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EFFECT OF COMBINED USING OF GnRH AND $PGF_2\alpha$ ON OESTRUS SYNCHRONIZATION AND PREGNANCY RATE IN BUFFALO-COWS

(With 2 Tables)

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تأثير استخدام الجمع بين محفز الهرمون الحاث للغدة المنسلية و البروستاجلاندين(ف٢ الفا) على تواقت الشبق و نسبة الحمل في إناث الجاموس

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تهدف هذه الدراسة إلى معرفة تأثير الحقن المتوالى بـــال Fertirelin acetate (محفـــز الهرمون الحاث للغدة المنسلية GnRH agonist) واللوتاليز (البروستاجلاندين ف ٢ الفـــا) على تواقت الشبق ونسبة الحمل في إناث الجاموس. تمت هذه الدراسة على عـــدد ثمانيـــة وخمسون أنثى سبقت لجميعها الولادة اكثر من مرة وكانت كلا منها تحمل علـــى المبيـــض جسم اصفر أثناء الجس المبدئي. وقد قسمت هذه الحيوانـــات عنـــد بدايـــة الدراســـة إلـــى مجموعتين. كانت المجموعة الأولى ضابطة (عددها ٢١ أنثى) والتي تم إعطائها ٢ ملى من محلول الملح الفسيولوجى. والمجموعة الثانية علاجية (عددها ٣٧ أنثى) والتي تم إعطائها ٢ ملى (١٠٠ ميكروجرام) من Fertirelin acetate. في اليوم السابع من بداية التجربـــة تـــم إعطاء ٥ ملى من اللوتاليز (٢٥ ملى جرام من البروستاجلاندين ف ٢ الفا) إلى الحيوانـــات التي لم تظهر عليها علامات الشبق خلال ٦ أيام الأولى من بداية التجربة وكان عددهـــا ١٦ حيوان من المجموعة الضابطة (سميت مجموعة SP) و ٣٣ حيـــوان مـــن المجموعـــة العلاجية . في اليوم التاسع قسمت المجموعة المعالجة وعددها ٣٣ حيـــوان عشـــوائيا إلـــى مجموعتين أعطيت المجموعة الأولى ١ ملى من محلـــول الملـــح الفســـيولوجى(وســـميت المجموعة GP وعددها ١٧ أنثى) وبينما أعطيت المجموعة الثانية ١ ملى (٥٠ ميكروجـــرام) من Fertirelin acetate (وسميت المجموعة GPG وعددها ١٦ أنثى). تم اخذ عينـــات من اللبن لقياس مستوى هرمون البروجستيرون لتحديد حالة الجســـم الأصفـــر قبـــل وبعـــد العلاج. تم ملاحظة حدوث الشبق في حيوانات المجموعـــة الضابطـــة (SP) والمجموعـــة المعالجة (GP) ذلك خلال الفترة من اليوم السابع إلى العاشر من بدايـــة التجربـــة لتلقيحـــها باستخدام طلائق جيدة الخصوبة. اما بالنسبة للحيوانات المجموعة المعالجـــة (GPG) فقـــد

لقحت في اليوم العاشر ٠ وجد ان معدل تواقت الشبق قد سجل اختلافا بسيطا خـــلال فـــترة التجربة (١٠ أيام) بالرغم من ان نسبة حدوثه كانت منخفضة في الحيوانات المعالجة بمحفز الهرمون الحاث للغدة المنسلية عن حيوانات المجموعة الضابطة ٠ وجد ان نســـبة حـــدوث الحمل بعد التلقيحه الأولى هى ٣ر٣١% من المجموعة الضابطـــة (SP) و٨,٨٥ % مـــن المجموعة المعالجة (GP) ٨ر٦٨ % للمجموعة المعالجة (GPG) وان هـــذا الاختـــلاف كان معنويا خاصة بين المجموعة الضابطة والمجموعة المعالجة (GPG)٠ وقد أظهرت هذه النتائج ان استخدام طريقة الحقن المتوالى لكل من محفز الهرمون الحاث للغدة المنسلية قبـــل الحقن ب ٧ أيام بالبروستاجلاندين ف ٢ الفا ثم الحقن مرة ثانية بعد يومين بمحفز الـــهرمون الحاث للغدة المنسلية طريقة مفيدة لتواقت الشبق وكذلك تحسين نسبة الحمل في الجاموس٠

SUMMARY

The objective of this study was to determine the effect of sequential injection with fertirelin acetate (GnRH agonist) and lutalyse ($PGF_2\alpha$ analogue) on oestrus response and pregnancy rate in buffalo-cows. A total of fifty eight pluriparous buffalo-cows (bearing luteal structure on ovaries at rectal examination) were initially assigned into two groups in this study. On day 0 (day of beginning of treatment) the animals in the control group [(SP group); n = 21)] received 2 ml of saline and the treated group (n = 37) received 2 ml fertirelin acetate (100µg). On day 7, the animals (n = 16 and 33, in the control and the treated groups, respectively) that had not exhibited spontaneous oestrus between days 0 and 6, were given 5 ml lutalyse (25 mg of $PGF_2\alpha$). On day 9 (two days after $PGF_2\alpha$), the animals in the treated group were divided into two subgroups: the first subgroup (n = 16, GP) received 1 ml of saline and the second subgroup (n = 17, GPG) received 1 (50µg) ml fertirelin acetate. Milk progesterone (P_4) levels were assayed for determining the luteal status of the animals before and after treament.The animals (SP and GP groups) were observed for oestrus from day 7 to day 10 and all animals in group GPG on day 10 were mated naturally by using fertile buffalo- bulls. Over the 10 days, the pattern of synchrony showed much less variation between treatment protocols. However, between day 0 and 7, the proportion of buffalo-cows exhibiting oestrus was lower (10.8%) in pre-treated animals with GnRH than in control (23.8%). The pregnancy rate to the first insemination were 31.3% (SP); 58.8% (GP) and 68.8% (GPG) and this difference was significant ($p<0.05$). These results indicated that the combine using of GnRH-$PGF_2\alpha$-GnRH appears

to be a useful synchronization method, as well as a tool for improving reproductive performance in buffaloes.

Key words: *Buffaloes GnRH PGF_2 & Oestrus Pregnancy.*

INTRODUCTION

In the current production practices, the application of reproductive technology, e.g. oestrus synchronization (OS) and standardization of this procedure, especially in buffaloes, has required considerable developmental work (Agarwal and Selvaraju, 2000). The OS is an important technique as well as an active area of research (Odde, 1990). Original methods of OS in bovine species were based on extending or reducing the luteal phase to produce a synchronize decline in P_4 levels (Macmillan and Burke, 1996). More recent attempts at creating tighter synchrony of oestrus have been primarily aimed at synchronizing both follicular waves and stage of CL (Thatcher et al., 1996; Adams, 1998). The most appropriate OS will vary with objectives in relation to breeding management, but should also take account to reduce some limitation of original ones (Macmillan and Burke, 1996). Prostaglandin's ($PGF_2\alpha$) products (luteolytic agents) has been recommended for inducing oestrus in bovines with a palpable CL but unobserved oestrus (Macmillan and Day, 1982; Dhoble and Gupta, 1987; Whittier et al., 1989; Chedc et al., 1996). Field results obtained after implementing protocols including single or double $PGF_2\alpha$ treatments indicated a large variations in response rates and conception rates (Strelow, 1993). The part of the variation in timing of oestrus is dependent on the day of cycle on which $PGF_2\alpha$ is injected (Jackson et al., 1979).

It has been known that administration of GnRH and its analogues are intended to: Induces a peak of LH within 2-3 hours in mature cycling cows (Chenault et al., 1990; Peters et al. 1999); Protects luteal function and maintains elevation of P_4 levels (White and Reimers, 1986); Induces either ovulation or atresia (regression) of dominant follicle (Webb et al., 1992; Peters et al., 1999). This is followed by the emergence of a new wave of synchronized follicular development with a new dominant follicle emerging synchronously in each cow (Macmillan et al. 1985a; Peters, et al. 1999). The ability of GnRH to inhibit oestrus and ovulation for several days has been applied to develop a new methods of OS in

combination with $PGF_2\alpha$ in bovine species. Therefore, if $PGF_2\alpha$ is given seven days later, luteolysis occur when there is a dominant follicle approaching maturity in each cow. Although numerous studies have been conducted using both GnRH and $PGF_2\alpha$ regimens to improve synchronization and fertility in cows (Macmillan et al., 1985a; Guilbault et al., 1991; Twagiramungu et al., 1992; Yamada et al., 1998; Mawhinney et al., 1999), but the effectiveness of these regimens for buffaloes has not become common yet. The objective of this study was to determine whether using a combination of fertirelin acetate (GnRH agonist), followed 7 days by $PGF_2\alpha$ and followed again by GnRH (2 days later) give an acceptable level of OS and fertility in comparison with $PGF_2\alpha$ alone in buffalo-cows.

MATERIAL and METHODS

Animals and Management:

This study was conducted at the buffalo dairy farm in El Hawataka station, Assiut Governorate, Egypt. The buffaloes were kept in an outdoor paddocks with access to an open-sided shelter and milked twice daily. A balance of nutritional diet including green fooder, dry fodder and concentrate mixture were fed to these animals. The herd participate in a routine reproductive health program. Monthly examinations were performed on animals, including rectal examination of the entire reproductive tract and a vaginal exploration of cows with vaginal discharges. The buffalo-cows selected for this study were: pluriparous; 4-8 years of age; calved normally; lactating with the body condition score range from 2.5 to 3.5 (on a1- 5 scale as previously described by Bhalaru et al., 1987) and in a good health and physical condition.

Treatment protocols and Sampling:

A total of 58 non-pregnant buffalo-cows were originally included in this study. These animals did not shown oestrus for about 60 days since the last parturition. The ovaries presented a well developed CL and possessed no gross or clinically detected abnormalities in the reproductive tract. The animals were randomly assigned to either control and treated groups.

The control group included 21 animals. They received a placebo i.m. injection (2 ml saline) on day 0. The animals which showed oestrus

signs (n = 5) between day 0 and day 6 were excluded. The rest (n= 16) received 25 mg $PGF_2\alpha$ (5 ml lutalyse) on day 7 and served as control group (SP = saline and $PGF_2\alpha$).

The treated group included 37 buffalo-cows. On day 0, all animals received i.m. 100 µg of GnRH agonist fertirelin acetate (2 ml Conceral, Takeda Chem. Co., Japan). The animals which showed oestrus signs between day 0 and day 6 were excluded from the experiment (n=4). The rest (n= 33) were injected with 25 mg $PGF_2\alpha$ (5 ml lutalyse) on day 7. These animals were randomly assigned into two subgroups. The first subgroup (n = 17) were injected with 1 ml saline. This group is named GP group (GnRH- $PGF_2\alpha$). The second subgroup (n = 16) were injected on day 9 a second dose (50 µg) of GnRH agonist fertirelin acetate. This group is named GPG group (GnRH-$PGF_2\alpha$-GnRH).

Oestrus was detected by a teaser buffalo-bull, in addition to visual observation. The animals detected to be in oestrus between day 7 to day 10 (from day 0) were mated naturally by using fertile buffalo-bulls. However, animals in the second subgroup (GPG) mated naturally on day 10 by a fertile buffalo-bulls. Pregnancy was determined by rectal palpation at 45-60 days after mating.

Milk samples were collected on each of the treatment days (day 0 and 7) of the experimental period to asses the luteal activity (milk P_4 level > 1 ng /ml, was considered indicative of a functional CL, Bulman and Lamming, 1978). At evening milking, after-milk (10 ml) was collected in test tube containing 100 mg potassium dichromate, then centrifuged (3000 r.min for 20 min.). The cream layer was discarded and the remaining skim milk was transferred to another test tube and kept frozen (- 20°C) until hormonal assay. Milk P_4 concentrations were determined by RIA method (Coat-A-count progesterone, Diagnostic Products Co. Los Angles, U.S.A.). All samples were analyzed in duplicate in the same assay.

Statistical Analysis:

The pregnancy rate was defined as the percentage of all animals pregnant at 45 to 60 days after mating. The effect of treatment on reproductive parameters such as luteal activity and pregnancy rate were analyzed statistically with SAS program (1985) as a complete randomized design. Differences in pregnancy rates due to treatment were analyzed by chi-square test. Mean milk P_4 levels between groups was compared by analysis of variance.

RESULTS

The effects of various treatment regimes on oestrus synchronization in buffalo-cows are summarized in Table 1. Administration of fertirelin acetate at day 0 tended to reduce (10.8%) the proportion of animals showing spontaneous oestrus between day 0 and day 6. In 4 days (day 7 to 10) following $PGF_2\alpha$ injection on day 7, the synchronization rate (93.8% vs. 84.8%) was almost similar between control and GnRH groups (Table 1).

For the buffalo-cows that did not exhibit oestrus between day 0 and 6; the overall synchronization rate tended to be higher for control animals (93.8%) than GnRH groups (88.2% and 81.3%) (Table 2). The reproductive parameters following different regimes are shown in table 2. Fertirelin acetate was effective in increasing significantly ($P < 0.05$) the pregnancy rate in buffalo-cows when administration two days later (day 9) after $PGF_2\alpha$ injection on day 7 (Table 2). Moreover, the pregnancy rate in GPG group tended to be higher (10.0%) when compared with GP group. Milk P_4 concentrations (mean ± S.E.) for buffalo-cows before and after treatment with GnRH are summarized in table 2. Milk P_4 levels on day 0 just before treatment were similar between treatment groups. From day 0 to day 6 after GnRH injection, the P_4 levels tended to be increasing (3.92 ± 1.52 and 3.14 ± 0.44 ng/ml), but these differences were not significant when compared with control ones (2.98 ± 0.84).

DISCUSSION

In bovine species, Rosenberg, et al. (1991) suggested that GnRH may affect several stages of the reproductive process and consequently different cows may benefit from GnRH administration through different mechanisms. The results of this study show evidence that administration of GnRH resulted in an almost complete inhibition of spontaneous oestrus for the next 6 days in buffalo-cows. This is in agreement with previous reports in cows (Twagiramungu, et al. 1992; Stevenson et al., 1996). Occurrence of oestrus is associated with development of a large follicles (Sirois and Fortune, 1988). Inhibition / or delay of oestrus in GnRH treated groups may be due to alteration in the formation of large follicles. Previous works by Guilbault et al. (1991); Twagiramungu et al.

(1992) and Yamada et al. (1998) reported disappearance of antral follicles by atresia and (or) lutenization after administration of GnRH. This reduced the follicular production of oestradiol (Tsonis et al. 1983) which in turn prevent an increasing in the concentration of endometrial oxytocin receptors. In addition, the oestradiol concentration affects the timing, magnitude and pattern of $PGF_2\alpha$ production.

In agreement with previous reports (Macmillan et al., 1985b; Twagiramungu et al. 1992) the administration of GnRH also seem to prolong CL lifespan and or partially protects the CL against spontaneous luteolysis. In the present study, the P_4 concentration on day 0 and in day 6 in GnRH treated groups tend to be higher than those of animals in control group (not exhibited oestrus between days 0 an 6). This suggested that luteal levels of P_4 was maintained in most of buffalo-cows (33/37) in GnRH treated groups. This may have also contributed to inhibition of oestrus in GnRH treated groups. Moreover, treatment with GnRH would not be effective in inhibiting oestrus if oestrus was imminent at the time of treatment (Peters and Ball, 1987). This is most likely what happened to the four buffalo-cows which showed oestrus during 6 days after GnRH treatment.

Although, the results of the luteolytic action of lutalyse imposed on day 7 after GnRH in this study corporate with those of Narasimha Rao and Venkatramiah (1989 and 1991), where about 88.0% of buffalo-cows were detected in oestrus. The synchronization rate tended to be lower (5.5 -12. 5%) when compared with control group. Previous study by Dobson et al. (1975) reported that GnRH treatment of animals decreased the response to a physiological dose of $PGF_2\alpha$ during what is normally, a $PGF_2\alpha$ susceptible part of the luteal phase. Recently, such effects were also reported by Birnie et al. (1999). This may be due to the luteotrophic effect of GnRH on the CL, thus affecting on the usual cascade of oxytocin stimulation and P_4 inhibition that occurs until completion of luteolysis. Mann and Lamming (1995) suggested that treatment with GnRH during the luteal phase depressed the luteolytic drive which start at the end of this phase.

The present study revealed that pregnancy rate was lower (33.3%) in control group (treated only with $PGF_2\alpha$). This is in agreement with previous studies by Landivar et al. (1985); Orihuela et al. (1983); Xu et al. (1997); Yamada et al. (1998); Porter et al. (2000) who reported lower fertility rates after OS using $PGF_2\alpha$ when compared with spontaneous oestrus. This could be partly explained by the facts: 1) when

a dominant follicle became persistent and failed to turn over in regular oestrus of two, three or four follicular waves (Lucy et al., 1992), the oocyte had the potential for aging. 2) CL drive from OS using $PGF_2\alpha$ has reduced P_4 production and short luteal phase (Hansen et al., 1987), a correlation between P_4 levels and conception rate has been reported (Rosenberg et al., 1990). This may have reduced fertility rates through its negative effect on the rates and subsequent embryonic survival. This hypothesis cannot be made for buffalo-cows treated by GnRH injection in the present study. It has been shown that the treatment of cows with GnRH during the luteal phase causes the ovulation / luteinization of dominant follicle (Yamada et al., 1998) and hence a) An increase of P_4 concentrations. b) Growth of a new cohort of follicles, one of which becomes dominant during the following seven days (Peters et al., 1999)

However, the pregnancy rate in GnRH treated group (especially GPG group) is nearly similar to that reported (59.1%) by Yamada et al. (1998) who used the same protocol during luteal phase in dairy cows. The pregnancy rate in the present study considered higher than those previously reported by Pursely et al. (1997a,b) who applied two different versions of GPG protocol (37% and 37.3%). The observed difference could be explained by the fact that buffalo-cows in our study were cycling with the presence of a palpable and active corpora lutea without any abnormalities of the reproductive tract. Moreover, the present study also indicated that administration of GnRH two days later after $PGF_2\alpha$ injections (GPG group) improved the pregnancy rate (10%) when compared with GP group. This is in agreement with recent study by Mawhinney and Biggadike (1998) who reported a strong correlation between 2^{nd} GnRH and maintained pregnancy rate close to control. Furthermore we allowed a time period of two days between $PGF_2\alpha$ and GnRH injections (GPG group), this was probably beneficial for oocyte maturation. Schmitt et al. (1996) have reported a significant improvement in conception rates when the time from $PGF_2\alpha$ to GnRH administration was extended to 48 hours. In addition, it has been shown that GnRH agonist given prior to or at time of the preovulatory LH surge cause an amplification of the surge which is likely to affect the process of oocyte maturation (Rosenberg, et al. 1991). This emphasizes the importance (improve the pregnancy rate in compared with GP and control groups) of giving of the 2^{nd} GnRH of this regime in the present study.

Thus it can be concluded that the use of GnRH (day 0) - $PGF_2\alpha$ (day 7) - GnRH (day 9) appears to be a useful synchronization method, as well as a tool for improving the reproductive performance in buffalo-cows.

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Table. 1: Effect of pre-treatment with GnRH (fertirelin acetate) or not (control) 7 days before $PGF_2\alpha$ on oestrus response in buffalo-cows[a]

	Control group		GnRH group		*P* value
	No./No.	(%)	No./ No.	(%)	
Animals in oestrus[b] (days 0 -6)	5/21	23.8	4/37	10.8	Ns[d]
Animals in oestrus[c] (days 7 - 10)	15\16	93.8	28/33	84.8	Ns
Overall oestrus (days 0 - 10)	20/21	95.2	32/37	86.5	Ns

a) All animals included. b) Sponatenous
c) Induced by $PGF_2\alpha$. d) Non significant ($p > 0.1$).

Table 2: Oestrus, pregnancy rate and progesterone (P_4) levels in animals[1] either pre-treated (subgroup I) and post-treated (subgroup II) or not (control) with GnRH (fertirelin acetate)

	Control group (SP)[2]	GnRHgroup	
		Subgroup I (GP)[3]	subgroup II (GPG)[4]
No. animals	16	17	16
Synchronization rate (%)	15/16 (93.8)	15/17 (88.2)	13/16 (81.3)
Pregnancy rate (%)	5/16 (31.3)[b]	10/17(58.8)[ab]	11/16 (68.8)[a]
P_4 conc. Day 0 (ng/ml)	3.20 ± 1.60	3.30 ± 1,53	3.12 ± 0.87
P_4 conc. Day 7 (ng/ml)[5]	2.98 ± 0.84	3.92 ± 1.52	3.14 ± 0.44

1): Only animals that did not exhibited oestrus between day 0 to day 6 are considered.
2): Saline and $PGF_2\alpha$.
3) GnRH and $PGF_2\alpha$.
4) GnRH- PGF2α-GnRH.
5) Just before $PGF_2\alpha$ injection.
a,b: values in the same rows with different superscripts are different ($p< 0.05$).