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Semen quality parameters of AI in cattle bulls related to different weather conditions

Az AI sperma minőségi paraméterei szarvasmarha bikáknál a különböző időjárási körülményektől függően

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Abstract

This thesis deals with the question of how weather conditions affect the sperm quality of bulls used for semen collection for artificial insemination. To investigate this, data from the Bos Genetic center in Martonvásár, Hungary is used. Data from eight bulls, without considering age, breeding or genetics, is examined on different days over a period from July 2023 to October 2023. This data is compared with the weather data from a nearby weather station in Ráckeresztúr on the respective collection days, as well as fluctuations in the previous week. The results show significant temperature differences as well as ejaculate differences. This study aimed to investigate a correlation between weather changes, particularly temperature fluctuations and ejaculate quantity and quality.

To summarize, this study confirms that weather conditions play a crucial role in the production of bull semen. Extreme temperatures negatively impact sperm quality, while moderate and stable temperatures favor optimal semen production.

Absztrakt

Ez a dolgozat azzal a kérdéssel foglalkozik, hogy az időjárási körülmények hogyan befolyásolják a mesterséges megtermékenyítéshez használt bikák spermagyűjtéséhez használt sperma minőségét. Ennek vizsgálatához a martonvásári Bos Genetikai Központ adatait használjuk fel. Nyolc bika adatait vizsgáljuk, a kor, a tenyésztés és a genetika figyelembevétele nélkül, különböző napokon, 2023 júliusától 2023 októberéig terjedő időszakban. Ezeket az adatokat összehasonlítjuk egy közeli, Ráckeresztúron található meteorológiai állomás időjárási adataival az adott gyűjtési napokon, valamint az előző hét ingadozásaival. Az eredmények jelentős hőmérsékleti különbségeket, valamint ejakulációs különbségeket mutatnak. A tanulmány célja az volt, hogy összefüggést vizsgáljon az időjárási változások, különösen a hőmérséklet-ingadozások és az ejakulátum mennyisége és minősége között.

Összefoglalva, ez a tanulmány megerősíti, hogy az időjárási körülmények döntő szerepet játszanak a bika spermájának előállításában. A szélsőséges hőmérsékletek negatívan befolyásolják a sperma minőségét, míg a mérsékelt és stabil hőmérséklet kedvez az optimális spermatermelésnek.

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1 Introduction of artificial insemination in animal breeding

1.1 Basics and History of AI

Nowadays, artificial insemination (AI) has become indispensable. However, what is such a procedure? AI is needed to bypass the natural reproductive process. In a nutshell, it is an activity to place the semen into the female reproductive tract to achieve fertilization and thus reproduction. How this procedure works exactly is described in detail in *chapter 2.3*.

This kind of process has been around for hundreds of years. There are records of a horse breeder in the 14th century, who is said to have taken the ejaculate from the vagina of a recently naturally served mare and inserted it into the vagina of another mare. Furthermore, from the year 1677 onwards, there are detailed records of sperm in an ejaculate by the Dutch scientist Van Loenhoek. In 1780, the first artificial insemination in dogs took place, performed by the Italian physiologist named Lazzaro Spallanzani. This progress inspired a new development for the preservation of semen in Russia in 1937. For the first time Cryopreservation was used, which is a preservation through freezing. [1]

1.2 Advantages of AI

First, artificial insemination is a very good way to preserve genetic material and also the improvement of a genetic pool. Furthermore, it is important to prevent diseases that can be transmitted and injuries that could happen during natural service. In addition, it is very useful to avoid possible long geographic distances.
Furthermore, this procedure is useful to control the herd as the breeder knows exactly which calf descends from what bull. [1]

1.3 Disadvantages of AI

Even though one of the biggest advantages of AI is to prevent genetic diseases, it cannot always be guaranteed. This is the case when the bull has not been tested sufficiently and thus genetic defects are passed on to numerous offspring.
Furthermore, artificial insemination is a very costly affair. As the cost of an AI can become higher than a natural service. This is due to all the observations that need to be done to determine the right timing of the AI as well as possible costs of a synchronized estrus and the semen. Therefore, the price of a calf is also increasingly. Another drawback is that as the artificial procedure increases, the demand for young bulls for the herd decreases.[1]

2 Artificial insemination in cattle

2.1 Estrus cycle in Cattle

The next section serves to summarize the cycle and the associated synchronization of the cow to understand the whole background of an artificial insemination. First, the cow is a polyestrous animal with a 21-day long cycle. Furthermore, this animal belongs to the spontaneous ovulatory group. The meaning of this statement is that ovulation is regulated by endogenous factors and mechanisms and takes place even without the presence of a bull. The cycle is split up in two major phases and four stages. The Luteal phase consist of the Metestrus and the Diestrus, whereas the Follicular phase is constituted by the proestrus and estrus. As in all living organisms with an estrous cycle, various hormones control the course of the cycle. These will also be of great importance later for artificial insemination. The largest command center for hormones is the HPA (Hypothalamus-Pituitary-Axis). The hypothalamus releases GnRH (Gonadotropic releasing hormone) and thus stimulates the secretion of LH (Luteinizing Hormone) and FSH (Follicle stimulating hormone) in the pituitary gland. While FSH is responsible for the maturation of follicles into both secondary and tertiary follicles and the stimulation of estradiol, LH plays a major role in final maturation and ovulation. Furthermore, LH ensures the production of estrogen and the maintenance of the corpus luteum after ovulation. This hormone is also responsible for the increase in progesterone produced by the CL. Progesterone is important for maintaining a possible pregnancy. It suppresses further ovulations and ensures that the uterus is prepared for possible implantation of the fertilized egg. If fertilization has not taken place, prostaglandin, which is produced by the endometrium of the uterus itself, leads to a regression of the corpus luteum and new follicular waves can take place. However, at least three follicular waves per cycle must take place before an ovulation occurs. [4]

2.2 Procedure of AI

To achieve optimal results in artificial insemination, it is imperative that the semen is of high quality and that the timing aligns with the cow's reproductive cycle. A specialized instrument known as a straw gun is typically employed for the insemination process. To thaw the semen from a temperature of -197 degrees Celsius to the desired range of approximately 35 degrees Celsius, warm water is commonly utilized. In certain instances, thawing may be accomplished using oral warmth or ambient air; however, research indicates that these methods are suboptimal, and that improper thawing can adversely affect conception rates.

To mitigate this risk, it is recommended to immerse the straw in warm water at a temperature between 34 and 36 degrees Celsius for a duration of thirty seconds, ensuring that no water enters the straw. After thawing, it is essential to avoid significant temperature fluctuations to protect the viability of the sperm. Once the straw has been gently twisted between the fingers to facilitate ejection, it is then placed into the insemination gun, ready for use. By adhering to these guidelines, the likelihood of successful insemination can be significantly enhanced.

The most used method of insemination is the recto-vaginal technique. In this case, the insemination gun is inserted into the vagina and at the same time guided through the other hand placed in the rectum. The gloved hand reaches through the rectum to the cervix and can thus ensure that it is fixed. After the gun has been inserted into the vagina, it is guided through the cervix folds by the hand in the rectum. if you are aiming for intrauterine insemination, the gun is guided all the way through the cervix. However, there are also methods in which the semen is placed in the middle of the cervix. this method is mainly used for so-called repeated cows, as some cows can cycle during the 3rd and 6th weeks of pregnancy. In this case, an intrauterine penetration could damage the embryo that has already been created. However, after the semen has been deposited, the pistol is removed, and the cervix can be massaged a little with the helping hand in the rectum to stimulate it. [5]

3 Bos Genetic Center Hungary

3.1 History

The History of “Bos Genetic” began in 1985, when the OHG (“Osnabrücker Herdbuch Genossenschaft” – “Osnabrücker Herd-Book cooperative”) founded the breeding bull station in cooperation with the Hungarian company “BosCooP”. After some contract negotiations and coordination in the ministry, the two parties signed the registration of Bos-Genetic Kft. in the commercial register on November 12th, 1985. Less than a month later, 12 bulls arrived in Martonvásár. While “OHG” was responsible for the purchase and transport of the tested breeding bulls, it was the task of “BosCoop” to organize the semen distribution. These 12 bulls mentioned above were already daughter-tested bulls and therefore entitled to be imported to Hungary. However, from 1991 onwards, there were first options to test promising young bulls bred in Hungary. This restructuring of the system made it possible for “OHG” to increase its share from 49%, at the time of signing the contract, to 72%.

Due to its great success, other companies also became aware of this company and Bos-Genetic gained further partners such as the companies “SRV” and “Genes Diffusion”. Bos Genetic had several breeding bulls that topped the list of the best bulls in Hungary for many years. One of them was the breeding bull named "Saphir", which for a long time was the best-selling bull in Hungary.

Figure 1: Breeding Bull "Saphir" [A]

From 2010 onwards, however, the breeding value estimation in the dominant countries of Holstein breeding increased. Unfortunately, this led to a reduction in the number of bulls and a reduction in progeny testing in “Bos Genetic”. As the Holstein breed is one of - if not the most sought-after - breed for milk production and Hungary, unlike other countries, counts as a small "Holstein country", it was a big problem that there was no own “genomic breeding

value estimation system" available. This procedure refers to the fact that an estimation that relies on an animal's performance and pedigree data, with genetic information obtained from the animal's genome. This approach allows for the calculation of breeding values, even for young calves that have not yet produced offspring.

As a result of all these circumstances, since 2010 there have been almost 100 fewer bulls in Bos Genetic compared to the years before and there was no longer any reason for partners to use the bulls from Martonvásár. Under the direction of Dr. Istvan Monostori, who has been the managing director since 1999, they tried to help themselves by expanding the sperm exports, especially to Turkey and Eastern European countries. Furthermore, a separate breeding program was set up to meet the needs of local cattle breeders. [2]

3.2 Education institute

Through the conversion of the economic benefit and through the imminent and deserved retirement, Dr. Istvan Monostori looked for a wide variety of new partnerships for the company. The university took notice and director Prof. Sotonyi saw this as an opportunity to introduce his students to the practical aspects of insemination stations and sperm production. Through this cooperation it became possible to keep Bos Genetic alive and to continue local breeding activities in Hungary. On March 9, 2022, contracts were signed for the transfer of Bos Genetic Kft. to the University of Budapest.

In addition to the aspect of teaching students, the university is also responsible for quality and quantity control. To guarantee the highest quality of semen, each ejaculate sample is tested at the University of Veterinary Medicine laboratory. This includes examining the sperm count and motility characteristics to ensure optimal results for artificial insemination.

3.3 Semen collection and deep freezing

This whole system of semen collection and cryopreservation was developed, as already mentioned in the section above, to make the spermatozoa largely stable over a longer period and to protect them until they are used in artificial insemination. To ensure this, storage in liquid nitrogen at -196 degrees Celsius is necessary. How to cool the seed samples to this temperature without damaging them is described in this section.

The whole process of insemination at the AI station Bos Genetic begins the evening before the actual day of semen collection. The so-called bull workers or grooms start by cleaning the prepuce and trimming long hair at this point so that the semen collection can be carried out as cleanly as possible. The next morning, the ejaculate of the bulls is collected after feeding at around 9 o'clock. The bulls are then led to the area where a dummy bull is already standing. The bulls are trained early on to get them used to this process. The teaser bull, which is to replace the cow, has been cleaned beforehand and sprayed with pheromones to arouse the bull's natural instinct. During the jump, the task of the trained worker is to grasp the penis and pull the prepuce backwards and insert the glans penis into the artificial vagina. This is provided with a lubricant and water at a temperature of 37 degrees is heated in the intermediate layer of the wall. These precautions are taken to give the bull the feeling of a real vagina and to make the process more comfortable for him. Usually, a bull must jump the teaser bull twice a day after a break. That is because various factors can influence the quantity and quality of the ejaculate. For example, loud noises as sirens, airplanes and so on. Beef cattle bulls are particularly sensitive to disturbances during semen collection.

Figure 2: Semen Collection 1

Figure 3: Semen Collection 2

Figure 4: Semen Collection 3

Figure 5: Artificial Vaginas

The next steps are carried out in the laboratory. First, the data of the bull from which the sample originates, and the exact time are documented. The ejaculate is analyzed for quantity and quality. The density is measured to calculate how many spermatozoids it contains. Furthermore, each sample is examined individually under the microscope to examine the motility and morphology but also to visualize the quantity. The motility of the sperm is examined and assessed by a trained eye of the expert laboratory staff. If the sperm have less than 70% motility, the sample is considered unusable and is destroyed. If both ejaculates are good enough, they are mixed. Otherwise only one ejaculate is used. The sample is then diluted in a 1:1 ratio with an extender. Depending on the density of the spermatozoa in the sample, a second dilution is added. The more spermatozoa there are in a sample, the more dilution can be used. As a result, more straws of sperm can be produced. The finished and diluted sample is now left to cool at room temperature for 10-15 minutes.

Now the samples are ready to be filled in straws. Therefore, a machine is used, which can print and fill the straws in the same procedure. The print consists of the name of the bull, the date and the company, where the straw was produced.

After each bull sample the machine must be cleaned and disinfected. As the last step of the filling procedure the laboratory staff examined the straws in a light source. In this process, the straws that were not completely filled or contain air are sorted out.

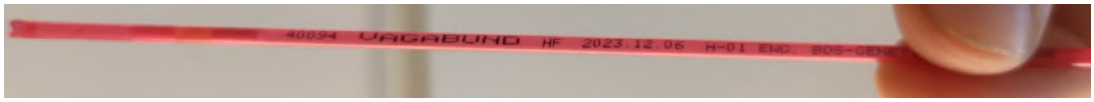


Figure 6: Straw with print

As the next step, the straws will undergo deep freezing. For this, the straws are placed in a refrigerator at four degrees Celsius for three hours. Meanwhile, the ultra-low-temperature freezer is prepared. First, it is heated to 20 degrees Celsius, and the machine is dried for 20 minutes. Then, the freezer is brought back to a temperature of four degrees Celsius, and the straws that have been stored in the refrigerator are placed inside. The setting of the deep freezer is then adjusted so that the temperature gradually cools down until reaching minus 196 degrees Celsius (-196°C). Meanwhile, styrofoam containers are being prepared and filled with liquid nitrogen. After the Deep Freezer has cooled the straws, they are filled into cups in liquid nitrogen. Now the straws can be stored for a long time.

Figure 7: Straws in liquid nitrogen

Figure 8: Deep Freezer with filled straws

4 Connection of semen quality, quantity and weather conditions in beef cattle bulls – Background information

4.1 Investigated Parameters of ejaculate

Various parameters are used to assess the quality of an ejaculate. Motility is an important factor for sperm to determine fertility. It determines the ability of a sperm to penetrate the female reproductive tract to fertilize the oocyte. Normal motility in the ejaculate is described as approximately 40% or more. Photometry is used to determine the concentration of an ejaculate. This method can be used to determine the number of sperm per milliliter of semen. A high number increases the chance of successful fertilization. The hypoosmotic swelling test is described next. This is used to determine sperm vitality and membrane integrity. The sperm are exposed to a hypoosmotic solution, and it is observed whether the sperm swell or not. The hypoosmotic swelling test is used to determine sperm viability and membrane integrity. Viable sperm with intact membranes absorb fluid through the osmotic reaction and swell, causing the spermatozoon's tail to coil. A bright field microscope is used to determine morphological abnormalities of the sperm. Normal sperm have an oval head and a long tail. With these methods, it is possible to determine a high-quality ejaculate.

4.2 Sperm abnormalities:

Sperm abnormalities can be broadly classified into two categories: head defects and tail defects. The most common types of sperm abnormalities are as follows:

Head defects:

- Giant head
- Round head
- Acrosome defect
- Acrosome swelling
- Narrow head
- Narrow at the base
- Double head

Tail defects:

- Proximal protoplasmic droplet
- Distal protoplasmic droplet
- Bent tail
- Folded tail
- Coiled tail
- Double tail

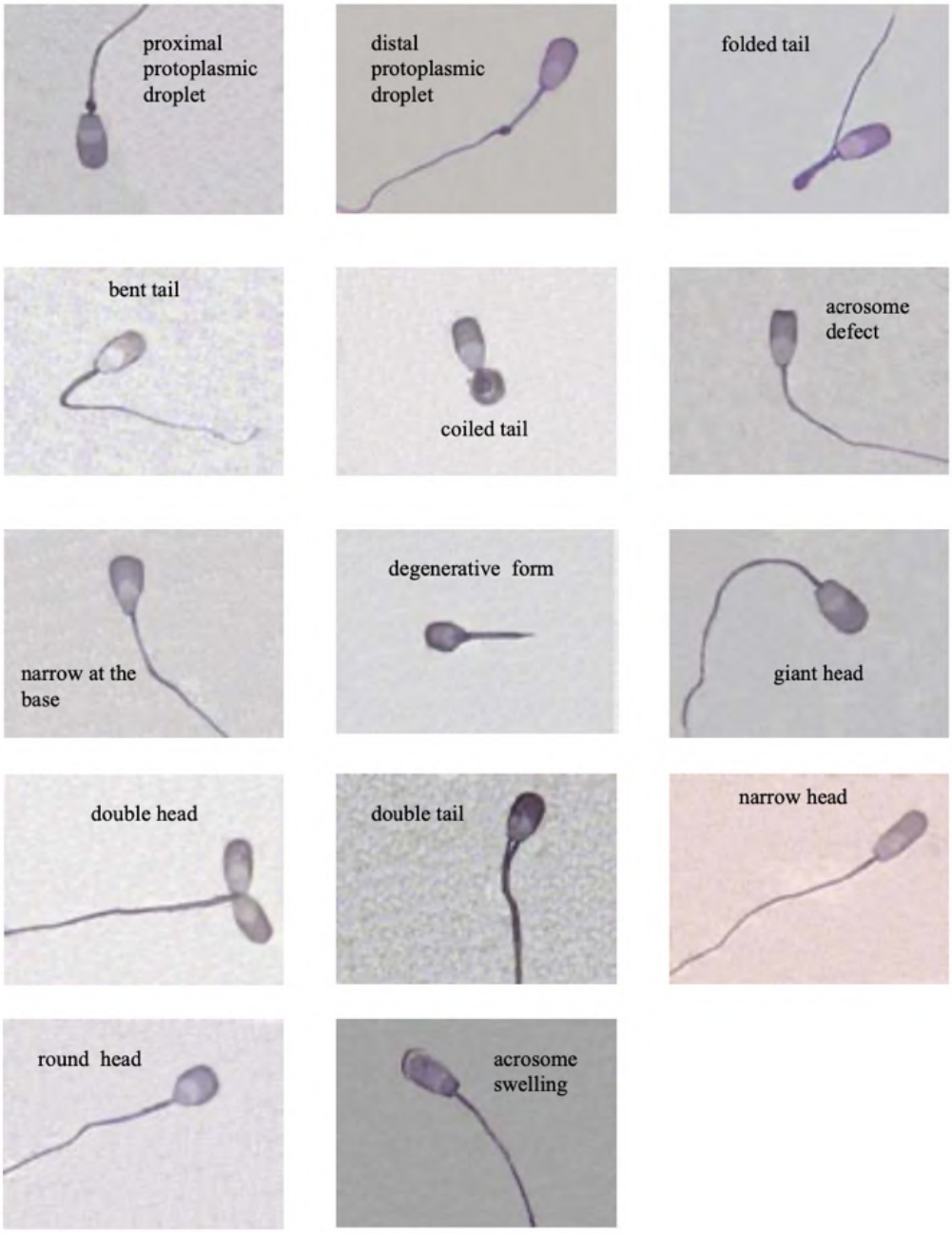


Figure 9: Sperm abnormalities [B]

4.3 Why might weather conditions be related to the semen production?

To elucidate the effects of temperature fluctuations on sperm production, this section will examine the conditions pertinent to sperm maturation and storage. The scrotum, which houses the testes and epididymides, is critical in regulating testicular temperature. It maintains a temperature that is approximately 4 to 5 degrees Celsius lower than that of the abdominal cavity. This temperature differential is essential for the proper formation and functionality of spermatozoa. The significance of this temperature regulation is further illustrated by for example the condition known as cryptorchidism, wherein the testes fail to descend into the scrotum. Animals afflicted with cryptorchidism exhibit infertility, despite normal hormonal levels. This observation implies that when the testes are retained within the inguinal canal or the abdominal cavity, the elevated temperature adversely affects sperm viability and functionality.

An optimal spermatogenesis needs a thermal environment of approximately 32 degrees Celsius. Any elevation in temperature or humidity can impact the evaporative processes of the scrotal tissue. Consequently, such changes may induce an increase in the animal's body temperature and metabolic rate, which subsequently leads to a rise in scrotal temperature. This thermal cascade can potentially compromise the efficiency and integrity of the spermatogenic process. For this reason, it is important to monitor heat stress. When an animal is subjected to elevated temperatures and humidity, the body may struggle to generate a normal physiological response. As a result of this imbalance, the male body may be unable to produce sperm of normal quality, leading to a significant reduction in fertility. The most critical components of heat stress are named under the term Thermal Heat Index (THI).

To understand the impact of heat stress on metabolism, regarding the formation of reactive oxygen species (ROS), it is essential to consider the physiological mechanisms which are involved. Reactive oxygen species are highly reactive molecules which are generated as by-products of cellular metabolism, especially during aerobic respiration. These species include superoxide anions, hydroxyl radicals, and hydrogen peroxide, which play roles in biological systems. However, at physiological levels, ROS are important for various cellular functions, including signaling pathways that regulate sperm maturation, acrosome reactions, and fertilization processes, for example binding to the zona pellucida. In case of heat stress, there is an increase in body temperature, which raises the temperature of the testes as well. This thermal elevation enhances the production of ROS apart of normal physiological levels. While ROS are very well necessary for sperm function, accumulation due to heat stress can

induce oxidative stress, which is characterized by an imbalance between ROS production and the antioxidant defenses. The effects of elevated ROS levels include for example: DNA Damage leading to mutations and potential embryo lethality if a damaged sperm fertilizes an oocyte. However, the damage may not manifest until after the first cleavage and the onset of genome activity in the embryo.

Furthermore, ROS can modify proteins, leading to structural changes and impairment of their function. This includes the oxidation of amino acids and the denaturation of enzymes critical for cellular processes

Additionally, sperm cells are rich in unsaturated fatty acids, making them particularly susceptible to lipid peroxidation induced by ROS. This process not only compromises sperm viability but also affects their ability to bind to the zona pellucida during fertilization.

In summary, while ROS are vital for normal metabolic functions and reproductive processes, excessive production due to heat stress can result in significant cellular damage. This oxidative stress can adversely affect sperm quality and viability, potentially leading to reproductive failures. Understanding the balance between ROS production and antioxidant defenses is crucial for mitigating the negative impacts of heat stress on male fertility. [3]

5 Results

5.1 Material and Methods

Various data were used to check the question of whether the weather is related to semen quality. The weather data were obtained from the weather station in Ráckeresztúr, Hungary, near the bull station Bos Genetica. For the ejaculate analysis, various bulls that were at Bos Genetic in Martonvásár for semen collection were used. In total, data from 8 bulls were collected and compared in the period July - October 2023. To examine whether there is a correlation between weather changes and ejaculate quantity and quality, the ejaculate quantities were considered with the weather on that day and the temperature fluctuations of the days before sample collection. It is assumed that all the ejaculates taken are high-quality samples. The reason for this is that, as already mentioned, the samples are discarded if the sperm motility is below 70% and each sample is examined separately under the microscope. The age, breed and genetics of the bull were not taken into account.

5.2 Weather data for given time period of evaluation

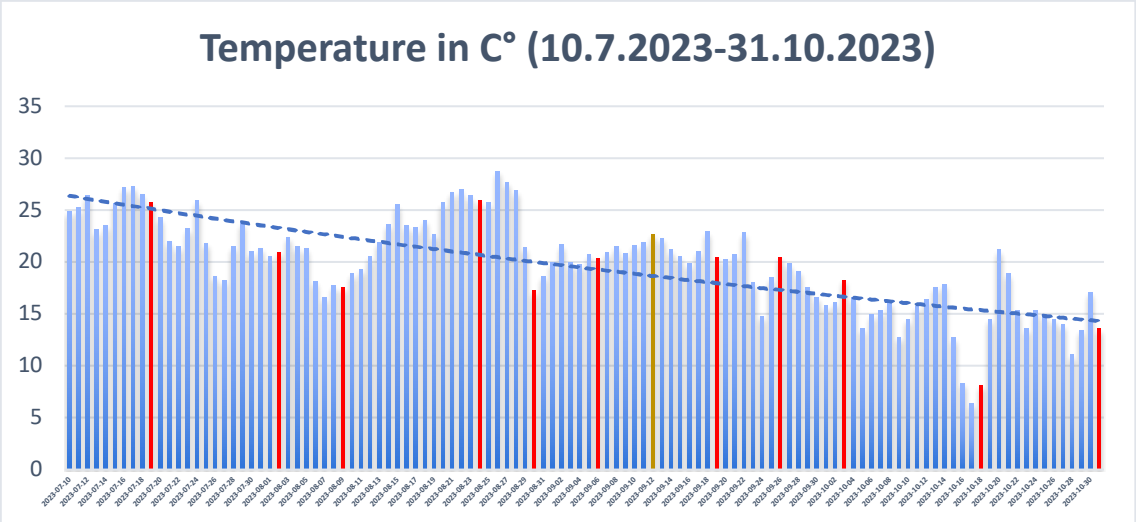
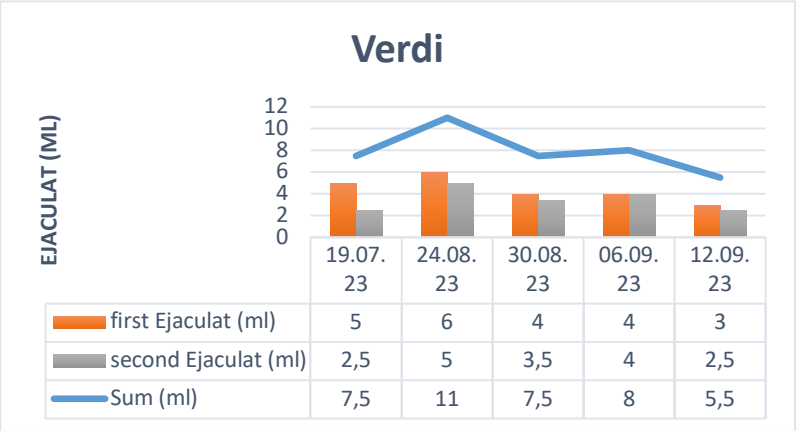


Figure 10: Temperature for given Dates. Red: All bulls that have been tested; Yellow: All bulls except Vagabund and Acta Limousin have been tested on this day

5.3 Overall average Results

5.4 Bull: Verdi



	19.07.23	24.08.23	30.08.23	06.09.23	12.09.23
first Ejaculat (ml)	5	6	4	4	3
second Ejaculat (ml)	2,5	5	3,5	4	2,5
Sum (ml)	7,5	11	7,5	8	5,5

Figure 11: Amount of Ejaculat Verdi

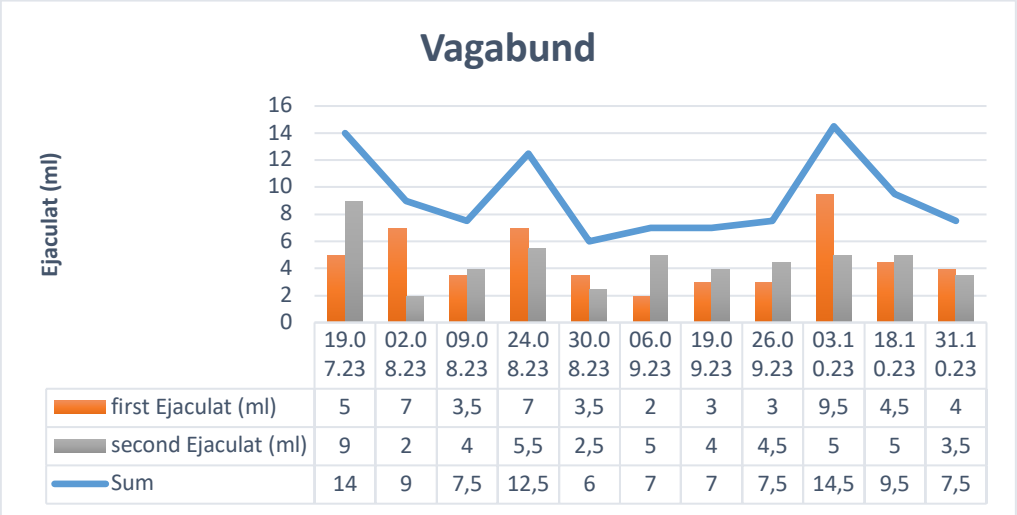
Figure 12: Comparison of quantity of ejaculate and temperature Verdi

The bull "Verdi" underwent a series of ejaculate sampling procedures over a three-month period. This period took from July 19th to September 13th, 2023. In total, samples were conducted on five separate days, with two collection runs performed on each of these days. The scheduling of these sampling days was influenced by the condition of the bull and the quality and quantity of the ejaculate. Only days with recorded results were included in the analysis, which indicates that the quality of the semen was acceptable. On July 19, 2023, the first collection yielded a total ejaculate volume of 7.5 ml, comprising 5.0 ml from the first collection and 2.5 ml from the second. The following sampling day was August 24, 2023, where the total ejaculate volume increased to 11.0 ml, with 6.0 ml collected in the first run and 5.0 ml in the second. On August 30th, 2023, the total ejaculate volume returned to 7.5 ml consisting of 4.0 ml from the first collection and 3.5 ml from the second. The following week, on September 6th, 2023, the total ejaculate volume increased slightly to 8.0 ml, with

both collections yielding 4.0 ml. Finally, on September 13th, 2023, the total ejaculate volume decreased to 5.5 ml, comprising 3.0 ml from the first collection and 2.5 ml from the second. The highest total ejaculate volume of 11.0 ml was recorded on August 24th, 2023, while the lowest total ejaculate volume of 5.5 ml was observed on the final recording day, September 13th, 2023. Conclusive, the first collection consistently yielded higher volumes compared to the second collection, except on September 6th, 2023, when both collections produced equal volumes.

To compare the amount of ejaculate and the temperature, we got a Temperature variety of the given sampling days. On July 19th, 2023, the total amount of ejaculate was 7,5 ml. The temperature on this day was about 25.8 °C. In the preceding week the average was 25.9 °C. On the next sampling day (August 24th, 2023) the temperature was at 26°C. In the previous week, the average temperature was 24.8°C. On the next collection day, August 30th, 2023, the temperature was 17.3°C, whereas the previous week's average was 25.2°C. The temperature remained at an average of 20.1°C that week and rose again the next week, reaching 20.4°C on September 6th, 2023. After this day, the average for the week remained at 21°C and reached 22.3°C the following week on September 13th, 2023. Based on this given data, a temperature fluctuation from 16.6 to 28.7°C can be seen. The highest amount of ejaculate collected was 11 ml on August 24th, 2023, when a temperature of 26°C was measured. This day was in a range where the temperature was constantly above 25°C. The lowest amount was 5.5 ml and was collected on September 13th, 2023. On this day the temperature was 22.3°C. The days before were all in a relatively stable range around 20-22°C. An extremely interesting fact can be seen at the end of August 2023. Between August 28th and August 30th, 2023, there was a drop in temperature from 26.9°C to 17.3°C. However, the amount of ejaculate was the same as on July 19th, 2023, when the temperature was 25.8°C. Furthermore, the highest amount of ejaculate was during a period when the temperature was more constantly warm, while the lowest amount of ejaculate was obtained with more variable temperature measurements.

5.5 Bull: Vagabund



	19.07.23	02.08.23	09.08.23	24.08.23	30.08.23	06.09.23	19.09.23	26.09.23	03.10.23	18.10.23	31.10.23
first Ejaculat (ml)	5	7	3,5	7	3,5	2	3	3	9,5	4,5	4
second Ejaculat (ml)	9	2	4	5,5	2,5	5	4	4,5	5	5	3,5
Sum	14	9	7,5	12,5	6	7	7	7,5	14,5	9,5	7,5

Figure 13: Amount ejaculate Vagabund

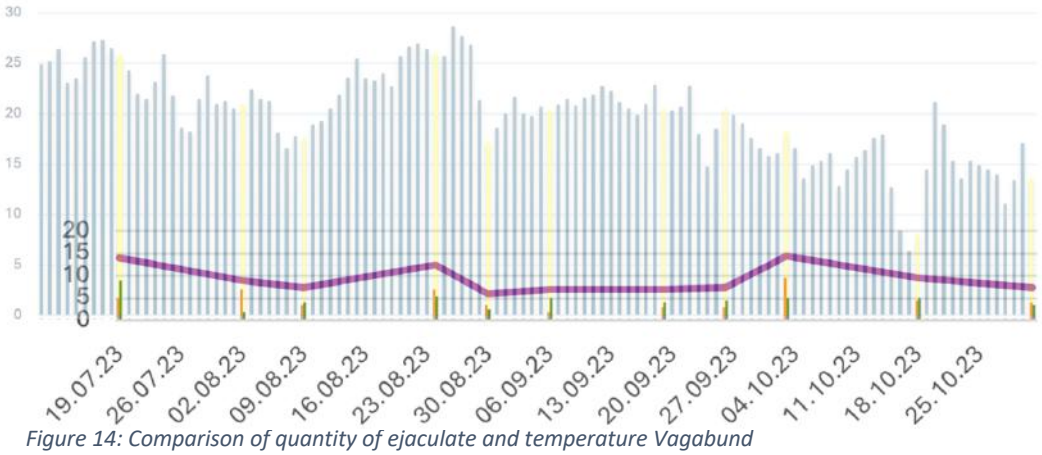


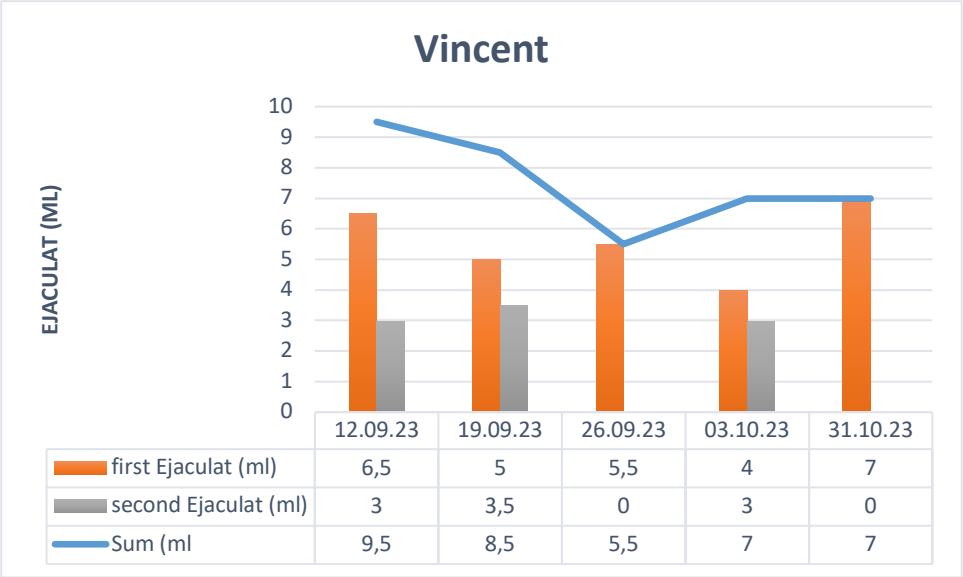
Figure 14: Comparison of quantity of ejaculate and temperature Vagabund

For the next bull named "Vagabund", data was collected over 4 months. Similar to the other bulls, the sample was not used and not documented if the quantity and quality was insufficient. Data collection began on July 19th, 2023, and totaled 14 ml. Two runs of 5 ml and 9 ml were also collected for this bull on each insemination day. The next collection day was on August 2nd, 2023, with a total of 9 ml (7 ml and 2 ml). In the following week, the samples were collected on August 9th, 2023, and a total of 7.5 ml (3.5 ml and 4 ml) was obtained. After this day, there was a break and it was not until August 24th, 2023, that the ejaculate was used again with a total of 12.5 ml (7 ml and 5.5 ml). In September 2023, the first insemination day was the 6th and yielded a volume of 7 ml (2 ml and 5 ml). The same volume was achieved on September 19th, 2023, with partial volumes of 3 ml and 4 ml. One week later, the total volume of 7.5 ml was measured (3 ml and 4.5 ml). In October 2023, the highest ejaculate volume of 14ml (9.5ml and 5ml) was recorded on October 3rd, 2023. Two

weeks later, on October 18th, 2024, the total volume was 9.5ml (4.5ml and 5ml). on the last and final day of Vagabund's recording, the total volume of ejaculate was 7.5ml (4ml and 3.5ml).

On July 19th, 2023, the first day of sowing, the temperature was 25.8°C. In the previous week, the average temperature was 25.9°C. On the following day of sampling, August 2nd, 2023, the temperature was 21°C, while the average temperature on the previous days was 21.6°C. In the following week, the average temperature dropped to 19.6°C and on August 9th, 2023, to 17.6°C. However, the average temperature became warmer again (24.8°C) and reached 26°C on August 24th, 2023. This temperature was maintained for a week until August 30th, 2023, when the temperature dropped again to 17.3°C. On September 6th, 2023, the daily value was 20.4°C. In the following weeks, the average was 21.2 °C, and the highest temperature was recorded on September 19th, 2023, at 20.5 °C. The same temperature was measured on September 26th, 2023, with a temperature fluctuation compared to the previous week and an average of 19.5°C. In October, the temperature dropped drastically to 18.3°C on October 3rd, 2023, with a daily average of 18.1°C for the previous week. Further fluctuations were observed until October 18th, when only 8.1°C was measured, with an average of 14.7°C. In the following days, the temperature remained around 14°C and on October 31st, 2023, only 13.6°C was measured.

5.6 Bull: Vincent



	12.09.23	19.09.23	26.09.23	03.10.23	31.10.23
first Ejaculat (ml)	6,5	5	5,5	4	7
second Ejaculat (ml)	3	3,5	0	3	0
Sum (ml	9,5	8,5	5,5	7	7

Figure 15: Amount ejaculate Vincent

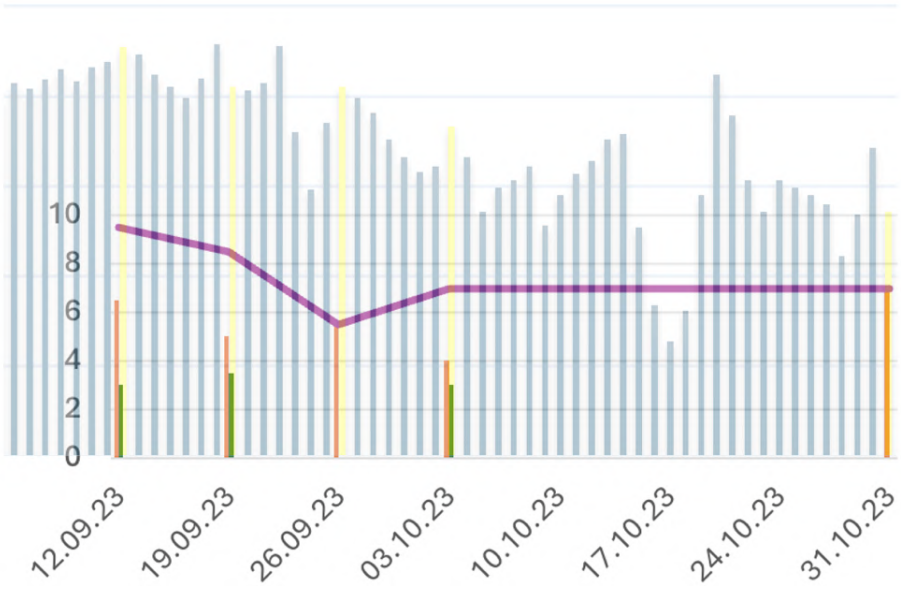


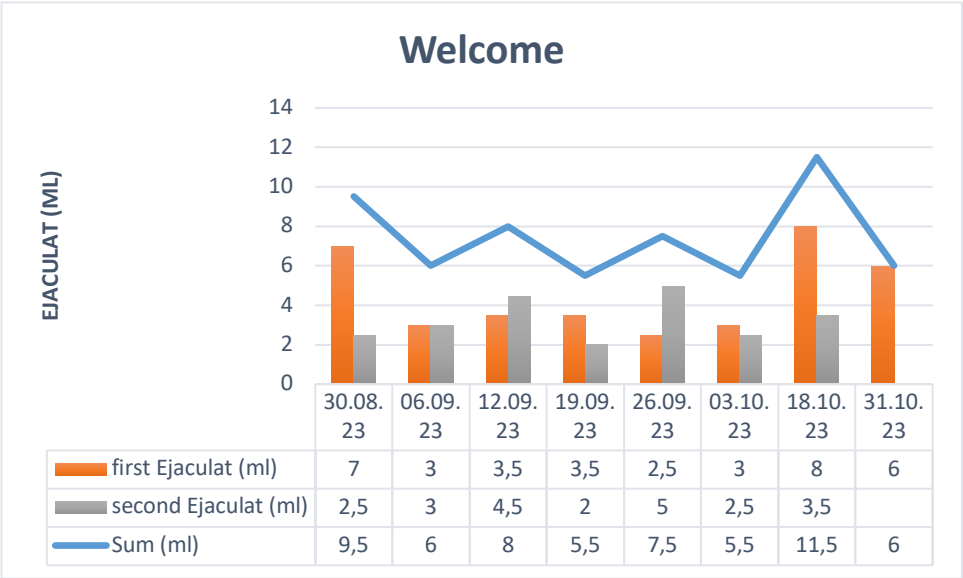
Figure 16: Comparison of quantity of ejaculate and temperature Vincent

For the bull "Vincent", 2 months and 5 days of sampling were evaluated. As with the other bulls, 2 rounds per day were recorded. The first sampling day was September 12th, 2023, and the total amount of ejaculate was 9.5 ml, divided into 6.5 ml and 3 ml for the two parts. One week later, on September 19th, 2023, the total amount decreased to 8.5 ml. The first run yielded 5 ml and the second 3.5 ml. In the following week, the amount decreased again to 5.5 ml. On this day, only the first sample was counted, the second sample was not taken. On October 3rd, 2023, the total amount of ejaculate collected increased to 7 ml (4 ml and 3 ml).

The last and final collection took place on October 31st, 2023, and contained a total of 7 ml. Again, only the first collection was usable.

The temperature aspect is like that of the other bulls. On September 12th, 2023, and the first day of the collection, the daytime temperature was 22.7°C. In the previous week, the average temperature was 21.1°C. One week later, the maximum temperature on the day of collection was 20.5°C. The average temperature of the days before was 21.5°C. On September 26th, 2023, the value was 20.5°C and the average temperature in the previous week was 19.5°C. In October 2023, the value dropped further to 18.3°C. In September 2023, the value was 20.5°C and the average temperature the week before was 19.5°C. In October 2023, the value dropped further to 18.3°C, with the average of the previous week being 17.8°C. The next and last recording was on October 31st, 2023, and the temperature dropped to 13.6°C.

5.7 Bull:Welcome



	30.08.23	06.09.23	12.09.23	19.09.23	26.09.23	03.10.23	18.10.23	31.10.23
first Ejaculat (ml)	7	3	3,5	3,5	2,5	3	8	6
second Ejaculat (ml)	2,5	3	4,5	2	5	2,5	3,5	
Sum (ml)	9,5	6	8	5,5	7,5	5,5	11,5	6

Figure 17: Amount ejaculate Welcome

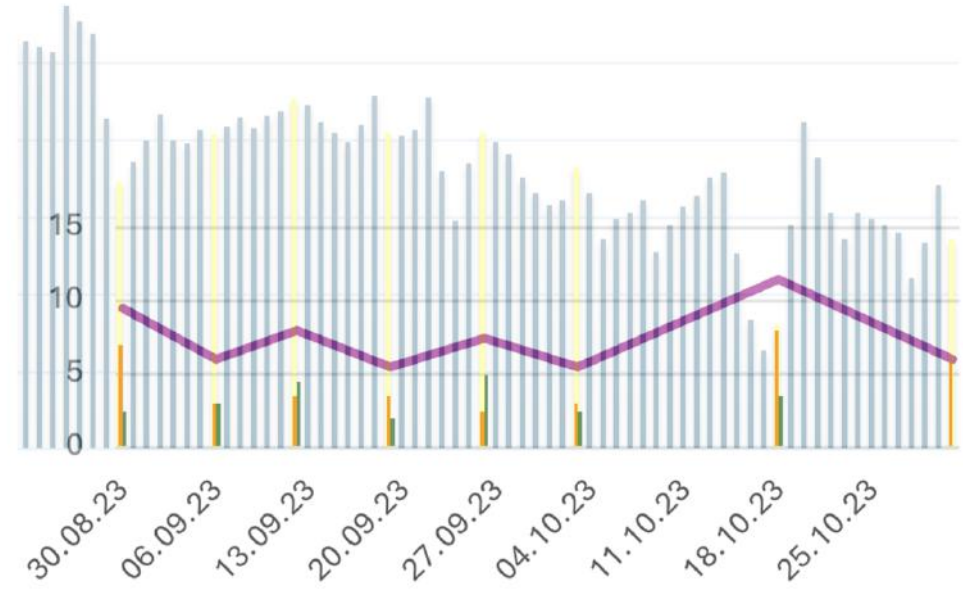
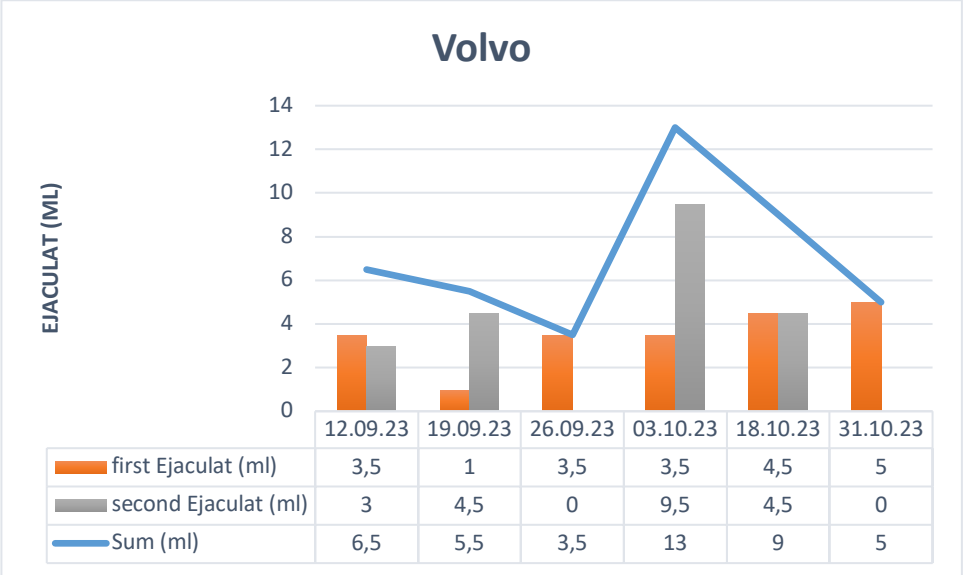


Figure 18: Comparison of quantity of ejaculate and temperature Welcome

A total of 8 days were analyzed for the bull "Welcome", which were recorded over a period of 3 months. On the first recorded day, August 30th, 2023, a total of 9.5 ml of ejaculate was recorded. This amount also consisted of 7 ml and 2.5 ml per semen collection. Next, on September 6th, 2023, a total of 6 ml of ejaculate (3ml and 3 ml) was evaluated. One week later, on September 12th, 2023, 3.5 ml was collected in the first round and 4.5 ml in the second round. Again, one week later, on September 19th, 2023, the total amount dropped to 5.5 ml (3.5 ml and 2 ml). On the last evaluated day in September, September 26th, 2023, a total of 7.5 ml (2.5 ml and 5 ml) was achieved. October 2023, on the other hand, probably

saw the greatest fluctuations. On October 3rd, 2023, a total of only 5.5 ml of ejaculate was again achieved, but on October 18th, 2023, the amount rose again to 11.5 ml. Even on this last evaluated day, October 31st, 2023, there was only one usable sample of 6 ml of ejaculate. For a detailed presentation of the temperature data, please refer to the sections on “Vagabund”, “Verdi” and “Vincent”. The following is just a summary of the temperature data from the previous bulls. The temperature on the first day of insemination was 17.3 °C. In the week before, temperatures were mainly above 20 °C. In September, the temperature also remained constant at 20 °C. In October, a drastic drop in temperature was observed. Between October 3rd, 2023, and October 31st, 2023, the temperature was between 18.3 °C and 8.1 °C.

5.8 Bull: Volvo



	12.09.23	19.09.23	26.09.23	03.10.23	18.10.23	31.10.23
first Ejaculat (ml)	3,5	1	3,5	3,5	4,5	5
second Ejaculat (ml)	3	4,5	0	9,5	4,5	0
Sum (ml)	6,5	5,5	3,5	13	9	5

Figure 19: Amount ejaculate

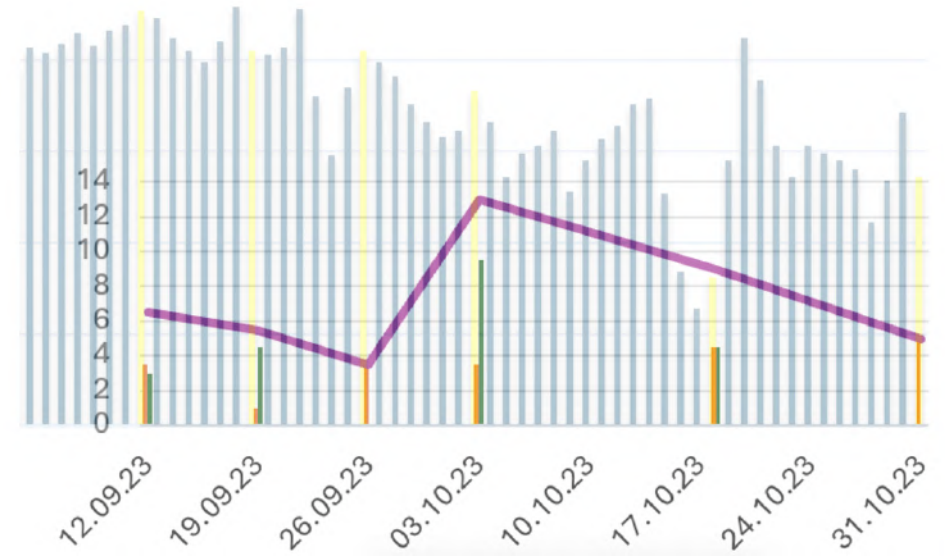


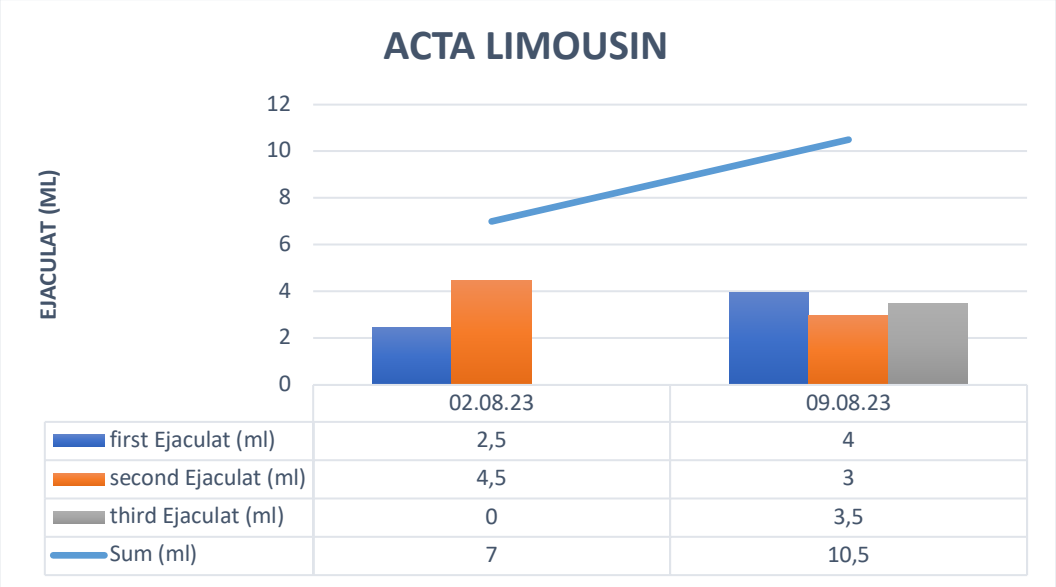
Figure 20: Comparison of quantity of ejaculate and temperature Volvo

The next bull described is “Volvo”. He also has six evaluated days in a period from September to October 2023. The first day is September 12th, 2023. On this day, a total volume of 6.5 ml was reached. Here too, the volume was made up of 3.5 ml and 3 ml. One week later, on September 19th, 2023, the ejaculate sample dropped to 5.5 ml (1 ml and 4.5 ml). On September 26th, 2023, the amount of ejaculate dropped further to 3.5 ml. In October, the total amount rose to a peak of 13 ml (3.5 ml and 9.5 ml) on October 3rd, 2023. However, on October 18th, 2023, the amount collected dropped again to 9 ml (4.5 ml and 4.5 ml). On

the last day of recording, October 31st, 2023, only the first semen sample of 5 ml was usable for this bull.

Similar, as with the Bull called “Welcome”, reference is made here to the detailed temperature descriptions already mentioned. In the interest of clarity, here are the most important points to remember. In September, the temperature was mostly around 20°C. In mid-October 2023, however, the temperature cooled down to 8.1°C. And rose again to 13.6°C by October 31st,2023.

5.9 Bull Acta Limousin



	02.08.23	09.08.23
first Ejaculat (ml)	2,5	4
second Ejaculat (ml)	4,5	3
third Ejaculat (ml)	0	3,5
Sum (ml)	7	10,5

Figure 21: Amount ejaculate Acta Limousin

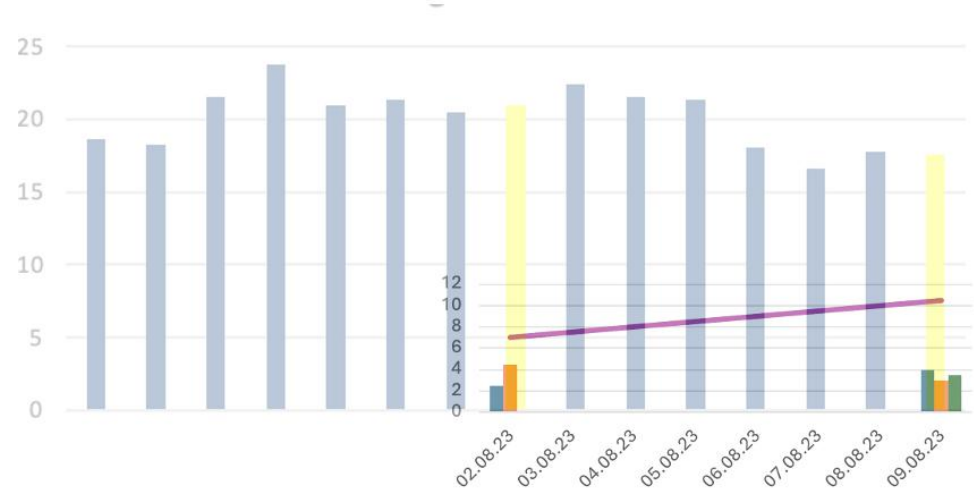
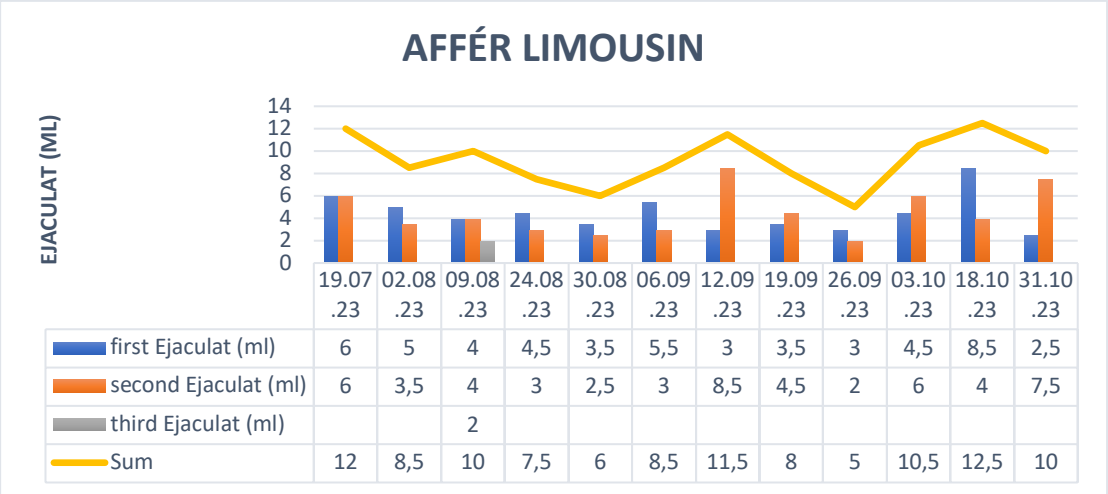


Figure 22: Comparison of quantity of ejaculate and temperature Acta Limousin

Only two days were recorded for “Acta Limousin”. The first day was August 2, 2023, on which three ejaculates were collected. The total amount was 7 ml, which consisted of 2.5 ml from the first and 4.5 ml from the second sample. The third sample was not counted on this day. On the second day of recording, August 9, 2023, a total of 10.5 ml was collected, which consisted of 4 ml, 3 ml and 3.5 ml.

For this bull, there are only two days for comparison. On August 2, 2023, the temperature was 21°C, which was also the average temperature for the previous week. It cooled slightly until August 9, 2023, reaching 17.6°C on that day.

5.10 Bull: Affér Limousin



	19.07.23	02.08.23	09.08.23	24.08.23	30.08.23	06.09.23	12.09.23	19.09.23	26.09.23	03.10.23	18.10.23	31.10.23
first Ejaculat (ml)	6	5	4	4,5	3,5	5,5	3	3,5	3	4,5	8,5	2,5
second Ejaculat (ml)	6	3,5	4	3	2,5	3	8,5	4,5	2	6	4	7,5
third Ejaculat (ml)			2									
Sum	12	8,5	10	7,5	6	8,5	11,5	8	5	10,5	12,5	10

Figure 23: Amount Ejaculate Affér Limousin

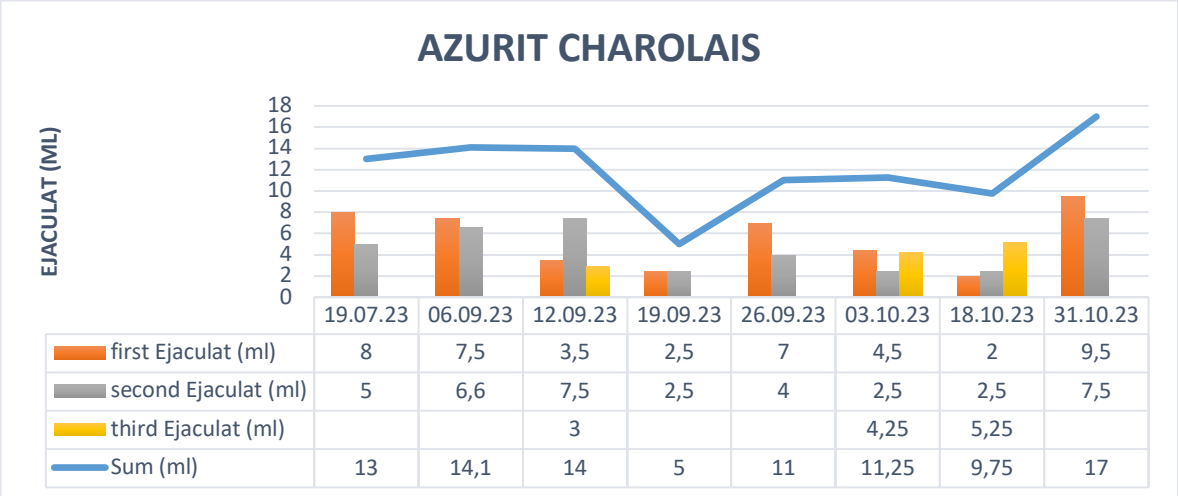
Figure 24: Comparison of quantity of ejaculate and temperature Affér Limousin

The recordings for the "Affér Limousin" bull were also evaluated from July to October 2023. On the first recording day, July 19th, 2023, a total of 12 ml was collected, consisting of 6 ml and 6 ml. On the following collection day, August 2nd, 2023, only 8.5 ml (5 ml and 3.5 ml) were collected. This was followed by August 9th, 2023, on which 3 collections were made. On this day, 10 ml of ejaculate was collected (4 ml, 4 ml and 2 ml). Two weeks later, on August 24th, 2023, a total of 7.5 ml of ejaculate (4.5 ml and 4 ml) was collected. In addition, on August 30th, 2023, a total amount of 6 ml of ejaculate was obtained after 2 samplings. In September 2023, ejaculate was regularly collected on four days. On September 6th, 2023, 8.5 ml (5.5 ml and 3 ml), on September 12th, 2023, 11.5 ml (3 ml and 8.5 ml), on September 19th, 2023, 8 ml ejaculate (3.5 ml and 4.5 ml) and finally on September 26th, 2023, only 5 ml (3 ml and 2 ml) were collected. In October 2023, the difference between the 3 ejaculate volumes collected was not quite as great. A volume of 10.5 ml was collected on October 3rd,

2023, 12.5 ml (8.5 ml and 4 ml) on October 18th, 2023, and 10 ml (2.5 ml and 7.5 ml) on the last day of recording. The highest value was reached on October 18th, 2023, and the lowest value on September 26th, 2023.

“Affér Limousin” is clearly referenced to the bull “Vagabund”. These two overlap on all days except on September 12th, 2023. On this day, the temperature was 22.7°C and the average of the previous week was 21.1°C.

5.11 Bull: Azurit Charolais



	19.07.23	06.09.23	12.09.23	19.09.23	26.09.23	03.10.23	18.10.23	31.10.23
first Ejaculat (ml)	8	7,5	3,5	2,5	7	4,5	2	9,5
second Ejaculat (ml)	5	6,6	7,5	2,5	4	2,5	2,5	7,5
third Ejaculat (ml)			3			4,25	5,25	
Sum (ml)	13	14,1	14	5	11	11,25	9,75	17

Figure 25: Amount of Ejaculate Azurit Charolais

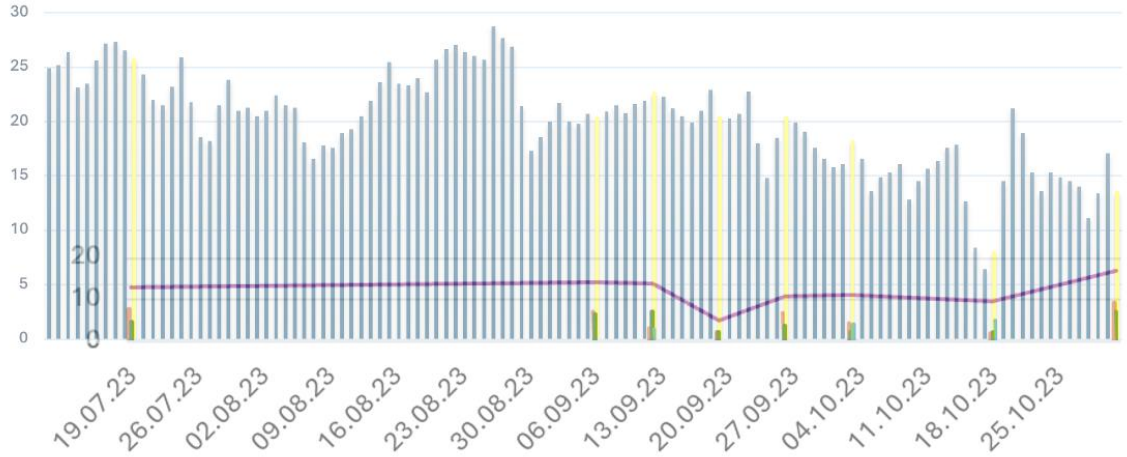


Figure 26: Comparison of quantity of ejaculate and temperature Azurit Charolais

In the case of "Azurit Charolais", a period from July to the end of October 2023 was examined. On some days, as many as three ejaculate samples were used. On July 19th, 2023, a total of 13 ml of ejaculate was collected. This total consisted of 8 ml and 5 ml from the second sample. The next recording took place on September 6th, on which day a total of 14.1 ml of ejaculate was collected. (7.5ml and 6.6ml). One week later, on September 12th, 2023, 3 samples were examined for the first time and a total of 14ml was obtained (3.5 ml; 7.5 ml and 3 ml). The next ejaculate collection took place on September 19th, 2023, and yielded a total of only 5ml (2.5ml and 2.5ml). One week later, this amount increased again to a total of 11 ml. In October 2023, the amount remained roughly the same as on the first collection day. Thus, on October 3rd, 2023, a quantity of 11.25 ml was obtained. This was again made up of three samples (4.5 ml; 2.5 ml; 4.25 ml). On October 18th, 2023, the total amount

obtained from three ejaculations also fell to 9.75 ml (2 ml; 2.5 ml; 5.25 ml). On the last day of recording, October 31st, 2023, the incredible amount of 17 ml (9.5 ml and 7.5 ml) was obtained after only two samples.

This data set also refers to “Welcome”, with one exception on July 19th, 2023. On that day, the temperature was 25.8 °C, which is a constant value compared to the previous week.

6 Conclusion

In general, the highest ejaculate volumes were recorded on days when the temperature was consistently around 25-26° Celsius. As an example, Verdi had his highest value of 11ml on August 24th, when 26°C was measured. Low ejaculate volumes tended to be seen on days when there had been more fluctuations or when it was generally cooler. Verdi's lowest ejaculate volume was 5.5 ml on September 13th, when 22.5 °C was measured. However, there were exceptions here as well. On July 19th, the temperature was 25.8 °C and on August 30th, it was 17.3 °C, and despite this temperature difference, the ejaculate volume remained unchanged at 7.5 ml. There were also clear differences in the way the samples were collected. Most of the bulls were sampled regularly at intervals of 1-2 weeks. Others, however, were tested rather sporadically. However, it seems that the consistency of the samples themselves are not really correlated with the ejaculate volume, but regular records are better for observing changes.

In summary, the highest ejaculate volumes vary between 11ml and 17ml. The absolute maximum was achieved by Azurit Charolais with 17ml at only 13.6°C. Although it is more likely to be concluded from the records that the highest ejaculate samples were obtained at a constant temperature of about 25 degrees Celsius. The lowest amount of ejaculate was achieved by Volvo with 3.5ml on September 26th at 20.5°C. Although it appears that average temperatures between 10° -30° Celsius have a positive effect on the amount of ejaculate volume, this connection is not entirely clear. The inconsistent temperature data collection does not seem to really affect the volume, but it would be interesting to observe the bulls over a longer period to get a more accurate temperature data collection and correlation of these factors. In conclusion, it seems that not only temperature but also many other factors, such as the time of collection, the age of the bull and the breed itself are responsible for the fluctuations in ejaculate volume.

7 Acknowledgements

I would like to express my gratitude to Dr. Rátky József for his invaluable support and assistance over the past months. I would also like to acknowledge the Bos Genetic team for their generosity in providing the data and for their kindness and openness during my visits.

8 References

8.1 Literature References

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8.2 Image References

Bull Saphir:

[A] 2024 - OHG Osnabrücker Herdbuch eG

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Sperm abnormalities:

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Thesis progress report for veterinary students

Name of student: Fankhauser Antonia Sophie

Neptun code of the student: EY40PA

Name and title of the supervisor: Dr. Rátky József

Department: Department of Obstetrics

Thesis title: Semen quality parameters of AI in cattle bulls related to different weather conditions

Consultation – 1st semester

	Timing			Topic / Remarks of the supervisor	Signature of the supervisor
	year	month	day		
1.	2023	4	12	First meeting	
2.	2023	4	17	Visit Bos - Genetic	
3.	2023	4	27	Feedback on the table of contents	
4.	2023	6	26	Discussion about progress	
5.	2023	8	17	Feedback on progress	

Grade achieved at the end of the first semester: ..... (pass) .....

Consultation – 2nd semester

	Timing			Topic / Remarks of the supervisor	Signature of the supervisor
	year	month	day		
1.	2023	10	25	Visit Bos Genetic	
2.	2023	11	14	Meeting, Discussion about progress	
3.	2023	12	6	Visit Bos Genetic, Semen collection, Deep freezing	
4.	2024	5	12	Feedback on for written chapters	

5.					

Grade achieved at the end of the second semester: .....5 (five).....

The thesis meets the requirements of the Study and Examination Rules of the University and the Guide to Thesis Writing.

I accept the thesis and found suitable to defence,

.....
signature of the supervisor

Signature of the student: .....

Signature of the secretary of the department: .....

Date of handing the thesis in..... 07.12.2024 .....