# SUMMARY

**Keywords**: boar, semen, processing, seasonal variations, CASA

The PhD thesis entitled "**Seasonal variations of seminal parameters in boars and their fertility**" in the Doctoral School of the University of Agricultural Sciences and Veterinary Medicine "Ion Ionescu de la Brad" University.

The thesis contains 214 pages and consists of two main parts, according to the currently regulations. A total number of 191 bibliographic titles from the national and international literature were used as a source of documentation. The data presented in the second part are supported by a total number of 66 figures and 45 tables.

The originality of the research resides in the comparison for the first time of three specific models of analyzing chambers designed for computerized analysis of boar semen. Also, for the first time in Romania, the seasonal variations of seminal parameters in boars held in standardized conditions were studied. Last but not least, the thesis contains the first study on the seasonal variation of motile sperm subpopulations in extended boar semen. All these will contribute to the improvement of boar semen evaluation techniques by choosing the adequate equipment, to the optimization of boar semen production by offering a solution against the "seasonal infertility" previously described in swine, and also to a better and more detailed understanding of the seasonal variations in boar semen.

The first part, having the title "CURRENT STATE OF KNOWLEDGE", is divided into three chapters (50 pages), presenting data from literature with respect to the morphology and physiology of the reproductive tract in boar, the seasonality of reproduction in swine and also the biotechnology of artificial insemination in swine, including data regarding the semen collection, examination using conventional and special methods, processing, storage and actual insemination.

The second part, with the title "PERSONAL CONTRIBUTIONS", contains a total of 101 pages and is divided into seven chapters, presenting the aims and importance of the research, the materials and methods used, the obtained results and their interpretation, related discussions as well as main conclusions of the research activities and practical recommendations.

The thesis contains original data published in scientific papers presented during scientific events organized by Faculty of Veterinary Medicine of Iași or other similar national institutions, as well as international conferences or scientific journals internationally indexed (B+ or ISI).

Within the first chapter of the second part (**Chapter IV** of the thesis), entitled "THE AIM AND OBJECTIVES OF THE THESIS", the main objectives of the research activities are presented, as follows:

- Optimizing the computer-assisted examination protocol for boar semen by evaluating the effect that the chamber type used for analysis can have on the obtained results;
- To strengthen the knowledge related to boar semen processing and storage by identifying possible differences in terms of sperm kinetic parameters between a short-term and a long-term extenders during a seven days period of storage, by observing the possible influence that automatic packing of boar semen can have on its quality, and also by detection of possible correlations between the seminal parameters of raw semen and its capacity of maintaining its quality during storage; all these could help in improving the boar semen processing protocols by choosing the right methods and ejaculates;
- To highlight the seasonal variations of the main seminal parameters in boars housed in usual conditions, both in raw and extended semen;
- To note the seasonal variations of the main seminal parameters in boars housed in standardized conditions;
- To identify the motile sperm subpopulations in boar semen and to note their seasonal variations;
- To study the relation of the variations in seminal parameters with the fertility of semen in boar;
- To suggest a solution against the "seasonal infertility in boars".

The **chapter V**, with the title RESEARCH FOR OPTIMIZATION OF COMPUTERIZED EXAMINATION OF BOAR SEMEN aimed to determine if the results of semen analysis using computerized instruments can be greatly influenced by the analyzing chamber and if yes, which of the compared chamber types offers the best accuracy.

Samples of semen were obtained from five adult and healthy Pietrain boars from a commercial unit in Germany. The examination was performed after dilution and two days of storage in liquid state at 17°C were examined by means of SpermVision version 3.7 using the following chambers:

Leja, MofA and Minitube, all of them having 20 µm in depth, at 0, 6 and 12 minutes after filling the chamber. The following parameters were determined: sperm concentration (Conc), total sperm motility (TMot), progressive sperm motility (PMOT), straight line velocity (VSL), average path velocity (VAP), curvilinear velocity (VCL), linearity (LIN), straightness (STR), wobble (WOB), amplitude of lateral head displacement (ALH), beat cross frequency (BCF). The sperm concentration was also determined by SQA-Vp, Nucleocounter NC-100 and hemocytometer. The results showed a significant difference between the MofA type and the other two chambers in terms of kinetic parameters, but also a significant difference between the concentration calculated by SpermVision, (regardless the chamber type), and the other methods ($p<0.05$). The main conclusion was that the output of CASA is influenced by the chamber type when examining liquid-stored boar semen. In this study the Leja and Minitube chamber types showed similar values, while the MofA chamber offered significantly lower results. Further studies on MofA chamber are needed, in order to confirm or to refute the negative effect exerted on the kinetic sperm parameters and to determine whether this effect (if confirmed) is due to chemical or mechanical factors. As regards the significant difference in calculated sperm concentration, we tend to think it was rather an underestimation by CASA system than an overestimation of the other devices, since all the other methods are widely recognized to be accurate and the differences among them were very small. Based on this, do not recommend the use of CASA for determining sperm concentration in liquid-stored boar semen, unless corrective measures are considered.

The **chapter VI**, "RESEARCH FOR THE IMPROVEMENT OF BOAR SEMEN PRESRVATION" is divided into three subchapters.

The first subchapter, with the title "Comparative study on the efficacy of two extenders from different classes (short-term and long-term)" aimed to identify the differences (if any) as regards the kinetic parameters within seven days of storage between a short-term and a long-term extender for boar semen.

Five ejaculates from five healthy and sexually mature Pietrain boars were collected by means of manual method and diluted with both BTS® (short-term) and DiluPorc™ (long-term). The extended semen was examined in the same day of dilution as well as after one, two, three, four, five and seven days of storage, by means of SpermVision 3.7. The following parameters were determined: total motility (TMot), progressive motility (PMot), straight line velocity (VSL),

average path velocity (VAP), curvilinear velocity (VCL), linearity (LIN), straightness (STR), wobble (WOB), amplitude of lateral head displacement (ALH), beat cross frequency (BCF).

Both extenders proved to be effective within the first seven days of preservation. However, significant differences ($p<0.05$), were revealed in terms of sperm velocity in the day of dilution, as well as in Days 2, 3, 4 and 7, with superior values in case of DiluPorc™. No significant differences were found for the other kinetic parameters.

In conclusion, the long term extender DiluPorc™ seems to provide slightly better results in case of longer storage of semen, but we think that both the examined extenders are suitable for semen preservation up to seven days without major differences in terms of kinetic parameters. Although BTS® guarantees the quality of sperm only for three days, in our study it maintained all the ejaculates within good values for a much longer period.

The second subchapter, entitled "Correlations between some Parameters of Raw and Stored Semen in Boar" aimed to identify the correlations between some parameters of raw semen, and those of extended semen, stored in liquid form, for three days at 17°C. Over 1000 ejaculates were collected by means of manual method and artificial vagina over a period of two years from 400 mature boars housed in a specialized unit in Germany. The semen was examined immediately after collection (before dilution) as well as after three days of preservation. All the parameters (except the volume) were determined by means of computerized instruments. The examination of raw semen was performed using SQA-Vp, while the stored semen was analyzed using Spermvision version 3.7. All data were processed with IBM SPSS Statistics version 21. The Pearson correlation revealed no strong correlation between any of the raw semen parameters when analysed together with stored semen parameters. However, some moderate or weak correlations were found, for example a positive correlation between motility of raw semen and motility of stored semen ($p<0.01$), a positive correlation between percentage of morphological normal sperm in raw semen and the motility of stored semen ($p<0.01$), a negative correlation between the concentration of raw semen and the linearity and wobble coefficient in stored semen ($p<0.01$) as well as a couple other weak correlations. We conclude that, despite the fact that some correlations were revealed, unfortunately the analyzed parameters of raw semen have low predictive value on the parameters of extended semen after preservation at 17°C, and that the parameters of semen will behave differently from one ejaculate to another.

The third subchapter has the title "The Type of Container and Filling Method Have Consequences on Semen Quality in Swine AI Doses" aimed to compare two types of containers for boar semen, namely the automatically-filled tube and the manually-filled bottle, in terms of preserving the quality of boar semen. Five ejaculates from five different boars were diluted with the same extender and then divided in two aliquots. First aliquot was loaded in tubes filled by an automatic machine while the second was loaded manually in special plastic bottles. The semen was stored in liquid state at 17°C, regardless of the type of container and examined daily, for five days of storage by means of a computer-assisted sperm analyzer. Both types of containers maintained the semen within acceptable values, but after five days of storage significant differences ($p<0.05$) between the container types were observed in terms of all selected kinetic parameters. The tube showed better values for sperm motility and velocity, while the bottle showed superior values for straightness and linearity of sperm movement. The automatically-filled tubes offered better sperm motility in every day of the study. Given the fact that sperm motility is still the main criterion in assessing semen quality in semen production centers, the main conclusion of this study is that the automatic loading in tubes is superior and recommended over the old-style manual loading in bottles.

**Chapter VII**, THE INFLUENCE OF THE SEASON ON THE MAIN SEMINAL PARAMETERS IN BOAR had the main objective of monitoring the seasonal variations of the seminal parameters in boars exploited in intensive conditions. It is structured into two subchapters.

The first subchapter is also divided into two sub-subchapters and aimed the study of the seasonal variations of semen in boars held in usual conditions, with high variations of the microclimate during the year, specific to each season.

The first sub-subchapter presents the results on raw semen. 11 476 ejaculates were examined, collected during eight consecutive seasons from healthy and sexually mature boars exploited within a commercial unit in Germany, specialized in boar and bull semen production. Only the ejaculates showing at least 65% total motility were recorded and processed. Semen examination was performed during the first 10 minutes after ejaculation. The following parameters were determined: ejaculate volume, sperm concentration (Conc.), total motility (TMot), total number of sperm/ejaculate (TS/Ej.), total number of motile sperm/ejaculate (TMS/Ej.), total number of motile sperm/ml (TMS/ml), the percentage of sperm with normal morphology (%Morph), total number of sperm with normal morphology/ejaculate (TMorphNS/Ej.) and total number of sperm motile

and with normal morphology/ejaculate (TMMorphNS/Ej.). The volume was determined by weight, while for the other parameters the SQA-Vp device was used. The values were statistically processed with IBM SPSS® Statistics. One-Way ANOVA analysis was applied in order to highlight the significant differences among seasons. The results showed a clear influence of the season on the semen quantity. The volume was higher during autumn and lower during spring, with significant differences among all the four seasons. Sperm concentration was higher during winter, but this could be correlated with the low volume recorded in the same period. Total number of sperm/ejaculate showed higher values during autumn and lower during spring. Sperm motility was the best in the winter and spring. The percentage of morphologically normal sperm also recorded higher values in winter and spring, and the lowest in summer. The number of motile sperm/ml was higher during winter and lower during summer and autumn. On the other hand, the total number of sperm/ejaculate showed superior values in the course of autumn and winter and inferior during spring and summer; the same variation was observed for total number of sperm with normal morphology/ejaculate and total number of sperm motile and morphologically normal/ejaculate. The obtained results are consistent with the most of the previous studies, according to which the raw semen quality is lower during the summer and good quantity of semen is produced during autumn.

The second sub-subchapter presents the data obtained when studying the seasonal variations of the kinetic parameters in extended boar semen after three days of preservation in liquid state at 17°C. The analysis included the examination of a total number of 4854 boar ejaculates, during two years, between 1$^{st}$ of March 2013 and 20$^{th}$ February 2015. The following parameters were determined: total motility, progressive motility, straight line velocity (VSL), average path velocity (VAP), curvilinear velocity (VCL), linearity (LIN), straightness (STR), wobble (WOB), amplitude of lateral head displacement (ALH), beat cross frequency (BCF). Semen was collected by manual method and by means of artificial vagina. The BTS® extender was used for dilution and the examination was performed using CASA system (SpermVision version 3.7). The results showed an evident influence of the season on the kinetic parameters, after preservation. During winter were recorded the highest values for progressive motility (75.27%), VAP (73.62 µm/s), VCL (141.86 µm/s), VSL (58.09 µm/s), ALH (3.32 µm) and BCF (37.29 Hz), during spring the highest values for total motility (80.18%), while the summer offered the best values for STR (0.80) and LIN (0.43). Interestingly, the differences were significant ($p<0.05$) only when comparing the winter-

spring season with the summer-autumn season, while the differences between summer and autumn and respectively between winter and spring were insignificant. The best season for the kinetic parameters was the winter. At the opposite pole were the autumn and the summer. The results of this study suggest that the seasonality of the seminal parameters in boars housed in normal conditions is also reflected in the values determined after dilution and storage.

The second subchapter of the seventh chapter presents the data of a study that aimed to observe the seasonal variation of the seminal parameters in boars held in standardized conditions. The study was conducted on a number of 537 ejaculates, from 31 Pietrain boars housed in standardized conditions in terms of temperature, humidity and light regime. Semen was collected by manual method, and examined using the beaker for ejaculate volume, and the CASA system (CEROS II) for the other parameters. The results largely contradict those of previous studies, which analyzed the effect of the season on seminal parameters in boars held in usual conditions. Expressed as mean values, the results were as following: Volume showed higher values in the summer (218.7 ml) and lower in the autumn (155.7 ml); Total number of spermatozoa/ejaculate was higher in the summer ($95.9\times10^9$) and lower in the winter ($74.3\times10^9$); Semen concentration was higher in the autumn ($571.6\times10^6$/ml) and lower in the summer ($454.8\times10^6$/ml); Total number of motile spermatozoa/ejaculate showed higher values in summer ($79.5\times10^9$) and lower in winter ($65.4\times10^9$); % of Total motility was higher in the winter (87.9%) and lower in the summer (82.3%); Total number of progressive spermatozoa/ejaculate was higher in summer ($52.7\times10^9$) and lower in autumn ($44.2\times10^9$); % of Progressive motility was higher in winter (61.4%) and lower in autumn (52.3%). The results contradict those of other authors, who studied the semen from boars held in usual conditions. This fact suggests that the standardization of exploitation conditions of boars by maintaining a controlled climate in the farm can reduce the effects of heat stress on spermatogenesis during summer, offering a great opportunity against the "seasonal infertility" previously described and the high variations of semen parameters during a year.

**The chapter VIII**, with the title "MOTILE SPERM SUBPOPULATIONS IN BOAR SPERM AND THEIR SEASONAL VARIATION" aimed to study the heterogeneous structure of boar semen, identifying the motile sperm subpopulations and observing the seasonal changes of their distribution. The study included the analysis of 4814 ejaculates collected during eight consecutive seasons form 700 healthy boars exploited in a commercial unit in Germany, specialized in boar and bull semen production. The examination was performed after three days of storage, by means

of a CASA system, software SpermVision 3.7. The following parameters were determined: average path velocity, curvilinear velocity, straight line velocity, straightness, linearity, wobble, amplitude of lateral head displacement and beat cross frequency. For each analyzed sample, individual data of the sperm were accessed thus obtaining the values for a total number of 433 145 analyzed sperm. All the retrieved data were included in a common database and processed with IBM SPSS® Statistics software in order to identify the sperm subpopulations. For that, a series of statistical analyzes were applied. First step was the Principal Component Analysis on the eight kinetic parameters based on Eigenvalues higher than 1. The variables showing the highest values in the component matrix were selected for further analyzes: VAP (0.973) and LIN (0.924). The values for VAP and LIN were then standardized and included in the „k-means cluster analysis" procedure, using the Euclidean distances. The requested number of clusters was 4, based on previous studies that analyzed motile sperm subpopulations. The membership of each spermatozoon to a specific cluster was saved in order to calculate the percentage of each identified subpopulation. After the clustering analysis, the heterogeneity of examined semen was obvious, with the existence of distinct subgroups characterized by specific values for velocity and linearity. Briefly, the main characteristics of the sperm subpopulations were as follows:

- Subpopulation 1 contained sperm with high velocity but low linearity. This group included 28% of the total sperm;
- Subpopulation 2, included sperm with low velocity and linearity. Approximately 26% of total sperm were part of this group;
- Subpopulation 3, represented by sperm with relatively high velocity and linearity. Approximately 20% of sperm were included in this group;
- Subpopulation 4 contained sperm with low velocity but high linearity. These sperm were approximately 26% of the total number.

The season showed a clear effect on the distribution of motile sperm subpopulations in extended semen. The spring was characterized by an approximately uniform distribution. The highest proportion was offered by subpopulation 1 (28.3%) and the lowest by subpopulation 3 (22.2%). During summer, subgroups 3 and 1 showed a decrease, reaching 16.5% and 23% respectively. At the same time, subgroups 2 and 4 increased. The highest proportion was showed by the subgroup of slow but linear sperm (31.4%). The autumn did not record major differences on the distribution of motile sperm subpopulation, when compared to the previous season. The rapid and linear sperm

were again the least numerous. The winter showed a substantial increase of the percentage of sperm with high velocity and high linearity and also a decrease of the percentage of slow and non-linear sperm.

The results led to the conclusion that extended boar semen is characterized by the existence of four distinct motile sperm subpopulation, defined by specific velocity and uniformity of movement and the collection season clearly affects the distribution of these subpopulations. The fact that the season modifies the distribution of sperm within motile subpopulations could change the perception on the seasonal variation of kinetic parameters in boar semen. In a previous chapter (chapter VII) it was shown that the season has a significant impact on the mean values of some parameters such as velocity and linearity/straightness. Thus, the seasonal variations of the mean values of these parameters could be caused by changes in the number of sperm included within specific subpopulations and not by increases/decreases in values of all ejaculated sperm.

**The Chapter IX**, entitled "FERTILITY OF SOWS IN RELATION TO RAW SEMEN PARAMETERS" aimed to assess the effect of some raw semen parameters in boar on the main indicators of fertility: farrowing rate and litter size.

The activities included boar semen collection and examination, artificial insemination of sows with extended semen that fulfilled the quality standards and calculation of farrowing rate, litter size, born alive/parturition and stillborn/parturition.

The semen examination consisted in the determination of ejaculate volume, sperm concentration, total number of sperm/ejaculate, total motility, progressive motility, total number of motile sperm/ejaculate and total number of progressive sperm/ejaculate. With the exception of ejaculate volume, for which a beaker was used, all the other parameters were determined by means of a CASA system (CEROS II, IMV Technologies, France).

The study detected weak positive correlations of farrowing rate with sperm concentration and total number of progressive sperm/ejaculate, of born alive/parturition with sperm concentration, and of stillborn/parturition with ejaculate volume; weak negative correlations of farrowing rate with ejaculate volume, of litter size with progressive motility, of born alive/parturition with total and progressive motility, and of stillborn/parturition with sperm concentration. Also, some correlations were detected among fertility indicators in sows, of which the most important were a strong positive between litter size and born alive/parturition and a moderate negative between born alive/parturition and stillborn/parturition.

As no strong correlation was detected between the main seminal parameters and fertility in sows, we conclude that the general exam of semen can not be used as a tool to predict the fertilizing capacity of an ejaculate. It can be useful in eliminating the ejaculates with certain low quality, but can not guarantee the selection of only good quality ejaculates. Also, it seems that the ejaculates with good fertilizing capacity that have great chances to induce gestation do not always provide a great litter size too, a fact suggested by the lack of significant correlations between farrowing rate and litter size.

The **Chapter X**, GENERAL CONCLUSIONS AND RECOMMENDATIONS highlights the main conclusions that can be noted after the research activities as well as a few suggestions for the specialists working in the field of boar semen production.