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EFFECT OF CIDR WITH OR WITHOUT GnRH AND DOUBLE $PGF_{2\alpha}$ BASED ESTRUS SYNCHRONIZATION PROTOCOLS ON ESTRUS RESPONSE AND PREGNANCY PER AI IN NON-DESCRIPT COWS OF THE PUNJAB

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ABSTRACT

Two experiments were conducted to analyze the effect of controlled internal drug release device (CIDR) containing 1.38 g progesterone (P4) with or without gonadotropin releasing hormone (GnRH) and double prostaglandin ($PGF_{2\alpha}$) on estrus response and pregnancy per AI in non-descript cows. In exp 1, cows in 2nd to 3rd lactation (n=80) were randomly divided into two groups as CIDR alone (n=40; CIDR inserted on D0, $PGF_{2\alpha}$ analogue administered on D6 and CIDR removed on D7) and CIDR along with GnRH (n=40; GnRH analogue administered at D0 and rest of the scheme was same as in CIDR alone) during the low breeding season. Whereas, in exp 2, cows in 2nd to 4th lactation (n=67) were distributed into two groups as CIDR (n=33; CIDR inserted on D0, $PGF_{2\alpha}$ analogue administered on D6 and CIDR removed on D7) and double $PGF_{2\alpha}$ group (n=34; $PGF_{2\alpha}$ analogue administered twice with 14 days interval) during the peak breeding season. Artificial inseminations (AI) were performed at 58 h after CIDR removal in all CIDR treated cows and upon detected estrus in the double $PGF_{2\alpha}$ group of exp 2. Pregnancy was diagnosed at 50 days post AI through rectal palpation. Estrus response did not differ significantly in both groups of exp 1(82.5 vs. 90%) and exp 2 (76 vs. 76%), however, estrus intensity differed (P=0.01; 3.02 ± 0.10 vs. 3.32 ± 0.12) between groups of exp 1. The pregnancy per AI did not differ (P>0.05) between CIDR alone (52%) and CIDR along with GnRH (58%) treated cows of exp 1; whereas, it tended to be higher (P=0.19) in CIDR treated cows (64%) as compared to double $PGF_{2\alpha}$ (46%) of exp 2. It is concluded that CIDR-based estrus synchronization protocol improves the reproductive efficiency of non-descript cows during the low and peak breeding seasons.

Key words: Non-descript cows; CIDR; $PGF_{2\alpha}$; Estrus synchronization; Artificial insemination; Pregnancy per AI.

INTRODUCTION

The zebu cattle (*Bos indicus*) are predominant in several tropical and subtropical regions of the world due to their ability to tolerate the thermal stress (Hansen, 2004) and resistance to ticks (Wambura *et al*., 1998). Pakistan mainly comes under the sub-tropical zoning, owns renowned breeds of zebu cattle *viz-a-viz* Sahiwal, Red Sindhi, Cholistani, Thari, Bhagnari and other are non-descript (do not fall in any defined breed). According to the livestock census of 2006, 11.7 million cows have been described as non-descript out of 29.6 million. Besides the aforementioned merits, *Bos indicus* especially, the non-descript has problems related to reproductive and productive traits (Turner, 1980). Milk production of non-descript cows is less than 1000 litre per lactation (Khan and Usmani, 2005). As non-descript cows make up the largest group of cattle in Pakistan, there is a dire need to work on genetic improvement of these animals.

In Pakistan, dairy and beef sector is developing and commercializing at a rapid pace to meet the increasing demand of milk and meat products (Dongre *et al*., 2011). Artificial insemination (AI) has been proved to be the major tool for genetic upgrading of *Bos taurus* cows, which may have the potential to genetically improve the *Bos indicus* as well through cross-breeding (Galina and Arthur, 1990; Larson and Ball, 1992; Nicholas, 1996). However, there are several factors that are responsible for slow reproductive efficiency with AI, such as poor estrus behaviour, difficulty in heat detection, poor semen quality and lack of skilled manpower (Anzar *et al*., 2003). Moreover, a previous study has reported that only 14% cows are inseminated artificially in the Punjab province, whereas the rest of the cows are bred either naturally or remain unmated due to the shortage of designated breeding bulls (Ahmad and Saji, 1997). The short estrus duration with a tendency to show heat signs at night in *Bos indicus* cows compared to *Bos taurus* also limits the possibility of efficient heat detection and AI (Pinheiro *et al*., 1998; Baruselli *et al*., 2004).

In order to improve the reproductive performance of a herd, a strategy of estrus synchronization has been used in temperate cows for the last six decades, which facilitates AI with or without heat detection (Nebel and Jobst, 1998; Patterson *et al*., 2003). Different protocols of endocrine hormones like GnRH, $PGF_{2\alpha}$ and P4 (CIDR; controlled internal drug release) alone or in combination have been used in temperate cows for estrus synchronization (Odde, 1990; Pursley *et al*., 1997). The exogenous source of P4 administered in a CIDR enhances plasma concentrations of P4, which increases the frequency of LH pulse and resumes the cyclicity in anestrous cows after its withdrawal (Stock and Fortune, 1993; Day, 2002; Rhodes *et al*., 2002). At present, the CIDR device is being extensively used in dairy and beef industry globally along with $PGF_{2\alpha}$ and GnRH irrespective of the stage of estrous cycle of cow (Colazo *et al*., 2004b; Lamb *et al*., 2010).

To date there is very scarce data available about the effectiveness of estrus synchronization protocols for heat induction and enhancing pregnancy per AI in zebu including the non-descript cows. Therefore, the objectives of this study were to evaluate the effect of CIDR with or without GnRH and double $PGF_{2\alpha}$ based estrus synchronization protocols on the estrus response and pregnancy per AI in non-descript cows during the low and peak breeding seasons.

MATERIALS AND METHODS

Animals and Management

Experiment 1: The first experiment was conducted during the low breeding season (September-November 2015) in non-descript cows (n=80) reared by small farmers in district Muzaffargarh, Punjab, Pakistan (30˚4' N, 71˚11' E). All the cows included in this study were non pregnant, having age ranges between 4-8 years, 2nd to 3rd lactation and 90 to 300 days postpartum with a history of normal calving. Body condition score (BCS) was recorded by visual assessment of animals before conducting the experiment by using a 5-scale scheme as 1= emaciated and 5= obese (Ayres *et al*., 2009), and cows having BCS between 2.75 to 3.25 were included in study. Animals were grazed on natural pastures of Kacha area of River Indus and offered water ad libitum. All the cows were rectally palpated to ensure that genitalia have no abnormality.

Experiment 2: The second experiment was conducted during the peak breeding season (March-June 2016) in lactating non-descript cows (n=67) kept under field conditions by farmers in district Mandi Bahauddin, Punjab, Pakistan (32˚35' N, 73˚29' E). Cows included in this study were non pregnant, having age between 4-8 years, 2nd to 4th lactation, 60 to 280 days postpartum and BCS ranges from 2.75 to 3.25. The animals were fed on green fodder mixed with wheat straw at the rate of 30-40 Kg per head per day and water was offered twice a day. Concentrate mixture (1.5 Kg) was fed at the time of milking to each lactating cow. The animals with normal history of calving included in this study and were rectally palpated to ensure that the reproductive tract had no abnormality.

Experimental Design:

Experiment 1: Cows were randomly allocated into two groups as CIDR alone group (n=40) and CIDR along with GnRH (n=40). In the CIDR alone group, an internal progesterone (P4) releasing device (1.38 g P4 in molded silicone; CIDR®, Pfizer, Auckland, New Zealand Ltd,) was placed in the vagina of all cows on day 0. Analogue of $PGF_{2\alpha}$ (Cyclomate injection: Cloprostenol Sodium 263 mcg; STAR Labs, Lahore, Pakistan; 2 ml, i.m.) was administered on day 6 and CIDR was removed on day 7. Whereas, in the group receiving both the CIDR and GnRH analogue (Lecirelin acetate 50 mcg; Dalmarelin TM Fatro®, Ozzano Emilia, Italy; 2 mL; i.m), the injection was administered on day 0 and rest of the design was the same as CIDR alone group. The timeline of experiment 1 is presented in Figure 1.

Experiment 2: Animals were randomly distributed into two groups as CIDR (n=33) and double $PGF_{2\alpha}$ (n=34). In the CIDR group the P4 releasing device (CIDR®: 1.38 g P4 in molded silicone; Pfizer, Auckland, New Zealand Ltd) was inserted in the vagina of each cow on day 0, irrespective of the stage of estrous cycle. After that a single injection of $PGF_{2\alpha}$ analogue (Dalmazin: Cloprostenol Sodium 0.150 mg; Fatro®, Italy; 2 ml; i.m) was administered on day 6 and the CIDR was removed on day 7. In the $PGF_{2\alpha}$ group, only cyclic cows having a functional corpus luteum (CL) on either left or right ovary were included (as $PGF_{2\alpha}$ has a limitation that it only works in cyclic animals). Therefore, transrectal ovarian scanning was performed through B mode ultrasonography by using an ultrasound device (Aloka SSD-500, Aloka Co., Ltd., Japan) equipped with a 7.5 MHz linear rectal probe to ascertain the presence of functional CL. All the cows received two doses of $PGF_{2\alpha}$ with an interval of 14 days between injections. The schematic representation of CIDR and double $PGF_{2\alpha}$ protocols is shown in Figure 2.

Estrus expression, AI and Pregnancy diagnosis: Animals were observed for estrus signs twice daily starting from 24 h after CIDR removal in all the CIDR treated cows of both experiments for 4 days; whereas in the second group of exp 2, estrus sign recording was started from 24 h after the administration of second $PGF_{2\alpha}$. The intensity of estrus was graded on estrus signs as 1=poor; no uterine tone and no behavioral signs, 2=satisfactory; mild uterine tone, slight mucus discharge and some restlessness, 3=good; intermediate uterine tone, mucus discharge, restlessness, nervousness, 4=very good; good tone, stand to be mounted, vulvar swelling, thick mucus discharge, restlessness; 5=excellent; high tone, stand to be mounted, thick mucus discharge, restlessness (Yousuf *et al*., 2015). Fixed time AI (FTAI) was performed by an experienced AI technician at 58 h after CIDR removal in all CIDR treated cows. Whereas, in double $PGF_{2\alpha}$ treated cows in exp 2, AI was performed upon detected estrus (12 h after standing heat) after second $PGF_{2\alpha}$injection. In exp1 and 2, inseminations were performed by using frozen thawed semen of Sahiwal and Friesian bulls, respectively, having more than 50% post thaw sperm motility. The AI doses were evaluated for quality assurance at Animal Reproduction Laboratory of Animal Sciences Institute, NARC, Islamabad (Pakistan). The AI doses of Sahiwal bull were procured from Semen Production Unit Qadirabad (Sahiwal, Pakistan), whereas semen of Friesian bull was prepared at Animal Reproduction Laboratory (NARC). Pregnancy was diagnosed through rectal palpation at 50 days post AI. The pregnancy per AI was calculated as: [(cows pregnant/cows inseminated) × 100].

Statistical Analyses: Estrus response and pregnancy per AI were statistically analyzed by using Chi-Square test of association. Kolmogorov–Smirnov test was used to analyze the normality of data for estrus intensity and estrus interval. The data for estrus intensity and interval to estrus after $PGF_{2\alpha}$ administration were compared by using the non-parametric Mann Whitney test. A probability level of $P<0.05$ was considered significant. All the data were analyzed by using Minitab (Release 17.3.1; MINITAB, Inc., State college, PA, USA) statistical package.

RESULTS

Experiment 1: Data on the effect of CIDR alone and CIDR along with GnRH protocols on estrus response, estrus intensity and pregnancy per AI in non-descript cows during the low breeding season are presented in Table 1. Estrus response did not differ between the CIDR alone (82.5%; $P>0.05$) and CIDR along with GnRH (90%) treated cows. However, estrus intensity differed significantly ($P=0.01$) between CIDR (3.02 ± 0.10) and CIDR along with GnRH (3.32 ± 0.12) treatment groups. The pregnancy per AI did not differ ($P>0.05$), and was calculated as 52% and 58% in the CIDR and CIDR along with GnRH treatment groups, respectively.

Experiment 2: Data on the effect of CIDR and double $PGF_{2\alpha}$ protocols on estrus response, estrus intensity, and pregnancy per AI in non-descript cows during the peak breeding season are presented in Table 2. The pattern of estrus response was similar ($P>0.05$) between the two groups (76% in each group). Similarly, estrus intensity remained the same in both treatments ($P>0.05$, 3.48 ± 0.12 and 3.31 ± 0.12 in CIDR and $PGF_{2\alpha}$ groups respectively). The mean intervals to estrus from $PGF_2\alpha$ administration did not vary significantly ($P>0.05$) in both groups (CIDR: 73.92 ± 1.79 vs. $PGF_2\alpha$: 75.23 ± 2.63 h). The interval to estrus from CIDR removal was 49.92 ± 1.79 h in CIDR synchronized cows. The pregnancy per AI tended to be higher in CIDR group (64%) as compared to double $PGF_2\alpha$ group (46%), however there was no statistical difference ($P=0.19$).

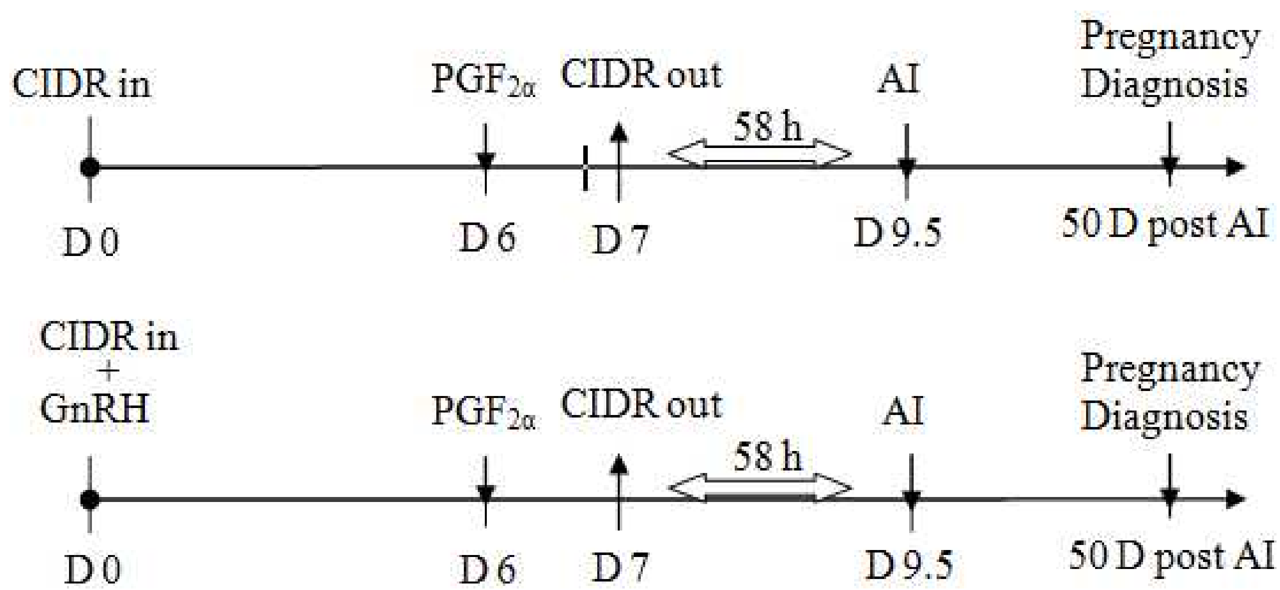

Fig. 1. Schematic representation to evaluate the effect of CIDR alone vs. CIDR along with GnRH on estrus response and pregnancy per AI in non-descript cows during the low breeding season. CIDR=Controlled internal drug release; $PGF_2\alpha$=Prostaglandin $F_2\alpha$; GnRH=Gonadotropin releasing hormone; AI=Artificial insemination; D=Day

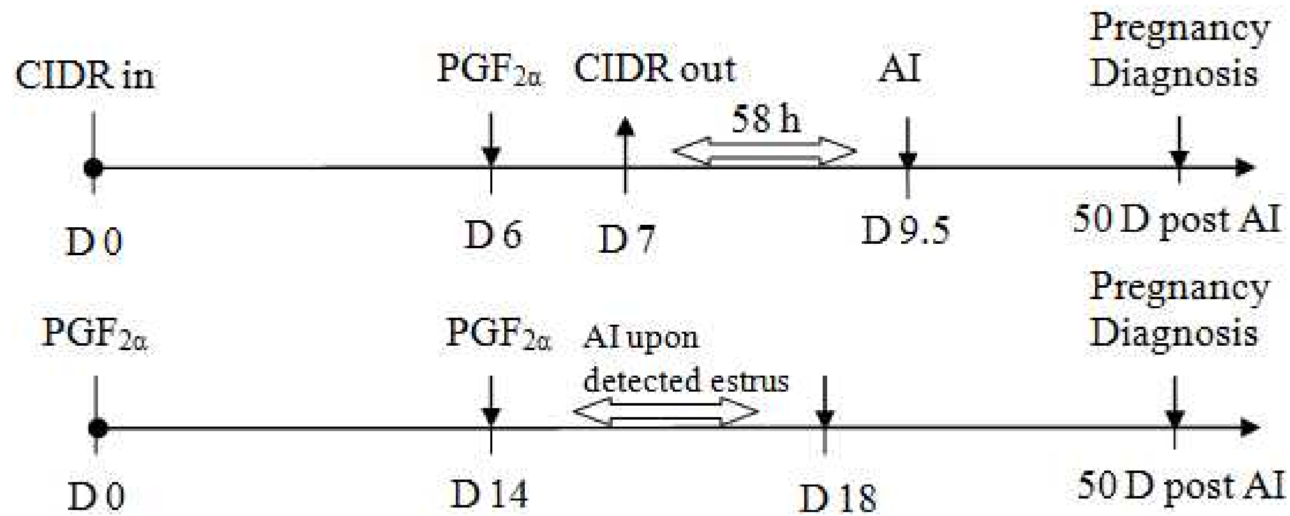

Fig. 2. Schematic representation to evaluate the effect of CIDR vs. double $PGF_{2}\alpha$ on estrus response and pregnancy per AI in non-descript cows during the peak breeding season. CIDR=Controlled internal drug release; $PGF_{2}\alpha$=Prostaglandin $F_{2}\alpha$; AI=Artificial insemination; D=Day

Table 1. Effect of CIDR alone and CIDR along with GnRH protocols on estrus response, estrus intensity and pregnancy per AI in non-descript cows during the low breeding season.

Variables	CIDR	CIDR-GnRH	*P* value
Estrus response (%)	82.5 (33/40)	90 (36/40)	0.327
Estrus intensity (score1-5)	3.02 ± 0.10*	3.32 ± 0.12*	0.017**
Pregnancy per AI (%)	52 (17/33)	58 (21/36)	0.569

* Values are mean ± SEM.
** $P<0.05$ shows significant difference.
The pregnancy per AI was calculated as: [(cows pregnant/ cows inseminated) × 100].
CIDR=Controlled internal drug release; GnRH=Gonadotropin releasing hormone; AI=Artificial insemination.

Table 2. Effect of CIDR and double $PGF_{2}\alpha$ protocols on estrus response, estrus intensity and pregnancy per AI in non-descript cows during the peak breeding season.

Variables	CIDR	$PGF_{2}\alpha$	*P* value
Estrus response (%)	76 (25/33)	76 (26/34)	0.945
Estrus intensity (score1-5)	3.48 ± 0.12*	3.31 ± 0.12*	0.355
Interval to estrus after $PGF_{2}\alpha$ (h)	73.92 ± 1.79*	75.23 ± 2.63*	0.509
Interval to estrus after CIDR removal (h)	49.92 ± 1.79*	_	_
Pregnancy per AI (%)	64 (16/25)	46 (12/26)	0.199

* Values are mean ± SEM.
The pregnancy per AI was calculated as: [(cows pregnant/cows inseminated) × 100].
CIDR=Controlled internal drug release; $PGF_{2}\alpha$=Prostaglandin $F_{2}\alpha$; AI=Artificial insemination.

DISCUSSION

To the best of our knowledge, this is the first study reporting on the efficacy of estrus synchronization regimens facilitating AI in non-descript cows. Two experiments were conducted to evaluate the efficacies of CIDR with or without GnRH and double $PGF_{2\alpha}$ based estrus synchronization protocols on the estrus response and pregnancy per AI in non-descript cows (*Bos indicus*) of the Punjab.

The P4 impregnated devices can be used for heat induction in both cyclic as well as in acyclic cows during breeding and low breeding seasons, respectively (Macmillan and Peterson, 1993; Xu and Burton, 2000; Lucy *et al*., 2001). Routinely the majority of cows are acyclic during the low breeding season (i.e. having non-functional ovaries). Therefore, in exp 1, cows were treated with CIDR alone and CIDR along with GnRH protocols to enhance the reproductive efficiency and ultimately improve the genetic makeup of herds by using semen from superior quality bulls through AI during the low breeding season. Both these protocols proved to be quite effective in inducing synchronized estrus in cows. The results showed that the estrus response did not differ significantly between groups receiving the CIDR alone (82.5%) and CIDR along with GnRH (90%); however the intensity of estrus remained higher ($P<0.05$) in cows receiving a CIDR along with GnRH. Similar ($P>0.05$) pregnancy rates per AI were achieved in CIDR (52%) and CIDR-GnRH (58%) treated cows. These results are consistent with a previous study conducted in dairy cows with an estrus response (92%) and pregnancy per AI (56%) (Xu and Burton, 2000). In contrast to these findings, estrus incidence (56%) recorded in Brahman cows treated with CIDR-$PGF_{2\alpha}$ was much lower (Flores *et al*., 2006). However, similar pregnancy rates documented in earlier studies in CIDR along with GnRH treated Holstein and beef cows ranged between 50 to 65% (Colazo *et al*., 2004a; Kim *et al*., 2005). The administration of GnRH along with CIDR insertion may help in the follicular wave emergence (Kim *et al*., 2005), however the findings of the present study indicated that the administration of only P4 in the form of a CIDR was efficient for heat induction in non-descript cows during the low breeding season.

The exp 2 was conducted to compare the efficacy of two fundamental estrus synchronization protocols (CIDR and double $PGF_{2\alpha}$) for heat induction and pregnancy rates achieved through AI in non-descript cows during the peak breeding season. The double $PGF_{2\alpha}$ protocol has a limitation that it only induces heat in cows having a functional CL on either ovary (Brito *et al*., 2002).The majority of such cows are available only during the peak breeding season. Both protocols were equally effective in inducing estrus, as the mean estrus response (76%) was similar ($P>0.05$) in both groups, which is in close agreement with the earlier findings in Sahiwal cows (Hassan *et al*., 2016). The interval to estrus after the second $PGF_{2\alpha}$ injection ranged from 48 to 96 h with a mean of 75.23 ± 2.63 h: this finding is comparable to the results of earlier studies (Aleem, 1998; Amjad *et al*., 2006; Gugssa *et al*., 2016). This broad window of the timing of estrus after the second $PGF_{2\alpha}$ restricts the possibility of using AI at a fixed time. Thus the use of AI upon detected estrus is favorable to attain reasonable pregnancy rates. The interval to estrus after CIDR removal was 49.92 ± 1.79 h, which is higher than the finding of an earlier study in Sahiwal cows (Singh *et al*., 2006). Although there was no statistical difference ($P>0.05$), the pregnancy rate per AI tended to be higher in CIDR treated cows (64%) than in cows receiving the double $PGF_{2\alpha}$treatment (46%). These results are in accordance with the fertility results (54%) of a previous study conducted in Holstein cows (Waldmann *et al*., 2006). The higher pregnancy per AI in CIDR treated cows may be due to the reason that P4 supplementation before the $PGF_{2\alpha}$ enhances conception (Xu *et al*., 1997; Niasari-Naslaji *et al*., 2001; Orozco *et al*., 2016). As non-descript cows represent about 40% of total cows in Pakistan, there is a great need to improve their genetic makeup on the large scale to meet the increasing demand of milk and meat. The information generated through these experiments is beneficial for commercial dairy and beef farmers to improve their profitability by producing genetically superior quality calves, during the low and peak breeding seasons.

Conclusion: The findings of these two experiments indicated that P4 based estrus synchronization regimens have the potential to improve the reproductive efficiency of non-descript cows in terms of enhancing estrus incidence and pregnancy rates through AI. As estrus synchronization facilitates the AI and pregnancy rates of up to 60% can be achieved, these protocols can be used on a large scale to improve the genetic potential of non-descript cows by using frozen-thawed semen of superior bulls during the low and peak breeding seasons.

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