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### Effect of Ecbolic Therapy on Postpartum Reproductive Parameters and Serum Hormonal Profile in Dangi Cows

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#### Abstract

The study was conducted on eighteen dystocia affected Dangi cows to evaluate the effect of ecbolic therapy on postpartum reproductive parameters as well as serum progesterone and estradiol-17 $\beta$  profile. The cows were randomly divided into three groups, each of 6 animals. The cows in Gr-I and Gr-II were injected with Methyl ergometrine (5 mg) and Dinoprost tromethamine (PGF<sub>2</sub>a, 25 mg) i/m, respectively, immediately after parturition and the cows of Gr-III received herbal ecbolic boli (2-4 boli, bid, PO) for first 10 days postpartum. The blood samples were collected on 0 day (day of calving), 7th day, 14th day, 21<sup>st</sup> day and 28<sup>th</sup> day postpartum from the jugular vein. The mean first postpartum estrus interval in Gr-I, Gr-II and Gr-III were 181.50 ± 44.73 days, 195.33 ± 23.38 days and 155.33 ± 27.81 days. The service period was 237.66 ± 28.01 days with 50 per cent (3/6) conception rate in Gr-I and 402.00 ± 46.12 days and  $368.66 \pm 53.46$  days with 100 per cent (6/6) conception rate in each Gr-II and Gr-III, respectively. The postpartum estrus interval and service period did not differ significantly (p>0.05) between Gr-I, Gr-II and Gr-III. A highly significant (p<0.01) decrease in both progesterone and estradiol-17 $\beta$  concentration was observed on 7<sup>th</sup> day postpartum and thereafter fluctuated non-significantly with increasing trend on 14th , 21st and 28th day postpartum in all groups.

#### Introduction

Dangi cow (*Bos indicus*) is one of the thirty eight (38) recognized breeds of cattle in India. It is found in Dangs district and adjoining areas of Navsari and Tapi districts in Gujarat and Nashik, Ahmednagar and Dhule districts in Maharashtra. It is primarily a medium-slow draft animal and well known for its hardy nature and ability to work hard under heavy rainfall conditions and subsist mostly on grazing alone. It provides steady and substantial regular income through production of 1-3 liters of milk, sale of male animals and manure to tribal people. To increase the productive performance of these animals, more emphasis should be given to their reproductive health to reduce the interval from calving to conception. The puerperal period till resumption of ovarian cyclicity is a critical phase in the reproductive cycle of dairy cows (Sheldon et al., 2008). Early postpartum activity, particularly in the ovary ipsilateral to the pregnant uterine horn hastened with improved reproductive efficiency in cows when treated with exogenous PGF<sub>2</sub> $\alpha$  or Methyl ergometrine during early postpartum period (Patel et al., 2014). The oral administration of herbal preparations with proven ecbolic and restorative actions appears to be a safe and effective to benefit the postparturient health and productivity of the animals (Ravi and Bhagwat, 2007). Moreover, the estrous cycle length is governed by the combined influence of uterine luteolysin and the steroid hormones estrogen and progesterone liberated from the ovary (Patel et al., 2013). Hence, study of such gonadal steroid hormones might help in diagnosing onset of postpartum ovarian cyclical activity and its aberrations. There is dire need to detect abnormal ovarian cycles earlier and their treatment for better reproductive efficiency. Hence, this study was aimed to evaluate the effect of  $PGF_{\alpha}\alpha$ , Methyl ergometrine and herbal ecbolic on reproductive parameters as well as serum progesterone and estradiol profile during postpartum period in Dangi cows.

## Materials and Methods

The present study was conducted on eighteen Dangi cows from the field conditions, with the symptoms of calving difficulties and successful parturition carried out with minor obstetrical assistance without any complications, in Dangs district, Gujarat, India. The cows were randomly divided into three groups consisting six cows in each. The study was carried out over a period of one and a half year from June, 2016 to November, 2017. The cows in Group I and II were treated intramuscularly immediately after parturition with 5 mg Methyl ergometrine maleate (inj. Nexbolic, Intas Pharmaceuticals Ltd.) and 25 mg of Dinoprost tromethamine, a natural PGF<sub>2</sub> $\alpha$ (inj. Lutalyse, Pfizer Animal Health Ltd), respectively. The cows in Group-III were treated with herbal ecbolic (Bol. Exapar, Ayurvet) 2-4 bolus twice a day (b.i.d) after calving till 10 days. The interval for first postpartum estrus occurrence

was recorded and cows exhibiting estrus were bred either by natural service or AI. The cows did not return to estrus following breeding were confirmed for pregnancy per-rectum 60-90 days later. The service period was recorded as time between calving to first successive conception.

Blood samples were collected by jugular vein puncture in the vaccutainers on 0 day (day of calving), 7<sup>th</sup>, 14<sup>th</sup>, 21<sup>st</sup> and 28<sup>th</sup> day postpartum. The serum was separated after clotting of blood and then centrifugation at 3000 rpm for 15 minutes and stored at –20°C in deep freezer until analysis. Serum progesterone and estradiol-17 $\beta$ concentrations were measured using ELISA based enzyme immunoassay kits and procedure described by Diatek Healthcare Pvt. Ltd. Hooghly, WB, India. Absorbance and concentration was measured using ELISA reader. A standard curve was obtained by plotting the concentration of standard versus the absorbance.

The data on reproductive parameters and hormone profile were analyzed by standard statistical methods using CRD and DMRT (Steel and Torrie, 1981).

## **Results and Discussion**

## First Postpartum Estrus and Fertility Response:

The mean first postpartum estrus interval in Gr-I, Gr-II and Gr-III were  $181.50 \pm 44.73$  days,  $195.33 \pm 23.38$  days and  $155.33 \pm 27.81$  days, respectively with overall  $177.38 \pm 18.48$  days which did not vary significantly (p>0.05). The present findings were little bit higher than  $159.2 \pm 8.1$  days documented by Janmeda *et al.* (2013) in Dangi cows. Although, too shorter interval as  $36.33 \pm 1.17$  days reported by Patel *et al.* (2016) in cows treated with methyl ergometrine; 28.14  $\pm 1.68$  days reported by Patel *et al.* (2013) in cows treated with 25 mg PGF<sub>2</sub> $\alpha$  immediately after calving and  $60 \pm 0.28$  days reported by Thakur *et al.* (2013) in cows treated with liquid Exapar orally.

The service period was 237.66  $\pm$  28.01 days with 50 per cent (3/6) conception rate in Gr-I and 402.00  $\pm$  46.12 days and 368.66  $\pm$  53.46 days with cent per cent (6/6) conception rate in Gr-II and Gr-III, respectively, which did not differ significantly (p>0.05). The overall service period

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and conception rate were  $355.80 \pm 31.62$  days and 83.33 per cent (15/18). The present findings were little bit higher than  $185.6 \pm 9.7$  days documented by Janmeda *et al.* (2013) in Dangi cows. The exceptionally higher service period as 249 days reported by El-Tahawy and El-Sharkawy (2014) in cows treated with methyl ergometrine, while, lower as  $60.00 \pm 4.81$  days and  $78.25 \pm$ 14.93 days reported by Patel *et al.* (2016) in HF crossbred cows treated with 25 mg PGF<sub>2</sub> $\alpha$ immediately after calving and herbal formulation Utrovet from calving till 10 days postpartum, respectively.

The longer postpartum oestrus interval and service period observed in present study in all the treatment groups might be due to keeping Dangi cows entirely on free ranging system apart from individual variation, breed specificity, location and parity of the animals, strong maternal behaviour and not to draw the milk and keeping their calves entirely on suckling, lightness in breeding i.e. settled tendency not to breed the cows so early as it should be and general ignorance of the reproductive status, lack of awareness and vigilance towards post partum breeding.

# Progesterone (P<sub>4</sub>) and Estradiol-17 $\beta$ (E<sub>2</sub>) Profile:

The mean serum progesterone and estradiol- $17\beta$  concentrations at different time intervals in Dangi cows are presented in Table 1. The mean serum progesterone concentration among individual cows on the day of calving ranged from 0.94 to 1.65 ng/ml indicating presence of an active CL. The serum progesterone concentration recorded on the day of calving in Group-I, Group-II and Group-III decreased significantly (p<0.01) on 7<sup>th</sup> day postpartum and further increased significantly (p<0.01) gradually on 14<sup>th</sup> day, 21st day and 28th day postpartum. The progesterone profile in the present study coincided well with Shah et al. (2012) in Surti buffaloes and Dhami et al. (2017) on day 0 (day of calving), 3<sup>rd</sup>, 14<sup>th</sup> and 28<sup>th</sup> day postpartum in HF crossbred cows. The mean serum progesterone concentration was found low in Dangi cows from 7<sup>th</sup> day postpartum to 28<sup>th</sup> day postpartum might be due to earlier regression of the previous pregnancy CL followed by late settlement of ovarian activity as compared to other breeds and species of cows and buffaloes.

Serum	Time intervals	Groups and hormonal profile			
Hormone	postpartum	Group-I	Group-II	Group-III	Overall
Progesterone (ng/ml)	0 Day (parturition)	$1.38 \pm 0.06^{a}_{w}$	1.36±0.11 <sup>a</sup> <sub>w</sub>	$1.40\pm0.03^{a}_{w}$	1.38±0.04 <sup>a</sup>
	7 <sup>th</sup> Day postpartum	$0.33 \pm 0.05^{b}_{w}$	$0.26\pm0.04^{b}_{w}$	$0.29 \pm 0.04^{b}_{w}$	0.29±0.02 <sup>b</sup>
	14 <sup>th</sup> Day postpartum	$0.57 \pm 0.04^{\circ}_{w}$	$0.53 \pm 0.05^{\circ}_{w}$	$0.49 \pm 0.02^{\circ}_{w}$	$0.53 \pm 0.02^{\circ}$
	21 <sup>st</sup> Day postpartum	$0.84{\pm}0.06^{d}_{w}$	$0.75 \pm 0.08^{\circ}_{w}$	$0.89 \pm 0.03^{d}_{w}$	$0.83 \pm 0.03^{d}$
	28 <sup>th</sup> Day postpartum	$1.15\pm0.02^{e}_{w}$	$1.08\pm0.06^{d}_{w}$	$1.19\pm0.07^{e}_{w}$	1.14±0.03 <sup>e</sup>
	F-value	63.887**	31.250**	106.405**	27.727**
	P-Value	0.000	0.000	0.000	0.000
	0 Day	$183.63 \pm 08.60^{a}_{W}$	$180.29 \pm 12.39^{a}_{w}$	$181.92 \pm 12.60^{a}_{w}$	181.95±6.16 <sup>a</sup>
	7 <sup>th</sup> Day postpartum	$032.03\pm00.37^{b}_{W}$	$032.55 \pm 01.56^{b}_{w}$	$031.67 \pm 00.81^{b}_{w}$	032.08±0.57 <sup>b</sup>
Estradiol-	14 <sup>th</sup> Day postpartum	$036.17 \pm 00.80^{bc}_{w}$	$036.25 \pm 00.85^{b}_{w}$	$035.11 \pm 00.71^{b}_{w}$	035.84±0.44 <sup>b</sup>
17β (pg/ml)	21 <sup>st</sup> Day postpartum	$043.06 \pm 01.75^{bc}_{w}$	$043.83 \pm 01.69^{b}_{w}$	$042.67 \pm 01.80^{b}_{w}$	043.18±0.95 <sup>b</sup>
	28 <sup>th</sup> Day postpartum	$046.87 \pm 01.80^{\circ}_{w}$	$047.31 \pm 01.91^{b}_{w}$	$045.76 \pm 01.78^{b}_{w}$	046.64±1.00 <sup>b</sup>
	F-value	257.839**	121.650**	124.056**	32.104**
	P-Value	0.000	0.000	0.000	0.000

Table 1: Serum progesterone and estradiol-17 $\beta$  profile at different time intervals in postpartum treated groups of Dangi cows (Mean ± SEM)

Group-I=Methyl ergometrine, Group-II=Dinoprost tromethamine (Natural PGF<sub>2</sub>a), Group-III=Herbal ecbolic

Means bearing different superscripts within a column (abcd) differ significantly (p<0.01) and means bearing common subscripts between a column (w) did not differ significantly (p>0.05).

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The other reason behind late ovarian activity attributed to entire suckling of young ones in these draft purpose breed lead to high prolactin concentration that suppresses the activity of hypothalamus to release the gonadotrophin hormone.

The mean serum estradiol-17 $\beta$  concentration recorded on day of calving decreased significantly (p<0.01) at 7<sup>th</sup> day postpartum and fluctuated non-significantly at 14<sup>th</sup>, 21<sup>st</sup> and 28<sup>th</sup> day postpartum in all three groups. The overall trend of estradiol-17 $\beta$  profile under the study coincided well with Sawada et al. (1988) who reported estradiol-17 $\beta$  at parturition, one hour postpartum and one day postpartum as 391 ± 42 pg/ml, 446  $\pm$  48 pg/ml, 34  $\pm$  9 pg/ml, respectively in Holstein cows. The present findings were in close agreement with Shah et al. (2012) in Surti Buffaloes who observed progressively increased estrogens towards the end of pregnancy and highest levels at 24-48 hours before calving thereafter fell down to remained at basal level (>12 pg/ml) till first ovulation. However, the longer first postpartum estrus interval and longer service period recorded in the present study might be due to non-significantly lower basal estradiol-17 $\beta$  levels after parturition till 28<sup>th</sup> day among all the groups as well as effect of suckling and its strong mothering behaviour leading to higher production of prolactin that probably imposed anti-gonadal activity.

## Conclusions

Very long first postpartum estrus interval and service periods were observed in the Dangi cows at farmer's doorstep. The ecbolics like methylergometrine, PGF<sub>2</sub> $\alpha$  and herbal drug were found effective to improve reproductive performances in Dangi cows. Higher progesterone levels at parturition reflect active nature of pregnancy CL and decreased after calving suggested subnormal gonadal activity entirely due to their suckling calves. Higher levels of estradiol-17 $\beta$  at parturition suggested its significant role during the process of parturition followed by markedly declining trend at a basal level deduced delayed follicular activity in all the Dangi cows.

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## **Conflict of Interest:**

All authors declare that they do not have conflict of interest.

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