SUMMARY

Key words: postpartum anoestrus, dairy cattle, persistent corpus luteum, ovarian cyst, ovarian inactivity.

The doctoral thesis entitled “Research regarding Anoestrus Syndrome in Dairy Cattle” is structured according to the regulations regarding the drafting of a doctoral thesis, in two main parts: the first part includes data in regard to the current stage of knowledge, and our own research for the established subject is presented in the second part.

First part of the thesis consists of 29 pages, and depicts in two chapters the reference data that refers to resuming the reproduction function during the postpartum period, what the conditions that favour the occurrence of the anoestrus syndrome in dairy cows are, as well as to information in regard to the classification, diagnosis, therapy and prevention of anoestrus syndrome in dairy cows.

The second part of the thesis has a total number of 142 pages, and it is structured in 7 chapters. They include the purpose and objectives of the thesis, the results of the studies carried out for the purpose of determining: the prevalence of ovarian disorders, what the main conditions that generate the anoestrus syndrome in cows are, data in regard to the ultrasound diagnosis and therapy of anoestrus, as well as offering a modern prophylactic method to prevent the anoestrus syndrome in cows.

Each chapter is structured in a part of material and method, where the organisational framework/population taken into study or the treatment protocol are presented, and a part where partial conclusions pertaining to each chapter are provided. The thesis includes a total number of 39 figures, 18 tables and 240 references.

Chapter III presents the ***“Purpose and objectives of the thesis”*** and aims at updating the information in regard to the favouring and determining factors in the occurrence of the anoestrus syndrome in dairy cows. We also tried to find modern methods to optimise the reproductive process in cow farms, and to create a smartphone application for the purpose of monitoring and managing the reproductive activity in cattle farms.

The main objectives of this thesis were:

- establishing the prevalence of ovarian disorders in dairy cows and the influence of hormonal treatments on the reproduction period;
- isolating and identifying the bacterial strains involved in uterine conditions, which represent infertility factors in dairy cows, with the help of microbiological tests;
- establishing the sensitivity of the bacterial strains involved in uterine conditions to antibiotics using the agar diffusion method;
- characterising the molecular substrate of the ESBL (extended spectrum beta lactamase) phenotype identified in *Escherichia coli* strains isolated from samples collected from cows with clinical endometritis;
- establishing the diagnoses of uterine and ovarian conditions in dairy cows with the help of complementary methods (echography examination);
- establishing the efficacy of some treatments applied in dairy cows diagnosed with postpartum anoestrus;

- identifying correlations between the conditions from the postpartum period and the influence of these pathologies on the reproductive performance in dairy cows;
- conducting an analysis from an economic point of view in regard to the losses caused by cows diagnosed with anoestrus syndrome;
- creating a smartphone application to monitor and manage the reproductive activity in cow farms, for the purpose of making the reproductive process more efficient.

Chapter IV entitled ***"Research regarding the determination of prevalence of ovarian disorders, and the influence of hormonal treatments on the reproductive period in dairy cows"*** includes two subchapters.

In the first subchapter, a retrospective study has been carried out in regard to determining the prevalence of ovarian disorders in dairy cows, on a total number of 834 cows from the Romanian Black Pied breed. The data came from a farm specialised in dairy production from the North-East of Romania, and included a period of four consecutive years (2010-2013).

The analysis of the data indicated that from the total of monitored cows, 38.49% had ovarian conditions which evolved with pathological anoestrus. From the total of animals taken into study, 18.70% were diagnosed with persistent corpus luteum, pathology followed by ovarian hypotrophy (11.03%) and ovarian cystic disease, 8.75%.

In regard to reoccurrence of ovarian conditions registered during the 4-year period taken into study, from the total of 321 cases of registered ovarian disorders, the highest value of prevalence of ovarian conditions was registered in 2010 (30.22%), and the lowest was registered in 2012, with a value of 20.56%.

Depending on the calving season, it has been noticed that during the four years, the persistence of corpus luteum registered two maximum levels, in winter and in spring, the lowest prevalence of this ovarian disorder being registered in cows which calved in summer. Depending on the number of lactation, the highest prevalence was registered in cows diagnosed with persistent corpus luteum, during the first lactation.

The next subchapter refers to the second retrospective study in regard to the effect of hormonal treatments on the reproduction period in dairy cows. Thus, it has been noticed that the existence of ovarian pathologies extends the reproduction period, resulting in a higher number of artificial inseminations to obtain a gestation, in extending the service period, the calving interval, and implicitly in increasing the economic losses within the farm.

Chapter V includes ***"Research regarding uterine conditions as infertility factors in dairy cows"***, it was also structured in two subchapters, and the research was carried out on a number of 285 cows.

A total number of 35 samples were collected, coming from cows with clinical endometritis, and the samples were processed at the Microbiology laboratory from the Faculty of Veterinary Medicine Iași. From the total of bacterial strains isolated from cows with clinical signs of endometritis, 64% were Gram-negative bacteria, and 36% of the strains were Gram-positive bacteria.

For Gram-negative strains, it has been noticed that all were resistant to Clavulanic Acid-potentiated Amoxicillin. It has also been noticed that a rather high percentage of isolated Gram-negative strains were resistant to Penicillin, Ampicillin, Oxacillin and Vancomycin. Similarly to Gram-negative strains, it has been observed that isolated Gram-positive strains had a high resistance to Beta-lactam antibiotics, Penicillin subclass: Clavulanic Acid-potentiated Amoxicillin,

Ampicillin and Penicillin, but also to Macrolide antibiotics (Erythromycin) or to Cyclic Polypeptide antibiotics (Colistin).

The second subchapter aimed at the phenotypic and molecular analysis of enterobacteriaceae strains associated with high resistance to beta-lactam antibiotics. The isolated strains that generated characteristic colonies on the Brilliance ESBL medium were presumptively considered to produce ESBL and then tested using the combination disk test to phenotypically confirm the presence of these enzymes.

The characterisation of the molecular substrate of the ESBL phenotype was carried out through the PCR technique, and aimed at identifying the main genes that codify the ESBL enzymes: CTX-M, SHV and OXA.

Following the investigations carried out in order to identify the main genes that codify the ESBL enzymes, our results were negative for all analysed isolated strains. The negative result that we obtained can be explained either by the presence at a molecular level of other genes that codify the ESBL enzymes, or by the presence of another resistance mechanism that can determine this type of resistance, such as the efflux pump mechanism.

Chapter VI entitled ***"Research regarding the main conditions that generate the anoestrus syndrome in dairy cows"*** aimed at identifying the correlations between certain postpartum conditions (dystocia, retained foetal adnexa, milk fever, metritis, endometritis, ovarian disorders) and other reproductive parameters, for the purpose of establishing the possible risk factors for the poor reproductive performance of cows, and the negative consequences that these pathologies have.

The data used in this exploratory study was collected from two farms of dairy cows from the East region of Belgium, for a period of two consecutive years (2014-2015), from the Holstein breeds.

Following the statistical analysis, it has been noticed that dystocic parturitions are predisposing factors for the occurrence of postpartum conditions (retained placenta, milk fever, metritis, endometritis). It has also been observed that retained placenta secondary to dystocic parturitions has represented a predisposing factor for the occurrence of endometritis and metabolic conditions (milk fever).

Presence of dystocia and retained placenta has contributed to the change of reproductive indices (reproduction period, service period, calving interval, increase of the waiting period - calving - first artificial insemination), thus causing delays in the onset of new gestations.

Of the followed postpartum conditions, those that were statistically significant for our study were retained foetal adnexa, metritis and endometritis, negatively influencing the resuming of ovarian cycle within the 60 postpartum days.

Although the calving season is mentioned by other authors as having a great importance in explaining the reproductive conditions in cows and particularly the postpartum anestrum syndrome, following the statistical analysis that was carried out, no statistically significant correlation has been obtained for this study.

Chapter VII, ***"Research regarding the ultrasound diagnosis and treatment of anoestrus syndrome in dairy cows",*** describes in the first subchapter observations regarding the ultrasound diagnosis of anoestrus syndrome in dairy cows, a study which was carried out in a cattle farm from the East region of Romania, on a total number of 450 cows, in the period February - September 2016.

A total number of 120 cows from the Holstein and Romanian Black Pied breeds were selected for the period of this study, depending on the experimental design that we approached.

Of all the pathologies that evolved in the period February - September, endometritis and persistence of corpus luteum were the most commonly diagnosed.

The second subchapter included observations regarding the evaluation of the response to hormonal treatments applied to cows diagnosed with postpartum anestrum, and the research was carried out on a total number of 120 cows (6 experimental batches).

For the cows from batch **L2** (cows with calm oestrum), it has been noticed that close monitoring of animals leads to a better detection of oestrous signs. Thus, of the 20 cows that were followed, 60% had oestrous signs and were artificially inseminated. Of these, 35% were diagnosed as being with calf without being necessary to administer hormonal treatments in addition.

For batch **L3**, oestrous signs occurred within an average number of 3.26 days, and of these, only 45% were diagnosed as being with calf. In batch **L4**, 90% of the 20 cows were diagnosed as being with calf following the performed treatment, and the gestation index and the conception rate had values of 90% and 55%, respectively.

Unlike batch **L4** (cows diagnosed with persistent corpus luteum), in which response to treatment occurred in all treated cows (20), for batch **L3** (cows diagnosed with persistent corpus luteum), response to the administered treatment was registered only in 75% of the treated cows.

In the cows from batch **L5** diagnosed with luteal cysts and treated with PGF2α + I.A. upon observation of oestrous signs, of the 20 treated cows, 85% responded positively to treatment, and only 70% of the cows were diagnosed as being with calf. In our study, for the batch of cows diagnosed with luteal cysts, oestrous signs occurred on average within 4.11 days, and the gestation index had an average value of 82.4%.

For the cows from batch **L6** diagnosed with ovarian hypertrophy, re-balancing the diet had results only for 30% of the 20 cows included in this batch. In these cows, oestrous signs occurred on average within 30 days after starting treatment, the gestation index was 66.7%, and the conception rate was 33.3%.

Chapter VIII referred to ***"Research regarding the economic implications and implementation of a modern measure to prevent anestrum syndrome in dairy cows"***.

The economic study carried out in this chapter revealed that following expenses made with fodder provision, maintenance and caring for infertile cows, the daily costs for each cow were €8.44. Non-productive expenses per year of study made with fodder provision, maintenance and caring for infertile cows registered for the entire farm were €30,443. The total losses registered for the entire farm for 3607 days of infertility were €38,012, and for each cow €10.53 were spent every day.

To treat infertility, the total expenses registered in one year of study were €589.9, and for each amount of money spent for the purpose of treating infertility, an efficiency coefficient of €85.64 was registered at the farm level.

In the second part of the chapter, we presented a **Smartphone Application (Android Operating System)**, "**Cattle365**", which represents a modern method to manage a group of animals from a reproductive point of view, thus being a useful instrument for veterinarians who want to supervise cows from a group more accurately.

The use of this application allows to manage the entire reproductive activity of cows from the farm, from the moment of introducing the animal in the group and until its reformation. It also offers the possibility to have access to all of the information stored in the database of the application, providing at the same time a perspective and real-time picture of the entire animal group.

Using the "**Cattle365**" application offers the possibility of a more precise identification of cows, which are about the have oestrous signs in the following immediate period (by notifications received on the mobile phone, three days prior to physiological occurrence of oestrous signs), thus warning false positive diagnosing with anoestrus of cows with calm oestrum.

Chapter IX, ***"Final conclusions, limits of the study and research perspectives"***, includes the main aspects of the study carried out on the established subject, which are presented in 35 main conclusions.