH was given in two doses the 1st dose at the day of CIDR insertion, the second dose 48hrs post CIDR removal. The pattern of estradiol and progesterone levels and peaks greatly varied among and between animals. Injection of $PGF_2\alpha$ (day 0) resulted in sudden decline in elevated P_4 level (luteal phase) as seen in figures (1-4). Progesterone started to elevate significantly 4 days after $PGF_2\alpha$ injection, then declined but above normal value and started to elevate post CIDR insertion in buffaloes numbers (1057); (1087) and (1125), while in animal No. (987) the level was fluctuated. A second peak of P_4(Figs. 1-4) was recorded after 25 days(Figs. 1&2), or after 35 days (Figs 3&4) post initiation of treatments. These animals conceived and became pregnant after treatments.

With respect to the second group of buffalo-cows (Figs. 5-8) in which animals received the same regimen of treatment, but not conceived 45 days post insemination, showed a decline in P_4 level at days 28(Figs 7&8),or at day 35(Fig. 6), while it remained elevated at day 30 (Fig.5) .

Estradiol peak and its amplitude varied from animal to another depending on P_4 level in a reverse relationship, except in animal No. 1050(Fig. 6).

Buffalo-cows infected with fasciola(Figs. 9&10), showed two peaks of P_4 , while estradiol was elevated post CIDR removal and injection of 2nd dose of GnRH.

The levels and peaks of E2 and P4 not differ significantly between animals that conceived or not conceived after treatment in infected buffaloes, only drop in P_4 level at day 2(Figs. 12&13), or 25 days (Fig. 11).

The overall mean of hormonal levels in fasciola free and infected buffalo-cows are summarized in table (3) and figure (14).

Estradiol: The estradiol level in the serum of *Fasciola* free buffaloes and treated with CIDR-OvSynch regimen, varied from animal to another and day to day according to the exogenous hormonal treatments and to the stage of estrus cycle at the onset of treatment. It averaged 39.61±8.23 pg/ml at the onset of $PGF_2\alpha$ injection and gradually increased to reach the peak (62.79±32.59 pg/ml) at day 7, then fluctuated along the estimation period (35 days) with a mean of 38.76±3.96 pg/ml (Table 3 and Fig. 14). While in buffalo-cows infected with *Fasciola*, it averaged 28.42±8.64 pg/ml at the onset of $PGF_2\alpha$ injection, and reach a peak (106.22±37.68 pg/ml)16 days post treatment with a mean of 73.05±9.63 pg/ml, which was differ significantly ($p<0.05$) than that in healthy treated buffaloes.

Progesterone:

The concentration of serum progesterone was averaged 3.05±1.29 and 3.95±0.93 ng/ml in healthy and *Fasciola* infected animals, respectively. Then declined to reach lowest value after 2 days in healthy animals (0.43±0.09 ng/ml) and after 7 days (0.77±0.05 ng/ml) in infected animals. Another peak of P_4 was observed at day 11 from beginning of treatments, it averaged 3.9±1.04 and 6.89±2.13 ng/ml in healthy and infected treated animals, respectively. The total average P_4 level was significantly elevated ($p<0.05$) in infected than in healthy buffaloes. It averaged 2.54+0.58 in healthy and 4.84±0.93 ng/ml in *Fasciola* infected buffaloes.

Some biochemical parameters:

-GPT(AST) levels: did not differ significantly between healthy and infected buffaloes, it averaged 40.41±2.04 and 41.39±3.05 u/ml, respectively; while GOT levels, were averaged 62.52±2.53 and 61.42 u/ml in the serum of healthy and infected buffaloes, respectively.

-Alkaline phosphatase(ALP), was significantly ($p<0.05$)decreased in infected buffaloes (117.86±11.18 u/ml) than healthy group (149.08±5.33).

-Total bilirubin, was elevated non-significantly in infected (1.6+0.1 mg/dl) than in healthy buffaloes (1.52+0.54mg/dl).
-Direct bilirubin was significantly ($P<0.05$) elevated in infected (2.98+0.17 mg/dl) than in healthy buffaloes (2.46±0.09mg/dl), while total bilirubin did not significantly differ between healthy and infected buffaloes.
Total protein was elevated non-significantly in infected (6.73±0.24 g/dl) than healthy cows (6.16±0.15g/dl).
Glucose: Decreased non-significantly ($p<0.05$) in infected (44.03±4.3 mg/dl) than healthy buffaloes (48.29±5.08 mg/dl).

Effect of CIDR OvSynch protocol on pregnancy rate:

As shown in table (5), the pregnancy rate in healthy treated buffaloes was 55.6%, whereas, the pregnancy rate obtained in case of *Fasciola* infected buffaloes was 30.8%.

4. Discussion

Modern estrus synchronization protocols involve either lengthening or shortening the animal's estrous cycle to achieve synchrony. A variety of techniques are available for producers to utilize and all are based on several strategies of hormonal supplementation including progestin, $PGF_2\alpha$ and gonadotropins **(Odde and Holland, 1994; Ryan, *et al.*, 1995).**

The results of the current study in buffaloes subjected to CIDR-Ovsynch protocol revealed to significant differences in progesterone level among different days of treatment either in individual animals or overall means of healthy and infected animals. The overall means of P_4 at day(0) averaged 3.95+0.93 ng/ml, then declined sharply 48 hrs post 1st $PGF_2\alpha$ injection to reach the lowest value (0.43+0.09 ng/ml), these results were partially in accordance with **Vijay *et al.* (2002)** who indicated that, the mean serum progesterone concentration in buffaloes subjected to Ovsynch were 2.70±0.18ng/ml at (0)h but the concentration were decreased ($p<0.05$) by (4)h post $PGF_2\alpha$ injection and they were 0.068±0.06ng/ml at (18)h. These findings may be attributed to incomplete luteolysis of CL, due to response of luteal tissues to drug, type of prostaglandin used or other human and chemical factors as reported by **Twagiramungu *et al.* (1995)** who indicated that in some cows subjected to Ovsynch, estrus is blocked due to incomplete luteolysis and the selected dominant follicle becomes persistent. Also results come in agreement with **Skarzynski *et al.* (2009)** who concluded that, pharmacological manipulation of the estrous cycle using a $PGF_2\alpha$ may cause lower progesterone secretion and inhibited CL sensitivity to luteotropic factors in cattle.

Serum progesterone concentration at days (23) and (35) after initiation of regimen (1st $PGF_2\alpha$ injection) showed significant elevation ($p<0.05$) particularly in pregnant buffaloes. Our data agree with **Gianluca *et al.* (2003)** who found that, progesterone level were elevated 10 days after AI in 81.1% of buffaloes treated with Ovsynch. Whereas, in some individual animals P_4 was decreased specially in animals not conceived after treatments, these findings may be attributed to low response of animals to the treatment because the protocol was applied during non breeding season (heat stress season), these results can be explained in the light of published reports of **Razdan *et al.*(1981); Rao and Pandey (1983); Kaur and Arora (1994)** they indicated that, from an endocrinological perspective, summer anoestrus in buffalo is characterized by low plasma circulating concentrations of pituitary and gonadal hormones .

The results of the present study in healthy and infected buffaloes, treated with CIDR plus OvSynch (GPG) protocol pointed to a high significant($p<0.05$) progesterone level after CIDR insertion compared to its level before insertion in buffaloes, these findings come in agreement with **Chenault *et al.* (2003)** who mentioned that the progesterone released from the CIDR inserted was sufficient to increase and maintain a progesterone concentration in blood high than 2.0 ng/ml in the absence of CL on the ovary .We can attribute this elevation to releasing of exogenous progesterone from CIDR to circulation and decreased after removal of CIDR and injection of $PGF_2\alpha$. These data are parallel to that achieved by **Perry *et al.* (2004) and Lamb *et al.* (2006)**, they indicated that Progesterone concentrations were shown to be rapidly increase blood concentrations peak within 1h after CIDR insertion and decrease rapidly to 0 from 12 to 24 h once the CIDR is removed.

The total means of progesterone level in all animals at three weeks after AI were 4.48±1.03 & 8.37±3.48 ng/ml in healthy and infected buffaloes, respectively, the former results were nearly similar to those recorded by **Han *et al.* (2006)** who found that at 15 to 32 days after AI (based on pregnancy status of dairy cows) was consistently higher in pregnant (> 4 ng/ml) than non-pregnant cows.

Concerning the exogenous sex hormones pattern in buffaloes infected with *Fasciola*, this study showed that there was a non-significant decrease in estradiol concentrations in the infected(28.42+8.64 pg/ml) than the healthy group(39.61+18.23 pg/ml). On the other hand, progesterone concentrations were increased significantly at P<0.05 in the infected (3.95+0.93) buffalo-cows than the healthy ones(3.05+1.29 ng/ml) before treatments. These findings matched with those of **El-Khadrawy *et al.* (2008)** who also measured lower levels of estradiol and higher levels of progesterone in infected than healthy animals. While after treatments with CIDR OvSynch regimen both estradiol and progesterone was significantly elevated in *fasciola* infected buffaloes than healthy group , this may attributed to the effect of fasciolosis on some liver enzymes that may inhibit degradation or metabolism of steroid hormones in the liver and tissues.

The results of the present study revealed that pregnancy rate in buffalos was 55.6%, these finding to some extent in agreement with data obtained by **Busch *et al.* (2007)** who recorded that pregnancy rate after CIDR protocol were significantly greater (62%) compared to other protocols, these results can be explained in the light reports **De Rensis *et al.*(2005),** who observed a high significant difference in conception rate when progesterone was used with the Ovsynch protocol in cyclic buffaloes. It is likely that the addition of progesterone to the Ovsynch protocol may be affected by a number of variables such as age, post-partum interval and ovarian follicle development.

The percentage of parasitic infection among all animals in the examined herd was 22.58% in younger animals (heifers) while, it was 26.5% in multiparous animals. The percentage of *Fasciola* infection was 6.45% in heifers and 6.82% in multiparous animals. These results agreed with **Ghirmire & Karki (1996) and Marques & Scroferneker (2003)** they noticed that a higher infection rate was recorded in older buffaloes than in younger ones. Also, **Molina *et al*. (2005)** found that the highest prevalence was observed in cattle and buffaloes more than 6 years of age, followed by those aged more than 3-6 years and then, the lowest prevalence was in animals aged 3 months-3 years.

It was of interest to clear that ELISA technique detected 12 buffalo-cows showing positive titers against *Fasciola gigantica* ES antigen from coprologically negative animals. Prevalence of infection in buffalo-cows examined by fecal analysis was 6.75% while, the incidence of infection had increased to 14.11% using ELISA method. This finding coincided with those of **Munguía-Xóchihua *et al.* (2007)** who detected a prevalence of 11.4 % using the sedimentation test and 24.4% for the indirect ELISA in bovines. Also, **Ferre *et al.* (1995)** detected the mean prevalence as determined by ELISA as 77.6% and as 23.7% by coprological examination. The lower prevalence detected by fecal analysis might be due to the length of the life cycle of *Fasciola Spp.,* which made eggs not to be detected in the faeces until 10 to 21 weeks post-infection after the immature fluke had reached the bile ducts, matured and reproduced **(Almazán *et al.,* 2001).** This condition could be explained that ELISA could detect antibodies to E/S products as early as 2 weeks post infection **(El-Ridi *et al.,* 2007).** ELISA was allowed for early detection of fasciolosis in animal herds and their owners so that humans and livestock could be treated prior to the development of liver pathology, thus minimizing morbidity due to this disease **(Kumar *et al.,* 2008).**

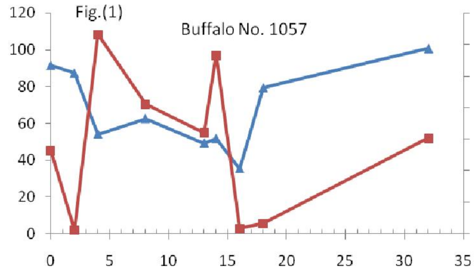
With respect to the relation between *Fasciola* infection and fertility of animals, the results revealed that the infection with fasciolosis disturbe the hormonal balance and liver enzymes (GPT, GOT and ALP) and some biochemical parameters which reflected on the response of buffaloes to synchronizing agents and decreased significantly the pregnancy rate compared with healthy animals. Other investigators reported prolonged anoestrus period in *Fasciola* infected mature animals **(Ahmed, 2006),** cessation of ovarian function (**Ahmed *et al.,* 2006**). Also, reported a following parasitic infection and reduce the lifetime reproductive and productive efficiency **(El-Wishy, 2007)**.

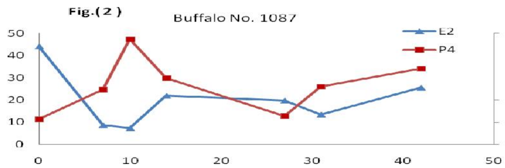
The pregnancy rate in the healthy treated animals (55.6 %) was decreased to (30.8 %) in the presence of *Fasciola* infection. These results indicated that the use of GPG (OvSynch) plus CIDR protocol improved the reproductive efficiency in the tested buffalo-cows. For the same reason, these treatments were tested by **Shah *et al.* (1990),** Rastegarnia ***et al.* (2004) and Stevenson *et al.* (2007)**. The difference in the resulted pregnancy rates between healthy and infected buffalo-cows might be due to the disturbance in sex hormones and biochemical parameters which in turn impaired fertility and produced a lower effect of the injected exogenous hormones.

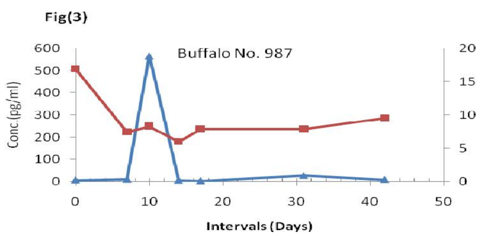
In the present work, GPT (ALT) and GOT(AST) concentrations were raised non-significantly in infected than the healthy animals while, ALP concentrations were decreased significantly (P<0.05) in the infected than the healthy ones. These results agreed with **Gonzalo-Orden *et al.* (2003)** found that AST activities did not significantly differ from the baseline after 15 and 12 weeks; and contradict with **Shaikh *et al.* (2007)**, **Pal and Dasgupta (2006), Değer *et al.* (2008), Molina *et al.* (2008) and Hutchinson *et al.* (2009)** who found that those enzymes were significantly increased in infected buffaloes and cattle.

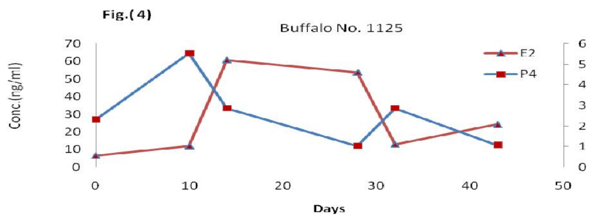
I-Concentration of Estradiol(pg/ml) and Progesterone(ng/ml) in the serum of buffalo-cows treated with CIDR plus OvSynch protocol for induction of ovulation.

1-Animals responded to the treatments and became pregnant after treatments (Figs. 1-4).

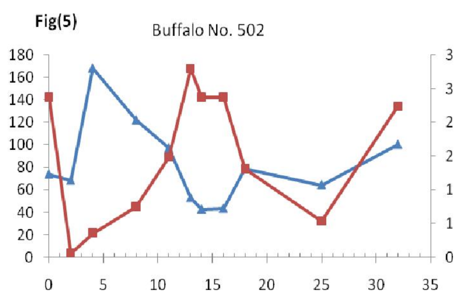


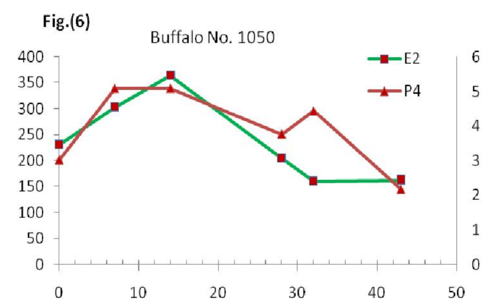


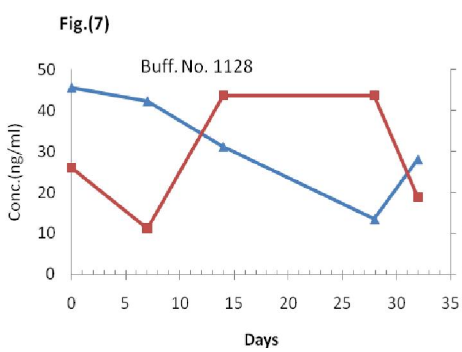


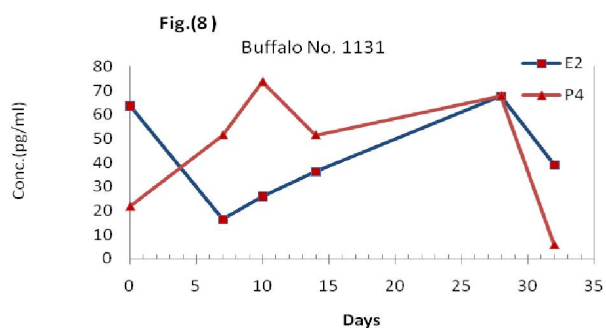


2-Animals responded to treatments(Exhibited estrus) and not conceived after treatments (Figs. 5-8)



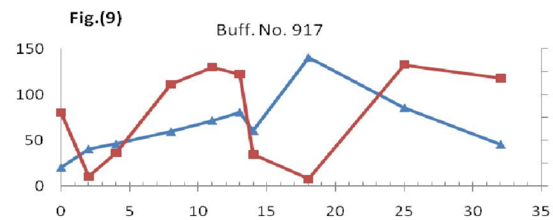


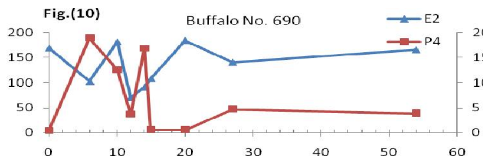




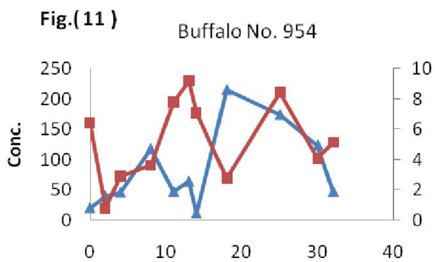
II-Concentration of Estradiol(pg/ml) and Progesterone(ng/ml) in the serum of buffalo-cows infected with *Fasciola* and treated with CIDR plus OvSynch protocol for induction of ovulation.

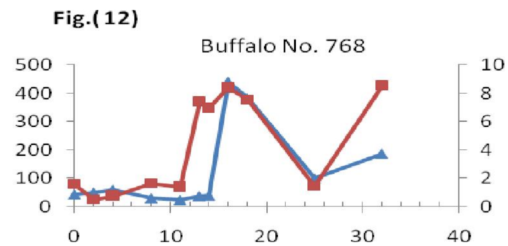
1- Buffalo-cows became pregnant after treatments (Figs. 9-10).

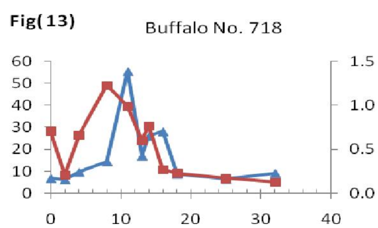




B-Animals responded to treatments(Exhibited estrus) and not conceived (Figs. 11-13).

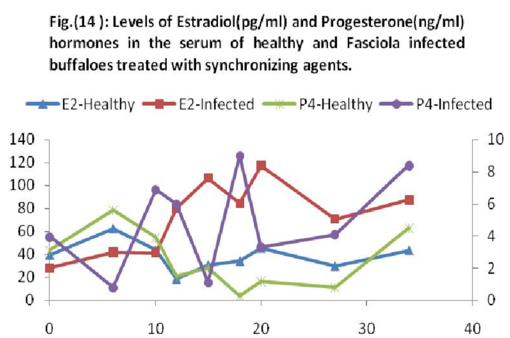


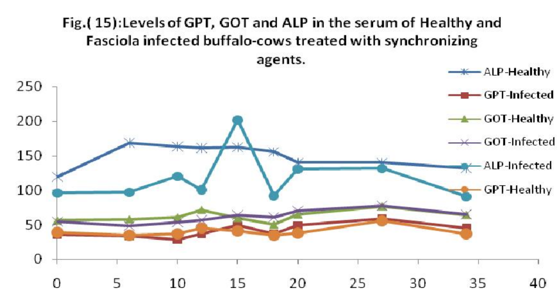


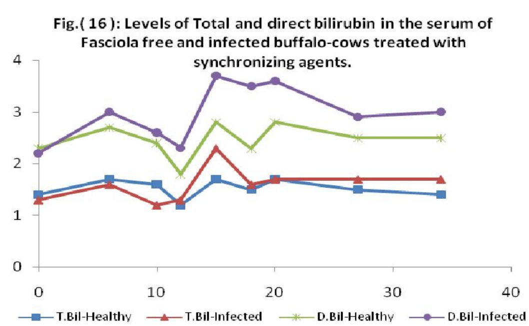


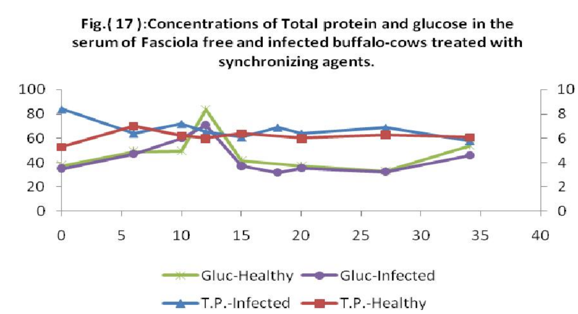
Intervals (Days)

III-Hormonal and biochemical changes in *Fasciola* free and infected buffalo-cows treated with synchronizing agents.









Ferre *et al.* (1995b) reported a significant elevation from weeks 6 to 14 in serum AST activities of experimentally infected sheep. While, **Bulgin *et al.* (1984)** reported that ALP concentrations were not significantly different between the control and infected calves. Increases in AST concentrations in blood serum had been associated with the migratory phase of infection and resultant parenchymal damage (**Wyckoff and Bradley, 1985 and Yang *et al.,* 1998)** or related to cellular tissue damage, such as skeletal tissue and cardiac muscle, possibly induced by handling, indicating a lack of liver specificity and a drawback for analysis of liver trauma **(Anderson *et al.,* 1977 and Wyckoff and Bradley, 1985)**. Changes in the antioxidant abilities of the liver and in the phospholipid structure of the cell membrane were accompanied by rising activities of ALT and AST as markers of liver damage **Değer *et al.* (2008)**. Serum enzyme concentrations and/or activity might be increased in response to liver trauma.

Both total and direct bilirubin were increased significantly ($P<0.05$) in the infected group compared to the healthy one. Similar results were obtained by **Kiladze *et al.* (2000), Sherwood (2001)**, **Pal and Dasgupta (2006) and Molina *et al.* (2008)** in ruminants. Also, this elevation in bilirubin concentrations was reported by **Lopez *et al.* (1994)** in rats and by **Ferre *et al.* (1995 b) and Mekroud *et al.* (2007)** in infected sheep. **Dalton (1999)** stated that increased bilirubin and globulin concentrations and decreased albumin concentrations were the common signs of chronic fasciolosis. However, minor differences between infected and non-infected calves for bilirubin concentrations were reported by **Wyckoff and Bradley (1985)**. High bilirubin excretion were maintained when the parasite migrated into the biliary ducts causing a cholestatic phenomenon responsible for changes in serum bilirubin levels. With the obstruction of the bile ducts, the yellow bile pigment was produced as the byproduct of degenerating haem groups in the RBCs.

There were significant increases in the total protein ($P<0.05$) concentration in the infected than the healthy animals. The results agreed with **Dalton (1999)**, **Pal and Dasgupta (2006), Shaikh *et al.* (2006), Shaikh *et al.* (2007) and Molina *et al.* (2008)** who stated that the estimated total protein in *Fasciola* infected buffaloes and cows were found significantly higher as compared to their control samples. On the other hand, **Wyckoff and Bradley (1985)** reported that there were minor differences between infected and non-infected calves for albumin and concentrations. Such a high level of protein content in the infected liver of buffaloes might be attributed to the marked fibrotic reactions. These changes in protein concentrations might be due to the increased production and secretion of some protein from hepatocytes which was called acute phase response or elevated after the damage of liver parenchyma.

A high significant decrease in glucose concentrations was found in the control infected than the control healthy animals ($P<0.05$). This result made an agreement with **Sheikh *et al.* (2006)** who reported a significantly low serum glucose concentration in *Fasciola* positive cattle.

Our findings confirm the important of stimulation of buffalo ovaries during summer season to resume ovarian activity and to overcome the inhibitory action of heat stress on reproductive system with role of progesterone (CIDR) in priming the follicle to respond to the Ovsynch protocol and that progesterone supplementation to the Ovsynch protocol stimulates ovarian activity in non-cyclic animals. Also, deleterious effect of fasciolosis on hormonal and biochemical imbalance of buffaloes, and the importance for treatment from fasciolosis before application of fertility programs.

Coresponding author
A. M. Hammam
Department of Animal Reproduction & A.I., Veterinary Research Division, National Research Centre, Dokki, Egypt
Hammam56@yahoo.com

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