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Influence of Tuberculin Test protocols on Semen Quality

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1. Abstract:

In the modern economy we struggle a big competition especially in the production animal sector therefore their performance which is especially defined by their reproduction status is of great value if we want to be able to face the competitive pressure at the modern market.

This is why this thesis deals with stressors influencing the sperm quality of beef and dairy bulls such as the intradermal tuberculin tests.

Some bulls may be more sensitive to just small environmental adjustments and stressors that's why an "easy" procedure such as the tuberculin probe can have a big influence on the following ejaculation quantity and quality.

As a consequence, will my following work deal with the impact of these protocols observed in the semen station at "Bos genetic Kft.".

What things, do we have to consider if we talk about the impact of tuberculin protocols? First of all, we need to think about the usage between different types of cattle f.e. a Holstein Friesian used for diary production is known to human contact whereas a Charolais living on the pasture has minimal human contact so the amount of stress felt in the same procedures can greatly vary in every individual.

To understand the importance of all these different factors we need to discuss the pathophysiology of stress and the general behaviors and sensitivity of cattle.

Therefore, it's important to understand are the desired sperm quality and the most common spermcell abnormalities.

The last chapter of my scientific work will contain the different country legislations, as well as the influence of tuberculosis in the economical animal production sector and the public health aspects as Tuberculosis is considered as a zoonosis and last but not least laboratory diagnostics used for detection of tuberculosis.

1. Összefoglaló:

Az modern mezögazdaságban különösen a haszonállat-ágazatban nagy versenyben vagyunk, ezért teljesítményük, amelyet különösen a szaporodási állapotuk határoz meg, nagy értéket képvisel, ha szembe akarunk nézni a modern piaci nyomással.
Ezért ez a dolgozat a marha- és tejelő bikák spermaminőségét befolyásoló stresszorokkal foglalkozik, mint például az intradermális tuberkulin tesztek.
Egyes bikák érzékenyebbek lehetnek kis környezeti változásokra és stresszfaktorokra, ezért olyan „egyszerű" eljárás, mint a tuberkulinszonda, nagy hatással lehet a következő ejakulátum mennyiségére és minőségére.
Következésképpen a következő munkám ezeknek a „Bos genetic Kft." Mesterséges termékenyítö állomásán megfigyelt protokolloknak a hatásával fog foglalkozni.

Milyen dolgokat, kell figyelembe vennünk, ha a tuberkulin protokollok hatásáról beszélünk?
Először is, gondolnunk kell a különböző szarvasmarha fajtacsoportok közötti használatra, pl. egy tejtermelésre használt holstein fríz szarvasmarha megszokta az emberi érintkezést, míg egy legelőn élő charolais szarvasmarha minimális emberi érintkezéssel találkozott, így ugyanazon eljárások során érzett stressz mértéke minden egyednél nagymértékben eltérhet.
Ahhoz, hogy megértsük mindezen különböző tényezők jelentőségét, meg kell vizsgálnunk a stressz patofiziológiáját és a szarvasmarhák általános viselkedését és érzékenységét.
Ezért fontos megérteni a kívánt spermiumminőséget és a leggyakoribb spermasejt-rendellenességeket.
Munkám utolsó fejezete tartalmazza majd a különböző országok jogszabályait, valamint a tuberkulózis hatását a gazdasági állattenyésztési ágazatban és a közegészségügyi szempontokat, mivel a tuberkulózist zoonózisnak tekinjük, és végül, de nem utolsósorban a tuberkulózis kimutatására használt laboratóriumi diagnosztikát.

2. History of “bos genetic”:

[1] The chief Ingenieur of the agriculture Dr. János Zsili was searching for a possibility to create a platform for breeding bulls in the Hungarian economy. That’s where the “Osnabrücker Herd-book cooperative” takes part in the future business management of „Bos genetic Kft.“ . The “Osnabrücker Herd-book Cooperation” (OHG) is one of the smallest but yet the largest firm, regarding their success in their breeding history in Germany. This cooperation was showing interest into an interaction with the Hungarian BOSCOOP cooperative joint enterprise which was the largest production system in the past in Hungary. The “OHG” agreed that their breeding bulls will participate in the establishment the new company. After an agreement with the ministries bos genetic was founded on the 12th of November 1985, where soon after the first twelve breeding bulls arrived on December 3rd. Because of the BOSCOOP company hungarian breeders where able to select semen from the breeding bull which they preferred for the first time. To select those, they got catalogues with pictures of which they could choose from.
The company BOSCOOP was able to provide many breeders at their region a few months after founding bos genetic. In 1993 the company finally got a remark for their successful management system as the got ranked in the BLUP (best linear unbiased prediction).

Picture 2: about a trophy of bos genetic. Photo taken at bos genetic.

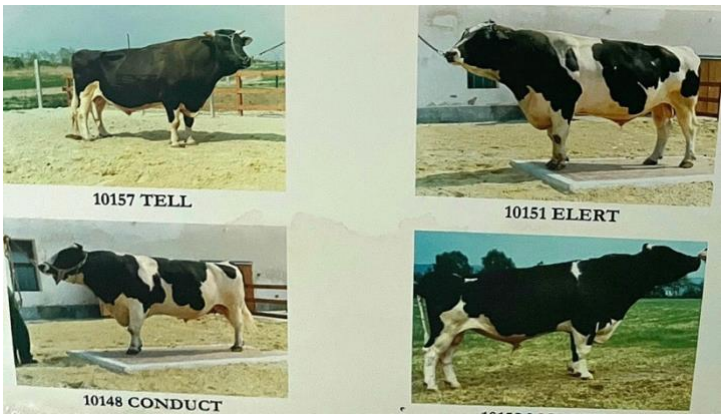
The further written passage will include the biggest challenges which „bos genetic“had to face over the past 39 years until it became a company to teach veterinary students at the university of veterinary science in Budapest.

First of all, I would like to present a few of the first bulls of “bos genetic” which where the key to success which the company experienced in the past.

The most successful sires between 1991 and 2010 included bulls like Chief Stic (Chief Mark x Sexation), Saphir (Storm x Prelude) and, above all, BG Shottle ET, born in 2005. These bulls dominated the breeding value top lists in Hungary for years. (See pictures below)

Picture 3: bull saphir which was hungarys most selled bull.

(Source: https://www.ohg-genetic.de/veterinaermedizinische-universitaet-budapest-uebernimmt-bos-genetic-kft/#



Picture 4: Seen can be four of the first bulls that have arrived from OHG to the bos genetic Kft.

In Hope to proceed the streak of success the leading manager of bos genetic changed on the 1st of January 1999 from the company's director Dr. János Szili, who successfully led the company for 14 years, to the new manager Dr. István Monostori, which has been the colleague of Dr. Janos Szili since 1991. Also, since 1999 the first opportunities arose to test interesting young bulls in Hungarian farms.

By restructuring the company (the OHG increased its share to 72%), Bos-Genetic itself was able to influence sales activities and concluded test agreements with partner companies. This gave Bos-Genetic's success story in Hungary an additional boost.

Due to the constantly growing offspring testing program and the entry of additional shareholders (SRV and Genes Diffusion), who were particularly interested in the excellent bull keeping and semen production on site in Hungary, it was decided shortly

after the turn of the millennium to build a new stable facility In 2004, the accommodation options for bulls were expanded to up to 140 places. [1] (https://www.ohg-genetic.de/veterinaermedizinische-universitaet-budapest-uebernimmt-bos-genetic-kft/#)

However, „Bos genetic Kft. "began to struggle adapting to the accelerating speed of progress in the economy, which makes it almost impossible to stay economically successful as a smaller company.

Nowadays every bull needs to be genetically screened for their genetic properties, even before we know if he will be a good breeding bull. Some screening methods are performed as described below.

In the modern breeding culture is the semen collected and stored and afterwards checked by investigations concerning the next generation that means we have to wait until F1 of the breeding bull gives birth to F2 and just if the properties of F1 are as desired of the modern economy will this semen be of a higher value.

But during that time the semen needs to be stored, the bull needs to be kept and shouldn't lose its reproductive value which is of course very cost intensive especially for smaller companies. But if this semen will produce an offspring with desirable features such as milk protein or fat ratio, an appropriate body size, teat length, just to point out some examples of what properties are considered for a good genetic value.

Although the biggest problem of such companies is not even the genetically screening or the reproductive behavior of the bulls, it's the speed of the changing demands as well as continuing progress which we face in the modern market. Furthermore, can't the prices of award-winning bulls be handled in smaller companies that's why the streak of success at bos genetic Kft. wasn't long lived.

[1] Because Dr. Istvan Monostori had already reached retirement age, he has worked intensively to establish new, strategic partnerships, including the University of Veterinary Medicine in Budapest. Prof. Sotonyi, rector of the university, would like to focus more heavily on the practical aspects of bull keeping and sperm production for students. In order to ensure both improved training for veterinary students and the continuation of breeding activities in the dairy and beef cattle sector in Hungary to continue Bos -Genetic Kft. under its own direction. The corresponding contracts were signed on site in Budapest on March 9, 2022, and the OHG remains a valued partner for Bos-Genetic Kft.

[1] (https://www.ohg-genetic.de/veterinaermedizinische-universitaet-budapest-uebernimmt-bos-genetic-kft/#)

Nowadays the leading manager of bos genetic Kft.is Dr. Józef Rátky he is the Head of the Reproduction and Obstetrics department at the university of veterinary medicine in Budapest.

Furthermore, is the veterinarian Dr. Meresz Lájos for their existence nowadays of great importance as their practicing veterinarian he as well had a great influence concerning our thesis research and the education Program of us students during our visits. Last but not least are the workers as well as their lab assistant of great importance for the successful running of the business.

3. General considerations:

3.1 desired sperm Quality and Quantity and the Anatomy of Spermatozoa:

Diagnostic methods for semen quality investigations: Macroscopic, Chemical procedures as well as Microscopic and Microbiological investigations

Macroscopic investigations: Subjective categorization by Volume, appearance and concerning color and smell.

Normal Findings: the detected Volume is considered small in ruminant species, but it should be in the range of 5-15ml. The appearance may not show any signs of contamination that means the color should be greyish–white or opalescent and homogenous, furthermore is the viscous „egg white–like “appearance and the non–odorous smell characteristic for healthy ejaculate.

Microscopic investigations: Motility, Morphology, Concerntration, total sperm output, Viability (ratio of dead and alive spermatozoa) as well as presence of cells other than spermatozoa. Mostly examined by microscopy.

Picture 5: microscopic investigations at bos genetic.

The microscopic investigations are of special importance for calculating the number of possible artificial insemination doses produced from one ejaculate as well as the amount of diluent used for the AI doses (further explanation in the following chapter). [2] For this examination is typically the gross motility checked by using an undiluted drop of semen placed on a slide examined at 1000x Magnification.

The Concentration of spermatozoa in a physiologic ejaculate is about 800 million up-to 2 billion spermatozoa/mL, with an average of 1 billion sperms per Milliliter.

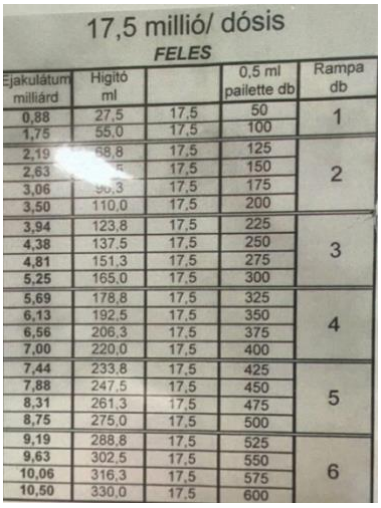
However, this can vary on the individual bull depending on its age, breed, and health status.

Total motile 6 X 109 X 0.7 (4.2 X 109 spermatozoa/ejaculate). Which gives us a minimum progressive motility around 70%. This feature is examined by a subjective rather than objective investigation since its confirmed under the microscope.

[2] (Quality in Young Bull Semen Can Be Improved by Single Layer Centrifugation

Isabel Lima-Verde [1], Emma Hurri [2], Theodoros Ntallaris [1], Anders Johannisson [1], Hans Stålhammar [2], Jane M Morrell [1,*]Editor: Clive J C Phillip, Copyright and License information! PMCID: PMC9494988 PMID: 36139296, https://pmc.ncbi.nlm.nih.gov/articles/PMC9494988/)

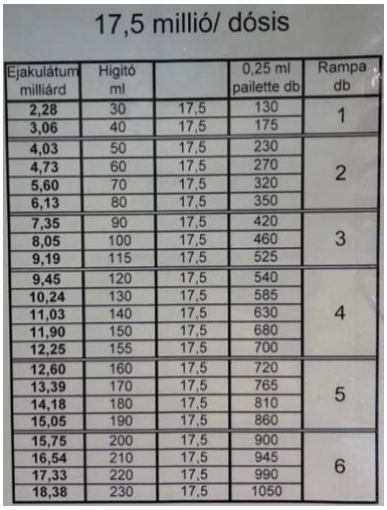
Concerning the above listed numbers the amount of diluent and the following AI doses can be calculated adequately as shown in the tables below:



17,5 millió/ dósis

FELES

Ejakulátum milliárd	Higító ml		0,5 ml pailette db	Rampa db
0,88	27,5	17,5	50	1
1,75	55,0	17,5	100	
2,19	68,8	17,5	125	2
2,63	[illegible]	17,5	150	
3,06	[illegible]	17,5	175	
3,50	110,0	17,5	200	
3,94	123,8	17,5	225	3
4,38	137,5	17,5	250	
4,81	151,3	17,5	275	
5,25	165,0	17,5	300	
5,69	178,8	17,5	325	4
6,13	192,5	17,5	350	
6,56	206,3	17,5	375	
7,00	220,0	17,5	400	
7,44	233,8	17,5	425	5
7,88	247,5	17,5	450	
8,31	261,3	17,5	475	
8,75	275,0	17,5	500	
9,19	288,8	17,5	525	6
9,63	302,5	17,5	550	
10,06	316,3	17,5	575	
10,50	330,0	17,5	600	



17,5 millió/ dósis

Ejakulátum milliárd	Higító ml		0,25 ml pailette db	Rampa db
2,28	30	17,5	130	1
3,06	40	17,5	175	
4,03	50	17,5	230	2
4,73	60	17,5	270	
5,60	70	17,5	320	
6,13	80	17,5	350	
7,35	90	17,5	420	3
8,05	100	17,5	460	
9,19	115	17,5	525	
9,45	120	17,5	540	4
10,24	130	17,5	585	
11,03	140	17,5	630	
11,90	150	17,5	680	
12,25	155	17,5	700	
12,60	160	17,5	720	5
13,39	170	17,5	765	
14,18	180	17,5	810	
15,05	190	17,5	860	
15,75	200	17,5	900	6
16,54	210	17,5	945	
17,33	220	17,5	990	
18,38	230	17,5	1050	

Picture 6 and 7:

Tables of AI doses Calculation depending on different straw sizes.

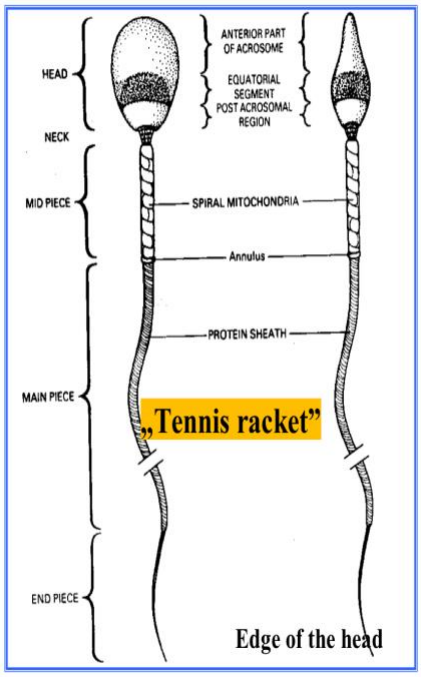
As seen from the following table, two different straw sizes are required, which is due to the various country preferences. The preferred semen straw size in the US, Canada as well as Australia are 0.5 ml. Whereas in most European countries, we use the 0.25 ml straw such as in Hungary, Germany, Austria and Switzerland. Following these different preferences the dilution amount has to be calculated accordingly.

Chemical investigations: Checking the pH as well as other properties concerning the seminal plasma.

Normal pH: is between 6.5 to 7.0.

Microbiological investigations: smears and bacterial culture to check for possible infections or contaminations.

Anatomy of spermatozoa:



Picture8: https://elearning.univet.hu/pluginfile.php/126688/mod_resource/content/1/stud_obstetrics3_1-2%20%20-%20%20Kompatibilitási%20mód.pdf

[3] 1.Head: Contains the nucleus and the genetic material. The head is covered by the acrosome, which is a specialized structure serving as the source of penetratation when entering the oocyte during the fertilisation procedure.

2. Neck: Connects the head to the midpiece and serves as a transition region.

3. Midpiece (body): Contains the mitochondria, which serve as energy source (in the form of ATP) and are essential for the sperm's motility.

4. Tail (Flagellum): is a long, whip-like structure that manoeuvres the sperm cell towards the oocyte. The tail contains protein fibres which helps it to approach the movement towards oocyte.

These anatomical features are essential for the sperm's ability to navigate through the female reproductive tract and fertilisation of the female oocyte.

[3] https://www.givelegacy.com/resources/overview-sperm-anatomy/

3.2 Typical abnormalities concerning Quantity, Quality and anatomical features of spermatozoa.

Classification: Defects can be categorized according to the location on the spermatozoa. Categorization: is based upon the location within the genital tract where the defect has arisen. Major and minor defects are based on their effects on the fertility.

[4] Primary defects:

1.Abnormal sperm morphology (for example miss shaped heads, or bent tails)

2. Poor sperm motility (for example low activity or even immotile sperm cells)
3. Low sperm output (oligospermia)
4. Reduced sperm viability (for example high levels of dead or moribund spermatozoa)
5. Sperm agglutination (clumping of sperm)
6. Sperm chromatin defects (DNA fragmentation)

[4] Secondary defects:

1. Acrosome abnormalities (for example incomplete or even absent acrosome)
2. Tail defects (for example shortened or coiled tails)
3.. Cytoplasmic droplets (abnormal structures on the spermtazoa)
4. Vacuoles in sperm heads (means fluid filled spaces within the head)
5. Abnormalities within the mitochondrial function (disturbs sperm energy production)
6. Sperm membrane defects (for example compromised integrity of the sperm membrane)
7. Sperm head deformities (for example fusion or multiplication of head)
8. Tailless or headless sperm (sperm lacking tails or heads)

[4] Possible Causes of the listed defects:

Primary defects:

1. Genetic abnormalities: Inherited gene mutations or chromosomal abnormalities can be the reason for primary defects on spermatozoa such as alterations in morphology, motility, or count.
2. Testicular pathological alterations: Abnormalities such as a varicocele (which is the enlargement of the veins within the testicular sac (scrotum)), or an infectious background following inflammation, or trauma to the testicles can impair sperm quantity and quality.
3. Hormonal imbalances: such as changes in hormone levels, especially testosterone or follicle-stimulating hormone (FSH), can affect sperm production as well as maturation.
4. Environmental factors: Exposure to toxins as well as medication, radiation, or even certain stressors can damage sperm DNA and causes typically primary defects.
5. Lifestyle factors: such as too high frequency of ejaculations as well as too low frequencies (as the employees at Bos genetica told us some bulls tend use objects in their surroundings for masturbation between the next service). [5] However, the importance of this factor is not fully understood yet. Whereas it is possible that too high masturbation frequency will negatively influence the quality of the semen other studies show that a higher ejaculation frequency caused by masturbation won't have a major impact on the Fertility and can sometimes even positively stimulate production and viability of the spermatozoa cells.

[5] (behavioral problems of cattle, by Gary M. Landsberg, BSc, DVM, MRCVS, DACVB, DECAWBM;Sagi Denenberg, DVM, DACVB, Dip. ECAWBM (Behaviour), MACVSc (Behaviour), https://www.msdvetmanual.com/behavior/normal-social-behavior-and-behavioral-problems-of-domestic-animals/behavioral-problems-of-cattle)

[4] Secondary defects:

1. Epididymal dysfunction: Issues with the epididymis, such as blockages or inflammation, can lead to secondary defects in sperm morphology and motility.
2. Seminal vesicle abnormalities: Problems within the seminal vesicles, which produce seminal fluid, can affect sperm viability and function.
3. Prostate disorders: Conditions affecting the prostate gland, such as infections or inflammation, may result in secondary defects in sperm quality.
4. Urethral abnormalities: Blockages or structural abnormalities in the urethra can impact the transport of sperm during ejaculation, leading to secondary defects.
5. Retrograde ejaculation: In this condition, semen enters the bladder instead of being expelled through the urethra during ejaculation, potentially causing secondary defects in sperm quality.

Abnormal sperm cells in general we call Teratozoospermia.

[4]Reviewed/Revised May 2014; defects Peter J. Chenoweth, School of Agricultural and Veterinary Sciences, Charles Sturt University, Wagga Wagga, NSW 2678, Australia, 2005 Elsevier Inc., https://cdn.ymaws.com/www.therio.org/resource/collection/4C617281-2F09-4B55-B6AA-29F092002BAC/2005_32-43.pdf)

Further Abnormalities considering quantity and quality of the semen.

Pathological changes in Volume: absence of spermcells in the ejaculate is called Aspermia, whereas too low output is called Hypospermia in contrast to that, is an increased sperm number of spermatozoa called Hyperspermia.

Pathological changes in Sperm concerntration: range between from Zero sperm Output to increased output, named scientifically Azoospermia, Oligozoospermia, Polyzoospermia and Normozoospermia which is, the physiologic and desired concerntration.

Pathological changes in the vitality are called Necrozoospermia whereas abnormal alterations concerning motility are described as Asthenozoospermia.

Concerning the macroscopic abnormalities, it is possible to observe changes in color, smell and consistency.

Picture 9: diluted semen probe, photo taken at bos genetic.

Discolorations in the ejaculate can often give us a useful hint for the diagnosis.

[6] For example, a yellowish watery ejaculate can be caused by urine inside the probe, whereas a yellow/green and thick semen output can indicate an infection or an inflammation which can be caused by severe or even zoonotic pathogens.

Whereas some yellowish discoloration can be also considered as normal for example if the bull consumed a lot of fresh grass before taking the probe a yellow discolouration can be caused by the excessive amount of riboflavin.

[6] (Publication: Nature, Volume 182, Issue 4636, pp. 667-668 (1958).Pub. Date: September 1958, DOI: 10.1038/182667a0, https://ui.adsabs.harvard.edu/abs/1958Natur.182..667W/abstract)

Whereas a reddish discoloration can be iatrogenically induced for example by a harmful collection method, or it's due to prostatic Hyperplasia, which is not a serious condition. Nevertheless, we also have to consider infections if we see discolouration and are dealing with food producing animals since the red discolouration can also be blood caused by an inflammation following an infection.

When we examine the colour of the semen we could also already determine an abnormal smell such as in case of a pyospermia, which is a very unpleasant smelly process often caused by infections which need further investigations. When considering infections and following inflammation of the abnormal sperm probe, we should as well put special interest in the pH because an acute inflammation typically causes an increase in pH up to

8.0 (alkaline) whereas a chronic inflammation causes a decrease of the pH under 7.2 can even go down to an acidic pH.

4. Country legislations concerning vaccination and tuberculin test protocols.

Tuberculin test protocols for animals, including cattle are mandatory in Germany and Hungary and subject to national legislation and regulations.
As Tuberculosis plays a major role concerning public health aspects the eradication program is of a strict value. Because, no Treatment nor vaccination is permitted, strict stamping out and regular testing is of great importance for their eradication. Furthermore, are general measurements taken such as meat inspection and postmortem investigations in case of any suspicion for Tuberculosis infection. As well meat waste products which come up from animals in risk of tuberculosis are destroyed as Category 1 waste product (EU Regulation No. 1069/2009). In case of any suspicion a sample has to be taken to national veterinary institute employees.
Hungary as well as Germany has eradicated Tuberculosis successfully, whereas some other European countries such as England did not reach tuberculosis free status yet.
[7] (https://eur-lex.europa.eu/eli/reg/2009/1069/oj)

Germany:
[8] In Germany, vaccination and testing protocols for cattle, including diseases as bovine tuberculosis (TB), are regulated by the German Federal Ministry of Food and Agriculture (Bundesministerium für Ernährung und Landwirtschaft - BMEL) in accordance with European Union directives. Farmers and livestock owners must adhere to these regulations, which may include requirements for mandatory vaccinations (in case of outbreak) for certain diseases such as foot-and-mouth disease, or as well Tuberculosis. Another very important regulation for the Maintenance of Tuberculosis free status is periodic testing for TB using the tuberculin skin test. These periods vary on the risk in specific regions or situations such cases, where TBC Testing is mandatory are for example in case of suspicion of an infected animal by mycobacterium bovis/tuberculosis or caprae. Another case of mandatory testing is the export of cattle into countries where a negative Tuberculin test is required or in case of a previous outbreak in the surveillance zone. Specific protocols may vary depending on the region and local health authorities. Nowadays Germany is considering free of the tuberculosis complex.

[8]
(https://www.bmel.de/SharedDocs/Downloads/DE/_Tiere/Tiergesundheit/durchfuehrungsverordnung-eu-2021-620.pdf?__blob=publicationFile&v=3)

Hungary:
Similarly, in Hungary, vaccination and testing protocols for cattle are governed by national legislation at the Hungarian Ministry of Agriculture. Like Germany, Hungary follows European Union regulations regarding animal health and welfare. This includes requirements for vaccinations against certain diseases and testing protocols, such as the tuberculin skin test, to monitor and control diseases like bovine tuberculosis. The orders in which Tuberculin tests are mandatory are pretty similar to Germany nevertheless Hungary does more frequent testing which is done in most of the herds on an annual basis. This Interval can vary depending on different National Programs. All Farmers and livestock owners in Hungary must comply with these regulations to ensure the health and safety of their animals and to prevent the spread of infectious diseases. Hungary is as well considered free of the tuberculosis complex.

4.1 Semen transmissible diseases:

Viruses: Picornavirus-Foot and mouth disease (FMD -Notifiable and Zoonotic), Bovine Herpes Virus 1-infectious bovine rhinotracheitis (IBR-Notifiable), Flavivirus- bovine viral diarrhoea (BVD- Notifiable), Orbivirus- Bluetongue (Notifiable in some areas), Retrovirus- Bovine Leukemia Virus (BLV), Bunyavirus-Schmallenberg disease, Orbivirus- epizootic haemorrhagic disease (EHD- Notifiable)
Parasites: Tritrichomonas fetus

Bacteria: Brucella abortus (Notifiable and Zoonotic), Leptospira species f.e. Leptospira interrogans (Notifiable and Zoonotic), Campylobacter fetus subspecies veneralis (zoonotic), Mycobacteria- Tuberculosis (Notifiable and Zoonotic), Chlamydia abortus (Zoonotic), Coxiella burnetti- Q- fever (Zoonotic), Mycoplasma bovis

4.2 Public health aspects

[9] „The worlds most infective killer “writes the world health organization. Mycobacterium tuberculosis causative Agent of the human Tuberculosis is a bacterium which can be treated with most Antibiotics nevertheless causing the “death of 1.5 million people per year” (WHO), which marks it one of the most important if not the most important zoonotic disease of our century.

A yearly average of 10 million people is infected with tuberculosis and become diseased as a consequence leading to a high antimicrobial resistance development especially due to the prolonged application time of the Antibiotics of around 6 months and its specific cell wall composition.

Especially harmed by this Gram-positive bacterium are people infected by HIV which leads to the major cause of death in these immune compromised people. The most affected countries are those with a generally low income for example Bangladesh, India or South Africa.

[9] (https://www.who.int/health-topics/tuberculosis#tab=tab_1

Nevertheless, is the “classical” Tuberculosis rather a contagion transmitted disease than zoonotically induced.

If we talk about the human-to-human transmitted disease which was described in the previous chapter, the causative agent is Mycobacterium tuberculosis, which is very rarely detectable in cattle. Mycobacterium tuberculosis is considered an Anthropozoonosis because bovine species serve as dead end hosts. In the Zoonotic Form of tuberculosis, it is typically induced by Mycobacterium bovis which shows more frequently extra pulmonary lesions in humans.

Cattle serve as the most important reservoir for mycobacterium bovis infection and can lead to great economic losses especially in already poor countries.

The numbers of zoonotic Tuberculosis are a lot smaller with approximately 147 000 cases yearly leading to the death of 12 500 people (estimation of the WOAH in 2016). Which doesn’t mean that cattle transmitted tuberculosis is of less importance since the huge economic impact which can also indirectly lead to the death of disadvantaged people which are very dependent of the production of their animals. Also, important, when dealing with zoonotic Tuberculosis is the pretty complicated estimation of an adequate

number of zoonotic cases, due to lack of surveillance data from the countries which are affected the most.
[10] (https://www.woah.org/app/uploads/2021/03/zoonotic-tb-factsheet-2.pdf)

Further subspecies which needs to be mentioned when talking about public health aspects in tuberculosis, in connection with ruminant species, are Mycobacterium africanum and caprae.
Mycobacterium africanum in cattle has no zoonotic potential, as cattle is considered a dead-end host, whereas M. caprae can be zoonotic especially in rural areas.

4.3. General features of tuberculosis in bovine species

[11] The "classical" bovine Tuberculosis is generally caused by M.bovis or caprae subspecies as M. tuberculosis is rarely affecting cattle since it's considered an Anthropozoonosis.
Carrier animals shed the disease via different discharges, like milk, faeces, tracheal discharge or semen. Affected animals often show no signs of the disease for a longer period of time. This is caused by the Generalization which is happening only in humans and cattle. That's what makes regular testing essential.
Bovine species are nevertheless very susceptible; to get infected even 10 inhaled bacteria are enough to cause disease. This infection can show very different lesions caused by the complex Pathogenesis.
The main Forms of the disease can be the tubercle (Granuloma) Formation, the proliferative or the exsudative type of lesions.
Most often we can detect the exsudative or proliferative type of lesion since the tubercle formation requires the strongest type of immune response. The Pathogenesis is divided in four different Phases, starting with the Primary Complex Formation where we usually detect lesions in the lungs and/ or regional Lymph nodes. At this stage the outcome of the disease can be even healing which can be followed by a possible reinfection afterwards. Other Outcomes are an inactive state or early Generalization, which marks the second Phase of the disease and can already result in clinical signs. Afterwards the progress of the disease can lead to the third state the Post Primary Phase.

If the cattle have not already died at this point the sick animal can also become again reinfected by external source (Superinfection), or is reinfected endogenously often leading to death, in case of survival it can reach the last stage.
The fourth Phase is called the exhaustion stage which pretty much explains what is about to happen here, affected animals die with the previously discussed Lesions.

Typically, the most prominent lesions are in the lungs, but every other organ system can be affected as well caused by the Generalization, these lesions are accompanied by general "flu-like" symptoms such as cough, fever, apathy, milk production drop, or even diarrhea.
[11]
(https://elearning.univet.hu/pluginfile.php/136661/mod_resource/content/1/Epi040%20Mycobactrial%20diseases%202024.pdf Source: 30.09. 13:01)

5. Procedure of the tuberculin probe

The most common used test for evaluation of a latent or active tuberculosis infection is the single intradermal test. This is a very simple, quick and cheap method by using a single injection of bovine tuberculin. This bovine Tuberculin is a purified Proteinderivate from a Mycobacterium bovis culture and causes a Hypersensitivity reaction in the tested animal.

In case of an inconclusive or a positive result, we have to perform an Intradermal comparative test. This is performed as listed below:

[12] You need one injection of bovine tuberculin as well as an injection of avian tuberculin given simultaneously. The quantity should not be less than 2 500 IU of bovine tuberculin and not less than 2 500 IU of avian tuberculin.

The volume of each injection dose shall not exceed 0,2 ml and is usually around 0,1 ml.

[13] Tuberculin tests shall be carried out by injecting tuberculin into the skin in a specific region of the neck. This can be performed, either in the cervical, or in the caudal region. In case of the cervical application shall the injection sites be situated at the border of the anterior and middle thirds of the neck. In Hungary they prefer doing the cervical injection and it's advised to perform this homogeneously not to cause any misunderstanding and therefore false results.

When both avian and bovine tuberculins are injected in the same animal, the site for injection of avian tuberculin shall be around 10 cm from the crest of the neck and as well from the site of the injection of the bovine tuberculin about 12.5 cm lower on a line parallel with the line of the shoulder.

Or in case of comparative tuberculin test on different sides of the neck, in very young animals on which there is not enough space to separate the sites sufficiently on one side of the neck, one injection shall be made on each side of the neck at identical sites in the centre of the middle third of the neck.

The injection sides should be prepared as followed: the fur shall be clipped; the clipped area should be cleansed to prevent development of an infection in the following which could gives us false positive results.

A fold of skin within each clipped area shall be taken between the forefinger and thumb and measured with callipers and recorded.

The dose of tuberculin shall then be injected by a method that ensures that the tuberculin is delivered correctly intradermically. A short sterile needle, with graduated syringe containing tuberculin, inserted obliquely into the deeper layers of the skin.

A correct injection shall be confirmed by palpating a small pea- like swelling at each site of injection. The skin-fold thickness of each injection site shall be remeasured 72 hours (± 4 hours) after injection and recorded. The following measurements will then give us either a positive, negative or an inconsecutive result, if the case of inconsecutive, or a positive test the procedure should be repeated either with a comparative or a repeated single intradermal test within the following 4 days.

The interpretation of the tuberculin test results should be done in the following 72 hours after injection.

Interpretation value is given by the appearance of clinical signs as well as the measurement of the skin fold according to the recorded previous results. To know what the results of our findings will be there is a list below with the interpretation of the findings and their interpretation.

Negative reaction: if there is only a limited swelling observed, with an increase of not more than 2 mm in the thickness of the skin fold, without clinical signs, such as diffuse

or extensive oedema, exudation, necrosis, pain or inflammation of the lymphatic ducts in that region or of the lymph nodes.

Inconclusive reaction: if no before mentioned clinical signs are observed and if the increase in skin-fold thickness is more than 2 mm and less than 4 mm.

Positive reaction: if the previously mentioned clinical signs are observed and/ or there is an increase of 4 mm or more in the thickness of the fold of skin at the injection site.

In case of a positive result a comparative intradermal test should be performed.

[13] https://elearning.univet.hu/pluginfile.php/135392/mod_resource/content/1/bovine%20tuberculosis%20state%20vet%202024.pdf

[12] https://portal.dimdi.de/amispb/doc/pei/Web/2612213-palde-20110801.pdf)

5.1 Further laboratory investigations

In the following chapter I will list and shortly describe further possibilities for checking of a possible Mycobacterial infection. The further listed are more likely to be used in small animal medicine or human medicine. But they are possible nevertheless to be used as comparative possibility for cattle as well.

Another pretty common and quick way of tuberculosis detection is the blood test with this we are searching for interferon gamma production which is produced as a reaction to tuberculosis infection. This test is very sensitive and can be used as an alternative in case of large groups of animals or in case of a doubtful test result.
The next two alternatives are used commonly not just for tuberculosis screening but as well for a lot of other infectious diseases. First of all, the PCR Test is a cheap and quick test to detect the nucleic acids of Mycobacterium bovis, PCR is specific and very sensitive method as well, even more specific is the culture of mycobacteria the only disadvantage is that its time consuming since mycobacteria a very slow growing (for weeks up to months).

The next method is very commonly used even on a daily basis since Tuberculosis is an important zoonotic disease which needs to be checked during Postmortem examination in slaughterhouses.
For further investigations in carcasses, we can use Histopathology to find the typical Granuloma Formation with typically a calcification center.

Further diagnostics which can be applied to viable animals are X-ray to find the Granulomas and the calcification center.
[14] page 1058 chapter 3.4.6., 30.09., 11:23 https://www.woah.org/app/uploads/2021/03/3-04-06-bovine-tb.pdf)

As evident from the previously described investigation methods, it is necessary to interact with each individual animal that requires testing. However, this can cause significant stress, particularly for more sensitive, shy, or unaccustomed animals that are not familiar with human contact.

6. The Pathophysiology of stress and the individual differences

[15] In General stress can be induced by certain activities which which can be different in every individual, these stressors can be either of physiological, biological or psychological nature. In the Animal production sector, it can be induced by different interventions or managements systems performed by people, but it is not solely attributed to human interaction. Stress and a following decrease in any kind of performance can also trigger the potential risk of infections which can be induced for example by the intradermal tuberculin test, as its success requires a specific immune reaction for adequate test results. During stressful situations the animal is not only dealing with the Stimulus itself, but also concurrently managing exposure to other ubiquitous pathogens, which can further strain its physiological systems.
This happens during activation of the different systems such as the autonomic nervous system and the hypothalamic- pituitary- adrenal axis (HPA), which produce adrenaline and cortisol and furthermore the sympathoadrenal medullary axis (SAM) and the following fight- or- flight response.

The release of cortisol which is performed by the change in the hypothalamic- pituitary-adrenal axis leads disruption of the metabolism, as well as physiological and immunological functions. These changes can lead to alteration of immune functions, metabolism and susceptibility of diseases.

These stressors, along with other factors, can significantly impact the productivity of livestock, particularly breeding bulls, where semen quality and production may be compromised. Although a localized immune response like the one caused by the tuberculin test typically should not affect systemic functions such as fertility. Research has shown that similar immune reactions can impair reproductive capabilities. The mechanisms behind this will be further explored in the following sections.

[16] The tuberculin test triggers a Type IV hypersensitivity reaction, which is mediated by T-cells and involves a delayed immune response, typically occurring 24 to 72 hours after exposure. This reaction can lead to inflammation and tissue damage, especially in cases of chronic infections or repeated antigen exposure. Systemic inflammation, often a result of chronic infections or immune responses, can negatively impact semen quality, motility, concentration, and morphology by disrupting the hypothalamic-pituitary-gonadal (HPG) axis. This disruption leads to decreased testosterone production, which impairs spermatogenesis and causes subsequent testicular damage.

However, there is no definitive evidence regarding the extent of the impact of specific immunological tests like the intradermal skin test on fertility. Repeated administration of such tests or as well the comparative intradermal tuberculin test might cause minor damages, but more research is needed to quantify these effects.

Nevertheless, we also have to interpret individual differences concerning specific stressor as already mentioned there is a huge gap between beef cattle and diary cattle concerning their sensitivity to stress. This was also already investigated considering heat stress. As detected the beef cattle showed an alteration in endocrine responses including reduced cortisol levels in several situations leading to the suggestion that the physiological reaction between beef and diary cattle to stress can differ significantly.
The differences in management systems for dairy and beef cattle significantly influence their stress responses and overall welfare. Dairy cattle are typically raised in intensive

management systems that involve daily human interaction for activities such as milking, artificial insemination, feeding, calving, and veterinary care.
This regular contact helps them adapt to human presence, reducing their stress levels. In contrast, beef cattle generally experience minimal daily human contact, leading to heightened stress responses characterized by acute spikes in cortisol rather than the minor chronic stressors seen in dairy operations.
[15] https://en.wikipedia.org/wiki/Stress_(biology) , (Ulrich-Lai, Yvonne M.; Herman, James P. (7 February 2017). "Neural Regulation of Endocrine and Autonomic Stress Responses". Nature Reviews Neuroscience. 10 (6): 397–409. doi:10.1038/nrn2647. ISSN 1471-003X. PMC 4240627. PMID 19469025.!
Biology of stress, CESH / CSHS. Retrieved 27 September 2022., Stephens, Mary Ann C.; Wand, Gary (1 January 2012). "Stress and the HPA Axis", Research: Current Reviews. 34 (4): 468–483. ISSN 2168-3492. PMC 3860380. PMID 23584113., Notaras, Michael; van den Buuse, Maarten (3 January 2020). "Neurobiology of BDNF in fear memory, sensitivity to stress, and stress-related disorders")

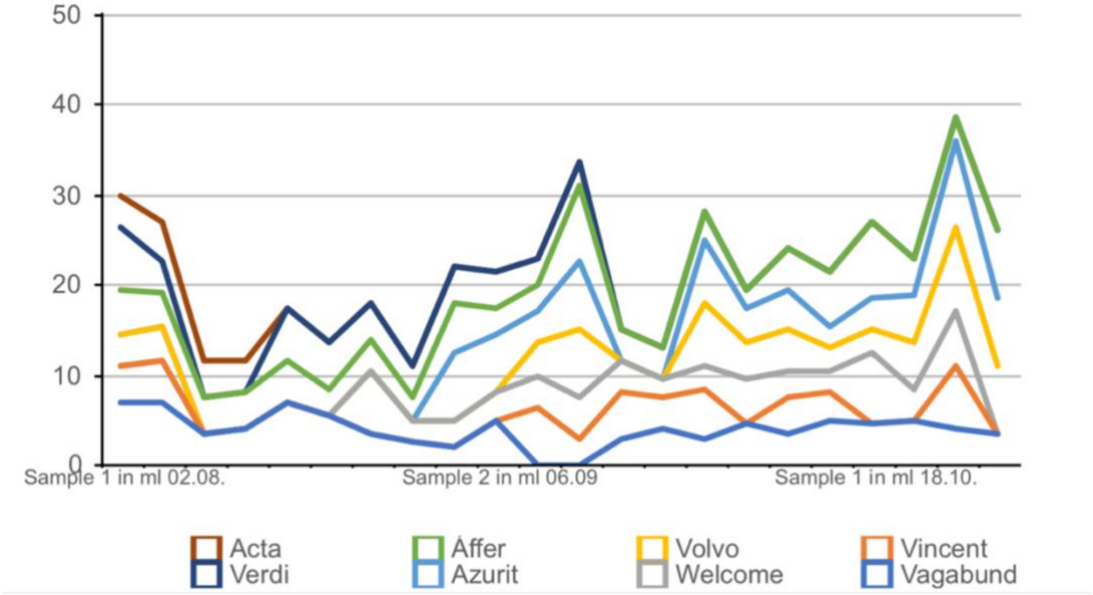
[16] Type IV Hypersensitivity Reaction, Khaled Marwa; Noah P. Kondamudi, Last Update: August 12, 2023., Bookshelf ID: NBK562228PMID: 32965899, https://www.ncbi.nlm.nih.gov/books/NBK562228/)

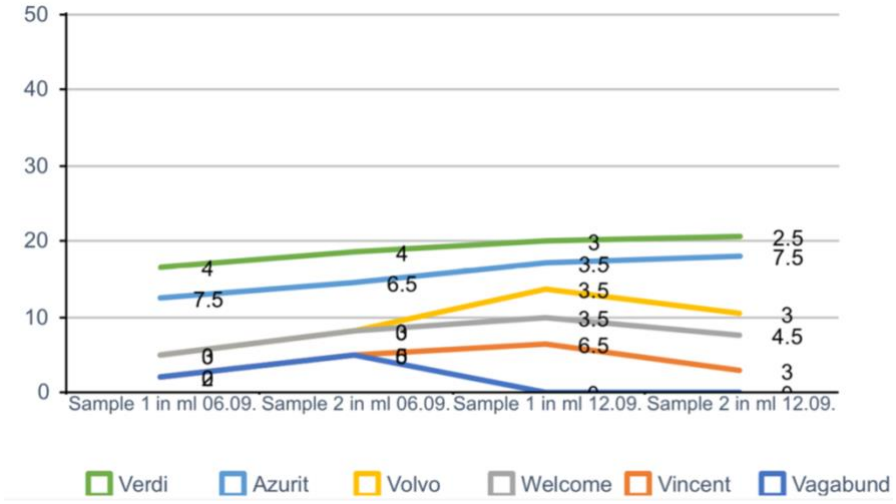
This lack of familiarity with human interaction can interfere with the immune responses of beef cattle, potentially resulting in prolonged inflammation and altered cytokine levels. Beef cattle are primarily managed through pasture grazing and are often kept in feedlots, where veterinary visits are infrequent. Consequently, they may exhibit increased nervousness and stress around humans.
This behavior and the before used natural type of breeding is particularly evident in breeding bulls at artificial insemination stations, where these animals often require extended periods to acclimate to the artificial vagina and to the handling by personnel. Similarly, during procedures like the tuberculin test, beef cattle tend to be more difficult to manage, which can lead to greater stress outputs.

These factors and many more can lead to huge difference during evaluation of the the sperm Quantity and Quality in general and also especially in consideration of previous veterinary interventions such as the intradermal Tuberculin Test.

7. Data Analysis

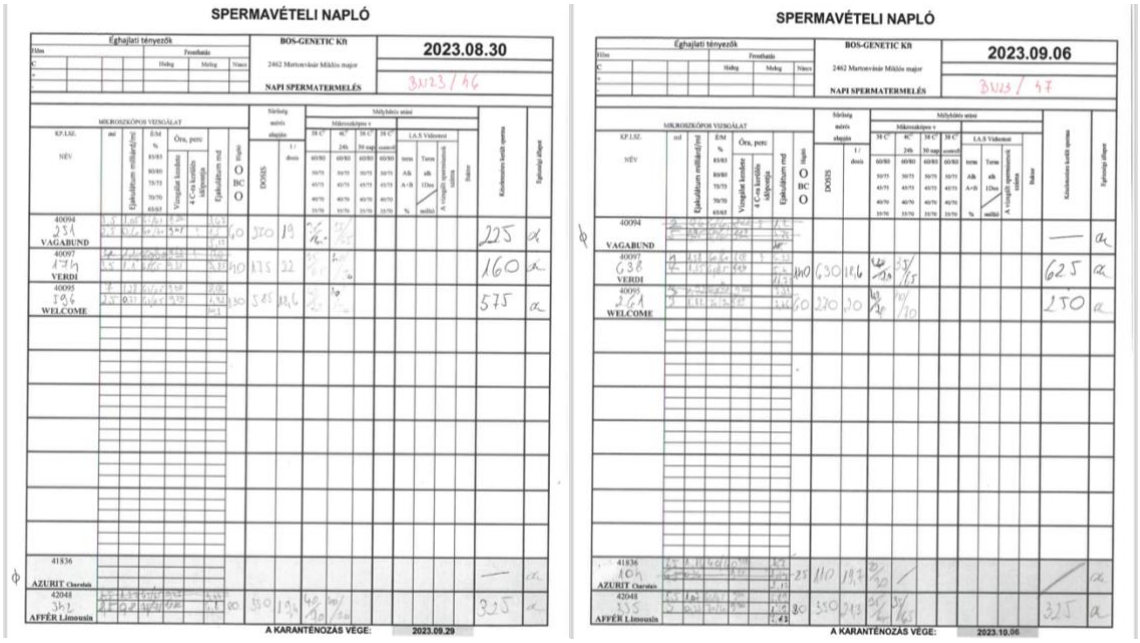
7.1 Table concerning Quantity:





Dates on which tuberculin tests have been performed, which are relevant for my scientific work is the 30th of August 2023 on which the bulls Azurit, Welcome, Vagabund, Volvo, Verdi, Vincent, and Gates have been tested.

The next semen collection after performing the tuberculin test is the 06th of September where we see the results of the quantity of the semen collection. The exact numbers can be found in the table above as well as a demonstration of the values in the graph above.



SPERMAVÉTELI NAPLÓ

BOS-GENETIC Kft

2023.08.30

NAPI SPERMATERMELÉS

SPERMAVÉTELI NAPLÓ

BOS-GENETIC Kft

2023.09.06

NAPI SPERMATERMELÉS

ÁLLATOK AZONOSÍTÁSA

Melléklet a 2023.08.30-én elvégzett gümőkor vizsgálatokhoz

		A1	A2	Kül.	B1	B2	Kül.
		cm					
1	41836 AZURIT	1,1	1,1	0,0	1,1	1,1	0,0
2	40095 WELCOME	1,3	1,3	0,0	1,2	1,2	0,0
3	40094 VAGABUND	1,2	1,2	0,0	1,1	1,1	0,0
4	40096 VOLVO	1,2	1,2	0,0	1,2	1,2	0,0
5	40097 VERDI	1,4	1,4	0,0	1,2	1,2	0,0
6	40093 VINCENT	1,3	1,3	0,0	1,3	1,3	0,0
7	35608 GATES	1,6	1,6	0,0	1,5	1,5	0,0

Dr. Schekk György

Dr. Merész Lajos

Ráckeresztúr, 2023. 09 02.

Picture 10, 11 and 12: showing an example of recorded data and the semen evaluation, as well as the performed tuberculin test.

7.2 Evaluation of the Quality of the ejaculate:

To make a proper qualitative evaluation of the sperm is difficult, when dealing with a company which is working economically, which means quick and effective. Therefore, the semen samples which are examined at bos genetic are not always fully interpreted. That's why the focus is primarily on the Vitality and the concerntration of the spermatozoa in the probe which will be checked under the microscope and if these parameters seem to be abnormal, it already leads to immediate disposal of the ejaculate. Without further notice beside crossing the probe on the documentary paper.

So, to perform a proper evaluation concerning quality we would have needed to save those disposed semen probe for further and more precise check ups.

Nevertheless, it is possible to list the most common causes for disposal of the probes.

Concerning abnormal morphological features, we most often find primary morphological features such as a small head (microcephaly) or a large head (macrocephaly).

Further abnormalities which are commonly detectable, concerning morphology are doubled heads or bent tails. Morphological abnormalities such as the bent tail can also have a huge impact on the motility of the spermatozoa, which will directly affect the capability to fertilize the egg the reduced motility is described as asthenozoospermia.

As already described in the chapter we primarily focus on the sperm count, which can be reduced. This feature is described as oligospermia and is also pretty commonly found and can be quickly detected by microscopic examination. The same is responsible for a moribund sperm probe with a lot of dead sperm cells described as necrozoospermia, further pathological changes can be found too but are sometimes not that quickly visible and can maybe just detected after a second check up after thawing, where can also find reduced vitality of the sperm indicating further changes such as DNA Fragmentation or damaged membranes etc.

8. Summary and conclusion

In order to evaluate my collected data and information I wanted to conclude my findings. As the table demonstrates on page 24 and 25 from the 30th of August 2023, a lower amount of ejaculate, in comparison to the collection of the following week (06.09.2023), can be noticed.
This finding is not matching the expectations of a decrease in the amount after performing the Tuberculin Test.
However, the table demonstrated on page 24 and 25, is showing, that the quality of the semen is decreasing since the samples from the bull „Welcome“ were partially destroyed, or in case of the bull „Vagabund“, even entirely destroyed because of the insufficient quality of the semen probe.
Unfortunately for my Thesis work qualitative results concerning morphological properties, vitality of the semen probe and other important characteristics which already mentioned in the previous chapters cannot be analyzed afterwards since they are not documented in detail.
Qualitative insufficiencies are noticed by crossing the probe from the paper without documenting the specific reason for the probe destruction.
Therefore to evaluate my Thesis work more correctly and detailed, more data is required, such as the bulls' history with tuberculin testing, consistent environmental factors, and more frequent and detailed semen evaluations which also needs to be interpreted from previous years.
Therefore my evaluation is also insufficient as the data, my work is referring to, is from one specific semen station, as well as the collected data is originating from one Tuberculin Test procedure of the year 2023.
Nevertheless, the most important thing for a more precise work, is probably the collection of detailed abnormalities of the qualitative parameters of the semen probes.
Both semen quantity and quality, including common defects, needs detailed reporting to assess the test's impact accurately.
Differences between beef and dairy bulls do also warrant further exploration, as discussed in earlier sections.
The limited data stems from the economic constraints of "Bos genetic Kft.," which challenges detailed record-keeping in order to work time and cost efficient.
However, tuberculosis remains a critical issue in the food production sector especially due to public health concerns, that's why more studies are needed to analyze the potential impact of the tuberculin tests on semen quality.
While conclusive evidence is lacking, minor negative effects of tuberculin tests on semen quality remain a plausible assumption based on collected observations.

9. Acknowledgements:

I want to especially thank all employees of „Bos Genetic Kft.“ and especially Dr. Joszéf Rátky and Dr. Meresz Lájos, for their help and cooperation with working out relevant information and data concerning my Thesis studies.

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[17] other information received during our visits at “Bos genetic Kft.” Our during previous studies or lectures

Pictures:

[18] Picture8: https://elearning.univet.hu/pluginfile.php/126688/mod_resource/content/1/stud_obstetrics3_1-2%20%20-%20%20Kompatibilitási%20mód.pdf