

STEROID HORMONES DOSING FROM SERUM, MILK AND BOVINE TISSUES FATED TO SACRIFICE AND THEIR IMPORTANCE ON HUMAN HEALTH

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Abstract. The researches were made on 45 subjects belonging to bovine and respectively buffalo species of both sexes and being dedicated to sacrifice. We drew samples of blood, milk, muscle, fat tissue, liver and kidney, from which we determined the level of the following steroid hormones: estradiol 17 β , progesterone and testosterone. The dosage were made by electrochemiluminescence technique based on the competition principle.

The utilization as analit of supernatant resulted after the ultrasonication and metanol and water dilution of tissue samples pointed out different data concerning the steroid hormones concentrations in liver, renal and muscle tissue samples. The fat tissue samples became solid in tubes, their interpretation being impossible.

High levels of estrogen hormones were identified in 15,55% of the cases (8,88% cows with cyst degeneration of the ovaries and 6,67% cows in the estral phase of the sexual cycle), respectively high levels of progesterone in 4,44% of cases.

The official data of Sanitary Statistics and Planning Center points out a raise of the neoplastic affections incidence from the genital zone, representing 23,89%.

INTRODUCTION

During the sexual life of the females and males there are moments when the plasmatic level of the steroid hormones reaches high values, which determines the diffusion in milk and also in the tissues of these hormones. The steroid hormones and their synthetic analogues are frequently used in the therapy of different reproduction disturbances, to manipulate the oestrus cycle and to develop (increase) the neovascular mass and to improve the carcass quality.

The European legislation recommends the exclusion from the public consumption of animal products that contain high levels of steroid hormones (67/548 CEE norm, regarding the classification of dangerous substances, 2001). The main aim of this work is to evaluate the sexual steroid hormones levels (17 β estradiol, progesterone, testosterone) from serum, milk, organs and tissues from bovines and buffalo, male and female fated to sacrifice from three districts: Cluj, Maramures and Alba. In addition, we want to establish the different reproduction disturbances, the level of steroidian tissue impregnation and the influence on consumer's health.

MATERIAL AND METHOD

The researches were made between May 2006 and April 2007, on 45 bovines and buffalo, males and females, 15 for each district.

The gathering of the biological samples (serum and milk) was preceded by the identification and clinical examination of each subject. The blood samples gathering was

made from the arterial ventral coccigian sinus, using vacutainer type tubes without anticoagulant. For drawing the milk sample, we used sterile and dry tubes. The first milk was removed. After the animal sacrifice we drew tissue and organ samples as follows: liver – 20 gram, kidney – 20 gram, muscle – 20 gram, adipose tissue – 20 gram.

The muscular tissue samples were gathered from the ventral abdominal level. The fat tissue was gathered from the adipose tissue accumulation of renal locus. The samples were packed separately and were numbered with the UE identification number of each subject. The technology used to draw the samples was the immunoassay by electrochemiluminescence (ECL). ECL is an immune dosing method based on luminescence and the mark is made with a light generating substance called luminifer. The luminescence term defines the light emission (photon), because of electron return from the energetic high levels to the stable low levels. The necessary energy to the luminescence can be generated as a result of some chemical reactions (chemiluminescence) an electrical stimulation (electrochemiluminescence) or some biological processes (bioluminescence).

The processing of the samples was made by 2 techniques:

- the first technique was used for the liver and kidneys; these were made small pieces until the complete destruction of the tissue. The paste so formed was processed with a mixture of metanol and water, in different dilutions which were immediately examined using ECL. We determined the dilutions corresponding to the interest steroid hormones reading and we established as optimum mixture volumes of 1:4 metanol/water in μl . The examination of fat, carbohydrates and proteins were made by heating, liquid-liquid extraction and solid phase extraction;
- the second technique was used for the muscle and adipose tissue samples. This technique was frequently used for the malignant tumours processing. After being weighted and macroscopically evaluated, the sample was made small pieces mechanically, until we obtained a homogenous cellular mass, which was then treated with metanol and water. All the samples were transferred in 2 ml Eppendorf tubes. The tubes were vortexed at high speed for 30 seconds after adding metanol and water. The supernatant was extracted and used in ECL determination. The samples which created problems in processing and determination were those resulted from the adipose tissue, their reading being impossible because of the solidification in tubes.

RESULTS AND DISCUSSIONS

The results obtained after the determinations were expressed in pmol/L in the case of estradiol and in nmol/L in the case of progesterone, and testosterone. Frequently, in specialized literature the 17β estradiol values are expressed in pg/ml, and those of progesterone and testosterone in $\mu\text{g/ml}$. In order to spread this information among the specialists we transformed the units of measure as follows: 17β estradiol: $\text{pmol} \times 0,273 = \text{pg/ml}$; progesterone: $\text{nmol} \times 0,314 = \text{ng/ml}$; testosterone: $\text{nmol} \times 0,288 = \text{ng}$.

Registration/sex	Clinical diagnosis	Samples				
		Serum pg/ml	Milk pg/ml	Liver pg/ml	Kidney pg/ml	Muscle pg/ml
0101(F)	Regressive CL.; Graaf Fol;	39,874	46,248	33,387	64,646	23,535
9352(F)	Regressive CL.; Graaf Fol;	29,211	27,081	24,627	29,238	5,520
9937(F)	Hemorrhagic CL.;Cav. Fol;	26,876	12,478	32,613	41,321	17,413
9921(F)	Cav fol.,right ov.;	18,432	11,523	36,199	39,621	18,738
9394(F)	Cav. fol., right ov.	31,176	-	65,721	16,355	7,723
4441(F)	Thecal fol.cysts;	62,462	-	74,317	79,468	21,41
2622(F)	Thecal fol.cysts;	29,429	146,409	148,075	94,376	15,124
2632(F)	Increase fol., left ov.;	23,242	134,370	110,07	124,06	43,21
2633(F)	Graaf fol., right ov.;	32,814	141,878	120,61	96,945	23,41
9926(F)	Cav. Fol., right ov.;	25,757	102,102	463,827	338,793	21,326
6981(F)	Uterine hipotrophy, first degree;	0,719	-	0,312	0,415	0,021
6993(M)	Clinically healthy;	0,374	-	0,165	0,072	0,0103
9934(F)	CL.,right ov.;	0,176	0,169	0,231	0,341	0,013
9344(F)	Hemorrhagic CL.,left ov.;	2,831	2,866	0,955	0,625	0,890
9930(F)	CL., right ov.;	2,784	1,256	3,473	4,513	0,951
6992(M)	Clinically healthy;	0,311	-	0,152	0,0616	0,236
6994(M)	Clinically healthy;	0,239	-	0,236	0,148	0,099
7732(M)	Clinically healthy;	0,589	-	0,144	0,093	0,058
6484(M)	Clinically healthy;	0,038	-	0,104	0,011	0,016
9395(M)	Clinically healthy;	0,236	-	0,495	0,075	0,049
6662(M)	Clinically healthy;	0,044	-	0,099	0,078	0,049
6991(M)	Clinically healthy;	0,038	-	0,221	0,058	0,047
0312(M)	Clinically healthy;	0,033	-	0,057	0,048	0,019
6986(M)	Clinically healthy;	0,037	-	0,147	0,071	0,046
6988(M)	Clinically healthy;	0,026	-	0,066	0,064	0,068
6943(M)	Clinicly healthy;	0,037	-	0,068	0,117	0,029
6936(M)	Clinically healthy;	0,033	-	0,099	0,044	0,061
6983(M)	Clinically healthy;	0,094	-	0,130	0,075	0,052
2415(F)	Pregnant, 45 days;	1,746	3,336	5,053	1,787	0,390
4257(F)	CL., right ov.;	2,312	3,341	6,120	1,649	2,271
4259(F)	Cav. fol., right ov.;	26,92	16,478	37,41	37,41	7,422
8700(F)	CL., left ov.;	3,862	4,432	3,158	3,158	2,282
0298(F)	Fol. left ov.;	27,027	56,57	76,433	76,433	12,810
2735(F)	CL. right ov.;	2,690	5,045	4,482	4,482	2,541
7504(F)	Cavitary CL.;	0,467	-	0,174	0,174	0,093
2801(F)	Hemorrhagic CL.;	5,031	2,831	2,574	2,574	1,696
9648(F)	Pregnanat, 45 days;	1,213	2,665	2,023	2,023	1,149
6987(M)	Clinically healthy;	0,386	-	0,128	0,128	0,085
9920(F)	CL, right ov.;	0,405	1,021	0,336	0,336	0,224
9906(F)	Regressive CL.;	0,877	-	0,816	0,816	0,492
2627(F)	Ut.-ov. Hipotrophy, first degree;	0,677	-	0,932	0,932	0,440
9396(F)	Thecal fol. cysts;	66,147	-	9,363	9,363	3,303
4669(F)	Graaf fol., right ov.;	50,395	-	72,781	72,781	25,318
3660(F)	CL., left ov.;	2,631	-	4,182	4,182	0,369
2307(F)	Thecal fol. cysts;	68,168	-	151,023	151,023	50,068

Tabel no.1- The estradiol values in serum, milk and tissues from bovines and buffalos;

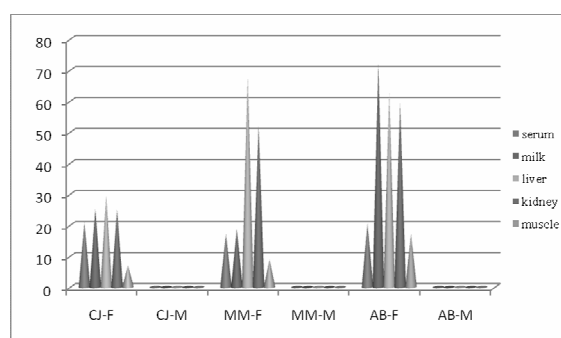
Analyzing the data in table 1, we point an important variation of the seric level of estradiol with values between 0,176 pg/ml respectively 68,168 pg/ml, in the case of female subjects. The highest values were recorded in the case of cows diagnosed with cystical ovary degeneration (a number of 4, respectively 8,88% from the total cases examined); also we identified 3 cases in the oestrus phase (representing 6,67% from the total cases examined), with the estradiol values superior to 30 pg/ml.

The thecal cystical degeneration of ovaries represents a disturbance of the reproduction function, in whose ethiology are involved genetic factors (a recessive gene with incomplete penetration) and also predisposing factors (hiperproteic alimentation, low concentrations of β caroten and vitamin A, altered fodder and hormonal equilibrium modification due to phitoestrogens). The internal of the cysts secretes important quantities of estrogen hormones for a long time, wich determines a powerful steroidian impregnation of the tissue. In the case of the male subjects, the plasmatic estradiol levels are subunitary. The estrogen hormones levels supervision in males is necessary, because the natural estrogen hormones and their synthetical analogues such as: the dietilstilbestrol (DES) and diacetatul de hexestral (DHS) are frequently used for the growth stimulation and for improving the qualitative parameters of the carcasa of the young bulls.

Analyzing the diffusion capacity in tissues of estradiol, maximum values were recorded in milk (38,770 pg/ml), then in liver (34,152 pg/ml), kidneys (29,911 pg/ml), the lowest levels being identified in the muscular tissue (6,927 μ g/ml).

	Serum (pg/ml)	Milk (pg/ml)	Liver (pg/ml)	Kidney (pg/ml)	Muscle (pg/ml)
Males					
Cluj	0,425	-	0,154	0,076	0,102
Maramureş	0,129	-	0,207	0,086	0,049
Alba	0,086	-	0,134	0,071	0,054
Females					
Cluj	20,899	25,260	29,565	25,123	7,008
Maramureş	17,178	18,774	67,871	52,219	8,918
Alba	20,475	72,312	62,615	59,523	17,110
Animales					
Cluj	16,804	25,260	23,683	20,114	5,627
Maramureş	11,495	-	45,316	34,841	5,962
Alba	10,960	-	33,457	31,778	9,151

Table no.2 - 17β estradiol comparative medium values at the animals from Cluj, Maramureş and Alba counties



Graphic 1 – 17β estradiol medium values comparative representation at bovines and buffalos, males and females, in Cluj, Maramureş and Alba districts

The analysis of comparative data, points superior medium values of 17β estradiol, in the case of female bovines and buffalos from Maramures and Alba districts, in the liver and kidney samples, fact explained by a raised frequency of the cases of ovaries cronical cystical degeneration, caused by a change in the hormonal equilibrium, duet o fito and micoestrogens from the fodder gathered in unfavorable pedoclimatic conditions (excessive rain), in the previous year. The progesterone level presented in table 2, points out physiological levelevels of this hormonal parametre in the samples examined in the laboratory. However after the clinical exam, made in the case of each subject before being sacrificed and on the genital examination after sacrificiation were indentified two bovines in an incipient pregnancy (pregnancy of 45 and 40 days). These two cases showed high levels of progesterone and an important impregnation of the analysed tissues.

Although the romanian legislation stipulates the compulsoriness of the pregnancy diagnosis specification onley at 90 days from the covering or the artificial insemination, the pregnancy, even in an incipient phase implies the significative growth of the progesterone in serum, milk and tissues, fact that influences the hormonal equilibrium of tha consumers of products from these animals, creating the premise of some disturbances of the endocrine status, with serous consequences on their health.

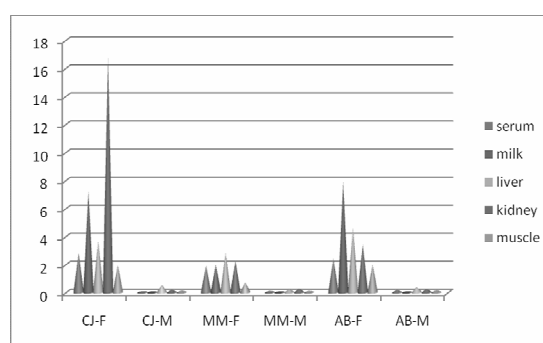
Registration /sex	Clinical Diagnosis	SAMPLES				
		Serum (pg/ml)	Milk (pg/ml)	Liver (pg/ml)	Kidney (pg/ml)	Muscle (pg/ml)
0101(F)	Regressive CL; Graaf fol;	4,631	6,431	5,446	4,912	1,46
9352(F)	Regressive CL.; Graaf fol;	0,737	2,791	3,231	1,695	12,553
9937(F)	Hemorrhagic CL; Cav. fol.;	3,849	1,905	3,992	4,431	0,632
9921(F)	Cav. fol., right ov.;	0,408	0,383	3,271	1,607	0,200
9394(F)	Cav fol., left ov.;	1,905	-	1,428	0,839	1,117
4441(F)	Thecal fol. cysts;	0,236	-	0,314	0,512	0,041
2622(F)	Thecal fol.cysts;	0,267	0,791	4,505	2,429	0,082
2632(F)	Increase fol., left ov.;	0,260	0,788	0,444	0,561	0,131
2633(F)	Graaf fol., right ov.;	0,300	0,671	0,218	0,431	0,049
9926(F)	Cav. fol., right ov.;	0,420	0,370	6,044	3,001	0,311
6981(F)	Ut.-ov.hipotrophy,gr.I	0,053		0,013	0,028	0,317
6993 (M)	Clinically healthy;	0,103	4,78	1,488	0,508	0,201
9934(F)	CL., right ov.;	3,21	2,863	4,61	3,96	0,873
9344(F)	Hemorrhagic CL., left ov.;	1,896	2,468	1,306	2,681	0,053
9930(F)	CL., right ov.;	3,786	-	3,612	2,43	1,889
6992 (M)	Clinically healthy;	0,071	-	0,082	0,044	0,192
6994 (M)	Clinically healthy;	0,120	-	0,150	0,074	0,092
7732 (M)	Clinically healthy;	0,064	-	0,094	0,46	0,028
6484 (M)	Clinically healthy;	0,124	-	0,099	0,057	0,048
9395 (M)	Clinically healthy;	0,134	-	0,122	0,113	0,055
6662 (M)	Clinically healthy;	0,822	-	0,931	0,505	0,283
6991 (M)	Clinically healthy;	0,091	-	0,211	0,203	0,123
0312 (M)	Clinically healthy;	0,048	-	0,096	0,048	0,037
6986 (M)	Clinically healthy;	0,110	-	0,167	0,073	0,95
6988 (M)	Clinically healthy;	0,123	-	0,075	0,536	0,461
6943 (M)	Clinically healthy;	0,092	-	0,574	0,671	0,173
6936 (M)	Clinically healthy;	0,0649	-	1,343	0,452	0,164
6983 (M)	Clinically healthy;	0,103	-	0,190	0,574	0,203
2415(F)	Pregnant, 45 days;	7,859	16,397	7,099	6,424	4,631
4257(F)	CL., right ov.;	3,403	13,363	18,739	0,029	3,731
4259(F)	Cav. fol., right ov.,	0,257	0,230	0,164	0,116	0,276
8700(F)	CL., left ov.;	9,402	16,420	3,257	6,733	1,454
0298(F)	Fol. left ov.;	0,115	0,892	0,436	0,203	0,116
2735(F)	CL.right ov.;	7,204	25,315	17,40	13,001	7,41
7504(F)	Cavitarry CL.;	4,783	-	5,642	2,62	2,413
2801(F)	Hemorrhagic CL.;	1,872	3,301	4,216	2,693	2,198
9648(F)	Pregnant, 45 days;	7,533	10,243	4,281	2,224	0,239
6987 (M)	Clinically healthy;	0,036	-	0,021	0,013	0,003
9920(F)	CL., right ov.;	1,973	3,159	2,459	1,871	2,003
9906(F)	Regressive CL.;	1,491	-	1,683	1,197	0,221
2627(F)	Ut.-ov. hipotrophy., gr.I;	0,946	-	1,957	0,500	0,485
9396(F)	Thecal fol. cysts;	0,321	-	0,095	0,185	0,074
4669(F)	Graaf fol., right ov.;	0,389	-	0,650	0,126	0,471
3660(F)	CL., left ov.;	20,241	-	2,258	1,298	0,459
2307(F)	Thecal fol cysts;	0,641	-	1,250	1,429	1,006

Table no.3- Progesterone levels in semen, milk and tissues from bovines and buffalos;

The medium values of progesterone in the case of each group points out the raised values of this hormone in milk (5,688 ng/ml), liver (2,544 ng/ml), kidney (1,647 ng/ml) and muscle (1,050 ng/ml).

	Serum (pg/ml)	Milk (pg/ml)	Liver (pg/ml)	Kidney (pg/ml)	Muscle (pg/ml)
Males					
Cluj	0,080	-	0,555	0,200	0,141
Maramureş	0,115	-	0,227	0,298	0,115
Alba	0,185	-	0,407	0,262	0,166
Females					
Cluj	2,822	7,196	3,685	16,81	1,964
Maramureş	1,918	1,990	2,872	2,25	0,734
Alba	2,419	7,878	4,637	3,434	1,999
Animals					
Cluj	2,274	-	3,059	1,385	1,599
Maramureş	1,317	-	1,991	1,602	0,528
Alba	1,377	-	2,663	1,954	1,144

Table no.3 – Progesterone comparative medium values at the animals from Cluj, Maramureş and Alba districts



Graphic 2- Progesterone medium values comparative representation at bovines and buffalos, males and females in Cluj, Maramureş and Alba districts;

The testosterone registers subunitary values in the case of female subjects, values considered negligible from the point of view of the impact on different categories of consumers. In the young bulls of different ages, the testosterone level varies significantly from subunitary levels to values superior to 15 ng/ml. The establishing of the maximum admitted levels of this hormone in the young bulls body is still a disputed subject among the endocrinologists, and there are therapeutic means capable to induce a growth of the testosterone endogenous synthesis.

Registration /sex	Clinical Diagnosis	SAMPLES				
		Serum(pg/ ml)	Milk (pg/ml)	Liver(pg/ml)	Kidney(pg/ml)	Muscle(pg/ml)
0101(F)	Regressive CL.; Graaf fol;	0,445	0,541	0,332	0,185	0,048
9352(F)	Regressive CL.; Graaf fol.;	0,427	5,108	3,190	2,326	2,042
9937(F)	Hemorrhagic CL Cav.fol.;	0,031	1,695	0,435	0,283	0,043
9921(F)	Cav. fol., right ov.;	0,053	1,353	7,878	3,768	0,348
9394(F)	Cav. fol.,left ov.;	0,146	-	3,913	2,515	2,125
4441(F)	Thecal fol. cysts;	0,176	-	0,158	0,287	0,041
2622(F)	Thecal fol. cysts;	0,139	1,855	11,424	2,408	0,637
2632(F)	Increase fol., left ov.;	0,148	2,047	0,841	0,449	0,133
2633(F)	Graaf fol., right ov.;	0,066	0,157	0,113	0,083	0,041
9926(F)	Cav. fol., right ov.;	0,078	0,860	1,234	0,963	0,430
6981(F)	Ut.-ov. hipotrophy, first degree;	0,395	-	0,694	0,783	0,104
6993(M)	Clinically healthy;	0,400	-	0,336	0,270	0,135
9934(F)	CL., right ov.;	0,69	0,153	0,082	0,102	0,001
9344(F)	Hemorrhagic CL., left ov.;	0,142	0,365	0,197	0,167	0,164
9930(F)	CL., right ov.;	0,029	0,167	0,039	0,281	0,042
6992(M)	Clinically healthy;	1,865	-	1,990	6,166	0,709
6994(M)	Clinically healthy;	0,708	-	1,783	1,361	0,993
7732(M)	Clinically healthy;	0,938	-	2,182	1,686	1,024
6484(M)	Clinically healthy;	16,343	-	3,318	16,342	9,642
9395(M)	Clinically healthy;	1,086	-	2,979	1,742	3,733
6662(M)	Clinically healthy;	0,891	-	2,339	3,592	2,434
6991(M)	Clinically healthy;	1,634	-	3,538	1,670	1,273
6984(M)	Clinically healthy;	1,537	-	2,962	2,031	1,543
6988(M)	Clinically healthy;	2,192	-	1,905	1,679	3,256
6943 (M)	Clinically healthy;	1,634	-	1,394	3,403	0,813

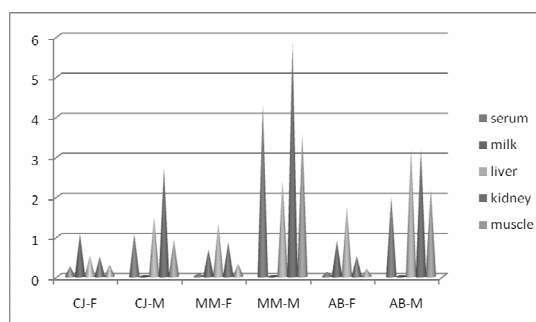
6936 (M)	Clinically healthy;	1,871	-	2,923	6,229	0,565
6983 (M)	Clinically healthy;	1,672	-	3,340	6,295	2,677
0312 (M)	Clinically healthy;	1,483	-	2,698	2,413	0,993
2415(F)	Pregnant,45 days;	0,293	0,636	0,379	0,481	0,365
4257(F)	CL., right ov.;	0,202	0,051	0,043	0,175	0,264
4259(F)	Cav. Fol., right ov.;	0,552	0,834	0,658	0,967	0,314
8700(F)	CL., left ov.;	0,509	0,256	0,302	0,368	0,163
0298(F)	Fol. left ov.;	0,074	0,225	0,314	0,201	0,134
2735(F)	CL, right ov.;	0,032	0,081	0,109	0,34	0,0213
7504(F)	CL.,cavitary;	0,5	-	0,083	0,023	0,031
2801(F)	Hemorrhagic CL.;	0,148	0,606	0,428	0,248	0,196
9648(F)	Pregnant, 45 days;	0,216	0,444	0,187	0,123	0,243
6987 (M)	Clinically healthy;	4,323	-	6,42	4,748	5,232
9920(F)	CL., right ov.;	0,047	0,113	0,088	0,045	0,02147
9906(F)	Regressive CL.;	0,098	-	0,196	0,186	0,054
2627(F)	Ut.-ov. hipotrophy, first degree;	0,041	-	0,324	0,253	0,075
9396(F)	Thecal fol. cysts;	0,042	-	0,078	0,019	0,024
4669(F)	Graaf fol., right ov.;	0,053	-	0,048	0,248	0,123
3660(F)	CL., left ov.;	0,166	-	0,324	0,602	0,124
2307(F)	Thecal fol. cysts;	0,143	-	0,304	0,165	0,093

Table no.5- Testosterone values in serum, milk and tissues from bovines and buffalos;

Medium testosterone values point out the same tropism for fat rich tissues of the steroid hormones.

	Serum (pg/ml)	Milk (pg/ml)	Liver (pg/ml)	Kidney (pg/ml)	Muscle (pg/ml)
Males					
Cluj	1,067	-	1,502	2,707	0,922
Maramureş	4,288	-	2,403	5,828	3,571
Alba	1,99	-	3,201	3,194	2,185
Females					
Cluj	0,261	1,065	0,521	0,501	0,301
Maramureş	0,086	0,672	1,322	0,859	0,326
Alba	0,126	0,897	1,74	0,515	0,195
Animals					
Cluj	0,261	1,065	0,521	0,501	0,301
Maramureş	1,487	-	1,682	2,516	1,407
Alba	0,996	-	2,421	1,765	1,123

Table no. 6- Testosterone comparative medium values at the animals from Cluj, Maramureş and Alba districts;



Graphic no. 3- Coparative representation of the testosterone medium values at males and females, bovines and buffalos in Cluj, Maramureş and Alba districts;

Analyzing the testosterone diffusion table, we observe a pronounced tropism of this hormone for the renal, hepatic and muscular tissue, maximum values being registered in the case of young bulls from Maramures district. The official data of the Sanitary Statistics and Estimation Centr points out a growth of the neoplastic affections incidence from the genitals, representing 23,89% (5813 cases) from the total neoplastic lesions of the woman in Romania. Among these, the cervix cancer represents 56,2% being followed by the ovarian cancer 22,5% and by the uterus cancer 16,7%. As the distribution of these affections in the domain studied, the cervix cancer was diagnosed in a percentage of 8,71% from the total cancer cases from Cluj district, 6,75% in Maramures and 4,69% in Alba district.

The breast cancer had an incidence of 12,47% from the total cancer cases from Cluj district, 11,17% from Alba district and 9,97% from Maramures district.

The maximum frequency of ovarian cancer was recorded in Maramures district with a 3,22% percentage from the total neoplastic cases diagnosed, followed by Alba district with 2,43% and Cluj district with 1,92%.

CONCLUSIONS

As a result of the clinical exam and of the hormonal determinations, we draw the following conclusions:

1. the 17β estradiol level registered superior value of 30pg/ml in 15,55% of cases (4 cases of cystical degeneration of ovaries and 3 in the oestrus phase). The literature notices these values as risk factors, for some consumers categories (boys aged 8-12 years);
2. 2 cases of pregnancy were identified, representing 4,44% from the total studied cases, in incipient phase (40, respectively 45 days) at which there were identified high levels of seric progesterone and an important gestagenic impregnation of analysed tissues;
3. in the case of females, the testosterone level registered subunitary levels, considered to be negligible from the point of view of their influence on the consumers health;
4. in the case of male subjects, there is an important variation of the androgen hormones level; the endocrinologists did not reach to an agreement regarding the maximum admitted level of the natural hormone; so we recommend supplementary exams in order to identify some hormonal substances (HCG), capable to induce an important growth of testosterone synthesis;
5. the ECL method of steroid hormones represents a convenient alternative to the gas-chromatographic and immunoenzyme techniques utilized in some laboratories from Romania;
6. in the case of fat tissues samples, the inconvenient consists in the solidification of the samples considered analysed; a solution for this technical difficulty could be represented by a dehydrogenation reaction of stearic and palmitic acids in fat;
7. we consider convenient a set of measures which form the supervision plan of animal origin products quality, the compulsory determination of hormonal residues from milk and tissues.

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