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## Effect of PUFA on ovarian morphometry and haemodynamics in repeat breeding cows

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**Abstract**

The objectives of the present study to investigate follicle and corpus luteum size and blood flow indices by ultrasonography in repeat breeding cows. Particularly polyunsaturated fatty acids (PUFA) in the form of crushed flaxseed @ 500 g per day for 30 days in addition to conventional feed, is a strategy to raise the nutritional status of the RBS cows to enhance follicular recruitment, growth, corpus luteum formation and its function.

**Keywords:** PUFA - Repeat Breeding Cow - Ovarian morphometry - Ultrasonography

**Introduction**

Reproduction is the backbone of animal production, the maximal production potential can be harvested from optimal fertility only. In lactating dairy cows there is a strong unfavourable genetic relationship between milk production and fertility (Oltenucu *et al.*, 1991) <sup>[1]</sup>. The reason is not only selection for milk production but a combination of variety of physiological, environment and management factors have an additive effect on reproductive efficiency (Santosh *et al.*, 2004; Chandra Shekher and Purohit, 2016) <sup>[2,3]</sup>.

In modern dairy practice, lactating cows experience a high level of conception failure or pregnancy loss, despite intensive efforts towards minimizing specific infectious disease by immunization. They are confronted with many reproductive problems, one of most commonly encountered but poorly understood sub-fertile condition in dairy animals is 'repeat breeding syndrome'. Many methods have been used to increase the pregnancy rate for repeat breeder cows such as intrauterine infusion of antibiotics, hormonal treatments for induction of oestrus, ovulation and progesterone supplementation, however the results were inconsistent between studies (Nowicki, 2021) <sup>[4]</sup>. Therefore, the best clinical approach in the reproductive programs is enhancing reproductive efficiency with minimal embryonic loss in repeat breeding cows. Hence, the present study reported that the dietary supplementation with various long chain PUFAs induced favourable changes in folliculogenesis including increase in total follicular numbers recruitment and size of the pre-ovulatory follicle, corpus luteum formation and its secretion (Ambrose *et al.*, 2006; Nugroho *et al.*, 2021) <sup>[5,6]</sup> in repeat breeding dairy cow.

**Materials and Methods**

The pluriparous repeat breeding crossbred cows (n=60) aged between 2<sup>nd</sup> - 4<sup>th</sup> calving and not greater than eight years old animals was selected for the experiment. The selected animals were subjected to Gynaecological examination and those cows without palpable abnormalities of the genital tract and negative for endometrial cytology test was utilized for the studies. All the selected cows could randomly divided into three experimental group *viz.*, Groups I (control group), Group II and III (Treatment groups), each group comprised of 20 repeat breeding cows. Group I (control) cows (n = 20) were orally supplemented rice bran @ 500 gram per day for 30 days from day 10 of the cycle and cows was observed for estrus signs and inseminated twice at an interval of 12 hours.

Group II and III cows (n = 20) was treated with crushed flaxseed @ 500 gram and groundnut seed @ 500 gram respectively per day for 30 days @ 10% of dry matter (DM) fed over and above the routine feed per day from day 10 of the cycle for 30 days and cows were observed for estrus signs and inseminated twice at an interval of 12 hours as per the standard procedure. The routine feed of cows comprised of 2/3<sup>rd</sup> roughages (2/3<sup>rd</sup> dry fodder+ 1/3<sup>rd</sup> green fodder) and 1/3<sup>rd</sup> concentrates.

All experimental cows were subjected to transrectal ultrasound examination on day 0 (estrus), 7 and 21 day post insemination with a color Doppler ultrasound device (Sonoray DS50 plus vet®). The diameters of follicles, corpus luteum and haemodynamics were evaluated by using grey-scale, B - mode, real-time scanner 5.0 to 7.5 MHz trans-rectal probe and statistically analysis by Snedecor and Cochran (1967)<sup>[13]</sup>.

**Results and Discussion**

The mean follicle diameters during estrus in the three groups after supplementation were presented in Table -1. According to (Nugroho *et al.*, 2021<sup>[6]</sup>) the follicles were measured and grouped into three classes according to their diameter size: Small (2-3 mm), medium (3.1-5 mm) and large (>5 mm). In addition, the preovulatory follicle diameter was determined on

the day before ovulation (Fig.1). As determined on day of estrus of the treatment period, the diameters of the small, medium and large follicles were not significantly different ( $p>0.05$ ) among all the groups. On day of estrus (Day 0) the numbers of large preovulatory follicles in the pregnant animals of the control, flaxseed and groundnut seed groups were  $10.65\pm0.36$ ,  $11.94\pm0.37$  and  $11.82\pm0.49$  respectively, with the high number in the flaxseed group being statistically significant ( $p<0.05$ ). However, the difference in the numbers of large preovulatory follicles between the group I and II was not statistically significant ( $p>0.05$ ). Although the diameters of all classes of follicles and the corpus luteum were not significant, that of the corpus luteum tended to be larger in the flaxseed group ( $p<0.05$ ) than in the groundnut seed and control groups. The present experiment reported that the mean diameter of preovulatory follicle were closely associated with Deshmukh *et al.* (2017)<sup>[8]</sup> who observed that the significantly increase in preovulatory follicle size those cows supplemented with soybean oil and crushed flaxseed groups compared to control animals. The similar results also have been described in cow observed that the significantly increases in preovulatory follicle size in fish oil and soybean oil groups as compared to control group of cows (Ghasemzadeh *et al.*, 2011; Ulfina *et al.*, 2015)<sup>[9, 10]</sup>.

**Table 1:** Preovulatory follicle and corpus luteum diameter in repeat breeding cows (Mean±SE)

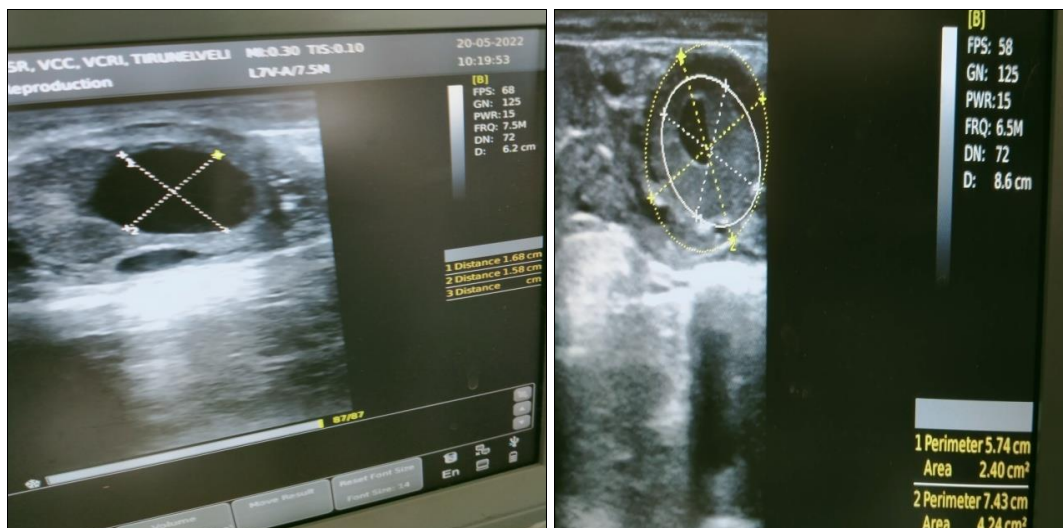
S. No.	Treatment groups	Pregnant/Non-pregnant	Preovulatory follicle diameter (mm)		Corpus luteum diameter (mm)	
			Day 0 (Estrus)		Day 7	Day 21
1.	Group I (Control)	Pregnant	10.65±0.36 <sup>b</sup>		13.6±1.3 <sup>a</sup>	13.9±0.7 <sup>b</sup>
		Non-pregnant	9.10±1.31 <sup>a</sup>		12.2±0.4 <sup>b</sup>	13.7±1.2 <sup>b</sup>
2.	Group II (Flaxseed)	Pregnant	11.94±0.37 <sup>a</sup>		17.3±1.6 <sup>a</sup>	19.1±1.4 <sup>a</sup>
		Non-pregnant	10.90±1.42 <sup>a</sup>		14.8±1.1 <sup>a</sup>	16.3±0.8 <sup>a</sup>
3.	Group III (Groundnut seed)	Pregnant	11.82±0.49 <sup>ab</sup>		16.4±1.8 <sup>ab</sup>	16.8±1.0 <sup>ab</sup>
		Non-pregnant	10.70±1.60 <sup>a</sup>		13.9±1.4 <sup>ab</sup>	14.6±0.6 <sup>ab</sup>

Mean with different superscripts (a and b) within row (CL) and Colum (Follicle) are significant at  $p < 0.05$ .

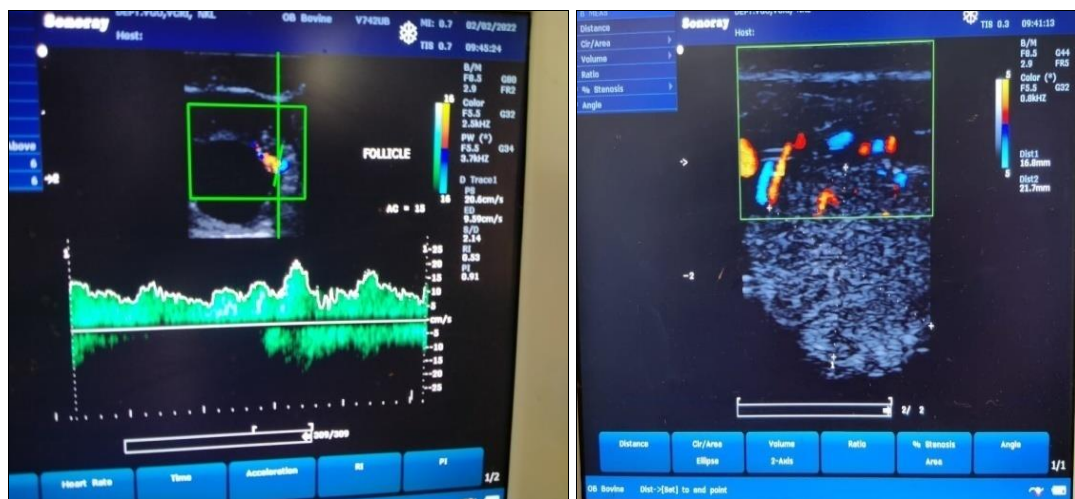
The follicle blood flow was greatest in large follicle when compared with the small and medium sized follicles. The pregnancy rates were greater for the large sized follicle compared to small and medium follicles. The present study reported for the first time in repeat breeding cows supplemented with PUFA treatment that cows had a greater follicle size, corpus luteum and blood flow than control animals (Table - 2). The RI of ovarian haemodynamics were not statistically significant ( $p>0.05$ ) between groups. PI, PSV, TAMV and BFV<sub>0</sub> were statistically significantly between

group I and II ( $p<0.05$ ) but no significant between group II and III ( $p>0.05$ ).

Similarly, the positive correlations between follicular blood flow and diameter and CL blood flow and diameter could be observed (Fig. 2). The present study agreement with De Tarso *et al.* (2015)<sup>[11]</sup> who reported that the significance difference in blood flow of the follicular size and CL diameter. In conclusion, Group II and pregnant cows had larger follicles and greater blood flow than Group I, III and non-pregnant repeat breeding cows.



**Fig 1:** Preovulatory follicle and corpus luteum diameter



**Fig 2:** Blood flow Indices in follicle and corpus luteum

**Table 2:** Haemodynamics of the Ovary during different days of treatment in repeat breeding cows

S. No	Parameters	Group I (Control)			Group II (Flaxseed)			Group III (Groundnut seed)		
		0	7	21	0	7	21	0	7	21
1.	RI	0.81±0.26	0.57±0.27	0.17±0.34	0.74±0.26	0.42±0.30	0.12±0.20	0.68±0.23	0.45±0.24	0.23±0.23
2.	PI	1.0±0.36 <sup>b</sup>	0.78±0.29 <sup>b</sup>	0.22±0.42 <sup>b</sup>	0.97±0.39 <sup>a</sup>	0.71±0.53 <sup>a</sup>	0.19±0.27 <sup>a</sup>	1.1±0.46 <sup>ab</sup>	0.99±0.55 <sup>ab</sup>	0.65±0.64 <sup>ab</sup>
3.	EDV	7.2±4.0 <sup>b</sup>	7.5±3.4 <sup>b</sup>	2.0±3.1 <sup>b</sup>	8.48±4.4 <sup>ab</sup>	8.08±5.2 <sup>ab</sup>	4.6±7.1 <sup>ab</sup>	9.8±4.1 <sup>a</sup>	11.0±5.5 <sup>a</sup>	7.15±7.7 <sup>a</sup>
4.	PSV	3.1±2.2 <sup>b</sup>	3.5±1.5 <sup>b</sup>	1.7±3.8 <sup>b</sup>	4.5±3.5 <sup>a</sup>	7.3±6.9 <sup>a</sup>	6.5±8.9 <sup>a</sup>	5.1±3.1 <sup>ab</sup>	5.3±2.6 <sup>ab</sup>	3.7±3.7 <sup>ab</sup>
5.	TAMV	3.1±2.2 <sup>b</sup>	3.5±1.5 <sup>b</sup>	1.7±3.8 <sup>b</sup>	4.5±3.5 <sup>a</sup>	7.3±6.9 <sup>a</sup>	6.5±8.9 <sup>a</sup>	5.1±3.1 <sup>ab</sup>	5.3±2.6 <sup>ab</sup>	3.7±3.7 <sup>ab</sup>
6.	BFVo	652.2±282 <sup>b</sup>	1246.5±562 <sup>b</sup>	822.4±385 <sup>b</sup>	1408.5±883 <sup>a</sup>	3205.1±2552 <sup>a</sup>	5126.3±2699 <sup>a</sup>	1179.8±381 <sup>ab</sup>	1537.0±455 <sup>ab</sup>	2150.0±475 <sup>ab</sup>

Mean with different superscripts (a and b) within row are significant at  $p < 0.05$ .

Blood flow indices were recorded: Resistance Index (RI), Pulsatility Index (PI), End-Diastolic Volume (EDV) (cm/sec), Peak-Systolic Velocity (PSV) (cm/sec), Time Averaged Maximum Velocity (TAMV) (cm/sec) and Blood flow volume (BFVo). TAMV was calculated from PI, PSV and EDV using the equation (Ginther & Utt, 2004<sup>12</sup>) TAMV=PSV – EDV/PI

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