# EFFECT OF EARLY POST-PARTUM GNRH AND $\mathrm{PGF}_{2}$ ALPHA ADMINISTRATION ON FOLLICULAR ACTIVITIES IN MURRAH BUFFALOES

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# ABSTRACT

The present study was carried out to examine the effect of GnRH and  $PGF_2\alpha$ administration during early post partum period (PP) on ovarian activities and initiation of PP estrus in Murrah buffaloes. A total of thirty six Murrah buffaloes were selected and divided equally into three groups comprising twelve buffaloes each. Buffaloes in Group I were i.m. injected with GnRH as busreline acetate (10 µg) while those in group II were i.m. injected with PGF<sub>2</sub> $\alpha$  as tiaprosttrometamol (0.750 mg) on day 14 PP.

The buffaloes in Group III were kept as control. The experimental buffaloes were examined with 7.5 transrectal probe on day 14, 21 and 28 PP. On day 14 PP, 14 (38.88%) ovaries showed small follicular development and 4 (11.11%) ones showed multiple follicular activity when observed ultrasonographically. On day 21 postpartum, 4 (11.11%) ovaries showed small follicular development, 11 (30.50%) ovaries showed multiple follicular activity and 18 (50.00%) ones indicated good follicular development when observed ultrsonographically. On day 28 PP, 3 (9.37%) ovaries showed small follicular development and 3 (9.37%) ones showed multiple follicular activities whereas 26 (81.25%) ones indicated good follicular development when observed ultrasonographically. The post partum ovarian activity was initiated with average 3.5±0.150, 3.25±0.130 and 4±0.467

weeks in Group I, II and III, respectively. From the present study it can be concluded that  $PGF_2a$ administration during early PP hastens the initiation of post-partum estrus in buffaloes and transrectal ultrasonography is good tool for monitoring the slight follicular developments.

**Keywords**: GnRH,  $PGF_2\alpha$ , ovarian activity, postpartum estrus

# **INTRODUCTION**

The buffalo has important role in livestock economy of Asia including India. Buffaloes are valued for milk, meat and draught power. Hence the importance of buffaloes to the economy of this country is considerable and cannot be underestimated (Madan, 2010). Low reproductive efficiency in general and buffaloes in particular remains a major economic problem globally, and its incidence is higher (4.66 to 12.66%) in our country (Tomar and Ram, 1993). Failure to resume ovarian activity after calving is the main reason for delay in conception in buffaloes (Parmar et al., 2012). Early post-partum (PP) breeding to reduce the calving interval in buffaloes would increase their reproductive efficiency (Shah et al., 2002). Thus PP period is regarded as an important in the reproductive life of bovines (Fonesca et al., 1983). The low reproductive efficiency of buffaloes, as

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evident from delayed first PP estrus and conception, prolonged service period resulting in extended calving interval (Khasatiya *et al.*, 2006).

Ovarian follicular growth resumes early after calving with the formation of first dominant follicle, detected by ultrasonographcally within 10.12±72 in Bulgarian Murrah buffaloes (Yotov and Atanasov, 2013). Although uterine involution begins and ovarian follicular waves resumes soon after parturition due to rise in FSH concentration (Schallenberger, 1985). However, dominant follicle of these waves fails to ovulate due to failure to undergo final terminal maturation. Failure of PP dominant follicles to undergo final maturation is due to inadequate LH pulse frequency, which result in low foolicular androgen production (Fortune, 1986) and inadequate oestrodiol positive feedback to induce LH surge (Peters et al., 1985), which is perquisite for follicular terminal maturation prior to ovulation. Absence of LH pulses in early postpartum is primarily due to depletion of anterior pituitary LH stores. Following replenishment of LH stores between days 15 and 30 PP absence of LH pulses is due to continued sensitivity of the hypothalamic GnRH pulse generator to the negative feedback effect of estradiol-17b which results in absence of GnRH pulses. The administration of GnRH will therefore overcome the inadequate secretion of pituitary LH in early post-partum period (Shah et al., 1990) and restore ovarian function earlier within PP period.

Prostaglandins plays major role in regulation of reproductive cyclicity (Singh and Madan, 1985). The reproductive cyclicity and its rhythm in terms of its reawakening during early PP period has been linked to temporal changes of prostaglandins in particular (Perera *et al.*, 1981). Lindell *et al.* (1980) reported that prostaglandin metabolites increased at the time of parturition and remained high for 8 to 16 days PP. So delay in involution of uterus was due to short period of high prostaglandin F2 alpha metabolite release whereas, long duration of PGF<sub>2</sub> $\alpha$  release resulted in short period for completion of uterine involution (Lindell, 1981). It has also positive effect on the uterine musculature tone (Lindell and Kindahl, 1983). So PGF<sub>2</sub> $\alpha$  injection in early PP period (day 14) enhances the uterine involution and reproductive efficiency in normal calved buffaloes (Nazir *et al.*, 1994). This is promoted us to study the follicular dynamics and initiation of PP ovarian activity ultrasonographically and per-rectally following 14 PP injection with GnRH and PGF<sub>2</sub> $\alpha$ in normally calved buffaloes.

#### **MATERIALS AND METHODS**

#### **Experimental animals**

The present research work was carried out using 36 post-partum (PP) Murrah buffaloes at M/S B.G. Chitale Dairy, Research and Development Farm, Bhilawadi in Sangli district, over a period of ten months. The buffaloes were housed in a loose housing barn with four groups of twenty-four buffaloes. The buffaloes were kept indoors and there was no open paddock in the barn. Each lot had twenty-six resting places (1.2x2m) on one side and a manure alley with Delta Master<sup>TM</sup> manure scraper (Delaval AB, Sweden) on the other hand side positioned towards the feed rack. Each lot had one automatic concentrate feeding station (AFS) and nine valve-controlled automatic water bowls. The ordinary routine in the barn was *adlib* feeding of roughages three times a day. The roughages fed during the experiment consisted of fresh, cut and chopped sugercane, alfalfa, napier grass, green maize and jowar straw which were chopped and transported to the barn in tractor trolley and dispensed manually into the feed troughs. A precalculated quantity of concentrate mixture was fed to each buffalo based on milk yield, body weight and pregnancy status. Concentrate was fed through the automatic concentrate feeding station (AFS) in the barn. If the pre calculated amount was not consumed, the residual was transferred to the next feeding. Residual amounts at the end of a 24 h period were transferred to the next 24 h period. During milking, an in-parlor feeding (IPF) system supplied a fixed amount of concentrates. The buffaloes were provided mineral mixture according to milk production and body weight of the buffaloes.

All the buffaloes were appropriately vaccinated against foot and mouth disease and haemorrhagic septicemia. They were also tested annually to detect possibilities of Brucellosis, Johne's disease and Tuberculosis and the positive reactors were suitably disposed off. The fecal samples and blood smears were also screened periodically for detection of parasitic infestations and protozoan parasites, respectively. As a routine, all buffaloes were dewormed biannually.

# Experimental design

Total 36 Murrah buffaloes with second to seventh lactation were selected. These buffaloes were divided into three groups comprising twelve bufaloes and following treatments were given.

Group I Buffaloes were intramuscularly injected with 10  $\mu$ g Busereline acetate (Intervet, India) on day 14 PP.

**Group II** Buffaloes were intramuscularly injected with 0.750 Tiaprost trometamol (Intervet, India) on day 14 PP.

Group III Buffaloes were kept untreated

as control group.

All the experimental were observed for ovarian activity on days 14, 21 and 28 PP perrectally and ultrasonographically. The ovarian follicular development, corpus luteum development and regression were monitored using real time, B-mode ultrasonograhy machine (Aloka-900) with 7.5 MHz linear array rectal transducer. The follicles appear as black anechoic, roughly circumscribed areas surrounded by hyperechoic ovarian stroma on the ultrasound image. The follicles observed ultrasonographically in the present study were classified according to

SFD (slight follicular development): F<0.5cm or follicles as small as 3-4 mm, but few (<5) in number.

MSF (Multiple small follicles): F<0.5 cm or follicles as small as 3-4 mm but many (>5) in number.

GFD (Good follicular development): Bigger follicles >0.8cm and or oestrual follicles.

NFD (No follicular development): Absence of the above picture within ovarian stroma.

The data was analyzed by employing statistical design as recommended by Snedecor and Cochran (1994).

# **RESULTS AND DISCUSSION**

The ultrasound imaging of ovaries in 36 PP buffaloes revealed ovarian activity with respect to follicular growth right from the first day of examination (day 14 PP). The ovaries were characterized by growth and regression of several small (up to 5 mm) and medium sized (>5 and <10 mm in diameter) follicles until the detection of first PP dominant ( $\geq$ 10 mm) and/ or ovulatory follicle during the study period.

# Follicular activities

A total 424 observations on ovaries were carried out by per-rectally and ultrasonographically. The manual observations were compared with the ultrasonographical observations to study the comparative detectability of ovarian follicles by palpation per-rectum and by ultrasonography. The overall observations during different periods for both the methods are presented in Table (1).

According to Table (1), 6 (16.16%) ovaries indicated small follicular development when observed per-rectally while 14 (38.88%) ovaries showed small follicular development and 4 (11.11%) ovaries showed multiple follicular activity when observed ultrasonographically on day 14 PP. Moreover, two (5.55%) ovaries showed good follicular activity when observed ultrasonographically on day 14 PP.

On day 21 PP, 15 (41.66%) ovaries indicated small follicular development when observed per-rectally while 4 (11.11%) ovaries showed small follicular development and 11 (30.50%) ovaries showed multiple follicular activity when observed ultrasonographically. Thirteen (36.11%) ovaries indicated good follicular development when observed per-rectally while 18 (50.00%) ovaries indicated good follicular development when observed ultrsonographically. On day 28 PP, 6 (16.16%) ovaries indicated small follicular development observed per-rectally while 3 (9.37%) ovaries showed small follicular development and 3 (9.37%) ovaries showed multiple follicular when noticed ultrasonographically. activity Furthermore, 26 (81.25%) ovaries indicated good follicular development observed per-rectally while 26 (81.25%) ovaries indicated good follicular development when observed ultrasonographically.

The above observations indicate that small follicular development and good follicular development is felt per-rectally, in all the ovaries. Follicular growth (small as well as good) is also noted on ultrasonographic examination in all ovaries. Besides that the follicular growth which was not palpated per-rectally was detected by

	Follicular Development	Day 14 PP		Day 21 PP		Day 28 PP	
Groups		Per- rectally	Ultraono- graphically	Per- rectally	Ultraono- graphically	Per- rectally	Utrasono- graphically
Group-I	SFD	3	5	6	1	0	0
	MSF	-	2	-	5	-	0
	GFD	0	1	5	6	11	11
Group-II	SFD	2	5	6	2	0	0
	MSF	-	2	-	4	-	0
	GFD	0	1	4	6	9	9
Group-III	SFD	1	4	3	1	6	3
	MSF	-	0	-	2	-	3
	GFD	0	0	4	6	6	6

Table 1. Ovarian activity monitored by per-rectally and ultrasonographically of Murrah buffaloes following 14 days PP GnRH and PGF<sub>2</sub> $\alpha$  administration.

ultrasonographic examination. This suggested that rectal examination is a reasonably fair indicator to judge the good follicular development. But ultrasound is better tool for classification of ovarian follicles depending upon its diameter. With the help of ultrasonographical examination it is possible to diagnose fairly good amount of follicular activity which otherwise would have missed on rectal palpation of the ovaries.

It is seen in the present study that higher percentage of follicles have missed or wrongly diagnosed by rectal palpation. The preference of ultrasonography method to compare with rectal palpation in current study is agreed with Pieterse (1990) who compared between trans-vaginal ultrasound and rectal palpation methods for diagnosis the ovaries in bovines. It is observed that ultrasonography permits a better estimation of number and size of the follicles, being similarly with observations in cows Hanzen *et al.* (2000).

The current observations that by manual diagnosis of smaller follicles was not detected and diagnose of follicles (<5mm) size was more accurate. The ultrasonographic appearance of ovarian structures in the present study was in line with those described by Honparkhe *et al.* (2003) and Lohan *et al.* (2004) in buffaloes. Ovarian activity is noted on day 14 post-partum by ultrasonography more effectively than by rectal examination.

It can be concluded that rectal palpation is fairly good technique to diagnose good follicular

activity or prominent follicular growth, but it is possible that slight follicular development or deeply situated follicles could be missed. Thus, rectal palpation is a fairly good indicator of ovarian activity only for routine observations. Ultrasonography should be considered as a tool when daily/frequent monitoring of follicular activity is required. Thus ultrasonographical ovarian scanning should be considered especially in the research techniques and therapeutic purposes.

# Initiation of post-partum ovarian activity

The week for initiation of ovarian activity was noted in the buffaloes from all groups (Table 2).

It was that PP ovarian activity was initiated earliest (P<0.01) in the PGF<sub>2</sub> $\alpha$  treated buffaloes (Group II) with the average of 3.25 weeks, followed by GnRH treated buffaloes (Group I) and control group (Group III) with averages of 3.50 and 4 weeks, respectively.

Observations regarding the initiation of ovarian activity in the present study was corroborated with those reported by Iqbal *et al.* (2003) who observed initiation follicular development at days  $21.20\pm5.71$  after PGF<sub>2</sub>a treatment as compared with days  $28.20\pm8.75$  in control Nili-Ravi buffaloes. Lohan *et al.* (2004) observed large follicle>8.5 mm in 75% buffaloes and increases to more than 8.5 mm between day 14-33 PP. Chaudhary *et al.* (1989) noticed that

Table 2. Average week of initi	ation of post-partum of	ovarian activity for a	ll groups in	Murrah buffaloes.
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Particulars	Group I	Group II	Group III	
1 al ticulai 5	(GnRH)	$(\mathbf{PGF}_{2}\alpha)$	(Control)	
Average	3.5±0.150	3.25±0.130	4±0.467**	

\*\* Significant at P<0.01

interval from calving to detection of first palpable follicle was averaged  $27.43\pm1.24$  days. Sheldon *et al.* (2000) showed >8 mm between days 14-28 day PP. Concomitant with present study, Bekana *et al.* (1994) observed the resumption of cyclical ovarian activity within a month in seven animals by rectal palpation as well as on ultrasound. More days required for initiation of follicular activity than present findings were reported by Baruselli (1991), Usmani (1992) and Shah (1999) in buffaloes.

The early return to active follicular development in PP buffaloes, and the fact that some buffaloes ovulated between days 15 and 20 PP, demonstrated the ability of the ovary to resume early PP activity. It suggested that ovarian responsiveness may not be the major reason for the variable duration of the PP anestrus period commonly observed in buffaloes. Usmani et al. (1985) recorded formation of first CL on day 23.8±1.7 after calving as indicated by plasma progesterone level in buffaloes. Arya and Madan, (2001) observed 19.67±3.23 and 19.17±4.53 days for first ovulation in suckled and non-suckled Murrah buffaloes. The small  $(8.10\pm5.67)$  and large number  $(1.00\pm0.00)$  of follicles was detected day six post-partum in buffaloes (Lohan et al., 2004).

The present finding of initiation of PP ovarian activity at comparatively earlier days might be due to careful monitoring of ovarian activity through ultrasound scanning of ovaries, good feeding, health and management practices of the farm as well as due to the  $PGF_2$  alpha treatment in early PP period.

Determination of ovulation by rectal palpation during earlier days after calving is tedious and it may be easily missed. Moreover, most of these studies were based on palpation of corpora lutea formed following ovulation. It is accepted that CNS requires prior exposure to progesterone to elicit behavioral signs of estrus (Noakes *et al.*, 2001). Behavioural signs of estrus did not accompany the first PP ovulation in majority of buffaloes. This finding agreed with the observation reported by Savio *et al.* (1990) who showed that first PP ovulation occurred without overt estrus behavior in 17 out of 18 dairy cows. Thus, the sign of first estrus is not a true reflection of onset of ovarian activity.

# CONCLUSION

It can be concluded that the slight follicular developments is easily detectable through transrectal ultrasonography being a good tool for monitoring of follicular activity. The  $PGF_{2}a$  administration during early PP hastens the initiation of estrus in Murrah buffaloes.

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