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**PhD THESIS**

***OPTIMIZATION THE DIAGNOSIS AND TREATMENT  
METHODOLOGY IN UTERINE AND VAGINAL  
INFECUNDITY IN COW***

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**SUMMARY**

The paper entitled "*Optimizing the diagnostic and treatment methodology in uterine and vaginal infecundity in cow*" contains a number of 183 pages, structured in two distinct parts.

The first part contains 46 pages and consist of "**The current stage of knowledge**". This is divided into three chapters describing the most current data regarding the subject, data intertwined with other consolidated information from the past.

Chapter I ("*Immunological status of bovine uterus during the puerperal period*") is divided in 3 subchapters which contain novelties about the immunity of the cow uterus during the

puerperal period. First was described the critical period through which cows pass near parturition in correlation with their nutritional needs. Also, contamination of the puerperal uterus with different germs is common because of the physiological phenomena that is happening at that time. The relaxation of vulva and cervical opening allow the passage of a significant population of germs with different pathogenicity such as *Escherichia coli*, *Truperalla pyogenes*, *Staphylococcus aureus*, *Fusobacterium* spp..

Another topic from this chapter was to identify the means in which the genital apparatus defends itself in the postpartum period from various pathogens. The barriers that are limiting the rise of pathogens has been described from macroscopic to microscopic level. The macroscopic barriers are represented by the components of the copulatory tract consisting in the cervical close delaying and the activity of the vulva.

At the microscopic level were described polymorphonuclear cells that promote non-self phagocytosis, immunoglobulins that help with direct neutralization of bacteria in the uterine lumen and at the molecular level has been described the connection between the pathogen-associated molecules and their recognition pattern. The last one is mainly represented by Toll-like receptors that are responsible for the recognition of various substances on the surface of the antigens.

In the second chapter, entitled “*Vulvo-vestibular-vaginal infecundity*”, were described vulvo-vestibular-vaginal duct disorders that can lead to infecundity.

In literature are few articles that mention the importance of these disorders in breeding activity in dairy cow.

Vulvovaginitis has been described with mechanical origin (which were consecutive to dystocia and infections) and with infectious origin (bacterial and viral).

The broadest chapter of the first part is the third one (“*Uterine infecundity*”), in which are described the links between production and breeding and the most important diseases that are the determinant factor of infectivity or uterine infertility in cow.

We discuss the relation between reproductive activity and nutrition which results from the statement that low rumen pH has been associated with dystocia, metritis and laminitis.

Another marker to diagnose uterine infections at 5 and 7 weeks postpartum may be the low level of glucose before parturition.

During the transition period, the importance of nutrition results from the fact that amino acids are needed to export fat from liver as lipoproteins. Over the years, the transition period has

been shortened by reducing the dry period to less than 60 days. This was beneficial in the short term by improving reproductive parameters. Beginning with the second lactation, this tactic resulted in reproductive failure.

Cervicitis were the first affections discussed, in this chapter. Through his topography, the cervix is accessible to many traumatic and infectious factors during parturition and in the postpartum period.

Few bibliographic sources have been described the clinical manifestations of cervicitis, their impact on the breeding activity and the diagnostic methods applied to this point.

In this chapter were exposed the current knowledge about mechanisms that promote fetal retention, such as: induction of parturition, shorten of gestation, abortion, gemelarity, dystocia, fetotomy, caesarean, nutritional deficiencies (such as deficiency in vitamin E, selenium and carotenes), infectious agents etc.. In close dependency with nutrition, placental retention was evaluated by some authors in relation with changes of certain metabolic markers (non-esterified fatty acids and  $\beta$ -hydroxybutyrate) and by their impact on suppression of immune response.

To minimize the negative impact of this disease on the cow productive and reproductive status various treatment protocols have been described.

Referring to the puerperal period, one of the most severe form of infecundity is toxic metritis, a disorder that affects cows systematically within the first 2 weeks postpartum. One of the most accurate methods of prediction is the ratio of non-esterified fatty acids and circulating cholesterol, which can be applied at least two weeks before the expected date of parturition.

A cow which do not have general signs of disease but have a large uterus volume and red-brown vaginal discharge 21 days postpartum are considered to have clinical metritis.

This disorder has a negative impact on the reproductive performance such as delay in resumption of cycles after parturition, uterine disturbance and embryo developmental disorders. At the same time, the decrease of feed intake will reduce the energy required for milk production.

Subchapters 3.6 and 3.7 are the most debated because they support the theoretical basis of research from the second part of this paper. In this subchapters are discussed the aspects of clinical and subclinical endometritis, aspects that refer to: etiology, severity of these diseases, clinical and paraclinical diagnostic methods and treatment methods.

The last chapter is dedicated to newness supporting the etiology, diagnosis and treatment of pyometra in cow. Compared to other disease, data about pyometra does not bring newness regarding diagnostic and treatment protocols.

In terms of content, the vastest part of this paper consist of **Personal Research** where the information were presented in order to meet the proposed objectives. The first chapter of this part is the fourth one (“*The purpose and description of the institutional research in which were carried*”), in which were detailed the purpose and the motivation of this study in the current context of global expansion.

The aim of this paper was to identify causes that lead to infecundity of the inferior genital tract, using different biotechnical methods of diagnostic. The same goal was to test and to implement different treatment methods, part of them innovative, to limit the negative impact on reproductive function.

We followed objectives such as: making a large and representative stock of animals necessary for study in order to extract relevant conclusions, the evaluation of puerperium in Montbéliard and Holstein Friesian cow, identifying the affections of the copulatory tract and highlighting their influence on breeding at primiparous and multiparous, diagnosis cervicitis by applying new protocols, some of them used for the first time in our country, diagnosis subclinical endometritis in Holstein cow through different techniques, applying innovative treatment protocols (for the first time in our country) in cow suffering from clinical endometritis and calculating reproductive indices to certify the diagnostic methods used, to evaluate the efficiency of the treatments used and to make the breeding function more efficient.

Also in this chapter were described the time slot, the place where research took place and the locations where the samples were processed.

The fifth chapter, entitled “*Researching the etiology and diagnosis of vulvo-vestibulo-vaginitis in cow*”, had the purpose to determinate the incidence and the negative impact on the reproductive function of the injuries that may occur in vulvo-vestibular-vaginal duct. A number of 1,772 cows (primiparous and multiparous) from three farms in the South of Romania were taken into study. The study was carried out between 2014 and 2017. We followed: the annual number of parturitions, assistance in parturition, the number of parturitions per female, the number of gemelarity and the fetal sex. These data were needed to identify the causes that produced vulvar, vestibular or vaginal disorders from mechanical causes.

After parturition, through inspection and palpation had been identified lesions such as: edema and infiltration, tears on this tract, necrotic vaginitis, rectovaginal fistula and granulous vulvovaginitis.

From 1772 cows included in study, only 7.95% were affected by one or more pathologies the vulva-vestibulo-vaginal (v.v.v.) duct. Different types of v.v.v. pathologies were associated with male fetuses in 5.75% of cases and 4.90% in cases of female fetuses. A percent of 0.16% of the parturition were reported as gemelarity.

The highest percent of the conception rate at first artificial insemination (over 30%) was achieved in farm II where the stock was represented by Montbéliard breed and the lowest percentage was achieved in farm I (20.49%).

Considering the incidence of v.v.v. we concluded that the most common affection was edema and v.v.v. infiltration, followed by v.v.v. injuries; the rectovaginal fistula had the lowest incidence.

We have been proposed recommendations such as: establishing a pair matching plan or providing assistance in parturition in order to prevent accidents and/or to solve them.

The sixth chapter (“*Researching the diagnosis of cervicitis in cow*”) aims to highlight the frequency of cervicitis and their diagnosis by different methods. The study was carried out on 113 Holstein cows belonging to a farm from Prahova district.

The cows were evaluated by transrectal palpation, vaginal speculum examination and endocervical cytology. These examinations analyzed cervix data such as: cervical consistency and diameter, appearance of the *fornix vaginalismucosa* and the number of polymorphonuclear cells in the cervical mucosa.

Also in this chapter was described the technique for epidural anesthesia in cow and the technique of cytological examination using the cotton swab and the brush to collect cells from the structure of the cervical mucosa.

Through transrectal palpation, a healthy cervix was estimated in 93.54% of the primiparous and 93.58% of the multiparous, respectively, and a rough cervix with the diameter below 7 cm was diagnosed in 1.83% primiparous and 4.58% multiparous.

Using vaginoscopy in 12.90% of primiparous was observed edema, hyperemia and prolabatation of the second fold respectively in 28.20% of multiparous, this being the worst situation.

Through cytobrush technique has been obtained 109 smears resulting that 28.44% (31) of the animals were diagnosed with cervicitis (PMN  $\geq$  5%) and the remaining 71.55% (78) were negative to cervicitis (PMN  $<$ 5%). Using the cotton swab technique, only epithelial cells and cervical mucus were exposed on the smears.

At the end of the chapter, the recommendations were related to application of diagnostic methods as soon as possible before the puerperium ends and the endowment of the farms with a minimum necessary in order to achieve the coloring and evaluation of the cervical smears.

The 7<sup>th</sup> chapter, entitled “*Researching the diagnosis of subclinical endometritis in cow*”, has proposed stadialing subclinical endometritis using different diagnostic techniques and also establishing a correlation between cows with cervicitis and subclinical endometritis.

In this study we subjected the same animals from the previous study and we evaluated: the uterine body and the uterine horns during the voluntary rest period and at the time of the artificial insemination using transrectal palpation test, endometrial cytology, biopsy exam, microbiological exam, transrectal ultrasound exam, exam of leucocyte esterase activity and the White side exam.

It has been observed that palpation of the cervix and uterus at primiparous has a correlation of 0.97 and it can be concluded that endometritis are associated with the cervicitis in the case of the Holstein multiparous but in the case of primiparous, cervicitis can occur independently of the uterine inflammation.

Concerning the endocervical cytology and endometrial cytology there is a low correlation between examinations of the primiparous cow (0.47) and an increased correlation in multiparous cow (0.99). By applying the bacteriological examination, there were identified 4 species of Gram-negative bacteria and 8 Gram-positive bacterial species, the most frequent being *Escherichia coli* followed by *Staphylococcus hyicus*.

If we evaluate all the results we can say that the most accurate method of diagnosis of subclinical endometritis remains the histopathological examination of the endometrium.

Regarding the reproductive indices, the voluntary waiting period was 84.72 days for cows with severe subclinical endometritis and the rate of conception at the first artificial insemination recorded a percentage of 36.50% for the same category of animals.

In the 8<sup>th</sup> chapter (“*Researching the treatment of endometritis in cow*”) were described five treatment protocols applied to a number of 133 cows diagnosed with clinical endometritis.

Efficacy results were compared to the results of 52 control cows using reproductive parameters.

The novelty of this chapter is the application of ozone and N-acetylcysteine treatment, alongside the use of traditional products such as antibiotic and antiseptic or plant extracts and proteolytic enzymes.

The most effective treatment was the combination of antibiotics, antiseptics and N-acetylcysteine, with the highest rate of conception achieved at the first artificial insemination (63.1%) and the shortest service-period (93 days) of the study. The most ineffective treatment was recorded in the group of cows that were treated with plant extract (37.2% first-rate conception rate and 121.5 days service period).

Among the recommendations of this chapter, we mention the implementation of antibiotic, antiseptic and N-acetylcysteine treatment as soon as possible for cows with clinical endometritis.

The ninth chapter contains *general conclusions and practical recommendations* and aims to synthesize as accurately as possible the results obtained from the research and to point out the achievement of the proposed objectives.

*The bibliography* of the present paper is revealed in the tenth chapter, where 294 bibliographic sources are listed. Of these, 285 sources are represented by articles published in various scientific journals, 6 are books in the field, and 3 are doctoral thesis. The share of articles published since 2016 is over 40%, and for those published between 2009-2015 is over 38% from the total of 285 articles. It means that over 78% of the bibliographic sources are from the last 10 years.

Elements of originality brought out by this doctoral thesis:

- Carry out a detailed study of the incidence of injuries from the vulvo-vestibular-vaginal duct and their consequences on reproduction;
- Presentation of data on the incidence of cervicitis at the end of the puerperal period;
- Development of diagnostic methods to identify subclinical cervicitis and endometritis in a modern manner;
- Implementing innovative treatment protocols such as the ozone spray or the N-acetylcysteine solution.