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Comparative Efficacy of Ovsynch and Heatsynch Protocols Assessed by Transrectal Ultrasonography and Serum Progesterone in Egyptian Buffalo Heifers

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Abstract

To evaluate the comparative efficacy of Ovsynch and Heatsynch protocols with respect to ovarian response, estrus, pregnancy rate and progesterone concentrations, 29 cyclic buffalo heifers were assigned randomly to one of four treatments: G-P-G treatment (n=8) received two GnRH doses, one seven days before and the other two days after a single dose of $PGF_{2\acute{a}}$; G-P-EB1 treatment (n=8) was given estradiol benzoate (EB) one day after $PGF_{2\acute{a}}$ treatment instead of the second GnRH dose; G-P-EB2 (n=8) and G-P-ECP (n=5) treatments received EB or estradiol cypionate instead of second GnRH in G-P-G protocol, respectively. Ovarian ultrasonography and expression of estrus signs were monitored twice daily until the fifth day after the last hormone dose. Our findings indicated a more obvious estrus response to estradiol than the second GnRH dose. The longer estrus duration observed in response to estradiol (32 to 34 h) could provide more time for estrus detection and insemination in these animals. The pregnancy rates to first insemination recorded for the different estradiol-based protocols (20.0 to 37.5%) indicate that estradiol esters may be used to replace the second GnRH injection of the Ovsynch protocol to induce ovulation and obtain a satisfactory reproductive outcome.

Keywords: Buffalo, Estradiol, Heatsynch, Ovsynch, Progesterone, Ultrasonography.

Introduction

Precise manipulation of follicular development is needed to achieve better synchrony of ovulation and improve fertility of a herd; therefore several estrus and ovulation synchronization protocols have been developed. The Ovsynch protocol, which was developed in the cow and successfully used in cyclic buffaloes (Paul and Prakash, 2005), is based on a three- injection schedule: one

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GnRH injection 7 days before and another 2 days after a single injection of $PGF_{2á}$ (Pursley *et al*., 1995). Recently, three-injection estrus-synchronization protocol Heatsynch was also developed in cattle based on the use of estradiol cypionate (Pancarci *et al*., 2002) or estradiol benzoate (Barros et al., 2000) in place of the second GnRH injection in the Ovsynch protocol. The main advantages of Heatsynch are reduced hormone costs (Lefebvre and Block, 1992) and somewhat easier scheduling and implementation. Both GnRH and estrogens can induce atresia or ovulation of dominant follicles and synchronize the emergence of a new follicular wave (Burk *et al*., 2003). An estradiol induced LH surge will last approximately 10 h, which is longer compared with a spontaneous LH surge induced by GnRH (Pancarci *et al*., 2002). In Egypt, more than 90% of buffaloes are raised in small holdings (1-4 animals) without bull, such that there is little opportunity for behavioral interaction among estrous animals (El-Kirabi, 1995) and consequently poor estrus detection, which limits the use of artificial insemination (AI). Singh (1990) has shown that fertility to natural mating is better than AI in terms of conception rates (60.2 vs. 39.7%), number of services per conception (1.7 vs. 2.5) and pregnancy rates (33 vs. 30%). The aim of the present study was to compare the effects of four synchronization protocols on the estrus and ovarian activity, serum progesterone and fertility to natural breeding. In particular, to determine the possible effects of using estradiol esters in place of the second GnRH injection of the Ovsynch protocol in buffalo heifers, because the former hormones are less expensive.

Materials and Methods

1. Animals

This study was conducted on 29 nulliparous, non-pregnant, cyclic buffalo heifers (as determined by the observation of CL in the ovaries in several ultrasound examinations) free from anatomical or reproductive disorders; kept at the Military farm, Tal El Keber, Ismailia, Egypt, during the period June to September, 2010. Age was 2 to 2.5 years and mean body weight (BW) was 399±8 kg. Body condition score according to Campanile *et al*. (9) was 3 to 4 (1=emaciated and 5=obese). Each animal was fed a daily ration comprised of 4 to 6 kg concentrate mixture (dry matter (TDN); 65%, ash; 9% , crude protein; 16%, lipids; 2%, dietary fiber; 15% and moisture 12%) in addition to 4 kg corn silage and 10 kg fodder corn, as green fodder, and was given free access to fresh water, wheat straw and mineral licks.

2. Experimental design and hormone treatment

Each buffalo heifer was assigned randomly to either of four treatments as illustrated in the timelines in Figure 1. Animals in treatment 1 (G-P-G) (n=8) were subjected to the Ovsynch protocol

as described by Pursley *et al*. (1995). In this protocol, on Day –9 before the last injection and on Day 0, the animals received a 10 ìg dose of GnRH analogue, iv (Buserelin acetate; Receptal®, Intervet International B.V.; equivalent to 2.5 ml), beside an intravulvomucosal (ivm) injection (Baruselli *et al*., 2003) of 12.5 mg natural $PGF_{2á}$ analogue (Dinoprost Tromethamine; Lutalyse®; Pfizer, Belgium NV/SA, equivalent to 2.5 ml) on Day –2. Treatment 2 (G-P-EB1) animals (n=8; BW 394±17 kg) were subjected to the Heatsynch protocol according to Patel *et al*. (2009). This consisted of an iv injection of buserelin acetate followed 7 days later by $PGF_{2á}$ analogue ivm, and then 24 h later (Day -1) by 5 mg of estradiol benzoate im (Folone®, Misr co., Egypt; equivalent to 1 ml). The heifers in treatment 3 [(G-P-EB2) (n=8, BW 393±15 kg)] and treatment 4 [(G-P-ECP) (n=5; BW 409±18 kg)] were treated as in treatment

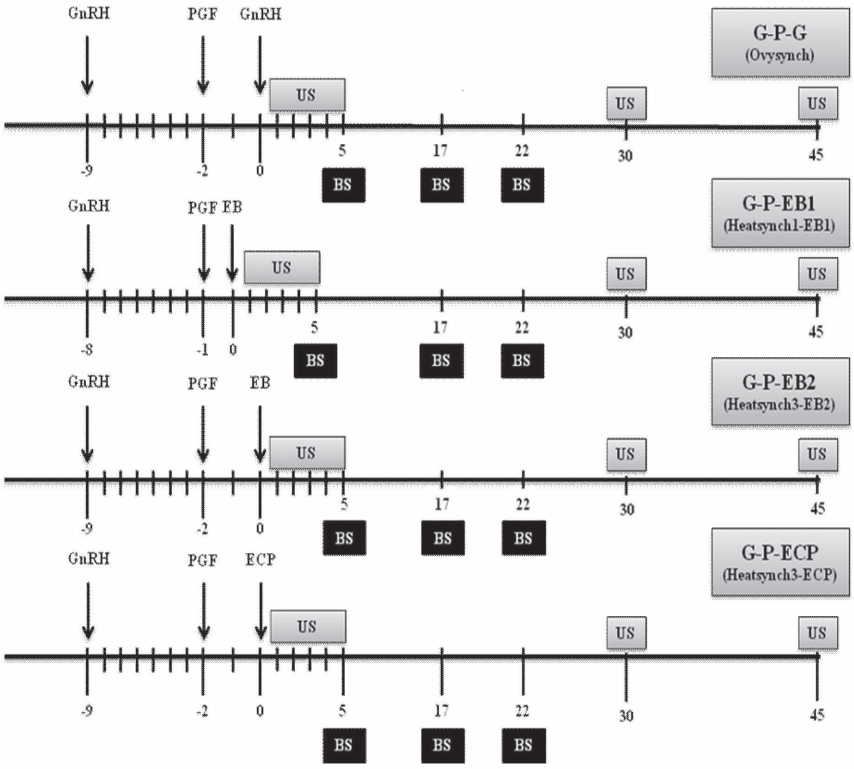

Fig. 1: Timelines (d) used for estrus-synchronization using GnRH=10µg (buserelin acetate), $PGF_{2á}$= 12.5 mg (dinoprost thromethamine), EB= 5.0 mg (estradiol benzoate), ECP= 3.75mg (estradiol cypionate. BS= blood sampling.

1, except that the 5 mg of estradiol benzoate or 3.75 mg estradiol cypionate (O.S.T.®, Vemedim, Vietnam, imported by Golf Vet. Stores, K.S.A., Riyadh; equivalent to 5 ml) was given i.m. on Day 0, respectively.

Estrus detection and breeding

All animals were monitored twice daily for estrus signs (standing to be mounted, bellowing, redness and swelling of vulva and clear mucous discharge) for five days after the last injection and those in estrus were mated naturally. Pregnancy was confirmed by ultrasound on 30 and 45 days after receiving the last hormone dose. The reproductive variables recorded were: i) the time to the onset of estrus (in h), ii) synchronized estrus duration (h), iii) synchronized conception rate (CR, i.e. the number of cows showing sustained serum progesterone > 1.0 ng/ml on Day 22 after the last hormone dose divided by the number of animals inseminated (Batra *et al*., 1979), and iv) synchronized pregnancy rate (number of pregnant heifers divided by the number of treated heifers).

Blood sampling and hormone assay

Serum progesterone (P4) concentrations in the synchronized animals were assayed on Days 5, 17 and 22 after the last hormone dose to confirm ovulation, luteal phase activity and pregnancy status, respectively. Blood samples (6 ml) were collected from the jugular vein and the harvested sera by centrifugation (1200 ×g for 5 min) were kept at -20 °C until assayed for progesterone in doubleton using the direct ELISA EiAsy ™ Way progesterone kit (DBC; Diagnostics Biochem Canada Inc®; London, Ontario, Canada) according to the manufacturer's instructions. The intra- and inter-assay coefficient of variation and assay sensitivity of P4 is 10.4%, 11.4% and 0.1 ng/ml, respectively.

Ovarian ultrasonography

The ovaries were assessed ultrasonographically using a 5 MHz linear transrectal transducer (Ultrascan 900®, ARI Alliance Medical Inc., Canada) one day after each hormone dose, and thereafter twice-daily for five days after the last treatment dose. The animal was considered to have ovulated or not in subsequent examination (s), the dominant follicle either disappeared as an ovulatory follicle or became atretic as a dominant non-ovulatory follicle (Taneja *et al*., 1996). After counting the total number of available antral follicles e" 3 mm, detected follicles were classified into small (< 0.5 cm), medium (0.5-1.0 cm) or large (e" 1.0 cm) as described previously (Wolfenson *et al*., 1994). Diameters (cm) of luteal structures and cavities were determined on the basis of CL size.

Statistical analysis

Data were tabulated and expressed as mean ± SEM. Significant differences between treatments were determined by Chi-square and one way ANOVA using the SPSS package (ver. 14) according to

Armitage *et al.* (2002). The level of significance was set at $p < 0.05$.

Results

Synchronized estrous response and estrus duration

Regardless of the estrus-synchronization protocol (Table1), an estrous response was detected in 23 out of the 29 buffalo heifers (79.3%). By treatment group, the estrus responses differed ($P<0.05$) between G-P-G, 4/8 or 50.0% and G-P-EB1, 8/8 or 100%; the corresponding values in G-P-EB2 and G-P-ECP were 7/8 or 87.5% and 4/5 or 80%, respectively. Durations of estrus also differed ($P<0.05$) according to the synchronization protocol, tending ($P=0.055$) to be shorter in the G-P-G group (14.4±2.4 h) than the other groups (Table 1).

Ovarian follicular activity

Monitoring of the ovarian activities before and following the last GnRH or estradiol ester dose indicated no significant differences in total follicle counts between the different synchronization protocols. However, follicle populations were slightly higher in response to EB treatment than pre-treatment values (G-P-EB1, 4.6±0.5 vs. 3.4±0.5; G-P-EB2, 4.8±0.6 vs. 3.1±0.6). The size of the pre-ovulatory follicle failed to differ among treatments (Table 1). Although similar ovulation rates were recorded in the four treatments, the time interval from the last hormone dose until the detection of ovulation was longer ($P<0.05$) in G-P-EB2 than G-PG and G-P-ECP treatments.

Ovarian luteal activity

The time (h) elapsed from ovulation to the detection of an early corpus luteum was shorter ($P<0.05$) in G-P-G and G-P-EB1 compared to G-P-ECP or G-P-EB2. The maximal diameters of the CL measured on Day 5 were similar in the groups G-P-G, G-P-EB2 and G-P-ECP. However, the CL was considerably smaller ($P<0.05$) in G-P-EB1 compared with G-P-G (17.2 ± 0.2 vs. 20.0±0.7 mm).

Serum progesterone concentration

Serum progesterone concentrations in responding animals are presented in Figure 2. Differences among treatments in progesterone concentration was not likely detected in the ovulating animals during the early- (Day 5), and mid- (Day 17) luteal stages but rather differences in pregnancy status (on Day 22) did vary among the treatments. Progesterone concentration was significantly ($P<0.05$) lower in animals of G-P-ECP group than that recorded in other treated groups on Day 22. (early-luteal), Day 17 (mid-luteal) and Day 22 (pregnancy) according to estrus-synchronization treatment.

Pregnancy status

Although no differences were detected by Chi-square analysis among the treatments, conception and pregnancy rates did differ numerically (Table 1). Thus, when arranged in decreasing order

Table 1: Behavioral and ovarian responses, and conception and pregnancy rates observed in Egyptian buffalo heifers subjected to different estrus-synchronization protocols

Factor assessed	Estrus-synchronization protocol			
	G-P-G	G-P-EB1	G-P-EB2	G-P-ECP
Synchronized estrus response	4/8[c]	8/8[a]	7/8[b]	4/5[b]
Synchronized estrus duration (h)	14.4±2.4 [b]	34.7± 5.8 [a]	32.0±5.1 [a]	33.0±5.8 [a]
No. of pre-treatment follicles	3.6±0.4 [a,A]	3.4±0.5 [a,B]	3.1±0.6 [a,B]	3.2±0.9 [a,A]
Size of pre-ovulatory follicle (mm)	13.3±0.6 [a]	14.3±2.3 [a]	13.3±0.9 [a]	13.0±1.3 [a]
Time of ovulation (h)	36.0±3.5 [b]	42.0±6.0 [ab]	53.0±5.8 [a]	40.0±4.0 [b]
No. of post-treatment follicles	4.3±0.5 [a,A]	4.6±0.5 [a,A]	4.8±0.6 [a,A]	4.0±1.0 [a,A]
Time of early CL detection (h)	66.0±6.0 [a]	66.7±9.6 [a]	90.0±6.0 [b]	96.0±3.9 [c]
Size of early detected CL (mm)	15.7±0.6 [a]	18.8±1.6 [a]	16.2±1.3 [a]	16.7±0.7 [a]
Size of CL on Day 5 (mm)	20.0±0.7 [a]	17.2±0.2 [b]	20.9±1.3 [ab]	18.4±1.1 [ab]
Synchronized ovulation rate	4/8 (50.0%)[a]	3/8 (37.5%)[a]	4/8 (50.0%)[a]	2/5 (40.0%)[a]
Synchronized conception rate	3/4 (75.0%)[a]	3/8 (37.5%)[a]	4/7 (57.0%)[a]	2/4 (50.0%)[a]
Synchronized pregnancy rate	3/8 (37.5%)[a]	2/8 (25.0%)[a]	3/8 (37.5%)[a]	1/5 (20.0%)[a]

Values with a different small (a, b,…) or capital (A, B,...) superscript within the same row or column differ significantly at $P<0.05$, respectively. All times are referred to the time of the last hormone injection. G = GnRH, P = $PGF_{2\acute{a}}$, EB= Estradiol benzoate, ECP= Estradiol cypionate, CL= Corpus luteum.

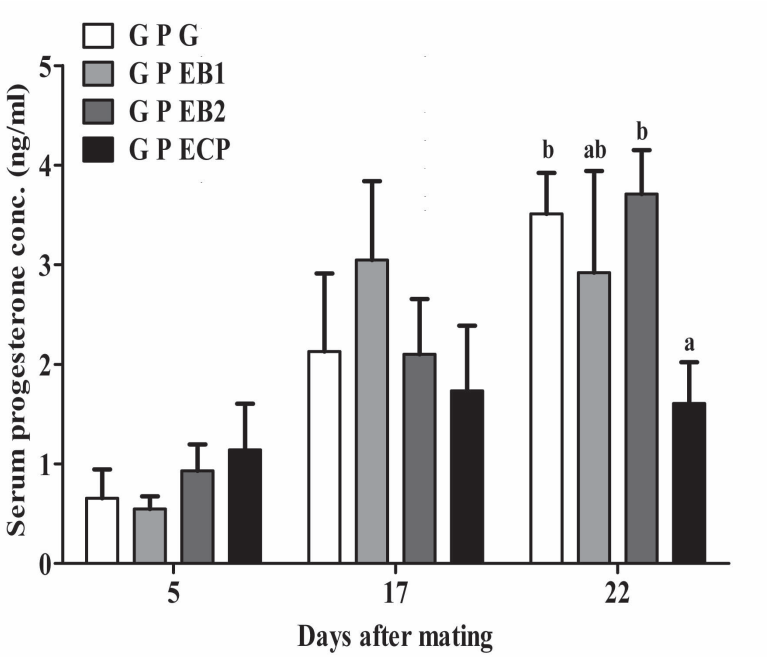

Fig. 2: Serum progesterone concentrations (ng/ml) recorded in the buffalo heifers on Day 5

of magnitude, the synchronized conception rate of the treatment groups was highest in G-P-G, followed by the groups G-P-EB2 and G-P-ECP, and finally, the G-P-EB1 group. In contrast, the highest synchronized pregnancy rate was recorded in G-P-G and G-P-EB2 while lower rates were recorded in G-P-EB1and G-P-ECP.

Discussion

The world buffalo population has been estimated at 194.1 million, of which 4.0 million are in Egypt (FAOSTAT, 2010), and figures continue to rise. This has determined an interest in using assisted reproductive biotechnologies to improve production and reproduction in this animal. In our study, we were able to detect an improved response in Egyptian buffalo heifers to the Heatsynch [GnRH-$PGF_{2á}$-EB or ECP] than Ovsynch [GnRH-$PGF_{2á}$-GnRH] protocol in terms of behavioral estrus, ovarian activity and pregnancy rate. Thus, the use of an estradiol ester (EB or ECP) to replace the second GnRH dose of the Ovsynch protocol managed to successfully induce estrus with a long duration in this animal (32 to 34 h), besides, the ability to induce ovulation (37.5-50.0%) with a satisfactory pregnancy rate (20.0-37.5%).

Our results indicate that the addition of estradiol esters to GnRH-$PGF_{2á}$ treatment increased the percentage of

animals expressing estrus, though some was false estrus. Improvements in the rates of heifers experiencing standing estrus or ovulation have both been linked to increased plasma estradiol concentrations after $PGF_{2á}$ injection (Borman *et al*., 2003). Dairy cattle revealed an improved synchrony of estrus when treated with EB within 24 h (100%) rather than 48 h (87.5%) of $PGF_{2á}$ administration and this was attributed to the timing of treatment in relation to the stage of follicle growth (Cavalieri *et al*., 2006). Hence, the stage of the estrous cycle when estradiol or GnRH treatment is given may be an important factor affecting the synchronization of follicular wave emergence (Lefebvre and Block, 1992).

Duration of estrus recorded in our buffalo heifers was approximately 35, 32, 33 or 14 h for the G-P-EB1, G-P-EB2, G-P-ECP and G-P-G treatments, respectively. This means we may expect to achieve considerably longer estrus duration in buffaloes if we use estradiol rather than GnRH after $PGF_{2á}$ treatment. The differences in estrus duration could be attributed to failure of buffaloes to display overt signs of estrus in association with a wide variation of estrus duration in these animals (Awasthi *et al*., 2007). Thatcher *et al.* (2002) reported that estradiol injection induces a longer duration LH surge than GnRH. Williams et al. (2002) reported that 28.9% of the Ovsynch-treated *B. indicus* heifers exhibited natural estrus between the first GnRH and the assigned time of fixed time artificial insemination, suggests that follicular development occurs faster in ovsynchronized heifers. Consistently, in our study, estrus duration failed to significantly vary between the estradiol treated groups, but was significantly shorter in the G-P-G treatment. The reason for G-P-G heifers showing estrus for a shorter period of time is that the second GnRH injection might induce an LH surge thereby down-regulating aromatase enzyme activity in follicular granulosa cells. Earlier work (Burk *et al*., 2003) established that the intramuscular administration of large doses (5 to10 mg) of ECP led to high plasma concentrations of estradiol prolonged from 98 to 170 h. This suggests that reduced ECP doses may be useful in estrus-synchronization regimens.

Here, we examined how the timing of EB or ECP administration might affect the synchronization of ovulation and observed that the time interval from estradiol treatment to the onset of ovulation was longer in G-P-EB2 than G-P-EB1 or G-P-ECP. These results indicate that the synchronization of ovulation can be enhanced by the use of estradiol (EB or ECP), but that the timing of treatment relative to the stage of follicular development can affect outcome (Cavalieri *et al*., 2006). Besides, it seems that the interval to the LH surge and consequently ovulation rather than the time between the onset of the LH surge and ovulation is crucial for the different effects of EB1, EB2 or ECP on the time of ovulation (Stevenson *et al*., 2004). Former studies have shown

that used in the Heatsynch regimen, EB induced ovulation at 66.5 ± 3.5 h in 5 of 11 dairy cattle heifers (Castilho *et al*., 2000) and at 45.38±2.03 h in 7 of 10 lactating cows (Barros *et al*., 2000), whereas the rate of ovulation in response to ECP in dairy cows was 86.5% and the mean interval to ovulation was 55.4±2.7 h (Burk *et al*., 2003). In Murrah buffaloes, EB induced ovulation at 50.0±2.0 h (Mohan and Prakash, 2010), while in animals subjected to Ovsynch, ovulation occurred 23.3±1.3 h after the second-GnRH dose (Paul and Prakash, 2005).

When we examined the changes in serum progesterone (P4) concentrations produced during the early- and mid-luteal phases and on Day 22, significant effects of the estrus-synchronization protocol were only observed 22 d after the last hormone injection, which confounded with pregnancy status. At this time, lowest P4 concentrations were recorded in the G-P-ECP group, while highest concentrations were detected in G-P-G and G-P-EB2. The explanation for this difference could be the development of a more functional CL that produces more progesterone, thus enhancing embryonic growth and fertility (Thatcher *et al*., 1994).

Finally, synchronized conception and pregnancy rates in buffalo heifers undergoing estrus-synchronization differed numerically yet not significantly among treatments probably because of the low numbers of animals in each group. The highest synchronized conception rate was recorded in G-P-G followed by G-P-EB2, G-P-ECP, and lastly by G-P-EB1. However, synchronized pregnancy rates to first insemination were 37.5% for G-P-G and G-P-EB2, 25.0% for G-P-EB1 and 20% for G-P-ECP. Such low pregnancy rates relative to conception rates could perhaps be the consequence of the reduced fertility of an aged follicle/oocyte induced by estradiol injection. Thatcher *et al*. (2001) added that the sub-luteal plasma progesterone concentrations after insemination is associated with lower pregnancy rates and therefore an injection of hCG on day 5 post-insemination resulted in induction of an accessory CL, increased plasma progesterone concentrations and increased conception rates. Our findings, however, also support the hypothesis that ECP may improve embryo survival over the use of EB given 24 h after the $PGF_{2á}$ dose although further work is needed to confirm this conclusion relative to the ECP dose used. Previous results have suggested the Ovsynch protocol (GnRH + $PGF_{2á}$ + GnRH) is effective at synchronizing ovulation and that its use in embryo transfer technology is likely to improve pregnancy rates in the buffalo (Awasthi *et al*., 2007). Bartolome *et al*. (2005) detected no difference in pregnancy rates on Days 27, 45 and 90 post-insemination between cows in the Ovsynch (25.2, 17.5, and 13.9%, respectively) and Heatsynch (25.8, 19.9, and 16.1%, respectively) groups.

In conclusion, estradiol can be utilized as an alternative to induce ovulation in

place of GnRH estrus synchronization treatment. The difference between the interval EB/ECP injection to insemination (32–34 h estrus duration) of the Heatsynch and GnRH injection to insemination (14.4 h estrus duration) of the Ovsynch protocol makes the Heatsynch protocol used here more easily applicable to buffalo heifers.

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