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The effect of GnRH analogue on the fertility of Prostaglandin F_2-α induced- oestrus cows

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Abstract

The effect of GnRH analogue (gonadorelin) was studied on the fertility of 35 postpartum anoestrus zebu cows and their crosses (Friesian, Sahiwal, Sindhi). Age (4-15 years), body weight (125-375 kg), body condition score (1.5-3; 1-5 scale), parity (1-9), postpartum intervals (4-12 months) of the cows were recorded. Oestrus was synchronized with two injections of PGF_2-α analogue (Luprostinol, 15 mg) intramuscularly (i.m.) 10 days apart. The cows were divided into Group I (n = 11), Group II (n = 12) and Group III (n = 12). In Group I, 8 cows with oestrus were inseminated without gonadorelin treatment. In group II, 6 cows in oestrus were treated with gonadorelin (0.25 mg) i.m. just prior to insemination. In group III, 3 days after the second injection of luprostinol, cows with smooth ovaries were treated with gonadorelin followed by luprostinol injection 9 days later, which resulted in oestrus in 8 of the 12 cows, which were inseminated. Cows without oestrus signs were inseminated at fixed-time (72 and 96 hours after the last luprostinol injection). In group I, II and III, 5, 6 and 8 cows became pregnant, respectively ($P>0.05$). More cows became pregnant at observed oestrus (68.2%) than at fixed-time insemination (30.8%; $P<0.05$). Insemination in observed oestrus is more effective than at fixed-time. GnRH analogue may have favourable effect on the induction of oestrus by PGF_2-α analogue. (*Bangl. vet*. 2014. Vol. 31, No. 2, 60 – 69)

Introduction

To maintain optimum fertility, calving interval should not exceed 365 days (Haresign *et al*., 1983; Coleman *et al*., 1985) and as such the average interval between calving to first service must not exceed 90 days (Madouasse *et al*., 2010). The interval between calving to conception depends on the resumption of ovarian cyclicity after calving (Law *et al*., 2009). It is possible to shorten the postpartum anoestrus period by using hormonal treatment but this may be successful only after improved management (Law *et al*., 2009).

The synchronization of oestrus using prostaglandin F_2α (PGF2-α) or its analogue has the advantages of facilitating artificial insemination (AI; Giordano *et al*., 2013). It is not always possible to determine the functional status of the corpus luteum (CL) by rectal

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palpation (Chowdhury *et al*., 1998). To avoid this problem two injections of PGF_2-α 10 days apart may be used for oestrus synchronization (Alam *et al*., 1993). Administration of GnRH after PGF_2-α, increases pituitary response to gonadotrophin release (Alam and Dobson, 1987; Sahu *et al*., 2014). Gonadotrophin resealing hormone (GnRH) treatment increases pregnancy rate (Shephard *et al*., 2014; Sahu *et al*., 2014). Pregnancy rate significantly increases when GnRH is given at the time of insemination in heifers and cows (Shephard *et al*., 2014; Heuwieser *et al*., 1995). Use of GnRH followed by an injection of PGF_2-α 6 or 7 days later is followed by good fertility at the induced oestrus (Sahu *et al*., 2014; Roy and Twagiramungu, 1997). Several workers investigated the effect of GnRH on fertility in *Bos taurus* cows. In Bangladesh, there are no reports on the use of GnRH analogue in zebu cows and their crosses during postpartum anoestrus. The present study was designed.

- To study the effect of GnRH treatment on fertility in anoestrus zebu cows and their crosses after synchronization of oestrus using PGF_2-α analogue and
- To evaluate the potential of fixed time AI (72 hours and 96 hours after the last PGF_2-α injection) in synchronized cows.

Materials and Methods

Animal selection

A total of 35 local zebu cows and their crosses belonged to private farms were included. Twenty five cows were from Sutiakhali of Mymensingh district, 10 were from Satpai of Netrakona district, 27 were crossbred between local indigenous with Holstein-Friesian, Sindhi or Sahiwal and eight were local indigenous. Age of the animals was determined from register or by dental examination. The body weight of animals was estimated by using a standardized tape (Swedish Association for Livestock Breeding and production, Eskilstuna, Sweden). The body condition score (BCS) of the cows was scored (1 – 5 scale) following modification of the method of Nicholson and Butterworth (1986). Cows that did not show oestrus by three months postpartum were selected. The cows were examined per rectum to confirm that they were not pregnant. The cows with puerperal complications were not included. Age, body weight, BCS, parity, postpartum intervals of cows varied from 4 – 15 years, 125 – 375 kg, 1.5 – 3.0, 1 – 9, 4 – 12 months, respectively.

Housing, feeding and management

The cows were housed in tied standings with limited floor space and good ventilation. Most of the houses were made of wood, bamboo, tin and straw: some were made of concrete. Some of the cows were turned out for grazing for six hours daily. Approximately 5 – 6 kg roughages and 1.5 – 2 kg concentrates were given daily to each animal with *ad libitum* water. The concentrate feed consisted of rice polish, wheat bran, oil cakes, molasses and salt. Lactating cows were milked by hand twice daily at an interval of 8-9 hours with their calves.

Synchronization of oestrus

All cows were clinically examined and cows found in normal health were included. The treatment schedules are presented in Fig. 1. Each animal was given two single intramuscular injections of 2 ml Prosolvin® (15 mg of luprostinol, 7.5 mg/mL, Intervet International B.V. Boxmeer, Holland) 10 days apart regardless of the stage of the oestrous cycle. Oestrus was detected twice daily at an interval of 12 hours for 5 days after each PGF_2-α injection. Induced oestrus was detected on the basis of standing to be mounted, redness and swelling of the vulva and genital discharge. After synchronization, the cows were divided into three groups.

Group-I: Eleven cows were inseminated without further treatment.

Group-II: Twelve cows were injected i.m. with GnRH analogue (single dose of 2.5 mL Fertagyl®, Gonadorelin; 0.1 mg/mL, Intervet International B. V. Boxmeer, Holland) just prior to insemination.

Group-III: Three days after second injection of PGF_2-α analogue, 12 cows with smooth ovaries were injected with 2.5 mL Fertagyl®. Nine days later the cows received 2 mL Prosolvin® and cows were observed for oestrus at 12-hour intervals for 5 days.

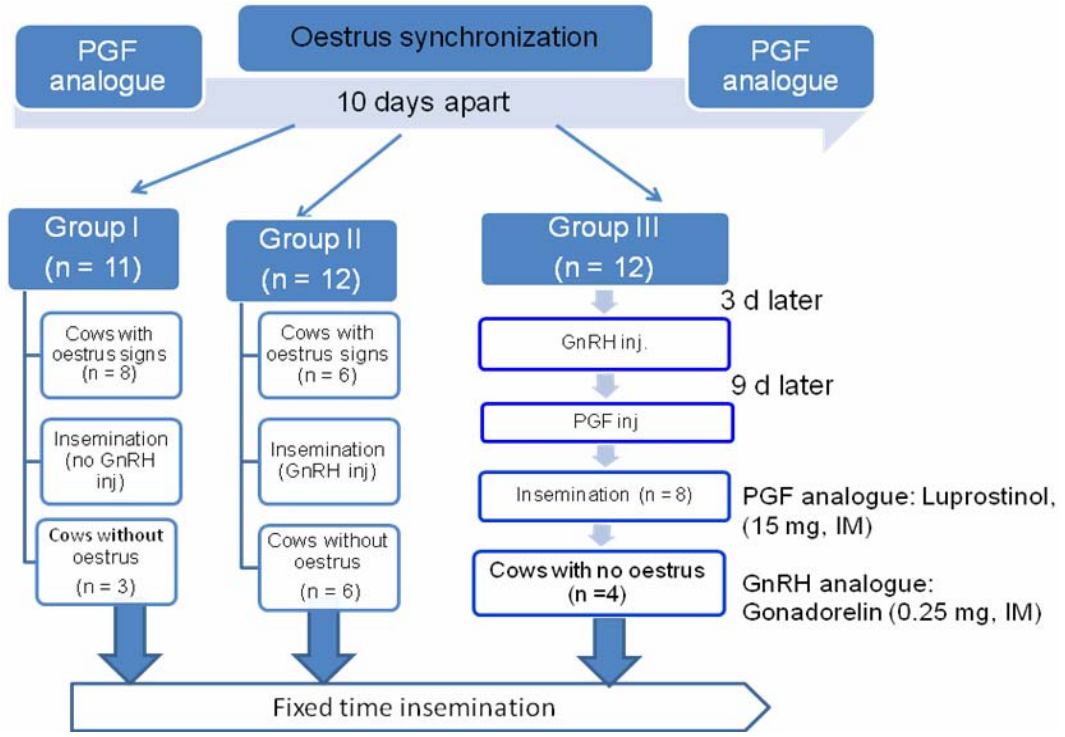

Fig. 1. Oestrus synchronization protocol with PGF2-α and GnRH analogues with fixed time insemination at 72 h and 96 hours.

Semen and insemination

The cows were inseminated 12 hours after the onset of oestrus or, when oestrus signs were not evident, fixed-time insemination was done at 72 and 96 hours. Twenty-five cows were inseminated with liquid semen from the University AI Center, Mymensingh, diluted with egg yolk citrate as described by Herman and Madeen (1963) and used within 24 hours of processing. Ten cows of Netrakona district were inseminated with frozen semen in 0.25 mL French straws (Central Cattle Breeding Station, Savar, Dhaka). In both cases, the inseminations were performed by trained AI technicians.

Pregnancy diagnosis

Cows were examined for pregnancy by rectal palpation between 60-90 days after insemination as described by Ball and Peters (2004).

Statistical analysis

Chi-square (χ^2) test (Gupta and Kapoor, 1993) was used to estimate the difference between observed oestrus AI and fixed time AI and between different groups with respect to the proportion of pregnant animals.

Results and Discussion

Outcome of different treatments with regard to the onset of behavioural oestrus is summarized in Table 1.

Table 1. Cows showing behavioural oestrus after treatment with Prosolvin® and Fertagyl®

Group	No. of cows treated	No. of cows in oestrus		
		Single injection of PGF_2-α	Two injections of PGF_2-α (10 days apart)	Two injections of PGF_2-α + GnRH + PGF_2-α (9 days later)
Group-1	11	5	8	-
Group-II	12	6	6	-
Group-1II	12	1	2	8

Second shot of PGF_2-α was given irrespective of occurrence of oestrus after first PGF_2-α injection. Out of 35 cows, only 12 showed signs of oestrus after a single shot of PGF_2-α and 16 cows showed oestrus after two injections of PGF_2-α 10 days apart. A regimen of PGF_2-α – PGF_2-α – GnRH – PGF_2-α, resulted in oestrus in eight of the 12 cows. The cows pregnant after different schedules are shown in the Table 2.

In group I, only 5/11 cows became pregnant. Six of 12 cows became pregnant in group II. In group III, 8 of 12 cows became pregnant. In total, 19/35 cows became pregnant. The response of the cows to different treatments did not differ significantly ($P>0.05$).

Table 2. Cows pregnant after different treatment schedules

Group No.	Treatments	No. of cows treated	No. pregnant
Group-1	PGF_2-α - PGF_2-α	11	5
Group-1I	PGF_2-α - PGF_2-α - GnRH	12	6
Group-1II	PGF_2-α - PGF_2-α - GnRH - PGF_2-α	12	8

Fifteen out of the 22 cows (68.2%) were pregnant after insemination at observed AI (Table 3). Thirteen cows were inseminated at fixed-time after the administration of PGF_2-α: only four cows (31%) became pregnant (Table 3).

Table 3. Number of cows pregnant after AI at observed oestrus or fixed-time insemination

Group No.	Treatments	Observed oestrus AI		Fixed-time AI	
		No. AI	No. P	No. AI	No. P
Group-1 (n = 11)	PGF_2-α - PGF_2-α	8	4	3	1
Group-II (n = 12)	PGF_2-α - PGF_2-α - GnRH	6	4	6	2
Group-III (n = 12)	PGF_2-α - PGF_2-α - GnRH-PGF_2-α	8	7	4	1
Total (n = 35)		22	15	13	4

n = Number of cows in each group; AI = Artificial Insemination; P = Pregnant; Fixed-time AI = AI at 72 and 96 hours after last PGF_2-α injection

The difference between the proportion of cows pregnant at observed–oestrus-AI (15/22, 68.2%) and fixed–time-AI (4/13, 30.8%) was significant ($P<0.05$), indicating that GnRH treatment initiates ovarian activity in cows without palpable follicle or CL, and with good management. AI at observed oestrus is more effective than at fixed-time. Energy balance is important in the resumption of postpartum ovarian activity (Law *et al*., 2009; Alam *et al*., 2009). The delayed onset of postpartum cyclicity in dairy cows often results from negative energy balance after calving, reflected by poor BCS (Alam *et al*., 2009; Crowe, 2008). In this study of zebu cows, more than three months postpartum anoestrus period with functional ovary can be common (Alam and Ghosh, 1988). The cows were fed with green grass, which might lack β-carotene, which is essential for luteal function (Arthur *et al*., 1989). It was not possible to estimate the amount of β-carotene. Suckling causes delayed resumption of ovarian cyclicity, which might be an additional factor for delayed onset of oestrus (Kawashima *et al*., 2008; Qureshi and Ahmad, 2007). However, PGF_2-α analogue may not always result in complete regression of the CL (Chowdhury *et al*., 1998). Although in Bangladesh Provolvin® is marketed by Intervet, a well known international company, there is no evidence in Bangladesh with respect to its effectiveness in indigenous cows.

The pregnancy rate (number of cows calved/number of cows serviced: 50%) after treatment with two injections of PGF_2-α at an interval of 10 days was much higher than Hardin *et al*. (1980) who recorded 30% in zebu cows and their crosses. However, the pregnancy rate was less than Bhuiyan (1990) data (57%) in local zebu crosses. Breeds, age and season can cause differences, but these were not statistically significant. PGF_2-α also has a myometrial contractile effect. PGF_2-α treatment might reduce the incidence of subclinical uterine infection and hasten the return to a suitable environment for pregnancy (Jeremejeva *et al*., 2012). It is not always possible to maintain hygienic procedures at calving in Bangladesh, which may result in uterine infection with the presence of a CL. However, this study supports the fact that local zebu and their crosses may be placed under synchronization programme using only PGF_2-α (Alam and Ghosh, 1994).

GnRH analogue is used to induce ovulation at AI (Sahu *et al*., 2014) for ensuring successful fertilization. At this investigation GnRH was given just prior to AI to synchronize the LH surge and ovulation. Previously several studies have tested the effect of GnRH given at the time of AI with conflicting results (Stevenson *et al*., 1990; Rosenberg *et al*., 1991). Our study supports the previous findings that pregnancy rate significantly increases when GnRH is administered at insemination in heifers and cows (Fukuda *et al*., 1984; Heuwieser *et al*., 1995). Pregnancy rate was higher (5 to 17%) in GnRH-treated cows where BCS was 1.5 to 3 (Burke *et al*., 1996). GnRH injected at insemination was effective, especially in cows at the first and third lactations, cows at 101 days postpartum or later, cows with daily milk yield of 26-30 kg, and also in cows from the area where a regional average fertility was relatively low (Nakao *et al*., 1983). GnRH was given 72 hours after 2nd injection of PGF_2-α, as insemination 80 hours after a 2nd treatment of PGF_2-α does not significantly influence the pregnancy rate (Lucy *et al*., 1986). The injection of GnRH closer to the onset of oestrus may increase pregnancy rate (Thatcher *et al*., 1993). Roy and Twagiramungu (1997) suggested 11 hours was the optimal time after PGF_2-α for the GnRH injection and for AI. In zebu cows and their crosses, the precise timing of AI in relation to the GnRH injection warrants further investigation. Conclusions on fertility after applying GnRH at AI in synchronized oestrus need to be cautious, because the number of cows used in this study was small.

In our study GnRH pretreatment resulted in oestrus in 67% of cows, compared with 56% in another study (Twagiramungu *et al*., 1992) confirming that synchronization is high after using GnRH as well as PGF_2-α (Thatcher *et al*., 1993; Roy and Twagiramungu, 1997). GnRH eliminates the large follicles by ovulation or atresia and induces emergence of a new follicle wave within 3-4 days after treatment at any stage of the oestrous cycle (Twagiramungu *et al*., 1995). In the present study 8/12 cows with smooth ovaries became pregnant. The pregnancy rate is encouraging in local zebu cows. In postpartum cows, large antral follicles may fail to ovulate due to low synthesis and/or secretion of hypothalamic gonadotrophin (McNatty, 1988). Although the cows were not in very early postpartum period, GnRH might ensure the ovulation of large follicles where the LH surge was absent. LH surge may be absent in

zebu cows more often than in *Bos taurus* breeds. Further work is needed with larger population of cows for definite conclusion. It seems that GnRH-PGF_2-α may initiate ovarian cyclicity in zebu cows. Proper herd management can raise the fertility in dairy herds (Butler, 2014). Veterinarians can improve herd reproductive performances by participating in oestrus detection and AI (Phatak and Whitmore, 1991). AI at observed oestrus resulted in 37% higher pregnancy rate than fixed time AI, which confirmed the beneficial effect of oestrus detection after PGF_2-α treatment and AI (Zeroual, 1994; Roy and Twagiramungu, 1997). It is necessary to develop a programme using exogenous hormones for fixed-time AI to eliminate the need for oestrus detection, especially in zebu cows and their crosses. It is not always possible to identify oestrus due to shorter duration (Galina *et al*., 1982). Hormones, especially the analogues of PGF_2-α and GnRH are expensive. GnRH- PGF_2-α-GnRH treatment at day 0, 7 and 8/9, respectively, regardless of the stage of oestrus and then fixed-timed AI is economically advantageous (Mawhinney *et al*., 1996; Britt and Gaska, 1998).

With this protocol the overall pregnancy rates are similar in beef cattle inseminated at fixed time (Twagiramungu *et al*., 1995). Pregnancy rates using GnRH-PGF_2-α-GnRH treatment and fixed-time AI in lactating cows were similar to those of controls (Pursley *et al*., 1994), whereas Schmitt *et al*. (1994) reported a reduction in pregnancy rates in heifers. Therefore, GnRH-PGF_2-α-GnRH may eliminate the need for oestrus detection (Twagiramungu *et al*., 1995). In this study 2nd dose of GnRH at AI was not given after synchronizing oestrus with GnRH-PGF_2-α (in group III). However, to get consistent results this protocol (GnRH - PGF_2-α - GnRH) may be tested with a larger population.

Conclusions

The present investigation reveals that GnRH analogue may have favourable effect on the induction of oestrus by PGF_2-α analogue in anoestrus zebu cows and their crosses with good husbandry practices. Moreover, AI at observed oestrus is more effective than at fixed-time in oestrus synchronized cows.

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