EFFECT OF LUTEOLYTIC HORMONES TREATMENT BEFORE AND DURING SUPEROVULATION ON ESTROUS SYNCHRONIZATION IN BUFFALOES

K.M.E. MOHAMMED[1], S.T. ISMAIL[2], M. Y. ABBOUD[3] AND M.S.TAWFIK[1]

1Animal Reproduction Research Institute, Agricultural Research, Centre, Dokki,Giza, Egypt.

2 Faculty of Vetrinary Medicine, , Cairo University.

3 Faculty of Veterinary Medicine, Alexandria University,

(Manuscript received 20 Octobre 1998)

ABSTRACT

Sixty-six trials of estrous synchronization were done in buffaloes (31, before superovulation and 35. during superovulation). The donors synchronized before superovulation were injected IM during luteal phase with a single dose of 5 ml Lutalyse, 5 ml Ilerin or 3.5 ml Prosolvin. Twenty-four hours after luteolytic drug administration, each animal was observed 3 times daily for detecting the onset of the estrus. If the animal was not noticed in estrus up to 5 days after Prostaglandin (PG) injection, luteolytic drug was repeated again with the same drug and dose, 11 days apart. Response traits for donor included time from treatment to estrus and duration of heat.All donor animals detected in heat were injected IM at mid luteal phase with superovulatory hormones (PMSG). Two days later, the animals received double doses with 12 hours interval of lutalyse (5 ml, each), Ilerin (5 ml, each), or Prosolvin(2 ml each) according to the luteolytic hormone used before superovulation.

Synchronization before superovulation indicated that, the mean time for the onset of estrus was 48.25±1.24, 43.83±4.12 and 50.24±1.05 hours, for Lutalyse, Ilerin and Prosolvin, respectively. The mean duration of estrus phase was correspondingly 19.75 ±1.82, 20.17±1.32 and 21.74±1.96 hours. The overall mean lengh of estrus phase was 20.40±0.88 hours. No significant difference between the there treatments. In the synchronization during superovulation, the mean time intervals from treatment to onset estrus was diminished to 31.0±3.03, 22.0±3.86 and 32.67±4.96 hours for Lutalyse, Ilerin and Prosolvin, respectively.The overall mean was 28.14±2.50 hours. No significant difference for the three treatments on the this criterion. However, the differences between these values before and during superovulation were highly significant ($P \leq 0.01$). Concerning the length of estrus phase, its means were22.2±2.41, 20.0±2.54 and 24.67±3.03 hours for Lutalse, Ilerin, and Prosolvin, respectively. The overall mean length of heat was 21.92±172 hours. No significant differenceses were obsered between the different treatments on the heat duration. Moreover, no significant differences were observed between these values before and during superovulation.

INTRODUCTION

Estrous synchronization can result in planned approach to breeding program me, increase the effectiveness of AI and overcome the difficulties in estrus detection. In addition, it is essential for the success of embryo transfer technique. Difficulty in detectecting buffaloes in estrus is one of the most important factors limiting the progress in the reproductive perfomance in this species. Estrous behaviour of bufflo differed from that of cattle in the lack of overt homosexual behaviour, and in the secretion of large amounts of clear estrual mucus. So,trials to detect estrus in buffaloes without the use of a teaser bull, by frequent observation or by the use of teaser bull are not reliable (Ishaq, 1956 and Drost and Cripe, 1985). However, the use of androgenized cow mounted like a bull when cows were in heat assisted in the detection of heat in 69% of the animals in the total herd heifers (Drost *et al.*,1985). One of the merits of the synchronization process is to overcome the difficulty in estrus detection, therefore, the use of synchronization technique followed by the use of a teaser bull did help in detecting 87% of estrous buffalo (Drost *et al.,* 1988).

The present investigation aimed to compare the influence of different types of synthetic PG analogues on estrous synchronization before and during superovulation. Three commercial compounds of synthetic PG (Lutalyse, Ilerin and Prosolvin) were used to study this purpose.

MATERIALS AND METHODS

A total of 16 adult buffalo-cows were used more than one time for estrous synchronization before and during superovulation, 10 of them were raised at ARRI, while, the others (6) were bred at EL-Khanka governmental farm. The age of these animals ranged from 5-11 years. Three hormonal treatments were used for synchronization of the etrous cycle in buffalo-cows. In treatment I, Lutalyse (Upjohn Co., Kalamazoo, USA; each ml contains 5 mg Dinoprost) was used as a luteolytic hormone (10 trials before and 11during superovulation). Tratment II, Iliren (Hoechst Veterinary Gm bH, Germany; each ml contains 0.150 mg Tiaprost) was used for synchronization (13 trials before and 15 during superovulation) and treatment III, Prosolvin (Intervet International, B. V., Holland; each ml contains 7.5 mg Luprostiol) used for estrous synchronization (8 trials before and 9 during superovulation).

Estrous synchronization before superovulation

In all trials, when CL could be palpated rectally, a single dose of 5 ml

Lutalyse, 5 ml Iliren or 3.5 ml of Prosolvin was injected IM. Two doses of each drug, 11 days apart, was used when a clear CL was not detected. Twenty-four hours after admininistration of the luteolytic drug, each donor was carefully noticed 3 times/day for detecting the onset of heat by aid of a teaser. Response traits for each buffalo included, time from treatment to heat (it is expressed as the period per hours from injection of the last dose of drug to the onset of symptoms of standing heat), duration of heat (calculated from the first to the last hour of standing heat).

Estrous synchronization during superovulation

PMSG was injected IM at the mid-luteal phase . Two days later, luteolysis of the old CL was aimed by injecting double doses, 12 hours apart of Lutalyse (3ml and 2 ml), Ilerin (5m each)or prosolvin (3.5 each). Twelve hours after the last treatment dose, the animal was isolated in a separate barn and carefully observed 3 times/day for the onset of standing heat by the aid of a fertile bull. Response traits for each animal included, treatment to estrus interval (period per hours from injection of the last dose of luteolytic drug to the first successful mount), duration of heat (from the first to last hour when a definite mounting occurred).All statistical analysis were done according to Snedecor and Cochran (1980).

RESULTS

The influence of Lutalyse (Dinoprost), Iliren (Tiaprost) and Prosolvin (Luprostiol) treatments on synchronization of the estrous cycle of buffalo-cows before and during superovulation is shown in Table1.

Estrous synchonization before superovulation

All animals showed estrus response following application of the three treatments. The average time for the onset of estrus was 48.25 ± 1.24 ± 43.8 3±4.12 and 50.24 ± 1.05 hours after administration of Lutalyse, Iliren and Prosolvin, respectively. The overall mean was 46.67±2.09 hours. The average length of the estrous phase was correspondingly 19.75±1.82,20.17±1.32 and 21.74±1.96 hours. The overall mean length of estrous phase was 20.40±0.88 hours. Statistical analysis revealed non-significant difference in the three treatments.

Estrous. synchronization during superovulation

All donor buffalo-cows that received Lutalyse, and Prosolvin showed estrus response, whereas, one of the buffaloes that were injected with Ilirin failed to show any estrual behaviour. The mean intervals from treatment to the onset of estrus

was diminished to 31.00 ± 3.03 ± 22.00±3.86 and 32.67±4.96 hours after administration of Lutalyse, Iliren and Prosolvin, respectively, with an overall mean of 28.14±2.50 hours. Statistically, there was no influence for the different treatments on this criterion. However, the differences between these values before and during superovulation were statistically highly significant ($p<0.01$). Concerning the length of estrous phase, it averaged 22.2±2.41 after application of Lutalyse, 20.00±2.54 after administration of Iliren and 24.67±3.03 hours after Prosolvin injection. The overall mean length of estrous phase was 21.92±1.72 hours. Analysis of variance revealed non-significant effect for the different treatments on the duration of the heat period. Moreover, its values before and during superovulation were non-statistically different.

Table 1. Influence of different treatments on synchronization of estrous cycle before and during superovulation in buffalo-cows

Treatment drugs	State of Synchronization	No.of Treated buffaloes	No.of buffaloes in estrus(%)	Treatment to onset of estrus interval (h)	Range (h.)	Duration of estrus(h)	Range (h.)
Lutalyse	Before Superovulation	10	10 (100)	48.25±1.24**	44-50	19.75±1.82	14-24
	During Superovulation	11	11(100)	31.00±3.03	17-37	22.20±2.41	17-32
Iliren	Before Superovulation	13	13 (100)	43.83±4.12**	26-54	20.17±2.54	15-24
	During Superovulation	15	14 (92.86)	22.00±3.86	8-34	20.00±2.54	16-30
Prosolvin	Before Superovulation	8	8 (100)	50.24±1.05	42-61	21.74±1.96	18-29
	During Superovulation	9	9 (100)	32.67±4.96	22-43	24.67±3.03	20-32
Overall mean	Before Superovulation	31	31(100)	46.67±2.09**	26-61	20.40±0.88	14-29
	During Superovulation	35	35 (97.14)	28.14±2.50	8-43	21.92±1.72	16-32

Tvalue :** Significant at P<0.01 h.:hours

DISCUSSION

In the present investigation, synchronization of buffalo-cows by the use of either Lutalyse, Iliren or Prosolvin followed by parading a teaser bull, assised in detecting 100% of the estrous animals. It was clear that, unless those buffalo-cows that were spotted by chance in estrus, all animals assigned for superovulation needed to undergo with two processess of synchronization. The first, before the start of the superovulatory regimen, to determine the midluteal phase for the beginning of the superovulatory treatment. The estrus of this synchronization pased without breeding. The second, synchronization was during the regimen of superovulation. This, usually started two days after the beginning of the superovulatory treatment, to induce luteolysis, and consequently, ovulation of the newly formed follicles. Breeding was allowed during this estrus.

Before starting the superovulatory regimen, all the buffaloes included in the present investigation, expressed estrual symptoms after the application of Lutalyse, Iliren and Prosolvin. Similarly, Ramamohana-Rao and Venkateswara-Rao (1978) observed that all buffalo-cows showed estrual response after the treatment with 2 doses of 500 µg Cloprostenol, 11 days apart. However, lower values (72-85%) were reported in the same species that received PG or its analogue (Prasad, *et al.*, 1979 and Jindal, *et al.*, 1988). During the regimen of superovulation, only one buffalo-cow from the Iliren group failed to show estrual behaviour. The overall mean (97.14%) of the buffaloes experienced estrus in this study is similar to the results (89.5-100%) of Sharifuddin and Jainudeen (1984), Vlahov *et al.* (1985), Karavinov (1986), El-Nahata (1989) and Ismail *et al.* (1991).

Based on the mean time interval from treatment to the onset of estrus, Iliren buffalo-cows expressed estrus 7 and 5 hours earlier than the Prosolvin and Lutalyse buffalo-cows, respectively. During the regimen of superovulation, Iliren buffalo-cows were also seen in standing estrus 11 and 9 hours before the Prosolvin and Lutalyse cows, respectively. On the other hand, before superovulation treatment, lutalyse buffalo-cows tended to be more tightly synchronized over time (44-50 hours) than the Iliren (26-54 hours) and Prosolvin cows (42-61hours). The variation in time to estrus as indicated by this wide range, especially, in the Iliren and Prosolvin buffaloes, is more likely due to the differences in the time of initiating synchronization during the estrous cycle. Mamond and Seguin (1982), Duan (1984) and Etherington *et al.* (1986) noted that the mean interval from treatment to observation of estrus was shorter in cows injected early (Days 6-8) or late (Days 13-17) as compared to the mid (Days 9-12) estrous cycle. These authors attributed

their findings to the presence of greatest number of large, well developed ovarian follicles (> 8 mm in diameter) on day 8, and again from day 16 until ovulation, and were less evident from days 9-15 of the estrous cycle. Moreover, the size of CL as indicated by serum progesterone concentration at the time of PG treatment was positively correlated to the interval from treatment to estrus (Greve *et al.*, 1983). However, during the superovulation regimens, all buffalo-cows expressed estrus over a period of approximately 20 hours in the three treatments of synchronization.

The mean time intervals from treatment to onset of estrus in the current study was significantly ($P<0.01$) influenced by inducing superovulation. It was diminished from 48.25±1.24 before superovulation to 31.0±3.03 hours during superovulation, 43.83±4.12 to 22.00±3.86 and 50.24±1.05 to 32.67±4.96 after administration of Lutalyse, Iliren and Prosolvin, respectively. Consequently, the overall mean of treatments to standing heat interval decreased from 46.67±2.09 before superovulation to 28.14±2.50 hours during superovulation. Correspondingly, the treatment to estrus interval in the same species decreased from 54 hours (Dharmawardena and Thamatharam, 1980), 75±3.5-77.0±3.8 (Jindal, *et al.*, 1988), and 48-96 (Ramamohana-Rao and Vehkateswera-Rao, 1978, Prasad *et al.*, 1979 and Eissa *et al.*, 1990) before superovulation to 66.00±3.3 (Sharifuddin and Jainudin, 1984) and 42.00±1.48 to 44.8±2.31 hours (Karavinov, 1986) during superovulation. A fairly close interval of 26 hours was reported in buffaloes during the regimen of superovulation by Lalita *et al.*, (1988) and EL-Nahat (1989). In a study on Bulgharian buffaloes, Vlahov *et al.* (1985) noted that average PG standing heat interval was reduced from 60.2±1.13 in rcipient buffaloes to 42.8±1.48 and 45.3±1.55 hours in the FSH and PMSG treatment buffaloes. The evident decrease of the interval from treatment to the appearance of estrous symptoms in superovulated donors is probably, due to the preovulatory increase in concentrations of estradiol which triggers the onset of LH surge (Hansel and Convey, 1983). An early built up of this hormone in superovulated cows, compared with that not superovulated, must contribute to a more rapid onset of estrus and the associated LH surge (Henricks *et al.*, 1973 and Yadov *et al.*, 1986). In the present study, the length of the estrous phase in synchronized buffalo-cows before the beginning of superovulation averaged 20.4±0.88 hours. Statistically, non-significant differences were noted between the Lutalyse, Iliren and Prosolvin applications in this trait. During the regimen of superovulation, the mean length of estrous period (21.92±1.72 hours) was close to that obtined before superovulation. This finding indicates that the lenghth of estrous sphase did not significantly change dut to

superovulation. Fairly similar values of estrous period (24 to 28 hours) were reported in the same species by Hafez (1954), Yadov *et al.* (1986) and EL-Nahata (1989). However, the latter author reported longer estrous periods (35.4±3.50 and 36.3±2.7 hours) in buffaloes superovulated with different doses of PMSG. the author attributed these long estrous phases to the treatments enforced during experimentation.

REFERENCES

1. Dharmawardena, J. and M. Thamatharam. 1980. " Estrous synchronization in Buffaloes (Bubabus bubali)". An. Reprod. Sc., 3(2): 107-112

2. Drost, M. and W.S. Cripe. 1985 " Superovulation and embryo transfaloes". Ist World Buffalo Cong., Cairo, Egypt. December, 27-31, pp., 498-505.

3. Drost, M., A., Alexieve Vlahov K., Karaivanov C.H., W.S., Cripe A.P., Leonards D., Kachera, Q., Palihronov N., Nicolov M. Petrov and A. Dragoev. 1988. Successful non surgical embryo transfer in buffalo. (Bubalus bubalis) in Bulgaria' . Theriogenology, 30: 659 -667.

4. Drost, M., W.S. Cripe and A.R. Richter. 1985. Estrous detection in buffaloes use of an androgenized female. Buffalo J., 1:159-161.

5. Duan, W. 1984. Estrous synchronization and AI in beef heifer with prostaglandin following rectal palpation of a functional corpus luteum. Proc Soc. Theriogenology, 249-252.

6. Eissa, H.M., I.M. Goneim and M.S.S. Abdou. 1990. Response of buffalo cows and heifers with or without palpable corpora to different doses of prostaglandin F2 alpha (Lutalyse). Egypt Soc. Anim. Reprod Fert., III Cong., Cairo. 141-152

7. EL-Nahat, S.S. 1989. Synchronization of estrus and trials on superovulation in buffaloes. Thesis, M. V. Sc., Cairo univ .

8. Etherington, W. G., B.A., Kilme J.E., Burke, M.E. Montgoery and D.C.Wilson. 1986. Pregnancy rates related to stage of estrous cycle at prostglandin treatment in embryo transfer recipient herd. Theriogenology, 25 (6):845-854.

9. Greve, T., Henrik C. and Hyttel. 1983. Endocrine profiles and egg quality in superovulated cows. Nord. Vet. Med., 35:408-421.

10. Hafez E. S. E. 1954. Gonadotropin-induced ovulation in the buffalo. Vet. Rec., 65 (24):364-385.

11. Hensel, W. and Convey E.M. 1983. Physiology of the estrous cycle. J. Anim. Sci., 57: 404-423.

12.Hénricks, D. M., J. R. Hill J.F. Dickey and D.R. Lamond. 1973. Plasma hormone

levels in beef cows with induced multiple ovulations.J. Reprod. Fert., 35:225-233.

13. Ishaq, S.H. 1956. Estrous and applied problems in buffaloesAgric. Pakist., 7: 361-365.

14.Ismail, S.T., M. Drost and W. Cripe. 1991. Ovarian response to different doses of FSH in water buffalo. Egypt Soc., Anim. Reprod. Fert., 303-313.

15. Jindal, R., S.P., Gill M.S. Setia. and P.J. Rettam. 1988. Estrous synchronization in buffaloes using lutalyse. Buffalo Bulletin, Vol. 7 (9)

16.Karavinov, D.H. 1986. Comparative studies on the superovulatory effect of PMSG and FSH in water buffalo (Bubalus bubalis)". Theriogenology, 26:51-59

17. Lalita, D. S.P., Singal I.S., Lohan G.C. Georgie and M.N. Razdan. 1988. Superovulation in Murrah buffaloes . II World buffalo Cong., NewDelhi, December, pp. 23.

18. Mamond, H. and E. seguin. 1982. Temporal factors affecting the response to prostglandin F2 alpha in cows. Proc. Soc. Theriogenology, 166-176.

19. Prasad, A., N.K., Bachlaus R. Arora and R.S. Pandey, 1979. Synchronization of estrous in buffalo heifers with estrumate and fertility with frozen semen. Indian J. Exp. Biol., 16: 801-802.

20. Ramamohana-Rao, A. and S. Venkateswara-Rao. 1978. Synchronization of estrus in buffalo with Cloprestenol. Vet. Rec., 9,23.

21. Sharifuddin, W. and M.R. Jainudeen. 1984. Superovulation and non surgical collection of ova in the water buffalo (Bubalus bubalis). 10 th Int. Cong. Anim. Reprod. Al., June 10-14, pp.240-242.

22. Snedecar, G. W. and W.G. Cochran. 1980. Statistical methods. 7th Ed., The Iowa State Univ., Press, Amer. Iowa, USA.

23.Vlahov, K., C., Karavanov M., Kacheva D.A., Alexiev O. Polihronov and A.Danev. 1985. Studies on superovulation and embryo transfer in water buffalo. Ist. World Cong., 510-512.

24. Yadov, M.C., J.S. Walton and K.E. 1986. Timing of the onset and duration of ovulation in superovulated beef heifer. Theriogenology, 26:509-521.

تاثير المعالجة الهرمونية على احداث التزامن الشبقى فى الجاموس قبل وأثناء التبويض المتعدد

كامل مصطفى السيد ١ , طه اسماعيل ٢, يحى عبود ٣ , محمود صبرى ١

١ معهد بحوث التناسليات - مركز البحوث الزراعية - الجيزة - مصر
٢ كلية الطب البيطري - جامعة القاهرة .
٣ كلية الطب البيطرى - جامعة الاسكندرية

أجريت ٦٦ محاولة لاحداث التزامن الشبقى للجاموس منها ٣١ محاولة قبل احداث التبويض المتعدد و ٣٥ أثناءه . تم حقن الحيونات فى العضل بجرعة من هرمون الليوتاليز والأليرين والبروسولفين. وبعد ٢٤ ساعة من حقن الهرمون لو حظت الحيوانات ثلاث مرات يوميا لمعرفة بداية الشياع. أما الحيونات التى لم تظهر أى علاقات من الشياع حتى ٥ أيام من الجرعة الأولى / فقد حقنت بجرعة ثانية من نفس الهرمون وبنفس الجرعة فى اليوم الحادى عشر من الجرعة الأولى . ثم لوحظت للشياع وسجلت الفترة ما بين حقن الهرمون وبداية ظهور علامات الشبق وكذلك مدة الشبق . بالنسبة للتزامن الشبقى قبل احداث التبويض المتعدد كان متوسط الفترة ما بين حقن الهرمون الى بدايةظهور علامات الشبق ٤٨,٢٥ ±١,٢٤ و٤٣,٨٣ ±٤،١٢و٢٤,٠ ±٠,٥١ ساعة للمجموعات التى حقنت باليوتاليز والألبرين والبروسولفين على التوالى . وكان المتوسط العام هو ٤٦,٦٧ ساعة. أما فترة الشياع المقابلة فكانت١٩,٧٥± ١,٨٢و ٢٠,١٧ ±١,٣٢و٢١,٧٤±١,٩٦ساعة . وكان المتوسط العام هو ٢٠,٤٠ ساعة. ولم تسفر النتائج عن وجود تأثير معنوى للأنواع الثلاثة من الهرمونات على الفترة مابين الحقن وظهور علامات الشبق وكذلك مدة الشبق . أما بالنسبة للتزامن الشبقى أثناء احداث التبيويض المتعدد فكانت الفترة ما بين حقن الهرمون وبداية ظهور علامات الشبق هى ٣١,٠ ±٣,٠٣و ٢٢,٠ ±٣,٨٦و ٣٢,٦٧ ±٤,٩٦ ساعة للمجموعات التى حقنت بالليوتاليز والألبرين والبروسولفين على التوالى. وكان المتوسط العام هو ٢٨,١٤ ساعة. أما متوسط طول فترة الشبق المقابلة فكانت ٢٢,٢ ±٢,٤١و ٢٠,٠٠ ±٢,٥٤ و ٢٤,٦٧ ±٣,٠٣ ساعة. قد بلغ المتوسط العام ٢١,٩٢ ساعة وأيضا لم تسفر النتائج عن وجود تاثيرمعنوى للأنواع الثلاثة من الهرمونات على الفترة ما بين الحقن وظهور علامات الشبق وكذلك مدة الشبق. وجدير بالذكر ان الدراسات الاحصائية أثبتت أن الاختلاف فى الفترة ما بين الحقن وظهور علامات الشبق وكذلك مدة الشبق قبل وأثناء احداث التبويض كانت على درجة معنوية عالية.