Himachal Journal of Agricultural Research 41(1): 55-60 (2015)

Response and quantification of certain milk attributes following artificial induction of lactation in Jersey crossbred cows of Himachal Pradesh

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Received: 23.12. 2014; Accepted: 25.05.2015

Abstract

The present study evaluated the efficacy, lactation response, certain attributes of the milk induced and plasma alongwith reproductive changes periodically from day 5 to 75 using a 13 day protocol (day of first injection as day 1) of diethylstilbestrol, hydroxyprogesterone caproate and dexamethasone. A total of 22 non-lactating and infertile/sterile Jersey crossbred cows (G1=19, in goshalas; G2=3, with individual farmers) were used. In addition, normal postpartum cows (C=5) were also used to compare normal and induced milk. The overall efficacy of the protocol was 31.8% (21% in G1; 100% in G2). The milk yield at all recordings (five) was significantly higher in G2 than G1, the average being 1.0 and 3.6 L/d, respectively. Except for low milk Ca, especially in G1, comparable Na, K and Mg, declining estrogen and IGF-1 (Insulin-like Growth Factor-1) by day 30-35 and normal specific gravity and fat percentage at different evaluation intervals (two to six) in the induced milk suggested it to be safe for consumption after one month of induction. There was a significant correlation in Ca and Mg concentrations between blood *versus* milk as well as with increase in milk quantity at different days of lactation in the induced cows. Further, 2 of the 3 G2 chronic repeat breeder cows became pregnant. Higher BCS in G2 *versus* G1 (3.3 ± 0.1 *versus* 2.7 ± 0.14) was the probable reason for superior efficacy, better lactation response and setting up of pregnancy in G2.

Key words: Cows, Induction of lactation, Diethylstilbestrol, Progesterone

Artificial induction of lactation revitalizes the production potential of the infertile/sterile and unproductive cattle. Earlier, estradiol-17β has been used in Indian cattle (Chakravarty *et al*. 1981a; Deshmukh *et al*. 1992; Mohan *et al*. 2010) other than Jersey, the mainstay breed of Himachal and other hilly Indian terrains. However, breed variation in lactation response (Jewell 2002) and minerals in milk (Chauhan 1999) underscores the need for systematic studies *per se* using easily accessible estrogen derivative. Further, induced milk becomes a concern, as dietary/milk estrogen increases the incidence of breast and genital cancers (Ganmaa and Sato 2005). Milk insulin-like growth factor-1 (IGF-1), not reported in Indian studies, may resist pasteurization and cross the intestines undigested to increase the propensity of colon, breast or prostate cancers (Epstein and Samuel 1990) in humans. Hence, with a holistic intent, the present study evaluated the response and milk quality in Jersey crossbred cows injected diethylstilbestrol, progesterone and dexamethasone.

Materials and Methods

The cattle selected were Jersey crossbreds (cows and heifers, unto 62.5% exotic germplasm) and were non-lactating, non-pregnant with adequately developed teats and udder. The cows belonged to gaushalas [G1; n=19] or individual farmers [G2; n=3]. A group of normal postpartum cows from the University Dairy Farm, at lactation stage corresponding to the induced cows (non-lactating), was kept as control [C; n=5]. The G1 cows were provided roughages with little or no concentrate which, however, was given in variable amounts to G2. One week prior to induction, the cows were given a dose of Albendazole (15 mg/kg Analgon; 1500 mg / bolus; Vetoquinol Indian Animal Health Limited, Mumbai) and provided 1.5 kg concentrates and 30 g mineral mixture during induction (13 days).

Before induction, BCS (Edmonson *et al*. 1989), live weight and reproductive status (recorded every 15 day interval) by transrectal palpation was recorded. The protocol was initiated during the luteal phase and comprised of intramuscular injections of diethylstilbesterol (Miss-Mating Veterinary; 10 mg/ml; Inmac Laboratories, Bangsipura, Punjab) - 0.07 mg/kg of b. wt.; hydroxyprogesterone caproate (P-Depot; 250 mg/ml; Sarabhai Zydus, Ahmedabad) - 0.2 mg/kg of b. wt. from day 1 to 10 and dexamethasone sodium phosphate (Dexagee; 4 mg/ml; German Remedies, Mumbai) - 14 mg/d from day 11 to 13. The day of first injection was day 1. All the cows were given 30g Galog (Indian Herbs, Saharanpur) orally for two weeks from day 1. Stripping of teats and udder massage began from day 5. The induction was considered successful in cows producing 1Litre or more milk/d during the investigation period of 75 day. The day of appearance of udder secretions, initiation of lactation and milk quantity (L/d) at different days (day 15, 30, 45, 60 and 75) was recorded.

Estimations of Na, K (flame photometry; Systronics Flame Photometer 129), Ca and Mg (Atomic Absorption Spectrophotometer; Perkin Elmer Analyst, 400) in blood plasma and milk (day 5, 15, 30, 35, 50, 75); total estrogen and progesterone in skim milk (day 15, 30, 50, 75) and IGF -1 in plasma and skim milk (day 5, 35) (ELISA; standard kits using DRG Instruments GmbH, Germany) were undertaken. The analytical sensitivity, the inter- and intra-assay coefficient of variation of estrogen was 9.714 pg/ml, 7.39% and 4.01%, respectively. The corresponding values for progesterone were 0.045 ng/ml, 6.63% and 5.7% and for IGF-1 were 1.29 ng/ml, 7.45% and 5.63%, respectively. The milk specific gravity (lactometer) and fat content (Gerber's method) were evaluated in day 15, 30 and 45 samples. The data were analyzed using Tukey-Kramer multiple comparison test of ANOVA or Student's t – test. A difference of $P<0.05$ (at least) was considered as significant, whereas $P=0.10$ was a tendency for difference. The entire statistical analysis was performed using SAS® (Statistical Analysis System).

Results and Discussion

Induction of lactation was successful in 21% G1 (4/19 cows) and all the cows in G2 (3/3 cows), the overall success being 31.8%. Using similar drugs, a much higher success of 82-100% in exotic (Sawyer *et al*.1986; Ryan *et al*. 1988) and a much lower success of 53% in Indian crossbreds (Chakravarty *et al*. 1981a) was reported. There was no difference in G1 *versus* G2 on the day of appearance of watery/honey like secretions (4.2 ± 0.2 *versus* 6.6 ± 1.8) and initiation of lactation (10.0 ± 1.7 *versus* 12.3 ± 1.4). Using a similar protocol, lactation was initiated between 9 to 14 days (Chakravarty *et al*. 1981b). The milk yield increased gradually and was higher ($P<0.05$) in G2 *versus* G1 at days 15 (0.5 ± 0.0 *versus* 0.2 ± 0.0 L), 30 (2.9 ± 0.2 *versus* 0.6 ± 0.1 L), 45 (4.5 ± 1.0 *versus* 1.1 ± 0.1 L), 60 (4.9 ± 1.0 *versus* 1.5 ± 0.1 L) and 75 (5.2 ± 1.5 *versus* 1.4 ± 0.1 L). Higher live weight and BCS in G2 *versus* G1, 346.7 ± 14.5 kg *versus* 269.0 ± 30.3 Kg and 3.3 ± 0.1 *versus* 2.7 ± 0.14,) respectively, could be the reason for difference in lactation response and induction failures in cows from goshalas that had lower live weight (259.46 ± 9.9 kg) and BCS (2.6 ± 0.2). The average yield for all the days in present study was 1.0 L/d in G1 and 3.6 L/d in G2, respectively. Much higher average of 7.83 L/d (Sawyer *et al*. 1986) and 5-6 L/d (Chakravarty *et al*. 1981a; Sawyer *et al*. 1986; Ryan *et al*. 1988) has been reported which may be due to an inherent potential of high yield. The peak milk yield in G1 (1.42 ± 0.15 L at day 60) was less than G2 (5.20 ± 1.50 L at day75). In the Indian crossbreds, a peak of 5.51 L at 21 weeks (Chakravarty *et al*. 1981a) and of 4.5 L at day 31 (Agrawal *et al*. 1993) have been reported.

The plasma Na concentration did not differ much either within or between groups and was comparable to normal range of 132 to 152 mEq/L (Kaneko 1989). The milk Na (range: 15.7 ± 2.1 to 23.1 ± 1.2 mEq/L) resembled more closely to Jersey (20.4 ± 0.4 mEq/L) than Jersey x Red Sindhi crossbreds (22.0 ± 0.4 mEq/L) (Chauhan 1999). Slightly higher milk K of 3.9 to 5.8 mEq/L (Kaneko 1989) and 4.97 to 5.07 mEq/L from early to mid lactation (Manzoor *et al.* 1994) has been reported. Milk is an intracellular fluid and therefore contains large amount of K compared to plasma (Schmidt 1971), which was evident in present study for most lactation days. Compared to C, the milk K in G1 and G2 was comparatively higher for nearly all lactation days, which augurs well with higher milk potassium in low producing cows (Harrison *et al.* 2007). The average milk yield in C group (6.62 ± 0.52 L/d to 7.10 ± 0.53 L/d from day 15 to day 75) was higher than G1 and G2 ($P<0.01$). Much higher milk K concentrations of 34.1 to 47.2 mEq/L (Manzoor *et al.* 1994), 31.8 ± 0.6 mEq/L in early lactation to 26.5 ± 0.8 mEq/L in later lactation (Chauhan 1999) have been reported. Variation among different studies could be due to difference in dietary K (Harrison *et al.* 2007). The plasma Ca in C, varyingly higher than G2 and G1 at most lactation days, was close to 97 to 124 mg/L in exotic cows (Kaneko 1989), but much higher than 12.4 ± 0.2 mg/dL (Hadzimusic and Krnic 2012). Plasma like trend was recorded in milk Ca with highest and lowest values in C and G1 leading to most of the significant differences. The Ca in milk comes from the blood Ca, which is derived from the feed and from the skeleton (Schmidt 1971). Hence,

Table 1. Average (Mean ± S.E.M) concentrations of Na (mEq/L), K (mEq/L), Ca (mg/L) and Mg (mg/L) in blood plasma and milk at different days of lactation in C (n=5), G1 (n=4) and G2 (n=3)

Mineral	Group	Plasma						Milk					
		Day of lactation						Day of lactation					
		5	15	30	35	50	75	5	15	30	35	50	75
Na	C	131.6 ±2.3	133.6±3.8	126.8±0.4	126.0±0.8	124.8±1.8	128.0±1.1	17.3±2.7	17.4±4.1	16.9±0.4^{1}	16.8±0.7^{5}	16.6±1.7	18.1±4.0
	G1	115.5±3.8	107.5±11.6	99.5±6.7	87.5±10.5	99.0±5.3	84.5±15.7	23.1±1.2^{b,2}	19.8±2.3	15.7±2.1	19.0±3.4	18.1±0.3^{a}	20.3±0.9
	G2	129.0±1.7	135.3±3.5	127.3±2.4	136.6±4.3	131.6±2.0	122.6±7.3	15.7±2.2^{a,1}	18.2±3.0	21.9±1.1^{2}	20.2±0.0^{6}	19.7±1.3	21.4±0.2^{b}
K	C	2.9±0.1^{4}	2.8±0.1	2.7±0.1	2.6±0.2	2.5±0.2	2.7±0.2	2.9±0.1^{5}	2.8±0.2^{5}	2.8±0.1^{i,5}	2.7±0.2^{5}	2.6±0.1^{5}	2.9±0.1^{5}
	G1	3.5±0.3^{2}	3.2±0.3	2.9±0.1	2.5±0.3	2.8±0.2	2.4±0.7	5.1±0.3^{6}	8.8±4.5	6.6±0.7^{6}	6.3±0.5^{6,7}	5.6±1.0^{6,7}	3.0±1.1^{5}
	G2	2.4±0.1^{e,1,3}	3.1±0.0^{f,h}	2.8±0.0^{f,h}	3.1±0.0^{f,h}	3.8±0.7	2.5±0.0^{g}	8.5±2.7^{a,c}	10.5±1.2^{c,e,6}	16.8±6.6^{2}	13.2±2.1^{6,8}	16.7±0.4^{d,6,8}	18.7±2.1^{b,f,6}
Ca	C	80.6±4.8	90.1±3.1^{6}	93.0±11.0^{2}	82.5±2.4^{6}	85.1±2.5^{6}	89.7±3.7^{2,6}	57.5±3.2^{6}	67.3±6.6^{6}	71.6±4.8^{6}	67.7±7.1^{6}	67.7±10.8^{6}	61.8±6.2^{2}
	G1	67.4±3.5^{f}	51.8±8.0^{5}	52.0±4.1^{1}	40.6±2.0^{5}	36.1±5.0^{e,5}	30.4±6.8^{e,5}	9.2±0.3^{b,e,g,5}	4.7±1.2^{a,e,g,5}	12.5±2.3^{b,g,5}	16.9±0.9^{f,g,5}	11.0±3.5^{g,5}	42.1±1.0^{h,1,5}
	G2	71.0±9.3	63.9±0.4^{5}	72.7±9.6	55.9±5.3^{5}	47.0±10.2^{5}	49.7±12.7^{1}	105.3±32.6^{6}	68.2±14.0^{6}	54.8±21.5	73.6±12.5	57.4±7.3^{6}	66.5±5.9^{6}
Mg	C	18.2±0.8^{2}	21.3±1.2^{2}	21.7±3.0^{2}	18.0±2.0^{6}	20.4±2.2^{6}	21.8±3.2^{6}	7.3±0.4^{6}	7.1±0.2^{6}	6.7±0.5	7.1±0.4	7.5±0.4	6.8±0.0
	G1	12.3±1.5^{b,1}	9.9±2.3^{1}	8.7±1.3^{a,1}	6.4±0.8^{a,1,5}	7.0±0.6^{a,1,5}	5.8±1.7^{a,5}	2.0±0.9^{5}	1.6±0.3^{5}	3.1±1.7	3.8±1.2	4.9±0.7	5.1±0.8
	G2	16.7±3.4	17.4±4.1	18.1±2.6^{2}	12.3±2.1^{2}	10.8±1.8^{2}	9.7±1.7^{5}	9.7±3.4^{6}	11.8±7.1	7.3±3.1	8.4±3.1	7.7±4.6	9.0±5.3

Values with different superscripts differed significantly ($^{ab,\ cd}$ within same row and $^{12,\ 34}$ within same column at P<0.05; $^{ef,\ gh}$ within same row and ^{56,78} within same column at P<0.01) for each mineral in plasma or milk consistently lower milk Ca in G1 compared to the G2 confirms a better nutritional status in the latter. The average milk Ca concentration ranged from 120.8 ± 2.6 mg/dl to 130.7 ± 2.5 mg/dl without much effect of breed and stage of lactation (Sen *et al.* 1989, Chauhan 1999). The plasma Mg, barring day 5, remained consistently lower in G1 and differed significantly from C and G2 at most days. The correlation of each mineral between plasma *versus* milk concentration and milk concentrations *versus* milk quantity in the induced cows, at different days of lactation, revealed significance for Ca and Mg. This implies (i) transfer of Ca and Mg from plasma to milk and (ii) sustained increase in milk Ca and Mg concentration with the increase in milk quantity and is in complete agreement with Nozad *et al*. (2012) recording a significant (P<0.01) positive correlation between the blood and milk parameters for Ca and Mg but not for Na and K.

Gradual decline in milk estrogen with progression of lactation in all groups of present study corroborated to 180 ± 73 pg/ml at d 15, reducing to 95 ± 32 pg/ml at d 42 (Erb *et al*. 1976). Relatively higher estradiol concentrations of 430 pg/ml and 330 pg/ml at day 16 and 32, respectively, have also been reported (Harness *et al*. 1978). In Indian cattle, estrogen concentration in induced than postpartum milk have been relatively higher (210.0 ± 50.0 versus 170.0 ± 20.0 pg/ml) (Narendran *et al*. 1979) or similar (59.1 pg/ml *versus* 54.7 pg/ml) (Mohan *et al*. 2010). In contrast, the postpartum milk had 30-80 pg/ml of estrogen that was undetectable in induced milk (Agrawal *et al*. 1993). Except for higher milk progesterone at day 15 in G1, it did not vary among different groups. Noticeably, milk progesterone in C was lower than induced cows (Table 2). The 15 day progesterone values simulated to a previous study, significantly higher progesterone in induced (1.5 ± 0.0 ng/ml) than normal (1.3 ± 0.0 ng/ml) milk (Mohan *et al*. 2010) as against nearly similar concentration of 2ng/ml from day 5 to day 30 in induced milk has been reported (Agrawal *et al*. 1993). In contrast, induced cows had relatively higher progesterone at day 15 (5.1 ± 1.2 ng/ml) and 42 (3.9 ± 1.5 ng/ml) (Erb *et al*. 1976) and still higher average of 15.1 ± 6.2 ng/ml to 18.1 ± 2.2 ng/ml over a 30 day period (Harness *et al*. 1978; Zhou *et al.* 2009). Hence, a precipitous decline in milk estrogen and IGF-1 by day 30-35 makes the induced milk to be safe for human consumption one month after induction. The available literature focusing exclusively on estrogen content indicate the induced milk to be safer for consumption after 2 to 3 weeks of induction (Mohan *et al*. 2010). Variation in estrogen and progesterone concentration in the induced milk among different studies may be attributed to differences in the molecules used *per se* (Harness *et al*. 1978), method of estimation (Narendran *et al*. 1979), duration of treatment (Deshmukh *et al*. 1993), endogenous contribution (Sawyer *et al.*1986), vehicles used, interval between two injections (Harness *et al*. 1978) and quantity of milk produced (Sawyer

Table 2. Average (Mean ± S.E.M) milk estrogen (pg/ml), progesterone (ng/ml) and IGF-1 (ng/ml) at different days of lactation in C (n=5), G1 (n=4) and G2 (n=3)

Hormone	Group	Day of lactation			
		Milk			
		15	30	50	75
Estrogen	C	233.8 ± 18.6[b]	114.2 ± 25.0[a]	99.0 ± 18.8	103.8 ± 63.6
	G1	311.5 ± 82.7[b]	81.7 ± 31.5[a5]	84.3 ± 36.6	121.0 ± 52.4
	G2	169.6 ± 41.9	354.3 ± 55.6[f,6]	109.0 ± 5.6[e,h]	51.6 ± 14.2[g]
Progesterone	C	0.9 ± 0.02[a]	0.8 ± 0.01	1.0 ± 0.1	0.8 ± 0.03
	G1	3.1 ± 0.5[b]	1.8 ± 0.6	1.7 ± 0.6	1.7 ± 0.5
	G2	1.0 ± 0.12[a]	1.0 ± 0 .02	1.7 ± 0.2	1.7 ± 0.3
		Day of lactation			
		Plasma		Milk	
		5	**35**	**5**	**35**
IGF-1	C	87.8 ± 22.4	67.8 ± 14.0	155.0 ± 3.6[1]	101.4 ± 23.4
	G1	87.3 ± 20.1	98.5 ± 19.0	190.1 ± 10.1[b,2]	142.0 ± 8.7[a]
	G2	44.7 ± 31.6	97.0 ± 28.0	144.0 ± 3.12[a]	112.6 ± 25.0

Values with different superscripts differed significantly ([a,b] within same row and [1,2] within same column at P<0.05; [e,f, g,h] within same row at P<0.01) *et al.*1986). The later factor appears to be of great significance as estrogen and progesterone in milk of induced cows were approximately twice as concentrated as in the normal post-partum milk (Harness *et al*. 1978; Deshmukh *et al*. 1993). The plasma IGF-1 concentrations in different groups did not differ, whereas the milk IGF-1 at day 5 was varyingly higher than the day 35 concentrations in all the groups (Table 2). The IGF-1 is a tissue mitogen and increases the number of milk secreting units (Schmidt 1971). Higher IGF-1 in milk than plasma is a testimony of its localized production in mammary tissue (Epstein and Samuel 1990).

The average fat percentage (range: 3.5 ±0.7 to 5.1±0.7) and specific gravity (range: 1.02±0.02 to 1.03±0.02) that depends on the amount of fat in milk, did not differ within and between groups at different days, which is an affirmation to earlier report (Deshmukh *et al.*1993). The induction protocol did not affect reproduction in any of the cows unlike aberrant estrus activity (Chakravarty and Razdan 1981) or ovarian cysts (Sawyer *et al.*1986) recorded earlier. Two cows of G2, however, became pregnant 90 to 120 days after the initiation of the treatment.

In conclusion, a moderate efficacy, normal milk quality and no aberrant reproductive change following induction by diethylstilbestrol, hydroxyprogesterone caproate and dexamethasone makes it a suitable preposition for use in unproductive and infertile cattle. However, a better body condition dictates a favorable outcome. Though there was precipitous decline in milk estrogen and IGF-1 by day 30-35, the use of induced milk after one month be recommended with caution.

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