Effect of Exogenous Progesterone in Treatment of Ovarian Inactivity in the Egyptian Dairy Parturient Buffalo-Cows

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ABSTRACT:

Inactive or nonfunctional ovaries are one of the most important reported causes of the prolonged postpartum anestrus that resulted to the delayed onset of estrus and inefficient breeding in buffaloes.

The suggested study of progesterone in the blood serum of buffalo could be potentially useful as far as the hormone content that might reflect the postpartum condition in the buffalo-cows.

This study aimed to evaluate the effect of exogenous progesterone in treatment of ovarian inactivity in the Egyptian dairy parturient buffalo-cows. This study was carried out on parturient buffalo-cows came to veterinary clinic and owned by small holders. These buffalo-cows ages ranged between 2-5 years. The animals were divided into 4 groups (28 animals). First group (7 buffalo-cows) as control group without any treatment. The second group (5 buffalo-cows) to study the effect of Progesterone (prontogest®) + PGF2α treatment trial. Third group (7 buffalo-cows) to study the effect of CIDR treatment trial. Forth group (9 buffalo-cows) to study the effect of CIDR+GPG protocol as a treatment trial. Progesterone level was measured before treatment (for diagnosis) and after treatment (to detect the response of treatment).

The results indicated that using CIDR+GPG gave good results for the treatment of ovarian inactivity in buffalo-cows, where increased the incidence of estrus and improved the reproductive parameters in dairy parturient buffalo-cows.

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1. INTRODUCTION:

Buffalo-cows are defined as poor breeder due to long days open after parturition and consequently long calving interval, a problem that reduce its reproduction and production. The River or Mediterranean type of buffalo is concentrated around the Nile delta.

The onset of estrus and regular cyclicity of estrus cycle and initiation of ovulation during the post-calving period in parturient buffaloes constituted a major problem and resulting into long postpartum anestrus and delayed breeding with consequent and serious economic losses in the milk production and in efficient reproduction. The problem received attention of many research works in countries where buffaloes raised. Results and reports of many authors revealed that the most important cause of absence or delay in the post-calving estrous cyclicity is the predominant incidence of ovarian inactivity or non-functional ovaries.

Diagnosis of this syndrome (due to a multiple etiology) was accomplished by transrectal palpation (the old and inexpensive technique), or by blood progesterone assay using RIA (Mettwelly, 2001 and Zakaria et al., 2001), or by ultrasonography scanning technique (Abuzeid et al., 2000), or laparoscopy (Gerstman and Cappucci, 1986). Great chances of missing C.L palpation (Usmani et al., 1990) by using transrectal palpation method and missing many palpable follicles rectally (Hanizen et al., 2000) may be due to the small size of the ovaries and slight protrusion of these structures over the ovarian surface or less marked crown of C.L or ovulation papillar, along with the relaxation degree of the rectal wall and the skillness of the examiner.
Parturient buffalo-cows should be bred within 80-90 days post-calving and the calving to calving (intercalving) period should not exceed 13-13.5 months (El-Wishy, 2007), the prolonged intercalving intervals are mainly due to prolonged postpartum anestrus which is mainly attributed to ovarian inactivity (Barile, 2005).

Ramoun and Darwish (2006) considered buffalocows were suffering from ovarian inactivity when structures cannot be detected on both ovaries by two ultrasonographic examinations along with two rectal palpations with 10 days interval between them.

High incidence of postpartum ovarian inactivity might be affected by several factors such as plane of nutrition, body condition score at calving, milk yield, parity, calving season and other factors (El-Wishy, 2007).

Elevated level of progesterone during pregnancy exerts a negative feedback effect on the hypothalamic–pituitary axis that lead to cessation of the cycle during pregnancy. After parturition the concentration of these hormones are withdrawn and emergence of follicular waves occurs with the transient rise of FSH, Follicle Stimulating Hormone (Noakes, et al., 2009). But subsequent growth of follicle is largely depends upon insulin and insulin like growth factor (IGF)-I whose concentration is greatly influenced by level of nutrition (Ramoun, et al., 2012). Negative energy balance during postpartum period reduces pulsatile secretion of luteinizing hormone (LH) that also affects the follicular growth (Noakes, et al., 2009).

Various progesterone interovaginal devices impregnated with different amounts of progesterone (0.5-1.99 g) are commercially available; the principle of its use is a key to subside the long postpartum anestrus in buffaloes due to ovarian inactivity. CIDR come to the fore front in various countries throughout the world cattle industry (Macmillan and Peterson, 1993) and buffalo’s postpartum anestrus (Andukar and Kadu, 1995; Andukar et al., 1997 and Singh, 2003a).

Zaabel et al. (2009) showed that estrus exhibition and ovulation in buffaloes after removal of intravaginally applied CIDR for 7 days with intramuscular injection of 25 mg PGF2α (Lutalyse) in the 6th day of CIDR insertion. The progesterone released from CIDR increased blood progesterone exerting negative feedback on hypothalamus and anterior pituitary. Removal of CIDR induces rapid drop in circulating progesterone associated with removal of progesterone block on hypothalamus and pituitary and consequently GnRH release followed by FSH and LH release and resumption of ovarian cyclicity (Zebra et al., 1999). Banuselli et al. (2003) and Carvalho et al. (2007) have used interovaginal progesterone device or norgestomet implant together with intramuscular injection of estradiol benzoate on random day of estrous cycle, 9 day later the device or implant was removed and i.m injection of PGF2 and eCG were given. At day 11, ovulation was induced by i.m injection of GnRH or HCG. Timed AI can be performed 16hours after induction of ovulation (last GnRH treatment).

Zaabel et al. (2009) concluded that the use of 25 mg PGF2α in the 6th day + i.m injection of 10 μg GnRH in the 8th day is successful in promoting start of postpartum ovarian activity in buffalo-cows. They also reported that a period of 14 days of CIDR was superior to 7 days period for resumption of estrous cyclicity in anestrous buffaloes.

El-Shaht and Badr (2011) concluded that, CIDR for 10 days plus i.m. injection of 2ml PGF2α (Estrumate) was the most effective treatment of smooth inactive ovary. Other regimens such as Logul’s solution for uterine infusion as well as ovarian massage could be considered. Moreover, PGF2α administration appeared to be efficient in treatment of suboestrus cows.

All authors have shown that the fertility in buffaloes can be improved by treatment of buffalo problems like providing genotypes matched to the environment and offering good management protocols like good programs of heat detection, properly balanced nutrition, hygienic births and good health care after parturition.

The aim of the work is to: study effect of Effect of exogenous progesterone in treatment of ovarian inactivity in the Egyptian dairy parturient buffalocows in relation to the progesterone assay in diagnosis and in response to the treatment.

2. MATERIALS AND METHODS:

2.1. Animals:

This work was performed during months of 2014 to 2015 (June to January). On the River (Mediterranean) type of dairy buffalocows (Bubalus bubalis). Buffaloes attending the main clinic in Damanhur city and in some of the commercial dairy farms of buffaloes in Behaira and Alexandria provinces, Egypt, are the main dairy animals included in this work.
Progesterone assay and rectal findings, by two examinations with ten days interval, were the procedures to detect the presence of ovarian inactivity.

Presence of ovaries in smooth condition and carrying no structures along the length of the two examinations and with absence of the visible or exhibited estrus behaviour have indicated to postpartum aneostrous.

Absence of ovarian structures (follicles or CL) in the two ovaries and presence of small-sized smooth ovaries have been diagnosed as case of postpartum inactive ovaries. The long absence of exhibited estrus signs with presence of persistent smooth ovaries (no ovarian structures) with p4 less than 1 ng/ml have indication to the postpartum ovarian inactivity.

2.2. Heat detection:
In all groups, heat detection was done by personal visual observation (twice daily, 6 am & 6 pm) for at least 30 minutes to detect signs of the estrus (heat signs, restlessness mount other animals and allow to be mounted) by the owner under farm condition. Standing heat is the true estrus sign in which animals in estrus stand firm when mounted by anther animal, and the estrus was confirmed by careful rectal palpation where estrus signs were observed, moreover, the cows show clinically transparent glassy estrus mucous without any turbidity or flacks.

2.3. Breeding: Breeding had done by using artificial insemination (AI) in all cases at the proper time and proper technique applied by well-trained inseminators.

2.4. Pregnancy diagnosis: Pregnancy diagnosis was done at day 45-60 post insemination by rectal palpation.

Treatment trials were evaluated in this study according to Hafez (2000) using estrus induction rate (EIR), treatment estrus interval (TEI), overall 1st service conception rate, number of services per conception (No. S/C) and overall pregnancy rate (PR).

2.5. Blood sampling:
Blood samples were collected from all animals before and after end of treatment program from jugular vein. Another blood samples were collected from animals showed response to treatment trail. Serum from each sample was separated and kept identified and kept in vials. A third and fourth blood samples were collected at 8th and 21st day post estrus.

The serum samples were obtained by centrifugation at 3000 rpm for 20 minutes and stored at -20 C until analysis.

The efficiency of treatment was judged by assay of progesterone levels by Radioimmunoassay (RIA) according to Abrah (1997) by using progesterone DPC kits (Los Angelus, USA).

2.6. Drugs used in treatment:
1- CIDR® (Controlled Internal Drug Releasing device) is impregnated with 1.38 g of micronised natural progesterone that is continually released into the vagina and absorbed by the blood stream. Pfizer Animal Health. One device = 75 L.E.
2- Cystorelin® (synthetic gonadorelin (GnRH) diacetate tetrahydrate 50 mcg). Ceva Sante Animal, France). Dose of 2ml = 15 L.E.
3-Estrumate® (synthetic prostaglandin analogue (PGf2α) each ml contains 263 μg of Cloprostenol Sodium BP (vet), equivalent to 250 μg of Cloprostenol, Schering-Plough Animal Health. Dose of 3ml = 25L.E.
5-Prontogest® (progesterone) i.m injection each one ampoule 2 ml contains 100mg progesterone. Produced by EIPICO for Marcyrl pharmaceutical industries. One ampoule = 8.5 L.E.

2.7. Treatment procedures:
I -control
II- Progesterone + PGf2α treatment.
III- CIDR treatment.
IV- CIDR+GPG protocol as a treatment

I -control:
This group included 7 buffalo-cows which affected with inactive ovaries as control group without giving any treatment.

II- Progesterone + PGf2α treatment:
This group included 5 buffalo-cows each was treated as the following protocol:
1st day: 1 ampule Prontogest® i/m injection. 2nd day: 1 ampule Prontogest® i/m injection.
3rd day: 1 ampule Prontogest® i/m injection. 4th day: 1 ampule Prontogest® i/m injection.
5th day: 1 ampule Prontogest® i/m injection. 6th day: 3ml Estrumate i/m injection

III- CIDR treatment:
This group included 7 buffalo-cows which treated as the following programe:
Zero day: administer CIDR. 6th day: 3ml Estrumate i/m injection.
7th day: remove CIDR. 8-11 day: heat detection and breed A.I.

IV- CIDR+GPG protocol as a treatment:
This group included 9 buffalo-cows which treated as the following programe:
Zero day: administer CIDR+2 ml Cystorelin® i/m injection.
6th day: remove CIDR+3ml Estrumate i/m injection.
9th day: 2 ml Cystorelin® i/m injection. 10th day: 12-16 timed A.I.

3. RESULTS:
I-The first treatment (Treatment by combined use of progesterone and PGf2α).
Treatment trial of combined use of progesterone and PGf2α.
This treatment was applied on 5 buffalo-cows. Each cow received daily for 5 consecutive days a single dose of i.m. prontogeste injection and on day 6 i.m. Estrumate was injected during the period of this trial.
Results of this were presented in tables 7 and 8; and when compared with the results of control buffalo-cows. The following observations can be recorded:
1-DIM length of the treated buffaloes was found longer than that of the control.
2-Before treatment serum progesterone conc. values were 0.23 ± 0.03 ng/ml in control and 0.38 ± 0.12 ng/ml in treated individual of the two groups.
3- Serum progesterone conc. during heat was comparatively nearly similar with control values.
4-Serum progesterone conc. during 8th and 21st day was higher than that of control values.
5- The number of days to induce heat was 9.40 ± 0.51 days in treated buffalo-cows compared to 28.67 ± 6.59 day in control buffaloes.
6-The number of s/c was lower in the treated than control buffalo-cows.
7-Pregnancy rate had higher average value than that in the control buffalo-cows. 80 % conception in treated compared to 57.14 % in control.

II-Second treatment:
Using CIDR in anestrous group of buffalo

In each of 7 dairy buffalo-cows CIDR was inserted into vagina of each animal using a special applicator with a string was attached for easy removal. When CIDR is inserted into the vagina the impregnated progesterone in the elastic silicon is slowly released over the 7 days period of its presence in the vagina, the time of its removal, Before CIDR removal on day 6, 3 ml Estrumate (PGf2α) i.m. injection was administered. After CIDR removal and throughout the following 3 days, heat signs were expected and A.I was done in suitable time after heat expression in each animal. The estrus induction rate achieved with this regimen was compared and evaluated by the assessment of serum progesterone assay carried out in this work. Results of this protocol were presented in tables 11 and 12. On comparing these results with control results the following items were noticed:-
1-DIM in treated buffalo-cows was found continued for longer days than control results (105.71 ± 6.89 days in control versus 122.71 ± 16.06 days of treated).
2-Before treatment Serum progesterone concentration was averaging 0.35 ± 0.07 ng/ml compared to 0.23 ± 0.03 ng/ml in control.
3-Mean serum progesterone level at heat was found higher in treated buffaloes (0.43 ± 0.06 ng/ml) than its mean in the control ones (0.31 ± 0.05 ng/ml).
4- Serum progesterone mean concentration was found higher in treated buffaloes at day 8 and day 21 post A.I.
5-The mean number of services per conception(s/c) was found lower in the treated group (1.57 ± 0.30) than in the control group (2.00 ± 0.37).
6-six buffaloes out of seven were found pregnant, while in control it was four out of seven indicating high pregnancy rates in the treated buffaloes.

Table 1: Serum progesterone assay of anestrus buffaloes treated with progesterone + PGf2α (Prontogeste® +3 ml Estrumate).

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>Before</th>
<th>Heat</th>
<th>8th day</th>
<th>21st day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7</td>
<td>0.23 ± 0.03 a</td>
<td>0.31 ± 0.05 a</td>
<td>1.46 ± 0.20 a</td>
<td>1.20 ± 0.50 a</td>
</tr>
<tr>
<td>Treated</td>
<td>5</td>
<td>0.38 ± 0.12 a</td>
<td>0.29 ± 0.08 a</td>
<td>1.37 ± 0.09 a</td>
<td>2.11 ± 0.60 a</td>
</tr>
</tbody>
</table>
Table 2: Reproductive parameters of anestrous buffaloes treated with progesterone + PGf2α (Prontogeste® +3 ml Estrumate).

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>DIM</th>
<th>Incidence of estrus</th>
<th>TEI (days)</th>
<th>SPC</th>
<th>1st SCR</th>
<th>TCR</th>
<th>Pregnancy No.</th>
<th>Pregnancy %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7</td>
<td>105.71 ± 6.89 a</td>
<td>6</td>
<td>85.74</td>
<td>28.67 ± 6.59 a</td>
<td>2.00 ± 0.37 a</td>
<td>33.33</td>
<td>66.67</td>
<td>4</td>
</tr>
<tr>
<td>Treatment</td>
<td>5</td>
<td>110.00 ± 12.41 a</td>
<td>5</td>
<td>100.00</td>
<td>9.40 ± 0.51 b</td>
<td>2.00 ± 0.32 a</td>
<td>20.00</td>
<td>80.00</td>
<td>4</td>
</tr>
</tbody>
</table>

DIM = Days in milk. TEI = Treatment to estrus interval.
Values are means ± standard errors. Means in a column without a common letter differ significantly (P<0.05).
SPC = Services per conception. 1st SCR = Conception rate to first service.
TCR = Total conception rate, number of pregnant buffaloes after three services as a percent of total number of buffaloes showing heat.
Pregnancy rate = Number of pregnant buffaloes as a percent of total number of buffaloes.

Table 3: Serum progesterone assay of anestrous cows treated with CIDR.

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>Before</th>
<th>Heat</th>
<th>8th day</th>
<th>21st day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7</td>
<td>0.23 ± 0.03 a</td>
<td>0.31 ± 0.05 a</td>
<td>1.46 ± 0.20 a</td>
<td>1.20 ± 0.50 a</td>
</tr>
<tr>
<td>Treated</td>
<td>7</td>
<td>0.35 ± 0.07 a</td>
<td>0.43 ± 0.06 a</td>
<td>2.24 ± 0.42 a</td>
<td>3.33 ± 0.97 a</td>
</tr>
</tbody>
</table>

Table 4: Reproductive parameters of anestrous buffaloes treated with CIDR.

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>DIM</th>
<th>Incidence of estrus</th>
<th>TEI (days)</th>
<th>SPC</th>
<th>1st SCR</th>
<th>TCR</th>
<th>Pregnancy No.</th>
<th>Pregnancy %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7</td>
<td>105.71 ± 6.89 a</td>
<td>6</td>
<td>85.74</td>
<td>28.67 ± 6.59 a</td>
<td>2.00 ± 0.37 a</td>
<td>33.33</td>
<td>66.67</td>
<td>4</td>
</tr>
<tr>
<td>Treatment</td>
<td>7</td>
<td>122.71 ± 16.06 a</td>
<td>7</td>
<td>100.00</td>
<td>10.00 ± 0.65 b</td>
<td>1.57 ± 0.30 a</td>
<td>57.14</td>
<td>85.71</td>
<td>6</td>
</tr>
</tbody>
</table>

III- third treatment:
Anestrous buffaloes treated by CIDR+GPG in 9 buffalo-cows having a long postpartum anestrous due to ovarian inactivity CIDR was intravaginally inserted in each buffaloes and left for 6 days and with CIDR 2ml i.m. Cystorelin® (GnRH) was simultaneously injected after 6 days, CIDR was removed and 3 ml Estrumate was injected and on day 9 (3 days after CIDR removal) 2 ml i.m. Cystorelin® (GnRH) was administered. Timed A.I was carried out on day 10 of the trial.

Results were presented in tables 13 and 14 comparative data revising was done with that of the control buffaloes. This lead to observations like:-
1- Longer period of DIM in treated than in control animals.
2-Higher serum progesterone during anestrous period than in control (0.50 ± 0.09 Versus 0.23 ± 0.03 ng/ml).
3-Higher serum progesterone during heat in treated animal than in control ones (0.39 ± 0.06 versus 0.31 ± 0.05 ng/ml).
4-The post A.I level at day 8 was also found higher in treated animals compared to control ones (1.88 ± 0.21 versus 1.46 ± 0.20 ng/ml in control).
4- A lower number of s/c was revealed in treated buffaloes (1.56 ± 0.24 versus 2.00 ± 0.37 in control).
5- Eight out of nine buffaloes were found pregnant in treated group compared to four out of seven in control group.

The following results have shown that the successful protocol was found that in which CIDR+GPG were used in inducing estrus in anestrous buffaloes suffering from ovarian inactivity problem (table 7 and 8).

Table 7 in results pointed to the serum progesterone concentrations along the length of the postpartum period in parturient buffalo dairy cows. These concentrations in serum capable of preventing estrous cyclicity in parturient dairy buffalo-cows. It was possible to conclude that drop from higher serum progesterone concentration was the only possible way that capable of creating a concentration capable of inducing a fast and efficient response to the releasing pituitary and ovarian hormones that capable of initiating estrous cyclicity . the blocking of the –ve feedback of progesterone capable of stimulating FSH & LH release which in turn inducing initiation of the other hormone responsible for the start of estrous activity after calving.

<table>
<thead>
<tr>
<th>Table 5: Serum progesterone of anestrous buffaloes treated with CIDR+GPG protocol.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group</strong></td>
</tr>
<tr>
<td><strong>Before</strong></td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>Treated</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 6: Reproductive parameters of anestrous buffaloes treated with CIDR+GPG protocol.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group</strong></td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>Treated</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 7: Comparison of serum progesterone assay of anestrous buffaloes in different treatments.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Trials</strong></td>
</tr>
<tr>
<td><strong>Before</strong></td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>1- (progesterone +PGf2α)</td>
</tr>
<tr>
<td>2- (CIDR)</td>
</tr>
<tr>
<td>3-(CIDR + GPG)</td>
</tr>
</tbody>
</table>
Table 8: Comparison of reproductive parameters of anestrous buffaloes in different treatments.

<table>
<thead>
<tr>
<th>Trials</th>
<th>Incidence of estrus</th>
<th>TEI (days)</th>
<th>1st SCR</th>
<th>TCR</th>
<th>Pregnancy No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>DIM</td>
<td>No.</td>
<td>%</td>
<td>SPC</td>
<td>TEI</td>
</tr>
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<td>6</td>
<td>85.74</td>
<td>28.67 ± 6.59 a</td>
<td>2.00 ± 0.37 a</td>
</tr>
<tr>
<td>1-(progesterone+PGf2α)</td>
<td>5</td>
<td>110.00 ± 12.41 a</td>
<td>5</td>
<td>100.00</td>
<td>9.40 ± 0.51 c</td>
<td>2.00 ± 0.32 a</td>
</tr>
<tr>
<td>2- (CIDR)</td>
<td>7</td>
<td>122.71 ± 16.06 a</td>
<td>7</td>
<td>100.00</td>
<td>10.00 ± 0.65 bc</td>
<td>1.57 ± 0.30 a</td>
</tr>
<tr>
<td>3- (CIDR + GPG)</td>
<td>9</td>
<td>131.44 ± 21.24 a</td>
<td>9</td>
<td>100.00</td>
<td>10.00 ± 0.00 bc</td>
<td>1.56 ± 0.24 a</td>
</tr>
</tbody>
</table>

4. DISCUSSION

The recent diagnostic tools of ovarian inactivity not depend only on rectal palpation but also can use blood progesterone profile by radioimmunoassay of serum (Mettwelly, 2001 and Zakaria et al., 2001), or by ultrasonography scanning technique (Abuzeid et al., 2000), or laparoscopy (Gerstman and Cappucci, 1986). Usmani et al (1990) have shown that using rectal palpation alone for diagnosis of post-calving anestrus in buffaloes, 50% missing of CL was possibly available. Generally, missing changes are higher due to the fact that the mature CL has less crown or don’t protrude sufficiently above ovarian surface. So far, the accuracy of rectal palpation of ovaries depends on the size of ovaries. the most important diagnostic method of ovarian activity by using the old rectal palpation technique with one or more of the recent aids e.g, serum progesterone assay or ultrasonic scanning.

Most investigators reported that this harsh climates result in a decreased expression of overt estrus due to ovarian inactivity prevailing as a consequence of the heat stress that possibly causes aberration in the endocrine profile, poor expression of estrus and poor conception rate and long calving interval (Singh et al., 1989 and Chaudhari et al., 2012).

The buffalo has been regarded as a poor breeder due to poor fertility in the majority of conditions under which they are raised (Jauindeen and Hfez, 1983 and Barile, 2005). Heat stress decrease thyroid activity which indirectly reduce the buffalo reproductive efficiency. Heat stress has a direct effect on neuroendocrine setup in buffalo (Ralzan, 1988). Heat stress causes hyper prolactinemia which reduce LH frequency poor follicle maturation and decreased estradiol production in anestrous buffaloes leading to ovarian inactivity (Palta et al., 1977). Heat stress also altered the oocyte or the reproductive tract so that normal embryonic development was compromised (Hansen, 2003), it was reported that heat stress can disrupts the spermatogenesis in the bull (Vale, 2004).

Hence, in many farming systems prolonged postpartum anestrus is a major problem, along with poor nutrition and stress due to harsh climate and improper management. Synchronization of time or induction of estrus can be done using the same regimens as applied in cattle. It is possible to use various combinations of PGf2α, progesterone, progesterone releasing devices (CIDR), GnRH alone or with PGf2α. All treatments in this investigation for avoiding long day's open and postpartum ovarian inactivity. Seven treatment trials were tested and compared with control in this work in order to achieve a correct hormonal treatment and this should be run side by side with the correct management procedures for achieving a correct farming system in buffalo breeding.

The first treatment (P2+PGf2α) for treatment of anestrous parturient buffalo-cows with ovarian inactivity was carried out on 5 buffaloes. Each injected with a daily i.m injection of prontogest®
(progesterone) for 5 consecutive days and on the 6th day with one Estrumate injection (PGf2α). A.I depended on true estrous signs detection. The treatment group was found having low serum progesterone (0.19-0.7 ng/ml) < 1 ng/ml. The treatment concept depend on the basis that low serum progesterone when supplemented by external source to increase its concentration, its heat response was found increased and this create a high –ve feedback when its concentration drop in the blood, which in turn stimulate pituitary gonadotropin faster than drop of low serum progesterone and the developed feedback. PGf2α in treated group helping inducing luteolysis of the pertaining c.l and in turn faster serum progesterone drop. The progesterone drop in serum will inhibit its action on ovarian activity and allow production of optimal LH surge. Singh and Chaudhry (1992) reported that progesterone and prolactin during summer months and its secretion is low during summer compared to winter.

Results of this trial was presented in table 5 and when the data of this treatment compared with parallel ones in control, the following notifications could be traced: (1) the response to no. of s/c was lower in treated ones. (2) Pregnancy rates were became higher in the treated cases. (3) Serum progesterone after insemination was found higher in its serum concentrations compared to control levels. (4) Decrease of embryo mortality rate. 4 out of 5 inseminated cows were showed higher +ve conception (80% conception) compared to 50% conception in control (not treated buffaloes).

The second treatment (CIDR) for inactive ovaries and anestrum manifested in buffaloes after calving was based on treatment by progesterone in the form of CIDR device inserted for 7 days intravaginally and on day 6 before remove 3ml i.m Estrumate (PGf2 α) was injected during 3 days post removal of CIDR detection of estrus signs were watched and A.I was done upon their detection in each animals. Serum progesterone assay was used for assessment of its levels before, during and after phases of estrous cycle in the treated buffalo-cows.

On comparing results in treated with control data through progesterone assay it was noticed that (1) serum progesterone in treated buffalo was higher (0.47 ng/ml versus 0.22ng/ml in control). (2) Progesterone concentration was higher and induced heat compared to that of control (0.37 ng/ml versus 0.19 ng/ml in control), and insemination pointed to possible successful embryonal development. (3) Pregnancy rate in the CIDR-treated buffaloes was higher than in control, 5 out of 7 were found pregnant (71%) in treated buffaloes compared to 3 out of 7 (40%) in control. Zaable et al. (2009) applied the same protocol in 7 buffaloes (CIDR for 7 days +5 ml Lutalyse on day 6). Three out of seven CIDR treated buffaloes (43%) exhibited estrus within 77.3 ±19.0 hrs after CIDR removal.

The response in the trial of this work (71%) was higher compared to Zaabel and others work Zaabel (2009) suggested in the same work that when CIDR left for 7 day +PGf2α on day 6 and GnRH on day 7 resulted into 100% of buffaloes exhibited estrus within 65.2±16.5 hrs (about 3.7 days) and 5 buffaloes were conceived at induced estrus (about 70%). They concluded that the GnRH injection with CIDR removal may induce ovulation at appropriate time relative to mating and thereby improving embryo survival. Many authors were found in agreement with Zaabel concept (Pineda, 2003; Herbert and Trigg, 2005, pawson and McNeilly, 2005 and Peter, 2005). The idea of CIDR use that was suggested in this work was based on that the progesterone released from CIDR and absorbed through vaginal mucosa capable of exerting a –ve feedback on hypothalamus and anterior pituitary which prevent their release and allow storage of GnRH, FSH and LH. The rapid drop of circulatory progesterone remove the –ve feedback and promotes the release of GnRH followed FSH and LH release with subsequent resumption of ovarian activity. Randel et al. (1996) explained injection of PGI2 near removal of CIDR increase pituitary responsiveness to GnRH with subsequent estrus induction in anestrous buffaloes.

The third treatment (CIDR+GPG) involved treatment of anestrous parturient buffalo-cows affected with ovarian inactivity, with applying CIDR device in each animal. CIDR inserted in each buffalo along with i.m 2ml Cystorilin (GnRH) on day 6 CIDR removal and 3ml i.m Estrumate (PGf2 α) were injected. On day 9 anther i.m Cysterolin was administered. Timed A.I was performed on day 10 of this trial.

This means that CIDR intravaginally inserted for 6 days and along with GnRH +PG f2 α +GnRH (GPG protocol) i.m given on days respectively 0, 6 and 9 of the trial.

Results have shown great improvements in no. of s/c and pregnancy rate (77.7%) . serum progesterone from estrus to days 21 post A.I showed good gradual rise indicating successful fertilization and embryonal development.

GPG protocol was used in the 5th trial and CIDR protocol in the 6 trial were gave pregnancy rates respectively 66.6 and 71.4% (table, 10).
Good results in trials 5&6 encouraged making a combination between them for a achieving more conclusive trial gave more successful results. Hence, a 7th trial was emerged in the 7th trial PG and CIDR were combined and results were presented in table 8. Results of each of these trials were presented in table 10 and for revising with control. In the 7th trial pregnancy rate increased to 77.7% and lower no. of s/c (2.1) was noticed.

The idea of the use of GPG protocol was based 1st use of GnRH to overcome high prolactin at time of CIDR use. GnRH will remove the block of prolactin which makes ovaries refractory to FSH and LH influences to leading more active ovaries and onset oestrous cyclicity and normal cycling buffaloes (Heranjal et al., 1979a). Optimal LH surge was absent in anestrous buffaloes and the decreased in LH level is attributed to the inhibitory action of progesterone and prolactin on LH secretion. therefore GnRH will mediate the return to normal optimal LH. CIDR will block the LH activity by progesterone released from CIDR and absorbed by vaginal mucosa and induce –ve feedback at both pituitary and ovarian levels. By CIDR removal progesterone–ve feedback will prohibited and more luteal activity will start and PGf2α at removal of CIDR and GnRH after removal will create a sort of ovarian estrous cyclicity and heat induction mechanism.

The high opportunity for a successful fertilization (table, 9) and embryo survival, high pregnancy rate (7 buffaloes pregnant out of 9) and the lower no. of s/c encouraging the use of the 7th trial, but one may still think it is more expensive and its use is not suitable to apply in the developing countries. However, the simplicity of the 4th trial which gave a better pregnancy rate(80.0%) and costs of drugs in this tail is less expensive and more economic than all other trials (table,10).

Collective data of the three trials for treatment of anestrous buffaloes due to ovarian inactivity are resented in one table with control (table, 10). Examination of these results revealed that any trial could be considered a successful one compared to the control. The pregnancy rate in these trials varied between 66.6% and 80% compared to 50% in control animals that give any treatment. however, among these trials, the trial that could be used in a short time program and more economic relative to prices of the medicines used in each trial and some other costs, in the view of these factors with the principle of good treatment, the 4 trial can be assumed the best one regarding the economy and the efficiency of the results and the schedule of time of the program. This trial (7th) revealed an 80% pregnancy rate. Similarity a high pregnancy rate was also obtained in the first trial (the cheapest trial) where ph was given alone and was capable of increasing the pregnancy rate to 75% and decreasing the no. f s/c to 2.6 (table, 10). This trial is very simple and did not cost the expensive medicines and more veterinary visits and can be allowed for breeders in the form of advices and with explanation about its value during summer calving as food supplement, and mean while paying his attention to watching carefully the results of its use alone and the importance of the follow up for possible change of the treatment program.

Results summarized in table,1 have shown that a single treatment can not be advised, but the inactive ovary is a syndrome having multiple branches for its etiology and the efficient treatment should depend on success of treatment depend mainly on efficiency of experience and learning about buffalo breeding. There is general agreement among authors and researches on buffalo breeding, that this animal can offer a nearly similar productivity like cattle. El-Wishy (2007) reported that in Egypt. India and Pakistan, 34-90% of buffaloes showed estrus during the 1st 90 days after calving, while 31-40%remained anestrous for more than 150 day. On the other hand Hassan (1995) was capable to obtain 30 days uterine involution and early onset of 1st postpartum obvious estrus signs within 76.3 after calving. The author having thus success with mineral mix composed of 1 gm/kg ration for 90 days after calving. On the opposite thinking it was and reported that the obvious postpartum estrus is not only enough for future breeding responses. De Rensis and Lopez-Gatius (2007) stated that the recent knowledge about follicular waves and attainment of the dominant follicular size (>10mm) and achieving better procedures for improving fertility in buffaloes is essential and still needed.

Many programs were assessed in the literature, but efficient single one can not be excersised. In 2012, in Egypt, El-Shaht and Badr simply obtained better solution for the problem. They used CIDR for 10 days plus i.m 2 ml Estrumate (PGf2α) and obtained a better solution for the smooth postpartum inactive ovaries, particularly when Lugol’s intrauterine infusion together ovarian manual massage per rectum.

5. CONCLUSION:

From the present study it is clear that the progesterone assay (by EIA) considers as a good diagnostic method and monitor of the ovarian activity in buffaloes and detect the estrus postpartum, also progesterone assay reveal the response to the treatment. It could be concluded that
high level of serum progesterone during heat period improve conception rate in buffalo-cows. Also it could be concluded that the using of CIDR + GPG treatment trial of postpartum ovarian inactivity in the Egyptian buffalo-cows achieve good results and enhance its reproductive efficiency in buffaloes.

6. REFERENCES: