SUMMARY

of the PhD thesis entitled:

RESEARCH ON SEMINAL MATERIAL INOCULATION IN CATTLE

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This PhD thesis aims to modify traditional artificial insemination procedures by suggesting the deposition of seminal material in different places. Although artificial insemination is a well-established and successful technique, the interactions between mammalian spermatozoa and the female genital tract have not been fully understood, therefore different sperm deposition has been attempted to improve the efficiency of the process.

In approaching the PhD thesis entitled "Research on seminal material inoculation in cattle" we sought improvement of fecundity, having a number of inseminations per gestation as small as possible, and also a shorter period between calvings. In addition to the fact that the artificial insemination technique employed is very important, we should also mention that the semen involved has a significant impact on the results, specifically the sperm quality. Moreover, the time when artificial insemination is performed is very important.

Artificial inseminations are used in our country both within farms and in private households. Unfortunately, cattle herds in households are dramatically decreasing. Such decrease is caused by the lack of dedicated pastures (grazing fields), the shortage of pasture keepers and the non-involvement of young people in zootechnical activities.

This doctoral thesis consists of two main parts.

In the first part, which is entitled BIBLIOGRAPHICAL REVIEW, we want to highlight matters in literature that are conclusive for the topic we have chosen.

Chapter I – entitled **CURRENT SITUATION AND EVOLUTION OF BOVINE BREEDING**, which presents the evolution of bovine breeding at European and national level, is structured in two sub-chapters:

1.1. Situation of bovine breeding at the European level, in which the livestock between 2014 and 2023 are analysed and from which the decrease in its number is observed. It is also mentioned that in 2023 the European country with the highest number of cattle is France.

1.2. Situation of bovine breeding in Romania from which it results that at national level we also had large decreases in the number of cattle, coming to a number of 1814700 livestock units, Romania being in eleventh place in the list of countries with the highest number of cattle in Europe.

Chapter II – entitled **MORPHOPHYSIOLOGY OF THE GENITAL TRACT**, which presents the morphology of male and female genital apparatus, as well as their morpho-physiological and reproductive characteristics, is structured in four subchapters:

2.1. Morphophysiology of the female genital tract

Both the components of cow genital system (vulva, vagina, uterus with cervix, uterine body and horns, oviducts and ovaries) and the components of bull genital system (testis, epididymis, vas deferens, ejaculatory duct, urethra, copulatory organ, seminal glands, prostate and bulbourethral glands) have been presented.

2.2. Morphophysiology of the male genital tract

In this sub-chapter, the component parts of the genital apparatus of the bull (testis, epididymis, vas deferens, ejaculatory duct, urethra, copulatory organ, seminal glands, prostate and bulbourethral glands) have been presented.

2.3. Sexual cycle (heats) and signs of heats

The length of the sexual cycle is presented in this sub-chapter in both cows and heifers. It should be noted that artificial inseminations are carried out in private households according to what the owner recounts, while in farms the signs of heat are noticed by the keepers or by sensors attached to the animals' ear tags. It is easier to monitor animals in farms as they are placed in groups.

2.4. Fertilization and ovulation

The process of the union of the male gamete with the female gamete is called fertilization and is detailed in this subchapter. Ovulation is the phenomenon of dehiscence of the Graafian follicle and expulsion of the oocyte from the follicle. It is an important and highly complex act in the reproductive process. There are also mentions about the timing of ovulation, which is still difficult to determine. Where the exact time of ovulation is not known, it is preferable to perform two artificial inseminations: the first when the first signs of estrus are noticed and the second 12 hours later.

Chapter III – entitled **ARTIFICIAL INSEMINATIONS** consists of seven subchapters:

3.1. History of artificial insemination

It can be noted that artificial inseminations have been carried out in horses by Arabian tribes since the very 14th century, spreading all over the world, while in our country the first attempts were made between 1948 and 1949 within the mating stations. It should be mentioned that at the moment it's hard to say whether there are any countries where artificial seeding is not carried out.

3.2. Advantages of artificial insemination

There are zootechnical, economic, sanitary-veterinary and scientific advantages of performing artificial inseminations. A very big plus is the fact that the semen of a male sire of high zootechnical value can reach several cows in different areas, even in different countries, resulting in offspring that adapt much more easily to local conditions. The process flow includes the following steps: semen collection, semen examination, semen dilution and preservation, and semen inoculation.

3.3. Collection of seminal material

Collection of seminal material is the biotechnical operation of obtaining semen by artificially inducing ejaculation without intromission and it can be achieved by several methods.

In order for males to be ready to be used for semen collection it is necessary that they are sexually mature, not allowed to mate, trained and accustomed to the method, and that training is carried out patiently and without rough handling. It is very important to take into account their feeding pattern, as they need good quality, balanced food and adequate housing conditions. Factors associated with breed, age, body condition, physical disability, dietary needs, libido and environment may influence both sperm quality and quantity.

Sperm collection with artificial vagina - this method is the most common and widely used as it reproduces the conditions existing in the vagina of a female in heat, thus triggering the ejaculation reflex. An artificial vagina consists of a vaginal tube, a vaginal cup, a vaginal liner, a collection cup, rubber rings, the opening in the wall of the vaginal tube, the metal faucet and the protective sleeve. The technique of semen collection is also presented.

Sperm collection with electroejaculation device - this method is performed by electrically exciting the ejaculatory nerve centre in the lumbar region using a 30V alternating current device and a pulse transformer. It is a method less widely used, although both in terms of quality and quantity the sperm is not much different from that obtained with the artificial vagina.

Sperm collection by massaging the ampulla pulla of vas deferens and seminal glands - this method is performed using 2 sterilized glass test tubes, each equipped with a glass funnel. It is a less commonly used method as the sperm thus obtained is rich in urine, which lowers spermatozoa viability.

3.4. Sperm exam

Both macroscopic and microscopic examinations are necessary to assess sperm quality. The macroscopic examination is carried out promptly after collection and observes the spermatozoa colour,

consistency, volume, density and pH. Semen color is different according to the age of the bulls, being pearly-white in bulls aged 1-2 years and whitish-white, pearly to yellowish in bulls aged 5-6 years. The microscopic examination is carried out using specialized equipment for observing the motility and number of, and distance between spermatozoa. The motility of spermatozoa is assessed by their mobility in the microscopic field. CASA system is used to assess concentration, morphology, motility, detached heads or abnormally shaped tails more accurately than conventional microscopy.

3.5. Methods and devices used for seminal material preservation

One of the most widely used methods for semen preservation is freezing. By preserving semen, it remains viable for a long period of time and can be transported anywhere, thus enhancing genetic progress. One of the most important steps in this process is dilution, which is divided into initial dilution, intermediate dilution and final dilution. The cryogenic agents are liquid nitrogen (-196°C), dry ice (-79°C) and liquid air (-183°C). Semen can be packaged and stored in straws, ampoules and beads, the most commonly used being the straws.

3.6. Inoculation of seminal material

Inoculation of seminal material is the last stage of the process flow of artificial insemination reproduction system and is represented by sperm inoculation into the female genital tract. The following methods are known to achieve inoculation: recto-vaginal method, vaginal speculum insemination, fractional cervical insemination and vaginal insemination by manual control.

Recto-vaginal method - This method is also called the bimanual method as it is performed with both hands. The inseminator's experience and knowledge about the topography and morpho-physiological changes of the genital organs are very important for the correct performance of artificial inseminations. In this sub-chapter, the steps to be followed in order to perform correct artificial insemination are mentioned. It should be noted that one of the advantages of using this method is the simple equipment required (pipette and Cassou pistol) and another one is that it can be used in all housing systems. The disadvantages of using bimanual insemination are the failure to carry out the specific steps and rough handling of the pipette, which can cause damage to the cervix or even the uterus.

The vaginal speculum method of artificial insemination - the equipment required to perform this method is: a vaginal tubal speculum, a headlamp, a glass or plastic pipette, a glass or plastic pipette, a spirit lamp, cotton wool, gauze, sanitary alcohol, grease and the metal stand we use to immobilize cows. The method steps are presented, and the advantages of using this method are that it is possible to localize the vaginal opening of the cervix and that we can easily detect any anatomical abnormalities or diseases of the vagina and cervix.

Vaginal insemination by manual control - this method is not commonly used, but it should be noted that if it is used, the most suitable inoculation place for the semen is in the body, about 0.5 cm beyond the uterine opening of the cervix at the base of the right horn at the first insemination and the second in the anterior half of the cervix.

3.7. Equipment and devices required to perform artificial insemination

The equipment used for preservation and thawing of semen is: one stationary point container and one small container, a measuring rule, a water heating source, hemostatic clamps, metal pliers, long gloves, Cassou type gun. The thawing steps are mentioned and described in the sub-chapter presented.

The second part of the paper is entitled **PERSONAL RESEARCHES** and includes the results of the studies carried out between 2017 and 2021 within the farms in Prahova county.

Chapter IV – entitled **RESEARCH PURPOSE AND GOALS** consists of six subchapters.

4.1. Research purpose

Artificial inseminations have a very important role in increasing productivity in cattle farms, therefore the purpose of this paper is to determine the optimal time of ovulation, the use of appropriate equipment and the optimization of the reproductive process by inoculation of semen in 3 different places. The purpose of scientific research is to determine the optimal time of ovulation in order to perform artificial insemination, appropriately use the relevant equipment, and optimize the reproductive process.

4.2. Place of research performance

The research was conducted within four farms located in Prahova county. Farms A and B are located in Bătrâni commune, Farm C in Drajna de Jos commune, and Farm D consists of animals from the households located in Drajna commune, Cerașu commune and Poseşti commune. According to DSVSA Prahova data, the number of cattle in the county is decreasing year by year, this is due to the aging population, the fact that young people are pursuing other activity areas, very few are interested in zootechnical sector, the lack of financial availabilities for animal care, and the lack of grazing fields. According to the census carried out in 2021 there was a decrease in the number of inhabitants in all the communes, with young people preferring urban areas.

Prahova County is characterized by the proportionality of landforms - mountains 26.2% (Omu peak - 2505 m), hills 36.5% and plains 37.3%. The areas where scientific research has been carried out are hilly.

Artificial seeding at local level is carried out by veterinarians and insemination operators - veterinary technicians.

4.3. Working method

Within the frame of the scientific research, artificial inseminations were carried out in bulls in the farms subject to the experiment. The artificial inseminations were carried out with seminal material from Semtest Craiova, of Malu Mare, Dolj county. Semen inoculation was carried out by the recto-vaginal method using the following equipment: a universal insemination pipette and a Cassou-type gun.

4.4. Material subject to research

This sub-chapter details information on the number of animals, feeding and housing in each farm.

The number of animals in each farm is as follows: in farm A a total of 60 animals of which 54 cows and 6 heifers, in farm B a total of 65 animals of which 58 cows and 7 heifers, in farm C a total of 49 animals of which 43 cows and 6 heifers, and in farm D (i.e. private households) a total of 60 units. The farms specialize in cattle of the breeds Brună, Bălțată with Negru Românească and Bălțată Românească, these breeds being specific to the area. Nutrition plays an important role in cattle breeding; in the farms where the study was carried out the animals are kept at pasture during the summer, while in winter they are stabled and the feed consists in hay, alfalfa and bran.

Artificial inseminations were carried out for 4 consecutive years in each farm, performing semen inoculation differently, specifically:

Control batch - inoculation was carried out at the end of the cervix towards the uterus

Batch 1 - inoculation was carried out in the uterine body, at the bifurcation of the uterine horns

Batch 1a - inoculation was carried out in the same way as in batch 1, with the exception that for this group a sanitary sheath was used to prevent contamination of the nozzle when passing through the vagina.

Batch 2 - inoculation was carried out in the uterine body, more precisely at the bifurcation of the uterine horns, half of the semen from a straw and the remaining half left by withdrawing the nozzle up to the cervix area.

Batch 2a - inoculation was carried out in the same way as in batch 2, with the same mention that a sanitary sheath was used to prevent contamination of the nozzle when the semen passed through the vagina.

The lots of animals chosen for the research are also described, i.e.:

- In farm A the control batch was a livestock of 30 cows where the semen was inoculated at the end of the cervix, towards the uterus, and batch 1, a livestock of 20 cows, where artificial insemination was performed in the uterine body, at the bifurcation of the uterine horns.
- In farm B the control batch - 30 cows and batch 2 - 20 cows
- In farm C the control batch - 20 cows, batch 1a - 10 cows and batch 2a - 10 cows
- In farm D the control batch - 20 cows, batch 1 - 10 cows, batch 2 - 10 cows, batch 1a - 10 cows and batch 2a - 10 cows.

4.5. Reproduction ratios

This sub-chapter describes the reproductive ratios: fecundity, service period, average number of inseminations/gestation and calving interval. Reproduction ratios are absolute or relative figures used to assess the reproductive activity of an animal or group of animals at a given point in time or over a given period of time.

4.6. Statistical ratios

This sub-chapter describes the statistical ratios, such as: mean, variance, standard deviation, coefficient of variability and mean error. Statistical indicators are used to draw relevant conclusions.

Chapter V – entitled **STUDIES CONDUCTED** consists of 4 subchapters.

5.1. Results in Farm A

Following the artificial inseminations carried out during the 4 consecutive years within Farm A in the batches chosen for research, the reproduction ratios were calculated. The control batch consists of 30 cows and batch 1 of 20 cows. It was proved that the highest average fecundity achieved during the years 2017 to 2021 is found in batch 1, i.e. 85% in 2018, and the lowest in 2019, recorded in the control batch, of 80%. The lowest service period average recorded between the years 2017 - 2021 was identified between 2020 and 2021 (75 days) in batch 1 and the highest between 2017 and 2018 in the control batch (79 days).

The lowest mean average number of inseminations/gestation recorded between 2017 and 2021 was observed in the second year of implantation performance (1.17) in batch 1 and the highest in the control batch (1.25). The lowest mean average of calving interval recorded between 2017 and 2021 was observed in the last interval (360 days) in batch 1.

5.2. Results in Farm B

Following the artificial inseminations carried out during the 4 consecutive years within Farm B in the batches chosen for research, the reproduction ratios were calculated. The control batch consists of 30 cows and batch 2 of 20 cows. The highest average fecundity achieved during the years 2017 to 2021 is found in batch 2, i.e. 87% in 2019, while the lowest was in 2018, recorded in the control batch, of 83%. The lowest service period average recorded between the years 2017 - 2021 was identified between 2018 and 2019 (77 days) in control batch and between 2020 and 2021 (also 77 days), while the highest was in the control batch (82 days) between 2017 and 2018.

The lowest mean average number of inseminations/gestation recorded between 2017 and 2021 was observed in the first year of implantation performance (1.13) in batch 2 and the highest in the control batch (1.20). The lowest mean average of calving interval recorded between 2017 and 2021 was observed in the last interval (362 days) in batch 2 and the highest in the control batch (367 days) in the first interval of time.

5.3. Results in Farm C

Following the artificial inseminations carried out during the 4 consecutive years within Farm C in the batches chosen for research, the reproduction ratios were calculated. The control batch consists of 20 cows, batch 1a of 10 cows and batch 2a of 20 cows. The highest average fecundity achieved during the years 2017 to 2021 is found in batch 2s, which was 92% in 2018, while the lowest was in 2018, recorded in the control batch, of 81%. The lowest service period average recorded between the years 2017 - 2021 was identified between 2018 and 2019 (76 days) in control batch, while the highest was in batch 2a (81 days) between 2018 and 2019, and also in the control batch between 2019 and 2020.

The mean average number of inseminations/gestation (0.15) was identified in the second interval between the control batch and batch 2a, while the smallest differences (0.04) are expressed in the fourth interval between the control batch and batch 2. The lowest mean average of the calving interval recorded between 2017 and 2021 was observed in the second interval (361 days) in the control batch and the highest in batch 2a (366 days) also in the second interval of time.

5.4. Partial results in Farm D

Following the artificial inseminations carried out during the 4 consecutive years within Farm D in the batches chosen for research, the reproduction ratios were calculated. The control batch consists of 20 cows, batch 1 of 10 cows, batch 2 of 20 cows, batch 1a of 10 cows and batch 2a of 10 cows. The highest average fecundity achieved during the years 2017 to 2021 is found in batch 2, which was 88% in 2021, while the lowest was also in 2021, recorded in the control batch, of 77%. The lowest service period average recorded between the years 2017 - 2021 was identified between 2020 and 2021 (76 days) in batch 1 and the same highest in the control batch between the years 2018 and 2019, in batch 1a between 2018 and 2019 and in batch 2a (80 days) between 2017 and 2018.

The lowest mean average number of inseminations/gestation recorded between 2017 and 2021 was recorded in the first year of implantation performance (1.13) in batch 2a and the highest in the control

batch (1.30). The lowest mean average of calving interval recorded between 2017 and 2021 was observed in the last interval (361 days) in batch 1, as well as in batch 2a, while the highest was in the second interval of time in batch 1a and in the first interval in lot 2a (365 days).

All these reproduction ratios have been analysed and compared for each farm separately as well as between farms. They can also be seen in the graphical representations attached hereto.

5.5. Final results

According to the results subject to analysis, the average fecundity reported for all farms between 2017 and 2021 is always lower in the control batch compared to the analysed batch in each farm. It was also observed that within farms fecundity was always higher irrespective of the place of inoculation of semen compared to the private households. In terms of service period the differences are small between the control and the other batches both in farms and in private households.

Within the private households we have the highest number of inseminations per gestation regardless of where the semen was inoculated. This is due to the fact that animals in private households are not monitored as well as in farms and thus it is quite difficult to determine the optimal time for artificial insemination.

As regards the calving interval achieved in the farms between 2017 and 2021 it was found that there are no major differences.

Chapter VI – entitled **GENERAL CONCLUSIONS AND RECOMMENDATIONS**

In the farms subject to analysis, any breeding system and any new technology should improve fertility and other reproductive indices, however the costs should also be reduced. The study revealed that the highest fertility was achieved in batch 2a, the place of semen inoculation being half the amount of straw inoculation in the uterine body at the bifurcation of uterine horns and the other half left by withdrawing the nozzle and using sanitary sheath to prevent contamination of the nozzle.

Recommendations made to farms are that they closely monitor animals for the purpose of noticing the first signs of estrus so that artificial inseminations will be carried out at the optimal time. It is also recommended to synchronize estrus and ovulation with prostaglandin F2α or progestogen administration in animals in which signs of estrus are faded in order to be able to schedule artificial insemination. Farmers have to take into account the feeding of the animals, which means that the rations given should be as balanced as possible and of good quality, that exercise is very important and even if it is winter, the animals must be taken out in the paddocks, and they also have to consider that moving and handling of the animals must be done in such a way as to avoid causing stress.

The recommendations made for the person performing artificial inseminations are: to check the nitrogen level in the container where the semen is kept so that the nitrogen level does not drop, not to thaw the semen earlier than 20 minutes before artificial insemination, and to perform two inoculations of semen 12 hours apart where animals have longer heat period.