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Effect of poly unsaturated fatty acid supplementation on luteal profile and conception in postpartum buffaloes

A A MALIK[1], V K GANDOTRA[2], P S BRAR[3], S P S GHUMAN[4], G S DHALIWAL[5] and M HONPARKHE[6]

Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab 141 004 India

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Studies regarding extent and timing of early embryonic mortality indicate that approximately 30% of bovine embryos are lost during the first 3 weeks of pregnancy (Vassilev *et al.* 2005). Experiments have indicated the potential use of polyunsaturated fatty acids (PUFA) in decreasing $PGF_{2\alpha}$ production from the uterus thereby reducing pregnancy losses (Ambrose *et al.* 2005). Fish meal (FM) besides being used in dairy cow rations as a source of rumen undegradable proteins (RUP), contains a relatively high concentration of 2 polyunsaturated fatty acids (PUFA) of the n-3 family, eicosapentaenoic acid (EPA, C20;5) and docosahexaenoic acid (DHA, C22;6). Bonnette *et al.* (2001) have reported improvement in pregnancy rates in cattle after supplementation with FM. Hence, the objective of present study was to assess the effect of FM supplementation on luteal profile and conception in postpartum buffaloes.

The study was carried out on 15 postpartum Murrah buffaloes maintained at dairy farm, Kapurthala (Punjab). All the buffaloes had normal calving and subsequent normal genital health as assessed gynaeco-clinically. The buffaloes were randomly divided into 2 groups. Group 1: Postpartum buffaloes (5) were kept on normal routine feeding without FM supplementation. Group 2: Postpartum buffaloes (10) were initially supplemented with FM @ 100 g/head/day for the first week postpartum (i.e. acclimatization period) followed by 250 g/head/day FM daily till 90 days postpartum. On dry matter basis, FM contained 25.40% crude protein, 6.29% crude fiber, 5.22% ether extract and 64.13% ash. The total lipid content (5.86%) and free fatty acids (1.06%) of FM was determined as per Folch *et al*. (1957) and Lowry and Tinsley (1976), respectively.

Since none of the animals in both the groups come into estrous up to day 60 postpartum, hence buffaloes were synchronized using Ovsynch protocol. The synchronization protocol was initiated with administration (i.m) of 20µg of GnRH analogue on day 0, followed by 500µg of $PGF_{2\alpha}$ (cloprostenol sodium) on day 7 and second GnRH treatment (20µg) on day 9. Fixed TAI was performed at 16 and 40 h after 2nd GnRH injection. Ovarian ultrasonography was carried out using a real time B-mode diagnostic ultrasound scanner equipped with a 7.5 MHz linear-array transducer on the day of estrus and on day 12 for assessing preovulatory follicular size and CL size, respectively. The venous blood was collected in heparinized vials by jugular veinupuncture on day of estrus (day 0) and days 5, 12 and 40 post-insemination. Plasma samples were harvested by centrifugation @ 3000 rpm for 15 min and stored at –20°C until analysis. Conception rate was recorded by diagnosing pregnancy status on non-return basis and with trans-rectal ultrasonography on day 60 post-AI. Plasma progesterone was assayed with modified liquid phase radioimmunoassay (Kamboj and Prakash 1993). Sensitivity of the assay was 0.1 ng/ml; intra-assay and inter-assay variation coefficients were 6.2% and 9.5%, respectively.

Numerical data obtained was represented as mean±SEM, and differences were considered significant at $P<0.05$. The data obtained were analyzed statistically by Students t-test and DMRT (Snecedor and Cochran 1994) using SPSS 16 software.

This was the first study to investigate the luteal profile in buffaloes after supplementation with FM during the postpartum period. There was nonsignificant difference in the plasma progesterone concentration between the FM supplemented non-pregnant and control buffaloes and up to day 12 in FM supplemented pregnant, non-pregnant buffaloes and control buffaloes (Table 1). Thus the plasma progesterone was similar ($P<0.05$) between control and treatment group buffaloes as observed by others (Mattos *et al.* 2002,

Present address: [1]M V Sc. Scholar (malikasloob @gmail.com), [2]Professor-cum-Head (vkgandotra @yahoo.com), [3,4]Associate Professor ([3]parkashbrar @gmail.com), [4](ghuman_s @yahoo.co.in), [6]Assistant Gynaecologist (honparkhem @rediffmail.com), Department of Veterinary Gynaecology and Obstetrics; [5]Professor (e mail: dhaliwal1964@rediffmail.com), Department of Teaching Veterinary Clinical Complex.

Table 1. Plasma progesterone concentration (mean±SE) on different days of estrous in different groups

Groups	Progesterone concentration (ng/ml) on different days			
	Day 0	Day 5	Day 12	Day 12
Treatment (n,5) pregnant	0.302±0.05[a]	0.34±0.04[a]	0.71±0.23[a]	1.4±0.21[bc]
Treatment (n,5) non pregnant	0.388±0.11[a]	0.38±0.07[a]	0.44±0.09[a]	0.338±0.06[ad]
Control (n,5) non pregnant	0.244±0.04[a]	0.37±0.14[a]	0.452±0.04[a]	0.52±0.11[ad]

Means having different superscript within a row and columns differ significantly (P<0.05).

Table 2. Effect of PUFA (FM) supplementation on follicular and corpus luteum size (mean±SE)

Parameter	Groups			
	Control (n, 5)	Supplemented (n, 10)	Supplemented pregnant (n, 5)	Supplemented non-pregnant (n, 5)
Follicle diameter (mm)	10.34±1.41	11.14±0.89	12.74±1.12	9.54±1.03
Corpus luteumdiameter (mm)	13.92±0.99	14.9±0.46	15.16±0.57	14.64±0.79

Wuenschel 2006). In contrast other researchers observed increased levels of progesterone in beef cattle in response to high fat supplementation. Staples *et al.* (1998) concluded that ruminants fed supplemented fat often have a slight increase in plasma progesterone concentration.

In present study, dietary supplementation of FM did not affect the size of largest follicle (Table 2). This is in agreement with the findings of Wuenschel (2006), who observed no significant effect of dietary treatment on the size of dominant follicle. Contrary to this several authors (Lucy *et al*. 1991, Petit *et al.* 2002) recorded increase in the size of dominant follicle after supplementation of EPA and DHA, either as a fish meal or as products containing their precursors.

There was nonsignificant effect of fish meal supplementation on the corpus luteum size (Table 2) as reported by Petit *et al.* (2001). Larger CL was detected by Petit *et al*. (2002) in lactating dairy cows fed high levels of n-3 fatty acids through a diet as a mixture of formaldehyde treated linseed and fish oil. Difference in this study was proposed to be due to differences in fatty acid profiles and intake of experimental animals as well as their lactation status. However, in the present study, the mean pre-ovulatory follicle size as well CL size was bigger in pregnant buffaloes compared to non-pregnant although statistically nonsignificant. The larger preovulatory follicle might have resulted in better CL formation subsequently following ovulation and thereby improved conception rate in these animals.

Of the 10 fish meal supplemented buffaloes, 5 animals conceived (50%) whereas no buffalo conceived in control group. First service conception rate was improved when cows were fed ruminally inert fat (Ferguson *et al*. 1990) or soybean oil (Cullens *et al*. 2004). Soybean oil infusion appears to decrease the prostaglandin concentrations in subfertile crossbred cows and improve pregnancy rates. Fish meal supplementation decreased the PGFM levels (over all and low release in response to oxytocin challenge, Malik 2009) that might have improved conception rate in the present study. Since $PGF_{2\alpha}$ is the main luteolytic hormone in cattle and PUFA supplementation has been proved to decrease the prostaglandin production from uterus, both in *in-vitro* and *in-vivo* studies (Mattos *et al.* 2002, 2003). It suggests that the dietary supplementation with rumen protected fish meal might have resulted in higher first service conception rate by attenuating PGFM as there was nonsignificant difference in progesterone concentration, CL and preovulatory follicular size between control non-pregnant, FM supplemented pregnant and non pregnant buffaloes. In conclusion, supplementation of FM in buffaloes did not result in improved luteal profile but can be used effectively to improve conception rate in postpartum buffaloes.

SUMMARY

Pluriparous postpartum buffaloes (10) belonging to organized dairy farm were supplemented with 250 g FM/ buffalo/day for 90 days postpartum and 5 buffaloes were kept as non-supplemented control. On day 60 postpartum, all the buffaloes were synchronized using Ovsynch protocol followed by fixed TAI at 16 and 40 h after second GnRH injection. Subsequent to Ovsynch protocol, all the buffaloes were subjected to ovarian ultrasonography on day 0 (day of estrus and AI) and day 12 post AI and blood sampling on days 0, 5, 12 and 40 post-insemination. There was no difference in the preovulatory follicle size and CL size in both the groups and plasma progesterone concentration in control and non-pregnant FM supplemented buffaloes. Of supplemented 10 animals, 5 conceived as compared to none in control group. It was concluded that FM supplementation had no influence on preovulatory follicle size, CL size and plasma progesterone concentration. However, FM induced

suppression in PGFM concentration might have resulted in better conception in the supplemented buffaloes.

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