# IMPROVEMENT OF FERTILITY IN EGYPTIAN BUFFALOES DURING SUMMER SEASON USING DIFFERENT PROTOCOLS FOR ESTRUS SYNCHRONIZATION

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## *ABSTRACT*

*This study aimed to determine the efficacy of different synchronizing protocols for improving the fertility in Water buffaloes.*

*A total of 53 anestrous buffaloes (24 cows and 29 heifers) were used in this study through 2 experiments, during summer season. In experiment 1, 24 buffalo-cows were divided according to the treatment protocol into 4 groups:- G1 buffalo-cows (n=7) were given* $PGF_{2\alpha}$ *followed 48 hrs later by injection of eCG; G2 buffalo-cows (n=6) were injected with* $PGF_{2\alpha}$ *followed 48 hrs later by GnRH; G3 buffalo-cows (n=5) were injected with GnRH , followed 7 days later by* $PGF_{2\alpha}$ *and G4 (control) buffalo-cows (n=6) did not receive any treatment. In experiment 2, 29 heifers were divided according to the treatment protocol into 3 groups: G1 heifers (n=10) were injected with GnRH followed 7 days later by* $PGF_{2\alpha}$, *and after 48 hrs injected with eCG; G2 heifers (n=11) were injected with GnRH,* $PGF_{2\alpha}$ *and GnRH at 0, 7 and 9 days, respectively and G3 (control), in which heifers (n=8) did not receive any treatment. Animals were gynecologically examined, by trans-rectal ultrasonography (8 MHz) to detect the ovarian and genital tract condition.*

*In experiment 1, the proportion of cows exhibiting estrus and the pregnancy rate after treatments were 85.75; 50% for G1, 67.0; 75% for G2 and 60; 66.6% for G3 compared to 16.7; 0% for control group.*

*In experiment 2, the proportion of heifers exhibiting estrus and the pregnancy rate after treatments were 70; 42.8% for G1 and 63.6; 57.1% for G2 compared to 0% for control non treated group.*

*It could be concluded that, during summer season, the use of* $PGF_{2\alpha}$ *and GnRH protocol in buffalo-cows and GnRH,* $PGF_{2\alpha}$, *and eCG or GnRH,* $PGF_{2\alpha}$, *and GnRH protocol in heifers could successfully improve their fertility.*

***Key words:*** *Fertility, summer, synchronization, buffalo-cows and heifers.*

## *INTRODUCTION*

Buffaloes play a prominent role in livestock production and the economy in most of developing countries. However, fertility in water buffalo (Bubalus bubalis) is considerably lower than that in cattle, and their productivity is limited by poor reproductive efficiency such as late onset of puberty, seasonality, poor estrus expression, and long calving intervals. Prolonged postpartum acyclicity and anestrum are major sources of economic loss to buffalo breeders **(El-Wishy, 2007)**. Anestrum is the most common cause of infertility in buffaloes **(Singh and Sahni, 1995)**. However, postpartum anestrus is prevalent in dairy **(Shah et al., 1990)** and suckled swamp buffaloes **(Jainudeen, 1988)**. In the few studies available on breeding and institutional herds **(Schmidt et al., 1963; Chauhan and Singh, 1979)**, the frequency of postpartum and post service anestrum was 64-76 and 24-36%, respectively.

Inactive or non-functional ovaries is one of the most important causes of anestrus in buffaloes **(El-Wishy, 1965; Baruah et al., 2000)**. In addition, silent estrus is also considered one of the most important factors responsible for poor reproductive efficiency in buffaloes **(Prakash , 2002)**. This characteristic factor, together with other reproductive peculiarities, such as a pronounced "bull effect" **(Zicarelli et al., 1997)**, variable duration of estrus and difficulty in predicting the time of ovulation **(Baruselli et al., 2001)**, need a global cooperation to improve the reproductive efficiency of buffaloes specially during summer season. In spite of some encouraging results obtained in the last years **(Neglia et al., 2001; Neglia et al., 2003; Campanile et al., 2005)** and the use of several different estrus synchronization protocols **(De Araujo et al., 2002; Neglia et al., 2003)**, the efficiency of synchronizing agents and success of artificial insemination (AI) in buffalo species is still low. Different protocols were evaluated to determine the efficiency of synchronization of ovulation and timed AI in buffaloes during the non-breeding season (spring and summer) **(Miyamoto et al., 1997)**.

Ovsynch protocol effectively synchronized ovulation in Murrah buffaloes and resulted in conception rates (to two fixed-time inseminations) that were comparable to those achieved with a single AI after detection of spontaneous estrus **(Vijay and Prakash, 2005)**.

However, there are only three recent reports using the Ovsynch protocol in buffalo: half-bred (Murrah-Mediterranean) buffaloes **(Berber et al., 2002)**, Mediterranean buffaloes **(Neglia et al., 2003)**, and Egyptian buffaloes **(Bartolomeu et al., 2002)**. It is noteworthy that the use of the Ovsynch protocol in Murrah buffalo in its native tropical environment has not been reported. The objective of the present study was to determine the efficacy of different synchronizing protocols for improving the fertility in Water buffaloes as indicated by expression of estrus and pregnancy rates after treatments.

## *MATERIALS AND METHODS*

**Animals :**

This study was carried out in a dairy buffalo farm belonging to Animal Reproduction Research Institute, Ministry of Agriculture at Beni Sweef governorate in Upper Egypt from June to September (37-41$^{o}$C). The average calving interval of the buffaloes was 446.02± 86.34 days. The animals were supplied daily with balanced ration and water ad libitum. These buffaloes had BCS ≥ 3 (scale 1 = thin to 5 = fat; **Bhalaru et al., 1987**). Animals were milked twice daily (7 a.m. and 7 p.m.) by using a milking machine. The total milk yield was 783.5 ± 576.01Kg/season, with a lactation period 176.58 ± 106.61 days. The milk yield was 3.97 ± 1.94 Kg/day in average.

**Gynecological examination :**

To detect the ovarian and genital tract conditions, all non-pregnant buffaloes were gynecologically and ultrasonographically examined before and after treatments. A real time B-mode ultrasound scanner (Pie Medical 240, Maastricht, The Netherlands) equipped with a 5- MHz endo-rectal linear-array transducer was used for ultrasound examination.

Before applying the estrus synchronization protocols, an ultrasound examination had been carried out to identify the cyclic and non-cyclic buffaloes, based on the presence of corpus luteum (CL) (Fig., 1A). To confirm an acyclic condition, a second ultrasound examination was carried out 10 days later on those animals having no CL (Fig. 1B) at the first monitoring **(De Rensis et al., 2005)**. Based on the ovarian cyclicity, the non-pregnant animals (n=53) were classified according to their reproductive status into cyclic and acyclic (smooth ovaries) animals.

**Synchronization protocols :**

A total of 53 anestrous buffaloes (24 cows and 29 heifers) were used in two experiments:

**Experiment 1 :**

Twenty four anestrous buffalo-cows were divided according to the treatment protocol into 4 groups:-

**Group (1) :** Seven buffalo-cows were administered PGF2_ (5ml of Lutalyse®, 25 mg dinoprost; Pharmacia N.V./S.A. Purus, Belgium) i.m., followed 48 hrs later by administration of 1000 I.U. equine chorionic gonadotropin (eCG; Folligon®, Intervet, Holland).

**Group (2) :** Six buffalo-cows were administered with 5ml of Lutalyse® i.m. followed 48 hrs later by i.m. administration of 10 $\mu$g of Buserelin (2.5 ml GnRH; Receptal®, Intervet international B.V., Boxmeer, Holland).

**Group (3) :** Five buffalo-cows were administered 2.5 ml Receptal® i.m., followed 7 days later by i.m. administration of 5 ml Lutalyse®.

**Group (4) :** Six buffalo-cows did not receive any treatment for estrus synchronization and was considered as control group

**Experiment 2**

Twenty nine anestrous heifers were divided according to the treatment protocol into 3 groups:-

**Group (1):** Ten heifers were administered 2.5 ml Receptal®, i.m., followed 7 days later by i.m. administration of 5 ml of Lutalyse®, and after 48 hrs they were administered 1000 I.U. eCG i.m.

**Group (2) :** Eleven heifers were administered with GnRH, PGF2_ and GnRH at 0, 7 and 9 days, respectively.

**Group (3)** (control):- Eight heifers were not receive any treatment for synchronization of estrus.

Buffaloes were observed twice daily at a 12-hr interval by experienced herdsman for at least one hour for estrous signs, especially the acceptance of buffalo-cows to the buffalo bull **(Rhodes et al., 2003)**. The day at which the female stand to be mounted was considered the day of estrus (Day 0). The females were checked for pregnancy 45 days after mating by palpation per rectum and ultrasonographic examination.

**Statistical analysis :**

Fisher`s exact test was used to compare the proportion of animals exhibited estrus and pregnancy rate among groups **(Petrie and Watson, 1999)**.

## *RESULTS*

The responses of the anestrous buffalo-cows and heifers to the hormonal treatments are presented in tables 1 and 2, respectively. The proportion of anestrous buffalo-cows exhibited estrus in G1 (PGF2_ + eCG) was significantly ($P<0.05$) higher than that in control group. The proportion of buffalo-cows exhibited estrus in G1 (85.7%) was none significantly higher than those in G2 (67%) and G3 (60%). However, the pregnancy rate after mating buffalo cows at induced estrus was higher in G2 (75%) and G3 (66.6%) than that in G1 (50%).

The proportion of anestrous heifers exhibited estrus in G1 (GnRH+PGF2_ + eCG) and in G2 (GnRH+PGF2_ + GnRH) was significantly ($P<0.05$) higher than that in control group. The proportion of anestrous heifers showed estrus in G1 and G2 was nearly similar (70 % vs. 63.6%), however, the pregnancy rate was higher in G2 (57.1%) than that in G1 (42.8%).

## *DISCUSSION*

The long calving interval is one of the major problems in buffalo breeding. The application of an estrus induction method offers the opportunity to increase fertility and reduce inter calving period. In the present work, multiparus buffaloes were treated with different hormonal protocols for induction of estrus and ovulation during summer season. Administration of $PGF_{2\alpha}$ plus eCG (G1) resulted in a higher percentage of buffaloes exhibiting estrus (85.7%) compared to other groups. This can be attributed to the formation of multiple follicles in the ovaries of buffaloes treated with eCG due to its long half life (Fig. 2). These follicles varied in size and secreting estrogens for long time, and thus the duration of estrus will be extended than normal, and exaggerate the symptoms of estrus. On the other hand the pregnancy rate after mating of buffalo-cows at synchronized estrus was lower in G1 (50%) when compared to G2 (75%) and G3 (66.6%). The lower pregnancy rate in G1 might be attributed to the presence of uterus under the effect of estrogens for long time **(Soumano and Price, 1995)** which consequently decrease the probability for embryo implantation. In addition administration of eCG resulted in formation of cystic ovaries due to deficiency or insufficient quantity of endogenous luteinizing hormone (LH) released from anterior pituitary to ovulate all follicles in the stimulated ovary. However, **Zaabel et al. (1997)** concluded that the best trial for treatment of anestrous balady cows with persistent CL and smooth inactive ovaries was exhibited by the administration of PGF2_ followed by eCG injection. This treatment resulted in the highest conception rate (70 and 60%) with the lowest number of services per conception (1.6 ± 0.22 and 19 ± 0.23) in both cows with persistent CL and smooth inactive ovaries, respectively. The difference between their results and those of the present work could be related to the species difference.

In the present study, administration of GnRH (2.5 ml Receptal) followed 7 days later by $PGF_{2\alpha}$ into buffalo cows (G3) induced estrus in 60% of anestrous animals and 66% of responded animals were pregnant after the first service. A higher estrus induction rate was reported by Narashima Rao and Venkatramiah (1991, 88.5%) and by **Heleil et al.** (2005, 100%). On the other hand a lower pregnancy rate (52.2% and 53.3%) was reported in both studies.

Since delayed puberty is a major constraint to reproductive performance in buffaloes **(Nanda et al., 2003)** we put in our consideration in this work to enhance the age of puberty of buffalo-heifers through hormonal manipulation. In the present work, two trials were done to stimulate the ovarian activity and synchronize estrus in buffalo-heifers during summer season, one by giving GnRH; $PGF_{2\alpha}$, and eCG and the other trial by giving GnRH, $PGF_{2\alpha}$ and GnRH (Ovsynch). The animals exhibiting estrus were 70.0 and 63.6%, respectively, while the pregnancy rate following treatments was 42.8 and 57.1%, respectively. Similarly, **Karen and Darwish (2008)** reported that 62.5% (5/8) of cyclic and acyclic heifers ovulated after administration of the $2^{nd}$ GnRH of the Ovsynch protocol in summer. Also, ovsynch protocol was efficient for synchronization of ovulation in 80% of cyclic heifers during breeding season and 40% pregnancy rate was obtained after a single fixed timed AI **(Presicce et al., 2005)**.

It could be concluded that, during summer season, the use of $PGF_{2\alpha}$ and GnRH protocol in buffalo-cows and GnRH, $PGF_{2\alpha}$, and eCG or GnRH, $PGF_{2\alpha}$, and GnRH protocol in heifers could successfully improve their fertility.

Table (1): Synchronization of estrus in buffalo-cows using different regimens of hormones.

| Exp. Groups | No. of Animals | Treatments | No. of animals exhibiting estrus (%) | Pregnancy rate (%) |
|---|---|---|---|---|
| Group 1 | 7 | $PGF_{2\alpha}$ + eCG | 6 (85.7)[a] | 3/6 (50)[a] |
| Group 2 | 6 | $PGF_{2\alpha}$ + GnRH | 4 (67.0)[ab] | 3/4 (75)[a] |
| Group 3 | 5 | GnRH + $PGF_{2\alpha}$ | 3 (60.0)[ab] | 2/3 (66.6)[a] |
| Group 4 | 6 | No treatment | 1(16.7%)[b] | 0 (0)[a] |

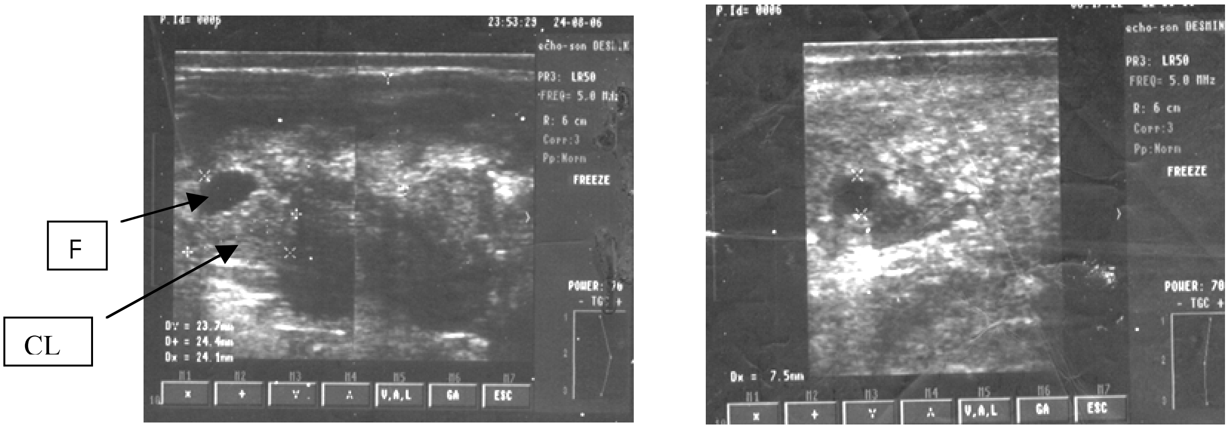
[a,b:] Values with different superscripts within the same column are significantly different at $P < 0.05$.

**Table (2):** Synchronization of estrus in buffalo heifers using different regimens of hormones.

| Exp. Groups | No. of Animals | Treatments | No. of animals exhibiting estrus (%) | Pregnancy rate (%) |
|---|---|---|---|---|
| Group 1 | 10 | GnRH + $PGF_{2\alpha}$ + eCG | 7/10 (70.0)[a] | 3/7(42.8) |
| Group 2 | 11 | GnRH+ $PGF_{2\alpha}$ + GnRH | 7 /11 (63.6)[a] | 4/7(57.1) |
| Group 3 | 8 | No treatment | 0 (0)[b] | 0 (0) |

[a,b:] Values with different superscripts within the same column are significantly different; $P < 0.05$.

a,b:




(A) (B)

**Fig. 1 :** Sonogram of an ovary of cyclic buffalo cow with a CL and small follicle (F) (A) and an ovary of acyclic buffalo-cow with small follicle (B) before treatment.

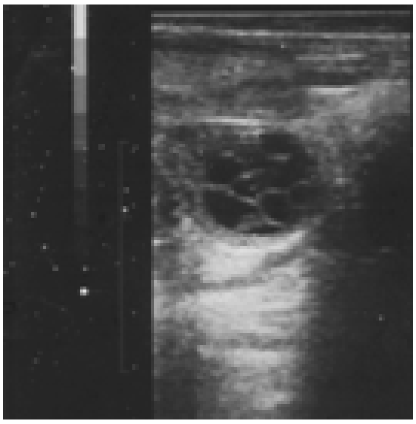


**Fig. 2 :** Sonogram of buffalo`s ovary showing multiple small follicles 3 days after administration of eCG

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## الملخص العربى

# تحسين خصوبة الجاموس المصرى فى فصل الصيف باستخدام بروتوكولات مختلفـــة لتزامن وإحـداث الشـبق

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هدفت هذه الدراسة لتحديد فاعلية مختلف البروتوكولات لتزامن وإحداث الشبق لتحسين الخصوبة فى الجاموس.

أستخدمت ٥٣ جاموسة تعانى من عدم الشياع (٢٤ جاموسة و٢٩ بكرية) من خلال تجربتين فى فصل الصيف من يونيو إلى سبتمبر.

التجربة الأولى : تضمنت ٢٤ جاموسة تم تقسيمها حسب بروتوكول العلاج إلى ٤ مجموعات :

المجموعة الأولى : تضمنت ٧ جاموسات تم إعطائها البروستاجلاندين تلاه ب٤٨ ساعة حقن الهرمون المحفز للحاثة المنسلية بمصل الأفراس الحوامل.

المجموعة الثانية : تضمنت ٦ جاموسات تم إعطائها البروستاجلاندين تلاه بـ٤٨ ساعة حقن الهرمون المحفز للأقناد.

المجموعة الثالثة : تضمنت ٥ جاموسات تم إعطائها الهرمون المحفز للأقناد تلاه بـ٧ أيام لهرمون البروستاجلاندين.

المجموعة الرابعة : تضمنت ٦ جاموسات لم يتم إعطائها شيء (مجموعة ضابطة).

التجربة الثانية : تضمنت ٢٩ بكرية تم تقسيمها حسب بروتوكول العلاج إلى ٣ مجموعات :

المجموعة الأولى : تضمنت ١٠ بكرية تم إعطائها البروستاجلاندين تلاه بـ٤٨ ساعة حقن الهرمون المحفز للحاثة المنسلية بمصل الأفراس الحوامل.

المجموعة الثانية : تضمنت ١١ بكرية تم إعطائها الهرمون المحفز للأقناد تلاه بـ ٧ أيام البروستاجلاندين تلاه بيومين حقن الهرمون المحفز للأقناد.

المجموعة الثالثة : تضمنت ٨ بكرية لم يتم إعطائها شيء (مجموعة ضابطة).

فى التجربة الأولى كانت نسبة ظهور الشياع بعد العلاج ٧٥ر٨٥٪ و ٦٧٪ و ٦٠٪ و٧ر١٦٪ فى المجموعات ١ . ٢ . ٣ . ٤ على الترتيب بينما كان معدل الحمل ٥٠٪ و ٧٥٪ و٦ر٦٦٪ و ٠٪ فى المجموعات ١، ٢، ٣، ٤ على الترتيب.

فى التجربة الثانية كانت نسبة ظهور الشياع بعد العلاج ٧٠٪ و ٦ر٦٣٪ و ٠٪ فى المجموعات ١، ٢، ٣ على الترتيب بينما كان معدل الحمل ٨ر٤٢٪ و ١ر٥٧٪ و ٠٪ فى المجموعات ١، ٢، ٣ على الترتيب.

مماسبق يمكن إستخلاص أن إستخدام بروتوكول إعطاء الهرمون المحفز للأقناد تلاه بـ ٧ أيام لهرمون البروستاجلاندين هو الأفضل فى الجاموس بينما إستخدام بروتوكول إعطاء الهرمون المحفز للأقناد تلاه بـ ٧ أيام البروستاجلاندين تلاه بيومين حقن الهرمون المحفز للأقناد هو الأفضل للبكريات لتحسين الخصوبة بفصل الصيف.

الكلمات الدالة : خصوبة – صيف – تزامن الشبق – جاموس.