Effects of Temporary Hormonal Suppression of Lactation on Milk Constituents, Clinical Mastitis, Colostrum, and the Estrous Cycle

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Abstract

Estradiol-testosterone injection had similar lactation suppressing effects in sheep, goats and cattle. The depression in milk production was generally in proportion to the dose, although the extent and duration was influenced by individual animal characteristics. Cows and goats responded within three days, whereas, sheep required eight days for maximum depression. The net synthesis of fat, protein and solids-not-fat following injection was reduced. As expected, when milk production decreased, solids-not-fat and generally per cent protein increased, whereas, fat was somewhat more erratic and generally depressed. A stimulatory effect of the hormone on milk synthesis following the initial depression was evident. In subsequent lactations, there was no altered production.

In addition, the milk was colostrum-like following smaller injections and was markedly altered (watery, clotted, etc.) following larger injections. The physical appearance was accompanied by relative increases in all whey proteins derived from blood (two times for albumin, and 20 times for immune and β-globulins). Those synthesized in the mammary gland decreased to one-third for α-lactalbumin and to one-half for β-lactoglobulin.

Possible effects of estrogen on suppressed lactation and altered composition during pregnancy were considered. Visible changes in the appearance of milk suggested that normal variations in endogenous hormone levels, especially during the estrous cycle, may influence milk leucocytes to a great extent.

The hormonal suppression of lactation has its forte in the human field. Estrogen, androgens and combinations of these hormones have been used with varying degrees of success by a number of authors, notably the studies of Kantor et al. (16); Robson (25); Dodek et al. (9); Markin and Wolst (19); and Foley (10). These authors were concerned mainly with suppressing lactation from the onset. Their results indicated that lactation could be suppressed with a minimum of discomfort if the hormone preparations were injected during the second or third stages of labor, which often means less than an hour before parturition. In addition, the solvent used, as well as the chemical derivative of the hormone influenced the degree and duration of action following parenteral administration.

A rather complete discussion and bibliography for the role of hormones in lactation, especially in ruminants, was published by Meites (21). The parenteral or oral administration of estrogens and androgens to lactating ruminants has been reported to increase, to decrease, and to have no effect on milk production. The responses noted, though not always explainable, are qualitatively dependent on dose.

Purposeful suppression of lactation in animals has not been advocated for obvious economic reasons; however, there are instances where it would be economically advantageous. The underlying motivation for our study was to test whether established lactation could be temporarily suppressed during lactation. In such a state of markedly reduced secretory activity, the mammary gland might be successfully treated for chronic mastitis which does not respond particularly well to treatment during lactation. However, our study deals only with suppression of lactation and not treatment. As preliminary trials unfolded, it became evident that milk from hormonally-suppressed animals shared many characteristics in common with milk from animals with so-called clinical mastitis, thus, this aspect was considered more closely.

Materials and Methods

Animals, feeding, and milking. A preliminary trial to indicate the expected response was conducted with two lactating goats and two lactating ewes. A further trial employed three

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lactating Holstein cows. All animals had completed at least one previous lactation so that the characteristics of their lactation curves were known. All animals were fed a standard dairy ration, and milked twice daily either by hand or with a quarter milking machine \(^3\) in the case of cows. Complementary milk was obtained following each milking and added to the normal milk. Both the amount of hay and grain fed and refused were recorded twice daily. Feeding and milk withdrawal were as reported by Caruolo and Mochrie \(^6\).

Chemical analysis. The preparation of whey and the use of gel-electrophoresis for quantification of whey proteins were according to the procedures reported by Caruolo \(^7\). Milk fat \(^27\), protein \(^29\), and solids-not-fat \(^30\) were determined. A strip cup score taken on fore-milk was assigned at each milking. The numerical values assigned to the strip cup ranged from 0 to 3; zero being negative, 1 being few flakes, 2 being flakes or clumps, and 3 being gross clumping and/or milk with milk serum characteristics. In addition, the occurrence of clinical mastitis was noted at each milking.

**Injection preparation and schedule.** The hormone preparation \(^1\) is described and the amounts given are outlined in Table 1. This particular preparation was used for its long duration of activity and because this combination of hormones in humans minimized discomfort when compared to estradiol only. Injections were made deep intramuscularly. The ewes and goats were injected once; the cows were injected up to three times. Where applicable, the second and third injections were given following recovery of milk production from the preceding injection. This timing will be shown in Figure 1. Since the dose and number of injections differed among animals, these values are presented in Table 1.

### Results and Discussion

**Preliminary Trials**

Generally, goats, ewes, and cows responded similarly to hormone injections; and, therefore, the results with ewes and goats will not be dealt with in depth. In general, milk production was severely reduced following hormone injection and then returned to expected levels within varying lengths of time following injection. Associated with this depression in production was a secretion having the appearance and consistency of colostrum. In addition, the relative percentages of whey albumin and globulins following hormone injection markedly increased when production was suppressed and decreased to pre-injection levels as production increased.

Some minor differences in response between sheep and goats were evident. Curiously, the production of both nannies decreased to zero by the third day postinjection, whereas it took eight days for the production to decrease to

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\(^3\) The use of DeLaval quarter milker is gratefully acknowledged.

\(^6\) Lactostat, courtesy of Charles E. Frosst & Co., Montreal, Canada.
zero for both ewes, which would indicate species influence. Moreover, the degree to which production recovered following suppression seemed to depend on the lactational persistency of a particular animal rather than to reflect either the amount of hormone injected or the stage of lactation. Both nannies and both ewes were injected midway through their lactations, and the body weights of all four animals were within five kilograms of one another.

The milk production of Animals 2 and 51 dropped off rather drastically toward the latter third of their previous lactation. On the other hand, the milk production of Animals 6 and 52 decreased slowly over their previous lactation. In the experimental lactation, the preinjection daily milk production was 0.5 kg for Number 2 and 0.6 kg for Number 51. Production of both animals postinjection decreased to zero and only returned to a maximum of 0.2 kg after 4 weeks post-injection. Conversely, the preinjection daily milk production was 0.7 kg for Number 6 and 0.4 kg for Number 52. However, after falling to zero post-injection, their daily production increased to 0.9 kg (at four weeks) and to 1.0 kg (at nine weeks) respectively. So, not only did production return to predictable levels, but there was evidence of a stimulatory effect on milk secretion. This effect could have been due to either the continuous low level of estradiol following injection or to the continuous removal of complementary milk at each milking.

Cow Trials

Milk production. Figure 1 represents the average milk production of each cow, daily for the first four weeks of the trial, and weekly thereafter through 33 weeks post-injection. It should be remembered that the number of injections and amount of hormone per injection varied (Table 1). In each instance, however, the response to injection was decreased milk production. The actual decrease was not strictly proportional to the amount of hormone injected, since Number 2078 was injected with half as much as Number 2115 on the first injection, yet reacted by a precipitous temporary drop in milk production. A comparison of the milk production curves following the first and second injections for Cows 2078 and 2115 indicated that an increase in amount of hormone injected, though not markedly changing the slope of the production curve following injection, did suppress milk production longer. However, it appears that the 15 ml given on the 14th day was an overwhelming amount as indicated by the precipitous reduction in production in all three cows. Cow 2005, though injected only once reacted by a large drop in production, and showed the most complete suppression. The explanation for this does not lie in stage of lactation since Cows 2078, 2005 and 2115 were in Day 222, 173 and 213 of their respective lactations. The degree of reduction in milk production, the immediacy of this response following hormone injection, and the length of time production is suppressed are somewhat characteristic of an animal. Cows 2078 and 2115 had an immediately previous lactation which exhibited extremely good persistency of milk production, whereas Cow 2005 had a somewhat less persistent lactation. It will be remembered that, in general, the responses of the goats and sheep also reflected the persistency characteristic of the respective animals. Meites and Turner (20) reported the lactation-depressing effects of large doses of estrogen were associated with loss of appetite in goats. This was very definitely not the case in our trials since there was no measurable reduction in food intake.

The question of what effect hormonal suppression would have on milk yield of the subsequent lactation was considered. It can be seen from Figure 2, which compares the first eight weeks of the experimental lactation with the first eight weeks of the subsequent lactation, that milk production was not adversely affected. As a matter of speculation, it is entirely possible that the hormone injection both initially suppressed lactation and subsequently stimulated milk secretion as the concentration of exogenous hormones was reduced with time (especially in Sheep 52, Goat 6 and possibly in Cows 2078 and 2115). The persistency of the effect following injection of the benzilic derivatives of hormones (as in the present study) was shown by Gleason and Parker (13) to last up to 20 weeks. They reported that estrus persisted for up to 20 weeks following injection in rats. In the present trial, the injection preparation exerted measurable influence for long periods of time following injection since cows exhibited continued estrus for up to seven weeks post-injection. Turner et al. (28) and Browning et al. (4) reported a beneficial effect of stilbestrol administration on milk production. However, Hutton (15) did not find enhancement of milk production when comparing doses of estradiol monobenzoate varying from 6.25 to 100 mg. Since the hormone preparation used in our study contained both testosterone and estradiol derivatives, it is impossible to compare directly the results noted with those from other trials where only estrogenic compounds
were employed. However, it is evident that the milk production response following hormone administration is not strictly dose dependent, but is to some degree influenced by individual animal characteristics.

Milk fat, protein, solids-not-fat. The effects of hormone injection on fat, protein and SNF for each cow are presented in Figures 3, 4 and 5, respectively. Both per cent and weight are plotted since one might wonder whether an increase in per cent of a constituent reflects a concentration resulting from decreased milk volume or synthesis de novo, whereas the comparison with weight takes cognizance of milk volume as well. It is important to remember that the secretion obtained at each milking more nearly reflects the actual synthesis than have other experiments, since in our trial, oxytocin was injected at the end of each milking and complementary milk was added to normal milk. In general, it can be seen that the weight of fat, protein and SNF decreased rather drastically following injection for a cow. This decrease paralleled the decrease in milk yield. Immediately following injection, per cents fat and SNF increased, and per cent protein either
increased or remained unaltered. The decrease in grams and per cent milk fat means that fat synthesis was affected more adversely than mere volume of milk per se. However, it can be seen (Fig. 3) that roughly four days after the third injection, there occurred a transitory increase in fat per cent while grams of fat remained fairly constant. At this time, milk fat synthesis was essentially unaltered, but total milk volume decreased. The over-all increases seen in per cent protein and SNF and the over-all decreases seen in grams of protein and SNF (Fig. 4 and 5) means that these were partially concentrated, where volume per se was reduced to a greater extent than synthesis of protein and SNF. Obviously, since protein is a portion of the SNF fraction, the changes in protein are to some extent reflected in the SNF fraction. However, when protein per cent took a transient but precipitous drop (No. 2078 and 2115), the SNF percentage increased. At this stage probably two things were occurring: Milk protein synthesis was impaired to a greater extent than milk lactose synthesis, or there was a large influx of inorganic ions and proteins from blood to partially replace the reduced synthesis of milk proteins and lactose to maintain osmotic homeostasis. The subsequent discussion of whey protein results would suggest an increase in permeability to blood proteins.

Folley (11) injected a total of 485 mg of estrone within 2.5 days to lactating cows and reported a net increase in amount of SNF and milk fat. Hutton (15) found that this so-called enrichment was dose-dependent, in that 100 mg of oestradiol caused a reduction, 50 mg and 25 mg an enrichment and 12.5 mg and 6.25 mg no effect.

Whey proteins. The changes in the mean relative percentages of whey proteins following hormone injection are presented in Figure 6. It can be seen that there was a slight response to the first injection, Period 1 vs. Period 2, and a marked and more lasting response to the large hormone dose, Period 4 vs. 5.

The albumin, immunoglobulin, and β-lactoglobulin, all blood fractions, increased in whey following hormone injection. This increase was probably due to a combination of altered capillary permeability and to a concentration effect due to a reduction in volume of milk secreted. Pappas and Blanchette (23) presented electron micrographs which showed that in the endothelial endometrium leakage of tracer particles occurred during prooestrus. Morgan (22) observed a protein increase in the endometrial interstices following estrogenic stimulation. Friederici (12) reported the intravital staining of endometrial connective tissue following injection of a small dose of estradiol. Moreover, the results from the present study agree with those of Zarkower (32) who reported an increase in serum proteins in milk following estradiol treatment of cows.

The β-lactoglobulin and α-lactalbumin fractions decreased. Since these proteins are synthesized by the mammary epithelial cells, this represents a suppression of protein synthesis in the mammary gland. These changes are reminiscent of changes occurring in mastitis as well as those following parturition. Lecce and Legates (18) have reported an increase in albumin and immunoglobulins and a decrease in α-lactalbumin and β-lactoglobulin with mastitis. Moreover, their values returned to normal following treatment of mastitis. Changes similar to those found in the present trial were reported by Bortree et al. (2) following experimentally induced acute mastitis. Shah and Morse (26) reported similar changes following intramammary infusion of live bacteria or sterile water.

In our study, the fraction migrating in the β₂ region appeared following injection of the large dose of hormone and then disappeared by four weeks post-injection. Larson and Ken-
Fig. 6. Mean relative percentages of whey proteins following estrogen-testosterone injection. Periods are averaged over cows and successive milkings, where: Period 1 represents four milkings prior to the first injection; Period 2 represents four milkings following the first injection; Period 3 represents four milkings prior to the second injection; Period 4 represents six milkings following the second injection; Periods 5, 6, 7, and 8 represent the 1st week, 2nd week, 3rd week, and 4th and 5th weeks respectively, following the third injection.
Caruolo and Mochrie (17) stated that β-globulins appeared in the mammary gland secretion simultaneously with their decrease in the blood stream at parturition. Carroll et al. (5) studied experimentally-produced mastitis and reported the appearance of a fraction which they called X. This was probably β since it migrated to the β region.

A different component, called X in our study, was a fraction which migrated between β-lactoglobulin and α-lactalbumin. A fraction with this mobility was noted by Lecce and Legates (18). It may be one of the α-globulins from blood, but at this time its identity is not known.

Generally, the whey components returned to normal values by the fourth week (Period 4) following injection of the small dose of hormone. Moreover, all of the components except the immunoglobulins and X fraction returned to normal by the fourth week (Period 8) following injection of the large dose of hormone. Curiously, Carroll et al. (5) reported that the immunoglobulin fraction remained elevated following experimentally produced mastitis.

In our study, changes in whey proteins are not only similar to the changes seen in infection or during sterile inflammation but also are similar to the changes reported following parturition. Porter and Conrad (24) followed the changes in whey proteins from parturition to three weeks postpartum. The milk content of whey proteins following hormone injection in the present study are quite similar to their values for colostrum. Moreover, the reversion in the present study is similar to the change from colostrum to normal milk. By analogy then, the results of the present study suggest that the hormone estrogen, either directly or indirectly, may play an important role in inhibiting the secretion of milk during pregnancy. They suggest further, that parturition and its effect on estrogen level may remove the block to allow increased synthesis within the mammary gland.

Of interest also, is the similarity between the chemical changes in mastitic milk and those noted in the present study. These chemical changes can include changes in milk enzyme concentration such as xanthine oxidase. The 5.5-fold increase in xanthine oxidase reported by Aurand et al. (1) was attributed to spontaneously oxidized milk; however, the two cows they sampled were those used in the present study after hormone injection. One then wonders what effect the normal estrous cycle has on milk constituents. This question has been partly answered by the report of Wells et al. (31) who stated that lipase activity of blood and milk was high during estrus and diestrus and low before and after estrus.

Clinical Observations

In the preliminary trials with ewes and nannies it was noted that following hormone injection the milk had visual colostral characteristics. This also was noted with cows. Interestingly enough, this observation is corroborated by De Fremery (8), who in 1936 reported the change in secretion from milk to colostrum in goats treated with estradiol. Moreover, chemical analyses further substantiate the colostral-like characteristic. The daily strip-cup analysis of separate quarters showed no marked response to the first two injections but a dramatic response within two milkings following the third and largest injection. For simplicity and ease in presenting the strip-cup observations, the strip-cup scores for a particular time interval were summed over quarters and milkings, and therefore represent the total divided by the maximum possible score for a given period. The minimum possible score for a day would be 0, and the maximum score would be 24 (3 for each of four quarters at both the AM and PM milkings). These results are presented in Table 2.

In comparing the two seven-day post-injection scores with their respective two-day preinjection score, it is evident that there was secretion of abnormal milk but not a dramatic change. However, following the third injection there was a markedly sustained abnormal secretion. Obviously, the largest injected dose gave the most dramatic response.

Bourland et al. (3) have suggested an interaction between estrogen and C. boris in causing mastitis as adjudged by increased California Mastitis Test reaction. However, in the present trial Cow 2005 secreted bloody milk yet repeated sampling and incubation for 72 hr on blood agar plates revealed no microorganisms. That this is not one isolated instance is substantiated

| Table 2. Total strip cup score of milk/total possible score following hormone injection. |
|-----------------------------------------|------------------|------------------|------------------|
| Animal | Post-injection* | 2 Days |  
| no. | preinjection | 0-7 | 8-14 | 15-45 |
| 2115 | 1/48 | 0/168 | 7/168 | 531/720 |
| 2078 | 2/48 | 15/168 | 25/168 | 559/720 |
| 2005 | 2/48 | 128/720 |

*Animals 2115 and 2078 were injected on Days 0, 8 and 15, whereas 2005 was injected only on Day 15.
by the work of Zarkower (32) who reported
that the abnormal secretion following estradiol
injection of one of his cows could not be explained
on the basis of detectable infection.
In addition, on ten occasions, Leece and Legates (18) noted the appearance of increased
amounts of serum albumin independent of their
ability to detect any bacteria. We concluded
from our study that this was a type of physio-
logical mastitis due in this case to estrogen
per se. Moreover, when considering the changes
seen in the strip-cup scores as well as in the
chemical components, and considering the clas-
sical effects of estrogen on leucocytosis of the
endometrium (Hooker (14)), it is feasible that
estrogen variation within the normal estrous
cycle exerts measurable effects on milk con-
stituents including milk leucocyte levels.

The fact that milk production and chemical
composition can be altered dramatically by
hormone injection means that a number of dif-
ferent parameters must be measured to dif-
ferentiate between physiologic and pathologic
alterations of mammary gland secretions.

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