

**RESEARCHES CONCERNING THE HISTOCHEMISTRY AND MICROBIAL
FLORA OF THE PUERPERAL UTERUS IN COWS**
**CERCETARI PRIVIND HISTOCHEMIA SI FLORA MICROBIANA A UTERULUI
PUERPERAL LA VACA**

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REZUMAT

Populatia microbiana reprezinta un grup de germeni heterogeni cuprinzând unele specii diferite care traiesc si interactioneaza împreuna în acelasi loc. Comunitatile de microorganisme, ca grupuri de populatii independente, sunt mai bine adaptate pentru crestere, decât o specie unica.

Puerperiumul reprezinta perioada cea mai critica din viata genitala a unei femele, deoarece este conditionat de oboseala gestatiei si a parturitiei, de nasterile laborioase sau distocice dar si de eventualele retentii placentare sau atonii uterine si nu rareori de infectia cu germeni bacterieni.

Dupa parturitie consumul energetic si plastic creste progresiv cu involutia uterina si volumul productiei, cu necesitatile determinate de autoapararea locala si generala. Scopul cercetarilor noastre a fost acela de a urmari flora microbiana ce populeaza uterul în puerperium, identificarea acestor agenti prin efectuarea de culturi si izolarea lor pe medii selective. De un real ajutor a fost si efectuarea antibiogramelor pentru a evidentia sensibilitatea agentilor microbieni la medicamentele ce vor intra în schema de tratament recomandata. Au fost studiate modificarile ce se produc la nivelul mucoasei uterine, refacerea acesteia prin urmarirea proceselor degenerative în prima faza si apoi a celor regenerative, evidentiata prin efectuarea de biopsii uterine si prelucrarea lor histologica la 12 h - 24 h - 48 h - 72 h - 7 zile - 21 zile post partum.

ABSTRACT

The microbial population represents a group of heterogeneous germs comprising several different species which live and act together in the same place. The communities of micro-organisms, as independent population groups, are better adapted for growing than a unique species.

The microbial flora of the puerperal uterus differs from case to case, being represented both as bacteria admitted as pathogenic and particular tropism for the uterine morphological structures and opportunist bacteria which are quite numerous.

Here are some of the microbial identified species: Streptococcus spp., E.coli, C.pyogenes . These exercise the pathogenic action under certain circumstances such as: the existence of these germs in big quantities, virulence and the high rate of pathogenity, the low resistance of the body.

The experimental and epidemiological observations have proved the fact that autochthonous microorganisms (the normal, permanent microflora), adapted to develop in certain natural habitats, exercise a barrier function which protects the animal organisms from the implantation of several microorganisms which penetrate in the outer environment.

The necrotic processes at the level of the uterine mucous membrane were at different stages of evolution in all the cases in question with big or even huge differences from one geographical area to another one.

In some geographical areas the alternative processes were obvious only at the surface of the mucous membrane, with the affecting of the cells and area placed close under the basic membrane, while the epithelium although it seemed obviously affected, was not detached from the basic membrane.

The cells of the uterine mucous membrane seemed detached here and there either under the form of groups taking up small areas, or in big areas alternating with zones where the epithelium was still partially preserved and, eventually extensive areas, completely without mucous membrane, where even traits of cells at the level of the mucous membrane could not be noticed.

KEY WORDS: histochemistry, microbial flora, uterus, cows

DETAILED ABSTRACT

The microbial population represents a group of heterogeneous germs comprising several different species which live and act together in the same place. The communities of microorganisms, as independent population groups, are better adapted for growing than a unique species. The puerperium represents the most critical period in the genital life of a female, because it is conditioned by the exhaustion of gestation and of parturition, by the laborious births, but also by the possibly placental retentions or uterine atony and not seldom by the infection with bacterial germs.

After parturition the energetic and plastic consumption increases progressively with the uterine involution and the production volume, with the necessities determined by local and general self-defence. The aim of our research was to watch the microbial flora which populates the uterus during the puerperium, the identification of these agents by accomplishing cultures and their isolation on selective media. The accomplishment of antibiograms has been of great help; they indicate the sensibility of microbial agents against the medicines that will be included in the recommended treatment scheme. The modifications which take place at the level of the uterine mucous membrane have been studied at the same time with its recovery by watching the degenerative processes in the first stage and then the regenerative ones, emphasised by the accomplishment of uterine biopsies and their histologic working out after 12-24-48-72 hours after 7-and 21 days post partum.

There are zones where the structural recovery and organisation process of the mucous membrane is in an incipient stage, materialised by active proliferation of the conjunctive stroma which is mostly formed of young collagen (thin fibres with low tinctorial affinity) which at the surface of the epithelium mucous membrane is recovering occupying the largest part of the mucous membrane surface, with very small areas where the epithelium is not yet present.

In other zones the process is more advanced in that the uterine mucous membrane epithelium is formed of one single layer of cubic and here and there prismatic cells, displayed on the basic membrane, without the existence of areas without cells, even of very small size. In other zones the uterine mucous membrane epithelium appears as something higher, being composed of prismatic cells; from place to place uterine glands in an initial stage of organisation, which occupy only a very thin area from the surface of the mucous membrane can be noticed. In all the areas described so far a mutual phenomenon can be noticed, namely that the blood vessels, especially the ones in the superior third of the mucous membrane have the lumen full of red cells.

On the sections of the uterine mucous membrane taken 7 days postpartum, the process of structural organisation is significantly more advanced than after 48 hours. The conjunctive stroma appears as better consolidated and the epithelium is formed of a layer of higher prismatic cells. The organisation process of uterine glands is more advanced, although it is far from its end.

In several zones the uterine stroma has a denser structure (it is in a slightly more advanced stage of organisation) and its glands are longer, without exceeding a superficial 1/3 of the mucous membrane. The uterine mucous membrane in endometritis appears as more modified compared to the normal state although there are sometimes quite big differences from one zone to the other one. Zones with superficial necrosis, which spread over the epithelium and the superficial corion, with a cauterizing aspect are to be noticed. The superficial zone of uterine stroma presents here and there a pronounced congestive hemorrhagic aspect, and the epithelium which covers these zones looks detached under the form of lambsours.

The elimination of the epithelium under the form of lambsours causes the appearance of the mucous membrane as bereft on large surfaces and with hemorrhages in the superficial zone. The cellular and tissue leavings resulted as a consequence of necrotic processes can be noticed in the lumen of the organ under the form of big or small conglomerates. Here and there the mucous membrane is completely bereft even without traces of epithelium and the necrosis processes can be noticed on extended areas in the depth of the uterine mucous membrane.

MATERIAL AND METHOD

Gathering, working out, and interpretation of microbial flora from the puerpera uterus in cows.

The gathering of samples has been done from 10 Holstein milk-cows, the property SC Agroindustrial SA Cluj-Napoca during their puerperium. The samples were gathered both from healthy cows and

from cows with endometritis signs. In order to gather the samples, test-tubes and sterile poads were used.

With the help of a speculum the vagina was penetrated and uterine secretion was gathered. For the identification of germs bacteriological and bacterioscopic examinations were done (table 1).

Table 1. The bacteriologic balance of the uterine content in the puerperal period

No	Isolated germs	No of positive samples	% of the total of samples
1	Streptococcus spp.	3	30
2	E.coli	2	20
3	E.coli + Streptococcus	2	20
4	C.pyogenes + E.coli + Streptococcus	2	20
5	E.coli + C.pyogenes	1	10

Gathering, working-out and interpretation of uterine biopsy from puerperal uterus in cows

The researches developed in the period 1999-2000 on 10 Baltata Româneasca milk cows in early puerperium. Uterine biopsies were gathered 12,24,48,72 hours, 7 and 21 days from parturition. The drawing technique of uterine biopsy is Popescu and colleagues (1966). The samples obtained were introduced in a solution of salin formol 10% and prepared to be coloured. The colouring method and technique were Masson modified by Goldner.

Table 2. The frequency of microbial infections according to the number of implied agents

Type of infection	No.samples	% samples
Monomicrobial infections	5	50
Polymicrobial infections		
• with 2 species	3	30
• with 3 species	2	20

RESULTS AND DISCUSSIONS OF RESULTS

After the bacteriologic balance we can assert that, irrespective of the evolution or involution of an inflammation process at the level of the uterus, both autochthonous (normal flora) and alothonous (invading) germs as, for instance, E.coli, Streptococcus, C.pyogenes can be made obvious.

These germs placed at the level of the uterus have a pathogenic action only under certain circumstances such as:

- the existence of the germs in a big quantity
- virulence and the high degree of pathogenity, low resistance of the organism (table 2).
- all these action cumulatively, in favour of the establishment and evolution of septical processes at the level of the uterus.

From the above table it results that in 50% of the cases one single microbial species was identified at the level of the puerperal uterus.

A polimicrobial population represented by two species is encountered associations with more than 3 microbial species.

After the accomplishment of Neosensitabs antibiogram more intense sensitivity of germs versus Cloramphenicol was noticed (table 3).

A better knowledge of the actioning mechanism of this product is imperative (the product is in discussion for its negative effects over the organism).

Taking into consideration the reserve concerning the utilisation of Cloramphenicol we resorted to - other medicine groups (table 4).

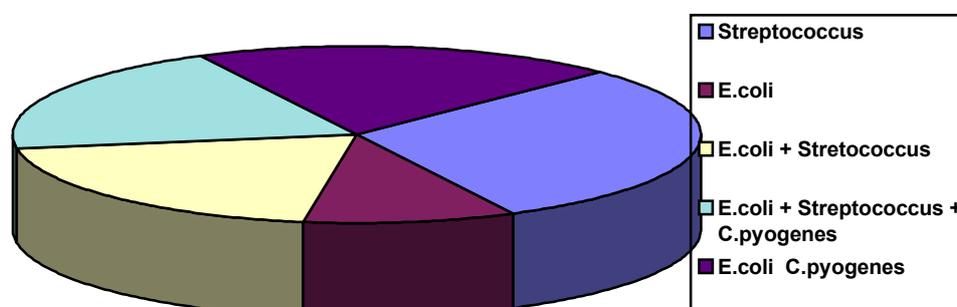
Table 3. Antibigram 1 - Neosensitabs

Antibiotic	E .coli Sample 1 (mm)	E.coli Sample 2 (mm)
Cloramphenicol MCG	60	30
Gentamycin 40 MCG	20	22
Tetracilin 80 MCG	R	20
Ampicilin 33 MCG	20	22
Cephalotin	20	20
Linco-spectin	22	22
Enrofloxacin	26	26
Trimetropine + Sulpha	14	16
Neomycine	18	16

Table 4. Antibigram 2 - SANOFI

Antibiotic	E.coli Sample 1 (mm)	E.coli Sample 2 (mm)
Flumeguain 30 MCG	26	24
Trime-sulpha 1,25-23,75 MCG	R	R
Anoxicicline 25 MCG	22	22
Colystine 50 MCG	16	14
Eritromycine 15 UI	R	R
Spectinomycine	18	16
Maxicycklin	28	30

Graphic 1. The bacteriologic balance of the uterine content in the puerperal period



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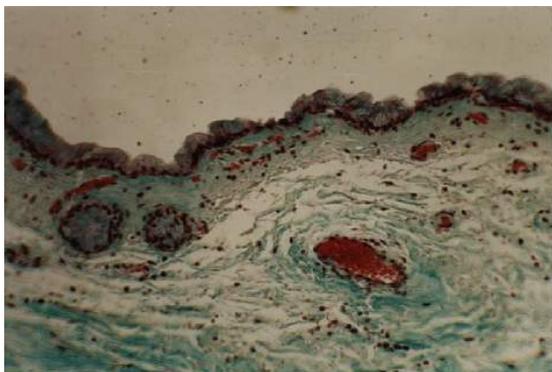
Actioning mechanism

Disturbs the synthesis of microbial proteins by linking the transfer ARN by the messenger ARN from ribosome complex. The actioning spectrum is wide, similar to cloramphenicol. It contains all the anaerobes and more, the protozoars (Toxoplasma, Anaplasma, Tripanosoma); it is more active compared to stafilococcus, streptococcus and other cocci, or compared to classical tetraciclins. It is similar to classical tetraciclins, being active against germs, which become resistant to these, as well. It is partially inactivated in the organism, the elimination is done through urine, excrements, milk. After the intramuscular administration the elimination through milk (in cows) lasts approximately 48 hours. The

microscopic examination of histological sections pointed out the fact that the uterine mucous membrane is at the moment of biopsy accomplishment in a full process of structural recovery and organisation.

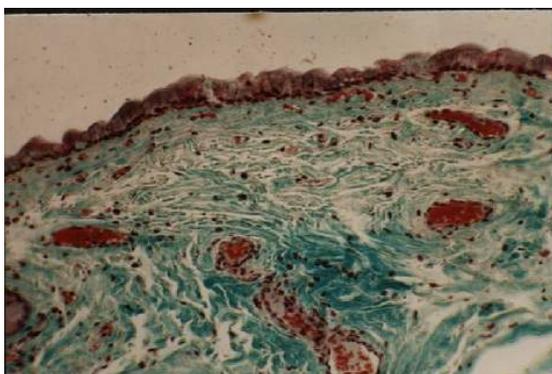
There are zones where the structural recovery and organisation process of the mucous membrane is in an incipient stage, materialised by active proliferation of the conjunctive strome which is mostly formed of young collagen (thin fibres with low tinctorial affinity) which at the surface of the ephitelium mucous membrane is recovering occupying the largest part of the mucous membrane surfac, with very small areas where the epithelium is not yet present (figure 1).

Figure 1. Stadiul de refacere a mucoasei uterine. Epiteliul lipsește pe zone mici (Col Goldner, 200X).



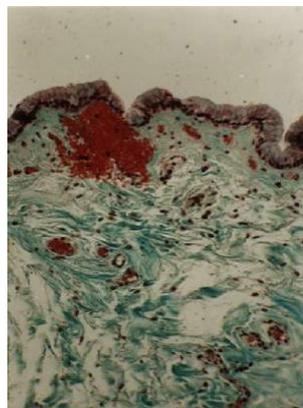
In other zones the process is more advanced in that the uterine mucous membrane epithelium is formed of one single layer of cubic and here and there prismatic cells, displayed on the basic membrane, without the existence of areas without cells, even of very small size (figure 2).

Figure 2. Epiteliul uterin în curs de refacere și organizare, ce acoperă complet membrana bazală. (col Goldner, 200 X)



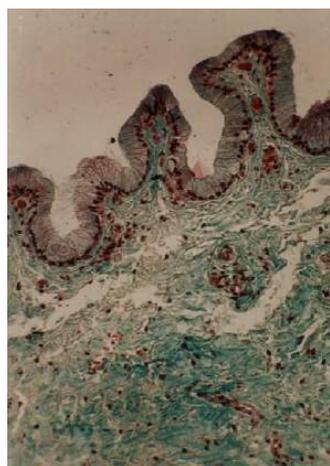
In other zones the uterine mucous membrane epithelium appears as something higher, being composed of prismatic cells; from place to place uterine glands in an initial stage of organisation, which occupy only a very thin area from the surface of the mucous membrane can be noticed (figure 3).

Figure 3. Stadiul incipient de organizare a glandelor uterine (col. Goldner, 200X)



In all the areas described so far a mutual phenomenon can be noticed, namely that the blood vessels, especially the ones in the superior third of the mucous membrane have the lumen full of red cells. On the sections of the uterine mucous membrane taken 7 days postpartum, the process of structural organisation is significantly more advanced than after 48 hours. The conjunctive stroma appears as better consolidated and the epithelium is formed of a layer of higher prismatic cells. The organisation process of uterine glands is more advanced, although it is far from its end (figure 4).-

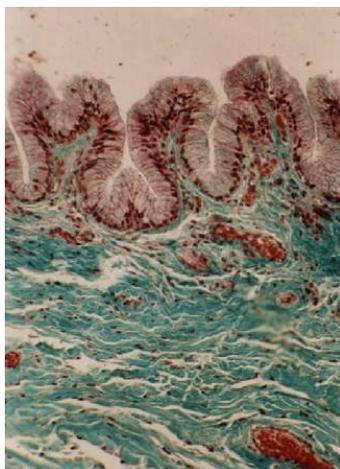
Figure 4. Aspectul epiteliului și al glandelor uterine la 7 zile postpartum (col. Goldner, 200X)



In several zones the uterine stroma has a denser structure (it is in a slightly more advanced stage of organisation) and its glands are longer, without

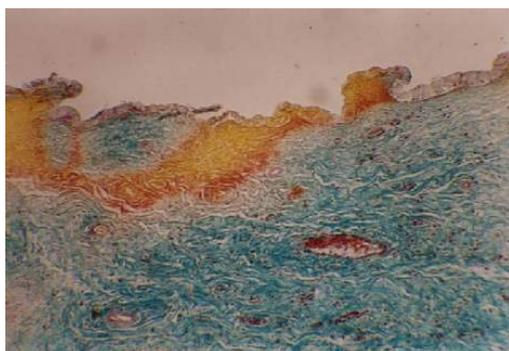
exceeding a superficial 1/3 of the mucous membrane (figure 5).

Figure 5. Consolidarea stromei uterine și organizarea glandelor la 7 zile postpartum (col. Goldner, 200X)



The uterine mucous membrane in endometritis appears as more modified compared to the normal state although there are sometimes quite big differences from one zone to the other one. Zones with superficial necrosis, which spread over the epithelium and the superficial corion, with a cauterizing aspect are to be noticed. The superficial zone of uterine stroma presents here and there a pronounced congestive hemorrhagic aspect, and the epithelium, which covers these zones looks detached under the form of lamboours (figure 6).

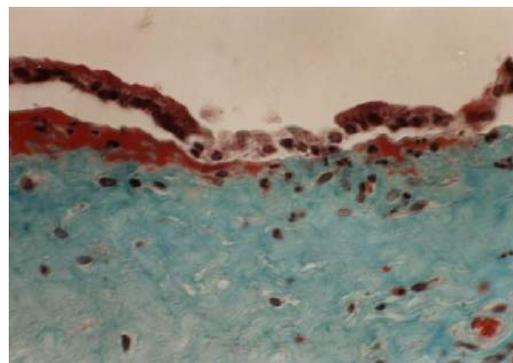
Figure 6. Necroza superficială a mucoasei uterine (col. Goldner, 200 X)



The elimination of the epithelium under the form of lamboours causes the appearance of the mucous

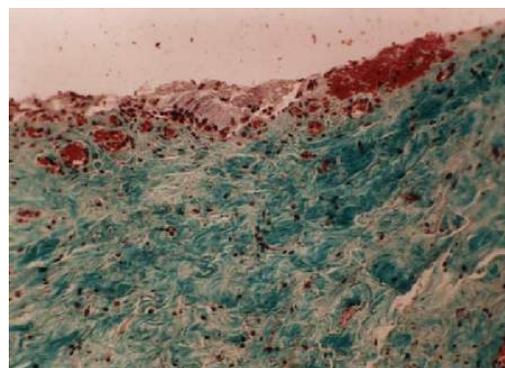
membrane as bereft on large surfaces and with hemorrhages in the superficial zone (figure 7).

Figure 7. Desprinderea epiteliului uterin pe zone întinse (col. Goldner, 200X)



The cellular and tissue leavings resulted as a consequence of necrotic processes can be noticed in the lumen of the organ under the form of big or small conglomerates. Here and there the mucous membrane is completely bereft even without traces of epithelium and the necrosis processes can be noticed on extended areas in the depth of the uterine mucous membrane (figure 8).

Figure 8. Denudarea zonală a mucoasei uterine (col Goldner, 200 X)



CONCLUSIONS AND RECOMMENDATIONS

A.1. The microbial flora from the puerperal uterus varies and is represented both by bacteria admitted as pathogenous and particular tropism for uterine morphological structures and oportunist bacteria, which are quite numerous.

A.2. From the microbial agents identified in the gathered samples both a 50% monomicrobial flora and a 50% polymicrobial flora with associations of two or more than two species have been emphasized.

B.1. The gathering of uterine mucous membrane biopsies with the help of Popescu, Boitor, Bogdan apparatus assure a drawing corresponding to the preserving of all the structural components at the time of the gathering.

B.2. The microscopic examination of the dynamically gathered biopsies allowed the watching of the recovering and organisation processes of the uterine mucous membrane for a period of up to 21 days postpartum.

In the case of gathered biopsies from cows suffering from endometritis, the necrotic processes in development 3-4 days postpartum can be watched from different zones of the uterine mucous membrane.

REFERENCES

- [1] ACIOCARLANOIE V., GROZA V., SUCIU A., CARMEN GUGA, COSTIN L., ICLOVAN F., OROS C. - 1989 - Observations concerning the use of colostrum and placental extract for the treatment of inflammatory injuries in cow. In "Simp" Present problems in amelioration and running of the farm animals. The pathology of Reproduction.
- [2] ADAMESTEANU I. - 1974 - The Yathropathies in Veterinary Medicine. Ed. Ceres, Bucuresti.
- [3] BARBU T., RUS I. - 1980 - New results in the control of the repeated hearts syndrome in cows. Rev. de cresterea animalelor., Bucuresti 30, nr.2, 39-42.
- [4] BERKECZI P. - 1980 - The treatment of endometritis and gynecological injuries in cows during all seasons. Rev. de cresterea animalelor, Bucuresti, 30, nr.5, 36-39.
- [5] BOGDAN A.T., BISTRICEANU M., MAJUNA C. - 1981 - The Reproduction of Farm Animal. Ed. Scrisul Românesc, Craiova.
- [6] BOGDAN INGEBORG - 1973 - Researches concerning therapy of endometritis in intensive growing cows.
- [7] BOITOR I., SOLTUZ V., PARAIPAN V., MUNTEAN M., BOGDAN A.T. - 1969 - Researches concerning the electrotherapy in some gynecological injuries in cow. Lucr. St. IA Cluj-Napoca, Seria Med. Vet. si Zoot., p.135.
- [8] BOITOR I., MUNTEAN M., MATES N., MOISE D., MARIA BOITOR - 1981 - Some aspects of the prophylaxis and control of main reproduction problems in cows. Rev. de cresterea animalelor., nr.23-28.
- [9] BOITOR I. - 1984 - The normal and pathological puerperium in farm animals. Ed. Ceres, Bucuresti.
- [10] BOITOR I. - 1988 - Tropical interest and prospect in biology and pathology of reproduction in bovine. Tipo Agronomia Cluj-Napoca.
- [11] BOITOR I., MUNTEAN M., GROZA I., MUSCA M., MOISE D. - 1988 - Observations concerning the efficiency of therapy with medicine associations of the endometritis in cow. Prod. Anim. Rev. Zoot. si Med. Vet., 38, nr.9, p.15-18.
- [12] BONCA GH., CERNESCU H., ARDELEAN V., BEDROSIAN ED., JOST A., FILIMON L. - 1988 - Preliminary datum concerning the efficiency of Germisan in therapy of endometritis in cow. Rev. Zoot. si Med. Vet., nr.9, p.19-23.
- [13] BONCA GH., CERNESCU H., MIRCUC C., ARDELEAN V. - 1992 - Researches concerning the therapy with antiseptics and sintetic analogues of prostaglandine in endometritis in cow. Rev. Româna de Medicina Veterinara, vol.2, nr.3-4, p.258-261.
- [14] CERNESCU H. - 1989 - The pathology of Reproduction and Obstetrical Clinic. Curs Lito IA Timisoara, vol.1.
- [15] CERNESCU H. - 1995 - Veterinary Gynecology. Ed. Helicon, Timisoara.
- [16] DAVID C. - 1987 - Decompensated reproduction metritis and chronic endometritis.

- Preluc.dupa Selez.vet.5,p.747-754 în Buletin informativ al SMVR nr.20, p.32-34.
- [17] DAWSON F. 1959 - Bovine endometritis: a review. Br.Rev.Annat.,5, p.73-79.
- [18] DECUN M.,ROSU MARIA - 1973 - Researches concerning microflora from normal and pathological cervical secretions in bovine. Rev.de Zoot.si Med.Vet.,nr.1,p.39-43.
- [19] DE COSTER -1 977 - L'etude histologique des en dometritis infections nespecificque chez les bovines. Ann.Med.Vet.,Bruxelles, 21,nr.7,p.491-501.
- [20] DOLEZEL R.,GROCH L.,KUDLAK E. - 1991 - Microscopic and biochemical changes of intercarencular uterin wall in first day after parturition. In Simp. "Topical interest in biology and pathology of reproduction. Cluj-Napoca,p.41-47.
- [21] GHERGARIU S. - 1981 - The metabolical profile in cow - clinical significance in infertility. Rev.de Cres.Anim., nr.2, p.28-35.
- [22] GROZA I.,MUNTEAN M. - 1998 - Elemente de fiziologia reproductiei la animale. Ed.Genesis, Tipo Cluj-Napoca.
- [23] 23.GROZA I.,MORAR I.,MOISE D.,BOGDAN L.,COCU F. - 1996- Researches concerning the use of PGF2alfa in therapy of reproduction disorders in cow. . Buletin USAMV, vol.50, p.141-147
- [24] MOCANU I. - 1985 -Aspects of incidence , evolution aand treatment results of the endometritis in cow. Simp. Reproduction and Pathoplogy of farm animals".vol.X, Cluj-Napoca
- [25] POPESCU P. - 1966 - The infertility and sterility brought about uterine disorders in cow. Rev.Zoot.si Med.Vet.,nr.4,p.14.
- [26] RAPUNTEAN GH, POP M.,MARICA D.,VASIU C.,POP I.,SPINU O. (1994) - A study about some lactozo-negativ strain of E.coli isolated from animals: variation of biochemical antigenical and pathogenical charachters.
- [27] Bul.USACN,-ZMV,48,290-293.

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