Assiut Vet. Med. J. Vol. 43 No. 86, July 2000

Dept. of Theriogenology,
Fac. Vet. Med., Zagazig University, Benha Branch (Moshtohor)

SOME TRIALS TO IMPROVE THE REPRODUCTIVE EFFECIENCY OF SUBESTRUS BUFFALO COWS USING $PGF_2\alpha$ AT MID LUTEAL PHASE

(With 3 Tables)

By

M.E.A. ABOU-EL-ROOS and A.E. ABDEL GHAFFAR

(Received at 22/6/2000)

بعض المحاولات العلاجية لتحسين الكفاءة التناسلية لإناث الجاموس ذات الشياع الصامت عن طريق أستخدام البروستاجلاندين ف ٢ ألفا في طور الجسم الأصفر

محمود السيد عابد أبو الروس ، علاء السيد عبد الغفار

أجريت هذه الدراسة على عدد ٣٣ من إناث الجاموس التى تعانى من مشكلة الشياع الصامت وعدد ١٠ من إناث الجاموس طبيعية الشياع كمجموعة ضابطة بدون علاج لمقارنة النتائج. المجموعة الأولى (١٢ حيوان) تم علاجها بوضع ١٠ مجم ($PGF_2\alpha$)في المهبل في اليوم الحادي عشر من الدورة ، المجموعة الثانية (١٠ حيوانات) حقنت ١٠ مجم ($PGF_2\alpha$) في شفرة المهبل على عمق ١ سم ، المجموعة الثالثة (١١ حيوان) حقنت ٢٥ مجم ($PGF2\alpha$) في العضل ، المجموعة الرابعة (١٠ حيوانات) استخدمت كمجموعة ضابطة. كل الحيوانات المعالجة التى ظهر عليها الشبق تم تلقيحها بطلوقة جاموس ذو كفاءة تناسلية عالية وكل الحيوانات التي لم يظهر عليها الشبق بعد العلاج تم تلقيحها اصطناعيا في وقت ثابت (٧٢-٩٦ ساعة) بعد الحقن. تم أخذ عينات بلازما لقياس هرمون البروجستيرون من كل المجموعات خلال طور الجسم الأصفر قبل الحقن مباشرة ، بعد الحقن بيوم وبعد يومين و خلال يوم ظهور الشبق (يوم التلقيح)، بعد ٥ ، ١٠ ، ١٥ ، ٢٠ ، ٢٥ ، ٣٠ يوم بعد التلقيح في كل المجموعات بما فيها المجموعة الضابطة. تم تحديد الحمل بالفحص عن طريق المستقيم بعد ٥٠-٦٠ يوم بعد التلقيح الطبيعي والاصطناعي . وأظهرت النتائج أن أعلى معدل لظهور الشبق كان في المجموعتين اللتين تم حقنهما في شفرة المهبل والعضل عنها في التي تم حقنها في المهبل. لم يكن هناك اختلافات معنوية بين كل المجموعات المعالجة في الزمن المستغرق من بداية الحقن حتى حدوث الشياع. كانت نسبة التبويض في المجموعات التى ظهر عليها الشبق بعد الحقن ١٠٠% وفي المجموعة الضابطة ٨٠% بينما كانت نسبته ٦٠ ، ٥٠ ، ٣٣,٣٣ % في الحيوانات التي لم يظهر عليها الشبق. بعد حقن البروستاجلاندين

في الحقن داخل المهبل ، شفرة المهبل والعضل. لم يكن هناك اختلافات في معدل الإخصاب بعد التلقيح الأول في كل المجموعات التي ظهر عليها الشبق والمجموعة الضابطة. بينما كان هناك زيادة طفيفة في معدل الإخصاب الكلى في المجموعات المعالجة عن المجموعة الضابطة. تم استنتاج أن الجرعات الصغيرة من البروستاجلاندين في الحقن داخل المهبل والشفرة يمكن استخدامه بنجاح لتحسين الخصوبة في إناث الجاموس التي تعانى من الشياع الصامت كذلك تم استنتاج أن قياس هرمون البروجستيرون في البلازما يفيد في تحديد الشياع الصامت وانعدام التبويض وكذلك تشخيص الحمل في تلك الحيوانات.

SUMMARY

Thirty three subestrus buffalo cows and 10-normal cycling females with apparent estrus symptoms were allocated to one of four treatment groups. The 1st group (n=12) received 10 mg of $PGF_2\alpha$ per-vaginum. The 2nd group (n=10) injected with 10 mg of $PGF_2\alpha$ at a depth of 1 cm on the labia majora. The 3rd group (n=11) treated with 25 mg of $PGF_2\alpha$ injected intramuscularly. The 4th group (n=10) with apparent estrous signs received normal saline and used as control group. Plasma progesterone were estimated in all groups under investigation before treatment at (luteal phase), 1st and 2nd days following treatment and at 5th, 10th, 15th, 20th, and 30th after insemination. In all treated groups, the females confirmed in heat mated with good fertile buffalo bulls. Animals failed to exhibit standing estrus were artificially inseminated twice at fixed time (72 and 96 hours after injection of $PGF_2\alpha$). Pregnancy rates were determined by palpation per-rectum between 50 and 60 days following insemination (natural or artificial). The incidence of subestrus buffalo cows exhibited standing estrus was found to be higher after intravalvular and intramuscular injection of $PGF_2\alpha$ (72.73 – 80.00%) than following administration of $PGF_2\alpha$ per vaginum (58.33%). The time elapsed until appearance of heat symptoms not varied among all treated subestrus females. All treated animals exhibited standing estrus and 80% of the control group were ovulated. While the ovulation rate was recorded to be 60, 50 and 33.33% of buffalo cows that not exhibited standing estrus following adminstration of $PGF_2\alpha$ per-vaginum, intra-vulvar and intramuscular, respectively. The 1st service conception rate not varied among all subestrus females that exhibited standing estrus (50-62.50%) and control group (60%), while the total conception rate slightly increased in treated groups (75-87.50%), than the control one (70%). In buffalo cows that not exhibited standing estrus after treatment

with $PGF_2\alpha$, The 1st service and total conception rates were observed to be 25 and 60%, respectively per-vaginum, 50 and 50% intra-vulvar, respectively and 33.33 and 33.33% intramuscular. Low dose of intravulvar or intra-vaginal could used successfully for imporvement fertility of subestrus buffaloes. In addition the plasma progesterone concentration can be used for detection of silent estrus as well as pregnancy diagnosis and anovulatory cycle in buffaloes.

Key words: *Peproductive effeciency, Bufflalo cow, $PGF_2\alpha$*

INTRODUCTION

Subestrus or silent heat is one of the major problems affecting the reproductive performance in buffaloes via increasing the claving interval and loss in milk production (Awastihi et al., 1998). The use of prostaglandin for controlling silent heat is a fairly recent development in buffaloes (Sarvaiya et al., 1995). Administration of $PGF_2\alpha$ produces luteolysis and decreased blood progesterone level (Abdel-Ghaffar et al., 1994); treated animals return to estrus within 3 days (Ibrahim et al., 1990). The present investigation was undertaken to assess the efficacy of different doses and routes of administration of $PGF_2\alpha$ for induction of estrus in subestrus buffaloes.

MATERIALS and METHODS

The present study was conducted on a farm of dairy buffaloes belonging to Horin village at El-Menofia province. Animals exhibiting post-partum or post-service anestrus were screened through repeated gynaecological examination three successive times at seven days interval. Animals, clinically free from genital infection and having a corpus luteum in one of the ovaries were selected. Subestrus animals (33) which did not shows clear external signs of estrus were injected with $PGF_2\alpha$ (lutalyse, Upjohn, Kalamazo, MI, USA) on day12 of the cycle. These animals were classified randomly into 3 groups. A 4th group (n=10) constituted the normal cycling animals.

Group 1: Consisted of 12 animals were treated with 10 mg of $PGF_2\alpha$ each per vaginum by filling the drug in 2 AI straws, 0.5 cc capacity and depositing it in the dorsal fornex of the vagina with the help of an artificial insemination gun.

Group 2: Consisted of 10 animals were injected each with 10 mg $PGF_2\alpha$ at a depth of 10cm on the labia majora through intra-valvosubmucosal route, ipsilateral to corpus luteum.

Group 3: Consisted of 11 animals were treated with 35 mg $PGF_2\alpha$ through intra muscular route.

Group 4 (control): This group consisted of 10 normal cycling buffaloes received normal saline and served as control.

All the treated animals were subjected to estrus detection by observation of signs of estrus twice every day. The changes in ovarian activity were monitored by rectal palpation. The estrus was confirmed by the presence of graffian follicle in the ovary and turgidity of uterine horns along with other behavioural signs of estrus.

Animals showing standing heat were bred naturally with a fertile buffalo bull in the second half of estrus. Females failing to exhibit standing estrus were artificially inseminated twice with frozen semen at 72 and 96 hrs following treatment (fixed time AI). All groups were examined per rectum between days 12 and 14 following treatment to detect the presence of corpus luteum to confirm ovulation. Pregnancy diagnosis was carried out in animals which did not return to estrus on days 50 - 60 post-breeding. Those which returned to estrus were re-inseminated. The response to treatment for induction of estrus, ovulation rate, 1st service conception and total conception rate were recorded in all groups.

Blood samples were collected by Jugular vein puncture in heparinized sterile evacuated tubes before treatment as well as on the 1st and 2nd days after treatment. In addition, blood samples were collected from all animals on the day of insemination (0 day), 5th, 10th, 15th, 20th, 25th and 30th days post insemination.

Radioimmunological estimation of plasma progesterone (El-Banna et al., 1985) were determined at the Laboratories of Endocrinology Research Unit. Belonging to Radiology Department, Nuclear Research Center. Atomic energy Authority, Enshas, Egypt.

Data obtained were statistically analysed using the statistical Analysis system SAS (1987)

RESULTS

Results presented in Table 1 indicated that, buffalo cows exhibited standing estrus was recorded to be significantly higher after

intravulvar and intramuscular injection of $PGF_2\alpha$ (80.00 and 72.73%, respectively) than post- administration of $PGF_2\alpha$ pervaginum (58.33 %) . Moreover, the time elapsed till appearance of standing estrus did not vary significantly among different groups, of treated animals.

As shown in Table (2), the ovulation rate was reported to be 100% in buffalo cows exhibited estrus after administration of $PGF_2\alpha$ with different routes and 80% for the control group. Further reduction in ovulation rate was observed in females not exhibited standing estrus after administration of $PGF_2\alpha$ - with different routes (33.33 – 60.00 %). The 1st service and total conception rates were recorded to be higher in females exhibited estrus (50.00 – 62.50 % and 75.00 – 87.50% respectively), than those not exhibited estrus (20.00 – 50.00% and 33.33 – 50.00%, respectively) after administration of $PGF_2\alpha$ with different routes.

Plasma progesterone concentration in subestrus buffaloes and normal cycling ones before and after treatment are presented in Table 3.

DISCUSSION

The present study revealed that 7 out of 12 buffaloes exhibited standing heat with a mean interval of 86.71 ± 5.55 hr post administration of $PGF_2\alpha$ per vaginum (Table, 1). This observation was in consonance with the reports of Subramaniam et al., (1991) who obtained similar results after per vaginal $PGF_2\alpha$ treatment in Indian buffaloes. The majority of animals exhibited estrus between 72 and 96 hours. Ten out of 12 animals revealed ovulation and corpus luteum formation. It is interesting to note that 7of 7 animals ovulated which exhibited standing estrus while 3 out of 5 animals ovulated silently. This finding explained that most of subestrus buffaloes did not express signs of estrus, ovulate silently following on prostaglandin treatment, therefore fixed time AI is a must. As shown in Table 3 a high level of progestrone was detected during estrus phase in females that not exhibited estrus (0.68 – 0.98ng / ml) and it was (0.43 - 0.66 ng / ml) in females exhibited estrus after treatment with $PGF_2\alpha$ while in normal cycling control buffaloes was (0.23 – 0.63ng/ml) during estrus phase. Therefore the high level of progesterone during estrus may explain the silent ovulation of subestrus buffaloes (Gupta and Dhoble, 1990). The 1st service conception and total conception rate with natural service buffaloes was 57.14 and 85.71% respectively, while it was 20% and 40% in fixed time AI females. Our

finding are in agreement with earlier reports (Pant and Singh, 1991; Singh and Dabas, 1996 and Awasthi et al., 1998).

In intravulvar group (Table, 1) was found that 8 out of 10 females exhibited standing estrus with a mean interval 81.82 ± 6.65 hours (70-90 hours) with 100% ovulation. Rectal examination confirmed ovulation in 9 out of 10 buffaloes from which one buffalo ovulated without expression of estrus signs. The time elapsed till appearance of estrus came in accordance with the observation of Singh et al. (1987). The 1st service conception and total conception rate in buffaloes with natural service was (62.52 and 87.50%) and with that of fixed time AI was 50% each for 1st service conception and total conception rate (Table 2) which approximates the finding of Gautam et al., (1990). However Singh et al., (1987) reported a higher conception rate.

In I / M group (Table 1) 8 out of 11 buffaloes exhibited estrus within 86.63 ± 4.09 hours with 100% ovulation rate, while rectal examination confirmed ovulation in 9 out of 11 animals from which one buffalo-cow ovulated silently. Most females exhibited estrus between 72 and 96 hours which is in conformity with Singh and Dabas (1996). The 1st service conception and total conception rate in females exhibited estrus was 50 and 75% and it was 33.3% each in females with fixed time AI (Table 2). A similar results obtained by Fulka et al. (1978).

In control group which represented the normal cycling animals 8/10 females exhibited estrus and the ovulation rate, 1st service conception, and total conception rate was 80%, 60%, and 70% respectively. A similar finding was reported by Bhela et al. (1996).

Non significant difference was observed between all treated groups with 10 mg $PGF_2\alpha$ and 35 mg $PGF_2\alpha$ regarding ovulation rate, 1st service conception and total conception rate. No significant difference between levels of progesterone during early pregnancy in all treated and control groups. A similar finding was recorded by (El-Belely et al., 1995).

The steady increase of progesterone level during pregnancy in non returned females of all groups can be used for pregnancy diagnosis in buffaloes. These finding came in accordance with other previous reports (Sharma and Kaker, 1990, Gupta and Dhoble, 1990 and Eissa et al., 1995)

In conclusion, low doses of prostaglandin $F_2\alpha$ given via in intravulvar or intravaginal routes could be used successfully and conveniently giving similar results as those obtained with high IM dose

of $PGF_2\alpha$. In addition plasma progesterone concentration can be used for detecting silent and anovulatory estrus as well as pregnancy diagnosis in buffaloes.

REFERENCES

Abdel-Ghaffar, A.E.; Shawki, G. and El-Sayed, A.I. (1994): Hormonal trial for predection of pulpable and unpalpable Corpora lutea in cattle and buffaloes. 2nd Vet. Med. Congress. Zag. Univ. Fac. Of Vet. Med., 11-13 October 1994-408-418.

Awasthi, M.K; Tiwarf, R.P. and Pagaokar, G.R. (1998): Induction of estrus and fertility with low dose of prostaglandin $F_2 \alpha$ in subestrus buffaloes. Indian Journal of Animal Science 68 (10): 1049-1050.

Bhela, S.L.; Kaker, M.L.; Lohan, I.S.; Singal, S.P. and Razdan, M.N. (1996): Oestrous cyclicity induction by folligon (PMSG) treatment in post partum Murrah buffaloes and their plasma progesterone profile. International Journal of Animal Science 11: 1, 121-124.

Eissa, H.M. El-Belely, M.S.; Ghoneim, J.M. and Ezzo, O.H. (1995): Plasma progesterone, oestradial – 17 beta, oestrone sulphate, corticosteroids and metabolite of PGF_2 alpha evolution throughout pregnancy,before and after parturition in buffalo cows. Veterinary. Research 26,310 – 318.

El-Banna, J.M. Asrgag, H.A.; Gado, M.S. and Gamal, M.H. (1985): Development of a homologus Iodine – 125 Labelled progesterone ralioimmunoassay. Economy versus sensitivity and precision. Alex. J. Agric. Res., 31 (3) : 101 – 113.

El-Belely, M.S.; Eissa, H.M.; Omaima, H.E. and Ghoneim, I. (1995): Assessment of fertility by monitoring change in plasma concentrations of progesterone, Oestradiol- 17 beta, androgens and Oestrone sulphate in suboestrous buffalo-cows treated with prostaglandin F2 alpha. Animal-Reproduction Science, 40: 1-2, 7-15.

Fulka, J.; Motlik, Janol Pavolk, A. (1978): Heat and conception rate after synchronization of oestrus with cloprostenol. Veterinary Record 103: 52-53.

Gautam, A.P.; Kaarche, K.G. and Thakur, M.S. (990): Comparative efficacy of prostaglandin $F_2 \alpha$ with two dose rates and routes of administration in subestrus buffaloes. Landian Journal of Animal Reproduction 11 (2): 164-167.

Gupta, S.K. and Dhoble, R.L. (1990): response of suboestrus rural buffaloes to $PGF_2 \alpha$ analogue in relation to levels of triiodothyronine (T3). Tetraiodothyronine thyroxione (T4) and progesterone. Proceedungs of II world buffalo congress held in India during 12 -16 Decembers 1998 (Volume III). Plupiology and Reproduction 1990, 162 –164.

Ibrahim I.A.; Khalifa, R.M.;El-Ghannam, A.A. and Dowidar, M.F. (1990): Effected of prostaglandin on some blood constituents in Egyptian female buffaloes.2nd Annual Congress of Egyptian Society for Animals Reproduction and fertility, held in the Fac. Vet. Med. Suez Canal Univ. Ismailia.

Pant, H.C. and Singh, B.P. (1991); Application of prostaglandin F2 alpha in the treatments of subestrus in buffaloes. Indian Journal of Animal Reproduction 12 (1): 55-57.

Sarvaiya, N.P.; Chauhan, F.S.; Mehta, V.M. and Dugvekar, Y.G. (1995): Effect of dose of prostaglandin $F_2 \alpha$ and season on induction of estrus in Surti buffaloes. XII National convention of Indian Society for study of Animal Reproduction pp. 91.

Sharma, Y.P. and Kaker, M.L. (1990): Monitoring ovarion cyclicity in post partum Murrah buffalo throush milk progesterone enzymeimmunoassay . Theriogenology 33 : 4, 915 – 923.

Singh, G.; Singh, G.B. and Sharma, R.D. (1987): Ovulation and fertility after an an inter-vulvo-Submucus injection of prostaglandin $F_2 \alpha$ in Subestrus buffaloes. Indian Journal of Dairy Science 11: 324-325.

Singh, O.V. and Dabas, Y.P.S. (1996): Augmenting milk production in lean period through controlled breeding in buffaloes. XIII National convention of Indian Society for study of Animal Reproduction PP 111.

Statistical analysis system (SAS) (1987): Mser's Guid " SAS" Institute Carry, North Cardina.

Subramaniam, A.; Mohanan, M. and Devarajan, K.P. (1991): Oestrus synchronization by vaginal, vulvar and intra muscular administration of $PGF_2 \alpha$ in buffaloes. Indian journal of Animal Sciences 61 (2): 183-184.

Table 1: Rate of response and time elapsed from treatment till appearance of standing estrus in different treated groups.

	Total treated females	Females exhibited standing estrus		
		n	%	Time elapsed
Group 1 (pervaginum)	12	7	58.33 [b]	86.71±5.55[a] (72-96 hr)
Group 2 (intravulvar)	10	8	80% [a]	81.82±6.65[a] (70-90 hr)
Group 3 (intramuscular)	11	8	72.73 [a]	86.63±4.09[a] (72-96 hr)

Means with different alphabetica superscripts are significantly different from each other at level ($P < 0.05$).

Table (2): Ovulation rate, 1st service conception and total conception rate in both treated and control buffalo – cows.

	Group 1 (pervaginum)		Group 2 (intravulvar)		Group 3 (intramuscular)		Group 4 (control)	
	exhibited estrus	not exhibited estrus	exhibited estrus	not exhibited estrus	exhibited estrus	not exhibited estrus	exhibited estrus	not exhibited estrus
Total number	7	5	8	2	8	3	10	-
Ovulation rate	7 (100%)	3 (60%)	8 (100%)	1 (50%)	8 (100%)	1(33.33%)	8 (80%)	-
1st service conception	4 (57.14%)	1 (20%)	5 (62.50%)	1 (50%)	4 (50%)	1 (33.33%)	6 (60%)	6 (80%)
Total conception rate	6 (85.71%)	2 (40%)	7 (87.50%)	1 (50%)	6 (75%)	1 (33.33%)	7 (70%)	7 (70%)

Table 3: Plasma progesterone profiles (ng / ml) in subestrus buffaloes and normal cycling ones.

	Subestrus buffalo-cows				Normal cycling buffaloes (control)	
	Exhibited standing estrus		Not exhibited standing estrus			
Before treatment at luteal phase	Returned (n = 5)	non returned (n=18)	returned (n = 7)	non returned (n = 3)	returned (n = 4)	non returned (n = 6)
0-day of treatment	2.89^{cd} ±0.83	2.99cb ±0.91	3.31bc ±0.87	3.73bc ± 0.73	3.11bc ± 0.98	3.33bc ± 0.73
1st day after treatment	3.10^{bc} ± 0.63	3.14 bc ±0.66	2.99c ±0.73	3.31bc ±0.63	2.81c ± 0.86	2.71c ± 0.73
2nd day after treatment	3.67 ^{bc} ± 0.68	3.88bc ± 0.37	3.11b ± 0.53	3.33b ± 0.67	2.92b ± 0.74	3.73b ± 0.44
0-day of insemination	0.66 ^{b} ± 0.05	0.43d ± 0.32	0.98d ± 0.33	0.68d ± 0.31	0.63d ± 0.13	0.23 c ± 0.07
5th day after inseminati on	3.34^{b} ± 0.77	3.63b ± 0.89	3.78ab ± 0.81	3.67b ± 0.73	4.15ab ± 0.35	4.13 ab ± 0.39
10th day after insemination	4.88^{ab} ± 0.65	3.93b ± 0.73	3.86b ± 0.55	3.81b ± 0.41	3.81ab ± 0.89	3.83b ± 0.31
15th day after insemination	3.73^{b} ± 0.93	4.40ab ±1.32	4.81ab ± 0.83	4.73ab ± 0.81	4.64ab ± 0.78	4.63ab ± 0.46
20th	0.68^{d} ± 0.05	6.84a ±1.35	0.73d ± 0.63	6.34ab ± 1.63	0.36d ± 0.03	6.11 a ± 1.34
25th	2.45^{c} ± 0.83	7.74a ± 1.43	2.11c ± 0.43	6.31a ± 1.31	2.31c ± 0.73	6.44a ± 1.60
30th	3.41^{bc} ± 0.93	7.77a ± 1.53	2.45c ± 0.73	6.93a ± 1.40	3.13bc ± 0.73	6.34 a ± 1.47

Means with different alphabetica superscripts are significantly different from each other at level (P < 0.05).