

USE OF NORGESTOMET AND OESTRADIOL VALERATE INJECTION WITH NORGESTOMET IMPLANT AND / OR PMSG FOR INDUCTION OF OESTRUS IN ANOESTROUS BUFFALOES

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SUMMARY

The study was designed to evaluate 5 hormonal treatments for induction of oestrus in anoestrous buffaloes . Treatment 1 of subcutaneous 3 - mg norgestomet implant and an intramuscular injection of 3 mg of norgestomet and 5 mg estradiol Valerate, with implant removed 9 days later (N=15; norgestomet - estradiol) ; Treatment 2 of norgestomet - estradiol, with an intramuscular injection of 500 IU of PMSG at the time of implant removal (n=13) ; Treatment 3 of intramuscular injection of 500 IU PMSG (n=10) ; Treatment 4 of intramuscular injection of 1500 IU PMSG (n=10) ; Treatment 5 of two intramuscular injections of PMSG (500 IU) 3 days apart (n=5). Ten animals received no treatment and used as a control group. The proportions of animals exhibited oestrus during 35 days after treatment 1 , 2 , 3 , 4 , and 5 were 6.7 , 76.9 , 60 , 30 and 100 % respectively. No animal in control group came in oestrus during this period . Conception rate after natural mating at induced oestrus in the five treatments

was 100 % . The highest oestrous rate (100%) and the shortest interval between treatment and displayed oestrus (within 5 days) was obtained after treatment 5 . Progesterone concentration was measured in all samples collected before treatment and sera collected from 5 animals did not exhibit oestrus after treatment 1 and from 5 animals exhibited oestrus 3 days after treatment 2 for monitoring ovarian status before and after treatment. Progesterone concentrations was < 0.22 ng /ml in two samples collected from all animals before treatment. Mean progesterone concentrations were maintained at low basal levels (0.18 + 0.09 - 0.21+ 0.07 ng/ ml) after treatment 1 however, it raised to 2.56 + 0.61 ng/ml at day 7 post treatment and maintained at high levles (2.80 + 1.68 - 4.41+ 0.94 ng/ml) during 28 days after treatment 2.

Keywords : Buffaloes; Progesterone; PMSG; Norgestomet; Estradiol

INTRODUCTION

Postpartum anoestrus in buffaloes due to ovarian inactivity is responsible for long calving conception interval (>90 days) and decrease income of the farmers. Several studies were conducted to induce oestrus in anoestrus cows and heifers due to ovarian inactivity using exogenous progestin. Ninety four percent of treated anoestrus heifers with Synchro - Mate B (Norgestomet - estradiol) were come in oestrus 4 days after norgestomet implant removal compared with 0 % for untreated animals (Gonzalez- Padilla et al ., 1975). Eighty five percent of the treated cows with norgestomet -estradiol combination evinced oestrus during the 5- day period after implant removal . Pregnancy rates to the 1st service were 60 for the treated and 69 % for control (Miksch et al ., 1978). The 1 st service pregnancy rate was 56% following implant (Synchr-Mate B) removal and insemination at 48 to 54 hours (Kaltenbach, 1980). 1st service pregnancy rate was 35 % in anoestrus cows and heifers treated with 9-day norgestomet implants (Hixon et al., 1981). Nine of 14 primiparous non lactating cows were treated for heat induction with norgestomet - oestradiol combination and 600 I.U. of PMSG on the day of implant removal responded to this therapy (Miguel et al., 1980) . The pregnancy rate was 65% followed treatment with norgestomet - oestradiol combination and 500 I.U. PMSG 2 days before implant removal (Chupin and Pelot., (1980). The same treatment induce oestrus and ovulation in 70 - 80 % of anoestrus cows and heifers (Aquer et al., 1980). Ovulation rate was 58.33 and 77.79 % in anoestrus Village and farm buffaloes respectively following the administration of norges-

tomet - oestradiol combination and 700 I.U. PMSG at implant removal . The conception rate was 40.16 and 61.11% respectively (Gurdial Singh et al., 1984). All anoestrus buffaloes were responded to norgestomet-oestradiol treatment and 1000 I.U. PMSG at implant removal within 2.0 - 0.71 days and had CL 10 -14 days after oestrus (Abdoon et al., 1994). Oestrus rate and conception rate were 45.45 and 40.0 % after removal of implant of Synchro-Mate B and injection of 1000 I.U. PMSG in anoestrus buffaloes (Younis et al., 1996). eighteen of 24 non cycling Surti buffalo- heifers came in oestrus after injection with 500 I.U. and 9 were diagnosed pregnant 45 days later (Shah et al., 1992). All the buffalo heifers treated with 700 - 1400 I.U. of PMSG exhibited oestrus following treatment compared with 42.85 % showed oestrus in non treated animals . The over all conception rate was 42.85 , 28.57 and 14.28 % in animals treated with 700 I.U. , 1400 I.U. of PMSG and non treated respectively (Khan et al., 1995). PMSG (1000 IU) lead to oestrus in 25 % of anoestrus buffaloes, although the conception rate was 0 (Younis et al., 1996) . The present study was conducted to evaluate the effect of 5 hormonal treatments on non cycling buffaloes . Progesterone concentration was determined in some serum samples for monitoring ovarian status of animals before and after treatment.

MATERIALS AND METHODS

Experimental location and Animals:

The study was conducted at some villages belonging to Kom Hamada Behira Egypt from February to May ,1998 . Sixty three buffalo cows (Bubalus

bubalis) aged 3 - 8 years with a history of clinical postpartum anoestrus of 6 - 12 months were used in this study . The animals had access to barseem pasture during the day and to dietary supplementation (1 - 2 Kg concentrate) at night. The animals were maintained in good condition . Before the treatment , the genitalia of the animals were examined per rectum twice with 10 days interval . The ovaries in both examinations were smooth ,small and had not any ovarian structures. There was not any detectable pathological abnormalities in the other examined reproductive organs. The treated animals were observed for oestrous signs 35 days after norgestomet implant removal and / or PMSG injection. The animals that came in oestrus were naturally mated by a fertile bull. The animals were examined rectally at day 45 post service for pregnancy diagnosis.

Treatment :

The animals were randomly allocated to 6 groups . Buffalo cows in group 1 (n = 15) were received an intramuscular injection of estradiol valerate (5 mg) with norgestomet (3mg) and at the same time 3 mg norgestomet implant (Intervet ,Netherland) was inserted subcutaneously in the outer aspect of the ear . On day 10 post implantation , the implant were removed . Animals in group 2 (n = 13) were received the same treatment of the animals in group 1 plus intramuscular injection of 500 I.U. PMSG (Chronogest, Intervet, Netherland) at the time of implant removal . Animals in group 3 (n = 10) were injected intramuscularly with 500 I.U. of PMSG . Buffalo cows in group 4 (n = 10) were received 1500

I.U. of PMSG intramuscularly . Animals in group 5 (n = 5) were received 2 doses of 500 I.U. of PMSG with 3 days interval . Ten buffaloes cows were received no treatment and used as a control group .

Blood Sampling :

Before the treatment , two blood samples were collected from jugular vein of each animal at 10 days interval . After administration of the treatment, five blood samples were collected from jugular vein of animals in group 1 and 2 at weekly intervals (day 7, 14, 21, 28, 35 post treatment). Blood samples were chilled in ice and centrifuged at 3000 x g for 15 minutes . Serum was kept at - 20 C until assayed.

Blood serum progesterone assay:

Progesterone was measured in all samples collected before treatment . Sera collected from 5 animals (group 1) did not exhibit estrus during 35 days post treatment and from 5 animals (Group2) displayed estrus 3 days post treatment were assayed for progesterone concentration.

Blood serum progesterone was measured using a commercial radioimmunoassay ,Coat-A-count Kit (Diagnostic Systems Laboratories , Inc. Corporate Headquarters, 445 medical Center Blvd. Webster, Texas 77598 - 4217 USA. Intra and interassay Coefficients of variation were 4.8 and 13.1 % . The Coat-A-Count progesterone antiserum is highly specific for progesterone , with particularly low cross reactivity to the other naturally occurring steroid.

RESULTS

Oestrus induction and conception rate:

All clinical data of the present study were presented in table (1). In treatment 1, one of fifteen cows oestrus signs at day 30 post removal of implant. The other treated animals in this group did not exhibit oestrus until 35 days post treatment.

When a 500 IU PMSG (i.m.) was administered at removal of norgestomet implant markedly increased the percentage of the animals exhibited oestrus to 76% . Moreover, it caused shortening to interval between the treatment and induced oestrus. In this treatment 5 animals exhibited oestrus at day 3 after removal of implant however , the other five came in oestrus at day 10 after treatment.

Six out of 10 animals in treatment 3 , exhibited oestrus during 35 days post treatment . Five of these 6 animals came into oestrus within 25 days post treatment . The use of high dose (1500 IU of PMSG) in animals of group 4 compared with 500 IU in animals in group 3 , lower proportion (30%) of the treated animals expressed oestrus and longer days interval between the treatment and displayed oestrus .

All animals (100%) in treatment 5, displayed oestrus within 5 days after the last injection. No animal in control group came in oestrus during the time of experiment .

All treated animals came in oestrus after treatment and naturally mated became pregnant.

Progesterone concentration:

Progesterone concentrations were very low (< 0.22 ng /ml) in all serum samples collected from animals before treatment.

Blood serum progesterone concentrations (ng/ml) in samples collected from five buffaloes did not exhibit oestrus after treatment with norgestomet -oestradiol combination treatment and five animals exhibited oestrus 3 days after norgestomet-oestradiol combination treatment and injection of 500 IU of PMSG at removal of implant were presented in Fig.(1).

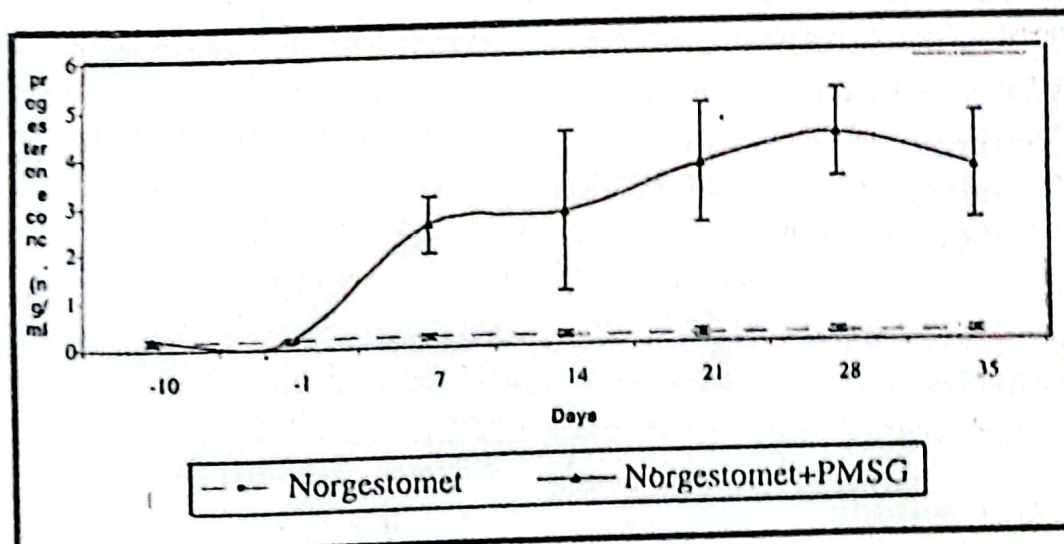
Mean progesterone levels in blood serum samples collected from five animals did not exhibit oestrus after removal of norgestomet implant were maintained at low levels (0.18 + 0.09 - 0.21 + 0.07 ng / ml) until day 35 post treatment . Non significant (P > 0.01) difference between the progesterone levels before and after treatment was reported .

intramuscularly injection of 500 IU of PMSG at removal of norgestomet implant caused increase in the mean progesterone concentrations from 0.18 + 0.06 to 2.56 + 0.61 ng /ml at day 7 post treatment. As shown in Fig (1) , mean progesterone concentrations were maintained at high levels. These levels were 2.81+ 1.68 , 3.80 + 1.28 4.40 + 0.94 and 3.7 + 1.14 ng /ml at day 14 , 21 28 and 35 post treatment.

Table (1): Effect of five hormonal treatment on non-cycling buffaloes.

TRT#	Treatment	No. of Animals	Animals exhibited oestrus during 35 days post treatment		Day of displayed oestrus after treatment		Pregnancy rate at induced oestrus
			No.	%	No. of animals	Day of oestrus	
	Control	10	0	0	0	0	0%
1	Norgestomet Oestradiol injection plus Norgestomet implant	15	1	6.7	1	30	100%
2	Norgestomet Oestradiol injection and Norgestomet implant plus 500 I.U. PMSG.i.m at implant removal	13	10	76.9	5 5	3 10	100%
3	500. I.U. i.m. PMSG	10	6	60	2 3 1	10 25 30	100%
4	1500. I.U. i.m. PMSG	10	3	30	2 1	15 35	100%
5	500. I.U. i.m. PMSG followed by the same dose after 3 days.	5	5	100	2 3	3 5	100%

Fig. (1): Mean progesterone concentration in five buffalo-cows did not exhibit oestrus after treatment with Norgestome - Oestradiol and five animals exhibited oestrus after treatment with Norgestomet - Oestradiol combination plus 500 I.u. PMSG (ng/ml)



DISCUSSION

Only one of fifteen anoestrous buffalo cows treated with norgestomet - oestradiol injection and norgestomet implant displayed oestrus at 30 days post implant withdrawal . This result indicate the failure of norgestomet-oestradiol treatment to induce oestrus in anoestrous buffaloes . In contrast to our data , Gonzalez - Padilla et al., (1975) induced oestrus in 94 % of anoestrous heifers treated with norgestomet - oestradiol treatment (Synchro Mate - B) 4 days after removal of norgestomet implant . Miksch et al ., (1978) used the same treatment for induction of oestrus in anoestrous cows and obtained markedly higher (85%) estrus rate as compared with our data (6.7%). The discrepancy between the results of this treatment in the this study and results of same treatment in previous studies could be attributed to the differences between the genetic makeup as well as the endocrinology of cattle and buffaloes .

Administration of PMSG at the end of a period of progesterone treatment has been shown to improve the oestrous response among animals that are anoestrous at the time of commencement of treatment (Munro ., 1988). In the present study, 10 (76.9%) of 13 treated anoestrous buffaloes exhibited oestrus followed injection of 500 IU PMSG at norgestomet implant withdrawal . 50 % of these animals displayed oestrus at day 3 and the other animals displayed oestrus at day 10 post - removal of norgestomet implant . Nearly similar to those proportions reported in primiparous cows (Miguel et al., 1980) and anoestrous cows

and heifers (Aquer et al, 1980) after treatment by the same above treatment. In buffaloes , Gurdial . Singh et al., (1983), Abdoon et al., (1994) and Younis et al., (1996) injected anoestrous buffaloes with 700 , 1000 and 1000 IU of PMSG at withdrawal of norgestomet implant and the oestrus rates were 58.33 , 100 and 45.45 % respectively. the observed variation in response of the animals to the treatment among authors may be attributed to the variation in the dose of PMSG , the ovarian condition at time of treatment or other environmental factors.

In the present experiments , 6 (60 %) of 10 treated anoestrous buffalo cows with a single dose of 500 IU PMSG exhibited oestrus during 35 days post treatment . However, only 3 (30%) of 10 treated animals with single dose of 1500 IU PMSG exhibited oestrus during the same period after treatment. Shah et al ., (1992) injected 24 non cycling Surti buffalo heifers with 500 IU PMSG , 18 (75%) of treated animals came in oestrus.

Younis et al., (1996) used 1000 IU of PMSG for induction of oestrus in anoestrous buffaloes . Poor percent (25%) of treated animals exhibited oestrus . This proportion is lower than ours (60%) by the use of 500 IU PMSG and nearly similar to the percentage (30%) after administration of 1500 IU of PMSG. Although , Bhela et al., (1996) treated anoestrous animals with 1000 IU of PMSG , the proportion (57.14%) of animals exhibited oestrus is similar to our data as a results of treatment of animals by 500 IU of PMSG

71.4% of treated anoestrous buffaloes with 1500 IU of PMSG exhibited estrus (Tiwari et al. (1995). This proportion is higher than our results (30%) by the administration of the same dose of PMSG.

Administration of anoestrous buffaloes with either 700 or 1400 IU of PMSG for induction of oestrus gave the same results. All animals (100%) treated with both treatments came in oestrus (Khan et al., 1995) . In contrast to our data , administration of 500 IU of PMSG gave more oestrus rate (60%) than oestrous rate (30%) resulted from administration of 1500 IU of PMSG . For explanation of these results, we need a further investigations particularly on ovarian receptors of hormones.

In the present work, 100% of treated anoestrous buffaloes with 500 IU of PMSG followed by the same dose after 3 days displayed oestrus within 5 days after the last dose . There is no available literature on the induction of oestrus by use of repeated doses of PMSG.

In the present investigation , Conception rates at induced oestrus with the five treatments after natural mating with fertile bulls were 100% . These data disagree with those from previous reports . In cattle, pregnancy rate was 60% after treatment with oestradiol - progesterone treatment and 69% for control (miksich et al., 1978). It was 56 % following norgestomet implant removal and insemination at 48 to 54 hours (Kaltenbach., 1980). First service pregnancy rate was 35%

followed treatment of anoestrous cows and heifers (Hixon et al., 1981) . In buffaloes, conception rate was 40.16 % after administration of 700 IU of PMSG at norgestomet implant removal (Gur-dial -Singh et al., 1983) . similar result (40%) obtained by Younis et al ., (1996) following injection of 1000 IU of PMSG. Conception rate following administration of 500 IU of PMSG was 50% (Shah et al.,1992) and were 42.85 , 28.57 and 14.28 following injection of 700 ,1400 IU of PMSG and control respectively (Khan et al., 1995). Conception rate was 0% after injection of 1000 IU of PMSG (Younis et al., 1996). The high conception rate in the present study agree with results of our work in the field . The high conception rate may owing to selection of animals with clinical healthy genitalia.

Progesterone concentration was determined in serum samples of animals treated with norgestomet-oestradiol treatment and did not exhibit oestrus . The object of progesterone determination was for monitoring the ovarian status before and after treatment . The low progesterone levels (< 0.22 ng /ml) in two samples collected from animals with 10 days interval and results of rectal palpation revealed that animals were non cycling before treatment. progesterone profile of non cycling buffaloes in the present study agree with those obtained by previous authors (Pahwa and Pandey., 1983; El-Belely., 1984 ; Khattab et al., 1988; Shah et al., 1992; Khan et al.,1995). After treatment with norgestomet -oestradiol , weekly progesterone levels were maintained at low levels (0.18 - 0.21 ng /ml) until day 35 post

treatment. The low levels of progesterone indicate the failure of this treatment to activate the inactive ovaries of the treated animals. On the other hand, the levels of progesterone were determined in five of animals treated with norgestomet - estradiol treatment plus 500 IU of PMSG at norgestomet implant removal and exhibited estrus 3 days after treatment. The observed increase in progesterone level at day 7 after treatment and maintain of high levels from day 7 to day 35 post treatment indicate that those animals become cycling as a result of treatment and then pregnant after natural mating. There is an agreement between our data and previous reports (Niansheng and Peichien 1988; Khattab et al., 1988; Khan et al., 1995).

On conclusion, The treatment of anoestrous buffaloes with two doses of 500 IU of PMSG at 3 days interval induced oestrus in all treated animals within 5 days after treatment. Injection of 500 IU of PMSG at norgestomet implant withdrawal induced oestrus in 76.9 % of treated anoestrous buffaloes within 10 days post treatment while administration of 500 PMSG induced oestrus in 60% of treated anoestrous buffaloes. Low oestrous rate was obtained by administration of anoestrous buffaloes with 1500 IU of PMSG. Failure of norgestomet - estradiol treatment to induce oestrus in non cycling buffaloes. The conception rates in all treated animals were 100 % . The authors recommended use of two doses of 500 IU of PMSG with interval 3 days for induction of oestrus and ovulation in anoestrous buffaloes.

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