ABSTRACT

The doctoral thesis with the title "THE INFLUENCE OF PUERPERAL GYNECOPATHIES ON THE DEVELOPMENT OF REPRODUCTIVE FUNCTION IN COWS" complies with the structure of the regulations regarding the elaboration of a doctoral thesis, being formed by two main parts: the first part, in which data are illustrated regarding the current state of knowledge and the second part where the results of own research for the topic addressed are reported.

The first part of the work includes 3 chapters in which data from the specialized literature are described regarding the morphophysiology of the genital system in cows, the events characteristic of the puerperium in dairy cows, the gynecological diseases that favor infertility and the monitoring of reproductive performance.

The second part of the thesis includes 6 chapters that describe the purpose and objectives of the research, the results of the studies regarding the monitoring of the puerperal period, the uterine conditions that generate infertility, the evolution of the microbiota at the cervico-vaginal level and the management of reproduction.

The thesis includes a number of 86 figures, 17 tables and 248 bibliographic titles.

Chapter 4 **"Purpose and objectives of the thesis".** The studies carried out as part of this research had the following objectives:

- monitoring the postpartum period and evaluating uterine involution in dairy cows based on the appearance of vaginal discharge; In this study, the vaginal discharge scoring system was tested in the diagnosis of clinical endometritis in dairy cattle;
- establishing the prevalence of the main uterine conditions, followed by diagnosis through the vaginal discharge score classification system plus complementary examinations (transrectal examination, vaginoscopy, endorectal ultrasound), and evaluating the results of the administered treatment and the consequences on reproductive performance;
- isolation of the bacterial flora from the cervico-vaginal level both in healthy cows and in those with subclinical endometritis, with the aim of identifying the bacterial species that were associated with the development of subclinical endometritis;
- establishing the diagnosis of subclinical endometritis with the help of cytological examination of the endometrium. Within this objective, the

prevalence and effect of cytological endometritis on reproductive performance in dairy cows was analyzed;

- following the evolution of the cervico-vaginal microbiota during the first 5 weeks postpartum, both in healthy cattle and in those with clinical endometritis, by means of microbiology techniques and confirmation of pathogens by the PCR technique.
- antimicrobial susceptibility testing for pathogens involved in uterine diseases of dairy cows;
- the economic evaluation of the reproductive management program addressed within the studied farm and the analysis of the losses produced by infertile females.

Chapter 5 **"The natural/organizational framework in which the research was carried out"**. This chapter describes the location where the research was carried out and the evolution of the cattle herds analyzed throughout the studied period.

Chapter 6 **"Research on the monitoring of the puerperal period and the influence of uterine conditions on the reproductive function in cows".**

The objective of this study was to compare three diagnostic approaches for postpartum uterine inflammation, namely the vaginal discharge scoring system, the quantification of the percentage of polymorphonuclear neutrophils (PMN) in cytological smears made from the endometrium and bacteriological examination of the cervico-vaginal microbiota.

Dairy cows (n = 100) were monitored starting 3 and up to $\geq$ 45 days after calving to identify females in which uterine involution did not proceed normally. The diagnosis of clinical endometritis was established based on the vaginal mucus score performed following weekly examinations between days 3 - 7, 9 - 14, 15 - 21, 21 - 28, 28 - 35, > 45.

Following the evaluation of the observations made during the monitoring, 21-28 days after parturition, the diagnosis of clinical endometritis was established for 34% of the herd analyzed based on muco-purulent and purulent discharges (score 2 and 3).

In the second part of this theme, the proposed objectives were; determination of the percentage of polymorphonuclear cells in cytological smears made from the endometrium of females suspected of infertility; evaluating the effect of Metricure therapy 12 hours after AI in dairy cows with subclinical endometritis $\geq$ 60 days postpartum; and the bacterial culture on the cervico-vaginal microbiota of healthy cows and those diagnosed with cytological endometritis.

To achieve these objectives, 54 lactating Holstein cows were selected that did not show estrous signs until 60 days after calving. In order to identify as many females with fertility problems as possible, a transrectal ultrasound examination of the uterus and a cytological examination of the uterine scraping were performed to detect subclinical endometritis.

Following the examination of cytological smears, a prevalence of subclinical endometritis of 40.7% (22/54) was established. The diagnosis was made when the PMN threshold of > 5% was exceeded

Infertile females diagnosed with cytological endometritis were divided into two groups, a control group (MES group, n = 11) and an experimental one (EES group, n = 11), with the intention of evaluating by comparison the results of therapy with 500 mg Cephapirin benzathine applied to the experimental group but also the ability to heal without therapy.

Following the treatment of cows from the EES group with Cephapirin 500 mg, there was no significant increase in the conception rate in females from this group compared to the MES group (36.4% vs. 27.3%; P > 0.05).

The degree of uterine inflammation was associated in the case of cows with cytological endometritis and the presence of numerous microorganisms from the genera *Bacteroides*, *Fusobacterium*, *Prevotella*, and *Proteus*.

At the end of this study it should be noted that a proportion of ≥ 5% PMN at more than 60 days postpartum had a high diagnostic value to distinguish cows with or without cytological endometritis. Cytological examination and determination of the percentage of polymorphonuclear neutrophils is a better predictor of reproductive performance than bacteriological examination of cervical microbiota or vaginal discharge score.

Chapter 7 **"Research on the evolution of the cervico-vaginal microbiota in the postpartum period".**

The objectives of this study were to describe and compare the diversity of the cervico-vaginal microbiota of clinically healthy Holstein dairy cows (n =18) and cows with purulent discharge (n= 18) for five weeks after parturition.

In the first sub-chapter, in order to achieve a comprehensive description of the diversity of the cervical microbiota, 180 samples were collected on which we performed a microbiological screening that included the isolation and identification of bacterial species and susceptibility testing for 11 antimicrobial agents used in the therapy of intrauterine infections.

After analyzing the samples, it was observed that, regardless of the health status of the females, the cervico-vaginal microbiota was mainly composed of members of the genera *Bacteroides*, *Bacillus*, *Staphylococcus*,

Streptococcus, *Enterococcus*, *Proteus* and *Escherichia*, but the bacterial diversity in the samples with purulent discharges gradually changed over time.

The second sub-chapter includes a study whose aim was to confirm the presence of resistance genes in the case of *Staphylococcus aureus* (MRSA) and *Enterococcus spp.* (VRE) species, but also to identify the bacterial genome of the microorganisms involved in the etiology of endometritis in dairy cows, with the help of molecular biology techniques.

Antimicrobial susceptibility tests were performed using the Kirby-Bauer method. The results showed that the isolated bacterial species showed resistance to important antimicrobial agents such as the class of penicillins, aminoglycosides and tetracyclines.

Gram-positive bacteria showed high sensitivity to antibiotics belonging to the class of beta-lactams (florfenicol, enrofloxacin and marbofloxacin) and quinolones (amoxicillin/clavulanic acid). Similar to Gram-positive bacterial species, Gram-negative strains also showed high susceptibility to beta-lactam agents (fourth generation of cephalosporins – cefquinome and amoxicillin/clavulanic acid) and quinolones (enrofloxacin, florfenicol and marbofloxacin).

Results of the present study show that the cervico-vaginal microbiota in dairy cows varies according to the health status and the number of days postpartum. In addition, it supports the hypothesis that there is uterine contamination with various bacterial species after parturition and highlights the role of microorganisms not identified by bacterial culture in this context.

Chapter 8 **"Research on reproductive index values and economic implications of infertility in dairy cows".**

This chapter evaluated the reproductive performance of a herd of dairy cattle over two consecutive years, 2020 and 2021. For this, comprehensive data was procured from a computerized farm database. The reproductive analysis focused on the individual records of each female that calved during 2020 – 2021.

The reproduction records on each animal were used to calculate the insemination index (Ii), the conception rate (CR) and the gestation index, but also the analysis of other parameters such as the distribution of heat cycles, the number of AI, the calving interval, the total of confirmed pregnancies, service period, average of open days but also average of wasted days.

Herd sizes ranged from 1295 females (435 – heifers, 688 lactating cows) in 2020 to 1442 females (423 – heifers, 1019 – lactating cows) in 2021.

The calving season recorded an increased incidence in winter and summer (38.46% and 30.21%) in 2020, and in 2021 the highest percentage was observed in autumn and summer (43.35% and 40.37%).

In the first year the seeding index was 1.97, followed by a slightly lower index of 1.95 during the second year. The average service period was 115 days for the entire workforce in 2020 and 111 days in 2021.

To evaluate the insemination management, the conception rate was evaluated, so that at the first I.A. there was a conception rate of 50.28% in 2020 and it varied between 36.36% and 54.41%. In 2021 the average conception rate was 49.7% at first insemination with a range of 40.12% to 56.47%.

The economic analysis was evaluated based on a milk price of 2/2.7 RON/liter in the years 2020/2021, using a feed cost value of 40 RON/animal and a calving cost of 500 RON. The value of the expenses borne by the farmer for the days of infertility recorded during the years 2020 - 2021, was 60,049 RON in 2020 and 139,986 RON in 2021 respectively. The treatments of postpartum uterine conditions reported at a minimum cost (96 RON/animal) were worth 53,376 RON during the first year and 52,704 RON in the second year.

The purpose of this study was to summarize the reproductive performance and management of the farm where the research took place, with the intention that this information will be used by veterinarians, farm managers and producers to improve management practices on these types of holdings.

Chapter 9 **"Final Conclusions, Recommendations and Research Perspectives"** includes the final research conclusions and suggestions for further studies.