

ABSTRACT

The doctoral thesis has a volume of 252 pages, presents 253 bibliographic titles and is structured in two parts: the „Knowledge Stage” of 96 pages and „Personal Contributions” that expands on 156 pages.

The script is illustrated with 28 tables and 129 figures, mostly in color pattern.

The first part, „Knowledge Stage” includes the following 5 chapters: 1. Morphophysiology of the genital apparatus of the cow; 2. Nutritional infertility of the cow; 3. Functional infertility of the cow; 4. Lesional infertility of the cow; 5. Infertility due to immune deficiency of the cow.

1. Morphophysiology of the genital apparatus of the cow

In this chapter the current state of knowledge is described based on synthetic data in the literature: the morphological components of the genital apparatus-genital tractus and ovaries, genital apparatus physiology, referring to oogenesis, folliculogenesis, neuroendocrine regulation of the sexual cycle, the sexual cycle of the cow.

2. Nutritional infertility of the cow

The most recent knowledge of the literature on the nutritional etiology of infertility in cows are presented in analytical and synthetic manner, with reference to etiopathogenic mechanisms of super-feeding, subfeeding, deficiencies in various nutritional principles, proteins, carbohydrates, lipids and vitamin-minerals involved in nutritional infertility in cows.

3. Functional infertility of the cow

In this chapter the latest research on the hormonal mechanisms involved in the etiopathogenesis of functional infertility in cows, forms of manifestation, symptoms, diagnosis, preventive and curative treatment of them are summarized. Different forms of pathological anestrus caused by persistent corpus luteum, ovarian hypotrophy, ovarian luteinic cysts and cysts of the corpus luteum are considered in this part.

4. Lesional infertility of the cow

There are references to puerperal endometritis as start point for chronic endometritis in dairy cows, presenting the most important forms of manifestation, retention of fetal membranes, puerperal catarrhal endometritis, necrotic and gangrenous metritis, in terms of etiopathogenesis, symptoms, diagnosis, preventive and curative treatment.

Also, the chronic endometritis in cow with its forms of manifestation, chronic latent catarrhal endometritis, chronic suppurative endometritis, piometra, perimetritis, parametritis are presented, recent research being concerned regarding etiopathogenesis, diagnosis, preventive and curative treatment of them.

5. Infertility due to immune deficiency of the cow

Correlations between the nervous, endocrine and immune systems are presented, which influence each other through the molecular factors that mediates them, with reference to endogenous and exogenous factors that determine variations in the immunological capacity of organisms.

The second part, “Personal contributions”, includes 3 chapters: 6. Natural/organisational and institutional framework of the research; 7. Material and method of the research; 8. Results and interpretation.

6. Natural/organisational and institutional framework of the research

In this chapter data on time and place of research are presented, referring to the number of animals taken in the study, their maintenance and feeding.

7. Material and method of the research

Research has been conducted in a breeding and exploitation dairy farm, the material being represented by cows of different age, Romanian Black and White breed.

Investigations were made on experimental groups consisting of cows with chronic suppurative endometritis and cows with chronic latent catarrhal endometritis and the results were compared with those recorded in the control group, consisting of healthy cows.

Blood samples were taken for the haematological, biochemical and immunological analysis, samples of genital secretions for bacteriological determinations and tissue samples of the endometrium and ovaries for histopathologic exams. Pelvic ultrasound examinations were conducted for the investigation of the ovaries and endometrium.

Analysis on the evolution of the reproduction processes in cows with endometritis were made, and prevalence of the endometritis was established in the general and genital morbidity pattern, several investigations on chronic endometritis etiopathogeny were conducted through epidemiological surveys, clinical and laboratory analysis. Zoonomic implications of the chronic endometritis were analyzed, as well.

Treatments to prevent and combat the endometritis were applied in different experimental variants to establish their effect on fertility parameters.

The results were statistically processed by Student test for determining the statistical meanings.

8. Results and interpretation

8.1. Clinical research in dairy cow chronic endometritis

Clinical research have shown various forms of manifestation of chronic endometritis in dairy cows, of which most common were chronic suppurative endometritis and chronic latent catarrhal endometritis. General signs from cows taken in the study were imperceptible or poorly expressed, and local signs were evident only from cows with chronic suppurative endometritis, manifested by purulent or mucopurulent genital discharge during estrus, with changes of the heats mucus.

8.2. Paraclinical research in dairy cow chronic endometritis

8.2.1. Metabolic profile research

Studies of metabolic profile revealed oscillations in the experimental groups compared to control group, as determined by the inflammatory process in the uterus, which are more severe as the intensity and extension of inflammatory process are greater.

The values of erythrocyte series taken from cows in the study were within the physiological range considered, with oscillations between the inferior level of the values (number of erythrocytes and hematocrit), the average level (hemoglobin, CHEM) and the superior level of the reference values (VEM and HEM), compared to the control group.

The total number of leucocytes was within the physiological range, being significantly increased ($p < 0,05$) in experimental groups towards the superior level of the reference values, comparing to control group.

Leucocyte formula had insignificant decrease of the lymphocyte, monocyte values and growth of the neutrophils, eosinophils and basophils for the experimental groups compared to control group.

Investigations on the biochemical profile in cows with endometritis showed different values for the main biochemical serum indices.

Total protein had lower values, statistically significant ($p \leq 0.05$), located to the lower limit of normal values, compared to control group. *Protein fractions* have varied between experimental

and control groups, with decreased serum albumin and increased serum globulin, statistically significant ($p \leq 0.05$) compared to healthy cows.

Serum ure, serum glicemia and serum ketone bodies recorded values located within the physiological limits in experimental groups compared to control group.

Serum cholesterol presented high level values, which exceeded the upper physiological limit in cows with chronic suppurative endometritis, compared to control group.

Alkaline reserve presented significant variations ($p \leq 0.05$) between experimental and control groups, below the lower limit of the species, indicating rumen acidosis.

The enzyme profile (TGP, TGO, GGT and FA) recorded increased average values, statistically significant above the upper limit of the physiological values, compared to controls.

FA-alkaline phosphatase changes indicated deficiencies of the calcium-phosphorus metabolism, particularly in relation to the calcium-phosphorus ratio.

The mineral profile, respectively calcemia and magnesemia, recorded average values situated within the physiological limits in all groups considered for the study.

Calcium-phosphorus ratio in all groups of cows taken in the study was lower than the level indicated by the literature.

8.2.2. Immune profile research

Analysis of the cellular immune profile showed increased number of leucocytes in experimental groups, mainly neutrophilic and serum globulin fractions involved in humoral immune response recorded significant increases of the γ globulins, the differences being statistically significant ($p < 0.05$) comparing to control group.

The opsonocytaphagic index showed insignificant decrease of the average values of cows in experimental groups compared with the control group of cows, showing a more intense phagocytic activity for cows with genital infections.

Serum complement showed decreases in average values of cows in experimental groups compared with the control group of cows, the differences being very significant statistically ($p < 0.001$).

8.2.3. Research on histological changes

Histopathological examination in cows with chronic endometritis revealed structural changes both at the level of the endometrium and ovaries.

The main histological uterine lesions were at the level of lamina propria of the endometrium, where many connective fibers, histiocytary infiltration and denuded mucosal epithelium were noticed in lamina propria of the endometrium among the uterine glands.

Changes of the ovaries were associated to uterine lesions, these being different depending on the type of ovarian affection. Pronounced degeneration, necrobiosis and compensatory fibroblastic hyperplasia have been noticed among luteal cells in the persistent corpus luteum associated to chronic purulent endometritis. Cystic corpus luteum had a cavity filled with citrine liquid, delimited by a thick, uniform and fully luteinized cortical. Changes were obvious in the hypotrophic ovaries: germinating epithelium was affected on stretched areas, being flattened; in the underlying area there were numerous mesenchymal cells, fibroblasts, fibrocytes, histiocytes, mastocytes and in the cortical area, abundant connective tissue was arranged in vortices, ovarian follicles stopping in the primary stages of development (atretic follicles).

8.2.4. Microbiological research

Microbiological examination of the genital discharge samples from cows with chronic endometritis revealed some particularities depending on the manifestation form, clinic or latent.

Staphylococcus aureus was isolated in pure culture in most of the cases from cows with chronic suppurative endometritis and the isolated bacteria from cows with latent chronic catarrhal endometritis were from the following species: *Staphylococcus aureus*, *Arcanobacterium pyogenes* and *Bacillus cereus*.

Bacteria in mixed cultures have been isolated in genital secretions from experimental cows with chronic endometritis: *Escherichia coli* and *Arcanobacterium pyogenes*, on the one hand, and *Staphylococcus aureus* and *Arcanobacterium pyogenes* on the other hand; *Arcanobacterium pyogenes* and *Staphylococcus aureus* in cows with latent chronic endometritis.

Conditional pathogenic bacteria were isolated in pure culture from cows in the control group, consisting of the following species: *Bacillus cereus* (20%), unpathogenic flora with *Lactobacillus* spp. (20%) and 60% of samples were sterile

The antibiogram showed that isolated bacteria from cows with chronic endometritis were moderately susceptible to Cefoperazone and Ampicillin and sensitive to Chloramphenicol, Ceftiofur, Kanamicin, Enrofloxacin, Lincospectin, Amoxicillin and Florphenicol.

8.2.5. Echographic aspects in chronic endometritis

Ultrasound examination in cows with *chronic purulent metritis with reduced collection* showed increased volume uterus, hypoechogenic thickened uterine wall, reduced uterine content and in cows with *chronic purulent metritis with massive collection (piometra)* showed increased volume uterus, hypoechogenic, irregularly thickened uterine wall, massive intrauterine content consisting of liquids (anechogenic) with a lot of particles in suspension, diffuse echographic aspect with disperse reflexions (spots in suspension).

8.3. Research regarding the evolution of the reproduction processes in cows with chronic endometritis

Manifestation of estrus in cows with chronic endometritis occurred after prolonged periods of postpartum anestrus, or repeatedly at different intervals in cows with chronic latent catarrhal endometritis.

Cows with endometritis had some variations on the type of sexual cycle: 34.4% regular sexual cycles, 11.72% irregular sexual cycles and 53.9% alternative cycles and regarding the length, 27.3% normal cycles and 72.65% long cycles.

8.4. Research regarding the incidence of chronic endometritis and its influence upon reproduction indices in dairy cows

8.4.1. The incidence of the chronic endometritis

The average value of the incidence of chronic endometritis in cows was 26.70% of the total genital diseases and 11.11% of the total group of cows, with annual variations ranging from 8.01% (2007) and 13.48% (2006). The incidence of chronic endometritis depending on the season recorded a maximum of 4.0% in the spring season and a minimum of 1.46% in autumn season.

8.4.2. The influence of the chronic endometritis upon reproduction indices

Studies on the main reproduction indices (the calving-first insemination interval, service period, and the number of inseminations per gestation) in cows with chronic endometritis had higher average values, with statistically significant differences compared with healthy cows.

- the average calving-first artificial insemination interval in cows with chronic purulent endometritis was higher by 96 days (181.3 ± 13.42 days), compared with the control group (85.3 ± 2.1 days), ($p \leq 0.001$),

- average calving-fecund insemination interval for the experimental group E was higher by 88.2 days (219.4 ± 14.89 days, compared to the control group (130.2 ± 9.8 days), ($p \leq 0.001$),
- insemination index showed average values for the experimental group of 5.0 ± 0.310 and for the control group of 2.3 ± 0.132 , ($p \leq 0.001$).

8.4.3. Zooeconomical implications of the chronic endometritis

Research on the zoeconomical implications of chronic endometritis in dairy cows have shown important losses for the dairy farmers.

Economic losses due to chronic endometritis in cows were estimated at 211.50 lei (52.9 euros)/cow/day and 5634.78 lei (1408.69 euros) for a total of 89.2 days of infecundity/cow, from which:

1. unproductive expenditure - 53,6 lei- 13,4 euro, cost/cow /day and 4781,12 lei (1195,28 euro), cost/ cow /total days of infecundity,
2. failure in the number of calves - 72,30 lei (18,10 euro), cost/ cow /total days of infecundity,
3. failure of milk production - 7,80 lei (2,0 euro), cost/cow /day and 695,76 lei (173,94 euro), cost/ cow /total days of infecundity,
4. loss by eliminating milk from animals treated with antibiotics, which have remanence - 21,60 lei (5,40 euro), cost/cow /day and 151,2 lei (37,8 euro), cost/cow/total days of treatment,
5. expenditure on treatment of cows with chronic endometritis - 64,00 lei (16,00 euro), cost/cow /day and 192,0 lei (48,0 euro), cost/cow/total days of treatment .

The losses due to early reformat of cows with chronic endometritis and to failure of the normal lactation can be added to previous losses.

8.5. Preventive and therapeutic conduct in dairy cow chronic endometritis

Studies regarding preventive conduct and endometritis therapy showed reduced incidence of inflammatory disease and improving the reproduction indices in dairy cows.

- *preventive treatments* applied antepartum in the last month of gestation with Sel-E-Vit (group A₁) reduced 100% the retention of fetal membranes and up to 6.7% the genital infections and treatments with PGF_{2 α} given at 14 and 28 days postpartum decreased their incidence up to 6.7%, compared to the control group which recorded 10.0% retention of

fetal membranes and 16.7% uterine infections, with levels 10% (A_1-M_1), ($p \leq 0.05$) higher compared to the control group,

- the average calving-first insemination interval in experimental groups recorded variations between 70 days (group A_1) and 81 days (group A_2), values being lower by 25 days and 14 days respectively, compared to control group (95zile),
- the fecundity rate at first artificial insemination recorded oscillations between 40% (group A_1) and 53.4% (group A_2), levels being higher by 13.3% (A_1-M_1), ($p \leq 0.05$) and 26.7% (A_2-M_1), ($p \leq 0.05$) compared to control group (26.7%),
- gestation percentages for the first two inseminations ranged between 73.4 (group A_1) and 86.7 (group A_2), being 16.7 (A_2-M_1), ($p \leq 0.05$) higher compared to control group,
- *curative treatments* performed in cows with chronic endometritis had variable results of the reproduction parameters:
- the treatment-fecund insemination interval recorded an average of 68 days for variant B, 27 days shorter than control group M_2 (95 days). The gestation percentage for total inseminations of 86.7 at experimental group B was 20.1 higher compared to control group M_2 . The average calving-fecund insemination interval was lower by 41 days in experimental group B (220 days) than in control group M_2 (261 days).
- the treatment-fecund insemination interval had an average value of 51 days for the variant C, 17 days shorter compared to control group M_3 (68 days). The gestation percentage for total inseminations of 90.0 for the experimental group C was 16.7 higher compared to control group M_3 , with a value of 73.3.