Maternal age, parity and nursing status at fertilization affects post-partum lactation up to weaning in horses

Emilie Derisoud, Juliette Auclair-Ronzaud, Delphine Rousseau-Ralliard, Sabrina Philau, Etienne Aujean, Alexia Durand, Michèle Dahirel, Madia Charlier, Marion Boutinaud, Laurence Wimel, et al.

Journal of Equine Veterinary Science 128 (2023) 104868

Contents lists available at ScienceDirect

Journal of Equine Veterinary Science

journal homepage: www.j-evs.com

Maternal Age, Parity and Nursing Status at Fertilization Affects Postpartum Lactation Up to Weaning in Horses

Emilie Derisoud[a,1,*], Juliette Auclair-Ronzaud[b,1], Delphine Rousseau-Ralliard[c,d], Sabrina Philau[e], Etienne Aujean[f], Alexia Durand[c,d], Michèle Dahirel[c,d], Madia Charlier[f], Marion Boutinaud[e], Laurence Wimel[b], Pascale Chavatte-Palmer[c,d]

[a] Department of Physiology and Pharmacology, Karolinska Institutet, Solna, Stockholm, Sweden
[b] IFCE, Plateau technique de Chamberet, Chamberet, France
[c] Université Paris-Saclay, UVSQ, INRAE, BREED, Jouy-en-Josas, France
[d] Ecole Nationale Vétérinaire d'Alfort, BREED, Maisons-Alfort, France
[e] Institut Agro, INRAE, PEGASE, Saint Gilles, France
[f] INRAE, AgroParisTech, GABI, University of Paris-Saclay, Jouy-en-Josas, France

ARTICLE INFO

Article history:
Received 14 January 2023
Received in revised form 24 May 2023
Accepted 12 June 2023
Available online 16 June 2023

Keywords:
Milk
Colostrum
Equine
Mare
Fatty acids

ABSTRACT

Nulliparity is associated with intra-uterine growth retardation and foal delayed catch-up growth. Older mares produce larger/taller foals than the precedents. Nursing at conception on foal growth had not been investigated yet. In any case, milk production conditions the foal's growth. This study aimed to determine effects of mare parity, age and nursing on subsequent lactation quantity and quality. Saddlebred mares and their foals (N = 43) run as a single herd over the same year were: young (6-7-year-old) primiparous, young multiparous, old (10-16-year-old) multiparous nursing at insemination time or old multiparous barren the previous year. No young nursing nor old multiparous mares were available. Colostrum was collected. Milk production and foal weight were monitored at 3-, 30-, 60-, 90- and 180-days postfoaling. The foal average daily weight gain (ADG) was calculated for each period between two measurements. Milk fatty acid (FA), sodium, potassium, total protein and lactose contents were determined. The primiparous versus multiparous colostrum was richer in immunoglobulin G, with lower production but greater FA contents in milk. The primiparous foals had a lower ADG for 3 to 30 days postpartum period. Old mares' colostrum contained more SFA and less polyunsaturated FA (PUFA) whereas their milk was richer in proteins and sodium and poorer in short-chain-SFA with a reduced PUFA/SFA ratio at 90 days. Nursing mares' colostrum was richer in MUFA and PUFA and late-lactation milk production was reduced. In conclusion, parity, age and nursing at conception affect mare's colostrum and milk production and foal growth and should be considered for broodmares' management.

1. Introduction

In the context of the Developmental Origins of Health and Disease (DOHaD), maternal parity has been shown to affect fetal growth in several species such as pigs [1], sheep [2], cattle, [3] or humans [4,5]. In horses, it is well-known that a mare's first foal is lighter and smaller than the next ones (reviewed in [6]).

In domestic animals, including horses, parity is often positively correlated with age. Mare age *per se* affects foal birthweight with foals from both very young and very old mares being lighter and smaller at birth (reviewed in [6]). Studies taking into consideration both age and parity independently reported that the lighter and smaller foals born to primiparous, youngest or oldest mares do not

Animal welfare/ethical statement: The experiment was performed at the experimental farm of IFCE (research agreement C1903602 valid until March 22, 2023). The protocol was approved by the local animal care and use committee ("*Comité des Utilisateurs de la Station Expérimentale de Chamberet*") and by the regional ethical committee ("*Comité Régional d'Ethique pour l'Expérimentation Animale du Limousin*", approved under N°C2EA - 33 in the national registry of French ethical committees for animal experimentation) under protocol number APAFIS#14963-2018050316037888 v2. All experiments were performed in accordance with the European Union Directive 2010/63EU.

* Corresponding author at: Emilie Derisoud, PhD.
E-mail address: emilie.derisoud@gmail.com (E. Derisoud).

[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.jevs.2023.104868

catch up to foals born to higher parity, middle aged mares, at least until weaning [7–9], suggesting that lactation is affected, as foal average daily gain (ADG) is positively correlated with milk intake for the first 2 months [10].

Horse's colostrum is the first feed of the neonate foal. It contains large amounts of immunoglobulins essential for the foal's passive immunity (reviewed by [11]) and is characterized by 15% to 18% of proteins while fat and lactose content both ranged between 2% and 4% [12–14]. From 24 hours after foaling, colostrum is progressively replaced by milk. Protein and fat contents are reduced in milk compared to colostrum while lactose contents increase up to 6% [12–16]. Unsaturated fatty acids are predominant (>50%) in fatty acid (FA) profile from milk of mares on pastures [17]. Both maternal age and parity seem to have limited, but visible, effects on colostrum and milk composition but available data are scarce with few animals involved [14,17–19].

In terms of milk yield, effects of mare's parity are controversial, partly due to the high variety of methods used for milk yield estimation (reviewed by [20]). Whereas some authors did not observe any impact of parity [21,22], others reported a greater milk production (+1.3kg/d) in the first month of lactation in dams with a parity of three compared with dams with a parity ≤2 [18,23]. With regards to age, effects on milk yield are poorly described but appear nonlinear with a peak production at 7-year-old [20].

Thus, colostrum and milk production are affected by maternal age and parity but their respective effects remained poorly defined. In addition, in the equine industry, multiparous mares are often bred when they are still nursing and the effect of successive pregnancies on lactation has not been explored.

The present work aims to better understand the respective effects of age, parity and nursing at insemination on milk yield as well as fatty acid, total protein and lactose, sodium and potassium contents of colostrum and milk in Saddlebred mares.

2. Material and Methods

2.1. Ethics

The experiment was performed at the experimental farm of IFCE (research agreement C1903602 valid until March 22, 2023). The protocol was approved by the local animal care and use committee ("*Comité des Utilisateurs de la Station Expérimentale de Chamberet*") and by the regional ethical committee ("*Comité Régional d'Ethique pour l'Expérimentation Animale du Limousin,*" approved under N°C2EA-33 in the national registry of French ethical committees for animal experimentation) under protocol number APAFIS#14963-2018050316037888 v2. All experiments were performed in accordance with the European Union Directive 2010/63EU.

2.2. Experimental Design

Forty-three Saddlebred mares (French Anglo-Arabian and Selle Français breeds) and their foals were included in this study. They were raised in the "*Institut Français du Cheval et de l'Equitation*" experimental farm (Chamberet, France, 45° 34′55.17″N, 1°43′16.29″E, 442m). The experiment took place in 2020. All mares and foals remained healthy during this period.

Mares were classified as "young" when they were <7-year-old at the time of insemination while 10 to 16-year old mares were considered "old." Multiparous mares had already foaled at least once before the current experiment. Mares were classified as "nursing" when they were nursing a foal at the time of insemination. In the nursing group, all mares were inseminated on the second heat after foaling and weaning took place when the foal was 6 months of age, that is, the mares had approximately a 6-month dry period before the next lactation. Multiparous mares that were non nursing at insemination in 2019 were mostly purposely not inseminated during the previous reproductive season. Dry periods ranged from 1.5 to 4.5 years in this group. To analyze effects of mare's age, parity and nursing, mares were allocated to one of 4 groups: Young Primiparous (YP, N = 15), Young Multiparous (YM, N = 10), Old Multiparous (OM, N = 13) and Nursing Old Multiparous (NOM, N = 5). Effect of maternal parity was studied in young mares through the comparison of YP versus YM, effect of age by comparing OM versus YM, and the effect of nursing at insemination through the comparison of NOM versus OM. Unfortunately, it was not possible to obtain a group of both old and primiparous mares, neither one of young and nursing mares for this study. Thus, the combined effects of old age with primiparity and of young age with nursing could not be evaluated. Characteristics of the mares according to the group are detailed in Table 1.

All mares were used to analyze milk production as well as milk potassium and sodium concentrations. For technical reasons, however, not all milk samples from all mares could be analyzed for FA. Thus, six animals were selected for FA analysis in the YP, YM and OM groups according to foaling date and mare size in the aim to maintain homogenous groups. Due to the limited number of samples in NOM group (N = 5), all were used.

The semen of one unique stallion was used to artificially inseminate all mares, and the mares were managed the same way during pregnancy (as previously described by [9]). Briefly, pregnant mares were kept in one herd from insemination to the October 20, 2020. During this period, grass was their only feed and grazing was managed as rotational grazing. From the October 20 to 3 days after foaling, they were housed in individual boxes in the same barn and fed same quantities of cracked barley, hay and haulage twice a day with free access to water until foaling (feed composition and quantities are presented in Supplementary Table S1). Foaling occurred from March 3 to May 4, 2020. Foals were weaned in two groups on September 23 and October 20, depending on their birthdate. For the 3 days following foaling, mares and foals had daily access to individual pasture. Thereafter until weaning, mares and foals were kept in one herd on pasture with free access to water as previously described [24]. Pastures were permanent grasslands (multi-species based with around 60% grass, mainly Ray-Grass, Fescue and Dactyl; 25% legume, mainly Clover, and Alfalfa; 15% of other plants). Rotational grazing was performed to ensure ad libitum feeding. As previously described [25], this enables access to grass of equivalent quality between pastures along the grazing season. Average available total energy was 0.66 ± 0.05 UFC (energy available French unit, 1UFC = 2,250 kcal) and quantity of digestible proteins was 83.61 ± 18.36 g/kg of DM throughout the season. Mares were weighed at least once a week (PUEC31, Radwag, Poland) and Body Condition Score (BCS) was evaluated by the same manipulator once a month using a 1 to 5 scale [25].

2.3. Colostrum Sampling and Immunoglobulin G Quantification

Quickly after foaling and before first foal sucking, a sample of around 5 mL of colostrum was collected from all mares for further analyses. Before the sampling, a few drops were directly used to determine immunoglobulin concentration using a refractometer (Colotest, IFCE, France) [26,27].

2.4. Lactation Monitoring and Sampling

Because the weight-suckle-weight method does not provide a reliable estimation of mare milk production unless it is performed within a 24 hours interval for all mares [24], it was not possible to perform this method due to the high number of animals involved. Thus a milking strategy was used. Milk quantity was eval-

Table 1
Characteristics of the groups analyzed for mare milk yield, Na^+ and K^+ dosages, fatty acid, protein and lactose content.

	Milk Yield and Na^+ K^+ Dosages				Fatty Acid, Protein and Lactose Content			
	YP	YM	OM	NOM	YP	YM	OM	NOM
Number of individuals	15	10	13	5	6	6	6	5
Mare age	6 ± 0.65	7 ± 0	13.15 ± 1.68	11.4 ± 0.55	6.17 ± 0.41	7 ± 0	12.67 ± 1.51	11.4 ± 0.55
Mare parity	1 ± 0	2 ± 0	3.92 ± 0.86	4.4 ± 0.89	1 ± 0	2 ± 0	3.83 ± 1.17	4.4 ± 0.89
Mare LW (in kg)	544.36 ± 30.68	548.52 ± 38.59	584.46 ± 48.99	553.58 ± 35.10	546.97 ± 32.55	537.49 ± 45.34	579.74 ± 47.16	553.58 ± 35.10
Number of males	6	1	7	2	2	1	5	2
Number of females	9	9	6	3	4	5	1	3
Period of foaling in 2020 (day/month)	08/03–30/04	12/03–18/04	17/03–03/05	07/04–04/05	12/03–21/04	22/03–18/04	22/03–03/05	07/04 – 04/05

"YP", "YM", "OM" or "NOM" standing for "young primiparous", "young multiparous", "old multiparous", and "nursing old multiparous", respectively and mare LW being the mare live weight 24 hours after foaling.

uated at 5 lactation times of lactation: at 3 (D3), 30 (D30), 60 (D60), 90 (D90) and 180 days (D180). The following protocol was applied each time, as previously described [24]. Foals were muzzled to prevent suckling (t0) around 8:30 AM and left with their dam. A first milking (M1) was performed 3 hours later to empty the udder. A second milking (M2) was performed half hour later. In all experiments, the right udder was emptied first. Milking was performed without oxytocin injection using a manual milker (Udderly EZ Mare Milker, EZ Animal Products) as oxytocin injection might lead to overestimation of the milk yield [20].

The collected milk was weighed on an electronic scale (0.1g of precision, SAUTER RE, KERN, Germany). The quantity of milk collected during M2 was used to estimate milk production and storage in half an hour for each mare, considering the very reduced cisternal capacity of mare udder [28]. Thus, M2 milk production is used here as a proxy for milk yield estimation and will be referred as M2 yield in the rest of this study. Milk samples were collected from M2 milk in 50 mL tubes (one for each side of the udder) after collection and stored at −20°C. Milk analyses were performed on a 1/1 mix of M2 samples collected from the right and left side of the udder.

2.5. Foal Weight Gain Monitoring

At each milk collection, foals stayed muzzled for 4 hours and then were weighted. The foal average daily gain (ADG) was calculated as the ratio of the weight difference between two consecutive milking days (i.e., between three and 30, 30 and 60, 60 and 90 and 90 and 180 days of milking) and the number of days during this period. In foals, regardless of the breed, growth is linear from birth to 6 months [9,29]. Thus, this weighing frequency was considered appropriate to obtain a good estimation of ADG.

2.6. Qualitative Analyses

Samples from D3, D30 and D90 were analyzed for lactose, protein and FA concentrations. FA contents were also determined in colostrum. Due to budget and time limitations, it was not possible to analyze all samples. Samples from randomly selected individuals (6 YP, 6 YM, 6 OM) and the 5 NOM individuals were evaluated. One YM sample at D90 was not available at all for assay, and one colostrum sample for YP was also not available for fatty acid analysis. Thus, altogether, 68 samples were analyzed for lactose and proteins and 90 were analyzed for FA.

2.6.1. Lactose and Protein Contents

Lactose concentrations were measured using a commercial kit for enzymatic assay of lactose/galactose (Megazyme, Libios, Pontcharra sur Turdine, France). Measures were performed at 340 nm on a Tristar 2S-LB 942 Microplate Reader (Bethold, Thoiry, France).

Protein concentrations were determined using the BCA Protein Assay kit (Thermo Scientific, France).

2.6.2. Fatty Acid Contents

Prior to lipid extraction, 100 µg heptadecanoic acid (C17:0) was added to full-fat samples as an internal standard to measure total FA. Total FA, quantified with this standard, can only be considered as a proxy for total fat, as FA are esterified with glycerol within phospholipids or triglycerids that make up lipid droplets.

Lipid extraction was performed on 200 µL of full-fat colostrum and milk using chloroform/methanol (2:1, adapted from [30]). Transmethylation of FA was made using 7% boron trifluoride methanol (Sigma-Aldrich, Saint Quentin Fallavier, France [31]). The resulting methyl FA esters were analyzed by gas chromatography (Auto Sampling 8,410 Gas Chromatograph 1,310, Thermo Fisher Scientific, Courtaboeuf, France) coupled to a flame ionisation detector on an Econo-Cap EC-WAX capillary column (30 m, 0.32-mm internal diameter, 0.25-μm film, reference 19654; ALLTECH Associates Inc., Templemars, France), using the Chromeleon software (Thermo Fisher Scientific, Courtaboeuf, France). FA identification was made referring to known FA profiles obtained from injection of standard FAME (FA methyl esters) mix (Supelco 37 components FAME mix, ref 47885-U, Sigma). Quantitative profiles were established for each sample whereas qualitative profiles were expressed as % of total FA. In the results, data are expressed after normalization to total FA in the sample.

2.6.3. Estimation of Mammary Epithelium Integrity

Mammary epithelium integrity was estimated by measuring the Na^+/K^+ ratio in colostrum and in D3 and D60 milk samples from all mares. Inductively coupled plasma optical emission spectroscopy (ICP-OES 5110 Agilent Technology, Les Ulis, France) was used for Na^+ and K^+ analysis in 200-µL colostrum and 500-µL milk samples. Samples were first diluted in H_20, 100 and 40 times, respectively, for colostrum and milk. Then, 2.5 mL of 0.01% Triton X100 (Sigma Aldrich, Saint- Quentin Fallavier, France) and 7.5 mL of 65% nitric acid were added and samples were completed to 50 mL with H_20. Analyses were performed in duplicate according to manufacturer instructions using calibration standards for ICP-OES Certipur Potassium and Sodium 1,000 µg/mL (Agilent Technology, Les Ulis, France) and a standard milk sample ERM-BD151 (European Reference Materials, milk sample with guaranteed contents, European Commission Directorate-General JRC - Joint Research Centre Brusels, Belgium).

2.6.4. Energy Content

Milk energy content was estimated based on M2 yield and milk qualitative analysis. It was estimated that 1g FA provided 9 calories, whereas 1g lactose and 1g protein provided four calories. Energy concentration (expressed in kcal/L) was calculated by dividing energy available in one milking by the quantity of milk collected.

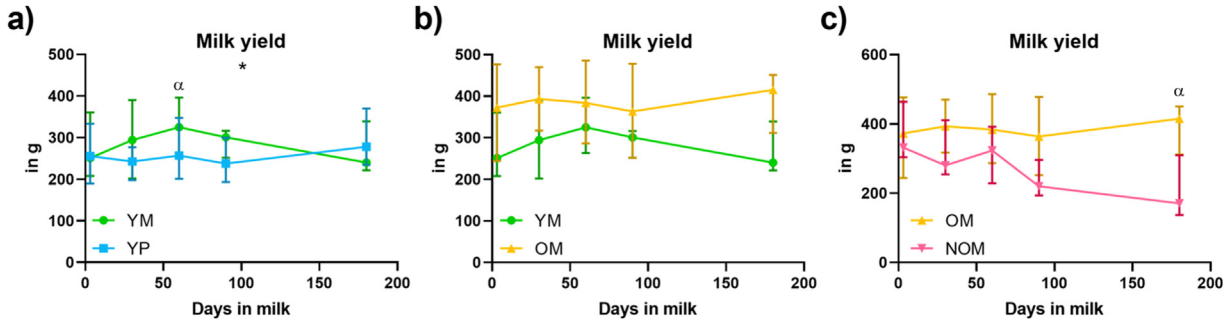

Fig. 1. Comparison of milk yield per milking at several lactation day according to mares' parity (A), age (B) or nursing status at insemination (C) YM: young multiparous; YP, Young primiparous; OM, old multiparous; NOM, nursing old multiparous. *indicates a $P < .05$ regarding group effect on the overall lactation and α indicates a significant difference ($P < .05$) between groups at specific lactation time. Results are presented as the median and interquartile range.

2.7. Statistical Analysis of Results

All statistical analyses were performed using R [32] on Rstudio software [33].

2.7.1. Differential Analysis

There were no significant differences in live weight between groups that have been compared at the same lactation stage nor within groups at different stages. Similar results were obtained for BCS and for ADG. Since weight, BCS and ADG are colinear, only live weight after foaling was finally considered as a cofactor in the models.

For all qualitative measurement, colostrum and milk were analyzed independently. For measurements in colostrum, linear models were performed with mare live weight 24 hours after foaling and group as fixed effects. Permutation tests were applied as data were not normally distributed using pgirmess package [34]. Results of these analyses were presented.

Linear mixed models were performed using nlme package [35] to evaluate differences in M2 yield and quality. Mare live weight 24 hours after foaling, group, lactation day (3, 30, 60, 90, and 180) and interaction between group and lactation day were set as fixed effects and individuals as random effect. For foal ADG, lactation period, mare live weight 24 hours after foaling and the average M2 yield for the corresponding period were considered (total of four periods, 3–30, 30–60, 60–90, and 90–180 days postpartum). Permutation tests were also applied for these analyses [34]. When interaction between period and group was significant, linear models followed by permutation tests were applied at each studied time with mare live weight and group as factors.

Factors effects were considered significant for $P < .05$ after permutation test.

In the results part, M2 yield, foal ADG and all qualitative measurements are presented as mean ± standard deviation (SD) in the text, in tables and in supplementary tables. According to the comparison (age, OM vs. YM; parity, YP vs. YM; lactation, NOM vs. OM), M2 yield, foal ADG and all significant qualitative milk measurements were graphically represented using the median and the interquartile range for each lactation day studied with GraphPad Prism software 8.0.1 for Windows (Graphpad Software, San Diego, CA, www.graphpad.com).

2.7.2. Correlations Between Mammary Gland Integrity, M2 Yield and Milk Composition

To study correlations between M2 yield and milk composition (total FA, total protein, lactose concentration, Na concentration, K concentration and Na^+:K^+ ratio), Kendall correlation tests were computed. To study the relationship between M2 yield and the total concentration of fatty acids, total lactose concentration or total proteins concentration, 64 individuals were available. To study the relationship between M2 yield and Na^+, K^+ concentration and Na^+:K^+ ratio, 84 comparison points were available as samples were collected at D3, D30 and D60 from 43 mares.

Following the same methodology correlations between total fatty acid concentration and Na^+, K^+ and Na^+:K^+ ratio were also studied.

3. Results

3.1. M2 Yield

In all comparisons, M2 yield was not different throughout the lactation period (YM vs. OM $P = .74$; YM vs. YP $P = .74$; OM vs. NOM $P = .46$) and was not affected by maternal weight (YM vs. OM $P = .12$; YM vs. YP $P = .74$; OM vs. NOM $P = .12$). Mean M2 yield over the whole lactation was significantly lower in YP versus YM (272.31 ± 84.42g vs. 303.39 ± 102.73g, respectively, $P < .05$) (Fig. 1A and Supplementary Table S2). There was also an interaction between parity and time on M2 yield with a significantly lower production in YP versus YM at D60 (268.62 ±72.51g vs. 342.39 ± 99.33g, respectively, $P < .05$). In contrast, it was similar between OM versus YM (382.71 ± 110.74g vs. 303.39 ± 102.73g, respectively, $P = 0.22$) (Fig. 1B and Supplementary Table S2) or OM versus NOM (382.71 ± 110.74g vs. 292.28 ± 105.83g, respectively, $P = 0.61$). Nevertheless, when comparing NOM and OM, the interaction between lactation period and group was significant ($P < .05$, Fig. 1C and Supplementary Table S2). NOM tended to produce less milk than OM at D90 (240.10 ± 71.14 and 359.79 ± 127.61g, respectively, $P = .05$) and produced significantly less milk than OM at D180 (212.80 ± 92.41 and 401.62 ± 100.64g, respectively, $P < .05$).

3.2. Foal Average Daily Gain

No effect of group only was observed, regardless of the comparison (YP vs. YM, $P = .26$; OM vs. YM, $P = .70$; NOM vs. OM, $P = .66$, Fig. 2). The mare live weight at 24 hours after foaling and the period had a significant effect on foal ADG in all comparisons (respectively $P < .05$ and $P < .0001$) but not the average M2 yield and for M2 yield (YM vs. OM $P = .15$; YM vs. YP $P = .52$; OM vs. NOM $P = .11$). Only for the comparison YP versus YM, the interaction of the period and the group was significant ($P < .05$). After further analysis, only the ADG over the period 3 to 30 days postpartum of foals from YM was significantly higher than the one from YP group (Fig. 2, $P < .01$).

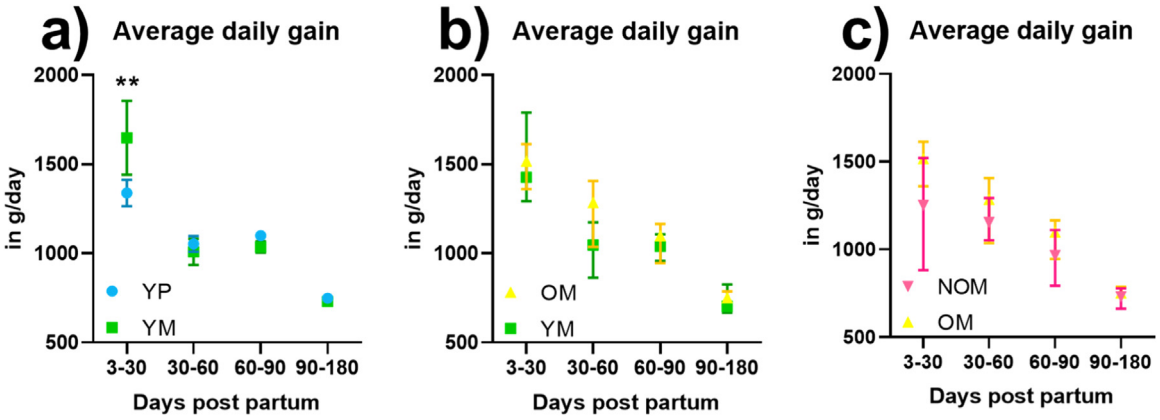

Fig. 2. Comparison of foal average daily weight gain according to mares' parity (A), age (B) or nursing status at insemination (C) The average daily weight gain was calculated as the ratio of the weight difference between two consecutive milking days (i.e., between 3 and 30, 30 and 60, 60 and 90, and 90 and 180 days of milking) and the number of days during this period. YM, young multiparous; YP, Young primiparous; OM, old multiparous; NOM, nursing old multiparous. ** indicates a $P < .01$ regarding group effect on the overall lactation and α indicates a significant difference ($P < .05$) between groups at specific lactation time. Results are presented as the median and interquartile range.

Table 2
Variables significantly modified in colostrum according to mare age, parity and nursing status.

	YP (n = 6)	YM (n = 6)	OM (n = 6)	NOM (n = 5)	*P* value		
					YP Versus YM	OM versus YM	NOM versus OM
Immunogobulins G	**77.69 ± 28.26**[a]	**42.50 ± 16.37**[a]	62.08 ± 31.87	89.00 ± 24.60	**0.01**	0.13	0.26
Palmitoleic acid (C16:1ω7)	3.91% ± 0.35	4.13% ± 0.87	**4.13% ± 0.45**[c]	**5.81% ± 1.34**[c]	0.55	1	**<0.0001**
Stearic acid (C18:0)	2.80% ± 0.47	**2.98% ± 0.55**[b]	**2.47% ± 0.42**[b]	2.08% ± 0.49	0.6	**0.01**	0.25
Linoleic acid (C18:2ω6)	13.65% ± 0.59	**14.51% ± 1.6**[b]	**10.48% ± 2.03**[b]	10.48% ± 1.68	0.31	**0.03**	0.62
α-linolenic acid (C18:3ω3)	13.36% ± 2.09	**15.98% ± 2.6**[b]	**12.33% ± 2.41**[b]	10.60% ± 1.52	0.19	**0.01**	0.35
Eicosadienoate (C20:2ω6)	0.58% ± 0.14	**0.57% ± 0.11**[b]	**0.39% ± 0.11**[b]	0.36% ± 0.08	0.84	**0.01**	0.80
Homo-gamma linolenate (C20:3ω6)	0.11% ± 0.01	**0.12% ± 0.02**[b]	**0.08% ± 0.02**[b]	0.08% ± 0.02	0.14	**0.01**	0.55
Arachidonic acid (C20:4ω6)	0.08% ± 0.02	**0.10% ± 0.04**[b]	**0.06% ± 0.02**[b]	0.07% ± 0.02	0.28	**0.02**	0.19
Docosapentaenoic acid (C22:5ω3)	0.17% ± 0.06	**0.19% ± 0.05**[b]	**0.12% ± 0.04**[b]	0.12% ± 0.02	0.48	**0.03**	0.71
Docosahexaenoic acid (C22:6ω3)	0.11% ± 0.03	**0.09% ± 0.05**[b]	**0.03% ± 0.02**[b, c]	**0.17% ± 0.10**[c]	0.51	**0.03**	**0.02**
Lignoceric acid (C24:0)	0.01% ± 0	**0.02% ± 0.04**[b]	**0.11% ± 0.04**[b]	0.06% ± 0.04	0.75	**0.01**	0.08
Nervonic acid (C24:1ω9)	0.03% ± 0.01	**0.10% ± 0.09**[b]	**0.35% ± 0.06**[b, c]	**0.11% ± 0.15**[c]	0.36	**0.02**	**0.04**
SFA	42.69% ± 4.32	**38.11% ± 7.27**[b]	**47.73% ± 5.54**[b]	45.51% ± 6.40	0.27	**0.01**	0.13
MUFA	28.43% ± 1.97	29.43% ± 3.91	**27.87% ± 2.71**[c]	**31.91% ± 3.70**[c]	0.65	0.38	**0.03**
PUFA	28.88% ± 2.56	**32.42% ± 3.92**[b]	**24.40% ± 4.27**[b]	22.58% ± 2.87	0.12	**<0.0001**	0.69
PUFAω6	14.62% ± 0.71	**15.49% ± 1.59**[b]	**11.30% ± 2.18**[b]	11.17% ± 1.85	0.26	**<0.0001**	0.77
PUFAω3	14.26% ± 2.11	**16.93% ± 2.47**[b]	**13.10% ± 2.52**[b]	11.41% ± 1.50	0.10	**0.01**	0.29
PUFAC22	0.32% ± 0.08	**0.28% ± 0.09**[b]	**0.16% ± 0.04**[b, c]	**0.34% ± 0.07**[c]	0.47	**0.02**	**<0.0001**
PUFAω3C22	0.27% ± 0.05	**0.27% ± 0.09**[b]	**0.16% ± 0.05**[b, c]	**0.29% ± 0.08**[c]	0.98	**0.03**	**0.02**
Total C16:1	4.88% ± 0.36	5.14% ± 0.99	**5.00% ± 0.49**[b, c]	**6.78% ± 1.37**[c]	0.55	0.73	**0.03**
PUFA/SFA	0.69 ± 0.12	**0.90 ± 0.32**[b]	**0.53 ± 0.15**[b]	0.51 ± 0.12	0.14	**<0.0001**	0.71
SFA/UFA	0.75 ± 0.14	**0.63 ± 0.18**[b]	**0.93 ± 0.20**[b]	0.86 ± 0.23	0.21	**0.01**	0.19
MUFA/SFA	0.68 ± 0.11	**0.82 ± 0.32**[b]	**0.59 ± 0.12**[b, c]	**0.72 ± 0.17**[c]	0.27	**0.04**	**0.03**
ETE/AA	7.71 ± 3.02	6.87 ± 4.23	**8.21 ± 0.58**[c]	**7.02 ± 3.02**[c]	0.75	0.55	**0.01**

All values are mean ± standard deviation; Percentage is defined as the ratio of the FA/total FA; Immunoglobulins in g/L; "YP", "YM", "OM" or "NOM" standing for "young primiparous," "young multiparous," "old multiparous," and "nursing old multiparous," respectively.
Abbreviations: AA, arachidonic acid (C20:4ω6); ETE, eicosatrienoic acid (C20:3ω3); FA, fatty acids; MUFA, monounsaturated FA;
PUFA, polyunsaturated FA; SFA: saturated FA
C followed by a number X indicates FA with X carbons. in each row,
[a] means that $P < .05$ in the comparison of YP to YM,
[b] in the comparison of OM to YM and
[c] in the comparison of NOM to OM

3.3. Colostrum and Milk Composition

3.3.1. Effect of Mare's Parity (YP Versus YM)

IgG concentrations were significantly higher in the colostrum of YP compared to that of YM ($P < .01$, Table 2 and Supplementary Tables S3) but there was no significant difference for all other components.

In milk, several differences in FA concentrations were observed (Supplementary Fig. 1 and Supplementary Table S3). Total FA concentration ($P < .05$) was increased in YP versus YM over the whole lactation period.

After normalization to the total FA concentration, only proportions of myristoleic (C14:1ω9), C18:3ω6 and total of C16:1 were significantly different ($P < .05$) according to group (Table 3 and Supplementary Table S4). After normalization, C14:1ω9 and total C16:1 concentrations were increased in YM milk at D3 ($P < .05$) while C18:3ω6 concentrations were increased in YM versus YP on D30 ($P < .05$).

In addition, Na^+ concentrations in milk were significantly lower in YM versus YP ($P < .05$, Supplementary Table S5 and Supplementary Fig. 1), with a trend towards a higher Na^+ concentration in YP on D3 ($P = .05$).

Table 3
Variables significantly modified by mare's parity in young mares on fatty acid percentage in milk at different lactation day.

	3 d		1 mo		3 mo		*P*.Value	
	YP	YM	YP	YM	YP	YM	Group	Group:Time
Myristoleic acid (C14:1ω9)	0.44% ± 0.22	0.88% ± 0.30	0.59% ± 0.17	0.52% ± 0.19	0.55% ± 0.10	0.54% ± 0.13	**.02**	**0.06**
C18:3ω6	0.26% ± 0.15	0.42% ± 0.22	0.21% ± 0.10	0.33% ± 0.11	0.13% ± 0.09	0.12% ± 0.03	**.01**	0.13
Total C16:1	5.01% ± 1.94	7.31% ± 1.73	7.48% ± 1.11	7.71% ± 0.81	8.55% ± 0.61	9.3% ± 1.13	**.02**	0.35

All values are mean ± standard deviation; Percentage is defined as the ratio of the FA/total FA; "YP" & "YM" standing for "young primiparous" and "young multiparous," respectively. FA, Fatty acids; Total C16:1 correspond to the sum of all monoinsaturated fatty acids with a length of 16 carbons.

Table 4
Variables significantly modified by age in multiparous mares on fatty acid percentage and total protein concentration in milk at different lactation day.

	3 d		1 mo		3 mo		*P*.value	
	OM	YM	OM	YM	OM	YM	Group	Group:Time
Lauric acid (C12:0)	10.82% ± 2.42	11.87% ± 2.29	5.99% ± 1.41	4.72% ± 1.8	4.49% ± 1.07	0.94% ± 1.09	.57	**0.03**
Homo-gamma linolenate acid (C20:3ω6)	0.06% ± 0.01	0.06% ± 0.01	0.06% ± 0.02	0.1% ± 0.03	0.06% ± 0.01	0.07% ± 0.02	**.02**	0.89
Cuplanodonic acid (C22:5ω6)	0.02% ± 0.02	0.02% ± 0.01	0.03% ± 0.01	0.02% ± 0.01	0.03% ± 0.01	0.06% ± 0.04	.62	**0.03**
scSFA	13.76% ± 3.11	18.89% ± 4.1	10.36% ± 3.2	5.47% ± 1.91	4.71% ± 1.2	0.96% ± 1.12	.26	**0.04**
Total protein (mg/ml)	**24.27 ± 4.32**	**18.57 ± 2.87**	**17.03 ± 2.11**	**17.7 ± 1.74**	**15.01 ± 0.97**	**14.75 ± 1.08**	**.02**	0.09
PUFA/SFA	0.39 ± 0.13	0.35 ± 0.14	0.94 ± 0.22	0.96 ± 0.23	0.95 ± 0.3	1.48 ± 0.38	.58	**0.02**

All values are mean ± standard deviation; Percentage is defined as the ratio of the FA/total FA; "OM" & "YM" standing for "old multiparous" and "young multiparous," respectively.
Abbreviations: FA, Fatty acids; PUFA, polyunsaturated fatty acids; scSFA, short chain satured fatty acids; SFA, Saturated fatty acids

3.3.2. Effect of Mare Age (OM Versus YM)

PUFA/SFA and MUFA/SFA ratios, were reduced and the SFA/unsaturated FA (UFA) ratio was increased in the colostrum of OM versus YM mares (Table 2 and Supplementary Table S6).

FA concentrations were normalized to total FA. The proportions of stearic acid (C18:0, $P < .05$), linoleic acid (C18:2ω6, $P < .05$), α-linolenic acid (C18:3ω3, $P < .05$), eicosadienoate (C20:2ω6, $P < .05$), homo-gamma linolenate (C20:3ω6, $P < .05$), arachidonic acid (C20:4ω6, AA, $P < .05$), docosapentaenoic acid (C22:5ω3, $P < .05$) and docosahexaenoic acid (C22:6ω3, $P < .05$) were reduced in the colostrum of old mares (Table 2 and Supplementary Table S7). In contrast, the proportions of lignoceric acid (C24:0, $P < .05$) and nervonic acid (C24:1ω9, $P < .05$) were increased in the colostrum of OM versus YM (Table 2). These changes induce a reduction in both PUFA ω3 and ω6 and, therefore, total PUFA was reduced in OM versus YM colostrum (respectively, $P < .0001$, $P < .05$ and $P < .0001$, Table 2). The 22 carbon PUFAs, particularly PUFA C22ω3, were also reduced in OM versus YM colostrum (both $P < .05$). Related to these results, proportions of SFA were increased in the colostrum of OM versus YM ($P < .05$, Table 2).

In milk, only the PUFA/SFA ratio was increased on D90 in OM versus YM ($P < .05$, Table 4, Supplementary Table S8 and Fig. 2). The proportion of small chain SFA was reduced on D3 ($P < .05$) in OM versus YM while on D30 and 90, the proportion of scSFA was increased in OM versus YM milk ($P < .05$, Table 4). The proportion of cuplanodonic acid (C22:5ω6, $P < .05$) was also decreased on D90 in OM versus YM milk (Table 4 and Supplementary Fig. 2).

Total protein concentrations were increased in OM milk on D3 ($P < .05$) compared to YM (Table 4), while total sodium was increased ($P < .05$) on both D3 and 60 in OM milk (Supplementary Table 5)

3.3.3. Effect of Nursing (NOM Versus OM)

In colostrum, the MUFA/SFA ratio was increased ($P < .05$) while the eicosatrienoic acid (ETE, C20:3ω3)/arachidonic acid (AA) ratio was reduced ($P < .05$) in NOM colostrum compared with OM (Table 2 and Supplementary Table S9).

Palmitoleic acid (C16:1ω7, $P < .05$) and, as a result, the total monounsatured C16 ($P < .05$) proportions were increased in NOM versus OM colostrum (Table 2 and Supplementary Table S10). Consequently, total MUFA levels were increased in NOM versus OM colostrum. Docosahexaenoic acid (C22:6ω3, $P < .05$) and the proportion of ω3 and total 22 carbons PUFAs were also increased in colostrum of NOM compared with OM. The proportion of nervonic acid (C24:1ω9, $P < .05$) was, however, reduced in NOM versus OM colostrum (Table 2).

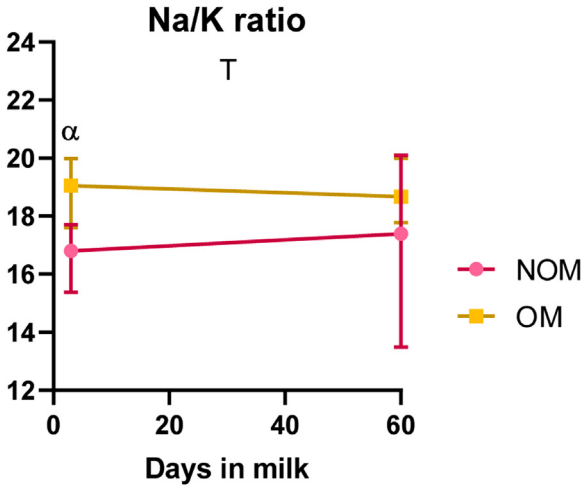

Fig. 3. Effect of mares' nursing status on milk Na/K ratio NOM: Nursing old multiparous; OM, Old multiparous; Na, Sodium; K, Potassium. T indicates a $P < .1$ regarding group effect on the overall lactation and α indicates a significant difference ($P < .05$) between groups at specific lactation time. Results are presented as the median and interquartile range.

In milk, a trend ($P = .05$) was observed towards an increased Na^+:K^+ ratio in OM versus NOM with a significant increase ($P < .05$) in OM at D3 (Fig. 3 and Supplementary Table S11). Other milk components were not affected by nursing at insemination.

3.4. Correlation Between Mammary Gland Integrity, M2 Yield Per Milking and Milk Composition

M2 yield was not correlated to any of the studied milk composition parameters (total FA concentration, $P = .37$; total lactose concentration, $P = .48$; total protein concentration, $P = .49$; Na^+ concentration, $P = .54$ and K^+ concentration, $P = .72$), except for

the Na^+:K^+ ratio that was significantly negatively correlated to M2 yield ($\tau = -0.15$; $P < .05$). Similarly, total fatty acid concentration was not correlated with NA+:K+ ratio ($P = .88$), indicator of mammary gland integrity.

3.5. Estimation of Energy Contents

Milk from primiparous mares provided more gross energy compared with milk from multiparous mares (278.63 ± 21.36 and 259.29 ± 15.78 kcal/L, respectively, $P < .0001$,). Old mares' milk was more energetic than that of young mares across the three first months of lactation (OM = 276.87 ± 26.60 kcal/L, $P < .05$).

Nursing at insemination, however, did not affect energy contents at any time in comparison to non-nursing mares (NOM = 283.99 ± 24.58 kcal/L).

4. Discussion

4.1. Colostrum

Colostrum IgG concentration was higher in primiparous compared to multiparous mares but did not vary between the other groups. In contrast, another study in eight mares did not report any difference according to parity [36]. In addition, no effect of mare parity was reported on the efficiency of IgG passive transfer in foal plasma [37–39]. One more recent epidemiological study on 192 Paso Fino foals demonstrated, however, a reduced risk of total failure of passive transfer in foals born to multiparous compared to primiparous mares [40], rendering it difficult to draw conclusions on the effect of parity on colostral IgG.

Effects of mare's age on colostrum FA content were previously highlighted in primitive Konik horses with an increase in PUFA in mares >10 year old [17]. Opposite effects were observed in the present study, with a decrease in PUFA. Both studies report, however, an increase of SFA in older mares. In humans, as in horses, the effect of maternal age on FA composition of colostrum remains controversial [41,42]. Pikul et al also studied effect of maternal parity on colostrum FA contents comparing mares with parities <5 versus ≥5. They demonstrated effects on a few FA which were not confirmed in the present study [17]. Both for age and parity, discrepancies between data reported by Pikul et al and the present work could be due to differences in age, breed, nutritional/environmental conditions or group constitution (only parity one and two were compared here). In addition, it is very likely that mares with a parity >5 were older than mares with a lower parity, and thus that age was confounding factor.

In terms of nursing effects, mares that were nursing at the beginning of their gestation had an overall increased proportion of MUFA. For both young and nursing mares, increased PUFA were observed. In humans, increased proportions of PUFA, especially of long chain PUFA, in colostrum, have been associated to benefits in child neurodevelopment [43] but potential effects in the foal remain to be studied.

4.2. Milk Fatty Acid Contents

Higher FA concentrations were observed in the milk of primiparous versus multiparous mares on D3 and D30. On D3, several C22 lcPUFA were increased while on D30, increased FA were essentially medium and long carbon chain SFA and medium carbon chain MUFA. In cattle, milk is fatter in the first lactation, with higher amounts of unsaturated FA [44,45] while in humans, maternal parity does not seem to alter milk FA contents [46]. Mares that foaled >5 times have been shown to produce a milk richer in short and middle chain saturated fatty acids (C10:0, C12:0, and C14:0) and poorer in UFA compared with mares with a lower parity [17].

Mare age barely affected milk FA contents. On D3 of lactation, young mares produced higher amounts of short chain SFA but this effect was reversed on D30 and D90 where older mares' milk was richer in SFA, with a reduced PUFA/SFA ratio on D90. In humans, there is no difference between mature and younger women for FA concentrations in milk collected within the first 2 weeks postpartum [47]. In horses, mares <10yo were reported to produce a milk richer in myristoleic (C14:1ω5), palmitic (C16:0) and palmitoleic (C16:1ω7) acids and poorer in arachidonic acid (C20:4ω6) [17] but this was not confirmed here, probably related to the two different populations and environments.

FA content in milk comes from diet, *de novo* FA synthesis and body fat mobilization (for review in ruminants [48] and in humans [49]). Since all mares were on the same pasture, results from the present study cannot be explained by differences in dietary FA but could be due to variation in feed intake in response to energy requirements [50]. The difference in FA composition in milk could also be at the origin/explained by the reduced milk production in young primiparous mares even though no correlation between FA content and M2 yield have been observed here.

Fatty acids in mare milk are also derived from body fat mobilization. In ponies, two of the main adipose tissue fatty acids are linoleic (C18:2ω6) and linolenic (C18:3ω3) acids, which are significantly influenced by parity, age and nursing [51]. In ruminants, the negative energy balance at the beginning of lactation leads to increased proportions of long chain FA in milk, released by adipose tissue [52,53]. As feed intake and body fat could not be monitored here, it cannot not be excluded that fat mobilization could have been more important in young mares, thus explaining the observed variations at 90 days. Similarly, primiparous mares could have mobilized more body fat for milk than multiparous mares at the beginning of the lactation period.

4.3. M2 Yield and Mammary Gland Epithelium Integrity

M2 yield was reduced in primiparous versus multiparous but not in young versus old mares throughout the lactation period.

The effect of parity on overall milk production is controversial, with studies reporting that multiparous mares produce more milk [18,23] while others do not report any difference [21,22]. Nevertheless, although no difference in milk yield was observed between first and second lactation, differences were reported with a greater number of lactations, and consequently with increased maternal age [18], which is consistent with the foal weight gain observed in the literature [7–9].

The effect of maternal aging on milk yield had been scarcely studied. Peak milk production was reported to occur at seven and 11 to 15 years of age in nursing and dairy mares, respectively [20]. In the present experiment, the overall production nor the production at specific lactation times were not influenced by age. Variations were according to the nursing status, with nursing mares producing less milk on D90 and D180, suggesting that successive pregnancies may impair mammary capacity to sustain a full lactation.

We observed a negative relationship between Na^+:K^+ ratio and M2 yield, as previously described for lactating goats [54]. The observed Kendall correlation, however, is weak ($r = -0.15$), thus the relationship might not be linear. This correlation may not be translated into biological effects and further analyses are needed. Nevertheless, in nursing mares a trend for a lower Na^+:K^+ ratio on D3 was observed which suggests that mammary epithelium integrity is reduced in mares with successive pregnancies. In ruminants, Na^+ and K^+ concentrations differ between colostrum and with an average 5 day transition from colostrum to milk [55,56]. In horses,

based on IgG concentrations, it has been suggested that the transition from colostrum to milk occurs in the 12 hours postpartum in nursing mares [57]. Nevertheless, protein and macro-element contents do not stabilize until 5 days [58]. Thus, the milk to colostrum transition is not over by D3 and results should be interpreted with caution.

4.4. Energy Content and Foal Weight Gain

Energy contents were higher in old versus young mares' milk and in primiparous versus multiparous mares. The increase was significant ($P < .05$) on D3 regarding both comparisons. The energy increase in primiparous milk is due to the increased fatty acids contents. Since primiparous mares produce less milk than multiparous mares, it might be hypothesized that the lower quantity is compensated by quality to cover the foal's nutritional needs. During the D3 to D30 period, however, foals born to primiparous mares had a lower ADG than those born to multiparous dams, in agreement with previous reports showing that foals born to primiparous mares do not catch-up after birth [8,9]. These results indicate that increased primiparous milk composition is not sufficient to fully compensate the lower milk yield.

In older mares, the increased energy contents seem to be related to increased milk protein contents when compared with younger mares, with no difference in milk yield. Thus, foals born to older multiparous mares might receive more energy from milk than their counterparts born to younger multiparous mares. This observation, however, did not lead to an increased weight gain. Without considering extreme ages, a positive relation has been reported between foal growth and mare age, but the confounding parity was not considered [8,59].

4.5. Limits and Perspectives

Due to the limited available literature on mammary gland function in equidae, most comparisons in the discussion were made with ruminants. Nevertheless, interspecific differences for fat synthesis and mammary gland function have been reported between cows, sows, and rats, with rats using glucose to form milk FA to a much greater extent than cattle, that have a lower glycemia compared to monogastrics [60]. Mammary gland data in monogastric animals is scarce and the omnivorous diet of many of them differs grandly from that of horses, hampering their use as models for equine.

In humans, colostrum and milk have been shown to be particularly responsive to maternal diet [61]. Here, mares were fed with the same diet during pregnancy and were in the same environmental conditions. All mares foaled within 57 days, thus limiting a potential seasonal effect although intra-group variation may have occurred. Only maternal weight after foaling, but not thereafter, was taken in account in statistical analyses because mares' live weights were not different in the comparisons. Thus, the observed differences are mainly due to the studied factors and to individual differences. Observed inter-individual variations might partially be explained by the absence of genetic selection based on maternal qualities in the studied herd, and more broadly in equine industry. Another limit of this study is that the young multiparous mares were all secondiparous. The first lactation, however, is the one shown to be reduced and different from subsequent lactations in cattle [62–66] and horses [24]. Furthermore, it is important to remember that these results have been obtained on limited number of samples, especially in NOM group and that more investigations are required to confirm them on a larger number of animals and on other breeds.

Finally, milk energy contents as calculated here is an approximation and, thus, is lower than observed in other studies. Indeed, the computation included only lactose for sugars and fatty acids without considering glycerol or other carbohydrate sources of energy. The comparison between groups, however, remains valid.

5. Conclusions

The present study demonstrates the importance of mare age, parity and of nursing during the periconceptional period, on subsequent colostrum and milk production. Although the mechanisms leading to these modifications in colostrum and milk composition and quantity have not been determined yet, it is possible to suggest that depending on mares' age, parity and lactating status, the differences observed here could be due to different capacity of the mammary gland to produce lipids as well as differences in body fat mobilization through the lactation period. Impacts of those changes on foal weight gain seemed to be limited and that the quantity of milk impacted more the weight gain. The long-term effects of these differences on the foal performance remains to be elucidated.

Data and Model Availability Statement

None of the data were deposited in an official repository but are available upon request.

Author Contributions

PCP obtained the funding. LW and PCP conceived the project. ED, JAR, LW and PCP supervised the study. ED, JAR, DRR, SP, EA, MD, MC, MB, LW and PCP adapted the methodology for the project. DRR, SP, EA, MD, MC, MB and LW provided the resources. JAR and AD performed experiments with animals and collected samples. DRR performed fatty acid analysis. SP and MB determined sodium and potassium concentration. EA and MC determined protein and lactose content. ED and JAR performed data curation. ED and JAR analyzed the data. ED and JAR wrote the original draft. All authors read, revised, and approved the submitted manuscript.

Financial disclosure

This work was supported by the "Institut Français du Cheval et de l'Equitation" (grant number CS_2018_23, 2018, obtained by PCP) and the National Research Institute for Agriculture, Food and Environment (INRAE) department Animal Physiology and Breeding Systems (no grant number, obtained by PCP in 2018).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors are grateful to the staff of the Institut Français du Cheval et de l'Equitation (IFCE) experimental farm (Plateau technique de la station expérimentale de Chamberet, Chamberet, France) for care and management of animals. The authors thank the "Institut Français du Cheval et de l'Equitation" (grant numbers CS_2018_23, 2018) and the National Research Institute for Agriculture, Food and Environment (INRAE) department Animal Physiology and Breeding Systems for funding this research.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jevs.2023.104868.

References

[1] Zotti E, Resmini FA, Schutz LG, Volz N, Milani RP, Bridi AM, et al. Impact of piglet birthweight and sow parity on mortality rates, growth performance, and carcass traits in pigs. R Bras Zootec 2017;46:856–62. doi:10.1590/S1806-92902017001100004.

[2] Gardner DS, Buttery PJ, Daniel Z, Symonds ME. Factors affecting birth weight in sheep: maternal environment. Reproduction 2007;133:297–307. doi:10.1530/REP-06-0042.

[3] Kertz AF, Reutzel LF, Barton BA, Ely RL. Body weight, body condition score, and wither height of prepartum holstein cows and birth weight and sex of calves by parity: a database and summary. J Dairy Sci 1997;80:525–9. doi:10.3168/jds.S0022-0302(97)75966-6.

[4] Shah PS. Parity and low birth weight and preterm birth: a systematic review and meta-analyses. Acta Obstetr Gynecol Scandinav 2010;89:862–75. doi:10.3109/00016349.2010.486827.

[5] Hinkle SN, Albert PS, Mendola P, Sjaarda LA, Yeung E, Boghossian NS, et al. The association between parity and birthweight in a longitudinal consecutive pregnancy cohort. Paediatr Perinat Epidemiol 2014;28:106–15. doi:10.1111/ppe.12099.

[6] Derisoud E, Auclair-Ronzaud J, Palmer E, Robles M, Chavatte-Palmer P, Derisoud E, et al. Female age and parity in horses: How and why does it matter? Reprod Fertil Dev 2021;34:52–116. doi:10.1071/RD21267.

[7] Hintz RL, Hintz HF, Vleck LDV. Growth rate of Thoroughbreds. Effect of age of dam, year and month of birth, and sex of foal. J Anim Sci 1979;48:480–7.

[8] Meirelles MG, Veras MM, Alonso MA, de Fátima Guimarães C, Nichi M, Fernandes CB. Influence of maternal age and parity on placental structure and foal characteristics from birth up to 2 years of age. J Equine Vet Sci 2017;56:68–79. doi:10.1016/j.jevs.2017.03.226.

[9] Robles M, Dubois C, Gautier C, Dahirel M, Guenon I, Bouraima-Lelong H, et al. Maternal parity affects placental development, growth and metabolism of foals until 1 year and a half. Theriogenology 2018;108:321–30. doi:10.1016/j.theriogenology.2017.12.019.

[10] Martin-Rosset W, Younge B. Energy and protein requirements and feeding of the suckling foal. Nutrition and feeding of the broodmare. Netherlands : Wageningen Academic Publishers; 2006.

[11] Perkins GA, Wagner B. The development of equine immunity: Current knowledge on immunology in the young horse. Equine Vet J 2015;47:267–74. doi:10.1111/evj.12387.

[12] Csapó J, Stefler J, Martin TG, Makray S, Zs Csapó-Kiss. Composition of mares' colostrum and milk. Fat content, fatty acid composition and vitamin content. Int Dairy J 1995;5:393–402. doi:10.1016/0958-6946(94)00008-D.

[13] Salamon RV, Salamon SZ. Csapó-Kiss Zs, Csapó J. Composition of mare's colostrum and milk I. Fat content, fatty acid composition and vitamin contents. Acta Univ Sapientiae. Alimentaria 2009;2:119–31.

[14] Barreto ÍM, Urbano SA, Oliveira CA, Macêdo CS, Borba LH, Chags BM, et al. Chemical composition and lipid profile of mare colostrum and milk of the quarter horse breed. PLoS One 2020;15:e0238921.

[15] Oftedal OT, Hintz HF, Schryver HF. Lactation in horse : milk composition and intake by foals. J Nutr 1983;113:2196–206.

[16] Doreau M. Le lait de jument et sa production : particularités et facteurs de variation. Lait 1994;74:401–18. doi:10.1051/lait:1994633.

[17] Pikul J, Wójtowski J, Danków R, Kuczynska B, Lojek J. Fat content and fatty acids profile of colostrum and milk of primitive Konik horses (Equus caballus gmelini Ant.) during six months of lactation. J Dairy Res 2008;75:302.

[18] Gibbs P, Potter G, Blake R, McMullan W. Milk production of quarter horse mares during 150 days of lactation 1982;54:496–9. https://doi.org/10.2527/jas1982.543496x.

[19] Ramin A, Bayrami M, Ramin S, Kalantry L. Relationships among urea, protein and macro-mineral concentrations in colostrums and whole milk in lactating mares. Int J Res Appl Basic Med Sci 2018;4:15–22.

[20] Doreau M, Martuzzi F. Milk yield of nursing and dairy mares, 120. Publication-EAAP; 2006. p. 57.

[21] Doreau M, Boulot S, Martin-Rosset W. Effect of parity and physiological state on intake, milk production and blood parameters in lactating mares differing in body size. Anim Sci 1991;53:111–18.

[22] Trottier NL, Stamper T, Moore J, Liesman J, Nielsen BD. A weigh-suckle-weigh approach for estimating milk yield in the Arabian mare. EAAP Publication 2006;120:163–5 2006-06-01.

[23] Pool-Anderson K, Raub RH, Warren JA. Maternal influences on growth and development of full-sibling foals2. J Anim Sci 1994;72:1661–6. doi:10.2527/1994.7271661x.

[24] Auclair-Ronzaud J, Jaffrézic F, Wimel L, Dubois C, Laloë D, Chavatte-Palmer P. Estimation of milk production in suckling mares and factors influencing their milk yield. Animals 2022;16:100498. doi:10.1016/j.animal.2022.100498.

[25] Arnaud MG, Dubroeucq MH, Rivot MD. Notation de l'état corporel des chevaux de selle et de sport: guide pratique. 1ère. Paris: Institut de l'élevage; 1997.

[26] Chavatte P, Clement F, Cash R. Field determination of colostrum quality by using a novel, practical method. Proceed Ann Convent Am Associat Equine Pract 1998;44:5.

[27] Clement F, Arnaud G, Chavatte-Palmer P. COLOTEST and colostrum bank for horse breeding - new data for 1999. Equ'idee - Bulletin d'Information Sur Les Equides 1999:38–43.

[28] Doreau M, Boulot S. Recent knowledge on mare milk production: A review. Livestock Product Sci 1989;22:213–35. doi:10.1016/0301-6226(89)90057-2.

[29] Morel P, Bokor Á, Rogers C, Firth E. Growth curves from birth to weaning for Thoroughbred foals raised on pasture. New Zealand Vet J 2007;55:319–25. doi:10.1080/00480169.2007.36788.

[30] Folch J, Lees M, Stanley GHS. A simple method for the isolation and purification of total lipides from animal tissues. J Biol Chem 1957;226:497–509. doi:10.1016/S0021-9258(18)64849-5.

[31] Morrison WR, Smith LM. Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride–methanol. J Lipid Res 1964;5:600–8. doi:10.1016/S0022-2275(20)40190-7.

[32] R Core Team A language and environment for statistical computing. Vienna, Austria: R foundation for Statistical Computing; 2020.

[33] Rstudio Team. RStudio: Integrated Development for R. 2020.

[34] Giraudoux P. PGIRMESS: spatial analysis and data mining for fiels ecologists 2021.

[35] R Core Team, Pinheiro J, Bates D, DebRoy S, Sarkar D, NLME: linear and nonlinear mixed effects models 2021.

[36] Kenzig AR, O'Meara KM, Kremer CJ, Jogan KS, Jack NE, Colostral Cole K. Milk and Serum Immunoglobulin G Concentrations in Quarter Horse Mares and Their Foals. J Equine Vet Sci 2009;29:486–7. doi:10.1016/j.jevs.2009.04.177.

[37] Morris DD, Meirs DA, Merryman GS. Passive transfer failure in horses: incidence and causative factors on a breeding farm. Am J Vet Res 1985;46:2294–9.

[38] Raidal SL. The incidence and consequences of failure of passive transfer of immunity on a Thoroughbred breeding farm. Australian Vet J 1996;73:201–6. doi:10.1111/j.1751-0813.1996.tb10035.x.

[39] Erhard MH, Luft C, Remler H-P, Stangassinger M. Assessment of colostral transfer and systemic availability of immunoglobulin G in new-born foals using a newly developed enzyme-linked immunosorbent assay (ELISA) system. J Anim Physiol Anim Nutr 2001;85:164–73. doi:10.1046/j.1439-0396.2001.00313.x.

[40] Franco Ayala MS, Oliver-Espinosa OJ. Risk factors associated with failure of passive transfer of colostral immunoglobulins in neonatal Paso Fino foals. J Equine Vet Sci 2016;44:100–4. doi:10.1016/j.jevs.2016.05.006.

[41] Argov-Argaman N, Mandel D, Lubetzky R, Hausman Kedem M, Cohen B-C, Berkovitz Z, et al. Human milk fatty acids composition is affected by maternal age. J Matern Fetal Neonatal Med 2017;30:34–7. doi:10.3109/14767058.2016.1140142.

[42] Sinanoglou VJ, Cavouras D, Boutsikou T, Briana DD, Lantzouraki DZ, Paliatsiou S, et al. Factors affecting human colostrum fatty acid profile: a case study. PLoS One 2017;12:e0175817. doi:10.1371/journal.pone.0175817.

[43] Guxens M, Mendez MA, Moltó-Puigmartí C, Julvez J, García-Esteban R, Forns J, et al. Breastfeeding, long-chain polyunsaturated fatty acids in colostrum, and infant mental development. Pediatrics 2011;128:e880–9. doi:10.1542/peds.2010-1633.

[44] Samková E, Špička J, Pešek M, Pelikánová T, Hanuš O. Animal factors affecting fatty acid composition of cow milk fat: a review. South Af J Anim Sci 2012;42:83–100. doi:10.4314/sajas.v42i2.1.

[45] Bilal G, Cue RI, Mustafa AF, Hayes JF. Effects of parity, age at calving and stage of lactation on fatty acid composition of milk in Canadian Holsteins. Can J Anim Sci 2014;94:401–10. doi:10.4141/cjas2013-172.

[46] Miliku K, Duan QL, Moraes TJ, Becker AB, Mandhane PJ, Turvey SE, et al. Human milk fatty acid composition is associated with dietary, genetic, sociodemographic, and environmental factors in the CHILD Cohort Study. Am J Clin Nutr 2019;110:1370–83. doi:10.1093/ajcn/nqz229.

[47] Lubetzky R, Sever O, Mimouni FB, Mandel D. Human milk macronutrients content: effect of advanced maternal age. Breastfeed Med 2015;10:433–6. doi:10.1089/bfm.2015.0072.

[48] Doreau M, Bauchart D, Chilliard Y, Doreau M, Bauchart D, Chilliard Y. Enhancing fatty acid composition of milk and meat through animal feeding1. Anim Prod Sci 2010;51:19–29. doi:10.1071/AN10043.

[49] Sauerwald TU, Demmelmair H, Koletzko B. Polyunsaturated fatty acid supply with human milk. Lipids 2001;36:991–6. doi:10.1007/s11745-001-0810-9.

[50] Heidler B, Aurich JE, Pohl W, Aurich CHR. Body weight of mares and foals, estrous cycles and plasma glucose concentration in lactating and non-lactating Lipizzaner mares. Theriogenology 2004;61:883–93. doi:10.1016/S0093-691X(03)00279-6.

[51] Robb J, Harper RB, Hintz HF, Reid JT, Lowe JE, Schryver HF, et al. Chemical composition and energy value of the body, fatty acid composition of adipose tissue, and liver and kidney size in the horse. Anim Sci 1972;14:25–34. doi:10.1017/S0003356100000246.

[52] Chilliard Y, Ferlay A, Rouel J, Lamberet G. A review of nutritional and physiological factors affecting goat milk lipid synthesis and lipolysis1. J Dairy Sci 2003;86:1751–70. doi:10.3168/jds.S0022-0302(03)73761-8.

[53] Chilliard Y, Ferlay A. Dietary lipids and forages interactions on cow and goat milk fatty acid composition and sensory properties. Reprod Nutr Dev 2004;44:467–92. doi:10.1051/rnd:2004052.

[54] Sorensen A, Muir DD, Knight CH. Thrice-daily milking throughout lactation maintains epithelial integrity and thereby improves milk protein quality. J Dairy Res 2001;68:15–25. doi:10.1017/S0022029900004593.

[55] Linzell JL, Peaker M. Changes in colostrum composition and in the permeability of the mammary epithelium at about the time of parturition in the goat. J Physiol 1974;243:129–51. doi:10.1113/jphysiol.1974.sp010746.

[56] Tsioulpas A, Grandison AS, Lewis MJ. Changes in physical properties of bovine milk from the colostrum period to early lactation. J Dairy Sci 2007;90:5012–17. doi:10.3168/jds.2007-0192.

[57] Rouse BT, Ingram DG. The total protein and immunoglobulin profile of equine colostrum and milk. Immunology 1970;19:901–7.

[58] Zs Csapó-Kiss, Stefler J, Martin TG, Makray S, Csapó J. Composition of

mares' colostrum and milk. Protein content, amino acid composition and contents of macro and micro-elements. Int Dairy J 1995;5:403–15. doi:10.1016/0958-6946(94)00014-G.
[59] Elliott C, Morton J, Chopin J. Factors affecting foal birth weight in Thoroughbred horses. Theriogenology 2009;71:683–9. doi:10.1016/j.theriogenology.2008.09.041.
[60] Bauman DE, Brown RE, Davis CL. Pathways of fatty acid synthesis and reducing equivalent generation in mammary gland of rat, sow, and cow. Arch Biochem Biophys 1970;140:237–44. doi:10.1016/0003-9861(70)90028-7.
[61] Fidler N, Koletzko B. The fatty acid composition of human colostrum. Eur J Nutr 2000;39:31–7. doi:10.1007/s003940050073.
[62] Jingar S, Mehla RK, Singh M, Roy AK. Lactation curve pattern and prediction of milk production performance in crossbred cows. J Vet Med 2014;2014:814768. doi:10.1155/2014/814768.
[63] Atashi H, Asaadi A. Association between gestation length and lactation performance, lactation curve, calf birth weight and dystocia in Holstein dairy cows in Iran. Anim Reproduct 2019;16:846–52.
[64] Sitkowska B, Kolenda M, Piwczyński D. Comparison of the fit of automatic milking system and test-day records with the use of lactation curves. Asian-Aust J Anim Sci 2019;33:408–15. doi:10.5713/ajas.19.0190.
[65] Masía FM, Lyons NA, Piccardi M, Balzarini M, Hovey RC, Garcia SC. Modeling variability of the lactation curves of cows in automated milking systems. J Dairy Sci 2020;103:8189–96. doi:10.3168/jds.2019-17962.
[66] Marumo J, Lusseau D, Speakman J, Mackie M, Hambly C. Influence of environmental factors and parity on milk yield dynamics in barn-housed dairy cattle. J Dairy Sci 2022;105:1225–41.