Role of suckling in regulating cell turnover and onset and maintenance of lactation in individual mammary glands of sows

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ABSTRACT: This study addressed the mechanisms by which suckling regulates cell turnover and onset and maintenance of lactation of individual mammary glands of sows. The effects of no, transient (through 12 to 14 h postpartum), or regular suckling of individual glands during d 0 to 6 of lactation were studied in 5 sows. Nonsuckling was obtained by taping the glands to prevent access to the nipples. Visual scores confirmed that regularly suckled glands maintained lactation, whereas transiently suckled and nonsuckled glands regressed during lactation. Mammary gland biopsies were collected on d − 5, 1, 2, 4, and 6 relative to farrowing in order to evaluate the cell turnover and to quantify the transcription of genes potentially involved in mammary cell turnover and function. The proportion of proliferating cells was greatest prepartum (13.1%). After farrowing, the proportion of proliferating cells declined in all glands, then remained low (5.6%) in nonsuckled glands and increased (P < 0.01) from an average of 7.5% on d 1 to 9.9% on d 6 in regularly suckled and transiently suckled glands. Transcriptional data were analyzed using a gamma-distributed, generalized linear mixed model. Abundance of α-lactalbumin mRNA (P < 0.01) increased in regularly suckled glands within the first day of lactation and remained elevated, whereas the expression in taped glands remained at the prepartum level. Prolactin receptor mRNA abundance decreased (P < 0.001), and IGFBP-5 mRNA abundance increased (P < 0.01) in nonsuckled and transiently suckled glands after parturition compared with regularly suckled glands. Mammary mRNA abundances of IGF-I, IGF-II, type I IGF receptor, and caspase 3 were minimally or inconclusively affected by the suckling regimens. In conclusion, suckling during the first 12 to 14 h postpartum is insufficient to initiate and maintain lactation until 24 to 36 h postpartum but sufficient to induce mammary cell proliferation for at least 6 d postpartum. Furthermore, a high prolactin receptor transcription and a low IGFBP-5 transcription seem important for maintaining a functional mammary gland during lactation.

Key words: cell turnover, gene expression, lactation, mammary gland, pig, prolactin receptor

INTRODUCTION

Suckling by piglets is a prerequisite to induce the second stage of lactogenesis (characterized by copious milk secretion) and to maintain lactation, whereas nonsuckling causes milk stasis (if lactation has been initiated) and gland involution (Kim et al., 2001). In spite of the profound effect of suckling on mammary development and function, the knowledge of these aspects of lactation physiology in pigs is sparse.

Initiation and maintenance of lactation is regulated in a concerted action of systemic and local factors (Hartmann et al., 1997). Prolactin (PRL) plays a key role in mammary growth, and thus mammary cell turnover, in sows during late pregnancy (Farmer et al., 2000). Prolactin is also essential for lactational performance (Farmer et al., 1998), but the circulating concentrations of PRL are not limiting for milk production (Farmer et al., 1999). Prolactin acts by binding to the PRL receptor in the mammary gland.

Mammary cell turnover is important because growth and involution are determined by the rate of cell proliferation and the rate of apoptosis (Capuco et al., 2001). Insulin-like growth factors I and II stimulate mammary

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growth of mice and cows (Sejrsen et al., 1999; Hovey et al., 2003). Several proteins, including IGFBP-5 and caspase 3, are important in the apoptotic process (Thornberry and Lazebnik, 1998; Schneider et al., 2002). Hence, altered mammary expression of apoptotic genes, PRL receptor, or selected members of the IGF system are potentially key factors in the local regulation of individual glands.

The aims of the project were to study the role of suckling (regular or no) in mammary cell turnover and in initiation and maintenance of lactation and to study whether transient suckling (12 to 14 h) is sufficient to initiate lactation. Transcription levels of selected genes were quantified to evaluate their potential roles for cell turnover or milk synthesis.

**MATERIALS AND METHODS**

**Health, Care, and Use of Experimental Animals**

Blood and biopsy sampling, housing, and rearing were in compliance with Danish laws and regulations for the humane care and use of animals in research (The Danish Ministry of Justice, Animal Testing Act (Consolidation Act No. 726 of September 9, 1993, as amended by Act No. 1081 of December 20, 1995)). Furthermore, the Danish Animal Experimentation Inspectorate approved the study protocols and supervised the experiment. The health of the animals was monitored, and no serious illness was observed.

**Animals**

Five multiparous sows (Landrace × Yorkshire) were randomly selected from the herd at the Research Center Foulum. On d 1 of lactation, litter sizes were standardized to 10 piglets. Sows were kept individually in farrowing pens and fed twice daily in accordance with Danish recommendations (Danielsen, 1988). The diet consisted of 40% barley, 32% wheat, and 22% soybean meal, on an as-fed basis, and contained 187 g of CP and 15.25 MJ of ME/kg of DM. The feeding level was gradually increased within the first 10 d of lactation until the daily supply of ME was 86 MJ. Throughout lactation, sows and piglets had free access to water. Piglets were given electrolytes (Dialyte, Wittfoss, Graasten, Denmark) in a bowl from d 6 onward and had free access to a starter diet based consisting of 32% barley, 32% wheat, 14% soybean meal, and 12% fish meal, on an as-fed basis (222 g of CP and 14.81 MJ of ME/kg of DM), from d 14 of lactation. To prevent infections due to biopsy sampling, sows received a prophylactic treatment of 10 mL of Engemycin i.m. (100 mg of Oxytetracyclin/mL, Intervet, Skovlunde, Denmark) on d 1 to 3. Piglets were weaned at 28 d of age.

**Suckling Regimens**

Three of the 5 anterior glands on the left side of the udder of each sow were exposed to 3 different suckling regimens from d 0 to 6 of lactation: 1) regular suckling (the gland was suckled regularly by the piglet throughout lactation); 2) transient suckling (the gland was suckled during colostrum secretion; i.e., until 12 to 14 h after birth of the first piglet, and then taped to prevent further suckling); or 3) nonsuckled (the gland was taped at farrowing so that no suckling could occur throughout lactation). Hence, nonsuckled glands were stimulated only by systemic (lactogenic) signals, whereas transiently suckled glands were stimulated by systemic signals and suckling stimuli only during onset of lactation, and regularly suckled glands were stimulated by systemic signals and suckling stimuli during onset as well as maintenance of lactation. The 2 remaining anterior glands on the left side of the udder were taped on d 1 for either 1 or 3 d to study lactation rescue and subsequent performance, as previously described (Theil et al., 2005). Nonsuckling was achieved by sealing the glands with tape, which was changed every other day. Treatments were randomly allocated to teat position 1 to 5 among the 5 sows. The piglets remained with the sow to fend for themselves during the time that their teats were taped. The sows had 7 or 8 pairs of mammary glands, corresponding to 14 to 16 teats. Hence, at least 10 glands were accessible for suckling at all times.

**Biopsies and Observations**

Blood samples and mammary biopsies were taken while the sows were held by snare restraint. Blood samples were drawn by jugular puncture 5 d before expected farrowing (d −5) and on d 1, 2, 4, and 6 of lactation. The biopsies on d 1 were drawn 24 to 36 h after the first piglet was born. Each sow and her piglets were separated 1 h before blood samples and biopsies were collected. Whole blood was collected in 10-mL Vacutainer tubes coated with sodium heparin (Hettich Instruments, Hvidovre, Denmark) and kept cold; plasma was harvested within 30 min by centrifugation at 2,100 × g for 10 min.

Biopsies were taken from glands selected for the regular-suckling and nonsuckling regimens on d −5 and from all 3 selected glands on d 1, 2, 4, and 6. Biopsies were taken with a Manan ProMag 2.2 biopsy gun (Medical Device Technologies, Gainesville, FL) loaded with a 14-gauge needle. A biopsy consisted of up to 3 shots in the same intrusion site, so that a total of 30 to 60 mg of mammary tissue was collected, as previously described (Theil et al., 2005). Approximately 5 to 10 mg of the first biopsy shot was placed in 4% formaldehyde for histological staining. The remaining tissue was immediately frozen in liquid nitrogen and stored at −80°C for later analysis of mRNA abundance by means of real-time reverse transcription PCR. In addition, on d 1, 2, 4, 6, 13, 20, and 27, glands were visually scored on a scale of 1 (involved gland) to 5 (fully developed gland). For consistency, visual scoring was done by the same person.
Analyses

Hormones. Free IGF-I was analyzed in plasma using a noncompetitive, time-resolved, immunofluorometric assay of the sandwich type, after acid hydrolysis and centrifugation, as described by Frystyk et al. (1994). The assay was previously validated for porcine samples (Carlson et al., 2004). All samples were run in a single assay, and the mean intraassay CV for the low and high standards were 4.9 and 1.4%, respectively. The assay sensitivity averaged 3.0 ng/mL.

Plasma PRL was analyzed using RIA, and the hormone was labeled with I-125. Samples were analyzed in triplicate, and 100 µL of sample was mixed with 100 µL of labeled hormone (10,000 CPM per tube). Plasma was mixed with buffer and incubated for 48 h at room temperature with the primary antibody (rabbit anti-porcine PRL; A. F. Parlow, National Hormone and Peptide Program, UCLA Medical Center, Torrance, CA) at a final dilution of 1:200,000. The secondary antibody (goat anti-rabbit) was added, and the assay was incubated for another 2 h at 4°C. Carrier (normal rabbit serum) was added, and the assay was further incubated for 1 h at 4°C. Test tubes were centrifuged at 1,800 × g for 30 min, the supernatant was discarded, and the radiation was counted for 2 min using a gamma counter.

Porcine serum depressed binding in parallel to the standard curve, as verified at 12.5, 25, 50, 100, and 200 µL of serum. Two assays were employed (low and high), and internal standards with concentrations of 1.1 and 17.5 ng/mL were used. The mean intraassay CV for the 2 assays were 3.3 and 9.6% (low and high), respectively, whereas the interassay CV for the 2 assays were 5.6 and 7.9% (low and high), respectively. Assay sensitivity averaged 0.85 ng/mL, and average recovery of PRL added to plasma pools was 95%. There was no detectable cross-reactivity to GH or IGF-I.

Immunohistochemistry and Morphometry. Tissue was fixed overnight in 4% formaldehyde, dehydrated in a graded series of ethanol concentrations, and embedded in paraffin. Sections (4 µm) were cut on a microtome. Proliferating cells were stained with monoclonal anti-human Ki-67 mouse antibody (clone mib-1) and Dako EnVision+ system (DakoCytomation Norden A/S, Glostrup, Denmark). Proliferating cells express the Ki67 antigen during the G1, S, G2, and M phases but not during the G0 phase (Duchrow et al., 2001). Apoptotic cells were stained using a terminal deoxynucleotidyl transferase, dUTP nick end labeling (TUNEL) assay (ApopTag Plus Peroxidase In Situ Apoptosis Detection Kit, Chemicon International, Temecula, CA). The TUNEL assay specifically labels DNA fragments, which are fundamental features of apoptotic cells (Wyllie et al., 1980). The manufacturer’s recommendations were followed, and a total of 1,500 cells were counted from at least 5 random fields within the same section. No distinction between cell types was done. Cell proliferation and apoptosis were expressed as the percentage of the total cells counted that were stained by the cell proliferation antigen (Ki-67) or with TUNEL assay, respectively.

Real time Reverse Transcription-PCR. Approximately 10 mg of mammary tissue was homogenized in 350 µL of RNeasy lysis buffer and diluted (1:1) with 70% ethanol. The RNA was purified using the RNeasy mini kit (Qiagen, Albertslund, Denmark) and reverse-transcribed with oligo-dT and Superscript II RNAse H reverse transcription kit (Invitrogen, Taastrup, Denmark) according to the manufacturer’s protocol. Reverse-transcribed material (1 µL) was amplified with TaqMan Universal PCR Master Mix (Applied Biosystems, Stockholm, Sweden) using gene-specific probes and primers. Probes were labeled with FAM fluorophore, and the PCR amplification signal was detected using an ABI PRISM 7900 detection system (Applied Biosystems). Data were normalized by the mean transcription level of 2 housekeeping genes (GAPDH and β-actin), as suggested by Vandesompele et al. (2002) and described by Theil et al. (2005). The oligonucleotide sequences for the genes were designed by using the Primer Express software, version 2.0 (Applied Biosystems). The forward primer, probe (Tamra or minor groove binder), and reverse primer sequences, respectively, were as follows:

α-Lactalbumin: 5′-acaatggcagcacagaaatgg, 5′-cttttcagatcaataat, and 5′-tcgtaagttcatcatacagatgtt;
Prolactin receptor: 5′-ggctccgtttgaagaaccaaa, 5′-caaggggccccc, and 5′-gctttctgcttatttgattcgtg;
IGF-I: 5′-gctggtgagcgttctcagt, 5′-cgtgtgaggagcagacag, and 5′-cgctacctggtggtgtgtt;
IGF-II: 5′-cgtgtcgttccggacactat, 5′-ataccccctgggcaagttcttccg, and 5′-cgctgggggaggtctt;
IGF-R: 5′-cagaagggacagtagcacttct, 5′-acctggggacagcgtcca, and 5′-tcgcctgtgctgactct;
IGFBP-5: 5′-gcgcaagggatcttacaagaga, 5′-aaaagctgtggaacggagctg, and 5′-tcgacgacagcagagat;
Caspase 3: 5′-gcattttcatcagcactggtcttatttct, 5′-ctggcaggaattcaaaaggaagctattg, and 5′-tcgacgacagcagagat;
β-actin: 5′-ccccatggctggctcctcctat, 5′-ctcgtggcagatgt, and 5′-cgaacctcatgcaggtgtgagaa;
GAPDH: 5′-gctggtgagcgttctcagt, 5′-cgtgtgaggagcagacag, and 5′-cgaacctcatgcaggtgtgagaa.

Statistics

Plasma concentrations of PRL and free IGF-I were analyzed by a mixed model using the MIXED procedure of SAS (Littell et al., 1996), where day of lactation was a fixed effect and repeated measurements within sow were taken into account by a random component. Standard contrasts, suitably defined for mixed models, were used for comparing stage differences. Cell proliferation was analyzed by a binomial mixed model (SAS macro...
GLIMMIX), and the repeated structure of measurements within sow and within gland × sow was accounted for by introducing 2 random components. A Friedman test (Siegel and Castellan, 1988) was used for comparisons of gland scoring between the different treatments (Figure 1C). The mRNA abundance was analyzed by fitting a suitable gamma mixed model (Littell et al., 1996), which took into account the kinetics involved in reverse transcription-PCR amplifications and accounted for repeated measurements within sow and within sow × gland. For a detailed description of the statistical procedures, see Theil et al. (2005). Statistical significance was set at $P < 0.05$.

**RESULTS**

**Gland Development**

All regularly suckled glands continued to be suckled normally until piglets were weaned 28 d after farrowing, whereas taped glands (Figure 1A) were of no interest for the piglets. When tape was removed from nonsuckled and transiently suckled glands on d 6 of lactation, the glands had become dry and partly involuted, and the involution continued with the progression of lactation (Figure 1B, 1C). Based on scoring of mammary glands, the involution was visible by d 4 or 6. Initially, the involution process occurred faster ($P < 0.05$) in transiently suckled glands than in nonsuckled glands (Figure 1C) as evaluated by visual scoring.

**Hormones**

Plasma concentrations of free IGF-I increased during the peripartum period, from 42 ng/mL 5 d before expected farrowing to 76 ng/mL by d 2; concentrations then remained stable until d 6 of lactation (Table 1). Prolactin plasma concentration was greater ($P = 0.02$) during the postpartum period (31 ng/mL) than in the prepartum period (12 ng/mL), and PRL concentrations tended to be greater ($P = 0.07$) on d 1 compared with d 2 to 6.

**Cell Turnover**

The proportion of proliferating cells was high before expected farrowing (13.1%) compared with early lactation, irrespective of the suckling regimen and day of lactation (range 5.0 to 10.4%, Figure 2A). During early lactation, the proportion of proliferating cells increased ($P < 0.001$) from 7.5% on d 1 to 9.9% on d 6 of lactation (mean of both glands) in regularly suckled and transiently suckled glands, although cell proliferation did not increase ($P = 0.51$) from d 4 to 6 in transiently suckled glands. Compared with regularly suckled and transiently suckled glands, the proportion of proliferating cells was less ($P < 0.001$) in nonsuckled glands throughout the first 6 d of lactation and remained at an average level of 5.6%. The statistical analysis of

![Figure 1](image-url)
Table 1. Plasma concentrations of free IGF-I and prolactin (PRL) in sows during the peripartum period

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Days relative to farrowing</th>
<th>SEM</th>
<th>P value</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>−5</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>IGF-I, ng/mL</td>
<td>42&lt;sup&gt;a&lt;/sup&gt;</td>
<td>68&lt;sup&gt;b&lt;/sup&gt;</td>
<td>76&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>PRL, ng/mL</td>
<td>12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>38&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27&lt;sup&gt;†&lt;/sup&gt;</td>
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<sup>a–c</sup>Means within rows with different superscripts differ (<i>P</i> < 0.05).

Apoptosis revealed a great overdispersion of this measure (<i>P</i> < 0.001 for testing equality of the overdispersion parameter to 1). The CV was in the range of 88 to 224%, confirming that the overdispersion was not only statistically significant but also of considerable magnitude. Consequently, no significant differences were found, and the proportion of apoptosis is given as median values (Figure 2B).

**mRNA Abundance**

The treatment × day interaction was significant for mRNA abundance of all genes studied (<i>P</i> < 0.001). The abundance of α-lactalbumin mRNA remained high throughout the study in regularly suckled glands, whereas it was reduced to prepartum levels on d 4 in transiently suckled (<i>P</i> < 0.001) and d 2 in nonsuckled (<i>P</i> < 0.001) glands (Figure 3A). On d 1, the abundance of α-lactalbumin was increased (<i>P</i> < 0.001) in all glands compared with the prepartum level, and the mRNA abundance of α-lactalbumin was greatest for regularly suckled glands, medium for transiently suckled glands (<i>P</i> = 0.08), and least for nonsuckled glands, although the transcription did not differ between transiently suckled and nonsuckled glands. Abundance of PRL receptor mRNA remained at prepartum levels during the first 6 d of lactation in regularly suckled glands, whereas it decreased (<i>P</i> < 0.001) in transiently suckled and nonsuckled glands (Figure 3B) during the treatment period. The abundance of PRL receptor mRNA decreased more rapidly (<i>P</i> < 0.05) in nonsuckled than in transiently suckled glands (<i>P</i> < 0.05) as evidenced by the difference on d 2, but PRL receptor mRNA abundance was least in transiently suckled glands on d 4. Mammary abundance of IGF-I mRNA remained at the level observed prepartum in regularly suckled glands on d 1 through 6 (Figure 3C). During the study period, transcription of IGF-I in transiently suckled and nonsuckled glands differed by less than 30% from that of regularly suckled glands. Mammary abundance of IGF-II mRNA remained at the level observed prepartum in regularly suckled glands on d 1 through 6 (Figure 3D). In contrast, IGF-II mRNA abundance transiently decreased (<i>P</i> < 0.001) in nonsuckled glands on d 4 in transiently suckled glands (<i>P</i> < 0.01). Mammary abundance of type I IGF receptor decreased transiently at d 2 in nonsuckled glands and at d 4 in transiently suckled glands (Figure 3E). The IGFBP-5 mRNA abundance increased slightly (<i>P</i> < 0.05) in regularly suckled glands during the treatment period but remained close to the prepartum level. In contrast, IGFBP-5 mRNA abundance increased considerably (<i>P</i> < 0.01) on d 1 through 6 in transiently suckled glands and increased (<i>P</i> < 0.001) on d 4 and 6 in nonsuckled glands (Figure 3F).

**Figure 2.** Cell proliferation and apoptosis in mammary glands in response to suckling regimen during the peripartum period (d −5, 1, 2, 4, and 6 relative to farrowing) in 5 sows. Mean proportion of proliferating cells stained by Ki-67 antibody (Panel A) and median proportion of apoptotic cells stained by TUNEL assay (Panel B) in glands exposed to regular suckling, transient suckling, or nonsuckling (No). Different letters indicate a significant effect of the suckling treatment within a day (<i>P</i> < 0.05).
Figure 3. Peripartum mRNA abundance in individual mammary glands in response to suckling regimen [regular, transient, or nonsuckled (No)] in 5 sows. Abundance of mRNA for α-lactalbumin (Panel A), prolactin (PRL) receptor (Panel B), IGF-I (Panel C), IGF-II (Panel D), type I IGF receptor (IGF-IR, Panel E), IGFBP-5 (Panel F), and caspase 3 (Panel G) was normalized to the mean abundance of GAPDH and β-actin and is expressed relative to prepartum levels [i.e., arbitrary (arb.) units]. Different letters indicate significant differences between suckling regimens within a day ($P < 0.05$). Note that the scales on the vertical axes are logarithmic and differ between genes.
Onset and Maintenance of Lactation

The abundance of PRL receptor mRNA was negatively correlated with that of IGFBP-5 (Pearson correlation coefficient was \(-0.27; P < 0.001\)). Compared with the prepartum level, caspase 3 mRNA abundance was increased in all glands in early lactation (Figure 3G). During the study period, transcription of caspase 3 in transiently suckled and nonsuckled glands differed by less than 22% from that of regularly suckled glands.

**DISCUSSION**

**Onset and Maintenance of Lactation**

Lactogenesis stage II, the onset of copious milk secretion, is regulated differently among species. In the pig, suckling, which includes both the suckling per se and the event of removal of colostrum/milk, is required to initiate lactation (Hartmann et al., 1997), whereas suckling is not required for inducing onset of lactation in many other species including humans (Kulski et al., 1978). In the current study, suckling for 12 to 14 h postpartum was insufficient to initiate and maintain lactation until 24 to 36 h postpartum, as indicated by the low expression of the \(\alpha\)-lactalbumin gene in transiently suckled glands compared with regularly suckled glands. It cannot be excluded, however, that lactogenesis stage II was induced to some extent in transiently suckled glands because \(\alpha\)-lactalbumin mRNA was not measured until 10 to 24 h after these glands were taped. Overall, regular suckling is required to maintain lactation, but the duration of suckling necessary to initiate lactation during the first 36 h remains to be determined.

It was shown that circulating PRL is essential for maintenance of lactation in the sow (Farmer et al., 1998). On the other hand, administration of additional PRL does not enhance milk production most likely because endogenous circulating PRL is not limiting the milk synthesis in this species (Farmer et al., 1999). Our finding that the abundance of PRL receptor mRNA is greater in suckled than in nonsuckled glands, and generally parallels that of \(\alpha\)-lactalbumin mRNA, lends credibility to the hypothesis that the impact of PRL on milk synthesis is to a great extent regulated at the PRL receptor level. In agreement with this, removal of milk has been reported to stimulate expression of the long form of PRL receptor in mice (Kim et al., 1997). Different species express one or more forms of the PRL receptor, but pigs are known to express only one form (Sakai et al., 1984; Bole-Feytsot et al., 1998).

**Mammary Cell Turnover**

Whereas the data clearly confirm that regular suckling is essential for the maintenance of lactation, transient suckling is sufficient to maintain a mammary cell proliferation rate similar to that of regularly suckled glands for at least 6 d postpartum. This indicates that suckling during the first few hours postpartum initiates some long term (at least lasting for 6 d) effect on mammary cell turnover. The nature of these effects, however, is not known because none of the genes for which mRNA abundance was measured show clear differences between nonsuckled and transiently suckled glands.

The absence of suckling clearly causes involution of individual mammary glands, whereas suckled glands remain fully developed. Thus, local factors are responsible for the different developmental patterns of individual glands, and systemic factors in mammary cell turnover such as circulating concentrations of PRL (Farmer et al., 2000) and IGF-I (Kleinberg et al., 2000) have to be modulated at the level of each individual gland. Analysis of selected members of the IGF system (abundance of mRNA for IGF-I, IGF-II, type 1 IGF receptor, and IGFBP-5) revealed that regular suckling of a gland maintains a low expression of IGFBP-5 compared with transient suckling and nonsuckling. This is in agreement with the finding that an increase in IGFBP-5 expression is an early event in the process of mammary involution (Tonner et al., 2000). Expression of IGFBP-5 in transiently suckled glands was greater than in nonsuckled glands on d 1 and 2, maybe indicating that apoptosis was stimulated more in glands with milk status than in glands in which lactation was not initiated. Evaluation of apoptosis by the TUNEL assay did not show significant differences between suckling regimens because of a high overdispersion (the CV ranged between 88 and 224%). An explanation for this might be that apoptosis is not evenly distributed among alveoli but may be prevalent in certain areas of involuting glands. In line with this hypothesis, Molenaar et al. (1992) demonstrated a great diversity in milk gene expression between different alveoli in pregnant, lactating, and involuting mammary glands of sheep and cows.

Prolactin seems to be involved in the regulation of mammary cell turnover by inhibiting IGFBP-5 expression (Tonner et al., 1997). The inhibition of gene expression for IGFBP-5 was previously shown to be important for the antiapoptotic effect of PRL in rodents (Flint et al., 2001, 2005), and this may explain the role of PRL in regulating mammary cell turnover. In support of this, the expression of IGFBP-5 was negatively correlated with that of the PRL receptor gene in the current study. Furthermore, the down-regulated expression of the PRL receptor gene and the up-regulated expression of the IGFBP-5 gene in response to milk stasis were recently demonstrated to reverse in glands where suckling was reinitiated and lactation of individual glands was rescued (Theil et al., 2005). The other measured factors in the IGF system, as well as caspase 3, did not seem to be strongly related to suckling-dependent changes in mammary cell turnover.

In conclusion, suckling during the first 12 to 14 h postpartum is insufficient to initiate and maintain lactation until 24 h postpartum and the extent of suckling necessary to initiate lactation remains to be determined. It therefore appears that regular suckling is necessary to maintain lactation. Furthermore, a high PRL receptor transcription and a low IGFBP-5 tran-
scription seem to be critical for a mammary gland to remain functional during lactation.

LITERATURE CITED


