

ABSTRACT

The aim of this research is related to the study of some reproductive processes in swine, using a new and modern biotechnical method for artificial insemination in sows, along with improving methods for appreciation the biological value of boar semen.

Following implementation in the practice of intrauterine artificial insemination (I.A.I.U.) in sows, is aimed to improve fertility, for as high economical efficiency. As general objectives were pursued: - knowledge of reproduction parameters from some pigs breeding farms; - creating a comparative experimental study on endocervical artificial insemination and intrauterine artificial insemination; - implementation of intrauterine artificial insemination into the management of an industrial complex.

In this thesis was chosen along with the usual parameters, as the main parameter of semen quality, the *microbiological load*, considered as qualitative and quantitative aspect. Thus, were followed some activities: qualitative and quantitative determination of both bacteria and fungi; identification of critical points of semen contamination during harvest; manners of reducing contamination in the critical points; and efficiency of antifungal aseptisation by dilution of boar semen.

Another criterion for evaluation the biological value of boar semen, is the degree of resistance of spermatozoons. Spermatozoons "*resistance*" is given by their property to resist the harmful action of a "toxic". Solution of sodium chloride, 1% concentration is used for this purpose. Some authors claim that spermatozoons viability and fecundity is as higher as the spermatozoons maintain their mobility in a volume greater than 1% NaCl solution.

This sentence sums a total of 225 pages and is illustrated in 47 tables and 56 figures.

Part I - Stage of Knowledge - consists of 65 pages representing 30,95% of the work, consists of 6 chapters which are summarizing selected data from 196 bibliographical sources of Romanian and foreign literature on biotechnology in breeding sows and biological evaluation of boar semen.

Part II - own research - is carried out over 145 pages (69,05% of the work) contained in 10 chapters, following the purpose and objectives, and in the final, the present thesis ends with final conclusions, recommendations and references. Each chapter is structured in the material and working method, results and discussion and partial conclusions.

Researches were conducted in three pig breeding farms, located in N-E and S-E of Romania. The biological material studied represented the sows and boars of high genetic value, stock lots that were purchased from specialty companies.

Reproduction parameters status from some pig breeding farms

Analyzing data, the endocervical inseminated sows (I.A.Ec.) with 5×10^9 spermatozoons per dose, were both fecundity values (71.85%), and prolificity (8.55 piglets / calving) lower. But the farms where they used I.A.Ec. with $4,5 \times 10^9$, and 4×10^9 spermatozoons/dose, the two key parameters had higher values: 78.8% with 9.06 piglets; respectively 84.27% with 9.6 piglets / calving.

We can not correlate fecundity growth with decreased number of spermatozoons per dose of insemination. The explanation would find its answer in the influence of different operating conditions and zoo-hygiene (between farm 1 and farm 2), correlated with specialized technicians operators - sows.

By using the I.A.Ec method, the number of seminal cells used must be high enough to be able to achieve a sufficiently population of oviduct towards fecundation. Is found by this method a waste of spermatozoons on the one hand, given by the repression of the semen, and on the other hand, - by spermatozoons death due to their inability to overcome the two critical thresholds (cervix junction and utero-lining)

Experimental study on intrauterine artificial insemination (I.A.I.U.)

By practicing I.A.I.U. it managed to improve the sows fecundity with 6.9%, differenced by insemination season. Also, at calving, were collected in addition from lot LE, 0.19 piglets, leading to weaning at a difference between lots of 0.12 piglets / sow.

Group witness (LM) was inseminated with 4×10^9 spermatozoons and the insemination method was classical, and experimental group (LE) with 3×10^9 using the intrauterine method. The fecundity of witness group (LM) was 81.7%, and for (LE) of 88.6%. The prolificity annual average of sows from LM was 11.27 piglets, insensitive lower than reported in LE of 11.46 piglets. Although the difference is small, in absolute terms, this represents 0.19 piglets / calving, what to practice in a complex where there are approximately 12000 calving / year, means an extra 2280 of piglets.

This seasonal increase of fecundity and prolificity can be correlated with the dynamics of spermatogenesis process, dependent on ambient temperature.

Analyzing fecundity of the two lots reported to the number of spermatozoons per dose, and correlated with the type of insemination, finding that fecundity does not depend on the number of spermatozoons from dose (3 billion), but instead of the place of filing it and the suffered losses after insemination.

**Implementation of intrauterine artificial insemination into the management
of an industrial complex**

To know in detail the reproduction activity and for explaining some of the intimate mechanisms of this complex process, was followed a whole cycle on technological flow starting with collection of semen until calving, or even up to piglets weaning, piglets obtained by intrauterine insemination.

Regarding the average number of doses (with 3×10^9) obtained from an ejaculation per year, the maximum value was 49 doses obtained from a PIC boar of 1.8 years old, and the minimum value, 32 doses in a Pietrain of 1 year old. Average was 39 doses, these oscillations are influenced by spermatogenesis and perfect adaptability of races. The prepared doses were inseminated to a total of 10713 sows, the average of inseminated sows with doses from a boar per a calendar year was 1339 sows per year. From the total I.A.I.U sows, were diagnosed pregnant, an average per working year of 84.37, with limits of 80.93% in hot season and 87.8% in winter.

Boars prolificity average was calculated on the basis of sows prolificity, so prolificity varied from 10.47 (PIC of 4.5 years old) to 11.15 (Pietrain 1 year old). Prolificity average per year was 10.72 piglets of which: P.Po. were 7.38, 2.88 - P. Sp, and a number of removed pigs of 0.43 (P. Mo, P. Mu. and P.Nv.).

Entering I.A.I.U. by flow technology of reproduction activity into a livestock complex for pigs breeding is a success, obtaining a saving of seminal cells, correlated with profitable fecundity and prolificity. We recommend therefore the implementation into the management of the breeding farms with industrial system, the I.A.I.U. with 3×10^9 seminal cells to increase economic efficiency.

Microbiological examination of boar semen

Quantitative microbiological examination, sought to identify the number of bacteria and fungi in the semen freshly collected, from the diluted doses and preserved ones.

The total number of bacteria per ml S.B. recorded an average value of $98,41 \times 10^3$ in the two farms. In the farm 1, NTG-ului/ml S.B. values were between 22.4×10^3 UFC / ml and $118.2 \times$

10³ UFC / ml with an average of 65.4 x 10³ UFC / ml. Values in the NTG-2 farm were between 74.6 x 10³ UFC / ml and 130.05 x 10³ UFC / ml, with an average of 99.33 x 10³ UFC / ml. After obtaining doses for insemination by diluting the semen, the average of NTG's farms was 0.354 x 10³ UFC / ml. On farm 1, NTG is drastically decreased from 65.49 x 10³ UFC / ml to 0.120 x 10³ UFC / ml. In the samples examined from farm 2 is also observed that the number of bacteria decreased after dilution (the average is 0.588 x 10³ UFC / ml NTG / ml), but their values remain high.

The total number of fungi per ml S.B. reached the average value of farms 0.1485 x 10³ UFC / ml. Mycological load has relatively constant values, between 0.116 x 10³ UFC / ml and 0.287 x 10³ UFC / ml, with an average of 0.194 x 10³ UFC / ml in farm 2. On farm 1, the average value was 0.103 x 10³ UFC / ml with a variation from 0.081 x 10³ UFC / ml and 0.130 x 10³ UFC / ml. After dilution, the number of fungi has remained approximately constant, with value of 0.1405 x 10³ UFC / ml. Their media, in the farm 1 was 0.089 x 10³ UFC / ml, and in farm number 2 of 0.192 x 10³ UFC / ml.

Viable bacterial populations in diluted semen are considered antibiotic resistant variants or antibiotics contained diluents do not have action spectrum of all possible existing bacterial genera and fungi population remains unchanged.

Qualitative examination of bacterial flora isolated from the boar sperm demonstrates the existence of a diverse polibacteria flora. Thus have been isolated a number of 14 genera. Most often were: *Escherichia coli* with 81.8%, followed by *Staphylococcus aureus* and *S. hyicus* by 72.7%, and *Pseudomonas aeruginosa* with 63.6% of total samples examined, with a lower frequency have been identified *Yersinia enterocolitica* and *Y. Rucker*, *Pantoea spp.*, *Shigella spp.*, *Serratia marcescens.*, *Tatumella spp.* Analysis of incidence of bacterial flora in semen shows that the predominant flora examined is Gram negative, as seen in a proportion of 71.4% and Gram positive flora in proportion of 28.6%.

Qualitative examination of the mycological flora has identified a number of 9 types of fungi, both filamentous fungi and levuriforms. The most frequent types of isolated filamentous fungi were *Penicillium spp.*, *Aspergillus spp.*, *Mucor spp.*, *Fusarium spp.*, *Cladosporium spp.*, *Alternaria spp.* Levuriform Fungi spp were found consistently in each determination and in a various degree of concentrations. The most common type of Candida levuri were *C. parapsilosys* and *C. Sake*.

Preservation of diluted semen takes place at a temperature of 17 ° C for one to 7 days; conditions of growth and development for microorganisms and through biochemical processes the substrate changes and also the biological parameters of semen. Insemination in sow with

such semen, frequently leads to infecundity and appearance of some occult gynecological problems. All these turn up, eventually, the economic aspect of the unit.

Identify sources of semen contamination and the critical points of contamination

Critical points of contamination for boar semen during harvest are considered to be the boars fur and the atmospheric air in the harvesting room, they had most bacterial and fungal load.

The harvest room atmosphere represents the major source of semen contamination. the the number of germs values in the atmosphere of harvest room before introducing boars recorded value of $309 \times 10^2 \text{ m}^3/\text{aer}$. Values lower compared with those obtained after harvest ($442 \times 10^2 \text{ m}^3/\text{aer}$). After introduction the fourth boar for harvesting, the microbiological air load increases by 43%.

Effect of contamination on semen quality

Glucose concentration, pH and NTG values were monitored in preservation dynamics for 96 hours (T4) at $+17^\circ \text{C}$. These parameters were interpreted and reported to the main indices of reproduction obtained by I.A. of semen from the time T1 of preservation.

On farm 1 glucose average was 527.5 mg / dl, after dilution (T0), 527.1 at 24 hours (T1), 517.7 at 48 hours (T2) 513.8 72 hours (T3), 509.5 at 96 hours (T4). In the farm 2, the averages were: 526.3 at T0, T1 at 505.2, 482.0 in T2, T3 to 463.8. Reducing the amount of glucose in the dynamics represents its consumption by the spermatozoons and this is intimately linked to the quality of the diluent used and the number of present germs.

In semen doses is a variety of microorganisms. NTG's varies from 11 UFC / ml (in T0 in farm 1), to 1926 UFC / ml (in T3, in farm 2). Most of the identified bacteria, and all fungi, have mainly biochemical character the consumption of glucose and fructose. This explains the decrease the glucose concentration and pH values of doses that have a higher NTG.

The average values of pH varied from 7.1 (at T0) to 6.6 (T4) in the farm 1; and from 7.1 (at T0) to 5.5 (at T3). After 72 hours after dilution (T3), during preservation, was obvious a decrease of pH value in samples collected from farm number 2, which reached up to 5.5 because of the metabolic activity of microorganisms which are in a continuous growth. There is a close correlation between NTG, the pH value and glucose consumption; so, as the NTG values are higher, the pH of the dosage is changed by acidification and the concentration of glucose decreases.

It is noted that the lowest fecundity correspond to doses that had the NTG's highest. This link between the NTG's value of the preserved doses and the percentage of fertility, maintained also in comparison the lots of sows from that farms. Average fertility in farm 1, was 83.33%, with NTG of 16.7 UFC / ml at the time of insemination T1. The values of fertility were lower in farm 2 (72.00%), where NTG had much elevated values (328 UFC / ml in T1).

We can say therefore that there is a correlation between the amount of NTG / ml of diluted semen and spermatozoons capacity to fertilize, expressed as a fecundity percentage of the studied ejaculate.

Possibilities to optimize the reduction of contamination in the critical points of contamination

This chapter has sought the reduction of NTG in gross and diluted semen, by application of measures that aimed the critical points of semen contamination.

By applying preventive decontamination with aerosols in the harvest room, the NTG from air decreased by 85% (from $841,2 \times 10^2$ to $114,8 \times 10^2$ UFC/m³ air). Noted that the number of bacteria decreased from 810×10^2 to 110×10^2 UFC/m³ air and the fungi from $31,2 \times 10^2$ to $4,8 \times 10^2$ UFC/m³ air.

Another objective which aims to reduce contamination in the critical points is the equipment of the operator-harvester with two gloves for harvesting, one over the other one. After practicing local toilet, the operator removes a glove and continues harvesting using the remaining glove.

Microbiological load of S.B. after application the hygiene protocol was $312,9 \times 10^2$ UFC / ml, compared with $623,38 \times 10^2$ UFC / ml initially identified. So we can say that the effectiveness of this measure is 50.19%, thus reducing the bacterial load of gross semen to half. After dilution, it was found that in the NTG from insemination doses recorded value of $0,156 \times 10^2$ UFC / ml. These obtained values are much lower than previous determinations and than those cited in literature. Note that some analysed samples of diluted semen have been sterile, out of germs. It has succeeded also the removal of filamentous fungi in diluted semen, the load of levuri had an average of $1,39 \times 10^2$ UFC / ml.

Antifungal aseptisation possibilities of semen

To achieve this goal it has been developed a protocol for aseptisation the boar semen by using of diluents with antimycotic. *Fluconazol* was introduced in the formula of two diluents commonly used in practice, in different concentrations. Were analyzed in dynamic its effects on the quality of spermatozoons preserved for 48 hours with C.A.S.A. analyzer.

At a concentration of 125 mg % Fluconazol introduced into the formula of diluting agent A, the spermatozoons progresivity value increased by 3.3%. We appreciate that, in this concentration Fluconazolul has a stimulating role on the spermatozoons at least for a period of 12 hours of preservation after dilution, at a temperature of +17 ° C. Analyzing the processes indices of reproduction obtained through comparative insemination was found that there are no changes between lots, both fecundity and prolificity are comparable to the same values.

Changing the diluent formula by adding fluconazol with concentration of 125 mg % is a win in that it offers better aseptisation for semen preservation and the antimycotic does not adversely affect sperm quality, nor the signs of reproduction.

Appreciation of boar spermatozoons resistance

Resistance is expressed by the number of ml of 1% NaCl that stop the movement of submission (rectilinear) of spermatozoons that are in 1 ml of semen. Some authors claim that the spermatozoons longiviability and fecundity is the higher as the spermatozoons keep their mobility in a higher volume of working solution. Resistance values obtained for spermatozoons from 23 boars were between 4500 and 17000, with an average of 11,564. In isothermal conditions, the highest values were obtained for raw semen with an average of 11,664; its value decreased when working on semen after dilution (with an average of 8031).

Mangalița breed boars had the highest values: the average of gross semen was 13500 at a temperature of 37 ° C and 11000 at a temperature of 25 ° C; 8666 on sperm after dilution and 7500 after preserving for 24 hours.

The highest percentage of fecunditz (88.09%) was obtained in breed Pietrain boars, RK line, where the average value of the spermatoyoons resistance was the highest in all categories of semen (13500 in S.B., 11750 in S.D, and 9000 in S.c).

We believe that the resistance of semen influences the reproduction indices produced by intrauterine artificial insemination (I.A.I.U.).