Clinical Department for Farm Animals and
Food System Science

Clinical Centre for Ruminant and Camelid Medicine
(Head: Univ.-Prof. Dr. med. vet. Thomas Wittek, Dipl. ECBHM)

Correlation between immunoglobulin concentration and somatic cell count in bovine colostrum

Thesis

University of Veterinary Medicine Vienna

Submitted by

Li Kayl

Vienna, April 2024

Supervisors:
Univ.-Prof. Dr. med. vet. Thomas Wittek, Dipl. ECBHM
Dr. med. vet. Katharina Lichtmannsperger, Dipl. ECBHM

Clinical Department for Farm Animals and Food System Science
Clinical Centre for Ruminant and Camelid Medicine
Veterinaerplatz 1
1210 Vienna

Assessor: Mag. med. vet. Viktoria Neubauer, PhD.

Declaration of independence

In this thesis no other resources and references than those mentioned have been included. The decisive work has been carried out by the student, Li Kayl, herself and all contributors to the work have been named. The thesis submitted for assessment has been written independently and has not been submitted or published elsewhere.

Vienna, April 2024 Li Kayl

Table of contents

1. Abstract

1.1 Abstract English

This study investigated colostrum quality in terms of immunoglobulin concentrations and the somatic cell count (SCC). The focus was on the SCC of the milk *ante* and *post-partum* as well as of the colostrum. The 947 colostrum samples were collected from 72 farms of different sizes in the Austrian province of Salzburg. Of these 72 farms, 47 were members of the National Milk Recording Association (LKV). In cooperation with the Salzburg Animal Health Service (TGD), the data from the farms with a LKV-membership was anonymised and used for further data processing. A mobile cell counter (DeLaval cellcounter DCC®, DeLaval, Sweden), based on the principle of optical fluorescence, was used to determine the SCC of the colostrum samples. The total protein content was measured using a digital Brix refractometer. Comparative and descriptive statistics were used to compare the results of the samples with corresponding milk recordings of the LKV database. The cows were divided into two groups based on milk quality. Animals with an SCC ≤400 cells/µl belonged to the good milk quality group. The lower milk quality group was composed of those with an SCC >400 cells/µl. The correlations between milk quality *ante* and *post-partum* and colostrum, and between immunoglobulin levels and SCC in colostrum were calculated. The latter was determined with the Spearman coefficient as -0.2. The value indicated a negative relationship, but was considered very weak. Further studies and more balanced cohorts are recommended to analyse this relationship in more detail.

1.2 Abstract Deutsch

Die vorliegende Arbeit beschäftigte sich mit dem Thema der Kolostrum Qualität in Bezug auf Immunglobulingehalt und somatischer Zellzahl (SCC). Der Fokus lag auf der SCC in der Milch *ante* und *post-partum* sowie jene im Kolostrum. Es wurden 947 Kolostrumproben von 72 Betrieben unterschiedlicher Größen im österreichischen Bundesland Salzburg gesammelt. Von diesen 72 Betrieben waren 47 Mitglieder des Landeskontrollverbands Salzburg (LKV). In Zusammenarbeit mit dem Tiergesundheitsdienst Salzburg (TGD) wurden die Daten der 47 LKV-Betriebe anonymisiert übermittelt und konnten so zur weiteren Datenverarbeitung herangezogen werden. Zu der Bestimmung der SCC der 1.051 Kolostrumproben wurde ein mobiles Zellzahlmessgerät (DeLaval cellcounter DCC®, DeLaval, Schweden) eingesetzt, welches auf dem Prinzip der optischen Fluoreszenz beruht. Durch vergleichende und deskriptive Statistik wurden die entsprechenden Resultate der Proben mit der Datenbank des LKVs verglichen. Die Kühe wurden basierend auf der Milchqualität in zwei Gruppen eingeteilt. Tiere welche eine SCC ≤400 Zellen/µl aufwiesen, gehörten zur Gruppe der guten Milchqualität. Die zweite Gruppe entsprach jenen Proben die sich >400 Zellen/µl befanden und somit einer niedrigeren Milchqualität entsprachen. Der Totalproteingehalt wurde mit einem digitalen Brixrefraktometer gemessen. Es wurden schließlich drei Korrelationen berechnet, betreffend der Milchqualität *ante* und *post partum* und dem Kolostrum sowie zwischen den Immunglobulinwerten und der SCC im Kolostrum. Letztere wurde mit dem Spearman Koeffizienten als -0,2 bestimmt. Der Wert deutete auf eine negative Beziehung der beiden Werte hin, war jedoch als sehr schwach einzustufen. Weitere Untersuchungen und ausgeglichenere Kohorten werden empfohlen, um diese Beziehung genauer zu analysieren.

2. Introduction and literature review

The first milk produced in the mammary gland around the event of birth is called colostrum. It plays an essential role for the newborn calf and its development later on [1]. It contains a wide range of important components, but in concentrations, which vary significantly in comparison to regular milk. Each group of components carries its own importance for the development and health of the neonatal calf, which are described in greater detail below.

2.1 Macronutrients

2.1.1 Fat

The fat content of colostrum can vary greatly, but in general it can be stated that the colostral fat content is higher compared to regular milk [2]. Expressed in figures, this means a fat content of 7.0% - 18.0% [3] compared to 3.6-4.0% in regular milk [1]. Colostrum and regular milk have specific fatty-acid (FA) profiles. It was found that long-chain FA are found in high concentrations in colostrum, in contrast to short-chain FA. More precisely, colostrum is over 25% richer in n-3 and n-6 polyunsaturated FA as well as palmitic, myristic and linoleic acid for 16%, 27%, and 13% respectively. There are 50% and 60% less C4:0 and C6:0 short-chained FA. Colostrum is also poorer in branched-chain FA by 13% and *trans*-monounsaturated FA by 15% compared to regular milk. It was also found that parity had a significant influence on the FA profile, possibly related to different energy requirements. In high-yielding cows, it is not untypical, that up to 40 kg of body fat is mobilised in the peripartum period, which leads to an elevated amount of pre-formed FA in the milk. The higher *post-partum* feed consumption boosts the production of volatile FA through ruminal microbial fermentation, which serves as a substrate for de novo FA production in the mammary gland [4]. Short-chain fatty acids, stearic acid and oleic acid are present in low concentrations in the first week *post-partum* and increase with the ongoing lactation [2]. In addition, lipids are said to have health benefits ranging from immunomodulation (e.g. oleic acid), neuronal development, to pathogen-binding (gangliosides, phospholipids) [1]. From a dietary point of view, colostrum is also richer in high levels of fat to compensate for the lack of thermoregulation capacity in neonates as well as an energy source [3,5].

2.1.2 Lactose

In contrast to other substances, lactose is found in lower concentrations in colostrum than in regular milk. Bovine colostrum contains 1.4-3.0% of lactose [6], whereas bovine milk is comprised of almost double as much lactose with 4.7-5.0% [1]. The quantity increases steadily as the lactation progresses and should generally return to normal levels within the first seven days *post-partum*. Due to the strong osmotic properties of lactose, it significantly influences the water content of the milk. This also explains the high viscosity of the colostrum. Due to the lower lactose content, less water is drawn into the milk and the colostrum acquires its typical thickness [2].

2.1.3 Proteins

The protein levels in bovine colostrum are higher (13.9-21.8%) [6] than in regular milk (around 3%) [1] , primarily due to the substantial quantity of immunoglobulins and caseins present. The proteins can be divided into two groups, the distinction between the two being in their solubility. The whey proteins are soluble and comprise the immunoglobulins, which will be described in further detail in a separate section, lactoferrin, α-lactalbumin, β-lactoglobulin, lactoperoxidase, glycol-macropeptide and a multitude of growth factors. Besides their nutritional benefit, they are biologically active, a selected few of them only being activated by exposition to acid or being partially digested. These biological functions range from reduction of inflammation to repair stimulation. The second group is composed of insoluble proteins called caseins. In bovine colostrum, they make up 4.8% and decrease *post-partum*. In regular milk they represent 2.5-2.6%. Important functions of caseins, besides being a provider of energy, are of the immunological sort, comprising antimicrobial, antiphlogistic and immunoregulating characteristics [1,2].

2.2 Micronutrients

2.2.1 Minerals

Colostrum is richer in minerals than regular bovine milk. Zinc, phosphorus, and calcium are represented in the highest concentrations with 11.6-38.1 mg/kg, 4.5 g/kg and 2.6-4.7 g/kg respectively in colostrum. In milk the same minerals are concentrated lower with values of 3.0-6.0 mg/kg, 0.9-1.2 g/kg, and 1.2-1.3 g/kg, respectively. The minerals iron, copper, magnesium, and manganese are also comprised on higher levels in colostrum than regular milk and contribute as well to the neonatal health [1].

2.2.2 Vitamins

The vitamin levels are also higher in colostrum compared to milk and are present in two different groups: fat-soluble and water-soluble vitamins [1]. Vitamins A, D, E and K are fat-soluble vitamins, Vitamin B1, B2, B3 and B12 are the water-soluble group. They play a significant role in relation to the immune system, bone growth, and are also associated with antioxidative properties [1,7].

2.3 Immunoglobulins

Due to the specific synepitheliochorial structure of the bovine placenta [8], the transfer of any immunoglobulins is almost impossible. Therefore, bovine neonates are born agammaglobulinemic, meaning without any immune defence passed on by the cow *in utero* [9]. Immunoglobulins are proteins in the colostrum which are absorbed through the mucosa of the intestine of the neonates upon digestion of the first milk [10] and are considered as a temporary immune defence passed on by the dam. The antibodies enter into the lymphatic system and continue into the bloodstream through the thoracic duct [10], providing protection against all sorts of pathogens [11]. This kind of immunity will persist for up to three weeks until the calf is able to develop its own active immune system [12]. Bovine immunoglobulins (Ig) can be sorted into three different types, carrying out different roles as antibodies in the neonates system: IgA, IgG and IgM. Immunoglobulin G represents by far the largest group, taking up approximately 88.0% [13] and can be distinguished into two subtypes IgG1 and IgG2,

immunoglobulin G1 being the dominant subgroup with 75.0% [13,14]. IgG can be measured in the calf's blood, more precisely in the serum, one day after feeding it colostrum, to check the success or failure of the passive-immunity transfer [12]. The concentration of IgG in colostrum can differ a lot depending on a multitude of factors like parity, breed, quantity of colostrum, herd size, and management [13,15,16]. Proper colostrum management is crucial for successful transfer of passive immunity. Starting with the collection of the first milk as soon as possible *post-partum* to ensure a maximum IgG concentration available to the neonate [16]. It is best for the newborn to have its first colostrum intake close after its birth or as soon as possible within the first few hours of life [10,16], as the absorption capacity of the intestinal tract decreases more and more rapidly after 6 hours of being born [17] and ends entirely at 24 hours after birth [10] . It is important that the neonate ingests the sufficient quantity of colostrum, at least 2 litres, to ensure the intake of enough Ig [18]. At the same time, the quality is a factor not to be neglected. It is equally relevant to administer good quality colostrum to assure a successful absorption of enough Ig and transfer of passive immunity [19].

2.4. Cells

Another important component of colostrum is its SCC. Langel et al. (2015), found that cells in colostrum do play a role in the calf's immune system. It was shown that calves fed cell-free milk had fewer CD4+ T-cells present [20]. The SCC, like the amount of Ig, is dependent on multiple factors such as infectious status, age days in milk, season, and multiple other reasons [21]. It is one of the indicators expressing the cow's state of health, more precisely the udder health. This parameter is widely used to determine the milk quality and is a very helpful tool to detect subclinical and clinical mastitis [22]. Furthermore it has been proven, that an increased SCC correlates with a decline in the amount of milk produced by the animal, which equals to a significant economic loss for the affected farmer [21]. The composition of the somatic cell population, also called differential somatic cell count (DSCC), can vary significantly depending on the status of the cow. The cells that are detected are primarily immune cells such as lymphocytes, macrophages, and polymorphonuclear neutrophils (PMN) [23]. The difference is made between cows in their lactating period and cows secreting colostrum. For the former, a conspicuous amount of the cells are of the macrophage type. In colostrum, a significant surge

of PMN can be observed without a simultaneous sign of infection. The cells that are omnipresent in both of these groups are the lymphocytes. The most important task of these cells is the local protection of the udder tissue and intestines of the newborn calf [24]. The DSCC can be used to differentiate between regular milk and mastitis milk. In an infected udder, a surge in PMN or, in the chronic stage, a constantly increased PMN level is measured. In a healthy udder of a lactating cow, lymphocytes and macrophages are dominant [23].

Objective

The SCC, immunoglobulins, and milk quality are frequently analyzed topics in bovine medicine. While there are several sources available for reference, there is limited information specifically on this subject area in Austria. The aim of this study was to measure the somatic cell count (SCC) and immunoglobulin concentration in bovine colostrum, and to investigate the association between the amount of immunoglobulins and SCC in bovine colostrum. Additionally, the study aimed to provide insights into the colostrum and/or milk quality management situation in Salzburg, Austria.

Hypothesis

The hypothesis of this study was that there is a correlation between the immunoglobulin concentration and SCC in bovine colostrum.

3. Material and methods

3.1 Study population

The study consisted of three distinct study populations. The first population was limited to 47 farms that were members of the National Milk Recording Association “Landeskontrollverband” (LKV), and data was analyzed on a herd-level. In this study, the term 'herd' refers to the sum of dried-off cows and lactating cows. The second population consisted of the same cows from the LKV-farms, that provided the colostrum samples, and associated SCC LKV-data. These SCC results are based on the LKV-database and correspond to the milk samples taken *ante* and *post-partum*. The third population consists of 947 colostrum samples, which were measured using the DeLaval cell counter (the method is explained in detail below). The samples were collected from 72 different farms, including the 47 LKV farms mentioned above, in the federal state of Salzburg, Austria. The animal health service Tiergesundheitsdienst (TGD) Salzburg kindly forwarded the data from the LKV recordings of the farms anonymously.

3.2 Sample collection

The farmer harvested the samples from the first milking of each cow post-partum, following the standard operating procedure provided by the TGD Salzburg. Each farm and cow were assigned a number, which was marked on the sample tube and recorded in the LKV database to ensure anonymity. The required 15.0 ml of colostrum were poured, not milked directly, into a sample tube and immediately frozen on the farm at -18.0°C. The samples were then transported and stored at -80.0°C at the University Clinic of Ruminants in Vienna. To prepare for testing, the samples were thawed in a refrigerator at a temperature between 4.0 °C and 8.0 °C approximately one day before testing.

3.3 Assessment of SCC and immunoglobulin levels

3.3.1 DeLaval measurement of SCC

For the measurement of the SCC contained in the assays, a portable cellcounting machine (DeLaval cellcounter DCC®, DeLaval, Sweden) based on the principle of optical fluorescence was used. The machine used propidiumiodide, which binds to the DNA present in the

colostrum. The somatic cells were then counted by Charged Coupled Devices. The Light Emitting Diodes (LED) emit a wavelength of 540.0 nm and the absorption wave length to 620.0 nm. Between the LEDs and the sensor an absorption filter is installed, enabling only emitting light to pass. The measurement process started with the defrosted test tube being vortexed for 3 seconds. After having made sure that the liquid is well homogenised, five to seven of the colostrum sample were put inside of the cap of the test tube. This method proved to be the most convenient for the following step. The sample was soaked up manually by the cassette corresponding to the cell counter. It contained approximately 60.0 µl of the sample as well as the necessary chemicals for the cell measuring. Here it was important that the tip of the cassette was put deep enough into the liquid, otherwise air would be sucked up and the results would be falsified and could not be measured. The tip of the cassette was cleaned off before putting it into the device. The measured volume was 1.0 µl, meaning 100 to 400 cells would be counted. As the results of the SCC measured by the DeLaval cellcounter DCC® were assessed in µl, it was decided to represent all the data in the unit cells/µl in this project. Some samples were too thick in consistency, which is not unusual for colostrum, and were impossible to measure. If after two measurements, the error message persisted, the sample was marked down as not measurable (n. m.) and thus would not be included in the results.

3.3.2 Immunoglobulin concentration

The measurement of the immunoglobulin concentration was part of another study that used the same colostrum samples. A digital Brix refractometer (0 to 85% Brix; HM-DREF-1®, Hebesberger Messtechnik, Neuhofen, Austria) was used for this purpose. Using a disposable plastic pipette with a capacity of 2.0 ml, the colostrum was applied to the prism and the Brix percentage was obtained for the total protein content, of which the IgG content could be deduced [25].

3.4 Statistical methods

3.4.1 DeLaval data

Overall, 1,051 samples were sent in, of which 1,010 were measured with the DeLaval cellcounter DCC®. There were 41 samples missing. They could not be located and thus not measured. During the measurement process, 63 samples were impossible to evaluate due to excessive thickness of the colostrum and were therefore excluded from the sample pool. This left 947 samples and respective measurements assessed by the DeLaval cellcounter. Descriptive statistics were carried out and values are shown as the median 25^{th} and 75^{th} percentiles, minimum and maximum. The results of the SCC were logarithmised with the basis of ten to better represent the large numbers of SCC values.

3.4.2. LKV data

As only 47 of the 72 the contributing farms were LKV-members, the data for comparisons between the SCC of colostrum measured by the DeLaval cellcounter DCC® and *ante* or *post-partum* milk samples was limited to the data sets of these 47 farms. The LKV data records contained numerous information on each cow regarding breed, calving date, number of lactations, milk composition of the sample milkings, as well as milk yield and SCC among other records. Only the data regarding the breed, number of lactations (primiparous vs. multiparous), milk yield (herd-level, selected individual cows), and SCC (herd-level, individual cows) were used in this study. The dry period was also recorded. This information was used to document the SCC determined in the last milk test before and the first milk test (German: Milchleistungsprüfung, MLP) after the calving, corresponding to four to five weeks before the dry period and six weeks after the calving. In addition, the average SCC of the three MLPs *ante partum* and of the three MLPs *post-partum* were calculated, each over a period of three months. The corresponding colostrum SCC measured in this study was then assigned to the appropriate cow.

The LKV results of 122 cows needed to be excluded from the analysis due to error in the data assessment. For ten of the 947 samples, the cow was dried up again before three consecutive MLPs could be carried out *post-partum* and hence disabling the possibility to calculate the

average SCC *post-partum*. In 86 cases, one or several MLP results were missing *ante* and/ or *post-partum*. One farm presented with data missing for one or more of the reasons listed above, for all 26 of its cows. The amount of the colostrum SCC data that ultimately remained for analysis, with corresponding LKV results to compare, equaled 332 samples. Another major reason for this was that 25 of the 72 farms that have sent in samples are not LKV-members.

According to the AgrarMarkt Austria (AMA), the highest quality level for raw milk, called S-class, is <250 cells/µl. The second highest quality level, allows up to 400 somatic cells/ µl, the third and last classification starts at >400 cells/ µl [26]. The cut-off point for good milk quality in this study, was set at 400 cells/µl, because it represented the limit for the second highest quality level of the AMA for raw milk. The study of Puppel and co-workers (2020) also used these cut-off levels [27].

In order to determine whether there was a correlation between the immunoglobulin concentration and the SCC in colostrum and what kind of association, if any, existed between the two variables, Spearman rank correlation coefficient was calculated, since the Brix values and the SCC were not normally distributed ($p < 0.05$, Kolmogorov–Smirnov test including Lilliefors correction). Overall, significant level was set at $p < 0.05$.

4. Results

4.1 DeLaval cellcounter results

The average SCC of the measured colostrum samples was to 1,091 cells/µl (SD= 1,052 cells/µl). In a comparative overview, Table 1 contains the data of all the 72 farms, that sent in greater or equal to 10 colostrum samples. Of the 72 farms in total, 39 farms provided a minimum of ten colostrum samples. The average SCC on each farm is presented, as well as the median, minimum, maximum and the 25th and 75th percentiles.

Table 1: Overview of SCC in cells/µl of farms that sent in 10 or more individual colostrum samples, sorted from lowest to highest median. The standard deviation is abbreviated as SD.

Farm	Mean	SD	Median	Minimum	Maximum	25 Percentile	75 Percentile
69	325	155	328	43	594	227	458
57	573	649	346	4	2,618	127	793
24	618	669	361	4	2,688	185	979
11	519	417	382	59	1,515	202	824
4	614	642	391	73	2,547	176	730
30	631	872	415	72	3,023	145	633
65	626	672	450	12	3,281	216	776
18	589	720	489	150	1,737	244	735
22	589	466	489	150	1,737	244	735
84	607	596	496	109	2,049	168	820
25	657	572	505	96	1,845	219	1,036
14	821	824	534	37	3,597	307	1,046
87	529	297	541	5	1,117	270	683
29	844	991	594	6	4,318	336	1,008
54	858	723	611	81	3,344	381	1,320
59	1,014	1,115	641	0	2,930	83	2,146
85	787	614	647	9	2,105	246	1,209
3	1,491	1,648	707	21	5,347	213	2,821
41	1,009	951	731	152	3,279	245	1,678
79	1,308	1,405	763	196	3,907	259	2,750
53	1,085	854	813	267	3,502	527	1,313
55	1,245	1,236	835	214	4,262	508	1,527

51	1,585	1,510	853	94	4,180	366	3,232
2	1,070	922	856	2	3,825	338	1,528
31	984	816	858	4	3,665	354	1,549
71	1,395	1,291	895	355	4,445	467	2,168
17	1,235	994	905	231	3,707	644	1,608
50	1,149	785	952	278	2,423	425	2,001
56	1,368	1,125	995	45	4,328	558	2,035
7	1,373	1,207	996	0	4,031	294	2,055
21	1,446	1,087	1,103	11	3,918	642	2,028
60	1,456	1,294	1,108	0	4,542	377	2,286
8	1,779	1,521	1,192	206	5,465	775	2,924
27	1,555	1,163	1,245	105	3,779	767	2,214
38	2,132	1,472	1,467	750	4,717	1,020	3,574
13	1,863	1,215	1,769	182	4,976	838	2,598
19	1,651	959	1,806	114	3,159	771	2,468
5	1,880	1,244	1,969	112	4,176	831	2,605
90	1,154	1,209	3,748	480	4,325	583	4,092

The minimal colostrum SCC that was measured was 0 somatic cells/µl for three different samples. One of these samples originated from a farm that was also a member of the LKV. This enabled insight into more data related to the farm and details of the individual cow. This farm took care of 42 cows of which 21 were having their first calf. The mean SCC of this farm amounted to 99 cells/µl. According to the LKV data, the sampled cow in question was of the Fleckvieh breed. It was in its first lactation period with a milk production of 4,988.0 kg. The maximal SCC measured amounted to 5,465 somatic cells/µl. The farm linked to this sample was also a member of the LKV and had a herd size of 21 cows with one being a dam. The cow that provided the sample was in its fifth lactation period and of the Holstein Friesian breed with a milk production of 8,810.0 kg. The mean SCC of the farm was 323 cells/µl.

4.2 LKV results: herd level

The database included information on the breed or breed-mix represented in the herd, including dry and lactating cows. The data of 696 Fleckvieh cows was available. The second most represented breed was Holstein Friesians, with red or black colouring, counting 61 animals. Coming third was the Pinzgauer breed with 39 cows. 12 of the cattle were Swiss Brown and 6 were other breeds that were not further specified such as Ayrshire or Jersey. There were no mixed breed cows among the data pool that were of interest. The average milk yield of these cows amounted to a performance of 7,460 kilograms.

From Table 2 (Tab.2) it was apparent, that the majority of the participating LKV-farms were smaller businesses keeping 1 to 30 cows.

Table 2: Herd size of the LKV-farms, lactating and dried-off cows included, listed from lowest to highest number of cows.

Number of cows	Number of farms	Percentage of farms (%)
1-10	10	21.3
11-20	11	23.4
21-30	13	27.7
31-40	6	12.8
41-50	4	8.5
51-60	1	2.1
61-70	1	2.1
71-80	0	0.0
>80	1	2.1
Total	47	100.0

In terms of the amount of first calvings on each LKV-farm, the groups were rearranged in the following Table 3 . There was no information available for three farms on their frequency of first calvings. More than half of the farms had between 21.0% and 40.0% of their cows having their first calf during the observation period. In Figure 1 , the 47 farms are sorted from smallest to largest herd size. The first calvings are visualized in proportion to herd size.

Table 3: Percentage of first calvings in the herd.

First calvings (in %)	Number of farms
<10.0	1
11.0-20.0	9
21.0-30.0	12
31.0-40.0	14
41.0-50.0	7
>50.0	1
no information	3
Total	47

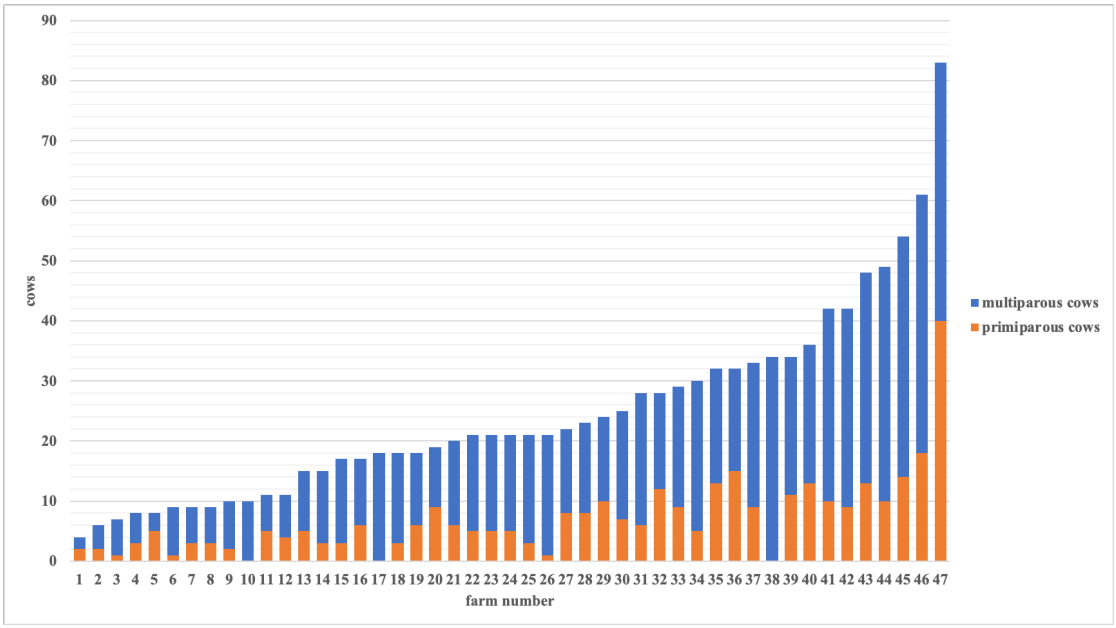

Figure 1: Parity sorted by herd size and for each LKV-farm, highlighting the diversity in the composition of the participating herds.

Taking into consideration the data on the herd SCC provided from the LKV data base, most of the farms didn't show elevated SCC results on a herd level. As shown in Table 4 (Tab. 4), 87.3% of the farms had an SCC of ≤ 200 cells/µl.

There were 41 of the 47 farms, which were assigned to the first category of the highest quality level of raw milk. The lowest result being 49 cells/µl and stretching up to 239 cells/µl. Two farms would be in the second highest quality class according to the AMA cut-offs, with values of 323 and 333 cells/µl. There was one single farm, that presented with an SCC of over 400 cells per µl, and thus falling into the last category.

Table 4: SCC at the level of the total herd of lactating cows.

Cell count (cells per µl)	Number of farms	Percentage of farms (%)
<100	16	34.1
100-200	25	53.2
200-300	5	10.6
>300	1	2.1
Total	47	100.0

4.3. LKV results: cow level

Regarding the data of the LKV, a few different results can be considered. The average SCC of the milk quality tests was calculated for the last three MLPs *ante partum* and the first three after the cow was being milked again *post-partum*. This data was set into relation to the SCC that was measured in the colostrum of the corresponding cow. There were a few outliers in both the MLP and colostrum count data. For Figure 2 the median and SD of the MLP data were 1.9 $\log_{10}$ (79 cells/µl) and 0.4 $\log_{10}$ (202 cells/µl) respectively. The MLP data in Figure 3 had a median of 1.7 $\log_{10}$ (51 cells/µl) and SD= 0.6 $\log_{10}$ (330 cells/µl).

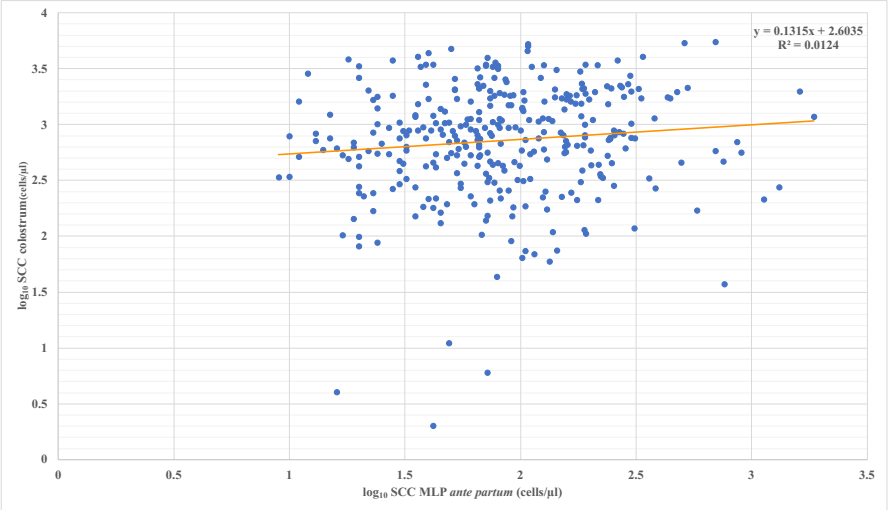

Figure 2: Scatterplot depicting the milk quality *ante partum*, calculated by averaging the last three SCC LKV-values before calving of each cow, to the corresponding colostrum sample.

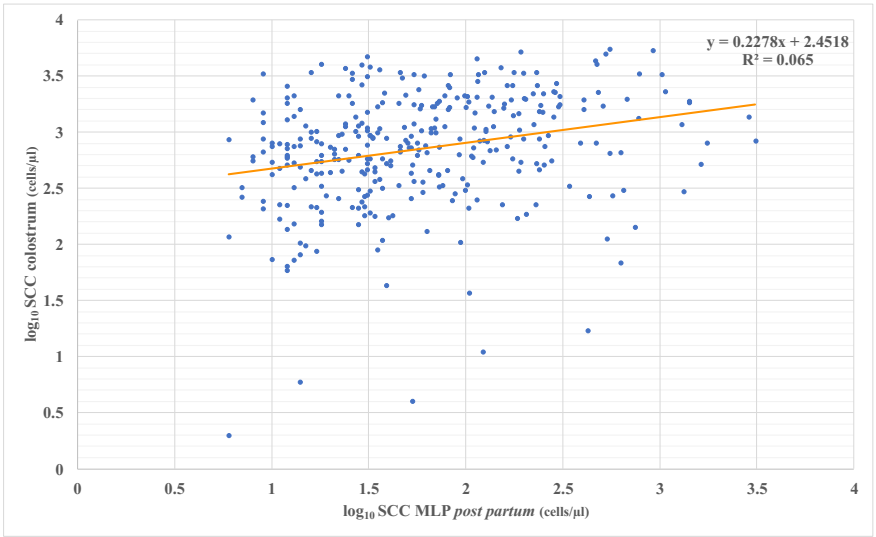

Figure 3: Scatterplot of the milk quality *post-partum* to the colostrum SCC.

By examining features such as dispersion and outliers of the data, the analysis highlighted the variability and patterns within the data pool (Fig. 4).

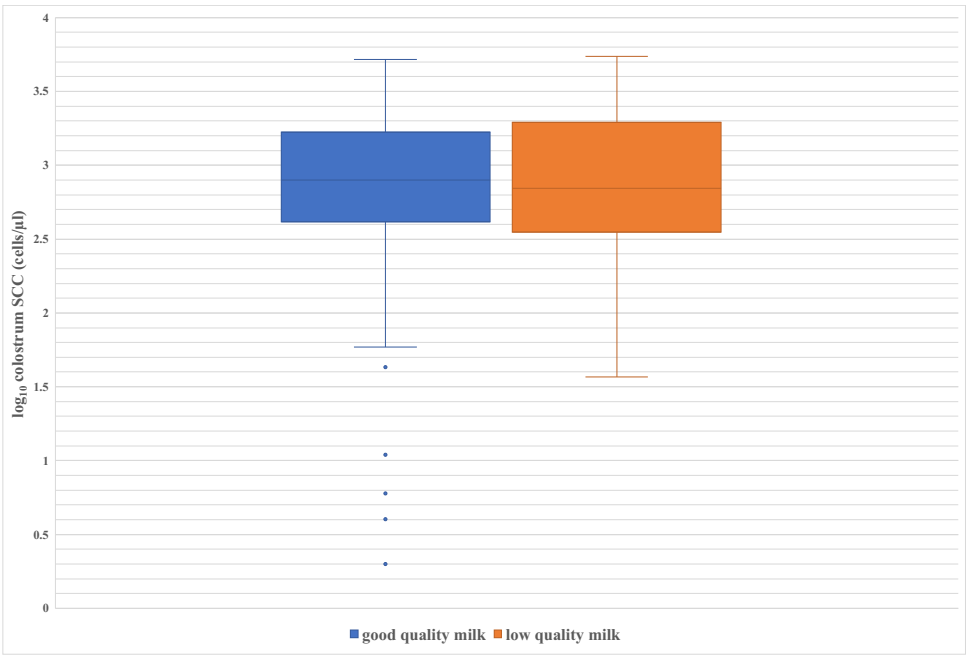

Figure 4: Boxplots with a median line inside the box, flanked by the first and third quartiles. The whiskers extend to the minimum and maximum data points within a specified range, excluding outliers. The boxplot analysis shows the distribution of colostrum SCC in two different categories of milk quality: a good milk quality group with an SCC *ante partum* of ≤400 cells/µl (GQM) and a second group with a lower milk quality (LQM) starting from >400 cells/µl *ante partum,* based on the MLP *ante partum* LKV-data.

Table 1: Characteristics of the two SCC groups in the boxplot (Fig.4), comparing LKV data of *ante partum* milk samples.

Group	GQM ≤400 cells/µl	LQM >400 cells/µl
n samples	315	17
median	796	697
25th quartile	394	363
75th quartile	1,676	1,960

In Figure 5 the data of the MLP SCC *ante partum* was reassembled with the groups being organized according to the colostrum SCC. For the group of good quality colostrum (GQC), the minimum and maximum cell counts were 9 cells/µl and 762 cells/µl, respectively. In group 2, with lower quality of colostrum (LQC), the minimum and maximum cell counts were 10 cells/µl and 902 cells/µl. For further details see Figure 5 and Table 6.

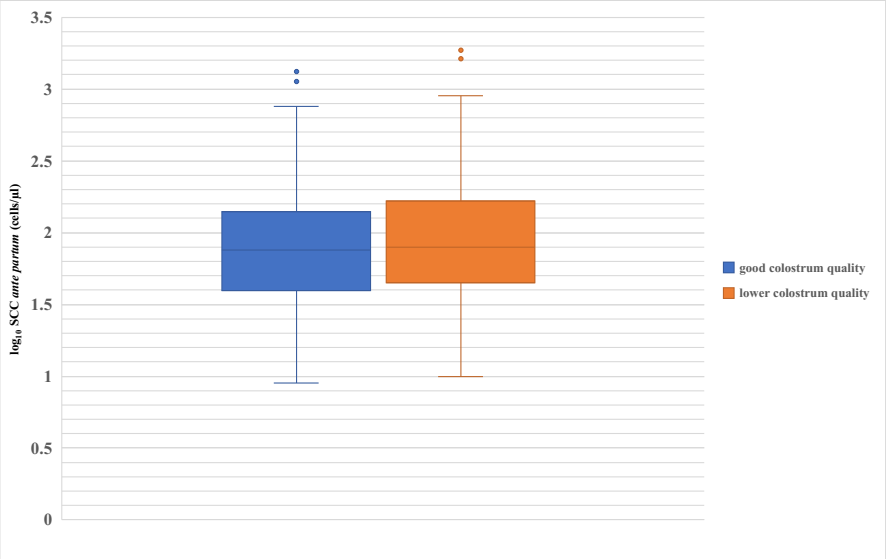

Figure 5: Boxplot, the colostrum SCC cut-off point was ≤400 cells/µl, the MLP SCC is now on the y-axis, the blue box represents the colostrum samples ≤400 cells/µl with better quality (GQC) and the orange box the group >400 cells/µl with lower quality (LQC).

Table 2: Characteristics of the two SCC groups in the boxplot (Fig. 5), comparing LKV data of *ante partum* milk data between the good (GQC) and lower colostrum quality (LQC).

group	GQC (≤400 cells/µl)	LQC (>400 cells/µl)
n samples	82	250
median	77	80
25th quartile	40	45
75th quartile	140	167

Lastly, a boxplot was created with the LKV data of the MLP *post-partum* and the colostrum SCC. For the first group (GQP), the lower whisker extended to 59 cells/µl and the upper whisker to 5,211 cells/µl. In the GQP group, there were 6 outliers outside the lower whisker with values between 2 and 43 cells/µl. The box or interquartile range of group 2 was larger than that of group 1. In group 2 (LQP), the lower whisker reached down to 69 cells/µl and the upper whisker up to 5,465 cells/µl. One outlier with the value of 17 cells/µl was identified. For more details, see Figure 5 and Table 7.

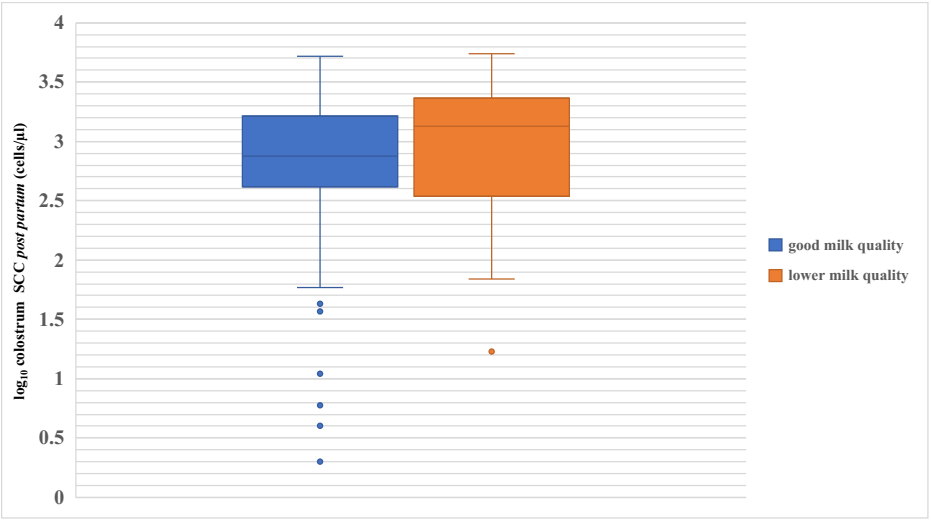

Figure 6: Groups were built with data of the MLP results *post-partum* and the matching colostrum sample SCC. The cut-off point was the same as in the other figures (Fig. 4 and 5) at 400 cells/µl. This separated the data into one group, in blue, with good quality of milk with an SCC *post-partum* of ≤400 cells/µl (GQP) and the second group with a lower quality of milk, in orange, starting at >400 cells/µl *post-partum* (LQP).

Table 3: Characteristics of the two milk quality groups based on MLP results *post-partum* in Fig. 6

Group	GQC (≤400 cells/µl)	LQC (>400 cells/µl)
n samples	299	32
median	757	1,347
25th quartile	413	347
75th quartile	1,638	2,304

4.3 Immunoglobulin results

In total, 1,045 of the 1,050 investigated samples revealed a readable result, five samples were excluded due to technical errors. The median Brix value was 22.0% (min =7.3%, max = 36.1%, 25th percentile = 19.0%, 75th percentile = 25.1%). The number of samples with good and poor-quality colostrum was 517 (49.5%) and 528 (50.5%), respectively. The cows in the first (N = 276), second (N = 224), third (N = 176), fourth (N = 115), fifth (N = 88), sixth (N = 62) and >6

(N = 83) lactations showed median Brix values of 22.7%, 20.8%, 21.3%, 22.1%, 23.3%, 23.1% and 24.1%. (Lichtmannsperger et al. unpublished)

4.4. Correlations

All correlations were calculated using log-transformed SCC results. The correlation between the mean SCC of the last three MLP ante partum results and the SCC of the colostrum samples was 0.04 ($p<0.05$). The correlation between the mean SCC of the first three MLPs post-partum and the colostral SCC was 0.3 ($p<0.05$). Additionally, a Spearman correlation was calculated between the Brix values and the SCC of bovine colostrum, resulting in a coefficient of -0.2 ($p<0.05$). The scatterplot in Figure 3 displays the Brix value as a percentage in relation to the SCC value of the 947 colostrum samples.

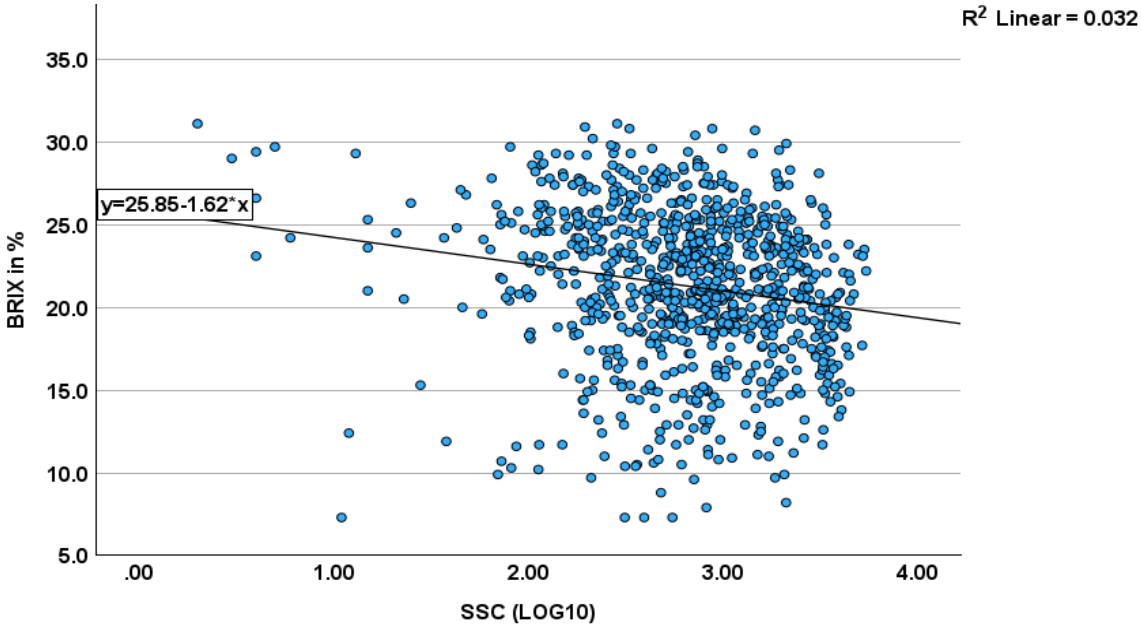

Figure 7: Scatterplot showing the relation of the Brix of the total protein to the colostrum SCC.

5. Discussion

This study presented data of the SCC and immunoglobulins in bovine colostrum and milk on selected dairy farms across the federal state of Salzburg Austria. In total 72 farms participated and sent in colostrum samples but only 47 farms (65.3%) were members of the LKV. For future studies in Austria, it would be advisable to create a more homogenous study population by sampling only LKV-farms as this allows quick access to a lot of useful data.

The average milk yield of an Austrian Fleckvieh dairy cow is 7,500.0 kilograms and aligned closely with the regional average of 7,460.0 kilograms. In this study, many farms took part that were operating on a smaller scale. According to the annual report of 2022 of the LKV Salzburg, the average herd size in that year was 20.6 cows per farm in the federal state of Salzburg and the mean herd size in Austria averaged to 24.2 milking? cows per farm [28]. The average herd size of the participating 47 LKV-farms was 24 cows per farm. Conclusively 44.7% of the LKV-farms were below average sized. Only three farms (6.3%) had herds of considerably larger sizes ranging from 51 to 83 animals. Therefore the milk yield was lower as in other studies [29] since milk production correlates positively with herd size. Smaller, often family-run, farms have a comparatively lower milk yield on average. In addition, a negative correlation was observed in a few studies, between herd size and SCC [30,31]. It could not be applied to every farm, but generally there was a tendency for the SCC to be significantly lower in larger herds compared to smaller herds, where the SCC of the herd tended to be elevated, possibly influenced by different milking techniques. This was also partially observed in this study.

Of the 47 LKV-farms, 87.3% fell into the S-class of raw milk quality (3), which can be assessed as positive. All LKV-farms, with the exception of one, were below 400 cells/µl in their MLP data. The farm with a SCC above 400 cells/µl therefore has milk of low quality. Here it would be important to eliminate the potential quality issues, emphasizing the need for investigation and corrective actions.

In this section, the materials and methods used are discussed to provide a clear insight into the experimental framework of this study. The selection of suitable materials and the precise application of the methods are of crucial importance to ensure the validity and reproducibility of the experiments carried out. The effective range of the DeLaval cellcounter DCC® expands

from 10 to 4,000 cells/µl. The variation coefficient is between 7.0% and 12.0% for repeated measurement. This made the accuracy of the results located beyond those limits questionable. It is worth mentioning that some samples showed 0 cells/µl, which is physiologically impossible as somatic cells are a constituent of colostrum and milk [20]. These samples should have been excluded or measured again for more accurate results. Hisira et al. (2023) compared three indirect measuring methods for SCC, among them the DeLaval cellcounter DCC®. They determined a sensitivity of 75.0% and a specificity of 97.5%, deeming the cell counting machine suitable for freshly taken and cooled tank milk samples [32]. Additionally, to the limitations of the used cell counting device, another aspect worth mentioning would be the storage methods. Barkema et al. (1997) froze their samples at -20.0°C and determined that the SCC was lower after freezing compared to the fresh samples SCC. The difference between fresh and frozen was more significant the longer the freezing period lasted but they deemed it still useful for detecting mastitis [33]. In this study, the samples were stored at -80.0 °C for various periods of time, of 6 to 18 months. In 2005, Sanchez et al. tested goat milk, using different treatments for storage freezing. They found that the SCC significantly decreased for the milk samples not treated with preservatives. The use of bronopol (BR) as a preservative resulted in unaltered SCC after freezing [34]. For reliable, accurate results, it would therefore make sense to measure the samples as fresh as possible or to keep the freezing period short and at best to use a preservative such as bronopol. The gold standard for measuring SCC is the direct microscopic counting method [35]. However, in a study of Hanuš et al. (2010) they found a strong agreement and consistency between the direct microscopic method and the DeLaval cellcounter DCC® aswell as the Fossomatic 90 [36]. The clear advantage of the DeLaval cellcounter DCC® is its portability making it suitable for quick on-farm measuring without needing a laboratory. In Tab. 4 the mean colostrum SCC of the farms that sent in at least ten samples are listed. It is noticeable, that colostrum samples showed much higher SCC results than milk, which corresponds with findings in other studies [37].

In this study, only the SCC was measured, but there are several papers that put their focus on the DSCC in relation to udder health. Macrophages are the dominant cell type with 50.0-90.0% followed by T-cells making up 16.0% of the cell population and only a few B-cells in

comparison. Polymorphonuclear cells, most present neutrophil granulocytes, and epithelial cells with 2.0-15.0% are also part of the DSCC[5]. The composition of the cell population changes depending on the health status of the cow as well as other factors like parity and age. It is assumed that leucocytes have a role to play in the calf's immune system[5]. However, this role is yet unclear, as calves that have been fed colostrum without leucocytes have nevertheless developed a competent immune system, so more research is needed in this field. For the measurement of the DSCC, electronic particle counting or fluorescence counting [38] and flow cytometry were used. The DSCC could be used as a complimentary method to SCC for mastitis screening [39]and could be of interest for further studies.

In Figure 2 and 3, the SCC values *post-partum* are lower than *ante partum*. It can only be assumed, since there is no data available, that this shift in SCC was due to the usage of antibiotic dry-off treatments on the farms and self-healing. Since it is a common practice for improving udder health and consequently lowering the SCC *post-partum*[40], this would be the most evident conclusion. The outliers indicate individual differences between cows in terms of general udder health, age or parity. Another reason could be external factors such as antibiotic dry-off or environmental factors, which were all a type of data that was not collected because it would go beyond the scope of this study. In general, it can be pointed out, that the outliers would need further investigation with possibly more specific diagnostic tests for the cows in question to make a well-founded interpretation. However, the accuracy of the outlier results should also be kept in mind in combination with the limits of the machine used for the SCC determination.

In the work by Puppel et al. in (2020), 40 Polish Holstein Friesian cows from one experimental organic dairy farm (n=250) were sampled. 40 cows were then selected to be separated into two groups. This explained why the two quality groups, were more balanced (n=18 good quality and n=22 low quality) in comparison to the groups in this study. Here, data from many LKV-farms (n=47) were used. The study population was also more heterogenous, consisting of different cow breeds, although the dominant breed was Fleckvieh. So, it was natural that larger differences occurred between the farms and the results looked different, which lead to more unbalanced quality groups, respectively fewer cows milk above the limit of 400.0 cells/µl. Some other studies have shown that the SCC of colostrum is related to the quantity of Ig and

other key components [27], consequently influencing the neonatal health and immunology status upon ingestion [11,41]. Puppel et al. (2020) conducted research on SCC as an indicator of colostrum quality. The study analysed various elements of colostrum and regular milk, including IgG and SCC. The results showed that the SCC of colostrum is higher than that of regular milk, which was also observed in our study. The SCC concentration gradually decreases, and the protein content, including IgG, was significantly affected by SCC. The sampled group with low SCC (≤400.0 cells/µl) in the colostrum, contained twice as much IgG as the group with elevated SCC >400.0 cells/µl [27]. Dunn et al. (2017) obtained a colostrum SCC mean value of $6.3\log_{10}$ (n=117), measured using the delta somascope lactoscope method [42]. This corresponds to 1,995 cells/µl. In the current study, a mean value of 1,091 cells/µl was calculated (n=947). This shows that SCC in colostrum can vary in a wide range, through the influence of a lot of factors already mentioned.

The median brix value was at 22.0% which corresponded to the cut-off value for good colostrum quality [43]. In the study of Lichtmannsperger et al. (unpublished), almost half of the colostrum was of good quality (49.5%). It was found that the cows in their second parity had the poorest colostrum quality at 20.8% and the quality steadily increased after that with each lactation. This is consistent with the literature on this topic[44].

The hypothesis of this study was that there is a correlation between the immunoglobulin concentration and SCC in bovine colostrum. In this project the determined correlations were weak and closer to 0.0 than to 1.0, leading to the conclusion that there was no relevant correlation existing between the SCC and content of immunoglobulins in bovine colostrum for our samples. The negative Spearman coefficient hints that the two values could be negatively related, signifying that a higher SCC in colostrum could entail lower levels of immunoglobulins. This would comply with results of other studies [27,41], that have proven that such a correlation exists. Nevertheless, the initial hypothesis is to be rejected and further work is needed.

Inherent to this study were certain limitations that warranted consideration, as they might influence the interpretation and generalizability of the findings. One of the limitations, as mentioned above, was the fact that the samples were measured after being frozen and then

thawed, having a possible influence on the results. The colostrum samples too thick for measurement could have been diluted to include in the results. Another source of error to consider would be the lack of knowledge of freezing the samples regarding the farmers, though they had a standard operating procedure. Furthermore, only the SCC was evaluated, but it might be of interest to put the focus on the DSCC as well.

6. References

1. Playford RJ, Weiser MJ. Bovine colostrum: Its constituents and uses. Vol. 13, Nutrients. MDPI AG; 2021. p. 1–24.
2. McGrath BA, Fox PF, McSweeney PLH, Kelly AL. Composition and properties of bovine colostrum: a review. Vol. 96, Dairy Science and Technology. Springer-Verlag France; 2016. p. 133–58.
3. Quigley JD, Drewry JJ. Nutrient and Immunity Transfer from Cow to Calf Pre- and Postcalving. In: Journal of Dairy Science. American Dairy Science Association; 1998. p. 2779–90.
4. Wilms JN, Hare KS, Fischer-Tlustos AJ, Vahmani P, Dugan MER, Leal LN, Steele MA. Fatty acid profile characterization in colostrum, transition milk, and mature milk of primi- and multiparous cows during the first week of lactation. J Dairy Sci. 2022 Mar 1;105(3):2612–30.
5. Ellis J. Review Article Compte rendu Passive transfer of colostral leukocytes: A benefit/risk analysis.
6. Soufleri A, Banos G, Panousis N, Fletouris D, Arsenos G, Kougioumtzis A, Valergakis GE. Evaluation of factors affecting colostrum quality and quantity in holstein dairy cattle. Animals. 2021 Jul 1;11(7).
7. Jensen SK, Johannsen AKB, Hermansen JE. Quantitative secretion and maximal secretion capacity of retinol, β-carotene and α-tocopherol into cows' milk. Journal of Dairy Research. 1999 Nov;66(4):511–22.
8. Peter AT. Bovine placenta: A review on morphology, components, and defects from terminology and clinical perspectives. Vol. 80, Theriogenology. 2013. p. 693–705.
9. Weaver DM, Tyler JW, VanMetre DC, Hostetler DE, Barrington GM. Passive transfer of colostral immunoglobulins in calves. Vol. 14, Journal of veterinary internal medicine / American College of Veterinary Internal Medicine. 2000. p. 569–77.
10. Godden SM, Lombard JE, Woolums AR. Colostrum Management for Dairy Calves. Vol. 35, Veterinary Clinics of North America - Food Animal Practice. W.B. Saunders; 2019. p. 535–56.
11. Jaster EH. Evaluation of quality, quantity, and timing of colostrum feeding on immunoglobulin G1 absorption in Jersey calves. J Dairy Sci. 2005;88(1):296–302.
12. Geiger AJ. Colostrum: back to basics with immunoglobulins. J Anim Sci. 2020;98:S126- S132.
13. Kehoe SI, Jayarao BM, Heinrichs AJ. A survey of bovine colostrum composition and colostrum management practices on Pennsylvania dairy farms. J Dairy Sci. 2007;90(9):4108–16.
14. Butler JE. Bovine Immunoglobulins: A Review. Vol. 52, Journal of Dairy Science. 1969. p. 1895–909.
15. Kessler EC, Bruckmaier RM, Gross JJ. Colostrum composition and immunoglobulin G content in dairy and dual-purpose cattle breeds. J Anim Sci. 2020 Aug 1;98(8).
16. Conneely M, Berry DP, Sayers R, Murphy JP, Lorenz I, Doherty ML, Kennedy E. Factors associated with the concentration of immunoglobulin G in the colostrum of dairy cows. Animal. 2013 Nov;7(11):1824–32.

17. Matte JJ, Girard CL, Seoane JR, Brisson GJ. Absorption of Colostral Immunoglobulin G in the Newborn Dairy Calf. J Dairy Sci. 1982;65(9):1765–70.
18. Immler M, Büttner K, Gärtner T, Wehrend A, Donat K. Maternal Impact on Serum Immunoglobulin and Total Protein Concentration in Dairy Calves. Animals (Basel) 2022.
19. Stott GH, Fellah A. Colostral Immunoglobulin Absorption Linearly Related to Concentration for Calves. J Dairy Sci. 1983;66(6):1319–28.
20. Langel SN, Wark WA, Garst SN, James RE, McGilliard ML, Petersson-Wolfe CS, Kanevsky-Mullarky I. Effect of feeding whole compared with cell-free colostrum on calf immune status: The neonatal period. J Dairy Sci. 2015 Jun 1;98(6):3729–40.
21. Salsberg E, Meek AH, Martin SW. Somatic Cell Counts: Associated Factors and Relationship to Production.
22. Deluyker HA, Gay JM, Weaver LD. Interrelationships of somatic cell count, mastitis, and milk yield in a low somatic cell count herd. J Dairy Sci. 1993;76:3445–52.
23. Halasa T, Kirkeby C. Differential Somatic Cell Count: Value for Udder Health Management. Front Vet Sci. 2020 Dec 23;7.
24. Lee CS, Kemp P. Identification, properties, and differential counts of cell populations using electron microscopy of dry cows secretions, colostrum and milk from normal cows. Journal of Dairy Research. 1980;47(1):39–50.
25. Lichtmannsperger K, Hartsleben C, Spöcker M, Hechenberger N, Tichy A, Wittek T. Factors Associated with Colostrum Quality, the Failure of Transfer of Passive Immunity, and the Impact on Calf Health in the First Three Weeks of Life. Animals. 2023 Jun 1;13(11).
26. Agrar Markt Austria. https://www.ama.at/marktinformationen/milch-und-milchprodukte/aktuelle-informationen/2019/aenderung-der-rohmilch-bewertung (Zugriff 13.12.2023).
27. Puppel K, Golebiewski M, Grodkowski G, Solarczyk P, Kostusiak P, Klopcic M, Sakowski T. Use of somatic cell count as an indicator of colostrum quality. PLoS One. 2020 Aug 1;15(8 August).
28. Landeskontrollverband Salzburg. https://www.landeskontrollverband.at/media/news/LKV-Jahresbericht-2023-web.pdf?m=1678092597& (Zugriff 13.12.2023).
29. Sipahi C. The Effect of Different Environmental Factors on Milk Yield Characteristics of Holstein Fresian Cattle Raised with Different Production Scale on Teke Region of Turkey. Sustainability (Switzerland). 2022 Nov 1;14(21).
30. Jayarao BM, Pillai SR, Sawant AA, Wolfgang DR, Hegde N V. Guidelines for monitoring bulk tank milk somatic cell and bacterial counts. J Dairy Sci. 2004;87(10):3561–73.
31. Norman HD, Miller RH, Wright JR, Wiggans GR. Herd and state means for somatic cell count from dairy herd improvement. J Dairy Sci. 2000;83(12):2782–8.
32. Hisira V, Zigo F, Kadaši M, Klein R, Farkašová Z, Vargová M, Mudroň P. Comparative Analysis of Methods for Somatic Cell Counting in Cow's Milk and Relationship between Somatic Cell Count and Occurrence of Intramammary Bacteria. Vet Sci. 2023 Jul 1;10(7).

33. Barkema HW, Van Der Schans J, Schukken YH, De Gee ALW, Lam TJGM, Benedictus G. Effect of Freezing on Somatic Cell Count of Quarter Milk Samples as Determined by a Fossomatic Electronic Cell Counter. J Dairy Sci. 1997;80(2):422–6.
34. Sánchez A, Sierra D, Luengo C, Corrales JC, Morales CT, Contreras A, Gonzalo C. Influence of storage and preservation on Fossomatic cell count and composition of goat milk. J Dairy Sci. 2005;88(9):3095–100.
35. Enger BD, Nickerson SC, Akers RM, Moraes LE, Crutchfield CE. Use of commercial somatic cell counters to quantify somatic cells in non-lactating bovine mammary gland secretions. Prev Vet Med. 2020 Jan 1;174.
36. Hanuš O, Sojková K, Hanušová K, Samková E, Hronek M, Hyšpler R, et al. An experimental comparison of methods for somatic cell count determination in milk of various species of mammals. Acta Univ. Agric. Silvic. Mendelianae Brun. 2014;59:67–82.
37. Maunsell FP, Morin DE, Constable PD, Hurley WL, McCoy GC, Kakoma I, Isaacson RE. Effects of Mastitis on the Volume and Composition of Colostrum Produced by Holstein Cows. J Dairy Sci. 1998;81(5):1291–9.
38. Liebler-Tenorio EM, Riedel-Caspari G, Pohlenz JF. Uptake of colostral leukocytes in the intestinal tract of newborn calves.
39. Damm M, Holm C, Blaabjerg M, Bro MN, Schwarz D. Differential somatic cell count—A novel method for routine mastitis screening in the frame of Dairy Herd Improvement testing programs. J Dairy Sci. 2017 Jun 1;100(6):4926–40.
40. Sigmund M, Egger-Danner C, Firth CL, Obritzhauser W, Roch FF, Conrady B, Wittek T. The effect of antibiotic versus no treatment at dry-off on udder health and milk yield in subsequent lactation: A retrospective analysis of Austrian health recording data from dairy herds. J Dairy Sci. 2023 Jan 1;106(1):452–61.
41. Ferdowsi Nia E, Nikkhah A, Rahmani HR, Alikhani M, Mohammad Alipour M, Ghorbani GR. Increased colostral somatic cell counts reduce pre-weaning calf immunity, health and growth. J Anim Physiol Anim Nutr (Berl). 2010 Oct;94(5):628–34.
42. Dunn A, Ashfield A, Earley B, Welsh M, Gordon A, Morrison SJ. Evaluation of factors associated with immunoglobulin G, fat, protein, and lactose concentrations in bovine colostrum and colostrum management practices in grassland-based dairy systems in Northern Ireland. J Dairy Sci. 2017 Mar 1;100(3):2068–79.
43. Buczinski S, Vandeweerd JM. Diagnostic accuracy of refractometry for assessing bovine colostrum quality: A systematic review and meta-analysis. J Dairy Sci. 2016 Sep 1;99(9):7381–94.
44. Sutter F, Borchardt S, Schuenemann GM, Rauch E, Erhard M, Heuwieser W. Evaluation of 2 different treatment procedures after calving to improve harvesting of high-quantity and high-quality colostrum. J Dairy Sci. 2019 Oct 1;102(10):9370–81.