



CONCEPTION RATE IN CROSSBRED COWS FOLLOWING OVSYNCH AND DOUBLE PGF₂α PROTOCOL

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ABSTRACT

Estrous synchronization is the manipulation of the reproductive process so that females can be bred with normal fertility during a short predefined interval. This control facilitates breeding in two important ways: it reduces, and in some cases eliminates, the labor of detecting estrus (heat), and it allows the producer to schedule the breeding. Ovsynch is one of the most "classical" and widely known systems. The present study was therefore, undertaken to compare the efficacy of Ovsynch protocol and double PGF₂α protocol, given 11 days apart on estrus synchronization and fertility response in non-inseminated, non-pregnant, anestrus (Pre-service or post service) or repeat breeding crossbred cows. The entire experiment was divided into three groups G-I (control) G-II (Ovsynch protocol) and G-III (Double PGF₂α protocol). In G-I, G-II and G-III animals gave a conception rate of 33.33%, 50% and 33.33% respectively. Present investigation reveals that Ovsynch protocol is more efficient than Double PGF₂α protocol in crossbred cows.

Key words: Crossbred, Ovsynch, PGF₂α, Estrus, GnRH.

Estrous synchronization is the manipulation of the reproductive process so that females can be bred with normal fertility during a short predefined interval. This control facilitates breeding in two important ways: it reduces, and in some cases eliminates, the labor of detecting estrus (heat), and it allows the producer to schedule the breeding. Other advantages of estrous synchronization include creating a more uniform calf crop, enabling more cows to be artificially inseminated (AI) to a genetically superior bull and reducing the length of the breeding season. Ovsynch is one of the most "classical" and widely known systems. The protocol consists of two injections of a GnRH analogue separated by a single administration of PGF₂α.

Twelve anestrus cows were randomly divided into two groups of six animals each and assigned to the treatment schedule with double PGF₂α and Ovsynch protocol. The cows were subsequently inseminated as per fixed time double insemination schedule at 72 - 96 hrs in Double PGF₂α and at 12 - 24 hrs in Ovsynch protocol, post treatment by recto-vaginal technique using frozen semen. All animals were observed for post treatment expression of estrus. In addition, six normal cycling animals served as control. These cows were palpated for a mature corpus luteum in one of the ovaries following one overt estrus and subsequently inseminated at observed estrus for comparing the fertility between fixed time double insemination and conventional breeding techniques.

Six animals from the control group were inseminated when observed in behavioral estrus. Two animals were found to be pregnant by performing rectal palpation after 60 days. This gave a conception rate of 33.33%. Out of six animals from Ovsynch protocol, three

animals were found to be pregnant. This gave a conception rate of 50%. In case of Double PGF₂α protocol, out of six animals, two were found to be pregnant with a conception rate of 33.33%. In response to the fixed time Double PGF₂α treatment, the conception rate of 33.33% was obtained which is almost similar to the conception rates of 21.4% to 32.5% obtained on timed A.I. at 72 to 96 hr in swamp buffaloes (Perera *et al.*, 1977; Kamonpatana *et al.*, 1987). The combined use of GnRH and PGF₂α has improved the efficiency and precision of estrus synchronization in beef cows without affecting fertility. This method eliminates estrus behavior for the six or seven day period preceding the PGF₂α treatment and enables the synchronization of estrus in approximately 80% females, during a period of less than four days following PGF₂α induced luteolysis. Besides inducing the synchronization of a new follicular wave, GnRH also produces an ovulation 24 to 32 hr after its administration, when the CL is regressing (Pursley *et al.*, 1995). This property of GnRH has been used to develop fixed-time A.I. with the so called GnRH-PGF₂α-GnRH (Ovsynch) programme. A second GnRH injection administered after the PGF₂α causes the release of LH thus synchronizing ovulation (Pursley *et al.*, 1995). In response to the treatment GnRH-PGF₂α-GnRH (Ovsynch) the conception rate of 50% in crossbred cows was obtained whereas Paul and Prakash (2005) obtained 33.3% conception rate. In the study of Bartolome *et al.* (2002) conception rates of 55.6 to 64.2 was reported after TAI, which is similar to that of present investigation. Pursley *et al.* (1995) reported that in dairy cows, pregnancy rates of 40 to 45 percent were obtained after a fixed time A.I. following Ovsynch treatment. Therefore, from the above findings, it can be concluded that higher conception rate

of 50% was recorded with timed artificial insemination (TAI) following Ovsynch treatment, in comparison to 33.33% obtained after TAI following double PGF₂ α treatment. Conception rate of 50 % and 33.33% were obtained after timed A.I. in Ovsynch and Double PGF₂ α protocol respectively. Ovsynch protocol is capable of inducing estrus cycle in a large percentage of dairy cows allows timed insemination and provides higher pregnancy rates than does PGF₂ α protocol. The Ovsynch protocol is also capable of inducing ovulation in cows that had not resumed estrus cyclicity at the time of synchronization.

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HEMATOLOGICAL CHANGES INDUCED BY JATROPHA SEEDS IN RATS

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ABSTRACT

Jatropha curcas (*Euphorbiaceae*) is a multipurpose shrub with varied medicinal uses and is of significant economic importance. In addition to being the source of bio-diesel, its seeds are also considered highly nutritious and could be exploited as a rich and economical protein supplement in animal feeds. However, the inherent phytotoxins present in the seed is the hindrance. The toxicity nature of the seeds of the local variety of *J. curcas* is not known. Therefore, investigations were undertaken to evaluate the short term oral toxicity of the seeds of locally grown *J. curcas*. Short term toxicity was conducted in rats by daily feeding the basal diet (Group I), and the diet where the crude protein requirement was supplemented at 25% (Group II) and 50% (Group III) levels through *Jatropha* seed powder. The adverse effects of *Jatropha* seed protein supplementation (JSPS) were evaluated by observing alterations in hematological profiles. Hematological examination of the rats under JSPS also showed significant changes in the haemogram characterized by reduction in total red blood cell count, packed cell volume and hemoglobin. The JSPS was also found to induce macrocytic-hypochromic anaemia in the rats. However, no significant changes were noted in total or differential leucocyte counts.

Key words: *Jatropha*, haematological, phytotoxins, wistar rats.

Jatropha curcas (*Euphorbiaceae*), locally known as 'Ratanjyot' is a multipurpose shrub with varied medicinal uses and is of significant economic importance. In addition to being the source of bio-diesel, its seeds are also considered highly nutritious and could be exploited as a rich and economical protein supplement in animal feeds (53-58% crude protein in defatted meal) if the toxins are removed (Becker and Makkar, 1998). A recent study (Aregheore *et al.*, 2003) revealed that *J. curcas* seed meal reduced of its phorbol ester level to a tolerable level of 0.09 mg/gm had 68% crude protein, much higher than most of the oilseed meals. The toxicity nature of the seeds of the local variety of *J. curcas* is not known. Therefore, investigations were undertaken to evaluate the short-term oral toxicity of the seeds of locally grown *J. curcas*, which in turn characterized by changes in hematological profiles. This toxicity study will help us to evaluate the possibility, if any, of their utility as a source of protein supplement in animal feeds.

The seeds of *J. curcas* were locally collected in bulk, properly cleaned to free from any extraneous dust, shade dried, reduced to fine powder with the help of an electrical grinder and stored in air tight containers. The control diet (standard diet) provided 22% crude protein (CP) containing normal feed ingredients. The modified diets were prepared by replacing the normal feed ingredients to an extent of 25% and 50% of the CP with *J. curcas* seed powder (CP =18%).

The present study was conducted on fifty-two weanling albino rats (60-75 gm) of either sex which were randomly assigned to three groups having 20 (10 males and 10 females), 16 (8 males and 8 females) and 16 (8

males and 8 females) animals respectively. The rats in Group I were given normal/ standard diet. The Group II & III rats were fed on diet where the crude protein requirement was supplemented at 25 % (Group II) and 50 % (Group III) levels through *Jatropha* seed powder for 21 days. The adverse effects of *Jatropha* seed protein supplementation (JSPS) were evaluated by observing the alterations in hematological profiles.

The blood samples were collected through cardiac puncture in properly heparinized vials on different intervals (i.e. on 0, 7, 14 and 22 days). The different hematological parameters *viz.*, TEC, Hb, PCV, MCV, MCH, MCHC, TLC and DLC were estimated in whole blood as per the standard procedure suggested by Jain (1986). Pre-treatment values (normal/ control) were determined in randomly selected five rats of control group.

All the rats fed 25% JSPS (Group-II) survived & were healthy till end of the experiment. However, mortality was recorded in rats fed 50% JSPS (Group-III). Four rats of Group III died during 13th day of the trial. Further, two rats of Group III succumb to death on 16th days of treatment. Table 2 summarizes the effect of JSPS on different hematological profiles of rats.

The mean RBC count in the control group (I) during pre-treatment and on different treatment intervals was insignificant. In Group II (25 %JSPS) the RBC counts on 14th and 22nd day were significantly lower than the pre-treatment count. Further, in this group the RBC count on 22nd day was also significantly lower than the count on 7th day. In Group III (50 % JSPS) the RBC count on 7th and 14th days were significantly lower than pre-treatment. The RBC counts at the three treatment intervals of Groups II

TABLE-1:
Effect of feeding *Jatropha* seed protein supplemented diet (JSPS) on haematological parameters (mean \pm SE) of wistar rats

Parameter	Period (Days)	Groups		
		Control	25% JSPS	50% JSPS
TEC (millions/cu mm)	Pre-treatment	7.50 \pm 0.38		
	7 th day	7.60 \pm 0.31	6.20 \pm 0.50*	5.60 \pm 0.21*
	14 th day	7.90 \pm 0.15	6.10 \pm 0.34*	5.10 \pm 0.17*
	22 nd day	8.00 \pm 0.26	5.20 \pm 0.24*	—
Hb (gm %)	Pre-treatment	13.70 \pm 0.57		
	7 th day	13.80 \pm 0.50	11.10 \pm 0.71*	9.60 \pm 0.23*
	14 th day	13.90 \pm 0.55	10.60 \pm 0.69*	8.80 \pm 0.43*
	22 nd day	14.10 \pm 0.55	8.50 \pm 0.29*	—
PCV (%)	Pre-treatment	41.40 \pm 1.93		
	7 th day	42.20 \pm 1.39	37.00 \pm 1.78*	32.00 \pm 0.89*
	14 th day	44.40 \pm 0.42	36.50 \pm 1.55*	31.70 \pm 1.18*
	22 nd day	45.40 \pm 0.40	31.60 \pm 0.88*	—
MCV(μ^3)	Pre-treatment	55.10 \pm 0.97		
	7 th day	55.20 \pm 0.75	57.60 \pm 1.31	56.90 \pm 0.65
	14 th day	55.60 \pm 1.32	59.70 \pm 1.22*	61.40 \pm 0.63*
	22 nd day	57.00 \pm 2.55	60.20 \pm 1.61*	—
MCH (pg)	Pre-treatment	18.10 \pm 0.23		
	7 th day	18.20 \pm 0.20	17.80 \pm 0.62	17.10 \pm 0.26
	14 th day	17.50 \pm 0.60	17.70 \pm 1.10	17.50 \pm 0.41
	22 nd day	17.60 \pm 0.45	16.10 \pm 0.21	—
MCHC (%)	Pre-treatment	33.20 \pm 0.18		
	7 th day	32.80 \pm 0.14	29.30 \pm 1.45*	30.10 \pm 0.22*
	14 th day	31.30 \pm 1.10	29.00 \pm 1.43*	30.50 \pm 0.55*
	22 nd day	31.10 \pm 1.43	26.90 \pm 0.62*	—
TLC (thousands/cu mm)	Pre-treatment	5.70 \pm 0.25		
	7 th day	5.80 \pm 0.21	5.60 \pm 0.24	6.00 \pm 0.28
	14 th day	5.80 \pm 0.24	6.00 \pm 0.22	5.30 \pm 0.29
	22 nd day	6.00 \pm 0.28	5.30 \pm 0.29	—
DLC (a) Lymphocytes (%)	Pre-treatment	74.60 \pm 1.24		
	7 th day	75.00 \pm 0.54	74.00 \pm 1.0 4	74.00 \pm 1.14
	14 th day	75.40 \pm 0.54	74.50 \pm 0.64	73.70 \pm 1.49
	22 nd day	75.20 \pm 0.59	75.00 \pm 1.15	—
(b) Neutrophils (%)	Pre-treatment	21.20 \pm 0.86		
	7 th day	21.00 \pm 0.4 4	22.20 \pm 0.80	23.20 \pm 1.01
	14 th day	20.20 \pm 1.15	21.50 \pm 0.95	20.00 \pm 0.70
	22 nd day	20.80 \pm 0.80	20.60 \pm 1.76	—
(c) Monocytes (%)	Pre-treatment	2.40 \pm 0.24		
	7 th day	2.60 \pm 0.4 0	2.20 \pm 0.37	2.80 \pm 0.37
	14 th day	2.80 \pm 0.37	2.50 \pm 0.55	2.50 \pm 0.28
	22 nd day	2.00 \pm 0.31	2.60 \pm 0.33	—
(d) Eosinophils (%)	Pre-treatment	1.80 \pm 0.37		
	7 th day	1.40 \pm 0.24	1.60 \pm 0.24	1.40 \pm 0.24
	14 th day	1.60 \pm 0.24	1.50 \pm 0.28	1.50 \pm 0.64
	22 nd day	1.20 \pm 0.37	1.60 \pm 0.33	—

(n = 5), * indicates significant (P \leq 0.05) difference level.

and III were significantly lower than the respective counts of control group. Similarly, the 14th day RBC count of Group III was also significantly lower than the 14th day count of Group II. The reduction in red blood cell count of rats on *Jatropha* seed protein supplementation suggests inhibitory effect on erythropoiesis by the phytotoxins and anti-

nutrients present in the seeds.

The mean hemoglobin level in the control group (I) during pre-treatment and at different treatment intervals was insignificant. The hemoglobin levels in Group II (25 %JSPS) during the three treatment intervals were significantly lower than the pre-treatment level. The mean

hemoglobin levels in Group III on 7th and 14th day intervals were also significantly lesser than the pre-treatment hemoglobin level. Different treatment interval hemoglobin levels of Group II were significantly lower than the respective hemoglobin levels of control group. Similarly, different treatment intervals of Group III were also significantly lesser than those of control (I) as well as 25% protein supplemented group (II). The reduction in hemoglobin of rats fed on JSPS feed may be due to reduction in absorption of dietary iron, since tannic acid and phytates interfere with absorption of minerals and metals in intestines (Salunkhe *et al.*, 1982).

The mean PCV values at pre-treatment and during the entire treatment interval were statistically non significant. JSPS at 25% and 50% levels resulted in significant reduction in the PCV, where the reduction in Group II was significantly different compared to control group and in Group III, as compared to respective PCV levels in control group or Group II. Moreover, the 22nd day level in Group II and 7th and 14th days level in Group III were also significantly lower than the pre-treatment levels. The reduction in PCV was due to reduction in red blood cells of the *Jatropha* seed protein rats. Oluwole *et al.* (1997) also reported progressive reduction in PCV, hemoglobin level and red blood cell count in rats fed methanolic extract of *J. curcas* seeds.

The MCV in control group (I) during pre-treatment and at different treatment intervals was insignificant. The MCV value in Group II (25% JSPS) on 14th and 22nd days of treatment were significantly higher than the pre-treatment. The MCV values in Group III (50% JSPS) 14th day was significantly higher than the pre-treatment MCV. The MCV levels of Groups II and III on 14th day were significantly higher than the MCV of control group.

The variation in MCH values between different intervals or between the groups was not statistically significant as compared to the pre-treatment MCH.

In both the *Jatropha* protein-treated groups, the MCHC values during different treatment intervals were lower than the pre-treatment value. The MCHC level on 7th day in

Groups II and III was lower than control group.

The data of erythrocytic indices clearly demonstrated increase in MCV and decrease in MCHC suggesting macrocytic-hypochromic anaemia in the rats following feeding diets containing 25% and 50% protein supplementation through *Jatropha* seeds. The present results are supported by an earlier observation of macrocytic-hypochromic anaemia in rats fed methanolic extract of *J. curcas* seeds for 8 to 10 days (Oluwole *et al.*, 1997).

The variation in TLC and DLC counts between different intervals or between the groups was not statistically significant as compared to the pre-treatment count.

The seeds of local variety of *Jatropha curcas* at 50% protein supplement level cause hematological alteration as well as mortality in rats during feeding trial. Hence, feeding of seed powder at 25% protein supplement level could be advocated with caution. Removal of anti-nutritive factors or detoxification of the seeds should be attempted before conducting further studies.

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