Abstract

Mammary gland histology and milk composition were studied in Holstein cows and heifers induced to lactate following 17β-estradiol and progesterone therapy for 7 days. The most notable gross histological observation was a large number of immature or developing ducts and alveoli. These ducts and alveoli were without lumen and consisted mainly of clumped columnar epithelial cells. No gross histological abnormalities were seen. The composition of milk from induced lactations with regard to fat, protein, and lactose percentages was not significantly different from milk from lactations following parturition during the first 21 days of lactation.

Introduction

In goats, daily injections of diethylstilbestrol and progesterone for 60 days produced tightly packed alveolar cells, similar to those of normally developed udders at mid-pregnancy (14). Benson et al. (1) noted a combination of hexoestrol and progesterone induced normal udder development in the goat whereas estrogen alone produced cystic alveoli, papillomatous outgrowths of the epithelium, and immature lobules. Cowie et al. (4) comparing the glands at the peak of lactation showed that most of the lobulo-alveolar growth and maturation occurred during lactation, i.e. after the hormone injections had been stopped. They postulated that this mammary growth was due to the release of mammogenic hormones from the anterior pituitary in response to the milking stimulus.

This explanation was confirmed by Cowie et al. (5), who showed that regular application of milking stimulus to nulliparous ovarietomized goats induced mammary growth and lactation without pretreatment with ovarian hormones. They demonstrated further that the mammogenic response was of anterior pituitary origin since surgical transection of the pituitary stalk completely abolished the response. Folley et al. (8) with twin goats and Folley and Malpress (9) with heifers demonstrated that secretion induced by treatment with estrogen remained colostral for several weeks and then changed gradually to milk of normal composition. In identical twin heifers brought into lactation following diethylstilbestrol and progesterone treatment for 40 days (18), milk secretion was initially colostral but as the milk flow became established, the composition gradually changed to that of normal milk.

Our aim was to study histology of the mammary gland and milk composition in nonpregnant nonlactating cows and heifers induced to lactate with 17β-estradiol and progesterone.

Materials and Methods

Hormones: 1, 3, 5 (10)-estratrien -3, 17β-diol (17β-estradiol), and 4-pregnene -3, 20 dione (progesterone) (Sigma chemical Co., St. Louis, MO, USA) were dissolved in absolute alcohol so that each milliliter of stock solution contained 20 mg of 17β-estradiol and 50 mg of progesterone (20, 21). The stock solution was stored at room temperature in the dark for no more than 14 days.

Injections: 17β-estradiol and progesterone were injected at .1 mg/kg and .25 mg/kg body weight, respectively, in divided doses mornings and evenings. The postcaulcular subcutaneous injections were begun in all animals on day 3 after observed estrus and were continued for 7 days, alternating between right and left sides (15, 16).

Animals: Animals for induction of lactation were purchased at the stock yards from a group designated for slaughter. All animals were Holstein and were housed in a tie stall barn. They received a ration of haylage plus...
grain mixture. All animals were nonpregnant and nonlactating. Estrus was characterized by vulval tumefaction, clear mucous vaginal discharge, and restlessness. Milking of treated animals was commenced when the mammary glands appeared full, plump, and turgid with the teats erect and taut (15).

Histological studies: Subsequent to epidural and local infiltrative anaesthesia with a 2% solution of procaine hydrochloride, a 5 cm skin incision deep enough to expose the mammary parenchyma was made at the middle of the posterior aspect of the right rear quarters and in one instance (Cow ±137) in both right and left rear quarters. The tissue was grasped with a haemostat and a 2 cm square block of tissue removed with the aid of a scalpel (12, 17). The block of tissue was further cut into 5 mm square blocks and put into Zenker-Formol (Helly’s fluid) fixative (6). The depth at which the tissue sample was obtained was regulated by the depth at which secretory parenchyma appeared.

All biopsies were prior to evening milking and between 1400 and 1800 h. One cow (+132) and a heifer (+145) producing 16 kg and 9 kg of milk per day at the time of the biopsy were selected from among the cows successfully induced to lactate. Both had commenced lactation on day 11 post treatment and were in day 53 and 57 of lactation. Biopsies were also taken from the right and left quarters of one cow (+137) which had responded to induction only on the two right quarters. This cow was in her 10th day of lactation at the time of biopsy and had come into lactation on day 21 post treatment. With continued application of the milking stimulus the two left quarters came into production. The mammary tissue was fixed for 24 h and stained with haematoxylin and eosin (6). Serial sections from each block of tissue were examined, and the gross histological appearance was evaluated.

Milk composition: Milk samples were obtained at both the AM and PM milkings for the first 21 days of induced lactation from 9 cows and 13 heifers. All samples were preserved with potassium dichromate during refrigerated storage, prior to being analyzed. Milk samples were similarly collected for 21 days postpartum from 10 naturally lactating Holstein cows belonging to the University research station herd. This group served as controls for the comparison of milk composition. The AM and PM milk samples were analyzed separately and in duplicate for fat, protein, and lactose percentages by Infra Red Milk Analyzer (IRMA) (2, 3, 19). Milk samples that showed fat and protein percentages over 10% were analyzed in duplicate for fat percent by Babcock test (subsequent to 1:1 dilution of the sample with distilled water) and for protein percent by the Kjeldahl method (11).

Statistical analysis: The following statistical model was used for the comparison of percentages of fat, protein, and lactose in milk from the two treatments (induced lactation and lactation following parturition) in periods 1 to 3 days, 4 to 9 days, 10 to 15 days, and 16 to 21 days of lactation.

\[ Y_{ijk} = \mu + t_i + c_{ij} + e_{ijk} \]

where,

\[ Y_{ijk} = k^{th} \text{ observation for percent fat, protein, or lactose in the } i^{th} \text{ period, } j^{th} \text{ cow, and } t^{th} \text{ treatment, } \]

\[ \mu = \text{overall mean for percent fat, protein, or lactose,} \]

FIG. 1. Mammary tissue from cow #132. Alveoli are well-developed and distended with secretion. The alveolar epithelial cells appear flattened and have indistinct nuclei. (480x).

(2, 3, 19).
Fig. 2. Mammary tissue from cow #145. A typical area showing a number of immature or developing ducts and alveoli. There is a large amount of connective tissue in proportion to the secretory parenchyma. (189x).

\[
t_{i1} = \text{fixed effects of the } i^{\text{th}} \text{ period on the } i^{\text{th}} \text{ treatment,}
\]

\[
c_{ij} = \text{random effects of the } j^{\text{th}} \text{ cow in the } i^{\text{th}} \text{ treatment, and}
\]

\[
e_{ijk} = \text{random error}
\]

Tukey's W-procedure (Honestly significant difference - H.s.d.) was used to estimate significance of differences between the two treatments (23). Regression analysis was done for milk yield on the different periods for induced lactations and lactations following parturition.

Results

Mammary gland histology, Cow #132. Distinct lobules were distended with alveoli. There was scanty intra- and inter-lobular connective tissue compared to the epithelial parenchyma, and alveolar lumina were distended with secretion (Fig. 1). The alveolar epithelial cells appeared flattened with indistinct nuclei. A few lobules with alveoli having little secretion were also seen. These alveoli had tall columnar epithelial cells with prominent nuclei. A few ducts and alveoli with no lumen and comprised of clumped columnar cells were a notable feature.

Cow #145. The amount of connective tissue as compared to secretory parenchyma (Fig. 2) was distinctly greater than in cow #132. Most alveoli showed a lumen and had tall columnar epithelial cells. A number of ducts and alveoli had no lumen and consisted of columnar epithelial cells. (Fig. 3).

Cow #137. Sections from the functioning mammary quarter showed a number of distinct lobules with distended alveoli. A few ducts and alveoli were seen with no lumen and consisting of columnar epithelial cells clumped together. The amount of connective tissue compared to the secretory parenchyma was small. Sections from the nonfunctional quarter had

Fig. 3. Mammary tissue from cow #145. Budding of tertiary duct or alveolus from a larger duct. The end bud consists of clumped epithelial cells with prominent nuclei. (480x).
Fro. 4. Mammary tissue from cow #137. Poorly developed lobules in nonfunctional quarter. The lobules are well defined and mainly consisted of ducts. (189x).

a few clusters of ducts separated from one another by connective tissue (Fig. 4). Alveoli were few and were mostly without a distinct lumen. The alveolar epithelium consisted of a group of tall columnar epithelial cells.

Milk composition and yield. Mean percentages and standard errors of fat, protein, and lactose in milk for induced lactations and lactations following parturition (control) are in Fig. 5, 6, and 7.

Error variance was tested for homogeneity in both treatments and was significantly different for protein and lactose percentages. However, treatment differences were analyzed with error variances for both treatments and pooled variances. Treatment differences were the same in both cases. The differences (Induced-Control), in fat, protein, and lactose percentages by pooled variances and corresponding standard errors are in Table 1. Differences in percentage of fat, protein, and lactose in milk between induced lactations and control lactations were not significant (H.s.d..05) in all periods. Means and standard errors of milk yield during periods are in Fig. 8. Average daily milk yields for the first 21 days of lactation for
the control and induced cows were 23.1 kg and 6.8 kg. Regression coefficients and standard errors of milk yield on the different periods of lactation and the entire 21-day period for both control and induced lactations are in Table 2. The overall regressions for induced lactations and control lactations were .28 ± .03 and .50 ± .07. This indicated that slopes of lactation curves were different (P < .05) in the two groups.

Table 1. Estimated differences in fat, protein, and lactose percentages (induced-control) from pooled variances.

<table>
<thead>
<tr>
<th>Days of lactation</th>
<th>Constituent in milk</th>
<th>Difference</th>
<th>SE</th>
<th>H.s.d.</th>
<th>b</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-3</td>
<td>Fat</td>
<td>-.99</td>
<td>.33</td>
<td>1.43</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Protein</td>
<td>-.31</td>
<td>.28</td>
<td>1.22</td>
<td></td>
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<tr>
<td></td>
<td>Lactose</td>
<td>+.67</td>
<td>.15</td>
<td>.64</td>
<td></td>
</tr>
<tr>
<td>4-9</td>
<td>Fat</td>
<td>-.97</td>
<td>.31</td>
<td>1.33</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Protein</td>
<td>+.57</td>
<td>.27</td>
<td>1.15</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lactose</td>
<td>-.01</td>
<td>.15</td>
<td>.63</td>
<td></td>
</tr>
<tr>
<td>10-15</td>
<td>Fat</td>
<td>+.23</td>
<td>.31</td>
<td>1.33</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Protein</td>
<td>+.59</td>
<td>.27</td>
<td>1.15</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lactose</td>
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<td>.15</td>
<td>.63</td>
<td></td>
</tr>
<tr>
<td>16-21</td>
<td>Fat</td>
<td>+.08</td>
<td>.31</td>
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<td>Protein</td>
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<tr>
<td></td>
<td>Lactose</td>
<td>-.08</td>
<td>.15</td>
<td>.63</td>
<td></td>
</tr>
</tbody>
</table>

a Standard error.
b Honestly significant difference, Tukey (23).

discussion

A number of ducts and alveoli with no lumen and consisting of clumped columnar epithelial cells was the most notable observation in the three cows biopsied. These ducts and alveoli were apparently in an immature or developing state and resembled alveoli described in the 12 day pseudopregnant rabbits (7). According to Leeson and Leeson (10) in the first half of pregnancy intralobular ducts undergo proliferation and form buds which enlarge into alveoli. The clumps of cells in the three biopsied cows may thus be the developing ducts and alveoli. The wide variations in lactational response hitherto observed in induced lactations in dairy cows subsequent to 17β-estradiol and progesterone therapy (21)

Table 2. Regression of milk yield on the first 21 days of lactation.

<table>
<thead>
<tr>
<th>Days of lactation</th>
<th>Induced lactation Regression coefficient</th>
<th>SE</th>
<th>Control lactation Regression coefficient</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-3</td>
<td>+.22</td>
<td>.49</td>
<td>+.44</td>
<td>.16</td>
</tr>
<tr>
<td>4-9</td>
<td>+.44</td>
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</tr>
<tr>
<td>1-21</td>
<td>+.28</td>
<td>.50</td>
<td>+.28</td>
<td>.07</td>
</tr>
</tbody>
</table>

a Intercept: 4 kg of milk.
b Standard error.
c Intercept: 19 kg of milk.
HORMONAL INDUCTION OF LACTATION

References


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seem to indicate a link between the degree of response and presence of these immature ducts and alveoli. The short hormone therapy and the rapid lactational response (15, 21) compared to earlier methods of hormonal induction of lactation (13) may be contributory causes. The more time it takes (30 to 50 days) for the induced lactations to reach peak production (21, 22) may be explained also on the basis of the time taken for a majority of such immature alveoli to reach maturity. There seems to be no plausible explanation for the differential response in Cow 137. The fact that the undeveloped quarters came into lactation subsequent to regular milking stimulus seems to confirm findings of Cowie et al. (4, 5).

Differences in milk yield between the induced and control lactation cows may account for some of the differences in fat and protein percentages in milk between the two groups. But when differences between cows were accounted for by the model as random variables, differences in percentage fat, protein, and lactose were nonsignificant. The H.s.d. was an appropriate test in this instance to compare more than one mean.

Subsequent to 17β-estradiol and progesterone therapy there is no gross histological abnormality in mammary structure except for immature or developing ducts and alveoli. Quantitative measurements of the proportion of such ducts and alveoli will help understanding of how their presence influences the success of induced lactations. Our results also show that induced milk composition of fat, protein, and lactose percentages is not significantly different from milk from normal lactations during the first 21 days when differences between cows are accounted for.
