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EFFECT OF GnRH ON REPEAT BREEDER BUFFALO-COWS AND DELAYED PUBERTAL BUFFALO-HEIFERS AND ITS EFFECT WITH PGF2∝ ON REPRODUCTIVE EFFECIENCY OF POSTPARTUM BUFFALO-COWS

(With 3 Tables)

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(Received at 1/6/2004)

تأثير الهرمون المحرر للحاثة المنسلية على ظاهرة الشياع المتكرر فى الجاموس والبلوغ المتأخر فى عجلات الجاموس ومصحوبا بالبروستاجلاندين ف٢ ألفا على كفاءة الجاموس التناسلية بعد الولادة

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تم إجراء ثلاثة تجارب الأولى استهدفت تأثير الهرمون المحرر للحاثة المنسلية GnRH عند اليوم ١١ بعد التلقيح على ظاهرة الشياع المتكرر فى الجاموس والثانية استهدفت دراسة تأثير GnRH علـى البلوغ المتأخر فى عجلات الجاموس أما الثالثة فقد استهدفت دراسة تأثير جرعتيـن من GnRH عند اليوم الأول والتاسع وجرعة من البروستاجلاندين ف٢ الفا عند اليوم السابع وإجراء التلقيح عند اليوم العاشر (GPG) على خصوبة الجاموس بعد الولادة. فـى التجربة الأولى تم استخدام ٤٦ جاموسة ذات شياع متكرر تم حقن ٣٤ منها بالهرمون المحـرر للحاثـة المنسـلية عند اليوم ١١ بعد التلقيح و تم حقن عدد ١٢ منها بمحلول ملح فسيولوجى. أما التجربة الثانية فقد تم استخدام عدد ٤١ من عجلات الجاموس أعمارها تعدت العاميـن ولـم تظهر عليها علامات الشياع تم حقن عدد ٣٠ منها بالهرمون المحرر للحاثة المنسـلية وتم حقن ١١ الباقين بمحلول ملح فسيولوجى. وفى التجربة الثالثة تم استخدام عدد ٥٠ جاموسـة بعـد الـولادة بمـدة لا تقل عن ٤٥ يوم تم حقن عدد ٣٦ منها بجرعتين من الهـرمون المحرر للحاثة المنسلية عند اليوم الأول والتاسع من التجربة وعند اليوم السابع تم حقنهم بالبروستاجلاندين ف٢ الفا أما ١٤ جاموسة الباقين فتم حقنهم بمحلول ملح فسيولوجى فـى نفس المواعيد وتم تلقيح الخمسين جاموسة فى اليوم العاشر من التجربة. تم حساب عدد التلقـيحات اللازمة للإخصاب والفترة من العلاج للإخصاب ومعدل الإخصاب فى التجارب الثلاث. أظهرت النتائج أن الهرمون المحرر للحاثة المنسلية أدى إلى تحسن الكفاءة التناسلية

للجـــاموس فى التجارب الثلاث. ففي التجربة الثانية قد أظهرت النتائج أن عدد ٦، ٨٦ % من عجلات الجاموس المعالجة قد شاعت فى حين أن ٥، ٥٤ % من المجموعة الضابطة قد شـــاعت . وفـــى المجموعـــة الأولى كانت عدد التلقيحات اللازمة للإخصاب فى المجموعة المعالجـــة أقـــل منها فى الضابطة. وكذلك أظهرت النتائج أنه فى الثلاث تجارب كان هناك نقص معنوى فى الفترة من العلاج للإخصاب وكذلك ارتفاع معنوى فى معدل الإخصاب فى المجموعات المعالجة مقارنة بالضابطة.

SUMMARY

Three experiments were conducted to study fertility of repeat breeder buffalo cows, delayed puberty in buffalo heifers and reproductive efficiency of postpartum buffalo cows after GnRH (at day 11 post service), GnRH and GnRH- $PGF_2\alpha$ - GnRH (GPG) administration in three separate experiments respectively. In experiment 1, 34 repeat breeder buffalo cows were injected (im) with 0.02 mg Buserelin (5 ml Receptal) at day11 post service (treated group). Other 12 repeat breeder buffalo cows were received (im) 5 ml saline and used as control group. In experiment 2, 30 delayed pubertal buffalo heifers (their ages above 2 years) were received (im) 0.02 mg Buserelin (treated group) however, 11 similar buffalo-heifers were injected (im) with 5 ml saline (control group). While in experiment 3, 36 of postpartum buffalo cows were received (im) 1st dose of GnRH (5ml Receptal) then injected (im) with PGF2∝ at day 7 after the 1st dose of GnRH. At 40 hours post PGF2∝ injection, animals received the second dose of GnRH (GPG treated group). Another 14 postpartum buffalo cows received saline and used as control. Cows in both GPG and control groups were naturally mated 24 hours after 2nd dose of GnRH or saline injections. Number of services per conception (S/C), treatment conception interval (TCI) and conception rate (CR) were calculated. The results indicated that, 26 (86.6%) out of 30 anoestrous buffalo heifers came in estrus after GnRH injection, while in control 6 (54.5%) out of 11 exhibited estrus signs. In addition, S/C was significantly lower ($P<0.05$) in treated than control animals in experiment 1. While, there were no significant differences in S/C between treated and none treated animals in experiments 2 and 3. There was a highly significant ($P<0.01$) reduction in TCI in treated (20.00+4.57, 21.68±3.06 and 23.62±3.26 days) than control (51.67±13.64, 46.40±8.06 and 49.13±7.62 days) in the three experiments respectively. Mean conception rate of the first 3 services was significantly higher (71, 80 and 72%) in the treated animals than in control (50, 66 and 57%) in the three experiments respectively. It was

concluded that, fertility parameters were improved after GnRH administration in repeat breeder buffalo cows, delayed puberty in buffalo heifers and in postpartum buffalo cows after GPG protocol.

Keywords: *GnRH, $PGF_2\alpha$, repeat breeder, anestrous, buffalo-cows*

INTRODUCTION

In buffalo-cows, poor reproductive performance is the major obstacle limiting their productivity and genetic progress. Although many variables can reduce fertility in buffaloes, the high incidence of silent ovulation (subestrus) either due to absence or reduced intensity of estrus behavior, or poor estrus detection is the most frequent cause of prolonged calving (Awasthi *et al.*, 1998; Barkawi *et al.*, 1998 and Singh *et al.*, 2000). One important approach to deal with reproductive inefficiency in buffaloes is the strategic use of hormonal agents to manage reproduction. Such programs are relatively recent in buffalo practice and their application is still and being refined in this species (Agarwal and Selvaraju, 2000 and Abou El-Roos and Abdel-Ghaffar, 2000).

GnRH is the key hormone that regulates the synthesis and release of LH and FSH from the anterior pituitary gland. The recent synthesis of GnRH has allowed its extensive use to initiate ovarian cyclicity (Roche and Mihm, 1996). The conception rate to first service after GnRH injection immediately following insemination was 53.4% compared with 32.33% in control non treated buffaloes (Rao and Rao, 1984). The cows that were injected with GnRH agonist on either day 11, 12 or 13 after insemination had higher pregnancy rates than control cows (72.4% versus 60.94%) (Macmillan *et al.*, 1986). GnRH treatment in cows on days 11 and 13 improved conception rate (Mann and Lamming, 1995). On the other hand, Jubb *et al.* (1990); Thatcher *et al.* (1993) and Ryan *et al.* (1994) were unable to show any improvement in conception rates when GnRH was injected between 11 and 13 days after insemination in repeat breeder cows.

Injection of anoestrous Egyptian buffaloes with GnRH improved their fertility (Nasr *et al.*, 1983, Hazzaa *et al.*, 1985, Aboul-ela *et al.*, 1988, Abdoon *et al.*, 1994 and El-Fadaly *et al.*, 1994). On the other hand, Khurana *et al.* (1982) found mild response after GnRH treatment in 3 of 21 anoestrous buffaloes. Similarly, Dhoble and Gupta (1988) reported that only 7 out of 34 anoestrous bufffaloes expressed oestrus

after GnRH injection. Abeygunawardena *et al.* (1996) found that all treated anoestrous buffaloes with 2 GnRH injections 24 hours apart did not become pregnant within 90 days of treatment.

Combined use of GnRH and PGF2∝ in the postpartum period might be helpful for early resumption of ovarian activity in buffaloes, which have prolonged luteal activity (Shah *et al.*, 1990). Induction and synchronization of oestrus with acceptable level of fertility was obtained in anoestrus buffaloes receiving a GnRH followed by PGF2∝ (7 days interval) compared with untreated controls (Narasimah Rao and Venkatramiah, 1991). Conception rate was 77.8% after insemination in synchronized buffalo cows with PGF2∝ (Bicuda and Oba, 1992). Buffalo cows that were treated with GnRH- PGF2∝ responded in 80% ovulation without oestrus express that indicated by progesterone level above 1ng/ml (Hattab and Zeitoun, 1999). Administration of GnRH followed on day 7 by PGF2∝ elicited a rather poor response in induction of behavioral oestrus in anoestrous buffalo-cows (Narasimha Rao and Venkatamiah, 1991).

The present study aimed to investigate the effect of GnRH on reproductive performance of repeat breeder buffalo cows and delayed puberty in buffalo heifers and GnRH- $PGF_2\alpha$ - GnRH (GPG) protocol on fertility of postpartum buffalo cows.

MATERIALS and METHODS

Animals:

The buffalo-cows and heifers that were used in the present study were selected sporadically from small holders in Etay El Barod district (El-Behira province). A total of 137 animals were used in this study. 96 buffalo cows, range in ages from 5 to 8 years and calved between October, 1999 and September, 2000. Other 41 buffalo heifers their ages were more than 2 years and they did not exhibit any signs of oestrus. The animals were kept in open shelters during daytime and were stabled by night allover the year. The feeding system during winter months included Trifolium plant (Barseem), rice straw and concentrate mixture. During summer months, Darawa was offered instead of Barseem. The animals were milked twice per day. Estrus signs were observed 3 times daily, at dawn, afternoon and evening for a period of 15 minutes per each time. The animals were naturally mated using fertile buffalo-bulls; pregnancy was diagnosed 60 days after mating by rectal palpation.

Treatment:

Experiment 1(Repeat breeder):

In this experiment, 46 repeat breeder buffalo-cows were divided into two groups:

Treated group:

Thirty-four buffalo-cows were mated naturally during oestrus period. They were injected (im) with 5 ml Receptal (0.02 mg Buserelin, a GnRH analogue manufactured by Intervet International B.V. Boxmeer – Holland) at day 11 post service.

Control group:

Twelve buffalo-cows received 5 ml saline at day 11 post natural mating.

Experiment 2 (anoestrous buffalo-heifers):

In this experiment, 41 delayed pubertal buffalo heifers, aged more than 2 years and their ovaries had no structures were divided into two groups.

Treated group:

Thirty delayed pubertal buffalo heifers were injected (im) with 5 ml Receptal.

Control group:

Eleven delayed pubertal buffalo heifers were injected (im) with 5 ml saline.

Fertile buffalo- bulls were used for natural mating of animals at oestrus.

Experiment 3 (GPG treated buffalo-cows):

This experiment was carried out on 50 lactating buffalo-cows, non pregnant during their postpartum periods (>45 days). The animals were free from any reproductive disorders. They divided into two groups.

Treated group:

Thirty six buffalo cows were treated according to the following Schedule (Jemmesonl, 1998).

At day 0: Animals were injected (im) with 1st dose of GnRH (5ml Receptal)

At day 7: Animals were injected by 2ml prosolvin (7.5 mg/ml luprostiol; a synthetic $PGF_2\propto$ analogue manufactured by Intervet International B.V. Boxmeer – Holland).

At day 9: Animals were injected (im) with 2nd dose of GnRH (5ml Receptal) 40 hours after $PGF_2\propto$ injection.

At day 10: All animals were naturally mated using fertile buffalo-bulls at fixed time 24 hours post 2nd dose of GnRH.

Control group:

Fourteen buffalo cows were given saline at the same time as those treated cows. These animals were naturally mated 24 hours post injection using fertile buffalo-bulls.

Number of services per conception (S/C), treatment conception interval (TCI) and conception rate (CR) were calculated in treated and control groups in each experiment.

Pregnancy diagnosis was carried out at day 60 post mating by rectal palpation.

Statistical analysis:

The data were analyzed by using SAS (1987). For analyzing the data of the treated and control group, T- independent test was used.

RESULTS

Results are presented in tables 1-3.

Experiment 1 (Repeat breeder buffalo-cows):

The effect of GnRH injection at day 11 post mating in repeat breeder buffalo-cows on S/C, TCI and CR are presented in table 1. The results indicated that, S/C was significantly ($P<0.05$) lower (1.61) in treated animal with GnRH than control (2.33). Moreover, GnRH injection resulted in a highly significant ($P<0.01$) reduction in TCI (20.00±4.57 days vs. 51.67+13.64 days) and significant increase ($P<0.05$) in the CR of the first three services (71% vs. 50%) in treated vs. control group respectively.

Experiment 2 (anoestrous buffalo- heifers):

Effect of GnRH on S/C, TCI and CR in anoestrous buffalo-heifers is presented in table 2. Twenty six (86.6%) out of 30 anoestrous heifers exhibited oestrus signs after treatment with GnRH. However, six (54.5%) out of 11 buffalo-heifers that injected by saline (control group) exhibited oestrus signs. S/C was non significantly lower (1.45) in treated animals than in control (1.8). The results also indicated that, there was a highly significant ($P<0.01$) reducing effect in TCI (21.68±3.06 days) than control group (46.40±8.06 days). In addition, CR of the first 3 services was significantly higher (80%) in treated animals than control group (60%).

Experiment 3 (GPG treated animals):

Results presented in table 3 indicate that, S/C in treated animals were not significantly lower (1.62) than in control animals (1.75). However, There was a highly significant ($P<0.01$) reduction in TCI in the GPG -protocol (23.62±3.26 days) than control (49.13±7.62 days).

Moreover, CR (72%) was significantly (P <0.05) higher in treated animals than in control (57%).

Table 1: Effects of GnRH injection at 11 days post mating on S/C, TCI and CR in repeat breeder buffalo-cow (mean ± SE)

Treatment	Number of buffaloes	S/C	TCI (days)	CR (%)			
				1st service	2nd service	3rd service	Total
Treated	34	1.61	20.00±4.57[b]	35.5	14.8	20.7	71[a]
Control	12	2.33	51.67±13.64[a]	8.4	25	16.6	50[b]
T-value		1.893	2.791**				219.953*

Means in the same column have different superscripts are significantly different
* P<0.05, ** P<0.01
S/C = No. of services per conception
TCI = Treatment conception interval
CR = Conception rate

Table 2: Effects of GnRH injection on estrus exhibition, S/C, TCI and CR in anoestrous buffalo-heifers

Treatment	No. of buffalo heifers	Buffalo-heifers exhibited oestrus signs	S/C	TCI (days)	CR (%)			
					1st service	2nd service	3rd service	Total
Treated	30	26 (86.6%)	1.45	21.68±3.06[b]	53.2	19.2	7.6	80[a]
Control	11	6 (54.5%)	1.80	46.40±8.06[a]	33.0	16.5	16.5	66[b]
T-value			0.862	3.865**				256.923*

Means in the same column have different superscripts are significantly different
* P<0.05, ** P<0.01

Table 3: Effects of GPG protocol on S/C, TCI and CR in post partum buffalo-cows (mean ± SE)

Treatment	Number of buffaloes	S/C	TCI (days)	CR (%)			
				1st service	2nd service	3rd service	Total
Treated	36	1.62	23.62±3.26[b]	38.8	22.2	11	72[a]
Control	14	1.75	49.13±7.62[a]	28.5	14.2	14.3	57[b]
T-value		0.424	3.545**				225.203*

Means in the same column have different superscripts are significantly different
* P<0.05, ** P<0.01

DISCUSSION

Reproductive inefficiency in buffalo-cows is a major problem in many herds and has been well documented (Gupta *et al.*, 1994; Dahama, 1995 and Singh *et al.*, 2000)

The reproductive parameters of the repeat breeder buffalo-cows that treated with GnRH at day 11 post service showed improvement where, S/C was reduced to 1.61 compared to control animals (2.33). TCI and CR were also improved in treated animals compared to control animals. These results are in agreement with that reported by Macmillan *et al.* (1986) and Mann and Lamming (1995). They reported that, administration of GnRH at day 11-13 after insemination in repeat breeder cows improved the pregnancy rates in treated animals. They suggested that GnRH treatment on days 11 and 13 causes a reduction in oestradiol secretion, which reduces the stimulus to the luteolytic mechanism, the chances of an embryo preventing luteolysis are increased and conception rate is improved.

On the contrary, Jubb *et al.* (1990); Thatcher *et al.* (1993) and Ryan *et al.* (1994) were unable to show any improvement in conception rates when GnRH was injected between 11 and 13 days after insemination in repeat breeder cows.

This study revealed that, GnRH injection in anoestrous buffalo-heifers resulted in 86.6% of buffalo-heifers came in oestrus, while in control, 54.5% of buffalo-heifers exhibited oestrus signs. An improvement was found in S/C, TCI and CR in treated compared with control. These results are in agreement with Pattabiraman *et al.* (1986). They found that, 60% of treated buffalo-heifers showed oestrus 10-26 days after treatment with GnRH while 20% from control animals came in oestrus by 35 days. Similarly, administration of GnRH in anoestrous Egyptian buffalo-cows improved their fertility (Nasr *et al.*, 1983, Hazzaa *et al.*, 1985, Aboul-ela *et al.*, 1988, Abdoon *et al.*, 1994 and El -Fadaly *et al.*, 1994).

On the other hand, Khurana *et al.* (1982) found mild response after GnRH treatment in 3 of 21 anoestrous buffalo-cows. Also, Dhoble and Gupta (1988) reported that, only 7 out of 34 anoestrous buffalo-cows expressed oestrus after GnRH injection. Abeygunawardena *et al.* (1996) found that, all treated anoestrous buffalo-cows with 2 GnRH injections 24 hours apart did not become pregnant within 90 days of treatment.

The close synchronization of ovulation produced by GPG technique reduced the need for oestrus detection and allowed optimal results with single fixed time insemination (Gordon *et al.*, 1996). The results in this study revealed that, there was a highly significant ($P < 0.01$) reduction in TCI and a significant ($P < 0.05$) higher CR in treated postpartum buffalo-cows with GPG protocol than in control animals.

These results are in agreement with that those published in cattle and buffaloes by Shah *et al.* (1990); Coleman *et al.* (1991); Guilbault *et al.* (1991) and Rao and Venkatramiah (1991). They reported that, a 6 or 7days interval between GnRH and PGF2∝ administrations was a satisfactory system for synchronization of oestrus with good fertility.

CR in GPG treated buffalo-cows in this study (72%) was nearly similar to that obtained by Bicuda and Oba (1992) who reported that, the conception rate was 77.8% after using GnRH immediately after insemination in synchronized buffalo-cows with PGF2∝. Moreover, Roche and Mihm (1996) reported that, GPG protocol induced synchronization of follicle waves with GnRH and control ovulation by using two days after PGF2∝.

El-Din Zain *et al.* (2001) and Metwelly and Abdelsabour-Elmaghraby (2001) concluded that, GnRH-PGF_2 α –GnRH is a useful synchronization method, as well as a tool for improving reproductive performance in Egyptian buffaloes.

On conclusion, GnRH administration at day 11 post service increased CR, improved S/C and reduced TCI in repeat breeder buffalo cows. It also improved fertility in delayed puberty of buffalo heifers. GPG protocol improved the fertility parameters of postpartum buffalo-cows.

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