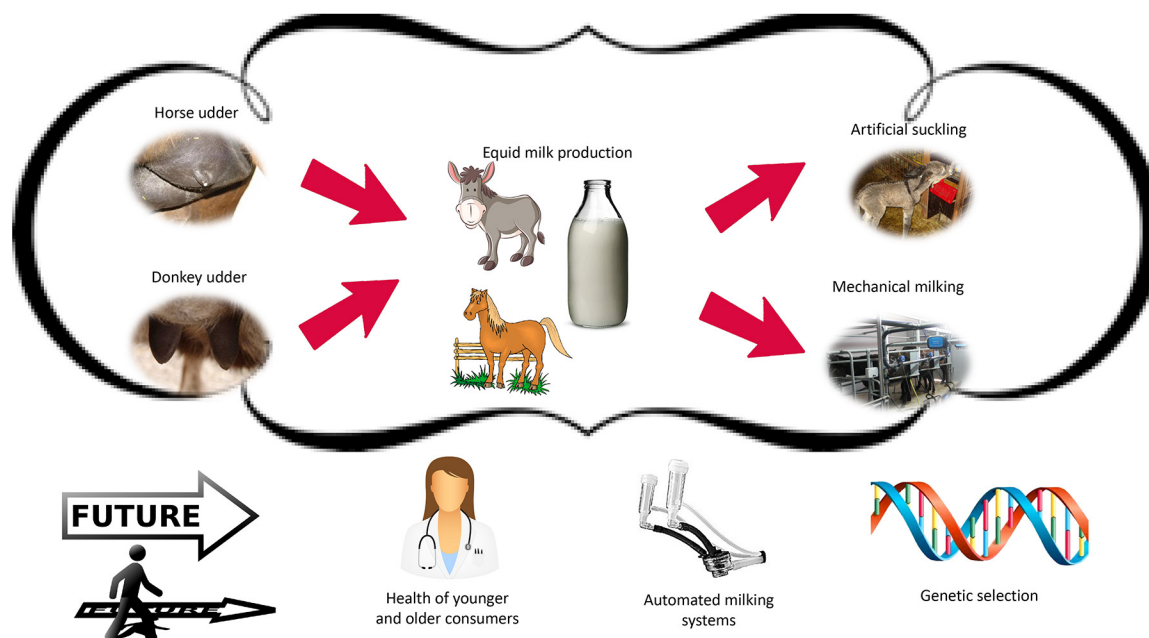


Mammary gland physiology and farm management of dairy mares and jennies*

Pasquale De Palo,^{1†} Juliette Auclair-Ronzaud,² and Aristide Maggiolino¹

Graphical Abstract



Summary

Both donkey and horse milk are arousing interest in consumers and researchers because of their similarity in composition to human milk; both milks are suitable for children with cow milk protein allergy. Despite this growing interest, the literature lacks good information on animal feeding and effects on milk production (both quality and quantity), as well as on the technologies that can be applied to equid milk production. It is important to study mammary physiology and anatomy to determine the correct approach to machine milking for these species, to maximize production, and to optimize mammary gland health and animal welfare.

Highlights

- The equid udder has a lower storage capacity than the ruminant udder.
- Milking frequency is one of the most important factors affecting milk yield.
- Foals can be partly artificially suckled during lactation.
- Mechanical milking has been used in equids but our understanding of machine milking remains poor.
- Automated milking systems for equids represent a great challenge for the future.



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Mammary gland physiology and farm management of dairy mares and jennies*

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Abstract: Equid milk is arousing increasing interest in consumers and researchers because of its similarity in composition to human milk. The low and different protein content makes equid milk it suitable for children with cow milk protein allergy. Both horse and milk production, in many farms, still follow a characteristic and traditional method of separating the foal from the mother to allow milking procedures. This separation lasts at least 2 to 3 h before milking, a time in which the foal remains fasting. This operation is repeated several times a day, as the equid udder has little collection capacity, and milking frequency is one of the most important parameters to increase milk production; it must be emptied often. New partial artificially suckling techniques have been developed that allow the foal to be separated from its mother for many hours without starving. Furthermore, mechanical milking has been introduced in equid milk production, although in-depth knowledge is lacking on milking parameters and how these aspects affect milk production and udder health. Moreover, in some farms, new milking parlors for Equidae have been developed, ensuring that stress is minimized and production and animal welfare are ensured. It is important to develop and apply technologies for equid milk production, evaluating potential effects on welfare, health, and milk production. This represents the broadest perspective and the greatest challenge because of the need to understand management best practices, thinking to the possibility to introduce as soon as possible automatic milking systems that could ensure a good milking frequency.

Equid milk is gaining interest in several areas worldwide for its properties as a food for human consumption (Matera et al., 2022). This has led several researchers to deepen their knowledge of milk production in mares and jennies; currently, several aspects need to be investigated and studied. Moreover, the wide knowledge on dairy ruminants cannot be translated to equids because of differences in morphological, physiological, and behavioral patterns. The equine udder is subjected to unusually high cellular replication and increase in gland volume in postnatal life, particularly after puberty and pregnancy (Hughes, 2021), as in other mammals. The equine udder is characterized by one pair of mammae, each with a teat. Each mamma is drained by 2 independent mammary ductal trees, although sometimes more than 2 can occur (Canisso et al., 2020). Each teat has 2 orifices, through which the main ducts discharge (Ofstedal and Dhouailly, 2013).

The equine mammary gland is characterized by a fibrous stroma in which the epithelial structures are arranged in terminal duct lobular units similar to those of the human breast (Howard and Gusterson, 2000). A lobular unit is composed of a group, or lobule, of blind-ending mammary acini and both intralobular and extralobular portions of the subtending terminal duct, which together comprise the functional unit of the mammary gland (Hughes, 2021). The “milk letdown” process is led by prolactin activity. After the high levels of estrogens and progesterones that characterize pregnancy (Chavatte-Palmer, 2002), prolactin rises in the last 7 d before foaling and remains high during the first 2 mo of lactation (Worthy et al., 1986). Prolactin activates the transcription factor signal transducer and activator of transcription 5 (STAT5), which initiates expression of milk protein genes in mammary epithelial

cells (Hughes, 2021). When foals suckle, oxytocin binds the receptor expressed on basal mammary cells, stimulating intracellular calcium signaling, which results in contraction of the myoepithelial cells and milk expulsion (Stevenson et al., 2020).

Unlike conventional dairy ruminants, no selection toward milk production has occurred in equids, although indirect selection based on the body weight gain of suckling foals in heavy horse breeds could be considered. Thus, when studying equine milk production, high inter-individual and inter-breed disparities are observed. Moreover, the small number of animals has negatively influenced the possibility of carrying out genetic selection. Although attention is drawn toward equine milk, few studies have assessed its qualities or the quantity of milk that can be produced throughout lactation. The mammary capacity of donkeys is lower than that of mares, probably because of the lesser milk production of jennies compared with mares and the different nutritional requirements of foals of these 2 species. During the whole lactation, for both species, lactation production and peak depend on environmental factors, lactation stage, and breed. In donkeys, depending on breed, lactation peak can occur between 40 and 60 d of lactation (Malacarne et al., 2019), whereas, in some breeds, milk yield appears constant throughout lactation (Martini et al., 2014). In horses, the peak of lactation occurs between 20 and 90 d depending on the breed (De Palo et al., 2017), with heavy breeds having a later peak than saddle horses. In fact, it seems that the growth rate of foals can affect milk yield, with higher production and delayed peak in breeds characterized by a higher growth rate in the first months of life (De Palo et al., 2017). For these reasons, horses used for meat production, due to their somatic precocity (De Palo et al.,

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2009, 2014), showed higher milk yield than donkeys, which are characterized by slower and longer growth (De Palo et al., 2016). Regarding milk quality, donkey and horse milks are similar in composition (Ofstedal and Jenness, 1988) and similar to human milk (Potocnik et al., 2011). Indeed, the low protein and casein contents of equid milk make these milks suitable for children with cow milk protein allergy (Vincenzetti et al., 2017). Equid milk has nutritional similarities to human milk in terms of protein content and caseins, the most allergenic cow milk components (Martini et al., 2021). Their primary structure is very similar to those of human milk (Cunsolo et al., 2017). Moreover, some *in vitro* studies on human digestibility have demonstrated that equid caseins degrade rapidly, which could explain the reduced allergenicity of equid milk, itself linked to survival of allergens in the gastrointestinal tract (Tidona et al., 2014; Aspri et al., 2018; Li et al., 2020). These similarities between equid and human milk underlie its application in the diet of children suffering from cow milk protein allergy, showing its tolerability and efficacy in these individuals (Monti et al., 2012; Barni et al., 2018; Sarti et al., 2019).

Equid milk is also close to human breast milk in terms of macronutrient content, such as Ca, Mg, and P, in both mares (Barreto et al., 2019) and jennies (Malacarne et al., 2019), although K and Na concentrations are reported to be higher in donkey milk, and Cu is highly concentrated in mares (Bilandzic et al., 2014). For both species, the high lactose content is noted, giving these milks good palatability, and sensory evaluators considering donkey milk to be slightly sweet (Nayak et al., 2020). Although no flavor tests have been conducted on mare milk, its similar composition to donkey milk indicates that similar findings would be present in horses. Equid milk has a different fatty acid profile compared with human breast milk, although the saturation index indicates that equid milk could be suitable for preventing diseases such as obesity (Michaelsen and Greer, 2014) and atherosclerosis (Salimei and Fantuz, 2012).

Several factors other than lactation stage and breed might affect milk production, although they are currently poorly understood. Effects of age and parity of the dam have not been widely studied, and few data are available for either species. Some authors have reported an effect of foal season on both milk yield and composition in donkeys (Cosentino et al., 2012). Contrasting results are reported on the effect of feeding strategies on milk quality and production. Although Doreau et al. (1992) showed slight effects when testing forage-based versus concentrate-based diets, more recent studies reported that milk yield and quality can be influenced by different pasture types (Minjigdorj et al., 2012), and that fatty acid content and composition in particular can change (Valle et al., 2018).

The milk fat content of equid milk is low and characterized by high multifactorial variability (Fox, 2003). However, considering milk fatty acid composition (Table 1), SFA are the most represented class in equid milk, although wide variability can be found, which is mostly related to dietary differences (Salimei and Chiofalo, 2006).

Despite the increasing interest in equid milk, there are some current limitations due to the lack of scientific research and innovation that aim to improve management techniques and efficiency, while ensuring health and welfare in these species. In milking mares and jennies, sharing milk with foals is one of the main problems. Traditional methods start milking 2 or 3 mo after foaling, with an evi-

dent reduction of milk production for human consumption or the daily separation of foals from their dams a few hours before each milking session. This is not a suitable technique to ensure the lack of behavioral disorders, particularly because it involves a fasting period for foals. This is particularly true during the first days of life when they are unable to eat enough solid feed to satisfy their energy requirements (De Palo et al., 2016). In fact, this practice leads foals to ingest water, hay, and concentrate before they can digest it in adequate quantities to meet their needs in the first months of life, resulting in a reduction in suckling frequency that could induce some alterations regarding animal welfare and physiology. The equid udder has a lower storage capacity (<2.5 L) than ruminants and is more adapted to frequent milk removal (D'Alessandro et al., 2015). This is tightly linked to the high suckling frequency of foals compared with other domestic ruminants (De Palo et al., 2018a) and could explain the lower SCC and the rare occurrence of mastitis in equines compared with ruminants (Hughes, 2021). All of these physiological peculiarities have encouraged researchers and farmers to introduce and study new techniques of artificial suckling in foal breeding. These new techniques could allow both natural and artificial suckling during the entire lactation period (avoiding letting dairy mares and jennies dry off). The introduction of a partly artificial suckling technique can improve foals' growth and productive performance (De Palo et al., 2016), including meat quality (De Palo et al., 2017), while ensuring their welfare (De Palo et al., 2018b). de Souza Farias et al. (2021) reported that separating foals from their mothers to milk them during lactation did not greatly affect foals' behavior. In fact, although vocalization and abnormal behaviors did not change, some agonistic behaviors may appear

Table 1. Fatty acid profiles of horse and donkey milk (expressed as % of FAME)

Fatty acid	Horse ¹	Donkey ²
C4:0	0.09–0.9	0.18–0.6
C6:0	0.21–1.4	0.11–1.22
C8:0	0.8–6.1	3.48–12.8
C10:0	2.3–16.07	10.15–20.42
C12:0	3.8–14.6	10.67–15.9
C14:0	4.7–19.2	5.77–10.59
C14:1n-5	0.1–2.6	0.14–0.88
C15:0	0.2–0.9	0.15–0.57
C16:0	12.4–28.5	11.47–29.17
C16:1	2.2–9.7	2.37–3.93
C17:0	0.3–1.2	0.10–0.52
C17:1	0.7–1.1	0.27–0.73
C18:0	0.3–3.00	0.73–3.91
C18:1n-9	9.4–31.6	9.7–24.33
C18:2n-6	3.6–20.3	8.15–15.17
C18:3n-3	2.2–26.2	2.16–16.33
SFA	43–49	46.7–67.7
MUFA	26.8–36.2	15.3–35.0
PUFA	19–20	14.17–30.5
n-3	8.66–11.97	2.16–9.64
n-6	7.06–11.77	11.57–13.26

¹References for horse fatty acid values: Doreau and Martuzzi (2006), Marconi and Panfili (1998), Salimei and Fantuz (2012), Devle et al. (2012), and Salamon et al. (2009).

²References for donkey fatty acid values: Chiofalo et al. (2005), Chiofalo et al. (2006), Salimei and Chiofalo (2006), Salimei et al. (2004), Salimei and Fantuz (2012), Devle et al. (2012), and Salamon et al. (2009).

initially due to socialization and hierarchy development with other foals. In equids, most of the milk is alveolar (70–85% of secreted milk), unlike in small ruminants, where most of the milk is cisternal (up to 75% in dairy breeds). Thus, milk production depends on the frequency of its removal from the mammary gland, generally by milking or suckling. Studies have reported that increasing milking intervals increased milk yield per milking, induced by the ability of the cistern to dilate; however, total production per day decreases (D'Alessandro and Martemucci, 2012). Although milk ejection is triggered by the foal sucking, milking procedures for jennies, in terms of both human and animal safety and for optimal milk extraction, are more manageable when foals are not physically present, so jennies are usually milked after 2 to 3 h of physical separation from their foals (Burden and Thiemann, 2015). Because of their small udder size, donkeys and horses must be milked multiple times a day. Long intervals between milking events may increase intra-udder pressure, inducing early cessation of glandular activity (Alabiso et al., 2009). Although these activities can stress foals, De Palo et al. (2018b) showed that if foals are properly separated and fed during this time, their welfare is ensured and sometimes improved. Very little is known about the partitioning of milk in the mammary gland, the efficiency of machine milking in donkeys, and the reactions of jennies to a milking parlor. Machine milking may be of crucial importance in improving milk yield, considering milk quality and animal health and welfare aspects. Machine milking is characterized by parameters linked to pulsation rate and negative pressure but they have been poorly investigated. In fact, it is not well understood how these parameters should be correctly applied in equid species. Moreover, milking parlors have been introduced on donkey farms, showing how important adaptation to the milking parlor and machine milking is. De Palo et al. (2018a) applied different habituation protocols (from the gentlest—letting jennies enter and pass by with the milking machine turned off for some days before the first machine milking—to the most drastic, directly milking the jennies) and observed that a training protocol before starting machine-milking in the milking parlor improves both animal welfare and milk yield, allowing jennies to habituate to novelty through good desensitization. Moreover, contact with the milking cluster seems to stimulate animal behavioral and physiological responses during milking procedures.




Applying milking technologies and evaluating their effects on donkey welfare, health, milk yield, and milk composition represent major challenges. It is necessary to understand the best machine milking parameters and the optimal daily milking frequency that can be applied. All milking parameters should be linked to health and productive aspects of foal management. Improving our knowledge in these areas will allow the future use of automated milking systems that could facilitate and improve milk production in equid species.

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