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THE EFFECTIVENESS ASSESMENT OF ANTAGONIST OPIOID – NALTREXONE IN COW INVOLUTION AFTER CALVING PERIOD

Xhavit F. Bytyçi

University of Agriculture and Veterinary, Prishtina
Str. "Skënderbeu" p.n.31000 Istog, Republic of Kosova;
Mob: +377 44 220 142
xhap2002@yahoo.com

Abstract

The main purpose of this study is to prove the hypothesis of Endogen Opioidantagonists by using Naltrexone at one organ with a dominant catabolic process in Organism like the uterus involution after calving. The results of involution rate in cow's uterus during this study was confirmed by using the laboratory method through measuring the level of Hydroxyproline (HYP) in cow's blood as well as clinical method through rectal examination. In order to perform this study, three groups of cows were devided from the same breed, age and with normal calving characteristics: Group F- control group (6 cows), treated with physiological dissolution; Group K – experimental group (6 cows), which were treated with calcium and Group N- experimental group (6cows) treated with calcium and after one hour with Naltrexone. All three study groups were treated in the following days: 1^{st}, 3^{rd}, 5^{th}, 7^{th} and 9^{th} after calving.

The average level of Hydroxyproline (HYP) in cows that belonged to group N (experimental), treated with Naltrexone + Ca++, resulted with 1.09 μg/ml. against 0.98 μg/ml. In those cows belonging to group K (experimental group) treated only with calcium and 0.95 μg/ml to cows of group F (control group) treated only with physiological water. Up to 30 days after calving, the clinical involution of uterus was achieved in almost 83.84% of cows to the group that was treated with Naltrexone and 66.7% to cows that belonged to the other two groups that weren't treated with Naltrexone. We think that the fastest process of uterus involution in group N comes by a positive effect of Naltrexone in the entire body metabolism, especially in the uterus as well as catabolism of uterine collagen.

Keywords: cow, uterus, involution, opioid, antagonist-opioid, Naltrexone

Introduction

Involution or the anatomic diminution of cow's uterus is a biological process, very apparent in clinical aspect. The anatomic Involution, parallel to sleet and endometrial regeneration, aims to turn the genital apparatus and the overall organism of the animal to its earlier state before the pregnancy. The post-pregnancy period continues to be an intensive study field for many authors that is expanded in some plans; anatomic, histological, bacteriological, metabolic, endocrinology, etc. (*Olson et al.* 1986, *Taverne,* 1992, *Gustafson et al.* 2004, *Degefa et al.* 2006).

The foundation of our study case stands mainly on catabolic processes of cow's genital apparatus and intermediation of opioids and "anti-opioids" in these processes. The catabolic processes that are developed in the genital apparatus, mainly in the uterus consist of its main bio-chemical mechanism. The biochemistry of uterus after calving is dominated by increasing the activity of collagen enzymes. This is because collagen is a main constructive protein in Uterus that consists about 25% of its dry substance.

Collagen fibres, by extracting the collagens and other proteins enable to decrease the uterus size up to 90% till 30 days after calving (*Boundurant,* 1999) or from about 9 kg weight falls to about 1 kg. (*Youngquist,* 1997, *Youngquis and Shore,* 1997, *Olson et al.* 1986, *Sali,* 1996, *Sulo,* 1990) etc. Thanks to Collagen fibres, the collagen is extracted to glycine, proline, hyrdoxyproline (HYP) and other peptides with small molecular diameter. Among the extracted products, HYP is the most important, which falls in the blood and throughout the urine is taken out (*Çela,* 2005, *Mane et al.* 2005, *Sulo,* 1990, *Breeveld-Dwarkasing et al.* 2003/a, *Breeveld-Dwarkasing et al.* 2003/b).

The Analyses and the HYP level in blood can be considered as an involutive matching indicator of uterus (*Robbe et al.* 1999). The highiest HYP value in the blood testifies for a faster involution of uterus (*Minoia et al.* 1994/ab, *Sciorsci et al.* 2000/ab).

Referring to the theoretical concept that the calving and post calving period makeup the organism also a strong stress at the same time, it gives the result that the endomorphs and calcium mediates also in activating the collagen fibres, extraction of collagen, endocrine activity, immunity, etc. in organism, thus in the normal or pathological development of the uterus involution. In many cases, the current high endomorph level also after the calving as well as the metabolic disorder of Ca++ can be a starting point for some pathologies after calving.

Concerning this issue, the literature emphasizes that treating the cow with calcium and Naltrexone may represent the first chosen therapy in order to normalize the metabolism of the animal after calving.

Based on these expositions and in edition of the third task, we raise the following experimental hypothesis: If the hormonal and enzymatic activity of uterus at involution phase depends from the normal concentration of calcium in uterine tissues and so on, then the high level of opioides after calving should be increased with a faster tempo as usual, throughout the treatment with Naltrexone - Ca ++, which would be represented with faster dissolution of collagen in uterus and showing the Hydroxyprole in animal's blood in bigger amounts that at those untreated ones..

Materials and methodology

In order to perform the third study case, we used 18 cows with normal calving from the same pen, race (Simental) and age (3 years old). They're split according to the occasional principle in three groups.

The first group or Group F (Physiologic Solution) (6 cows). Control:

Cows of this group were "treated" with physiological solution (NaCl 0.9%), 20 ml / 100 kg. weight), given the medicine orally in days 1, 3, 5, 7 and 9 after calving.

The second group or Group K (Proprietary Calcium) (6cows). Experiment:

Cows of this group were treated with pharmaceutical preparation (**Calcoral Forte-Gel)**, taken orally with 20 ml doses (2.7 g calcium) per 100 kg body weight in days 1, 3, 5 ,7 and 9 after calving.

The third Group or Group N (Naltrexone Tablet) (6 Cows). Experiment:

Cows of this group were treated initially with calcium, giving them **Calcoral**, 20 ml (2.7 gr. Calcium për 100 kg body weight) orally. One hour after treating them with calcoral, we treated them with Naltrexonee chlorhydrate (50 mg tablets), calculating 40 mg doses per 100 kg. live weight. This double treatment (Naltrexone + Ca^{++}) is done also in days 1, 3, 5, 7 and 9 after calving.

The blood is taken from all of three groups in days 1, 2, 4, 6, 8, 10, 12 and 14 after calving. The blood taken in the 1^{st} day has ensured the starting point analyses before experimental treatments. Up to the 10^{th} day after calving, the blood is taken 24 hours after each treatment. The blood is analysed for Hydroxyproline levels (HYP).

Meanwhile, the involution dynamics of uterus is followed through rectal examination in time periods between 18-20, 28-30, 38-40 and 56-60 days after calving.

We've decided to consider cows with completed involution, those to whom when their uterus draws in pelvix, when both horns of the uterus can be seized with hand palm whereas the horn diameter was gravid and should be 3-5 cm and with clear vaginal secretions. In practice, we proofed this when while holding the gravid horn, the tip of the first finger touches the tip of the thumb (5 cm) or when the tip of the first finger touches the articulation between the second and third falange (3.5 cm).

The main part of study result is processed with statistical analyses method. In this context, the arithmetic average was determined (M), the fault of arithmetic average (m), the criterion of different authenticities according to the Student (t) and Fisher (F). The level's authenticity of changes was given as follows: P = 0.05, P = 0.01, P = 0.001.

Results and discussions

Results, statistical analyse and discussion for levels of Hydroxiproline (HYP)

The results are given in *Table 1.*

***Table 1*. Values of Hydroxyproline in cows' blood of Group F (No. 6)**

No. of cows	**344**	**610**	**368**	**637**	**604**	**9580**	**Average**
Cypher	Pn- F/1	Pn-F/2	Pn-F/3	Pn-F/4	Pn-F/5	Pn-F/6	(μg/ml)
day 1 pp	0.97	1	0.96	1.08	1.07	0.98	1.01
day 2 pp	0.95	1.01	1	0.87	0.88	0.95	0.94
day 4 pp	0.9	0.9	0.85	0.97	0.85	0.95	0.90
day 6 pp	0.93	0.97	0.93	0.93	0.96	1.02	0.96
day 8 pp	1.06	1	0.93	0.97	0.95	1	0.99
day 10 pp	0.93	0.93	1.12	0.86	0.95	1.17	0.99
day 12 pp	1.07	0.86	0.9	0.97	0.86	0.86	0.92
day 14 pp	0.87	0.92	0.88	0.9	0.92	1	0.92
Average	0.96	0.95	0.95	0.94	0.93	0.99	0.95

The average HYP levels from one cow to the other one in group F (control group), varies between 0.93-0.99 μg/ml whereas by daily analyse to the next day analyse, they vary between 0.90-1.01 μg/ml. The character of this variation is clarified by the analyses shown in *Table 2*.

Table 2. **Hydroxyproline Variation analyse of Group F (No. 6)**

Source of change.	**Quadratic sum**	**Shkallët e lirisë**	**Quadratic average**	**F-calculated**	**F-critical**
Rows	0.05	6	0.01	1.97	2.51
Column	0.03	4	0.01	1.67	2.78
Fault	0.10	24	0.04		
Sum	0.18	34			

Table 2 shows the variation between days (rows) and the variation between cows (columns), according to Fisher. As it looks, the coefficient F-calculated (1.97) is smaller than the coefficient F-tabular or F–critical (2.51). It means that HYP variations from one day to the other one are not significant (statistically not proofed) (F.calc. 1.97 > F. critical 2.51).

HYP variations from one cow to the other one are not proved statistically, too (F.calc. 1.67 > 2.78). (It'd be significative if F- calculated would be bigger than F-critical).

Variations are noticed also in two other groups: K (calcium) and N (Naltrexone) that are also put into the variation analyse as shown below (*Table 3, 4, 5 and 6*).

Table 3. **Values of Hydroxyproline in cows' blood of Group K (No.6)**

No. of cows	**517**	**O52**	**6387**	**934**	**O97**	**347**	**Average**
Cypher	Pn-K/1	Pn-K/2	Pn-K/3	Pn-K/4	Pn-K/5	Pn-K/6	µg/ml
day 1 pp	1.1	0.86	1	1.2	0.98	1.17	1.05
day 2 pp	0.85	0.87	1.22	0.87	1.08	0.77	0.94
day 4 pp	0.92	0.88	0.87	0.87	1	0.81	0.89
day 6 pp	0.87	0.92	0.95	1.06	1.08	0.83	0.95
day 8 pp	0.85	1.03	1.25	1.02	1.22	0.87	1.04
day 10 pp	1.17	0.85	1.03	1.08	0.88	0.93	0.99
day 12 pp	0.93	0.97	0.85	0.92	1.27	0.92	0.98
day 14 pp	1.12	1.11	0.97	1	0.95	1	1.03
Average	0.98	0.94	1.02	1.00	1.06	0.91	0.98

***Table 4*. Hydroxyproline Variation analyse of Group K (Nr. 6)**

Source of change.	Quadratic sum	Shkallët e lirisë	Quadratic average	F-calculated	F-critical
Rows	0.12	7	0.02	1.15	2.29
Column	0.11	5	0.02	1.48	2.49
Fault	0.54	35	0.02		
Sum	0.78	47			

***Table 5*. Values of Hydroxyproline in cows' blood of Group N (No.6)**

No. of cows	579	188	496	o68	4221	O22	Average
Cypher	Pn-N/1	Pn-N/2	Pn-N/3	Pn-N/4	Pn-N/5	Pn-N/6	µg/ml
day 1 pp	0.8	1.13	0.92	1.08	1.12	1.08	1.02
day 2 pp	0.92	1.32	1.28	1.31	1.08	1.13	1.17
day 4 pp	1.25	1	1.01	0.97	1.02	1.08	1.06
day 6 pp	0.95	0.95	1.13	1.11	1.05	1.12	1.05
day 8 pp	1.18	1.13	1.18	1.07	1.06	1.22	1.14
day 10 pp	1.2	0.97	1.37	1.02	0.97	0.97	1.08
day 12 pp	1.12	1.02	1.07	1.11	1.25	1.27	1.14
day 14 pp	1.12	1.01	1.07	1.03	1.07	1.08	1.06
Average	1.07	1.07	1.13	1.09	1.08	1.12	1.09

***Table 6*. Hydroxyproline Variation analyse-Group N (No. 6)**

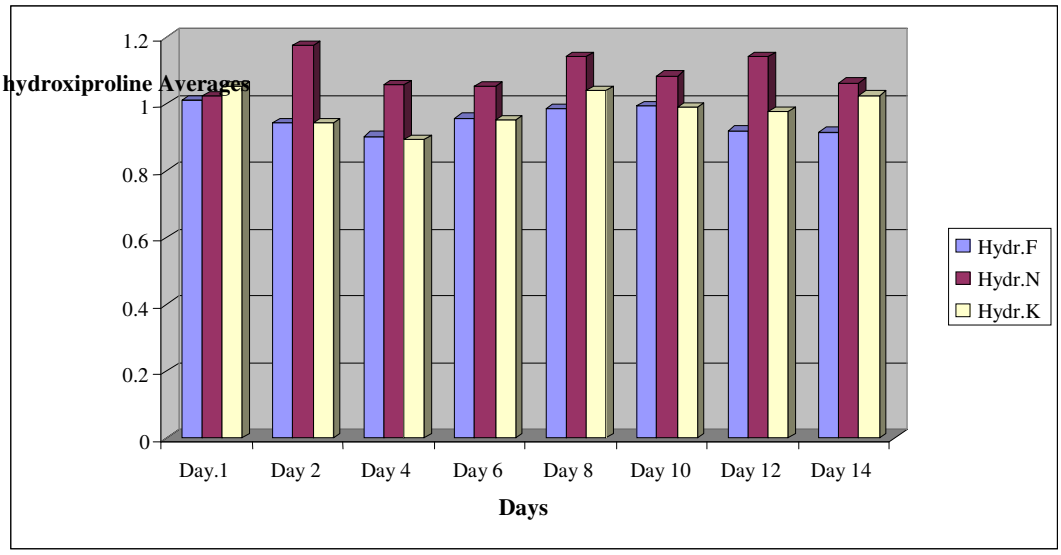
Source of change	Quadratic sum	Shkallët e lirisë	Quadratic average	F-calculated	F-critical
Rows	0.150	6	0.03	2.68	2.51
Column	0.048	4	0.01	1.27	2.78
Fault	0.225	24	0.01		
Sum	0.423	34			

Also the changes in group K are not proven statistically like between days (F-llog.1.15>F-critical 2.29), and also between cows of group (F-llog.1.48>F-critical 2.49).

Table 6 of variation analyse gives evidence about the variation of HYP levels from one day to the next day and the variations are dynamic or has increasing character that are statistically confirmed (F-calc. 2.68<F-critical 2.51). HYP level from day 2-14 after calving correspond with the time of treating the cows with Naltrexone, which vary from 1.05-1.17 µg/ml and it has never resulted as the first day level or less than the day before starting the treatment (1.02 µg/ml) as shown in Tab.5.6. HYP values in *Table 6* are similar with other authors, varying from 0.75-2.02 µg/ml (*Campanella,* 1998).

We think that this HYP addition in cow's blood of group N is a result of impact of study factor, meaning Naltrexone. This opinion is also confirmed by group F (control- without Naltrexone), where the level of HYP from day 2-14 after calving has never been increased over 1 µg/ml, as noticed in *Table 1.*

***Figure 1*. Graphical Dynamics of HYP average in groups F, N, K**



The metabolic fast normalization of Ca++ after calving surely had activated the dissolving mechanism of collagen in uterus (fibroblasts, leukocytes and their collagen fibres) for whom we talked in the front side of this study.

We see here the "key" of HYP addition in cow's blood of group N, emphasized earlier. Normal level of Ca++ in organism is necessary for the rhythmic involution of uterus as well as fast ovary reactivation after calving (*Campanella,* 1994, *Canfield and Butla,* 1991) etc. These two processes are stopped by opioid overproduction.

Clinical evaluation of uterine involution in groups N, K, F

The results are given in *Table 7, 8 and 9.*

Table 7. **Dynamics of uterus involution in group N**

Control(day p.p)	**18 - 20**	**28 - 30**	**38 - 40**	**56 - 60**
No. Of cows	–	5	1	–
% of cows	–	83.83	16.66	–
Conclusion	–	–	100 %	–

Table 8. **Dynamics of uterus involution in group K**

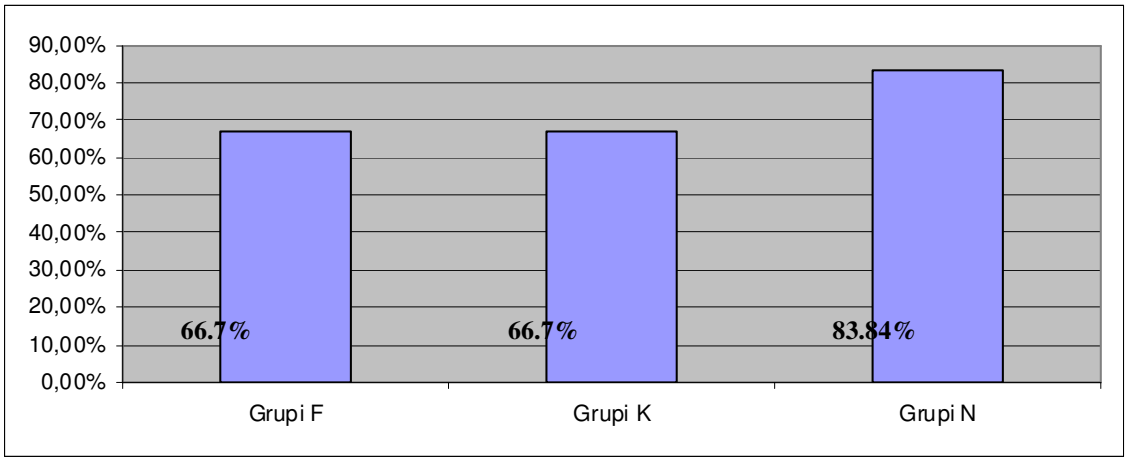
Control(day p.p)	**18 - 20**	**28 - 30**	**38 - 40**	**56 - 60**
No. Of cows	–	4	2	–
% of cows	–	66.66	33.33	–
Conclusion	–	–	100 %	–

Table 9: **Dynamics of uterus involution in group F**

Control (day)	**18 - 20**	**28 - 30**	**38 - 40**	**56 - 60**
No. Of cows	–	4	1	1
% of cows	–	66.66	16.66	16.66
Outcome	–	–	–	100 %

In the edge up to 30 days after calving, group N has around 17 % advantage towards group K and F regarding the involution rate of uterus.

***Figure 2*. Graphical comparison of uterine involution rates in three study groups until 28-30 after calving**



We think that a better involution or anatomic uterus diminution in cows in group N is part of Nalaxon's antagonist-opioid effect or as a result of Collagen's catabolism improvements, calcium metabolism as well as the catabolism in general as it resulted from the above-mentioned analyse of this task.

From the clinical perspective, it seems that the bio-chemical surrounding of the uterus changed by Naltrexonei avoid the relative atony in uterus that intrude opioids (mainly beta-Endorfina).

It is evidenced that endomorphines decrease uterus contractions in early purperium stage (*Robbe et al.,* 1999). We believe that Naltrexone given up to 10 days after calving has kept the uterus a quite active.

The results gained and their analyses prove the initial hypothesis raised that if hormonal and enzymatic activity in uterus during involution depends from normal Ca++ concentrations in uterine tissues and furthermore, then the opioides high level would decrease with a faster tempo as usual between the treatment between Naltrexone + Ca ++ that would be effected by a faster disolution of collagen in uterus and finding a larger amount of Hydroxyproline in cows' blood rather that at those untreated cows, indeed, which is true.

Conclusions

1. The methodical treatment of cows with antagonist-opioidin Naltrexone: (Naltrexone 4 mg.+Calcium-Oral 20 ml/100 kg.) changes the hydroxiproline profile (HYP) in the circulating bllod.

2. Naltrexone+Ca++ treatment stimulate the uterus involution that is indicated through HYP in animal's blood in a bigger amount that in those untreated ones. The average Hydroxyproline level to the cows treated with Naltrexone + Ca++ (group N) result 1.09 µg/ml.(microgram/ml.), against 0.98 µg/ml. that result to the treated cows only with calcium (groupi K) and 0.95 µg/ml. to the treated cows only with water (group F). The advantage of the Hydroxyproline level in group N comes by positive effect of Naltrexone in the entire metabolism of the body and the uterus in particular as well as in the catabolism of the uterine collagen.
3. The HYP level changes in cows' blood in our experiment (group N) against experimented cows (group F) are statistically evidenced: through days ($P<0.05$, $P>0.01$ and $P<0.001$), whereas between groups $P<0.05$. The changes among group N against group K and the last one against group F are statistically not evidenced.
4. Until 30 days after calving, the clinical uterine involution is achieved in 83.84% in the group with treated with Naltrexone and in 66.7% to cows of two other groups untreated by Naltrexone.
5. The biologic processes of uterine involution seem to be strongly interposed by endogen opioids with which Naltrexone antagonizes and competes persuasively..

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