Steroids 78 (2013) 973–981

Contents lists available at SciVerse ScienceDirect

Steroids

journal homepage: www.elsevier.com/locate/steroids

Changes in mammary secretory tissue during lactation in ovariectomized dairy cows

L. Yart [a,b], V. Lollivier [a,b,c], L. Finot [a,b], J. Dupont [d], S. Wiart [a,b], M. Boutinaud [a,b], P.G. Marnet [a,b,c], F. Dessauge [a,b,*]

[a] INRA, UMR1348 Pegase, F-35590 Saint-Gilles, France
[b] Agrocampus Ouest, UMR1348 Pegase, F-35000 Rennes, France
[c] Université européenne de Bretagne, France
[d] INRA, UMR85 Physiologie de la Reproduction et des Comportements, F-37380 Nouzilly, France

ARTICLE INFO

Article history:
Received 10 January 2013
Received in revised form 4 June 2013
Accepted 7 June 2013
Available online 27 June 2013

Keywords:
Mammary gland
Estradiol
Progesterone
Lactation
Involution
Dairy cow

ABSTRACT

In dairy animals, the milk yield (MY) changes during a lactation and is influenced by several physiological, livestock management and environmental factors. The MY produced by a mammary gland depends on synthetic activity of mammary epithelial cells (MECs) as well as MEC number and mammary secretory tissue organization. It has been suggested that ovarian steroids (estradiol and progesterone) have a negative effect on MY in lactating cows. In a previous study, we showed that the suppression of ovarian secretions by an ovariectomy improved lactation persistency in dairy cows. Here we were interested in the effects of ovariectomy on plasma estradiol and progesterone concentrations and on changes that occur in mammary secretory tissue during lactation. We demonstrated that the ovariectomy of lactating cows at the time of the lactation peak induced a rapid and dramatic drop in plasma progesterone and a smaller reduction in plasma estradiol. Interestingly, the study of the changes in mammary secretory tissue over time revealed that the improvement of MY measured in the ovariectomized cows was associated with a limited increase in estradiol receptivity in MECs, a reduced mammary tissue remodeling and reduced blood protein concentration in milk, in late lactation. These results suggest that ovarian secretions, particularly estradiol and progesterone, act to enhance processes for mammary gland involution in late-lactating dairy cows.

1. Introduction

In a lactating cow, the daily milk yield (MY) changes during lactation. After calving, the MY increases rapidly until the peak of lactation, a phase which is then followed by the declining phase of MY. Lactation persistency is characterized by the rate of decline in MY after the peak of lactation [1]. Several environmental and livestock management factors are known to influence MY and lactation persistency [1], such as feeding level [2], health status [3], photoperiod [4] and endocrine status [5]. The variations in MY during lactation result from changes in the mammary secretory tissue that produce the milk [6]. Milk yield notably depends on the number and activity of mammary epithelial cells (MECs) [7] as well as minimal mammary tissue remodeling [8]. In early lactation, the proliferation rate of MECs is higher than the apoptosis rate, thereby increasing the number of MECs. The secretory activity of MECs also increases and the tissue remodeling rate is very low, allowing a rapid increase in MY. During the course of lactation, the apoptosis rate in the mammary gland progressively increases until it becomes higher than the proliferation rate, which initiates the decline in MY. The increasing loss of MECs by apoptosis is followed by an increase in mammary tissue remodeling, which results in alveolar regression during involution [6].

In the involuting mammary gland, the cell-extracellular matrix and cell–cell interactions decrease due to the degradation of extracellular matrix by matrix metalloproteinases [8] and the disruption of epithelial tight junctions [9]. This disruption of the tight junctions in the mammary secretory tissue is responsible for the loss of epithelium integrity, which is characterized by an increase in the concentration of blood components in milk [10] and the passage of milk components into blood [11]. Mammary epithelium integrity is influenced by milking frequency and milk accumulation in the udder [12], but it is also influenced by endocrine factors such as sex steroids. Athie et al. showed that the administration of exogenous estradiol in late-lactating cows induced a rapid drop in MY that was associated with decreases in α-lactalbumin, lactose and potassium concentrations and increases in lactoferrin and

* Corresponding author at: INRA, UMR1348 Pegase, F-35590 Saint-Gilles, France. Tel.: +33 223485097.
E-mail address: Frederic.Dessauge@rennes.inra.fr (F. Dessauge).

0039-128X/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.steroids.2013.06.003

sodium in the milk suggesting an acceleration of normal involution with more leaky tight junctions [13].

Estradiol (E_2) and progesterone (P_4) are mainly produced by the ovaries in non-pregnant females and negatively affect mammary gland secretory activity and MY in mid- or late-lactation cows [13–15]. Delbecchi et al. reported a 15% and 82% decrease in MY at 3 and 11 days, respectively, after the first E_2 administration in lactating cows [14]. In a previous study, we showed that, in non-pregnant lactating cows, suppression of the main source of E_2 and P_4 by ovariectomy improved lactation persistency by limiting the decline in MY after the peak of lactation. The ovariectomy also reduced apoptosis and tissue remodeling in the mammary glands after 14 months of lactation [16]. Ovariectomy has been widely used as an experimental model, especially to investigate the role of ovarian steroids on mammary gland development in young ruminants [17–19]. It is assumed that ovariectomy rapidly reduces plasma E_2 and P_4 and that concentrations remain very low. However, few data about the levels of circulating E_2 and P_4 after ovariectomy are available.

There were two objectives of this study: (1) to follow the progress of the effects of ovariectomy on both E_2 and P_4 circulating levels and lactating mammary secretory tissue and (2) to test the hypothesis that an ovariectomy would delay mammary gland involution in dairy cows.

2. Experimental

All of the animal procedures were discussed and approved by the CNREEA No. 07 (Local Ethics Committee in Animal Experiment of Rennes – File number: R-2012-FDLY-01) in compliance with French regulations (Decree No. 2001-464, May 29, 2001).

2.1. Animals and experimental design

Fourteen Prim'Holstein multiparous cows (lactation rank 2 and 6) that were calving between October 18 and November 15, 2010 were used in this study. The cows were housed at the experimental farm of Méjusseaume INRA-Rennes (France). During the entire experimental period, the cows were collectively housed in barns during the winter and in fields during the summer, individually fed from October 2010 to March 2011 and from October 2011 to slaughter and milked twice daily. The cows were divided into two equivalent groups based on parity, calving date and previous lactation performances (total MY ± S.E.M. on d 280 of lactation). One group was ovariectomized (Ovx, $n = 7$, MY 280 d = 8840 kg ± 399 kg) approximately 60 d post-partum (±13 d), whereas the other group received a sham-operation (Sham, $n = 7$, MY 280 d = 9102 kg ± 542 kg). The Ovx cows were ovariectomized as previously described [16]. In this procedure, ovarian pedicles and blood vessels to the ovaries were bound. Thus, ovaries can no longer function but they remained in the animals. The ovarian pedicles of the Sham cows were not bound, but the presence of the ovaries was verified by an intra-abdominal palpation. All of the cows were maintained in lactation and kept non-pregnant for 52 weeks. The sham-operated cows did not receive any treatment for estrus synchronization and were maintained non-pregnant during the study.

After 52 weeks of lactation, the cows were slaughtered at the experimental slaughterhouse of INRA Tours (UMR 6175, INRA Tours, France). At slaughter, the uterus and udder (including teats, skin and lymph nodes) were removed and weighed before processing.

The first 15 weeks of lactation were used as a reference period for data analysis.

2.2. Milk production recording

The MY was recorded daily for 52 weeks. Milk samples were collected weekly during the study for somatic cell counting and to determine the milk composition (fat, protein and lactose contents). The milk protein, fat and lactose contents were determined by an independent laboratory using an infrared method (Lillab, Chateaugiron, France).

2.3. Blood sampling and hormone assays

Blood samples were obtained from the tail vein one week before surgery, weekly during the 3 weeks after surgery and then every 4 weeks for the rest of the study to determine the plasma E_2 and P_4 concentrations. Sampling was performed using Monovette syringes coated with lithium heparin (Sarstedt, Nümbrecht, Germany). The plasma was immediately separated by centrifugation at 3000*g* for 15 min at 4 °C and was stored at −20 °C until the assays were performed.

Plasma P_4 concentrations were determined with the AIA 1800 robot (Kitvia, Labarthe-Inard, France) using the Kitvia Progesterone assay kit (sensitivity: 0.1 ng/mL, 0025281, Kitvia). The CV between assays was to 5.5% and the CV within assays was to 11.2%. Samples were measured as single samples.

Plasma E_2 concentrations were estimated using the HRP-E2 DIASource immunoassay ELISA kit (E2-EASIA/KAP0621) (DIASource immunoassay SA, Louvain la Neuve, Belgium), with a modification for the analysis of E_2 concentrations in bovine plasma. Three hundred microliter aliquots of plasma samples and references were extracted into glass tubes with 3 mL of ethyl acetate/cyclohexane (V/V) and mixed for 5 min (extraction yield: 80%). After 2 h, the tubes were centrifuged for 15 min and frozen in liquid nitrogen. The solvent layer was decanted, transferred into new glass tubes and evaporated under nitrogen. The samples and references were reconstituted in 160 μL of 0.1 M Tris, 1 mM EDTA, pH 7.4 and 150 μL of the mixture was incubated in an ELISA plate with E_2 antibody diluted at 50% of the concentration recommended by the distributor for 24 h at 22 °C on a horizontal shaker set at 700 rpm. In each plate, 150 μL of standards from 0.78 to 12.5 pg/mL that were prepared in 0,1 M PBS, 150 mM NaCl, 1 g/L gelatin, pH 7.4 was distributed in duplicate. The HRP-E2 (50 μL) was added to each well at the recommended concentration for 1 h at 22 °C on a horizontal shaker set at 700 rpm and then incubated for an additional 23 h at 4 °C without shaking. The plate was washed 3 times with the washing solution and dried on absorbing paper. Next, 200 μL of TMB substrate was distributed into each well of the plate, and the plate was incubated for 30 min at 22 °C on a horizontal shaker set at 700 rpm. Finally, 50 μL of the stop reagent was dispensed into each well. The absorbance of each well was read at 450 nm wavelength (with the reference wavelength at 620 nm) immediately or within 1 h after the addition of the stop reagent. Calculations were performed with the RIA SMART program (Canberra Packard,-Packard Instrument Co, Meriden, Connecticut USA) with a 4PL logistic regression. Cross-reactivities were found to be less than 2% for estrone, estriol, E_2-3-glucuronide and E_2-17-glucuronide and less than 0.1% for E2-17-valerate, cortisol, progesterone, DHEA-sulfate, testosterone, androstenediol, norgestrel, premarin and equilin. The quantification limit was calculated in 0.78 pg/mL for Bo-2SD. The CV between assays was to 7.9% and the CV within assays was to 17.4%. Samples were measured as single samples.

2.4. Mammary tissue and milk sampling

Mammary tissue samples were collected by biopsy at weeks 6, 16 and 34 of lactation (adapted method from [20]) and at slaughter