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Comparison of Milk Estrogen and Progesterone Concentration in Induced Heifers and Normally Calved Lactating Cows

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Abstract: The present study was designed to investigate and compare levels of estrogen and progesterone secreted into milk of crossbred heifers that were artificially induced into lactation with that of naturally calved cows. Group I animals comprised of ten crossbred Holstein Friesian cows which were in first lactation served as control. Twenty repeat breeding non-pregnant, non-lactating crossbred heifers with a history repeat breeding were divided into two equal groups (group II and III). Group II animals received two doses of PGF_{2α} at 10 days apart (1st dose on day-10 and 2nd dose on day 0). In addition, these animals (group II) received 17β-estradiol (0.1 mg kg⁻¹) and progesterone (0.25 mg kg⁻¹) twice subcutaneously daily for 7 days (day 1 to 7). Metoclopramide (0.1 mg kg⁻¹) and dexamethasone (20 mg animal⁻¹) were administered for 4 days (day 14 to 17) by intramuscular route. Group III animals received 17β-estradiol (0.1 mg kg⁻¹) and progesterone (0.25 mg kg⁻¹) twice subcutaneously daily for 7 days (day 1 to 7) and metoclopramide (0.1 mg kg⁻¹) and dexamethasone (20 mg animal⁻¹) intramuscularly on day 14 to 17. Milking was initiated on day 19 in group II and III. The mean milk estrogen and progesterone concentrations were estimated during the 15 days in Holstein Friesian crossbred cows (n = 10) by radioimmunoassay. The mean milk estrogen concentration was significantly higher (p<0.05) in induced animals (group II and group III) on day 5 of lactation. From day 15 onwards, the milk estrogen concentrations did not differ significantly. Milk progesterone concentration significantly decreased (p<0.05) on day 1, 3, 11 and 13 in induced heifers (group II) as compared to normally calved cows. The concentrations of estrogen and progesterone in milk during the 15 days sampling period were comparable to those observed in normally lactating cows. This study suggested that estrogen and progesterone concentration of the induced milk was in similar pattern to that of normal lactating animals. Hence it can be hypothesized that consumption of milk from such induced animals may not pose any health hazard in human beings.

Key words: Induction of lactation, repeat breeder heifers, hormone assay

INTRODUCTION

It has been well established through *in vivo* studies that hormones play a pivotal role in the development and function of the mammary gland (Erb *et al.*, 1976; Tucker, 2000;

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Verma *et al.*, 2004; Mohan *et al.*, 2009). Many techniques over the past 60 years have utilized the ovarian hormones estrogen or progesterone, alone or in combination, to develop the mammary gland and initiate lactation (Verma *et al.*, 2004). Further, researchers have tried to quantify the concentration of various hormones, which had administered exogenously to induce lactation (Sawyer *et al.*, 1986; Deshmukh *et al.*, 1993; Dang *et al.*, 1997). The milk estrogen or progesterone concentrations in lactation induced cows differed markedly as reported by many workers. It has been reported that concentration of hormones in induced milk becomes normal within 2 to 3 weeks of milking (Dang *et al.*, 1997). However, studies regarding the use of PGF_{2α} for synchronization prior to initiation of steroid hormone (estrogen and progesterone) therapy for lactation induction and quantification of estrogen and progesterone concentration in the milk of such induced animals are rarely reported (Shridhar *et al.*, 2006; Mohan *et al.*, 2008). Keeping these facts in view, the present study was designed to induce lactation in repeat breeding Holstein Friesian (HF) crossbred heifers by two different protocols and to compare the concentration of the estrogen and progesterone excreted in milk from induced animals with that of naturally calved crossbred Holstein Friesian cows.

MATERIALS AND METHODS

Hormones and Drugs Used for Induction of Lactation

Progesterone (Sigma Chemicals, USA), 17β-estradiol (Sigma Chemicals, USA), metoclopramide (Perinorm, 5 mg mL⁻¹, Ipca Laboratories Ltd., Mumbai, India), dexamethasone (Dexamethasone, 4 mg mL⁻¹, KAPL, India) and (PGF_{2α}) Cloprostenol sodium (Vetmate, 250 μg mL⁻¹, Vetcare, India).

Preparation of Hormone Mixture

The common stock solution contained 17β-estradiol and progesterone (Sigma Chemicals, USA) with 1:2.5 ratios. This was dissolved in absolute ethanol (Merck, India) on magnetic stirrer until it was dissolved completely. The quantity of the stock solution required for an animal was determined based on the body weight of the animal. The prepared hormone mixture was stored in air tight amber colored glass container under refrigeration (4°C) until further use.

Experimental Animals

The animals for the present study were selected from various parts of Shimoga and Uttar Kannada districts of Karnataka State, India and the study was conducted during the period January 2007 to August 2007. Group I (n = 10) animals comprised of crossbred HF cows which were in first lactation served as control. Whereas group II (n = 10) and group III (n = 10) consists of repeat breeding crossbred HF heifers.

Treatments

Group I animals did not receive any treatment and served as control. Lactation induction protocol for group II and group III with treatment days and hormones or drugs administered is given in Table 1. Group II heifers received 2 doses of PGF_{2α} (Vetcare, India) intramuscularly at 10 days apart prior to initiation of hormonal therapy. Common stock solution containing 17β-estradiol (0.1 mg kg⁻¹) and progesterone (0.25 mg kg⁻¹) was administered twice subcutaneously at 12 h interval from day 1 to 7. Metoclopramide (Ipca Laboratories Ltd., Mumbai, India) (0.1 mg/kg/day) and dexamethasone (KAPL, India) (20 mg day⁻¹) were

Table 1: Lactation induction protocol for group II and group III with treatment days, hormones or drugs administered and milk samples collected

Day-10	Day 0	Day 1-7	Day 14-17	Day 19	Day 21	Day 23	Day 25	Day 27	Day 29	Day 31	Day 33
PGF _{2α}	PGF _{2α}	17β-E+P ₄	Dexamethosone metaclopramide	Initiation of milking 1st	2nd	3rd	4th	5th	6th	7th	8th

PGF_{2α} - Prostaglandin F_{2α}; 17β-E - 17β-Estradiol; P₄ Progesterone

administered separately on day 14 to 17 once a day through intramuscular route. Group III animals also received similar therapy except PGF_{2α} was not administered. Milking was initiated on day 19 for group II and III.

Collection and Storage of Milk Samples

Milk samples were collected on alternate days from the day of parturition to day 15 from the normally calved animals (Group I). Milk samples from induced animals were collected in 10 mL vials containing preservatives (15 mg of potassium dichromate and 10 mg of sodium chloride) on alternate days for 15 days (Table 1). Thus collected milk samples were stored at -20°C until analysis for concentrations of 17β-estradiol and progesterone.

Radioimmunoassay Procedure

Radioimmunoassay methods were based on the procedures adopted by Ramachandra and Narayana (1996) for measuring 17β-estradiol and progesterone concentration. Radio labeled hormone used was (2, 4, 6, 7, 16, 17-³H) estradiol, GE Healthcare, Amersham Biosciences International, Amersham, UK, specific activity 154 Ci mmol⁻¹ and (1, 2, 6, 7 ³H) progesterone (GE Healthcare, Amersham Biosciences International), with the activity of 99 Ci mmol⁻¹. 17β-estradiol and progesterone standard was procured from Sigma Chemicals, USA. For the purpose of RIA, 17β-estradiol and progesterone antisera at the rate of 1:10000 dilutions were used in the present study. The working stock solution of both the labeled hormones were evaporated separately and stored at 4°C. While performing the assay, the evaporated stock was reconstituted with required amount of 0.1% Gelatin Phosphate Buffered Saline (GPBS) to give about 10,000 Counts per Minute (CPM) per 100 μL. The radioactive counts were obtained by placing the tubes containing labeled hormones in the Liquid Scintillation Counter (Hydex, Netherlands) for 60 sec, supported with data reduction and analysis software Saprical (2007) for PC based RIA counter model PRIA-1 (Para Electronics, Mumbai).

Statistical Analysis

The experimental data obtained in this study were analyzed statistically using the General Linear Model procedure of Statistical Analysis System (SAS®) software (SAS Institute, USA, 2000). Day wise data were analyzed by 3×3 factorial manner using repeated measurement design (Gill, 1985). A one-way analysis (ANOVA) was employed and the data were compared by Duncan multiple range test at 0.05 probability level (Duncan, 1955).

RESULTS

In both treatment groups (group II and III), lactation was successfully induced by administration of hormones or drugs exogenously. Mammary gland of lactation induced animals showed marked enlargement in size of udder by day 7. As the days advances mammary gland became fully distended with fluids and the teats were elongated, full and turgid in both the treatment groups. Dropping of milk from teats was noticed on day 17 and

Table 2: Milk 17 β -estradiol concentration (pmol L⁻¹) of control and treatment groups (Mean \pm SE)

Day	Group		
	I (Control)	II	III
1	2042.91 \pm 116.10 ^a	2380.28 \pm 176.78 ^a	1431.32 \pm 91.54 ^b
3	1245.57 \pm 100.28 ^b	1642.41 \pm 83.76 ^c	1118.92 \pm 77.81 ^b
5	935.73 \pm 79.84 ^c	1786.68 \pm 133.70 ^a	1354.60 \pm 95.74 ^b
7	814.22 \pm 47.24 ^b	1457.75 \pm 92.91 ^a	888.74 \pm 61.52 ^b
9	479.79 \pm 37.95 ^c	1185.37 \pm 76.83 ^a	948.58 \pm 51.26 ^b
11	443.08 \pm 26.98 ^c	660.41 \pm 65.39 ^a	454.46 \pm 19.66 ^b
13	307.62 \pm 11.98 ^c	317.54 \pm 10.13 ^a	198.96 \pm 15.22 ^b
15	217.32 \pm 15.04 ^c	190.15 \pm 20.67 ^a	201.17 \pm 10.38 ^b

Means within each column bearing common superscript do not differ significantly ($p < 0.05$)

Table 3: Milk progesterone concentration (ng mL⁻¹) of control and treatment groups (Mean \pm SE)

Day	Group		
	I (Control)	II	III
1	2.60 \pm 0.05 ^a	2.12 \pm 0.05 ^b	2.67 \pm 0.05 ^a
3	2.20 \pm 0.06 ^b	1.98 \pm 0.04 ^c	2.44 \pm 0.04 ^a
5	1.88 \pm 0.07 ^b	1.82 \pm 0.01 ^b	2.18 \pm 0.03 ^a
7	1.64 \pm 0.03 ^b	1.70 \pm 0.01 ^b	1.90 \pm 0.04 ^a
9	1.47 \pm 0.04 ^c	1.74 \pm 0.01 ^b	1.98 \pm 0.03 ^a
11	1.69 \pm 0.03 ^a	1.46 \pm 0.10 ^b	1.73 \pm 0.02 ^a
13	1.63 \pm 0.05 ^b	1.46 \pm 0.02 ^c	1.79 \pm 0.03 ^a
15	1.31 \pm 0.06 ^b	1.37 \pm 0.01 ^b	1.53 \pm 0.03 ^a

Means within each column bearing common superscript do not differ significantly ($p < 0.05$)

18 in three and four animals respectively in group II and III. In both the groups of animals milking was initiated on day 19. The first milk obtained from induced animals was slightest yellowish to white, subsequently; milk secretions in all animals become normal within 3 to 5 days of milking.

The mean milk 17 β -estradiol concentration from day 1 through day 15 in control and treatment groups are presented in the Table 2. The mean milk 17 β -estradiol concentration was significantly higher ($p < 0.05$) on day 3, 5, 7, 9 and 11 in group II and on day 5, 9 and 11 in group III respectively as compared to control group.

The mean milk progesterone concentration from day 1 through day 15 in control and treatment groups are presented in the Table 3. Significant decrease ($p < 0.05$) in the milk progesterone concentration on day 1, 3, 11 and 13 in group II was recorded as compared to the control group. And significant increase ($p < 0.05$) in mean milk progesterone concentration was observed in group II on day 9 and in group III on day 3, 5, 7, 9, 13 and 15 as compared to control group.

DISCUSSION

The observed variations in the mean 17 β -estradiol concentration in milk of treatment groups as compared to control group might be attributed to the secretion of endogenous estrogen from active follicles in addition to exogenous estrogen source. Further significant increase in the estrogen concentration in milk of group II might be ascribed to luteolysis caused by PGF_{2 α} which might have lead to emergence of new follicular wave and co-relates with results regarding the estrus synchronization by (King *et al.*, 1982; Stevenson *et al.*, 1984; Beal, 1998).

However, from day 15 onwards estrogen concentration in milk was identical in both control and treatment groups. The concentration of estrogen in cows' milk after calving was

550 pmol L⁻¹ (Harness *et al.*, 1978; Narendaran *et al.*, 1979), which are comparable to the values reported in the present study, for both induced and normal milk. However, the concentration of estrogen in milk reported in the present study is higher than the values (1284 to 1468 pmol L⁻¹) reported by Sawyer *et al.* (1986) and lower than the values reported by Fleming *et al.* (1986).

Milk progesterone in induced lactation by the combination of hormones reported by Erb *et al.* (1977) was lower than 2 ng mL⁻¹ and was lesser than that of normal cows' milk during the estrous cycle (0.5-15 ng mL⁻¹). Furthermore, progesterone in milk following induced lactation was lower than normal cows' milk (Erb *et al.*, 1977; Sawyer *et al.*, 1986).

Agarwal *et al.* (1993) reported that ovarian steroids used for induction of lactation were not excreted in the milk in any marked amount and consumption of such milk may not pose health hazard. Steroid hormones in induced milk become normal within 2 to 3 weeks of milking (Dang *et al.*, 1997). They have quoted 281.4 pmol L⁻¹ of estrogen concentration in induced milk, which was 6 times higher than normal milk (46.9 pmol L⁻¹) and became normal by day 15 of milking.

Further, the concentrations of estrogen and progesterone in milk during the 15 days sampling period from the induced cows averaged 433.06 and 1.6 ng mL⁻¹, respectively and were comparable to normally lactating cows (Deshmukh *et al.*, 1993).

The results of the present study also correlate with earlier studies (Sawyer *et al.*, 1986; Agarwal *et al.*, 1993; Deshmukh *et al.*, 1993) that consumption of milk from hormonally induced animals may not pose any health hazard to human beings. Further, systematic experimental feeding of hormonally induced milk to rats and mice also provide conclusive evidence that the milk is free of hormone residues (Sinha *et al.*, 1985).

CONCLUSION

Extensive mammary uptake and metabolism of estrogen and progesterone by the mammary gland is one of the routes of steroid hormone excretion in the milk. The concentrations of estrogen and progesterone of milk induced in repeat breeding heifers is comparable to that of normal lactating cows.

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