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Fertility response in postpartum anoestrous cows using Ovsynch and double PG protocols

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Abstract
Administration of GnRH and PGF2a to postpartum anoestrus cows induces precise synchronization of estrus by controlling ovarian follicular and luteal functions. A total of 35 cows having body condition score >2.5 and healthy reproductive organs were selected and divided into two groups. Group I and II cows administered with Ovsynch and double PG protocols to assess the estrus induction, intensity of estrus, time required for onset of estrus and duration of estrus. The mean diameter of follicle 48 h after prostaglandin was 9.91±0.32 and 8.80±0.30 mm in Group I and II, respectively with a significant (p<0.05) difference. The mean time (h) required for the onset of induced estrus and duration of induced estrus were 54.038 ± 1.05; 22.93 ± 0.84, respectively in Group I and 52.15 ± 1.12; 21.15 ± 1.05, respectively in Group II. The conception rate was 93.3 and 84.6% in Group I and II, respectively with overall success rate of 80.0%. The difference in the time required for the onset of estrus (hrs), duration of estrus (hrs), estrus response (%) and conception at first AI (%) were not significant (P>0.05) between two groups.

Keywords: Postpartum anoestrous cows, Ovsynch, double PG protocols

Introduction
Postpartum anestrum is the most prevalent reproductive disorder in dairy cattle due to poor nutritional status, negative energy balance, hormonal imbalance and uterine pathology (Gilbert *et al.*, 2011) [6] and affect the reproductive efficiency and economy of milk production (Yadav *et al.* 2018) [25]. Several hormonal therapies are being used to combat the problem of anestrum through synchronization of estrus and ovulation (Pursley *et al.*, 1995 and Vasconcelos *et al.*, 1999) [15, 23]. Therefore, present study to understand the efficacy of Ovsynch and double PG protocols in the management of postpartum anoestrous based on the ovarian response by ultrasound scanning, time required for induction of estrus, estrus response, its intensity, fern pattern, conception rate was taken up.

Materials and Methods
A total of 35 crossbred cows with body condition score of >2.5 having healthy reproductive organs and history of minimum 2 to 3 months anestrus after calving were included in this study. Animals were provided with 2-4 kg high protein feed containing 20% DCP and 70% TDN and mineral mixture @ 50 grams per day for 15 days before starting the treatment. All the cows were randomly assigned in to two groups and Group I cows (n-19) were administered with Ovsynch protocol i.e., 10 ug of GnRH (Buserelin acetate) at any stage of estrus cycle (day 0) followed by 500 ug Cloprostenol (Pragma, Intas pharmaceuticals Ltd.) (day 7) and second GnRH inj. 48 hrs after PGF2α administration and after 18 to 24 hrs of second GnRH administration fixed time A.I. was done. Group II cows (n=16) were administered with double PG (500 ug of Cloprostenol, Pragma, Intas pharmaceuticals Ltd.) injections 11 days apart. After 48 h of PGF2α administration, induced estrus response (exhibition of estrus symptoms), time required for estrus induction, duration of estrus were recorded. Cervical mucus was also collected in a petri dish before insemination from estrus cows to study fern pattern. Pregnancy diagnosis was done after 60 days of AI by using ultrasonography (Fig. 7). The data were tested for significance by analyzing the means and comparing by independent samples t-test using SPSS V. 23.

Results and Discussion

In the present study, Group I protocol induced estrus in 15 out of 19 cows (78.90%) and Group II protocol in 13 out of 16 cows (81.30%) indicating higher induction rate (Table 1 and Fig. 1). Similar to the present study induction rates with Ovsynch were 80.00 to 83.33% in indigenous Gir cows (Hirole *et al*., 2018) [8] and 77.70% (Richardson *et al*., 2002) [18] in cows. While, higher induction rate (100%) observed by Deshmukh *et al.* (2017) [2]. The GnRH agonist action on ovarian follicular development and CL formation via release of pituitary LH and FSH might have caused large follicles to ovulate and induce emergence of a new follicular wave within 3 to 4 days after treatment. The Group II cows, findings are in accordance with Dherange (2000) [4] (90%) in Red Khandari cows while the same was slightly lesser (100%) than the findings of Hirole *et al*., (2018) [8]. PGF2α in early postpartum cows would have caused luteolysis and hasten the resumption of cyclic ovarian activity (Dudhatra *et al*., 2012) [5].

The average time required for onset of estrus after $PGF_2\alpha$ injection was 54.038 ± 1.05 and 52.15 ± 1.12 in Group I and Group II cows, respectively (Table 1 and Fig. 1) without any significant difference. Similarly, Hirole *et al.* (2018) [8] and Ratnaparkhi *et al.* (2020) [17] reported average time of 53.20±1.8 and 54.60 ± 2.44 h, respectively in dairy cows subjected to Ovsynch protocol. However, Prajapathi *et al.* (2019) [14] reported slightly longer estrus induction response of 62.1±2.26 h. Same in Group II cows is in accordance with Sahatpure and Patil (2008) [20] (54.40±2.60 and 55.58±3.28 h in non-descript and crossbreed cows, respectively). While, Ahlawat *et al*. (2015) [1] and Ratnaparkhi *et al.*, (2020) [17] reported slightly longer time (56.86±1.96 and 56.40 ± 2.22 h, respectively) than results of current study. Both Ovsynch and Double PG protocols might have induced estrus with more or less similar timings through wave-like patterns of ovarian follicular development (Sirois *et al*.,1988) [22] and stage (i.e., early, mid and late luteal phase) of the cycle (King *et al*., 1982) [9].

In present study, the average estrus duration was 22.93 ± 0.84 h and 21.15 ± 1.05 h in in Group I and II cows, respectively without any significant difference (Table 1 and Fig. 1). Several workers reported that Ovsynch protocol revealed the average duration of estrus within the range of 20.50±2.50 to 22.80±0.44 hrs (Sathiamoorthy and Subramanian 2003 and Hirole *et al.*, 2018; Ratnaparkhi *et al.*, 2020) [8, 17]. The present findings in double PGF2α group is in accordance with Hirole *et al.* (2018) [8] and Ratnaparkhi *et al.* (2020) [17] who reported 21.33± 0.49 and 23.80±0.55 h, respectively in crossbred cows.

Conception rate (%)

Although statistically not different, the conception rate after first service is higher in ovsynch group (53.33%) than in double PG group (46.15%) (Table 1, Fig. 1). Nevertheless, the conception rate is not constant and varies from 33.3% (Hirole *et al.*, 2018) [8] to as high as 50% (Ratnaparkhi *et al*., 2020) [17] in ovsynch protocol. In accordance with present study Dhami *et al.* (2015) [3] in crossbreed anestrus cows and Ratnaparkhi *et al.*, (2020) [17] reported 50% first service conception. Whereas, lower first service conception rate (16.66%) reported by Naikoo *et al.* (2016) [11] in Kankrej cows and 38.3% by Peters and Pursley (2002) [13]. Same in double PGF2α is in accordance (40%) with Ratnaparkhi *et al.*, (2020) [17], while Hassan *et al.* (2017) [7] and Hirole *et al.*, (2018) [8] lower (31% and 33.3%, respectively) conception rate. But higher (67%) conception rate recorded by Venkataramana *et al.* (2013) [24] in Ongole cows. The higher pregnancies in Group I might be due to the prevention of delayed ovulation as well as anovulatory defects and improved synchrony of emergence of a new follicular wave with a homogenous ovarian follicular status at induction of luteolysis. (Rhodes *et al.*, 2003) [19].

Fern pattern

The current study recorded 80.0%, 84.6% of typical and 20.0%, 15.4% atypical fern patterns in Group I and II, respectively (Fig. 3 and 4). These percentages are in corroboration with the results of Rao and Rao (1982) [16] and in contrast, Layek *et al.* (2013) [10] and Parikh *et al.* (2018) reported lower typical fern pattern (57.9 and 46.8% in Sahiwal and Gir cows, respectively).

Ultrasonographic evaluation of Follicular dynamics

The mean diameter of the follicle during the period of synchronization of postpartum anestrus cows was 7.47±0.34, 9.08±0.23 and 9.91±0.32 mm on the day of initiation of protocol (day 0), on the day of prostaglandin (day 7) and on the day of last injection (day 9), respectively in Group I, while the mean diameter of follicle was 6.43±0.31 and 8.80±0.30 mm on the day of first (day 0) and 48 hours after last (day 13) injection of prostaglandin in Group II cows (Table 2, Fig. 4, 5 and 6). There is no significance (P > 0.05) difference between diameter of follicle after 48 hours of Prostaglandin in Group I and II. Administration of first GnRH might have caused emergence of follicle to induce ovulation of the dominant follicle followed by wave emergence 1-2 days later (Pursley *et al.*, 1995) [15].

Table 1: Estrus and conception related parameters

S. No.	Parameter	Treatment		P value
		GPG (Group I)	Double PG (Group II)	
1	Time required for onset of estrus (hrs)	54.038 ± 1.05	52.15 ± 1.12	0.229
2	Duration of estrus (hrs)	22.93 ± 0.84	21.15 ± 1.05	0.193
3	Estrus response (%)	78.90	81.30	0.420
4	Conception in first AI (%)	53.33 (8/15)	46.15(6/13)	0.743
5	Overall conception (%)	93.30	84.60	0.457
6	**Fern pattern**			
	Typical	80.00	84.60	0.750
	Atypical	20.00	15.40	

Table 2: Follicular dynamics

S. No.	Parameter	GPG (Group I)	Double PG (Group II)	t value
1	No. of animals	19	16	
2	Diameter of follicle on the day of initiation of protocol	7.47±0.34	6.43±0.31	-2.22^{NS}
3	Diameter of follicle after 48 hours of PG injection	9.91±0.32	8.80±0.30	-2.47^{NS}

NS: Non significant (p > 0.05)

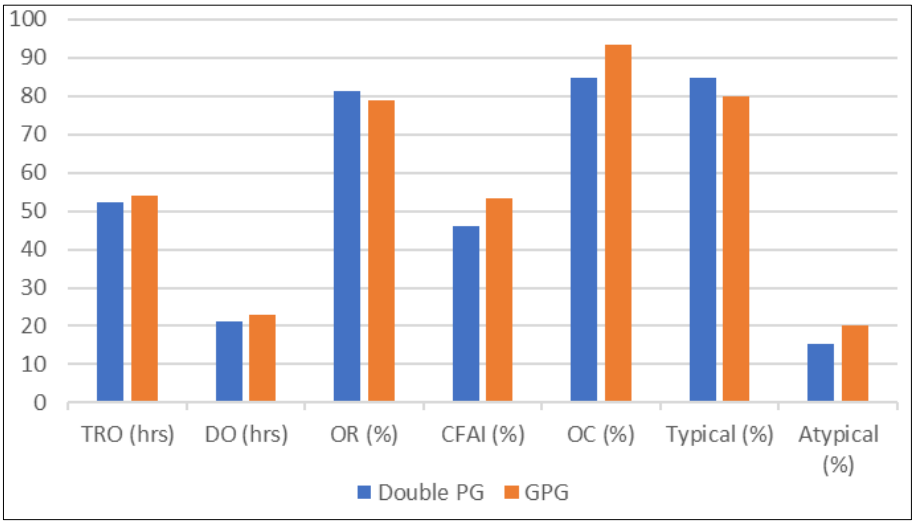

Fig 1: Estrus and conception related parameters

Fig 2: Typical fern pattern

Fig 4: Ultrasonographic image showing small follicles and CL

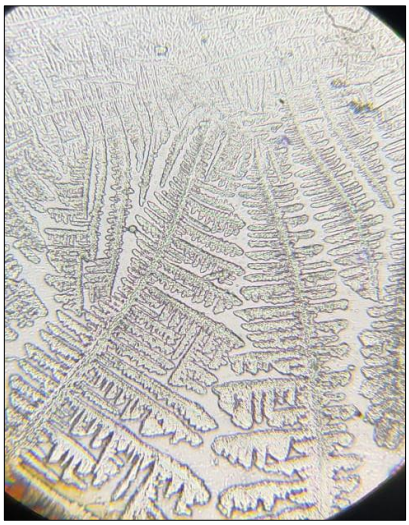

Fig 3: Atypical fern pattern

Fig 5: Ultrasonographic image showing small follicle along with CL

Fig 6: Ultrasonographic image showing dominant follicle after 48 hours of prostaglandin injection

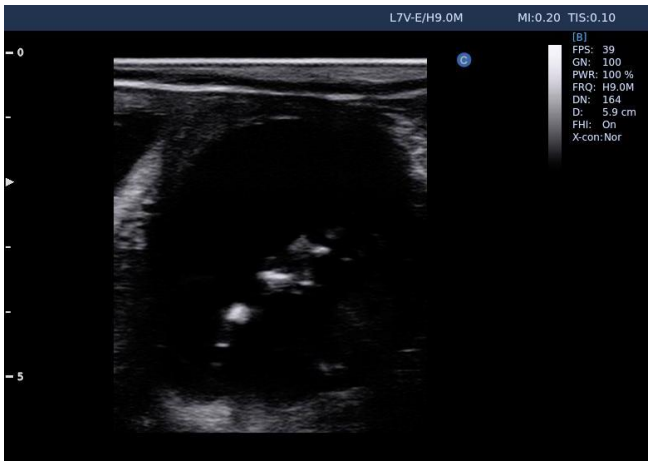

Fig 7: Ultrasonographic image showing Amniotic vescicle

Conclusion

From the present study it can be concluded that the application of Ovsynch and prostaglandin protocols can serve as a good tool to induce estrus and ovulation as well as enhancement of conception rate in postpartum anoestrous cows. No significant differences were observed for the conception related parameters in between two protocols.

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