INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
18F–0370/2018/20–6–1433–1436
DOI: 10.17957/IJAB/15.0000
http://www.fspublishers.org

Full Length Article

Effect of GnRH, hCG and P_4 Therapy on Pregnancy Rate of Crossbred Cattle

Muhammad Rizwan[1], Anas Sarwar Qureshi[2*], Muhammad Farooque Hassan[3], Muhammad Usman[2] and Muhammad Saleem[4]

[1]*College of Veterinary Sciences, Bahadur Campus, Bahauddin Zakariya University, Layyah, Pakistan*
[2]*Department of Anatomy, University of Agriculture, Faisalabad, Pakistan*
[3]*Department of Pathology, Shaheed Benazir Bhutto University of Veterinary and Animal Sciences, Skrand, Pakistan*
[4]*Military Dairy Farm, Lahore, Pakistan*
[*]For correspondence: anas-sarwar@uaf.edu.pk

Abstract

To elucidate the effect of GnRH, hCG and P_4 on conception and pregnancy rate in crossbred cows after artificial insemination (AI), a total of 40 lactating crossbred (Friesian × Sahiwal) cows were randomly divided into 4 groups (A, B, C, D) such that each group comprised of 10 animals. At 7th day after AI, group A was given GnRH (100 μg/animal IM), group B was given hCG (3300 IU/animal IM), group C were given P_4 (0.5 mg/animal IM) and group D was given no treatment and served as control group. P_4 estimation of all cows was done at the start of treatments and 7th day after treatment. All cows were examined for pregnancy by rectal palpation and uterus contents for detection of an embryonic vesicle on 40 ± 1 day after AI, and pregnant cows were re-examined four weeks later, on 68 ± 1 day after AI. The mean pregnancy rate was significantly higher ($P < 0.05$) in cows of group B and C as compared to control whereas non-significant differences ($P > 0.05$) were observed between group A and D. There was non-significant difference in serum progesterone concentrations between pregnant animals of group A, B, C and D during 7 and 14 days of AI, whereas 7 days after treatment, there were significant differences in serum progesterone concentrations between pregnant and non-pregnant animals of all groups (A, B, C and D). It is concluded that hCG or progesterone could be used as a method for improving pregnancy rate in crossbred cattle. © 2018 Friends Science Publishers

Keywords: Progesterone; hCG; GnRH; Artificial insemination; Conception; Pregnancy

Introduction

Conception failure in cattle is related with less concentrations of progesterone (P_4) than normal level as early as 6th day after insemination (Yildiz *et al.*, 2009). Development of the embryo is associated with concentrations of P_4 in the blood and the ability of the conceptus to secrete the anti-luteolytic hormone (De Rensis *et al.*, 2007). Exogenous P_4 has been shown to stimulate embryo development. A number of hormonal treatments could be used to enhance peripheral concentrations of P_4 after AI including those which increase endogenous function of the existing corpus luteum (CL), or supplement progestin or P_4 directly. Recent interest has been focused on the effects of GnRH and hCG hormones on pregnancy rates. GnRH and hCG may either increase conception rate (Anjum *et al.*, 2009; Olfati and Moghaddam, 2013) or has no effect on pregnancy rate (Coleman *et al.*, 1991) in lactating dairy cattle. This effect may occur through GnRH-stimulated leutinizing hormone surge, stimulating production of P_4 by CL (Gaja *et al.*, 2008). The use of GnRH or hCG after AI increases P_4 secretion due to the luteotropic effect (Santos *et al.*, 2001) and induction of formation of an accessory CL (Fonseca *et al.*, 2001) thus increasing P_4 concentration and conception rate. Administration of GnRH or its agonists induces accessory CL when administered while a dominant follicle will be viable on days 5 to 12 of the estrous cycle (Olfati and Moghaddam, 2013), hCG (3300 IU) on day 5th increased conception rates at 28, 45, and 90 days after first postpartum AI. The hCG also induces accessory corpus leuteum in 86% of cows and increases the concentrations of blood P_4 by 4 mg/mL (Wiltbank *et al.*, 2012). This will allow running a program where animals can produce offspring at a higher frequency and in consequence, farmers can obtain higher and sustainable economic returns. There is scanty information regarding the effect of administration of GnRH, hCG and progesterone after AI in crossbred (Friesian × Sahiwal) cattle so the present study was designed.

To cite this paper: Rizwan, M., A.S. Qureshi, M.F. Hassan, M. Usman and M. Saleem, 2018. Effect of GnRH, hCG and P_4 therapy on pregnancy rate of crossbred cattle. *Int. J. Agric. Biol*., 20: 1433–1436

Materials and Methods

Experimental Design

The study was conducted at Military dairy farm, Multan, Pakistan. A total of 40 lactating crossbred (Friesian x Sahiwal) cows in their 3rd to 5th lactation was selected and divided into 4 groups (A, B, C, D) each comprising of 10 animals. The composition of the ration fed to animals is shown in Table 1. All the cows were in their first AI postpartum and interval from calving to treatment was approximately 90 days. After seven days of AI, GnRH, hCG, P_4 hormone administration was made in the group A, B and C, respectively while group D was control group as depicted in Table 2. All the semen doses used for AI were from Sahiwal breed bull.

Serum Sample Collection

The blood samples were taken at 7th and 14th day post AI for the estimation of progesterone concentration in the blood. On day 7, blood samples from all the cows were collected just before hormonal treatment. The serum was taken from the blood for further processing.

Determination of Progesterone (P_4) Levels

Progesterone level was determined through ELISA by using a commercially available kit (Bio Check, Inc., Lot. RN-36893). The progesterone EIA is based on the principle of competitive binding between progesterone in the test specimen and progesterone-HRP conjugate for a constant amount of rabbit anti-progesterone. In the incubation, goat anti-rabbit IgG-coated wells is incubated with 25 μL progesterone standards, controls, sample, 100 μL progesterone-HRP Conjugate reagent and 50 μL rabbit anti-progesterone reagent at room temperature (18–25ºC) for 90 minutes. During the incubation, a fixed amount of HRP-labeled P_4 competes with the endogenous P_4 in the standard, sample, or quality control serum for a fixed number of binding sites of the specific P_4 antibody. Thus, the amount of progesterone peroxidase conjugates immunologically bound to the well progressively decreases as the concentration of P_4 in the specimen increases. Unbound progesterone peroxidase conjugate is then removed and the wells washed. Next, a solution of TMB reagent is added and incubated at room temperature for 20 min, resulting in a blue color. The color development is stopped with Stop Solution, and the absorbance is measured spectro-photo-metrically at 450 nm. The intensity of the color formed is proportional to the amount of enzyme present and is inversely related to the amount of unlabeled P_4 in the sample.

Statistical Analysis

The data regarding pregnancy rate were analyzed by Chi-Square Test, while P_4 concentration data by ANOVA (Steel *et al.*, 2006).

Results

The results indicated that pregnancy rate was significantly higher ($P < 0.05$) in cows of group B treated with hCG and C treated with P_4 as compared to Control (group D), whereas non-significant differences were observed between group A treated with GnRH and D (Control) as depicted in Table 3. There was non-significant difference ($P > 0.05$) in pregnancy rate between group B and C. Pregnancy rates were higher for hCG treated cows (Group B) on day 28, 45 and 90 after AI. Treatment with hCG on day 5 after AI enhanced pregnancy rate of high producing dairy cows. The pregnancy rates in cows treated with 100 μg GnRH (Group A) on day 7 after AI and in control group (Group D) cows were similar. Only one cow from GnRH treated group (Group A) and one cow from control animals (Group D) became pregnant. Pregnancy rates following the treatment with GnRH after AI have been demonstrated to be decreased in some experiments. The supplemental progesterone during early gestation resulted in 40% pregnancy rate, which was a significant improvement over the control (10%) cows. These results show that all the hormones used in the present study did not have similar effect (Table 3). In group A, there was significant ($P < 0.05$) difference in serum progesterone concentrations between pregnant and non-pregnant cows at day 14 after artificial insemination. At day 14 after artificial insemination, the progesterone concentrations were significantly ($P < 0.05$) higher in pregnant cows of group B in comparison with non- pregnant cows of this group. The mean serum progesterone concentrations in pregnant cows of group A treated with GnRH were not significantly higher as compared to pregnant cows of group B which were treated with hCG. In group C treated with P_4, there was significant difference ($P < 0.05$) in serum progesterone concentrations between pregnant and non-pregnant cows at day 14 after artificial insemination. At day 14 after artificial insemination, the progesterone concentrations were significantly higher in pregnant cows of control group D in comparison with non-pregnant cows of this group. The progesterone concentrations differed non-significantly between pregnant cows of group C and D. The mean serum progesterone concentrations in pregnant cows of all four (A, B, C, D) groups were significantly ($P < 0.05$) higher in comparison with non-pregnant cows of that particular group at day 14 after artificial insemination (Table 4).

Discussion

In the current study, the pregnancy rate was found significantly higher ($P < 0.05$) in cows of group B treated with hCG and C treated with P_4 as compared to control animals (Group D), whereas non-significant differences were recorded between group A treated with GnRH and control group D (Table 3).

Table 1: Quantity of ration fed to experimental groups of crossbred cattle

Sr. No.	Feed stuff	Quantity (kg)
1	Cotton seed cake	0.79
2	Maize oil cake	0.79
3	Wheat bran	1.59
4	Wheat straw	4.55
5	Green fodder	9.09
6	Common salt	30 grams

Table 2: GnRH, hCG, P_4 hormone administration in the group (A, B, C) while D as control group in crossbred cattle

Group	Hormone	Dose	Route	Stage of administration
A	GnRH	100 μg/animal	IM	Day 7th after AI
B	hCG	3300 IU/animal	IM	Day 7th after AI
C	P_4	0.5 mg/kg	IM	For 7 days starting on day 7th after AI.
D	No treatment, Control group			

Table 3: Effect of exogenous GnRH, hCG and progesterone hormones on pregnancy rate after artificial insemination in cross bred cattle

Hormones	Groups	Total animals	Pregnant animals	Pregnancy rate
GnRH	A	10	1a	10a
hCG	B	10	5b	50b
Progesterone	C	10	4b	40b
Control	D	10	1a	10a

Different alphabets indicate significant difference at 0.05 level

Table 4: Mean (± SE) serum progesterone (ng/mL) concentration at day 7 and 14 after artificial insemination in pregnant and non-pregnant cows. Different alphabets indicate significant difference at 0.05 level

Days after AI	Group A		Group B		Group C		Group D	
	Non- Pregnant	Pregnant	Non- Pregnant	Pregnant	Non- Pregnant	Pregnant	Non- Pregnant	Pregnant
Day 7	4.4± 0.2	4.6± 0.3	4.5± 0.3	4.8± 0.2	4.7± 0.3	4.4±0.3	4.6±0.6	4.6±0.5
Day 14	7.5± 0.2a	16.6±0.3b	7.3± 0.3a	15.8±0.3b	7.3± 0.3a	16.6±0.3b	7.5±0.3a	16.4±0.3b

Stevenson *et al.* (2007) also reported higher pregnancy rate in Holstein cows treated with controlled internal drug release (CIDR) or hCG at day 4 to 9 after AI, on day 0 and again 7 days later. Pregnancy rates, following the treatment with GnRH after AI, are reported to decrease in some experiments (Ryan *et al.,* 1991). However, a contradictory report has been published recently in crossbred dairy cows by Hamid and Kamruzzaman (2017). Among all groups under study, there were significantly higher mean serum progesterone conc. (P_4) in pregnant cows compared to non-pregnant cows at day 14 after AI (Table 4). The mean serum P_4 conc. in pregnant cows of group A was not significantly higher as compared to pregnant cows of group B which were treated with hCG. The pregnancy rate differed non-significantly between pregnant cows of group C which were treated with hCG and control animals (group D). In the current study, pregnancy rate in cows of Group A was similar to Group D on day 7 after AI. Only one GnRH treated cow (Group A) and one cow from control group (Group D) became pregnant (Table 3). Supplemental P_4 during early gestation resulted in 40% pregnancy rate, which was a significant improvement over the control (10%). The similar results were reported by Robinson *et al*. (1989), who recorded 60% pregnancy rate in Holstein cows when treated with progesterone releasing device (PRID). Supplemental P_4 also suppressed endogenous production of P_4 when administered during day 10 to 17 after insemination. Santos *et al.* (2001) reported the effects of hCG administered on day 5 after AI on CL number, plasma P_4 concentrations, conception rate and pregnancy loss in high-producing dairy cows. Pregnancy rates were higher for hCG treated cows (Group B) on day 28, 45 and 90 after AI. The results are also in agreement with Yildiz *et al.* (2009) that there were no differences in mean P_4 concentrations regarding, B, C but different from control. The results of present study for P_4 concentrations are also in agreement with Beltran and Vasconcelos (2008) as these authors reported higher P_4 concentrations in cows treated with GnRH or hCG at day 5 after AI.

Conclusion

The findings of present study can be directly applied to the cattle kept under rural subsistence small holding production system. The use of hCG, 7 days after AI, may be a beneficial tool to increase the pregnancy rate in cattle

under field conditions. It is concluded that hCG or P_4 could be used as a method for improving pregnancy rate in crossbred cattle.

References

Anjum, I.A., R.H. Usmani, M.T. Tunioand and S.H. Abro, 2009. Improvement of conception rate in crossbred cattle by using GnRH analogue therapy. *Pak. Vet. J.*, 29: 93–94

Beltran, M.P. and J.L.M. Vasconcelos, 2008. Conception rate in Holstein cows treated with GnRH or hCG on the fifth day post artificial insemination during summer. *Arq. Bras. Med. Vet. Zootec.*, 60: 580–586

Coleman, D.A., F.F. Bartol, T.E. Spencer, J.G. Floyd, D.F. Wolfe and J.P. Brendemuehl, 1991. Effect of potent GnRH agonist on hormonal profiles, synchronization of estrous and fertility in beef cattle. *J. Anim. Sci.*, 69: 396–401

De Rensis F., G. Ronci, P. Guarneri, B.X. Nguyen, G.A. Presicce, G. Huszenicza and R.J. Scaramuzzi, 2007. Conception rate after fixed time insemination following ovsynch protocol with and without progesterone supplementation in cyclic and non-cyclic Mediterranean Italian buffaloes (*Bubalus bubalis*). *Theriogenology*, 63: 1824–1831.

Fonseca, J.F., J.M. Silva-Filho, M.S. Palhares, J.R.M. Ruas and A.P. Neto, 2001. Concentracao plasmatica de progesterone emnovil has receptorassubmetidasa administracao de rbST, GnRHouhCG no quintodia do cicloestral. *Arq. Bras. Med. Vet. Zootec.*, 53: 451–458

Gaja, A.O., K. Hamana, C. Kubot and T. Kojima, 2008. Evaluation of the effect of a 3rd GnRH injection administered six days after the 2nd GnRH injection of Ovsynch on the reproductive performance of Japanese black cows. *J. Vet. Sci.*, 9: 273–279

Hamid, S.A. and S.M. Kamruzzaman, 2017. Effects of GnRH on conception rate at the time of artificial insemination in crossbred dairy cows. *Int. J. Anim. Sci. Technol*., 1: 19–34

Olfati, A. and G.H. Moghaddam, 2013. Effects of GnRH agonist (Cinnarelin) on Reproductive Performance in Synchronized Iranian Crossbred Ewes during the Breeding Season. *Slovak J. Anim. Sci.*, 46: 1–6

Robinson, N.A., K.E. Leslie and J.S. Walton, 1989. Effect of treatment with progesterone on pregnancy rate and plasma concentrations of progesterone in Holstein cows. *J. Dairy Sci.*, 72: 202–207

Ryan, D.P., E. Kopel, M.P. Boland and R.A. Godke, 1991. Pregnancy rates in dairy cows following the administration of a GnRH analogue at the time of artificial insemination or at mid-cycle post-insemination. *Theriogenology*, 36: 367–377

Santos, J.E.P., W.W. Thatcher, L. Pool and M.W. Overton, 2001. Effect of human chorionic gonadotropin on luteal function and reproductive performance of high-producing lactating Holstein dairy cows. *J. Anim. Sci*., 79: 2881–2894

Steel, R.G.D., J.H. Torrie and D.A. Dickey, 2006. *Principles and Procedures of Statistics*, 3rd edition. A Biometrical Approach. McGraw-Hill Publishing Co., New York

Stevenson, J.S., M.A. Portaluppi, D.E. Tenhouse, A. Lloyd, D.R. Eborn, S. Kacuba and J.M.D. Jarnette, 2007. Interventions after artificial insemination: conception rates, pregnancy survival and ovarian responses to gonadotropin-releasing hormone, human chorionic gonadotropin and progesterone. *J. Dairy Sci.*, 90: 331–340

Wiltbank, M.C., A.H. Souza, J.O. Giordano, A.B. Nascimento, J.M. Vasconcelos, M.H.C. Pereira, P.M. Fricke, R.S. Surjus, F.C.S. Zinsly, P.D. Carvalho, R.W. Bender and R. Sartori, 2012. Positive and negative effects of progesterone during timed AI protocols in lactating dairy cattle. *Anim. Reprod.*, 9: 231–241

Yildiz, H., E. Kaygusuzoglu, M. Kayai and M. Cenesizi, 2009. Effect of post-mating GnRH treatment on serum progesterone, luteinizing hormone levels, duration of estrus cycle and pregnancy rates in cows. *Pak. Vet. J*., 29: 110–114

(Received 09 March 2018; Accepted 14 March 2018)