Hormone release and behavior during suckling and milking in Gir, Gir × Holstein, and Holstein cows¹,²

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ABSTRACT: There are several different milking management systems in Latin America, because Gir cattle are reputed to be easily stressed and not well adapted to machine-milking. This paper, therefore, provides an overview of hormone release and behavior during suckling and milking in Gir cows and their crossbred offspring. Several experiments were performed to study oxytocin release during exclusive suckling or exclusive hand- and machine-milking, oxytocin, and prolactin release during a mixed suckling-milking system and oxytocin release after weaning. Cortisol concentrations and behavior were also examined. Concentration of oxytocin, released during suckling, and both types of milking were high, but the maximum concentration measured during suckling was significantly greater than that observed during exclusive milking. In the mixed suckling-milking system, the greatest oxytocin and prolactin releases were measured during suckling. Cortisol concentrations measured before, during, and after milking demonstrated that Gir × Holstein and Holstein cows were not stressed. On the other hand, although Gir had greater concentrations of cortisol, the percentage of residual milk for Gir cows was less than for dairy cows exposed to different stressful situations. In general, Gir cows and their crossbred offspring adapted to machine-milking, although these breeds can react negatively to milkers. Gir, Gir × Holstein, and Holstein cows all had similar cortisol levels during and after milking.

Key words: cortisol, Gir, milk ejection, milking, oxytocin, prolactin

INTRODUCTION

In Latin America, it is possible to classify dairy farmers into 2 distinct systems: intensive and extensive (Madalena et al., 1990; Magana-Sevilla and Sandoval-Castro, 2006). In the intensive system, the principal activity is milk production, and farmers maintain Holstein or Jersey cows in barns. In general, these specialized animals are fed in stalls with high-concentrate diets and are submitted to exclusive machine-milking (Madalena et al., 1990; Souza et al., 1996). In contrast, extensive farmers keep both Gir cows and Gir crossbred offspring with their calves in pastures, because these animals are very well adapted to tropical conditions (Madalena, 1995; Souza et al., 1996; Sandoval-Castro et al., 1999). Although this extensive system primarily produces calves and heifers, milk is also sold when it fetches a good price. As a consequence, Gir cows and their crossbred offspring are usually suckled by calves and only occasionally milked (Alvarez et al., 1980; Sandoval-Castro et al., 2000; Junqueira et al., 2005). In similar tropical conditions, Gir cows produce more milk on pasture than Holstein cows, and this is true of 70% of all Brazilian lands. However, when maintained in a climate-controlled barn and fed with a high-concentrate diet, Holstein cows produce more milk than Gir cows. The challenge of intensive dairy farmers is to select Holstein cows that are better adapted to the tropical environment so that they can be maintained on tropical grassland pasture throughout the entire year (Madalena, 1995; Souza et al., 1996; Negrão and Marnet, 2002). In contrast, many farmers that are now attempting to specialize in milk production with large herds of Gir × Holstein cows need to improve milk yield and adaptation to machine-milking (Souza et al., 1996). In the extensive system, thus, it is necessary to standardize suckling and milking management of Gir cows to, first, increase milk

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production when cows are maintained on tropical grassland pasture during spring and summer and, second, improve quality of the milk produced (Madalena et al., 1990; Junqueira et al., 2005).

Crossbred cattle are very important to the dairy industry of Latin America. However, Gir cows and their crossbred offspring are reputed to not adapt well to machine-milking, and so these cows are suckled by their calves before machine-milking (Sandoval-Castro et al., 1999, 2000; Negrão and Marnet, 2006). The purpose of this review is to examine hormone release caused by suckling and different milking management systems to examine whether Gir cows and their offspring are well adapted to machine-milking and to understand whether Gir cows differ from Gir × Holstein and Holstein cows in response to different milking systems, especially from a stimulatory and an endocrine point of view.

**OXYTOCIN RELEASE DURING EXCLUSIVE SUCKLING OR HAND- AND MACHINE-MILKING**

Normally, teat stimulation causes oxytocin release via a neuroendocrine reflex (Schams et al., 1984; Gorewit et al., 1992). It is well established that oxytocin release during udder stimulation is necessary for milk ejection from mammary alveoli and is essential for maintenance of lactation (Tancin et al., 1995; Bruckmaier and Blum, 1996; Marnet and Negrão, 2000). Milking and suckling also induce the release of several other hormones and growth factors (e.g., prolactin, insulin, GH, IGF-I, ACTH, and cortisol), and increases in these caused by milking and suckling are fundamental to continuous milk synthesis (Bar-Peled et al., 1995; Knight, 2001; Lupoli et al., 2001). Consequently, it is important to understand the relationship among these hormones and growth factors during suckling or hand-milking and machine-milking, because different modes of milk removal cause different levels of stimulation that could result in different endocrine profiles.

Initially, several experiments were performed to verify oxytocin release during exclusive suckling, hand-milking, or machine-milking of Gir cows (Negrão and Marnet, 2002). Oxytocin was released during both suckling and milking when compared with concentrations measured before and after udder stimulation (Figure 1). Maximum oxytocin concentrations during exclusive suckling were significantly greater than those observed during exclusive hand- or machine-milking. Although the durations of hand- and machine-milking were shorter when compared with suckling, hand- and machine-milking caused a significant increase in oxytocin concentrations. Oxytocin also remained high throughout both types of milking, which is necessary for continuous milk ejection (Bruckmaier and Blum, 1996). However, a previous study demonstrated that small increases in oxytocin concentrations, reaching a threshold level, are effective in inducing milk ejection in cows (Schams et al., 1984). Those results indicated that machine-milking induces oxytocin release in Gir cows, as previously described in Holstein (Gorewit et al., 1992; Bar-Peled et al., 1995), Brown Swiss (Tancin et al., 1995), and Brown Swiss × German Braunvieh cows (Weiss et al., 2003), all of which are considered to be well adapted to milking when compared with Gir cows. Other investigators have also demonstrated that udder stimulation induces oxytocin release in Shami cows (Kaskous et al., 2006) and Murrah buffaloes (Thomas et al., 2005). In general, our results support the hypothesis that oxytocin release was not a restrictive factor in submitting these farm animals to machine-milking. These results are in contrast with other studies on milk yield and weight gain of calves (Sandoval-Castro et al., 1999, 2000; Junqueira et al., 2005). In general, Gir cows show reduced milk yield during milking when compared with suckling (Negrão and Marnet, 2006), and, thus, Gir cows may be considered poorly adapted to exclusive milking. However, Holstein cows, which are considered to be well adapted to exclusive machine-milking, also demonstrate increased oxytocin and prolactin release and produced more milk when suckled (Bar-Peled et al., 1995; Negrão and Marnet, 2006) or when milked in the presence of a calf (Akers and Lefcourt, 1984; Tancin et al., 2001).

**OXYTOCIN AND PROLACTIN RELEASE DURING A MIXED SUCKLING AND MILKING SYSTEM**

The degree of adaptation to milking by Gir cows was not studied until recently, and so oxytocin and prolactin release by Gir × Holstein cows during a mixed suckling and milking system was examined by Negrão and Marnet (2002). Both suckling and machine-milking induced a significant increase in oxytocin (Figure 2, panel A) and prolactin (Figure 2, panel B) concentrations compared with basal levels. Other experiments have reported that the greatest oxytocin concentrations are ob-
It can be observed in Figure 2, panels A and B, that suckling caused greater peaks in oxytocin and prolactin levels than those observed during milking. However, oxytocin and prolactin were released during both suckling and milking when comparing concentrations measured before udder stimulation. Consequently, during a mixed suckling and milking system, the total amount of oxytocin and prolactin released was greater than during exclusive milking, because suckling and the cow-calf bond apparently influenced release of both hormones. Although these results agree with those of others (Bar-Peled et al., 1995; Marnet and Negrao, 2000), the effect of the cow-calf bond on oxytocin and prolactin release is controversial, because both hormones and milk yield were influenced by calf presence alone (Akers and Lefcourt, 1984) and by suckling by adopted calves (Bar-Peled et al., 1995). In many cases in the literature, the milking management system was altered for experimental reasons, and, consequently, the hormones and behavior measured could be influenced by differences in experimental procedures.

Several studies demonstrated that cows submitted to a combined milking and suckling system produced more milk than during exclusive milking (Alvarez et al., 1980; Bar-Peled et al., 1995; Sandoval-Castro et al., 2000). The same relationship was observed in our studies (Marnet and Negrao, 2000, 2002), because calf stimulation followed by milking induced complete milk ejection and a significant weight gain in calves. Although this kind of management can be used in many situations, this approach would increase the labor of milkers and is not recommended for specialized farmers, particularly those with large herds.

One should note that our experiments (Negrao and Marnet, 2000, 2002) were carried out to study the effect of the combined suckling and milking management system on endocrine responses. The importance of prestimulation and feeding in the milking parlor, in terms of oxytocin release, has been demonstrated in several situations for specialized breeds of cows (Weiss et al., 2003; Weiss and Bruckmaier, 2005) and Murrah buffaloes (Thomas et al., 2005). Consequently, it is possible that Gir cows submitted to prestimulation and feeding would show improved milk ejection.

**OXYTOCIN RELEASE AFTER WEANING**

In practice, weaning of Gir cows and their crossbred offspring by farmers is related to loss in milk production. In Figure 3, the effect of the transition from suckling to milking on oxytocin release is shown. During the first 5 d after the transition, none of the Gir × Holstein cows had detectable increases in oxytocin release, but after this period, oxytocin concentrations increased slowly during milkings. It was only after 15 d that several experimental cows showed a normal oxytocin profile during milking. In general, it was believed that transition to exclusive machine-milking is associated with inhibi-
tion of oxytocin release and decreased milk production (Tancin et al., 1995, 2001; Marnet and Negrão, 2000).

Some studies demonstrated that Gir and Gir × Holstein cows can adapt to machine-milking if they are exposed to exclusive milking from the beginning of lactation (Negrão and Marnet, 2002, 2006). Furthermore, many specialized dairy farmers that have dairy cows with varying percentages of Gir genetics wean their calves after birth and submit Gir and Gir × Holstein cows to exclusive machine-milking. However, 40% of Gir × Holstein cows studied did not have oxytocin release, milk yield was restored to previous levels when machine-milking replaced suckling, and 15% of experimental animals occasionally did not show effective oxytocin release and milk ejection (Negrão and Marnet, 1998, 2000). For these latter animals, suckling restored milk yield (J. Negrão, unpublished results), and similar results were reported previously for beef cows of Bos taurus breeding (Lamb et al., 1999). Consequently, suckling would be an economic alternative for Gir cows to improve milk for farmers that produce calves and heifers (Madalena, 1995; Souza et al., 1996; Junqueira et al., 2005).

CORTISOL RELEASE AND BEHAVIOR

Milking management may be stressful for cows, and some behaviors are considered indicators of stress (Grandin, 1993; Rushen et al., 2001). Consequently, it is important to understand the relationships among behavior, oxytocin (Figure 4, panel A), cortisol (Figure 4, panel B), milk yield (Figure 4, panel C), and residual milk (Figure 4, panel D). During our study, cow behavior was monitored in the milking parlor using direct observation and subsequent analysis of video recordings. Simultaneously, the opinions of 3 different milkers were obtained regarding cow behavior in the milking parlor. The interviews were performed using scientific methodology to determine the opinion of the milkers concerning adaptation to milking and behavior of Gir, Gir × Holstein, and Holstein cows. Milkers were instructed and trained to have actions and behavior as consistent as possible during experimental milkings.

In the opinion of the milkers, the Gir group contained a high percentage (60%) of agitated and very agitated cows when compared with other groups (26%). Video recordings generally showed that Gir cows and their crossbred offspring had a more active reaction, kicked more frequently, moved their head rapidly, and made small jumps in response to human contact when compared with Holstein cows. Usually the incidence of movement is a sign of agitation and the basis of temperament tests (Grandin, 1993; Rushen et al., 2001). Thus, there was a close relationship between the observations of the milkers and behavioral measurements, except for frequencies of urination and defecation. Previously, these 2 behaviors were associated with stress in the milking parlor (Rushen et al., 2001). However, further studies are required concerning behavior of Gir cows and their crossbred offspring. Our results show that it is necessary to distinguish different types of movement and measure their combination in different breeds, because Gir cows and their offspring could be more agitated and reactive to human contact but present a normal behavior profile and milk yield (Figure 4, panel C).

During experimental interviews, milkers recognized that Gir cows and their offspring were more difficult to milk and spent more time in the milking parlor. In fact, hand-stripping is a common practice at the end of conventional machine-milking of Gir cows and their offspring. Although Gir and Gir × Holstein cows are reputed to be not well adapted to machine-milking, Gir cows and their offspring had similar levels of oxytocin release (Figure 4, panel A) and percentages of residual milk (Figure 4, panel D) when compared with Holstein cows or with specialized breeds of cows (Bruckmaier et al., 1993; Tancin et al., 2001). Also, Gir and Gir × Holstein cows showed lower cortisol release at milking (Figure 4, panel B) and percentage of residual milk (Figure 4, panel D) than those described for dairy ewes (Negrão et al., 2003) and Holstein cows submitted to different stressful situations (Bruckmaier et al., 1992, 1993; Rushen et al., 2001).

Cortisol concentrations measured before, during, and after milking demonstrated that Gir × Holstein and Holstein cows were not stressed (Negrão and Marnet, 2006). On the other hand, although Gir had greater levels of cortisol, the percentages of residual milk for Gir cows were lower than dairy cows exposed to different stressful situations (Bruckmaier et al., 1993; Rushen et al., 2001). This confirms that cortisol release alone is often a poor predictor of stress during milking.

It is important to note that the majority of the published studies concerning hormone release and milk ejection discussed previously were conducted with animals that were exposed to changes in their typical manage-
Suckling and milking management

Figure 4. Total oxytocin (panel A) and cortisol (panel B) released, milk yield (panel C), and percentage of residual milk (panel D; means ± SEM) measured during machine-milking of Gir, Gir × Hostein, and Holstein cows. Total amount of oxytocin and cortisol released was calculated as the area under the curve (measured during machine-milking and corrected for basal levels of each hormone). a–c Means bearing different letters differ \( P < 0.05 \). Data are from Negrão and Marnet (2006).

The management practices for experimental reasons. Thus, in many cases it is not possible to directly compare those results to our study. For example, our experimental suckling group was exposed to exclusive suckling during their entire productive life (including our study) and never were exposed to hand- or machine-milking. Similarly, the exclusive milking group was never exposed to suckling or a mixed suckling and milking system. Furthermore, our discussion concerning the effects of weaning and the transition from suckling to milking demonstrated that these changes in typical management were associated with inhibition of oxytocin release and decreased milk production, as previously demonstrated in Holstein cows exposed to stress (Bruckmaier et al., 1992; Tancin et al., 1995).

Under the conditions we used, Gir cows and their offspring released oxytocin, exhibited effective milk ejection, and showed a lower percentage of residual milk than other stressed dairy cows (Tancin et al., 1995; Ruschen et al., 2001). Thus, despite the small number of animals studied, it appears that the Gir breed is not poorly adapted to machine-milking, especially from the standpoint of endocrine responses to a milking stimulus. Furthermore, within each of the Gir, Gir × Holstein, and Holstein groups studied, in all experiments there were individual cows characterized by better milk production,
more complete and rapid milk ejection, lower residual milk, or greater milk flow rate than the other cows within the same group. This variability makes it possible to identify and select Gir cows or to improve adaptation of their crossbred offspring to machine-milking.

CONCLUSIONS

In general, our results indicated that Gir and Gir × Holstein cows could adapt to exclusive machine-milking. In a combined suckling and milking system, however, these cows produced more milk than in other systems. It was also possible to identify the types of animals that were best adapted to milking. This indicates that it is possible to increase their dairy performance by selecting for adaptation to machine-milking.

LITERATURE CITED


