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Evaluation of Crestar® and modified Crestar programs for timed insemination in lactating Egyptian buffaloes (Bubalus bubalis) under intensive production system

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The current study was conducted on a total of 204 Egyptian, lactating buffalo cows. These animals were in the second or third parity, of good body condition scores and apparently healthy. The animals were raised in intensive production system on a private farm. The buffalo cows were allotted into three groups, two of these groups were experimental and the third was a control group. The first experimental group included 30 buffalo cows were undergo ovulation control by Crestar® a subcutaneous ear implant (3mg norgestamet) plus Crestar® injection i.m. (3mg norgestamet + 5mg estradiol valerate) at zero day. At the 7th day of implantation, PGF2α was injected i.m., then Crestar® implant was removed at the 9th day with injection of PMSG 400 iu. Timed insemination was conducted 56 hrs later. The second experimental group (24 buffalo cows) was treated by the same program, moreover they injected with GnRH at the time of insemination .The third group (150) buffalo cows was bred naturally and used as a control group. For serum progesterone assay blood samples were collected from the animals of the two experimental groups at day 0, 7 and 9 of the Crestar program. The buffalo cows of the experimental groups were closely observed for estrus signs and were rectally palpated at the time of insemination for detection of the internal estrus changes. At day 50 post insemination all animals were rectally palpated for pregnancy diagnosis. The result of the current study revealed that the visibility of estrus signs were 20 %, 16.7% and 22 % for the first, second and third group respectively. Pregnancy rate was much higher in the second group associated with the injection of GnRH at the time of insemination. Two animals of the second group were carrying twins (11 %). Serum level of progesterone was significantly higher in the 7thday in comparison with those recorded for 0 and 9th day.

Buffaloes were introduced into Egypt from India, Iran and Iraq approximately during the middle of the 7th Century. It is the most important and popular livestock for milk production in Egypt. Population size is 3 717 000 and resemble 55 % of total bovine population in Egypt (El-Kirabi, 1995; FAO 2003).

Reproductive efficiency is the primary factor affecting productivity and is hampered in female buffalo by inherent late maturity, Poor estrus expression, Distinct seasonal reproductive patterns and Prolonged intercalving intervals (Madan, 1988; Madan and Raina, 1984).Moreover, prolonged postpartum acyclicity (absence of ovarian cyclic activity) and anestrum (absence of overt estrous signs) are major sources of economic loss to buffalo breeders (Singh *et al.,* 2000; El-Wishy 2007).

Improvement of reproductive efficiency in buffalo requires the identification of specific limiting factors under a given situation and the development and field testing of strategies for improvements and interventions that are sustainable with available local resources (Perera, 1999).

In Egypt, more than 90 % of buffaloes are raised as small holder's.The traditional farmer possesses only one to four buffaloes and usually no bull; hence, there is little opportunity for behavioral interaction among estrus animals ((El-Kirabi, 1995)). Artificial control of the estrous cycle has provided an efficient means of increasing

the reproductive capacity of buffalo by obviating the need for frequent visual inspections (Madan, 1988).

Prostaglandins have been used to induce estrus in buffalo, but they work if a corpus luteum is present and therefore they can be useful in sub-estrus animals, having a synchronizing more than an inducing effect (Dhalival *et al.,* 1988 Chohan *et al.,* 1995; Sahasrabudhe and Pandit, 1997; Awasthi *et al.,* 1998; Kharche and Srivastava, 2001). The use of gonadorelin (GnRH), given by multiple injections or in microencapsulated form, did not appear efficacious and moreover their administration times are not of practical use (Shah *et al.,* 1990; Fateh *et al.,* 1999; Takkar *et al.,* 1999). More useful and efficacious have been the treatments using progesterone associated with gonadotrophin or gonadorelin (Zicarelli and Boiti, 1982; Rao and Sreemannarayana, 1983; Singh *et al.,* 1983, 1984, 1988; Borghese *et al.,* 1993; Shanker *et al.,* 1999; Hattab and Osman, 2000; Hattab *et al.,* 2000).

Borghese *et al.,* (1993) ,reported that, Italian Buffalo cows raised under intensive production system when treated with subcutaneous implants of Norgestomet + PMSG and Buserelin (GnRH-analogue) released by subcutaneous osmotic pump; or Progesterone + Buserelin i.m. have been able to reduce the intercalving interval and increase the fertility of the herd out of the breeding season. Better results have been obtained using progesterone - releasing intravaginal device (PRID) associated with PMSG and prostaglandin (Zicarelli *et al.,* 1994).

The aim of the current study was to evaluate the use of Crestar® and modified Crestar program for control of ovulation and timed insemination in the lactating Egyptian buffaloes under intensive production system in well managed dairy.

Material and methods

Animals and management. This study was conducted from December, 2004 to December 2005, using lactating (n = 204) second or third-parity, Egyptian buffaloes (*Bubalus bubalis*) from the herd maintained on El-Komy Farms (Kilo 138 Cairo-Alexandria Desert Road- Egypt). The buffaloes used were of good body condition scores, weighed from 500-600 kg and free from any apparent anatomical or reproductive disorders. All of these buffaloes were in the breeding period (60-120 days post partum) .The buffaloes were kept under loose housing conditions in clean, hygienic opened yard system with sandy-muddy flooring, asbestos roofing equal to 30 % of floor square area, and sufficient space for the free movement of the animals (40 square meters for each animal). All buffaloes were fed a ration with total mixed ration (TMR), consisting of concentrates (maize grain, Soya been cake, wheat bran), roughages (either Barseem or wheat straw), a mineral mixture, and salt .The concentrate/ roughage ratio was 55 / 45 %. Fresh water was available ad libitum.These animals were milked twice daily by milking machines with special bucket adopted for buffaloes .The average daily milk production was 10 kg /head /day. Calf rearing was depending on natural suckling of colostrum for five days and then isolated from his dam and raised on natural buffalo milk with bucket feeding.

Synchronization of ovulation. Buffaloes were randomly assigned into three groups, two experimental groups (group A and B) and control one (group C). The two experimental groups were collected randomly from different yards in two yards free from bulls. The first experimental group (group A) included 30 buffalo cows and were undergo ovulation control by Crestar® (Intervet, Boxmeer, The Netherlands). Crestar® consists of 2 components: an ampoule of 2 ml injection containing estradiol valerate (5 mg) with norgestomet (3 mg) and a silicone ear implant containing3 mg norgestomet (17a-acetoxyl-l l g-methyl- 19-nor-pregn-4-ene-3, 20-dione). At zero day, the injection was administered im and the implant was inserted subcutaneous (SC) at the outer edge of the ear in all animals. After 9 days the norgestomet implants were removed. Two days before implant removals 2ml Prosolvin (a synthetic analogue of PGF2α containing 15 mg of luprostinol, Intervet, Boxmeer, The Netherlands) was injected i/m. At the ninth day, 400 I.U. PMSG (Folligon, Intervet, Boxmeer, the Netherlands) was injected i.m. Artificial insemination was conducted, 56 hours after implant removal.

The second experimental group (group B) of 24 buffalo cows was treated with modified Crestar program. It was including Crestar® plus the injection of 100 µg GnRH (Gonadorelin Intervet, Boxmeer, the Netherlands) at the time of insemination. The third group (Group C), including 150 buffalo cows was bred naturally and used as a control group.

Table 1: Estrus activities and reproductive traits in timed insemination and naturally bred Egyptian buffalo cows.

Traits / Animal groups	No.	Visible estrus signs % (N)	Internal estrus changes			Pregnancy rate % (N)	Twinning % (N)	Still birth % (N)	Abortion % (N)
			Vulval changes % (N)	Uterine changes % (N)	Ovarian changes % (N)				
Group A	30	20 % (6)	60 % (18)	73 % (22)	80 % (18)	60 % (18)	_	_	_
Group B	24	16.7 % (4)	54 % (13)	79 % (19)	83 % (20)	75 % (18)	11 % (2)	5.5 % (1)	5.5 % (1)
Group C	150	22 % (33)	_	_	_	56 % (84)	2.3 % (2)	4.7 % (4)	3.5 % (3)

Group A: Timed insemination with Crestar ® **Group B: Timed insemination with modified Crestar**
Group C: Naturally bred Animals

Table 2: blood serum Progesterone profile in controlled ovulation Egyptian buffalo (Mean ± SE ng / ml).

Progesterone	At 0 day N (54)	At 7th day N (54)	At 9th day N (54)
Mean ± SE	3.75± 0.53[a]	6.75± 0.73 [b]	2.68± 0.32 [a]
Min. Value	.09	2.09	0.14
Max. Value	12.07	18.07	7.01

Means within the same raw with different alphabetical were significantly different at p≤ 0.01.

Artificial insemination and natural mating. Animals of the two experimental groups (group A and B) were artificially inseminated by a good quality of frozen semen produced by a world wide company imported from Italy. Meanwhile animals of the third group (control group of 150 buffalo cows) were bred naturally by good fertile bulls (one bull per 25 buffalo cows in separate yards).

Estrus activities. Animals of the two experimental groups were palpated per rectum to assess the internal estrus changes at the time of insemination (presence of follicle > 1 cm, tonic uterus and presence or absence of mucous vaginal discharge). Estrus detection for the animals of the control group was performed twice a day in early morning and at the afternoon by visual observation of well trained workers.

Collection of blood samples. Blood samples (5 mL for determination of progesterone concentrations) were collected by jugular venipuncture into polystyrene tubes at 0, 7 and 9 days from insertion of Crestar implant.. Blood samples were chilled on ice, transported to the laboratory and centrifuged at 3,000 x g for 15 minutes. Serum was kept at -20" C until assayed. Progesterone assay was measured using FERTIGENIX PROG-EASIA by BIOSOURCE EUROPE S.A.Kit according to Matthiews (1986).

Pregnancy diagnosis. Pregnancy diagnosis of the two experimental groups was assessed at 50 days after artificial insemination and confirmed at 90 days by rectal palpation of the uterine contents. Pregnancy rates were calculated for animals that were still pregnant at 90 days.

Regarding the control group (group C) , first rectal examination was performed after 40 days for animals with detected estrus and then at 90 days .Meanwhile animals without detected estrus were checked for pregnancy every three weeks. Statistical analyses. Differences between serum progesterone concentrations between the two experimental groups during treatment were analyzed using repeated measure ANOVA using PC-STAT (1985).

Results

Responce to synchronization.

Visible estrus signs. On the day of AI, 20% of animals in group A and 16.7 % of group B showed estrus behavior. Meanwhile the percentage of animals showed estrus signs were 22% in group C at the time of natural mating (table 1). Frequent urination is the most observed symptom of estrus and bellowing. Mucus vaginal discharge in quietly

sitting animals was observed. Many buffaloes showed clear or transparent cervical mucus at the onset of estrus.

Internal estrus changes. Edema of the vulva was observed in 60 and 54 % in the animals of group A and B respectively .Uterine tone and ovarian changes were more obvious in group B than group A (table 1).

Pregnancy rates and twinning. As shown in table 1, closed values of pregnancy rates were recorded for the animals treated with Crestar® program and the control group (60 and 56 % respectively).Meanwhile a higher value (75 %) of pregnancy rate was recorded for animals treated with modified Crestar program. Two animals of group B were carrying twins; abortion was recorded for one of them at the 7th month of gestation and the second buffalo gave still birth.

Serum concentration of progesterone. Summaries for serum concentration of progesterone were presented in table 2.A highly significant value (6.75± 0.73 ng / ml) was recorded for all treated animals in group A and group B at the 7th day of insertion of Crestar implant , with mean values averaged from 2.09 and 18.07 ng / ml). Meanwhile closed values were recorded for all animals in day 0 and day 9 0f treatments (3.75± 0.53 and 2.68± 0.32 ng / ml, respectively).

Discussion

The failure of buffaloes to show overt signs of estrus, together with the wide variation in duration of estrus, are major constraints to the proper adoption of AI for genetic improvement in buffaloes (Bruselli *et al.,* 2001).There is a requirement, therefore, to identify an estrus synchronization treatment that results in reliable and consistent synchronization of stage of the estrous cycle and associated with a relatively high pregnancy rate when combined with fixed – time AI in buffaloes. Previous studies in estrus synchronization in dairy buffaloes undergoing commercial milking have tended to utilize small numbers of animals and it has been difficult to identify a preferred estrus synchronization protocol (Hattab *et al.,* 2000).

In this regard, an important feature of the present study was the relatively large numbers of buffaloes used to evaluate the progesterone (Crestar®) and a new modification of this program by injection of GnRH at the time of insemination in synchronization of ovulation and timed – insemination. It was found that treatment with Crestar® and modified Crestar program both achieved good synchrony in ovulation in buffaloes as judged by the high pregnancy rates especially with the modified Crestar program. The overall pregnancy rate after synchronization with Crestar® was 60%.This study was consistent with previous reports in Italian buffaloes (Rao and Rao, 1983; de Araujo *et al.,* 2002).They reported pregnancy rates 0f 50% and 56%, respectively. Meanwhile the present study was inconsistent with the study of Neglia *et al.,*(2003) who reported that ,pregnancy rate was 28% after synchronization with PRID® .The later study was conducted during the transition to seasonal anestrus for Italian Mediterranean buffaloes in Southern Italy. Moreover it was proven that the use of PRID together with PMSG treatment is able to induce fertile estrus in non-cycling buffalo heifers (Barile *et al.,* 2001; Pacelli *et al.,* 2001). The high pregnancy rate in the current study in treated and control groups may be referred to the high body condition scores and all of these animals were not suckling animals. Body condition score (BCS) plays an important role in the reproductive performance of post-partum buffalo cows. Baruselli *et al.,* (2001).They reported that, first post-partum estrus was influenced by BCS at calving; cows with high BCS had an earlier first post-partum estrus and a shorter service period than cows with lower BCS.

Suckling significantly increases the interval from parturition to first estrus in buffalo. Jainudeen *et al.,* (1983) found that in Malaysian Swamp buffaloes that suckled their calves, had showed a significant increase in the interval from parturition to first ovulation than milked buffalo cows. An earlier resumption of ovarian activity in milked rather than suckled buffaloes was found by El-Fouly *et al.,* (1976). These authors report that only 38 percent of suckled buffaloes restored ovarian activity within 90 days from parturition. This result disagreed with that reported by Janudeen *et al.,* (1983). The extension of anoestrus period due to calf suckling is also reported by Usmani *et al.,* (1990). They found a post-partum estrus cyclicity resumption delayed by three to four weeks due to the practice of let buffaloes be suckled by their calves, before each milking, to stimulate milk let down. Arya and Madan (2001) also found a longer interval from parturition to

first observed estrus and a longer service period in suckled than weaned buffaloes

In the current study ,a high percentage of pregnancy rate was recorded for the second group (group B) .This is consistent with Rastegarnia *et al.,* (2004) who concluded that injection of 100 microgram of Gonadorelin is the most effective dose to induce ovulation in river buffalo (Bubalus bubalis) .

Regarding progesterone concentration, in the current study, it was 3.75± 0.53, 6.75± 0.73 and 2.68± 0.32 ng / ml at zero, 7th and the 9th day respectively. This value agreed with the report of Hattab *et al.,* (2000); Campanile *et al.,* (2005) .Peak progesterone values have been recorded about 15 days after estrus (Bachlaus *et al.,* 1979; Arora and Pandey, 1982; Takkar *et al.,* 1983).

In the current study the highly significant increase was recorded after 7 days of Crestar implant, this is consistent with Ahmed *et al.,* (1977), who reported that, the first significant increase in progesterone concentration occurs about 7 days after estrus in normal cycling buffaloes.

Conclusion

The use of Crestar® as progesterone implant was useful as an aid for ovulation control and timed insemination in the Egyptian buffalo cows .However GnRH injection at the time of insemination significantly improved the pregnancy rate.

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تقييم برنامج غرسة كرستار و كرستار المعدل كإحدى وسائل توحيد التلقيح الموقوت فى الجاموس المصرى الحلاب تحت ظروف الإنتاج المكثف .

أجريت هذه الدراسة على عدد ٢٠٤ جاموسة حلابة موزعة على موسمى الإنتاج الثانى والثالث فى إحدى المزارع الخاصة فى مصر وكانت هذه الحيوانات فى حالة صحية وجسمانية جيدة ، هذا بالإضافة إلى خلوها الظاهر من الأمراض التناسلية ، قسمت هذه الحيوانات إلى ثلاث مجموعات كالتالى :- المجموعة (أ) و تشتمل على ٣٠ حيوان و تم معاملتها بوضع غرسة الكرستار التى تحتوى على هرمون البروجستيرون تحت سطح الجلد الخارجى للأذن و فى نفس الوقت حقنت هذه الحيوانات بحقن الكرستار و هو عبارة عن ٢ ملل تحتوى على خليط من هرمونى البروجستيرون و الاستروجين ، فى اليوم السابع تم حقن هذه الحيوانات بالبروستاجلاندين F2 و بعدها بيومين ، أى فى اليوم التاسع تم إزالة الغرسة من الأذن و فى نفس الوقت تم حقن ٤٠٠ وحدة دولية من هرمون مصل الفرس الحبلى ثم لقحت هذه الحيوانات بعد ٥٦ ساعة من إزالة الغرسة ، المجموعة (ب) و تشتمل على ٢٤ حيوان تم معاملتها بنفس المعاملة السابقة مع حقن الهرمون المنبه للغدة النخامية أثناء التلقيح .

المجموعه (ج) و تشتمل على ١٥٠ حيوان و هى عبارة عن مجموعة قياسية و لقد تركت هذه الحيوانات مع الطلائق فى الحظائر دون أية معاملات هرمونية ، تم أخذ عينات دم من هذه الحيوانات فى اليوم الأول و اليوم السابع واليوم التاسع من المعاملات لقياس مستوى هرمون البروجستيرون فى مصل الدمِ، لوحظت هذه الحيوانات لإستبيان حالات الشياع الظاهر بها وتُلمّستْ بشكل مستقيمي لتشخيص الحمل بعد ٥٠ يوما من التلقيح لتحديد العشار من عدمه ، كَشفتْ نتيجةُ الدراسةِ الحاليةِ بأنّ رؤية إشاراتِ الشياع الظاهر فى المجموعات أ ، ب ، ج هى ٢٠ ، ١٦,٧ ، ٢٢ % على التوالى ، بينما سجلت المجموعة (ب) زيادة معنوية فى نسبة العشار عن باقى المجموعات ، بالنسبة إلى مستوى البروجستيرون سجلت أعلى القراءات فى اليوم السابع من الغرسة مقارنة بساعةِ الصفرو اليوم التاسع ، نستنتج من هذه الدراسة ١ – برنامج غرسة الكرستار هو برنامج فعال لإستخدام التلقيح الموقوت فى الجاموس وهو ما يساعد كثيرا فى إجراء عملية التحسين الوراثى . ٢ – حقن الهرمون المنبه للغدة النخامية أثناء عملية التلقيح يحسن من نتائج العشار .