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EDITORIAL

I. THE MAN-ANIMAL RELATIONSHIP IN FINE ARTS

Professor **Dorel MOISE***, PhD

In the fine arts, the relationship between man and animal has represented in ancient times a source of inspiration for artists in the visual arts. Following the scientific study of the anatomy of the animal body, masterpieces of art were made, using the animal symbol as a source, but also corporeal combinations between humans – animals, animals – animals, creating through art a number of surreal characters that were implemented in everyday life.

A number of professional artists from Cluj were highlighted by exceptional artworks using in their art, as inspiration, the animal world.

If the goal of visual arts translates into activities which manage to convey feelings and emotions to the viewer, for the artist it remains only to choose the topics or ideas which might offer, through creation, a specific thrill, thrill given by understanding the artistic message transmitted to those who admire the artist's work.

One much discussed topic in all branches of the visual arts is the animal theme. Creators of beautiful arts belonging to the great tradition of illustrating images of animals in the arts have created impressive artworks with images of strange animals, inspired by reality and transformed by figurative language in images, whether real or mystic, with great expressiveness.

Artists from all visual art branches interpret forms of reality that are visually perceived through the eye of talent, performing plastic arts transposition through sensitivity, skill and sophistication. In this respect, the study of animal figurative is a necessity so that the work produced is supported by a scientific support and not based on observing actual shape which interpreted intuitively would lead towards less professional art. The figurative animal study in artistic higher education has become the norm. Surface morphology of the body of an animal is known by studying artistic anatomy. If one does not understand animal anatomy, its reproduction, either static or moving through line, color and space may not be scientifically supported, which is required to be made by a creative professional with studies in visual plastic creation.

After the long process of domestication of animals and man's relationship with the animal kingdom, the man-animal bond has not escaped unnoticed to the artist who besides illustrative talent proved to be one of the most fertile and profound philosophers of the world in which they lived.

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It was found that in many subjects in people's life along their historical evolution, the artist began using the animal as a symbol and depending on the meaning that he wants to convey, the image goes in antithesis from beautiful to ugly. It is difficult to interpret in art what is beautiful and what is ugly because it all leads to the aesthetic obtained by metamorphosis of real form through works that illustrate combinations and by dendromorph and zoo-antropomorph hybridization. Thus, from ancestral art up to today, the artistic currents known as: symbolism, expressionism, surrealism, have born outstanding works translated through symbol by their metamorphosis based artistic expressiveness. We meet a lot of animal art symbol animals: lion, tiger, bear, animals endowed with various virtues of strength and courage, bravery, wisdom. The elephant is a symbol of wisdom, longevity and happiness, but also a symbol of good luck. Deer stags are recognized as a symbol of virility and wisdom, and their horns represent the tree of life, like the natural phenomena of rebirth and regeneration. The wolf and coyote embody the evil, greed, cruelty. The fox is a symbol of cunningness and rats and mice symbolize hypocrisy and destruction etc.

Bird as a symbol, such as the eagle and falcon who have an imposing appearance suggest the power over the air inspiring victory, courage, sunlight. The owl is considered sacred, a symbol of wisdom in Greek mythology, but also a symbol of death, bad luck, the darkness, in other people's cultures. The peacock portrays beauty. The swan is considered a symbol of love, it represents music, poetry, feminine grace. The stork bird is considered a symbol of luck that brings children and also expresses vigilance, purity, chastity and victory over evil. The magpie is the symbol that foretells misfortune and death. The turkey is an important symbol of female fertility and masculine virility etc.

Figure 1. Frank Fazetta
"The beauty and the beast", 1995

Of the symbols given to animals and inspired from people's lives, the creative mind of the artist creates new figurative appearances, the result of combining corporeal fragments of human and animal body together. Thus, monsters appear in art or fantastic animals, due to anatomic abnormalities with a studied and interpreted scientific support as a curiosity of nature, such as dragons, mermaids, cyclops (Figure 1)

A special chapter in visual art is physiognomy, considered a pseudoscience that connects human physiognomy and aspects of animal figure, this imaginary combination leads to particularly impressive illustrations such as a lion-like man or donkey-man, sheep-man, bird-man, a philosophy that addresses the fact that divine power transforms human appearance with symbolic character traits of the animal kingdom. Issues illustrated by Umberto Eco in "On Ugliness" (Figure 2).

In art evil creatures appear, casting spells and charms that were supported by the devil or witches and healing souls and diseases, an aspect of the subculture of people, remarkably reflected in masterpieces of art.

I will illustrate the interest of professional artists from Cluj on animal representation by a brief review after the acquired specialization in painting, graphics, sculpture. Fine art artists representative of Cluj use the integrated animal figurative integrated in a philosophical concept. In this realm, in painting, the following stand out: Ioan Sbârciu – much appreciated artist through his dramatic series of equestrian knights in his "Don Quixote" cycle. In achieving remarkable compositions as an

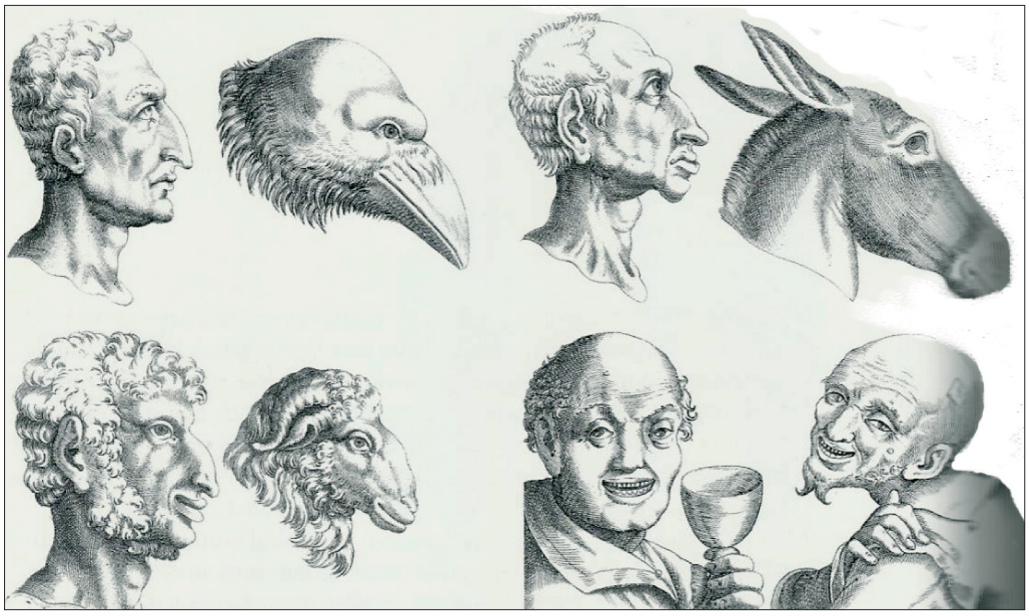

Figure 2. Giovan Battista della Porta, "On human physiognomy"

exquisite colorist, we find Andor Kömives that relate to an animal poetic image, a vision that slides from gentle to dramatic irony. Thanks to much appreciated work in the country and abroad, the artist is the guest of honor of the Cluj Veterinary Journal, to present a small part of his work exhibited at various art events.

Ioan Aurel Muresan presents himself in art with a fantastic bestiary seemingly taken from Oriental miniatures.

Theo Sandu Muresan, affirms himself through a cycle of dream animals painted on wood cut panels.

Adriana Elian by animal images, amplifies a feminist poetic perspective in the world.

Mariana Bojan is a poet and painter alike who through special coloring creates scenes of a poetic dream animal world, surrealist creations combined with everyday reality.

In graphics, the distinguished illustrator Ioan Horvath Bugnariu presents a wide range of graphic techniques, highlighting in many works, particularly, the ironic revaluing of certain animal clichés – fairy tales, and numerous animal themed illustrations translated into literary works.

Radu Solovăstru – through a design worthy of a master presents illustrated characters often accompanied by a grotesquely bad bestiary.

Ladislaus Feszt, the late illustrator, known as an animal lover, has produced masterpieces of artistic animal theme works with philosophical and satirical twists by the appearance of animal characters which transformed people's lives.

In sculpture, the artist Radu Moraru creates horse heads out of various materials in a post-modern dialogue with the ancient Greeks.

Mihai Barbu was a sculptor passionate about the anatomical expression of horses in motion.

And of course the list could continue with Cluj artists, many of them with livestock themed artistic masterpieces.

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II. ANATOMY OF FASCINATION – THE ANIMAL AS A CHARACTER

Professor **KŐMIVES Andor****, PhD

The animal and his image continues to exert on us a perpetual fascination, from fear to admiration. Our whole imaginary is impregnated by the bestiary, symbolically harnessed in a positive or negative register. This article highlights the importance, richness and diversity of animal representation within art history from prehistory to the present. Personal creation on the bestiary and death theme is a particular case study on a series of works within the artistic residency at IFITRY, Essaouira/ Morocco, 2013, in which mechanisms are dissected, the intimate composition of the "anatomy" of the pictorial animal image, the rich ambivalent meanings attributed to deer / stags. All these elements structure the work of art and visual emotional energy emanates.

The animal continues to exert on us a perpetual fascination, from fear to admiration. In time he has been worshiped and demonized alike. Man wanted to possess its qualities, to measure it, to obey and to exploit the "rational" and arrogant, and now is forced to save it and protect it. Our whole imaginary is impregnated by the bestiary, which is present in various forms and symbols in the myths of all cultures. The image of the animal was symbolically harnessed in the collective imagination, as positive or negative in terms of imaginary regime type: diurnal or nocturnal (Durand, 1998).

A simple glance at the history of art is enough to sense how rich, complex and diverse the animal is represented. Consider for example the magic ritual of prehistoric hunting, representations of deities and mythical antiquities, the human-animal monsters in Greek-Roman times, the whole Far Eastern or Christian bestiary in the Middle Ages, Romanesque capitols in Western churches, the grotesque fantasy of Hieronymus Bosch, sumptuous hunting scene paintings and tapestries of the Renaissance and Baroque periods, the decorative art of mural painting in palaces to manuscripts and book illustrations, irrational monsters of a Goya and JH Fussili, the oneiric surrealist Salvador Dali or Max Ernst, Picasso's bullfights or the new contemporary ecological sensitivity bestiary up to a contemporary painter, specializing in animal art, such as Walton Ford.

Even if sporadic, the animal theme of my work in painting and drawing has a certain poetic state, like exquisite fabrics, with a suave irony with a certain severity. This poetic dimension is essential, as is the vital energy that feeds my imagination. Labor at the artistic residency in IFITRY, Essaouira/ Morocco in the period August-September 2013, was a tribute to my father, Miklós Komives, who died just two days before I arrived there. I had to come to Morocco to rediscover an important part of my soul, like the oriental story about searching a treasure in your own backyard.

My father was a self-taught artist located with humor and poetry in the naive art. His works are small painted wooden sculptures. One of them – *a mouse who dreamed he is a stag* – in which he carved and intervened in a pictorial way with great imagination, on roots found in the woods – is emblematic of his destiny and inspired me to work in Morocco. At first glance, the combination

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between deer and mice has a touch of irony, but in depth it is about the desire to accede from a minor destiny marked by fear, to a major strong destiny. The set of works made in Morocco (painting on canvas, metal or ceramic) stood under the sign of symbolic exorcism of death, in a sort of Dantesque journey of dramatic memory recovery. The choice of the stag / deer motif does not fall only in the interest of a fashionable environmentalist, where artists return to nature, but has a strong personal emotional substrate

All images are in sober color register, the black and white tones dominate, contain a leitmotiv of androgynous characters, the woman-stag-deer, which symbolically slides between angel, victim, or messenger of death. Personal meanings attributed to the stag / deer enroll intuitively in the symbolic constellation that also exudes an aura around this animal. The anatomy of the character-animal is

Figure 1. Andor KŐMIVES, *The heart as a sacrifice,* 2013, acryl / canvas, 100x100 cm

Figure 2. Andor KŐMIVES, *The Centaur-Deer,* 2013, acryl / canvas, 100x100 cm

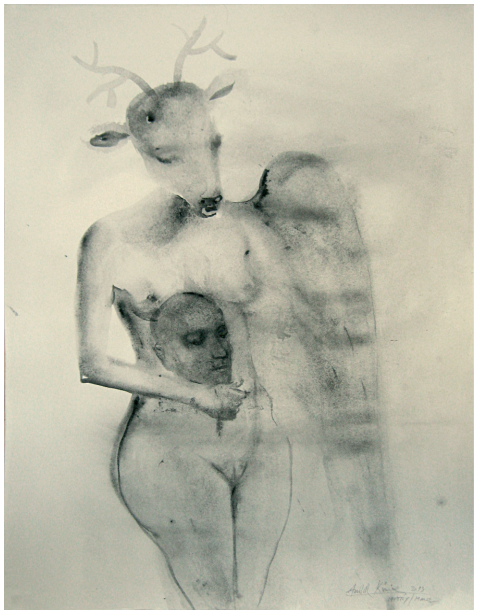

Figure 3. Andor KŐMIVES, *The Angel-Deer,* 2013, Watercolor on Arches paper, 65x46 cm

Figure 4. Andor KŐMIVES, *Vanitas,* 2013, Watercolor on Arches paper, 65x46 cm

not an assemblage of forms, but is organically and plastic integrated, opening the gate of imagination and the possible (Figures 1-6).

Figure 5. Andor KŐMIVES, From the series *In Memoriam: Kőmives,Miklos,* 2013, acryl/metal, Ø 31 cm

Figure 6. Andor KŐMIVES, From the series *In Memoriam: Kőmives,Miklos,* 2013, acryl/metal, Ø 31 cm

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EFFECT OF CYSTEAMINE SUPPLEMENTATION ON IVM, IVF AND SUBSEQUENT DEVELOPMENT OF OOCYTES IN AWASSI SHEEP

O. MARDENLI*

H. ARYAN

Abstract

The effects of supplementation of in vitro maturation (IVM) with cysteamine on IVM, IVF, cleavage rate, and subsequent stages of cleavage of oocytes were examined. A 942 Oocytes obtained from slaughterhouse, ewes ovaries were subjected to IVM and IVF. Oocytes were matured in TCM199 and fertilized in TALPm media, data were analyzed by contingency tables of chi square, TCM199 media were supplemented with 100 (group A), 200 (group B), 400 (group C) and 0 µm cysteamine (control group D). Total rates of maturation, fertilization and cleavage in present research were 68.26, 42.14 and 36.53 respectively. Total rates of (2-8 cell stage), morula and blastocysts were 70.83, 12.50 and 16.67% respectively. Supplementation of IVM medium with 100 µm cysteamine (group A) increased (P <0.05) the IVM rate compared with group C (80.57% vs.65.56%), there were no significant differences noticed at M-II and cleavage phases rates among groups, supplementation of IVM medium with 100 µm cysteamine increased (P <0.001) the (2-8 cell stage) rate (70.83% (group D) vs.17.86% (group A) but differences among groups were significant (P <0.05) at the rates of morula and blastocyst yield among groups, highest values were in group A and B (39.29 and 42.86% vs.14.81 and 18.52%) compared with C and D groups (15 and 15% vs.12.50 and 16.67%). The results of the present study suggest that supplementation of IVM media with cysteamine improves the rates of IVM, IVF, the yield of morula and blastocysts in IVP programs of sheep whereas gives the good chances for other aims like sexing and cloning of embryos.

Keywords: oocytes, IVM, IVF, cysteamine, morula, blastocyst.

Introduction:

Amino thiols and amino acids plays an important role in culture media as principle source of energy, regulating pH (Balasubramanian *et al.* 2007), base for proteins and nucleic acids, osmotic regulation inside cells, development the embryonic cells division and high ability for embryos culture inside receiver females while transferring (Bannai, 1984).

Synthesis of amino acids in IVM and IVF release Ammonia in culture media which is very harmful to embryos so modernizing of culture media every 3 days to get rid of Ammonia is very important procedure (Downs *et al.* 2003). Oxidative stress results in lipid peroxidation of membranes, amino acids, proteins, and nucleic acids; it also has the potential to cause cellular death, depending on the cell type, origin, and speed of oxidative stress production (Fukui *et al.* 1996). As the production of free radicals normally occurs in vivo, cells develop a mechanism called "antioxidant defense system"

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to neutralize the reactive oxygen species and their effects (Gardner, 1998). Catalase and superoxide dismutase, as well as the "thiol" components act as metabolic lids to neutralize the reactive oxygen species (Geshi *et al.* 1999). Cysteamine is one of low molecular weight amino thiol compounds with the formula: $HSCH_2CH_2NH_2$ and has important role in subscription actions and maintaining oxidative reaction at low levels in mice oocytes, cysteamine known to be a scavenger of hydroxyl radical, and may contribute to maintaining the redox status in oocytes (Guérin *et al.* 2001, Halliwel *et al.* 1989).

The Aim: experiment were designed to evaluate the effects of cysteamine supplementation to the in vitro maturation medium on maturation of ovine oocytes, fertilization, cleavage and subsequent embryo development in Awassi sheep.

Materials and Methods:

The research was conducted in 2009, using total number of 942 ovine oocytes obtained from ovaries collected from slaughterhouses in Damascus and Aleppo, Syria.

Ovine ovaries were obtained from a local abattoirs of Aleppo and Damascus cities and transported to the laboratories in phosphate-buffered saline (PBS) at 35–37° C within 2 h of slaughter. The cumulus oocyte complexes (COCs) were recovered by slicing method of follicles at all diameters at 32–36° C using an specific blade, pooled in 50 ml conical 4-wall plate (Fig. 1).

In vitro maturation (IVM)

COCs were assessed morphologically and only those that had a compact, three or more complete layers of cumulus cells and fully grown oocytes with homogenous cytoplasm were selected for the experiments. All selected COCs were washed three times thoroughly in HEPES-buffered tissue culture medium TCM199; washed once in IVM medium, placed in 4 wall-dishes (10-15 oocytes/wall) of the same medium under sterile silicone oil (SIGMA) and matured for 27 h at 38.5° C in an atmosphere of 5% CO_2 in humidified air. The basic medium used for IVM was 25 Mm HEPES-buffered TCM199 supplemented with 2 mM sodium pyruvate, 1mM l-glutamine, penicillin (75 mg/ml), streptomycin (50 mg/ml), 10% steer serum, after 27 hours oocytes were examined under inverted microscope for first polar body formation (Fig. 2).

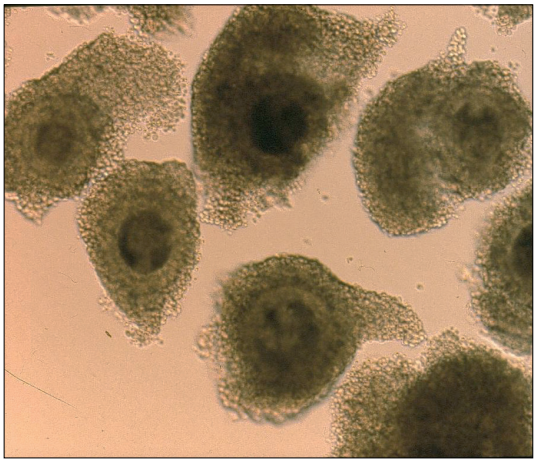

Fig .1. Ovine oocyte at GV stage (COCs)

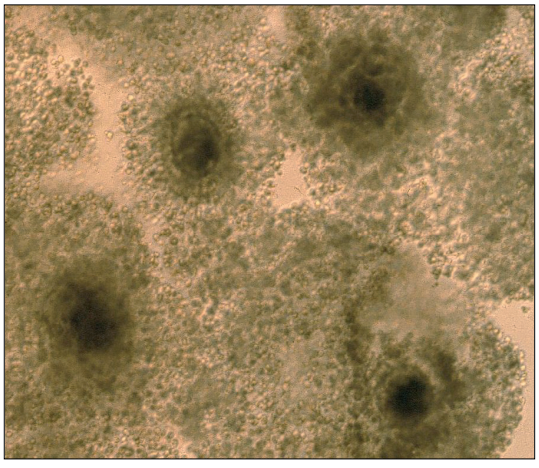

Fig. 2. Matured ovine oocytes at M-I stage with clear comolus cells. expantion

In vitro fertilization (IVF)

After maturation, COCs were vortexed for 90 s in HEPES-TALP medium to remove most of the cumulus cells and rinsed three times with HEPES-TALP supplemented with 2% bovine serum albumin (BSA, Fraction V) and an additional three times with IVF-TALP medium. After washing, 5-10 oocytes were transferred to fertilization medium to each wall of 4-wall plate under sterile mineral oil. In experiment, frozen-thawed semen from a single ram of proven fertility was utilized. Spermatozoa were rinsed twice with 8 ml HEPES-TALP medium by centrifugation at 900 rpm for 10 min and then resuspended in 2 ml of HEPES-mTALP medium for 1.5 hour at 38° C for the swim up of sperms, The sperms suspension was evaluated and being added to maturation medium of sperms a final sperm concentration of about $1.5*10^6$ sperms/ml. for 4–5 h at 38.5° C in an atmosphere of 5% CO_2 in humidified air. Each 5-10 matured and denuded oocytes added to 1 ml of fertilization medium in 4-wall plate and incubated at 38.5° C in an atmosphere of 5% CO_2 in humidified air for 17 hours, fertilized oocytes rinsed with PBS and examined under inverted microscope for second polar body formation (Fig. 3).

In vitro culture (IVC)

The presumptive zygotes were rinsed with serum-free TCM199 medium, and cultured in 50 μl droplets (20 oocytes/drop) serum-free TCM199 medium at 38.5° C in an atmosphere of 5% CO2 in humidified air. Cleavage was ssessed on days 2-8 of IVC (Fig. 4,5,6).

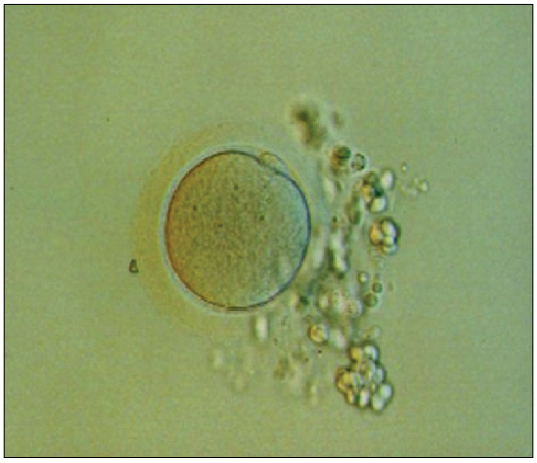

Fig .3. Ovine oocyte at M-II stage with clear second polar body

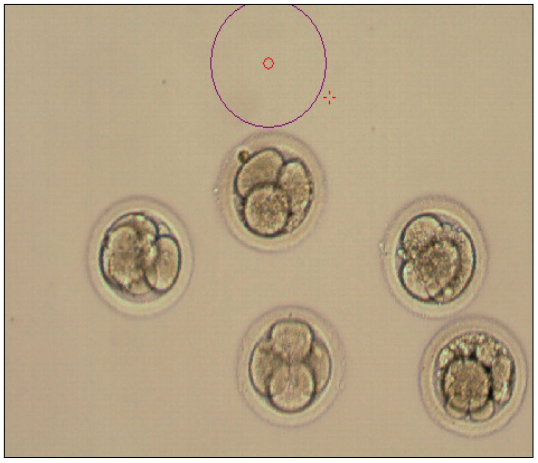

Fig .4. Cleaved ovine oocytes at (2-8) cell stage

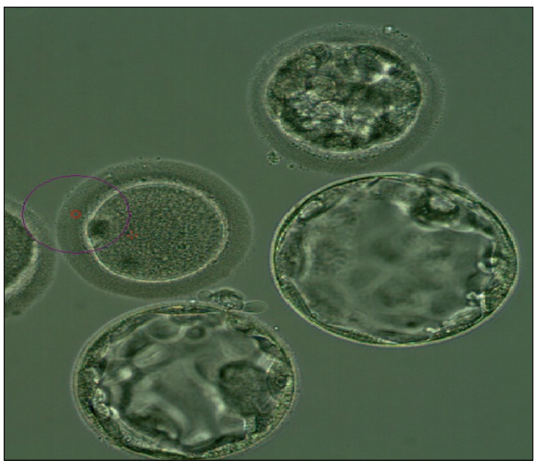

Fig .6. cleaved ovine oocytes at blastocyst stage.

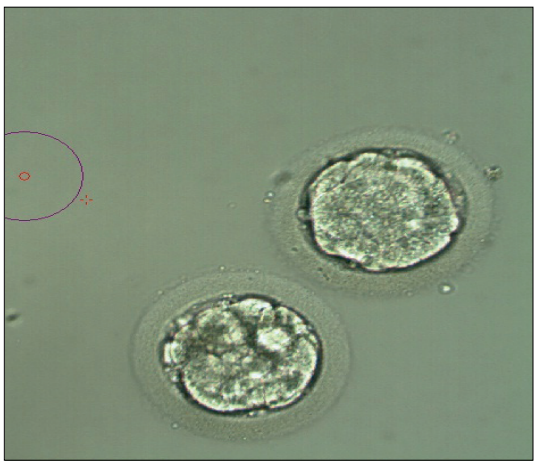

Fig .5. Cleaved ovine oocytes at morula stage.

Experimental design and Statistical analysis:

Cysteamine supplementations were added to maturation medium TCM-199 as following concentrations : (A) 100 μM, (B) 200 μM, (C) 400 μM, (D) control, Pearson Chi-square of contingency table test was used to analyze the data among treatment groups for matured oocytes, fertilized oocytes, cleaved oocytes and the morula and blastocyst total count among groups. A probability value of $P < 0.001$ and $P < 0.05$ was considered a significant difference by using SAS, 9 software.

Results and Discussion

Table 1 shows the oocyte maturation rates after 27 h of IVM, The oocytes at the MII stage and cleavage rates according to Cysteamine concentrations. Total rates of maturated, fertilized and cleaved oocytes were 68.26 %, 42.14% and 36.53% respectively. The study showed significant differences ($p < 0.05$) in IVM phase at different concentrations of Cysteamine where the highest value was in group A (80.57%) comparing with groups B, C, D (67.63, 61.04 and 65.56%) respectively. The study didn't show significant differences for cysteamine supplementation in IVF and cleavage phases among groups ,the values were closed and swing between 37.50% (group C) to 44.12% (group A) for IVF and 35.09% (group C) to 37.33% (group A) for cleaved oocytes.

Table 1. Effect of cysteamine supplementation on maturation stage, fertilization and cleavage of in vitro matured oocytes

Cysteamine oncentration µM	(Incubated oocytes GV) No.	Matured oocytes		Fertilized oocytes		Cleaved oocytes	
		No.	%	No.	%	No.	%
(A)100µM	211	170	80.57	75	44.12	28	37.33
(B)200µM	241	163	67.63	73	44.79	27	36.99
(C)400µM	249	152	61.04	57	37.50	20	35.09
(D) control	241	158	65.56	66	41.77	24	36.36
total	942	643	68.26	271	42.14	99	36.53
Sig		*		NS		NS	

* (0.05>P)
NS =Non-significant

Table 2 shows the oocyte cleavage rates at (2-8) cell stage, morula and blastocyst according to cysteamine concentrations, total rates of (2-8) cell stage, morula and blastocyst phase of embryos were 53.53%, 21.21% and 24.24% respectively. The study showed high significant differences ($p < 0.001$) in (2-8) cell stage at different concentrations of Cysteamine where the highest value was in the control group D (70.83%) comparing with groups C, B, A (70.00, 62.96 and 17.86%) respectively. At morula stage supplementation of cysteamine affected significantly ($p<0.05$) among groups by trend for group A (39.29%) comparing with groups C, B and D (15.00, 14.81 and 12.50%) respectively, effect of cysteamine supplementation seemed to be clear ($p < 0.05$) for embryos reached the blastocyst stage at group A comparing with groups B, C and D (42.86% Vs. 18.52, 15.00 and 16.67%) respectively.

Table 2. Effect of cysteamine supplementation on different subsequent stages of in vitro cleaved oocytes

Cysteamine concentration µM	Cleaved oocytes No.	(2-8)cell stage		morula		blastocyst	
		No.	%	No.	%	No.	%
µM 100 (A)	28	5	17.86	11	39.29	12	42.86
µM 200 (B)	27	17	62.96	4	14.81	5	18.52
µM 400 (C)	20	14	70.00	3	15.00	3	15.00
control (D)	24	17	70.83	3	12.50	4	16.67
total	99	53	53.53	21	21.21	24	24.24
Sig		***		*		*	

(0.05>P) *
(0.001 > P)***
NS =Non-significant

Conclusions

Results of This study agreed with the most reaserches established to investigate cysteamine supplementation in IVM for various spicies of animals which in turn emphasized the great role of cysteamine in IVEP programs especially the media contains 100 μM of cysteamine.

The results of study showed significant effects ($p < 0.05$) when adding low concentrations of cysteamine (groups A and B) in IVM of ovine oocytes comparing with high concentrations (groups C and D), adding 100 μM cysteamine to culture media during IVM was the optimum one (group A), cysteamine drove the rates of IVM to highest value significantly (80.57%), the present results support the observations of (Yamauchi et col 1999) in mice meanwhile (Thompson, et col 2000) showed that adding 50 μM of cysteamine led to better results in cattle, but the study didn't support observations of (Teleford, N.A et col 1990) which showed adding of 50 μM in TCM-199 had significant effects on IVM, IVF and cleavage rate up to blastocyst stage in buffalo, the differences in this study and the results of (31,32) may be related to the difference in animal species thus the positive role of cysteamine in IVM can be explained by increasing GSH which in turn control ROS (Reaction Oxidative Species) in embryos the main factor causes much damage, degeneration and finally the death for embryos cells at subsequent stages during IVF.

TCM-199 as maturation culture media may have an important role in illustrating the positive action of cysteamine compared with other culture media showed TCM-199 supplemented with cysteamine led to better results comparing with MEME media with same cysteamine concentrations in mice oocytes because of the richness of MEME with high rates of glucose and glutamine which considered as poor resources for energy related to cumulus cells during IVM.

Although non-significant effects in IVF and cleavage rates of ovine oocytes among different concentrations of cysteamine compared with control group which came in the contrary to results of (Perreault, et col 1988) at $p < 0.05$ in mice oocytes the results of this study came compatible with (Guérin et col 2001) with high rates in fertilized (44.12 and 44.79%), cleaved oocytes (37.33 and 36.99%) respectively in groups A and B, this can explain the positive action of cysteamine in subsequent development of embryos to the blastocyst stage.

Results of development of oocytes after fertilization to the subsequent divisions in groups A and B came compatible with most studies emphasized the great role of cysteamine in cleaved oocytes after fertilization to reach subsequent phases of in vitro division and finally the increase in embryonic cells included the morula and blastocyst stage at most animal species, a high significant differences ($p < 0.001$) noticed in cleavage rates (groups A and B) 42.86 and 18.52 % respectively taking into consideration the decrease in 2-8 cell stage in group A on the increase of morula and blastocyst rates in same group (17.86 % Vs. 70 and 70.83%), low molecular weight for amino thiol compounds like cysteamine affect significantly the development of fertilized oocytes by IVF because of the decrease of GSH in intracytoplasm, (Del Corse et col 1994) showed adding 100 μM of cysteamine to maturation media in horses led to significant increase in glutathione related to the effect of animal species and to the differences in culture media used.

Many compatible studies emphasized GSH resulting from cysteamine supplementation to culture media had another important action in spermatozoa thus a sufficient amount of GSH participates in sperm decondensation in parallel to oocyte activation, and in the transformation of the fertilizing sperm head into the male pronucleus (PN).

In conclusion, the results of the present study suggest that the supplementation of cysteamine during ovine IVM did not influence the rate of cleavage but enhanced the embryo development.

Further, cysteamine enhanced the morula and blastocyst stage of in vitro matured and fertilized ovine oocytes.

Therefore, the addition of cysteamine may improve the IVEP programs for ovine oocytes associated with methods of slicing technique.

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COMPARATIVE STUDY
ON THE DRUG INDUCTION ABORTION IN BITCH

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Abstract

Pregnancy interruption is one of the most common requests from dog owners, the main reasons being too young or too old females, the partners being disproportionate in size or accidental mating. The main porpouse of this study was to establish protocols to induce medical abortion in bitches, the effectiveness, side effects and costs. The research was conducted in March 2010 - May 2013 in the Clinic of Reproduction, Obstetrics and Gynecology, Faculty of Veterinary Medicine, Cluj Napoca, using a total of 75 female dogs, from 12 months to 8 years of age, different breeds and a body weight between 5 and 80 kg. The females were divided into five equal numerically groups. The use of estradiol benzoate, in the first group, resulted in implantation blocking in 100% of females exposed to the treatment. Long term side effects were observed and thus it is recommended to completely avoid the application of this protocol for induction of abortion in bitches. By using aglepristone, in the second group, in 4 to 45 days period of gestation had an efficiency of 100% in the studied group, side effects were discrete and relatively high cost price are consider surmountable obstacles to widespread use of this drug. By using cabergoline (third group), the success rate was in 86.66% of the cases studied. Complications occurred using this protocol. Side effects were reported from the use of cloprostenol, but the efficiency is high (100% of treated cases) as well as the minimal and transient neuro-hormanal impact it causes. Comparative evaluation of methods, taking into account the efficiency, the consequences they generate, side effects and cost price, it is recommended the aglepristone abortion in the first 26 days of gestation (in small females) and prostaglandin abortion in the second half of gestation, advantages being high efficiency, the possibility ultrasound confirmation of the unwanted pregnancy and of a transient neuro-hormonal impact.

Introduction

Pregnancy interruption is one of the most common requests from dog owners, the main reasons being too young or too old females, the partners being disproportionate in size or accidental mating. In the last 20 years various drugs have been used to induce abortion in bitches. There are various therapeutic protocols such as treatment with estrogens, PGF2α and its analogs, dopamine antagonists, prostaglandin association with dopamine antagonists, anti gestagens etc. Although most of these substances have been used in other species, only a few are widely marketed or approved for use in bitches (RS Abhilash *et al.*, 2012).

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The first stage of pregnancy begins with fertilization and ends a few days after implantation. During this time, the pregnancy cannot be confirmed, and the induction of abortion (blocking the implantation) is difficult because the corpus luteum is refractory to the exogenous luteolytic medication. Pregnancy interruption in this stage can be performed with the use of pharmacologic substances such as estrogens, prostaglandins and anti progestagens. In the second period, pregnancy can be confirmed; the abortion may be induced with prostaglandin anti-prolactin agents (bromocriptine or cabergoline, metergoline), combinations of prostaglandins or progesterone secretion inhibitors (epostane) or anti progestagen (mifepristone or aglepristone). The 3rd stage of pregnancy starts with calcification of fetal structures and the abortion is always associated with the expulsion of the fetus. At this stage premature parturition may induce abortion with the expulsion of live puppies. Therefore, the best time to initiate abortion in bitches is between 30 and 35 days after the last mating (RS Abhilash *et al.*, 2012).

The main porpouse of this study was to establish protocols to induce medical abortion in bitches, the effectiveness, side effects and costs.

Materials and methods

The research was conducted in March 2010 - May 2013 in the Clinic of Reproduction, Obstetrics and Gynecology, Faculty of Veterinary Medicine, Cluj Napoca, using a total of 75 female dogs, from 12 months to 8 years of age, different breeds and a body weight between 5 and 80 kg.

The females were divided into five equal numerically groups:

GROUP I – consisted of 15 females, between 1.5 and 6 years of age, who received estradiol benzoate, 0.01 mg/kg on days 5, 7, 9 after the unwanted mating, by intramuscular administration. Estrogens prevent the implatation by changing the uterine environment or they prevent migration of the embryo in the uterus by closing the uterotubale junction. In GROUP I, after the estradiol benzoate administration, was observed heat extension by 5 days in 3 bitches of different breeds, namely heat extension by 7 days and nipple swelling to one American Staffordshire Terrier breed bitch.

GROUP II – consisted of 15 females, between 9 months and 6.5 years of age, were administered subcutaneously in deposits up to 5 ml with a booster at 24 hours, aglepristone at a dose of 10 mg/kg, in the range of 4 to 45 days after the unwanted mating. The aglepristone mode of action is based on the blockage of the endometrial and myometrial progesterone receptors, with the plasma levels of progesterone remaining unaltered, so that the metabolism of progesterone secretion is not influenced in any way (aglepristona has no luteolytic effect). 4 females, Cane Corso Italiano breed (two bitches), Romanian Raven Shepherd and Romanian Carpathian Shepherd breeds (large females to which is necessary the administration of a high volume product) we found growth of nodules and even some induration plaques in the subcutaneous connective tissue at the site of inoculation of the product.

GROUP III – consisted of 15 females, between 1 and 7 years of age, receiving 5 mg cabergoline/kg body weight once daily for 4-6 consecutive days according to the clinical condition of the animal. They were administered orally alone or mixed with food, between 24 and 40 days of gestation. Cabergoline acts as a direct stimulant of dopamine receptors in the adeno-hypophysis, resulting in the inhibition of prolactin release. In the third group, after the product administration, metrorrhagia was diagnosed post-treatment in three bitches from different breeds. In two females belonging to this group: a 5 years old Belgian shepherd (Tervueren variety) female was diagnosed with partial retention of a fetus in the cervix, and partial retention of a fetus in the cervix, endometritis, cervicitis, recurrent catarrhal vaginitis to a female English Cocker Spaniel age 4.5 years.

GROUP IV – consisted of 15 females, between 1 and 5 years of age, underwent treatment with cloprostenol in a dose of 2 mg/kg, minimum 4 consecutive days administration/day, between 32-48 days of pregnancy. Cloprostenol induces abortion due to its luteolytic and uterotonic properties. In group IV after treatment with cloprostenol, tachycardia, tachypnea, salivation, vomiting, diarrhea, pollakiuria for 27-50 minutes, were observed. Abortion in the 15 females occurred after 4-6 days of treatment.

GROUP V – was the control group. The control group consisted of 15 females, between 1.5 to 6 years of age, diagnosed with pregnancy at 24 days after mating, by ultrasound. No females were administered medicinal products in the control group, for which they have led the pregnancy to term. All 15 females gave birth to alive, medically healthy puppies.

Results and discussions

Analysis of the results obtained for GROUP I (females who underwent treatment with estradiol benzoate) shows a high efficiency of the method, thus the blocking implantation, or the prevention of pregnancy occurred in 100% of the cases studied. Four of the females belonging to the experimental group, representing 26.6% of all cases of this group, presented heat extension 5 to 7 days. Sero-bloody secretions were resumed in these cases and the expression of secondary sexual characteristics specific to the estrous period was clear and complete, which caused discomfort to the owners of these females. Turgid mammary glands and nipples, their erythema and inter-oestrus shortening by about 60 to 90 days were also recorded. Safety of the product is also satisfactory, no local reactions were observed after the the deep intramuscular administration. Initially recorded cycle disorders (inter-oestrus shortening), then continued for four females belonging to this group, with prolonged duration of heat over 24 days. Numerous studies, since 1982, have discussed the genotoxic and carcinogenic effects of estradiol on breast tissue cells and even liver (Heitzman R.J., 1983; Pop Al., 2009). From the viewpoint of the owners this method is very commonly accepted and specifically requested due to relatively low cost price (around 60 USD/female) and the fact that implantation blocking does not require the expulsion of the conceptus, eliminating the elements that can cause social discomfort to the owners. However, with regard to the cycle disorders arising from a high proportion of patients (26.6%) and the involvement demonstrated of estrogens in the genesis of conditions such as pyometra, in the case of counter-phase treatment (the use of estrogen or synthetic compounds with estrogenic effect during the stage of sexual cycle LH), salpingian ovarian cysts, glandular cystic degeneration of the uterus and mammary tumors, we recommend avoiding the use of this method of inducing abortion (specifically blocking implantation) both for female pets and those for breeding and selection in the respective breeds.

Johnson (1995), stated that 25-50% of the bitches with pyometra were treated with estrogens during the last six months. It seems that estrogen receptors anomalies play a role in the development of cystic hyperplasia – pyometra complex in bitches. The use of estrogens is associated with side effects, the most important being bone marrow aplasia leading to thrombocytopenia, leukopenia, severe anemia and death. In case of bone marrow aplasia, even with blood transfusion and if the use of estrogen therapy stops, the prognosis remains grave.

Using estrogens the development of similar behavior during heat with vulvar swelling, attraction of males, mating and coitus is important. Irreversible infertility is another possible side effect of multiple treatments with estrogens (Verstegen J., 2000). Elderly bitches appear to be more likely to the side effects of estrogens than the younger ones (Shila V., 1982), probably due to prolonged and repeated exposure to endogenous estrogens.

For GROUP II (treatment with aglepristone) we found an efficiency of 100% of the cases treated in the range of 4 to 45 days of gestation. An occurrence, in four females, of nodules or induration plaques in the subcutaneous tissue at the product inoculation site, is recorded. Rubefaction with iodine (carried out over a period of 5 days) resulted in complete resorption of these lesions in a range between 10 and 22 days. No changes were observed of the inter-oestrus interval in any of the females belonging to this group.

For the females whose pregnancy was in the fetal stage, fetal expulsion was not accompanied by colic nor produced changes of the general condition or their behavior. Abortion has occurred in a period between 5 and 6 days from the start of tratament. In females to whom the treatment was applied in the embryonic stage of pregnancy, was found no vaginal or uterine discharge, behavioral or general condition changes.

The only detectable drawback of this method is represented by the relatively high cost price, especially for large females (even prohibitive in females with body mass over 45 kg). Corroborating the clinical data obtained from patients in this group, this method is effective and consider specific, local side effects observed in 26.6% of pacients being reversible. The cost is reduced inversely proportional to the size of the animal, thus is recommended to use this method in small females. In contrast, other authors have achieved comparable effectiveness using this method of inducing abortion.

Aglepristone acts as a progesterone antagonist in the uterus and does not have direct and immediate luteolytic properties. Abortion induction takes place in the presence of high concentrations of progesterone (Baan *et al.*, 2005). Administration of aglepristone 26 to 45 days after mating induced the resorption or abortion within 7 days in 96% of cases. Aglepristone can be administered at a dose of 10 mg / kg body weight subcutaneously twice every 24 hours. The efficacy of this treatment is reported to be 95%. No adverse reactions were observed. It is assumed that the aglepristone's mode of action is primarily through its direct effect on the uterus by suppressing the biological effect of progesterone (Van Look P. *et al.*, 1989). Several studies have been conducted to investigate the presence and distribution of estrogen (ER) and progesterone (PR) receptors during the estrous cycle and during physiological pregnancy in bitches (S. Johnston *et al.*, 1985). However, relevant publications are not available in the literature about changing the number and distribution of PR and ER after applying aglepristone during resorption, respectively abortion.

Analysis of data obtained from patients in GROUP III (treatment with cabergoline) were recorded occurrences of post-abortion complications that consisted of: post-therapeutic essential metrorrhagia (3 cases representing 20% of cases), partial retention of a fetus in the cervix (2 cases representing 13.33% of cases) followed by the installation of septic complications (endometritis, cervicitis, vaginitis recurrent catarrhal) in one case. The efficiency of the method was of 86.6%, and in both cases was partly abortion (13.33%). These results combined with the relatively high cost price, this method makes use of cabergoline to induce medical abortion in the bitch, not the first option recommended to the owners.

Cabergoline is highly effective, when administered either oraly or parenteral. A dose of 5 mg/kg once daily, sudden decreases prolactin in serum, resulting in abortion, with few side effects. These side effects are less severe (compared to those of bromocriptine) probably because it has a lower capacity for crossing the blood-brain barrier and affecting the CNS.

The method using cloprostenol at a dose of 2 mg/kg, daily, intramuscular administration, resulted in abortion in 100% of patients belonging to GROUP IV, 4 to 6 days of treatment. In all females, after this protocol was used, the side effects have been characterized by tachycardia, tachypnea, salivation,

vomiting, diarrhea, pollakiuria, clinical signs during the event being between 27 and 50 minutes. The side effects listed are considerable intense, generating reactions of sympathy among owners (aspect worth taking into consideration for pets in general). Side effects manifestations and their intensity do just that the method being denied by some owners even in the context of efficiency of 100% of the cases treated. Side effects, in medium term period, reduce the range on the first post-abortion heat cycle at the rate of 15% of bitches. Subsequently inter-oestrus interval remains in physiological limits. Another considerable advantage of the method is the possibility of specifying the state of gestation, by ultrasound, avoiding situations, where the treatment is established relying only on the information or the susceptibility of the unwanted mating, in which case the gestation installation does not generate an absolute percentage. Because of the reduced neuro-hormonal impact due to short half-life and the mode of action of natural and synthetic prostaglandins (abortion is due to the oxytocic and luteolytic affect of prostaglandin), both short term, this method of inducing abortion in bitches is recommended, mainly those for subsequent use in breeding and selection.

Concannon and Hansel have shown that repeated PGF2alfa injections are likely to induce luteolysis in bitches (Concannon P. and Hansel W., 1977). Repeated doses of prostaglandins have also been used to prevent unwanted pregnancies. The administration is typically initiated after the fifth day of diestrus (about 10 to 15 days after the LH peak). Many researchers have attempted to induce abortion using multiple administrations of prostaglandins during the first half of gestation, but with conflicting results and frequently partial abortions (Oettle E. *et al.*, 1988). However, Romagnoli et al. (Romagnoli S. *et al.*, 1996), induced abortion on a regular basis from 5 to 19 days of gestation, the administration of 150-200 mg of PGF2alfa twice a day for 4 days.

Lange et al. (Lange K. *et al.*, 1997) have evaluated the effects of low doses of PGF2alfa at the beginning of pregnancy (20 to 50 mg/kg for 7 days, on days 5 through 11 of diestrus) or immediately after the detection of pregnancy (20 to 21 days after ovulation). Luteolytic effect of PGF2alfa low doses was generally insufficient due to the recovery of the yellow bodies in almost all bitches. Given these results, the best approach is to confirm the pregnancy before using PGF2alfa or its analogues.

Analyzing the problems related to the drug induced abortion in the bitch, significant differences were observed in terms of advantages and disadvantages. Thus, aglepristone and estrogens are substances which may be used to induce abortion in the first period of gestation in female dogs. Of these two, aglepristone is the best choice. Because of the negative side effects of estradiol benzoate, it is less recommended. Starting with 28-30 days of gestation, synthetic prostaglandins, anti-prolactins and anti-progestational therapy prove to be most effective. The combination of prostaglandin and anti-prolactins (cabergoline) is considered to be very effective in inducing abortion. This treatment has the advantage of being free of side effects, induce abortion by resorption and works from day 25 of gestation.

The overall analysis of the results showed maximum efficacy to induce abortion when administered: estradiol benzoate, aglepristone, cloprostenol, while cabergoline was effective in only 86.66% of cases. Side effects induced by estradiol benzoate and aglepristonă reached a level of 26.66% , and cabergoline produced side effects in 33.33 % of cases. Cloprostenol caused side effects in all LOT IV females. Please note that the cloprostenol induced to estrogen induced side effects can not be compared.

Table 1. Efficiency and side effects of the used drugs

estradiol benzoate		aglepristone		cloprostenol		cabergoline	
efficiency	side effects	efficiency	side effects	efficiency	side effects	efficiency	side effects
100%	26,66%	100%	26,66%	86,66%	33,33%	100%	100%

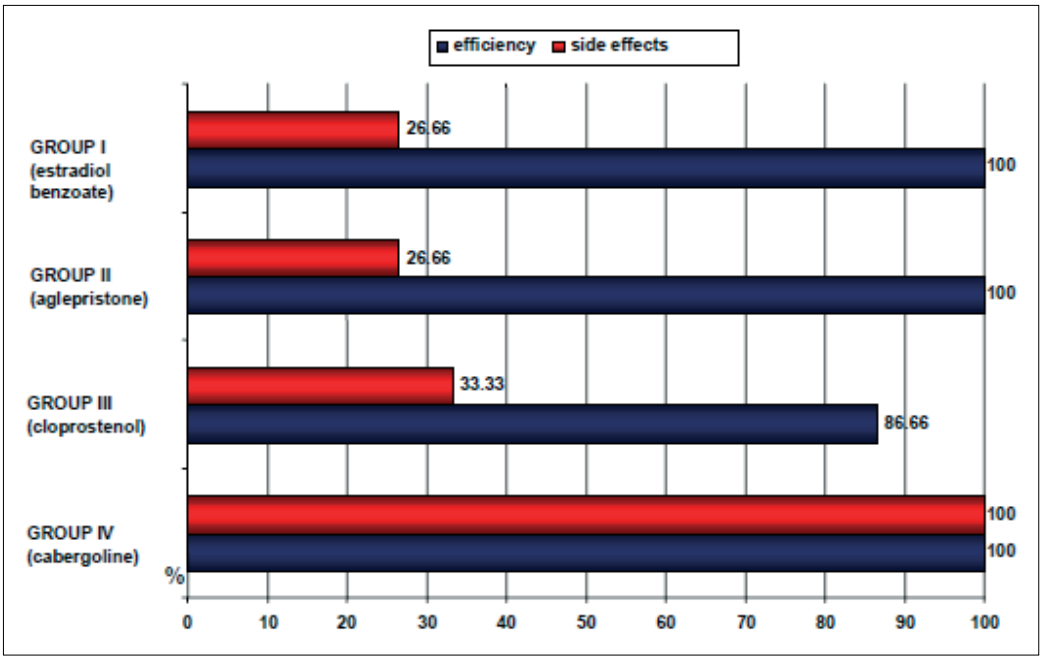

Fig.1. Comparative representation of the efficiency and side effects of the drugs

Conclusions

The use of oestradiol benzoate resulted in implantation blocking in 100% of females exposed to the treatment. Efficacy in conjunction with a relatively low cost price and discrete short term side effects causes owners to accept unreservedly this protocol for induction of abortion. Long term side effects observed: genotoxic and carcinogenic effect of 17β-estradiol and synthetic estrogenic compounds on the mammary gland and some reproductive disorders such as prolonged oestrus occurrence and/or anovulation, ovarian and pavilion cysts, pyometra, uterine glandulo-cystic degeneration, recommend to completely avoiding the application of this protocol for induction of abortion in bitches. Induction of abortion by using aglepristone in 4 to 45 days period of gestation had an efficiency of 100% in the studied group, side effects were discrete (lumps at the site of inoculation in 26.6% of cases) and relatively high cost price are consider surmountable obstacles to widespread use of this drug. By using cabergoline, drug induce abortion in the bitch was recorded at a rate of 86.66% of the cases studied. A post-therapeutic essential uterine bleeding occurred at a rate of 20% and 13.33% of cases presented fetus retention in the cervix which generated subsequent septic complications. These complications corroborated with a relatively high cost price gives rise to the recommendation regarding the use of this protocol. Side effects reported from the use of cloprostenol to induce abortion in bitches are: tachycardia, tahisfigmia, tachypnea, salivation, vomiting, diarrhea, pollakiuria, manifestation duration of these clinical signs ranging between 27 and 50 minutes. This drawback is offset by the high efficiency of the method (100% of treated cases) as well as the minimal and transient neuro-hormonal impact it causes. This method is not recommended to be used on older females with a cardio-pulmonary history. Comparative evaluation of methods of inducing abortion in the bitch, taking into account the efficiency of the methods, the consequences they generate, side effects and cost price, it is recommended the aglepristone abortion in the first 26 days of gestation (mainly in small females) and prostaglandin abortion in the second half of gestation, advantages being high efficiency, the possibility ultrasound confirmation of the unwanted pregnancy and of a transient neuro-hormonal impact.

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A LISTERIOSIS OUTBREAK IN GOATS

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Abstract

Listeriosis is a serious disease that causes encephalitis in ruminants, abortions or endophthalmitis. Usually one form evolves. This paper presents the clinical, diagnostic and treatment behavior into a listeriosis outbreak in goats. The disease has evolved in a group of six adult goats in March, favorized by the fact that the animals had free access to silage. Clinically, there were only nervous signs expressed by facial paralysis, incoordination in walking, spins in circles, swallowing difficulties, salivation, with no abortions. The disease remained localized in this group and no illnesses have been reported in the other animals of the farm. For the diagnosis, bacterioscopis and bacteriological examination were performed, pure culture of the strain was obtained streaking samples from the brain and cerebral bulb. Characterization of isolated strains was performed using Palcam and Oxford media, biochemical examination, both by classical tests (indole, hydrogen sulfide, catalase, hemolysis) and with the API Listeria Biomerieux. Isolates presented typical spects of *Listeria monocytogenes* to all tests performed (morphological, cultural and biochemical). Definite pathogenicity was determined by the guinea pig conjunctival test, which was positive. The specimen proved to be more sensitive to amoxicillin (32 mm), florfenicol (30 mm), gentamicin (28 mm), oxytetracycline (26 mm), erythromycin (26 mm), spectinomycin (24 mm), amoxicillin clavulat (24 mm), lincomycin (22 mm), flumequine (16 mm). Treatment using Penstrep, Gentamicin, duphalite and vitamin C did not work, sick goats died or were slaughtered. No disease has been furthermore reported, that was the end of the outbreak.

Keywords: clinical aspects, diagnostic, goats, listeriosis.

Introduction

Listeriosis is prevalent in many countries on all continents, with the natural geographic and climatic conditions vary widely. Basically, today can not discuss the existence of free areas of listeriosis. This spatial spread of the disease through large agglomerations was influenced by large animals, some special conditions and operating food and germ ability to have outstanding resistance to various environmental factors. In favorable conditions of temperature and humidity, the germ can survive long in soil, hydro sources, manure, feed and plants, and in some circumstances has the ability to multiply in these environments.

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Of the species from genus *Listeria*, *L. monocytogenes* exhibit definite pathogenic capacity, being considered as main pathogen *Listeria* infections in most species of animals and humans. However, in sheep is noted that *L. ivanovii* may cause septicemic forms (Sergent *et al.*, 1991) (Chand and Sadana, 1999) (Vasuţ, 2012). Isolation of listeria and cases of illness have been reported in many species of domestic mammals (cattle, sheep, goats, buffaloes, pigs, horses, dogs, cats), poultry (chickens, ducks, geese, turkeys, pigeons), wild mammals (rodents, carnivores, herbivores etc.) wild birds, laboratorz animals and zoological parks (rabbits, guinea pigs, mice, monkeys etc.) water vertebrates (fish, frogs), insects (ticks, flies, lice, fleas etc.), as well as insect larvae (larvae of *Oestrus ovis*), as well as from numerous sources of external environment (water, soil, plant, animal feed etc.) (Darie and Haroviuc, 1975), (Merck Manual, 2012).

Responsiveness to infection in the same animal species varies quite broad, being conditioned by extrinsic factors related to the external environment (especially climate and food) and intrinsic factors related to the affected host (age, physiological status, individual resistance, immune status). To these was added the factors relating to the etiological agents, especially pathogenic mechanism, and affinity for certain tissues, and especially the ability to travel from one cell to another without having to go into the extracellular space.

Sources of infection are extremely varied and plays an important role in the emergence, persistence, spatial and temporal distribution of disease cases. It is considered that *L. monocytogenes* is a bacterium that can survive telluric and even multiply in soil, water, forage, silage, within certain limits of temperature and pH.

Goats are susceptible to the disease especially towards the end of pregnancy, hormonal changes reducing the overall level of immunity. The germ can be eliminated via faeces and milk, intermittent or persistent. Clinic forms can evolve with septicemia, meningoencephalitis and nervous-abortive form (Perrin, 1996). It should be noted that in recent years, the importance of *Listeria* on public health has acquired a special significance, their isolation is frequently reported in various animal and vegetable products, making them important sources for the occurrence of disease in humans. These findings caused the listeriosis to become a notifiable disease and therefore in many countries have been initiated and implemented surveillance programs and prevention methodology and standardization of diagnostic and screening methods for high sensitivity. In our country the program of supervision, prevention and control of animal diseases, of diseases transmissible from animals to humans, animals and environment protection are surveilled in accordance with ANSVSA Order no. 79/2008.

Evolution of a listeriosis outbreak in goats, determined us to present the data found, especially since the disease in this species is rarely reported. On the other hand, milk and goat milk products, are getting an expansion in products and by increasing thus the risk to public health must be known if the cases of listeriosis occur in this species.

Materials and methods

Number of animals at the time the disease was suspected, consisted of 300 goats of different ages and races. Care was taken to shelters, respecting the technology for housing, feeding and biosecurity standards. A group of six goats, considered sparse, were kept in a makeshift shelter, where they had free access to silage. The epidemiological investigation s found that diseases occurred only in this group. They were clinically examined, the initial symptom in two animals found subsequently. Although it was subjected to treatment, two of goats diseased die. A body was sent to the Faculty of Veterinary Medicine in Cluj-Napoca, in order to perform necropsy and laboratory investigations to confirm the diagnosis.

Samples of brain, liver, lung and long bone unopened, were colected for the following exams: bacterioscopic (smears stained by Gram and methylene blue metho) bacteriologic (broth and streaks on blood agar plates, Palcam and Oxford culture media) biochemical examination (indole, hydrogen sulfide, catalase and API - BioMerieux SA for Listeria). A 24 hours colony was introduced in 2 ml sterile distilled water. From the suspension 100 μl were placed in a tune (the DIM test) and in other minitubes was distributed 50 μl/each tube. Gallery thus prepared was placed in moist chamber and incubated at 37°C for 18-24 hours. After incubation in DIM microtube test, Zym reagent is added and checked after 3 minutes. Interpretation of reactions was performed using read table provided by the manufacturer, and identification and confirmation with APIWEB software program. Mobility examination was performed on culture maintained at 37°C and 20-22°C, and by inoculation in semisolid agar. To verify pathogenicity, isolated strain was tested on guineea pig (conjunctival test). Antibiotic susceptibility was tested by disc diffusion method on nutrient agar using Mueller- Hinton medium and a set of microtablets with the following antibiotics: flumequine (UB), spectinomycin (SPT), oxytetracycline (OT) clavulat amoxicillin (AMC), ceftiofur (EFT), lincomycin (LS), florfenicol (FFC), amoxicillin (AX), gentamicin (GM).

Results

By conducting epidemiological investigation, it was established that illness occurred in March 2012, corresponding to the period of calving. Goats were affected only in the separate group that had access to silage. On examination of sick goats, was found salivation, loss of appetite, lack of mastication (the animals kept food in the mouth), thirst, difficult walking, paraplegia. There were no disease found in sheep, goats or the other animals in the shelters, although they feed silage too. Following the analysis of epidemiological data it was found excessive intake of silage and clinical development of nervous symptoms similar to listeriosis. Group was isolated and goats were treated with gentamicin, tonic (duphalite), vitamin C, but had no sensitivity. To eliminate a potential source of risk for the entire population, it was decided to slaughter and destroy by incineration the corpses.

Laboratory examination. Researchers unanimously considered absolutely necessary bacteriological examination to confirm the diagnosis, knowing that in clinical aspects can be confused with other diseases.

Bacterioscopic examination. In the smears made from the brain were found rare Gram -positive, thin, measuring 0.5/2 to 3 μm, isolated or diplo groups.

Bacteriological examination. Sowings made from the brain and bulb were positive and one strain of Listeria was isolated in pure culture. The development of the culture was performed at 37°C in aerobic conditions, for 18-24 hours.

In glucose broth was found moderate turbidity, which after maintaining the tubes several days at room temperature, lead to the formation of gray color sediment, which when stirred up lead to the appearance of a spiral and remains adherent to the bottom of the tube.

Examination of mobility in slide and coverslip highlighted that Listeriae culture maintained at 20-22°C were motile, while those maintained at 37°C were non-motile. Sowing on semisolid agar lead to the development of culture characteristic to listeria growth.

On nutrient agar was observed the formation of small colonies with a diameter of 0.2-0.5 mm, in the form of drops, round, translucent, regular edges and smooth surface. Examined in a beam of light at an angle of 45°, the colonies presented characteristic iridescent greenish blue reflections.

On blood agar (sheep) colonies had the characters described above, and were surrounded by a narrow zone of β-hemolysis.

On Oxford culture medium colonies developed within 24 hours, they were small and surrounded by a black area (Fig. 1). Within 48 hours the entire dish was black colored, colonies with a gray appearance, shiny. Examined using the magnifier, the colonies were round, cream-colored, granular surface with a central denser black background environment.

On Palcam (selective medium for isolation) was found after 24 hours of incubation, the formation of colonies of olive-green or gray-green color, with dimensions of 1.5-2 mm, sometimes with black center, but always surrounded by a black halo (Fig. 2).

In general the size of colonies growing on solid media depends on the degree of dispersion on the surface of the mediu, the larger the distance between the colonies is, observation time is prolonged to several days.

L. monocytogenes: colonies developed on solid culture media

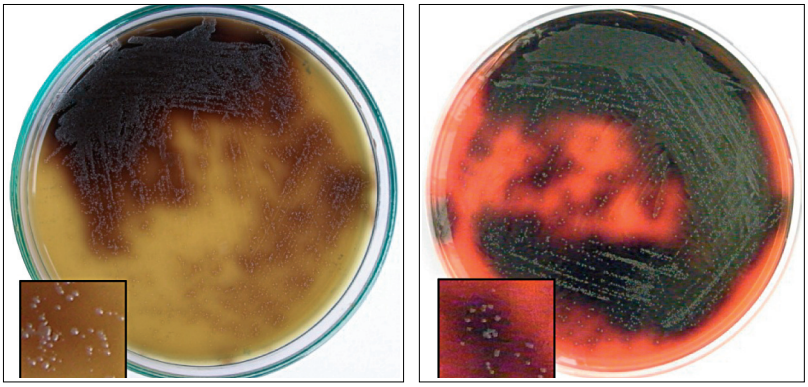

Fig. 1. Colonies on Oxford medium. Fig. 2. Colonies on Palcam medium

In smears stained by Gram method, was found the presence of germs with typical morphology of *Listeria monocytogenes*. The cells were Gram positive, intensely purple colored and and specifically arranged in the form of letter V and L, diplo, palisades or irregular arrangements (Fig. 3 and 4).

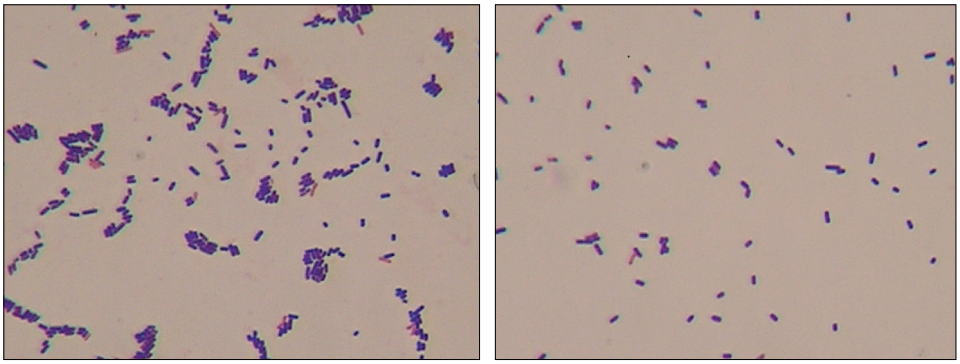

Fig. 3 and 4. *L. monocytogenes*: microscopic apparence (Gram stain x 1000)

Biochemical test on API Listeria system (BioMerieux), SA (standardized) allows the identification of *Listeria* species from mixed cultures and subcultures within 24 hours. The isolated strain had the following behavior: DIM (-), aesculin hydrolysis (+) α-manozidse (+), D-arabitol (+), D-xylose (-), L-rhamnose (+) methyl α-D-glucopyranoside (+), D-ribose (-), glucose-1-phosphate (-) D-tagatose (-). Analysing the diagram classification result, it was found that isolates behavior were characteristic to *Listeria monocytogenes* strains (Fig. 5).

Fig. 5. Biochemical exam result: API Listeria Biomerieux◊

Experimental infection on guinea pig performed by conjunctival test was positive. After 48 hours, it was found local reaction manifested by serous conjunctivitis that in the coming days became purulent, and complicated with keratitis (Fig. 6). The test is positive and so is the strain pathogenicity.

Fig. 6. Conjunctival test on guinea pig: purulent conjunctivitis

Antibiotic susceptibility testing revealed that isolates were susceptible to amoxicillin (32 mm), florfenicol (30 mm), gentamicin (28 mm), oxytetracycline (26 mm), erythromycin (26 mm), spectinomycin (24 mm), amoxicillin clavulat (24 mm), lincomycin (22 mm) and flumequine (16 mm).

Discussions

Listeria monocytogenes is the most important specie of the genus *Listeria*, having certain attributes of pathogenicity, being involved in the production of disease both in humans and in many animal species. Moreover, it is estimated that the number of cases has increased in recent years, having increased public health implications, which led to the design and implementation of epidemiosurveillance programs and compulsory declaration of the disease in many countries. The fact that the incidence is increasing is because the germ has outstanding resistance to environmental factors, can survive in different environments and under certain conditions of temperature and humidity can even multiply. In the opinion of many researchers, the multiplication of germs occurs most often in living organisms. Animals and people who have experienced illness or with clinical signs or unapparent may be carriers of *Listeria* for long time, and they multiplied in their body can be removed in the external environment through secretions (nasal discharge, milk) and excretions (faeces, urine). In animals infected, germ is localized and lives as a saprophyte in the digestive tract, airways, lymph nodes and other organs, exctering *Listeria* for a long time, especially in the faeces.

Is estimated that carriers of *Listeria* are numerous in both animals and humans. The natural reservoir appears to be the soil and mammalian digestive tract, especially cattle, which contributes to the amplification and dispersion of the germ in the farm. Some ribotypes may differ by their abilities

to infect animals and survive in the farm (Nightingale *et al.*, 2004). Grazing, animals ingest *Listeria* and contaminates the soil and vegetation. Transmission from animal to animal is made by fecal-oral route (Merck Manual, 2012). In diseased goats antibodies can be detected by agglutination tests, whose titer is within the 1/100 to 1/400 (Börku *et al.*, 2006). Additional information are mentioning antibodies identification using ELISA technique with monoclonal antibodies, test with a high specificity, sensitivity and can be distinguished from other pathogens (Bourry *et al.*, 1997).

Animal products (milk, meat, eggs) and some contaminated vegetables (lettuce, melons etc.) became important sources of infection for humans. In slaughtered goats, even clinically healthy, strains of *L. monocytogenes* are isolated (Ivanović *et al.*, 2009). Moreover, on various food-processing surfaces biofilms can form, thus increasing the strength and viability (Blackmann *et al.*, 1996). *L monocytogenes* can be isolated from mastitic cows milk, abortions and apparently healthy cows. Excretion in milk is commonly intermittent, but may persist for several months. Infected milk is a risk because *Listeria* can survive in certain types of pasteurization. Listeria has also been isolated from sheep, goats and women milk (Merck Manual, 2012). It is also noted that the goat milk can be infected in the absence of a history of listeriosis (Ménard *et al.*, 1993).

An important role for diseses incidence is represented by nutrition, with an equally important role on the onset of disease, as the virulence of the germ. Although listeriosis has a number of features related to the pathogenetic mechanism or influence of various predisposing factors related to climate, food, body resistance, outbreaks is sometimes surprising. In the case of small ruminants an important role is represented by the administration of silage, many data showing the direct correlation between the use of silage and disease frequency. In sheep, the use of poor quality silage in excessive amounts, is one of the main factors increasing receptivity to infection and the onset of explosive outbreak, sometimes with significant economic losses (Răpuntean *et al.*, 1999).

In goats an important role is represented by immunodeficiency states, hormonal changes occurring in pregnant goats and the possibility to induce acidosis as a result of feeding poor quality silage (Perrin, 1996; Braun *et al.*, 2002). This is incriminated in the production of disease in the outbreak studied, they almost exclusively feed silage with free and permanent access to the forage source. Moreover, the excessive consumption, lead to forestomach overload, which favored the pathogenic activity of *Listeria*. Clinical signs observed in diseased goats were dominated by nervous disorders, facial paralysis, motion changes, spins in circles, gripping and swallowing difficulties, salivation. There were no abortions, although one of the affected goat was pregnant.

Diseased goats have been treated with antibiotics (Gentamycin and Penstrep) for 5 days, associated with duphalite and vitamin C, but did not obtain any clinical improvement, although isolate proved to be sensitive to these antibiotics. The literature shows that the treatment involves the use of antibiotics, however, rarely produces improvement or cure, especially in the case of nervous type. Some data from the literature mentions improvments after combined treatment of gentamicin and ampicillin or penicillin G (Braun *et al.*, 2002; Merck Manual, 201) (Carp-Cărare, 2006). The lack of efficacy of antibiotics can be explained by the fact that *Listeria* are localized intracellularly and can even move from one cell to another, without persisting in the extracellular space and, on the other hand, damage caused to the nervous system are often irreversible.

Finally we quote Perrin, (1996), from his work on listeriosis in goats: At the end of the last century Louis Pasteur said "germ is nothing, the terrain is everything" and another eminent parasitologist, Professor Marotel said "take care of your sheep they will take care of their parasites". Today in the field of listeriosis, we might say "take care of your goats, they will take care of their *Listeria*".

Conclusions

1. Listeriosis evolved is a group of six adult goats in the spring season (March), the disease is favored by excessive silage feeding.
2. Clinically, there were only nervous signs expressed by facial paralysis, motion incoordinations, spins in circles, swallowing difficulties, salivation, there were no abortions.
3. The diagnosis was confirmed by bacterioscopic, bacteriological and biochemical exams. Pathogenicity was determined by conjunctival test on guinea pigs, which was positive.
4. Isolates proved to be sensitive to most of the antibiotics tested, the zones of inhibition ranging from 16-32 mm in diameter.
5. Treatment with Penstrep, Gentamicin, duphalite and vitamin C did not work, diseased goats died or were slaughtered.

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PHENOTYPIC ASSESSMENT OF ADIPOSE-DERIVED ADULT HUMAN STEM CELLS

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Introduction

Mesenchymal stem cells (MSCs) have been isolated from various tissues. These cells have the potential to differentiate to lineages of mesenchymal tissues, including bone, cartilage, fat, tendon, muscle, and marrow stroma. Adipose tissue provides a large numbers of stem cells as compared to bone marrow, with the ability to differentiate along multiple lineage pathways (Gimble *et al.*, 2007). The aim of this study was to isolate and characterized multipotent MSCs from human subcutaneous adipose tissue harvested after varicose vein surgery.

Materials and methods

Biological materials

Human MSCs were enzymatically (3 mg/mL collagenase type I) released from adipose tissues from adult human donors, the stromal vascular fractions were separated by centrifugation. $3,5x10^2$ cells/cm^2 were expanded and cultured in monolayer in DMEM/F12 (Gibco) culture medium supplemented with 10% fetal calf serum (FCS, Sigma), 5% horse serum (Gibco) 100 U/ml penicillin (Sigma) and 0.1 mg/ml streptomycin (Sigma). The cultures were maintained at 37° C with 5% CO2 in humidified atmosphere. The cell surface phenotypes were characterized at the 5^{th} passage using fluorescence-activated cell sorting. All flow cytometry measurements were made using a FACS Canto II flow cytometry system (BD Biosciences, San Jose, CA, USA) and analysed using the DIVA program.

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Differentiation assays

Osteogenic differentiation

For the osteogenic differentiation, $5x10^3$ cells were grown in DMEM/F12 supplemented with 50 μM L-ascorbic acid 2-phosphate (Sigma), 10 mM of β glycerophosphate (Sigma), 100 nM of dexamethasone (Sigma) 100 U/ml penicillin (Sigma) and 0.1 mg/ml streptomycin (Sigma). The cultures were kept in humidified incubator and the culture media were changed every three days. After 21 days, the cultures were fixed with 0,4% paraformaldehyde (Sigma) and were stained with alizarin red solution (40nM, pH 4.1) (Sigma) for 20 minutes.

Chondrogenic differentiation

For chondrogenic differentiation, $1x10^5$ cells were cultured in DMEM/F12 supplemented with 50 μM L-ascorbic acid 2-phosphate (Sigma), 10 μM insulin, 0.1 μM dexamethasone and 10ng/ ml TGF-ß1 (Sigma), 1% ITS-Premix (Sigma). The cultures were kept in humidified incubator and the culture media were changed every three days. After 25 days, the cultures were fixed with 0,4% paraformaldehyde (Sigma) and were stained with alcian blue solution (40nM, pH 4.1) (Sigma) for 20 minutes.

Adipogenic differentiation

For chondrogenic differentiation, $1x10^5$ cells were cultured in DMEM/F12 supplemented with 10 insulin (Sigma), 250 μM isobutylmethylxanthine (Sigma) for 24h. After this period the culture media were changed. The cultures were grown in αMEM supplemented with 10%FCS, 10 μg/μl insulin, 100 μM indomethacin for 21 days, the cultures were fixed with 0,4% paraformaldehyde (Sigma) and were stained with Oil red O solution for 10 minutes. The control cells were cultured in maintenance media.

Results and conclusions

The adipose tissue cells were isolated after enzymatic digestion with collagenase. The morphology (fig. 1.) of the cultivated cells was evaluated over the 6 passages. After 1st passage culture showed a heterogeneous morphology (spindle, stellate-shaped, round, flattened cells), but after passage 4 cultures were showed homogeneous morphology (fibroblast-like shape morphology, with the ability to form visible colonies after 3 days of plating).

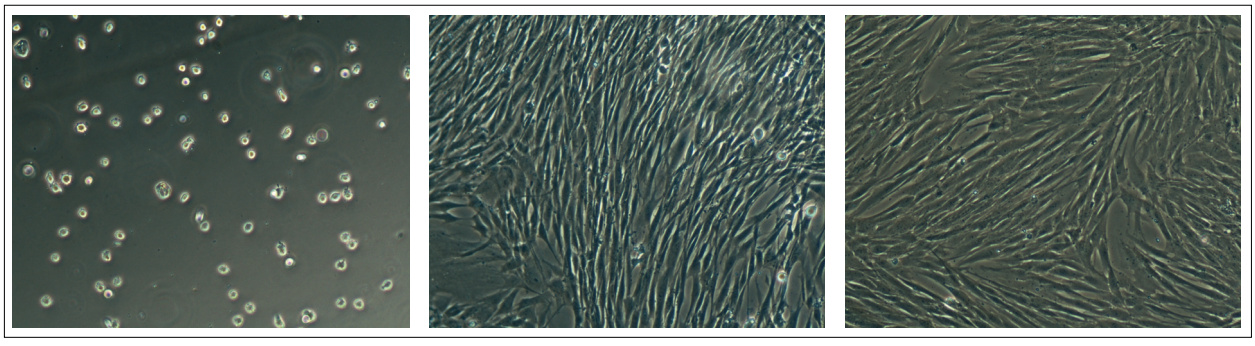

Fig. 1. The morphology of cells after isolation and the morphology of confluent cultures

The cultures were expanded for 5 passages in normal culture medium and were analyzed for the expression of cell surface molecules by flow cytometry (fig. 2).

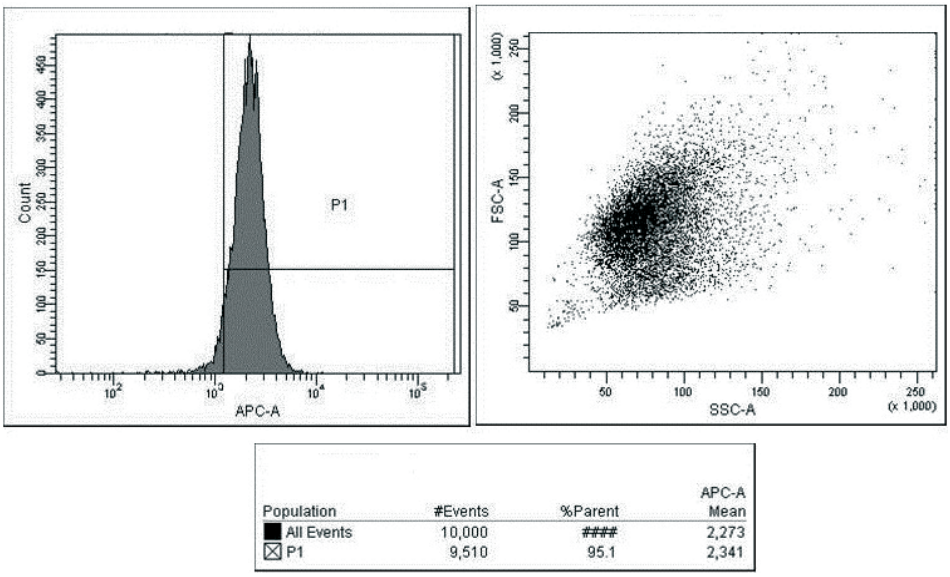

Fig. 2. Immunophenotypic profile of adipos tissue derived stem cells

After 5 passages the adipose tissue derivate stem cells cultivated in osteogenic medium showed morphology similar to osteoblasts after 21 days of directed differentiation, with the presence of osteogenic nodules and calcium precipitates confirmed by alizarin red staining (fig. 3).

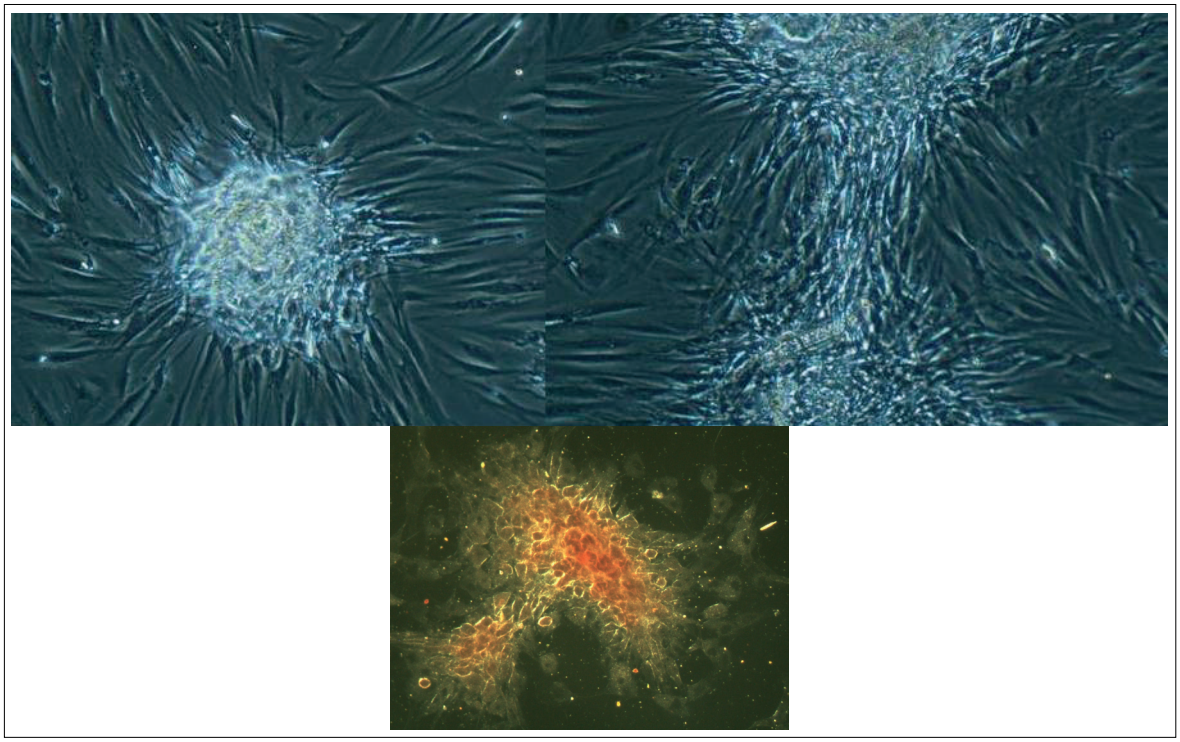

Fig. 3. The morphology of differentiated cells - osteogenic nodules

After chondrogenic induction, the cells showed similar morphology to chondrocytes, evidenced by Alcian Blue staining specific for the sulfated proteoglycans of cartilage matrices (fig. 4).

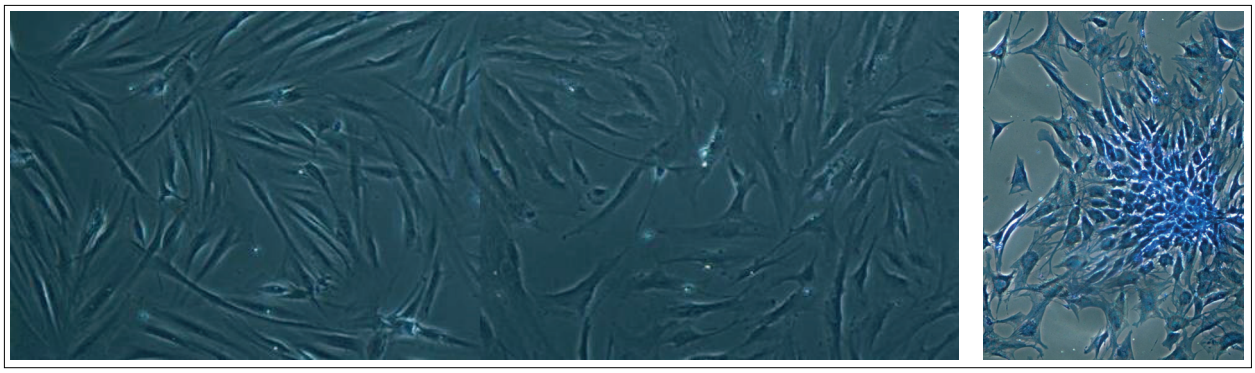

Fig. 4. The morphology of differentiated cells – chondrogenic differentiation

After adipogenic differentiation, the characterized cells containing small lipid vesicles exhibited an expanded morphology with the majority of intracellular space, after the terminal differentiation big lipid vesicles inclusions were found in the cytoplasm, indicating the adipogenesis, confirmed by Oil red O staining.

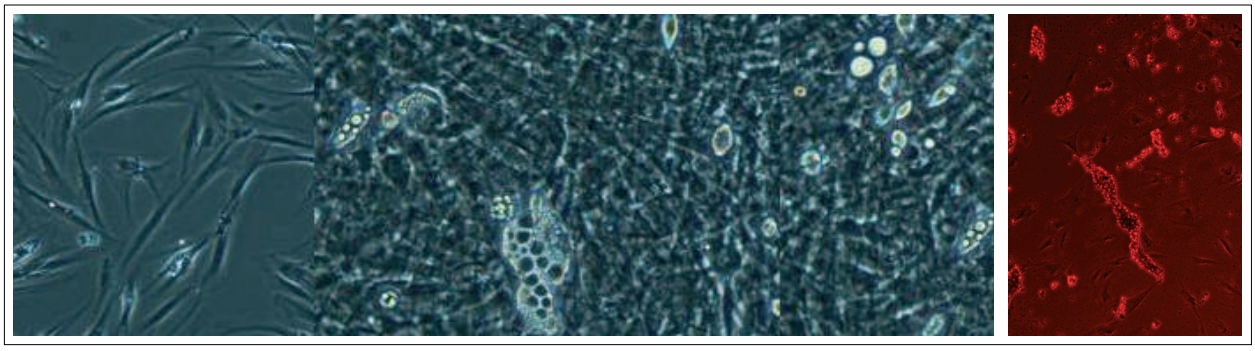

Fig. 5. Adipogenic differentiation of adipose tissue derivated stem cells – lipid droplets

Our data confirmed that the adipose derivate MSCs cells have multipotent (Jaiswal *et al.*, 2000) character based on specific surface antigen expressing (CD44, CD34/45) with capacity of differentiation on multiple lineages *in vitro.* Adipose tissue stem cells can be easily harvested from adipose tissue and expanded, this cells may be an major and less-invasive source of mesenchymal stem cells (Katz *et al.*, 2005, Dicker *et al.*, 2005, Zhu *et al.*, 2008). This biological material as a source for obtaining adult stem cell has been described earlier (Zuk *et al.*, 2002, Aust *et al.*, 2004, Estes *et al.*, 2004, Dubois *et al.*, 2005, Mitchell *et al.*, 2006). A wide range of transcription factors are responsible for regulating the biology of stem cells (Zhu *et al.*, 2008). Also molecular markers identified in embryonic stem cells are involved in maintaining the characteristic features of these cells (Remenyi *et al.*, 2003, Zhu *et al.*, 2008).This study indicate that adipose tissue-derived stem cells represents a very promising cells sources for regenerative therapy and for clinical applications . Using autologous adipose tissue for isolation and expansion of adult stem cells may reduce the side effects that may occur in heterologous transplantation.

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STUDY OF LINGUAL RHABDOMYOMA COMPLICATED BY GLOSSITIS IN SHEEP (CASE REPORT)

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Abstract

Rhabdomyomas are rare, benign straight muscle neoplasm . We report the clinical and morphological feature of Rhabdomyoma apparently present in the left anterior tongue of ewe.

The animal presented for surgical removal of an enlarging lingual neoplasm . Histological examination of this lesion confirm the diagnosis of Rhabdomyoma with glossitis and no evidence of recurrence of this neoplasm after initial resection

Keyword: Rhabdomyomas, muscle neoplasm, glossel surgery

Introduction

Rhabdomyoma is a benign mesenchymal tumor of skeletal muscle, which mainly occurs in the head and neck (Boysen *et al.*, 2007), separated into two major categories based on site: Cardiac and extracardiac. They are further separated by histology: fetal (myxoid and cellular), juvenile (intermediate), and adult types. Genital types are recognized, but are often part of either the fetal or juveline types. The fetal type is thought to recapitulate immature skeletal muscle at about week six to ten of gestational development.(Kapadia *et al.*, 1993)

Rhabdomyomas are benign solitary or multiple neoplasms that originate from striated muscles (Meuten, 2002). Rhabdomyomas can occur in the myocardium, skeletal muscles of the larynx, and in the head region in both humans and animals (Meuten, 2002; Radi, 2006) In domestic animals, cardiac rhabdomyoma has been reported most frequently in swine and rarely in cattle, sheep, and deer (Bradley *et al.*, 1980; Kolly *et al.*, 2004; Meuten, 2002; Tanimoto and Ohtsuki, 1995).

In gross pathology The tumor may be seen within the subcutaneous tissues (below the skin), (Walsh and Hurt, 2008) mucosal surfaces or in soft tissue. Within the head and neck, the posterior ear region, skin of the face, and the tongue are the most commonly affected sites (about a 2:1 ratio of soft tissue to mucosa) (Kapadia *et al.*, 1993; Willis *et al.*, 1994). The tumors are well defined, non-specific usually solitary masses, but when seen in the head and neck (or genital region), they may be

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polypoid. Tumors range in size from a few millimeters up to 12.5 cm, with a mean of about 3.0 cm. Although there are isolated case reports, multifocality is very rare. (Kapadia *et al.*, 1993)

In human Adult rhabdomyoma can be seen in all ages but these tumors are most common in adults older than 40 years old and they are three times more common in male (Kapadia *et al.*, 1993). Occasional cases are seen in children (Huang *et al.*, 2012). Adult rhabdomyoma are most frequently seen in the head and neck area (Hansen and Katenkamp, 2005; Vuong *et al.*, 1990) and rare cases occurring in mediastinum (Zolota *et al.*, 2006; Sidhu *et al.*, 2002) and other locations have also been reported. Although most cases are solitary tumors, multifocal cases can occur (Liess *et al.*, 2005; Koutsimpelas *et al.*, 2008) and the tumors are found within the head and neck area. Clinically, they are painless mass with a slow growth rate. Incidental findings during autopsy can occur. As may of them are found in the larynx and base of tongue, obstruction of the airway and difficulty in swallowing are common manifestations (Hassell *et al.*, 2012). These tumors has characteristic histologic features. Adult rhabdomyomas have similar features among tumors and composed of a checker board arrangement of large polygonal cells with solid amphopilic to eosinophilic cytoplasm and cells with a large cytoplasmic vacuole. Striations reminiscent of striated muscle can be seen (Hassell *et al.*, 2012).

Glossitis is inflammation of the tongue. It causes the tongue to swell and change color. on the surface of the tongue (papillae) may be lost, causing the tongue to appear smooth. (Liran and Yehuda, 2007)

Materials and method

Ewe 3 years old was transferred from a clinic from Qurna city for evaluation of a large mass in the left anterior tongue (Fig. 1 and Fig. 2), difficult to feed. Total surgical excision was made, representative tissue samples were collected, fixed in 10% buffered formalin, routinely processed to prepare hematoxylin and eosin (H&E) slides, and evaluated by microscopic examination.

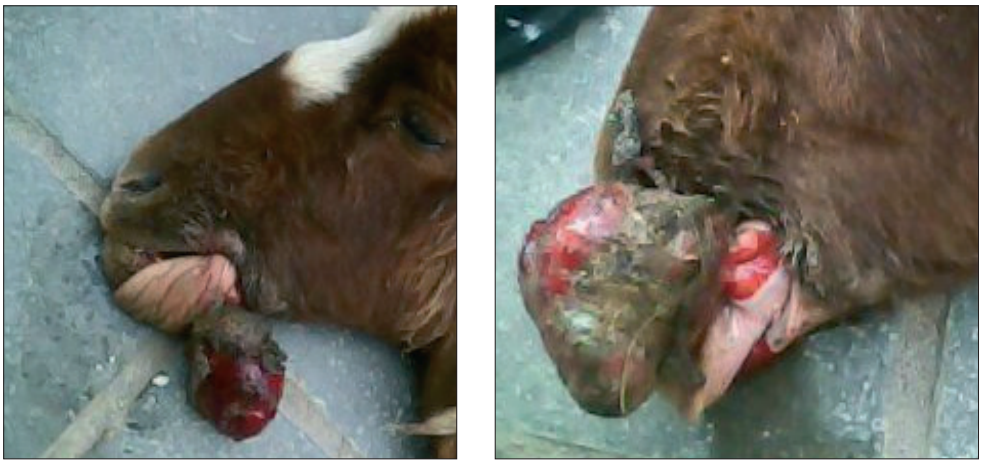

Fig. 1-2. A large mass on the left interior tongue

Results

The neoplasm consisted of tightly arranged, large, variably sized, ovoid to irregular, swollen myocytes The cells had variably distinct cell borders with a deeply eosinophilic cytoplasm and varying degrees of cytoplasmic vacuolation. The nuclei were single, oval to elongate, peripherally located (Fig. 3 – Fig. 11).

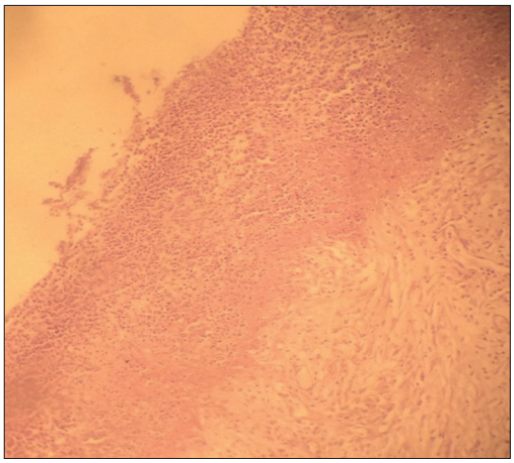

Fig. 3 Tongue a mass of lingual skeletal muscle associated with infiltration of inflammatory cells (glositis) 10X H & E

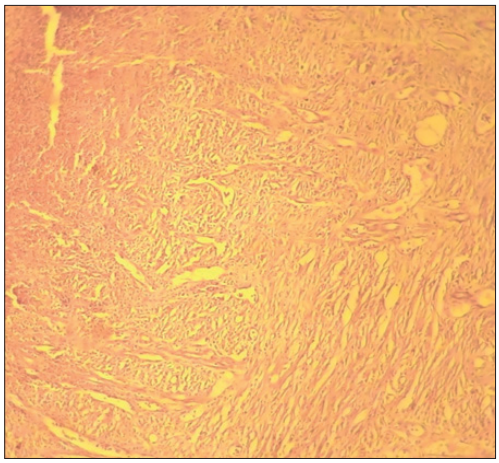

Fig. 4 Tongue a mass of lingual skeletal muscle associated with infiltration of inflammatory cells (glositis)10X H & E

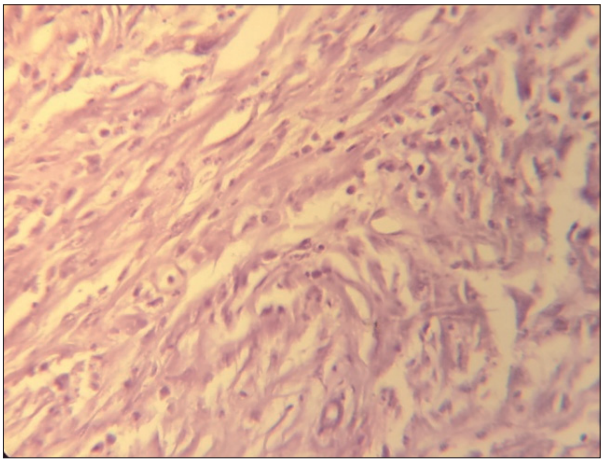

Fig. 5 The musculoskeletal structure of tumor 100 X H & E

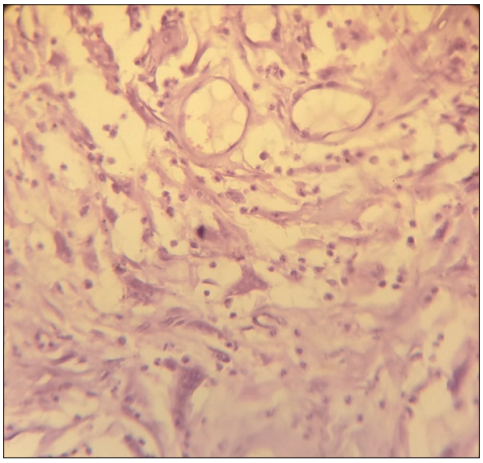

Fig. 6 High power skeletal muscle cells with some vaculation other with prominent nuclei also infiltration of inflammatory cells and high vascularization 100X H & E

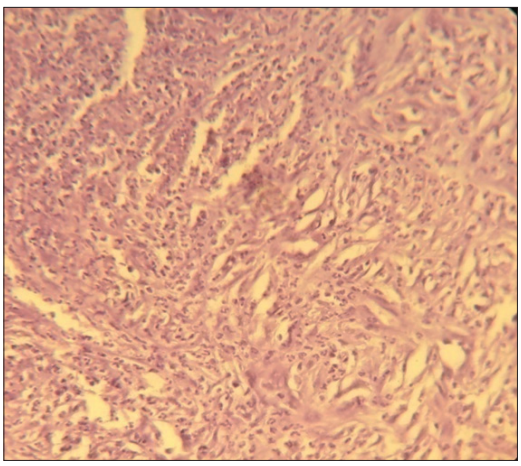

Fig. 7 High power heavy infiltration of inflammatory cells between skeletal muscles cells of the tumor 40X H & E

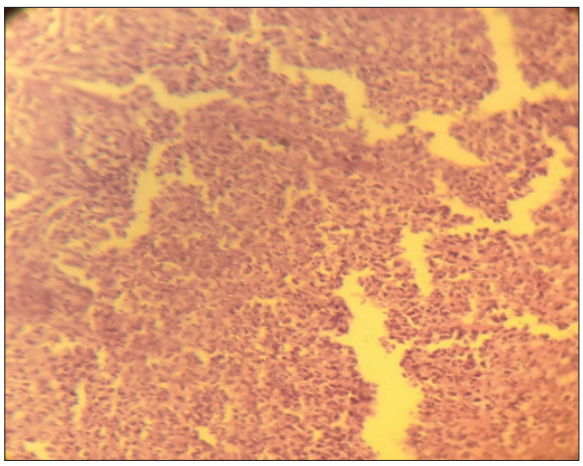

Fig. 8 Tongue mass of lingual skeletal muscle associated with infiltration of inflammatory cells (glossitis) 10X H & E

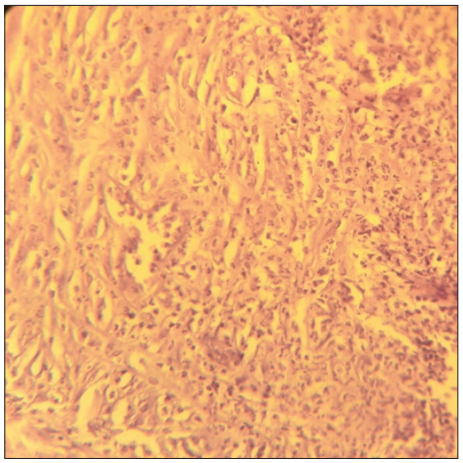

Fig. 9 High power of musculoskeletal structure of the tumor tongue mass of lingual skeletal muscle associated with infiltration of inflammatory cells (glossitis) 40X H & E

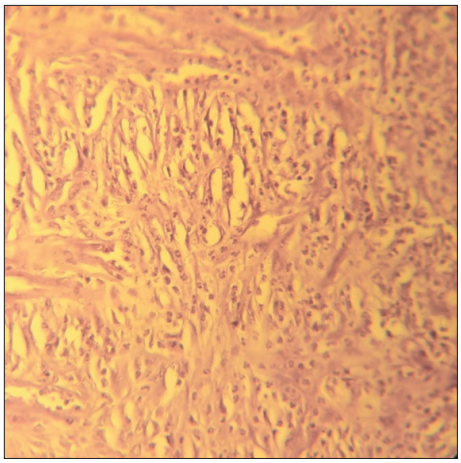

Fig. 10 High power of skeletal muscle cells with some vacolation other with prominent nuclei also infiltration of inflammatory cells and high vascularization 40 X H & E

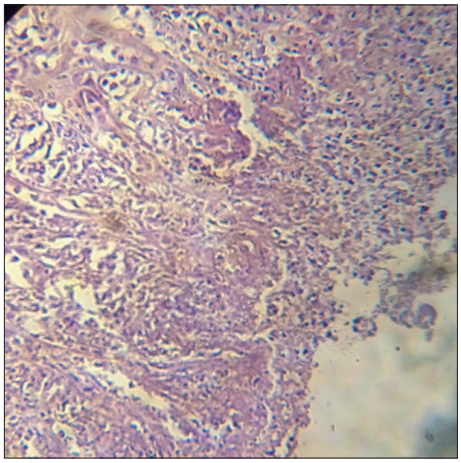

Fig. 11 High power heavy infiltration of inflammatory cells between the skeletal muscle of the tumor40X H & E

Discussion

The rare occurrence of rhabdomyoma and its relatively benign nature makes a histological diagnosis very necessary, though often difficult to make

Before we make histological section, Grossly, this tumor (rabdomyoma) is benign, it is in accordance with (Hassell *et al.*, 2012) who reported benign tumors are in centimeter range and have well circumscribed pushing margins. It can occur as a pedunculated mucosal or multinodular mass. The cross section is solid and finely granular with a tan to red-brown color. Necrosis should not be seen.

Histopathological study shows skeletal muscle cells with some vaculation other with prominent nuclei of skeletal muscle cells this findings agree with (Vuong *et al.*, 1990) who reported rhabdomyomas are composed of bland, primitive spindled cells. The spindle cells are haphazardly arranged primitive, elongated skeletal muscle cells. There are often large ganglion cell-like rhabdomyoblasts showing prominent nucleoli within nuclei that show vesicular chromatin distribution. tumors show short to more sweeping fascicles of spindled rhabdomyoblasts. The tumor cells may infiltrate into adjacent skeletal muscle or fat (vaculation).

Histological section of the tongue mass show this tumor with heavy infiltration of inflammatory cells between the skeletal muscle of the tumor (glossitis) this result in opposite to (Yehuda *et al.*, 2010) and (Rivera and Carlton, 1992) who reported Poor hydration and low saliva in the mouth may allow bacteria to grow more readily.

Conclusion

Among laboratory animal species, no reports of rhabdomyomas are available in sheep. Spontaneous lesions in the tongue of dogs are rare in studies. This report represents the first description of rhabdomyoma in ewe . Surgical excision is the treatment of choice. There is no role for chemotherapy or radiation therapy.

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TECHNIQUES OF ARTERIOPLASTY USING PATCHES MADE FROM SYNTHETIC PROSTHESIS IN SWINE

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Abstract

In the present study are described possibilities to intervene in case of substance loss at a vascular level by replacing a segment of the vessel with a synthetic graft. Synthetic grafts are used more and more in modern medicine. The most used grafts remain the ones made out of EPTFE and Dacron. Our study presents the utilization of grafts in angioplasty who work similar to patches. Five pigs were used for this study, three for Dacron and two for EPTFE. For the visualization of how the patches work, a linear arteriotomy was performed on the aorta artery situated infra-renal followed by fixating the patch with a continuous suture and minimizing the loss of blood. Postoperative, the pigs were observed for 30 days. The tolerance of each pig towards the patches was good, we didn't have any accidents postoperative. Some modifications were seen at the hind limbs extremities in the first 12-16 days, those were edemas, hemiparesis, prolonged decubitus.

Keywords: synthetic patch, pig, angioplasty, artery.

Introduction

There are numerous situations of loss of vascular tissue (wounds, septic processes, tumoral processes) when is required the substitution of a piece of the vascular wall. The piece that was replaced can be large or small, depending on the dimension of the vessel, the localization and the properties of the material used for the substitution. The prosthesis used have to respect some demands to be able to substitute the healthy tissue that was replaced.

For this, some aspects must be known, such as: biocompatibility with the new organism, elastic properties must be similar to the one of the vessel wall that was replaced, resistance to infections, to

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not cause thrombus, to have the possibility to be fixated to the vessel wall affected. Our study was done using 5 pigs of 6,5 months of age from the great white breed, weighing 60 kg, on which were used Dacron prosthesis (3 individuals) and EPTFE (2 individuals).

Before the fixation of the patches, arteriotomy was done on a length of 3-5 cm along the vessel with an intrarenal localization. The patch was made and fixated on the certain place using a continuous suture from one of the edges until the complete cover of the defect produced. A careful hemostasis is done, the abdominal cavity is closed, and after the surgery the animals were observed for 30 days and monitored for general condition and local modifications. In the first days after surgery, we observed local modifications manifested trough difficulties in movement, the appearance of edema of the hind limbs extremities, prolonged decubitus, uncertainty on the hind limbs in standing and in walking, for a period of 12-16 days in all individuals.

The purpose of this study is to appreciate and verify the behavior of patches made out of synthetic material in swine arterioplasty by using two synthetic prosthesis, Dacron and EPTFE.

Method and material

The study was conducted in the surgery clinic of FMV Cluj-Napoca in the period 2012-2014 on a number of 5 pigs from the Great White breed with a weight of 60 kg each, clinically healthy. On 3 of them were used prosthesis made out of Dacron (polyethylene terephthalate). For the fixation of the patches was used suture wire of Prolene 5-0. For the other 2 individuals we used prosthesis made of EPTFE (polytetrafluoroethylene) with a thick wall. For the fixation was used suture wire made of Gore Tex 5.0.

From all the subjects were taken blood samples for hematological examination.

The instrumentation used was the adequate one: vascular forceps, vascular needle holder, dissecting forceps, nontraumatic vascular clamps, autostatic clamp, spreaders, etc.

For the surgery the pigs were prepared by diet of 12 hours, 30 minutes befor the surgery was applied premedication with Atropine 0,2ml s.c., Diazepam 2mg/kg i.v. and Ketamin 2mg/kg i.v. trough an intravenous catheter placed in the external auricular vain. General anesthesia was mentained by narcosis with 2% Isofluran. During surgery and after, the animals were treated preventively with heparin serum i.v. 30 microL/kg of heparin.

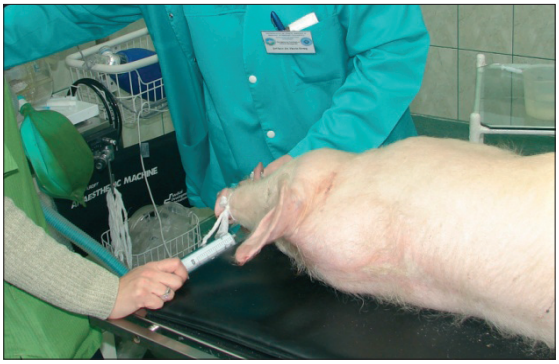

Fig. 1. General anesthesia by narcosis

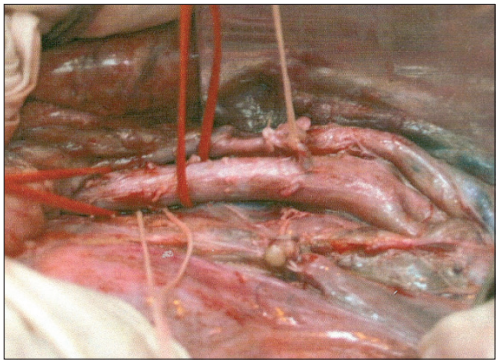

Fig.2. Isolation of the infra-renal aortic artery

The abdomen is prepared for the surgery through applying the rules of asepsis and antisepsis (clipping, washing, disinfection). The animal is placed in dorsal recumbency and the abdominal cavity is opened trough an incision on the midline, going trough the skin, fatty tissue, abdominal wall and the parietal peritoneum. The organs from the abdominal cavity are protected with sterile fields as to identify the infra-renal region of the abdominal aortic artery (Fig. 2.). With great care is done

the incision of the retro-peritoneum and the aortic artery is isolated distally from the renal arteries. Before clamping the aorta, the prosthesis are prepared by taking 5ml of blood and purging it in the gaps. After that the aortic artery is clamped with vascular forceps and the arteriotomy is done with a Potts scissor (Fig. 3.), in a straight line on the long axis of the vessel on a width of a few millimeters. After creating the defect, the patch is prepared according to the shape, size and elasticity (Fig. 4.).

For both methods were used the classic technique and the "parachute" technique.

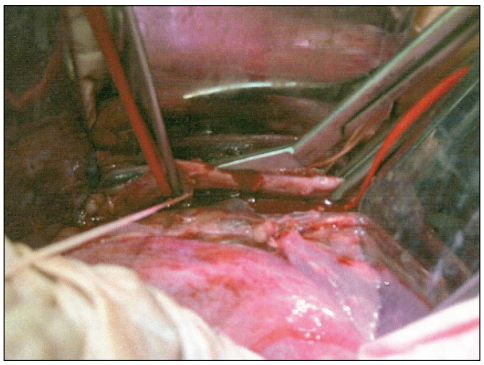

Fig. 3. Arteriotomy using the Potts scissor

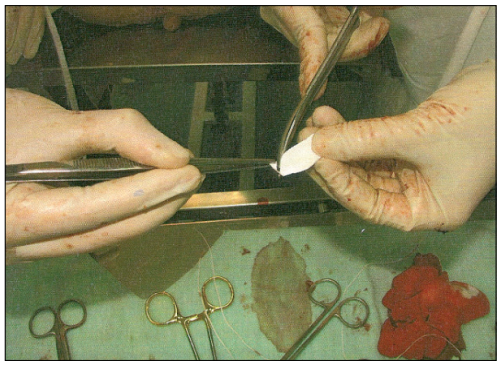

Fig.4. Preparation of the synthetic patch

The classic technique follows the fixation of the patch at the level of the two angles trough 4 quarters of continuous wire suture. The wires were tied two by two at the middle of anastomosis trance. The "parachute" technique implies forming the patch's width considering the extremities. The suture started by placing the first wire 4-5 mm above the superior angle of the arteriotomy. We made 3-4 suture points on each side of the trance. The patch was left longer and the distal extremity was shaped according to the defect. This method is preferred, because it allows the keeping of the round distal extremity of the patch and the precise fixation of suture points on the sides of the distal edge. We continued in this way with 5-7 suture points around the distal extremity before the descent of the patch and the tightening of the wire. The suture on the two edges of the anastomosis trance follows and when the suture is 5-10 mm away from the back angle, the artery is unclamped gently, with great care as to observe if bleeding appears or if there are any other local lesions. The abdominal cavity is closed backwards, ending with the suture of the skin in separate points. The operated animals were supervised 14 days trough measurement of the physiological indicators and local modifications appeared.

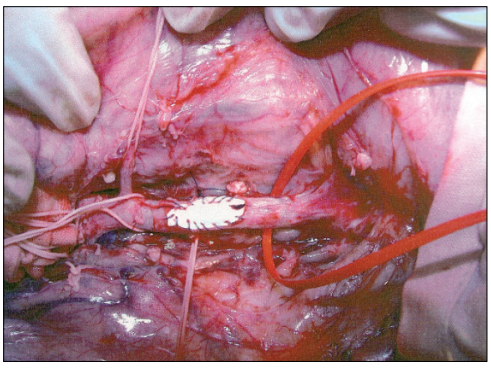

Fig. 5. Angioplasty with synthetic made prosthesis made from EPTFE

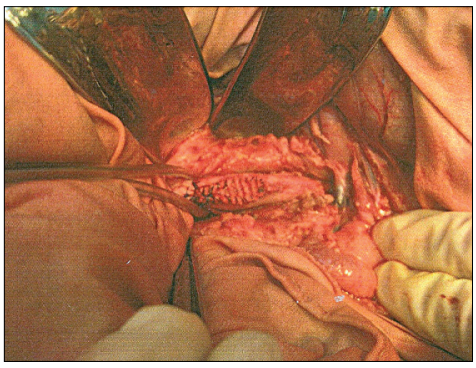

Fig. 6. Angioplasty with synthetic prosthesis made from Dacron

Results and discussions

The response of the organism after the implant of the vascular prosthesis takes place right after the restore of blood flow trough the interference of the tissue with the prosthesis and the blood

with the prosthesis. This interference can be responsible of the prosthesis's permeability because it represents a complex microenvironment where the physical and chemical properties represent safety elements at this level.

In accepting the prosthesis by the organism the plasmatic protein absorption phenomenon must be known, followed by the deposit of plaques, white cells, migration of endothelial cells and the smooth muscle cells, the presence of the fibrin network that contains plaques and which fill the holes of the prosthetic wall. In the case of our study arterioplasty with patches at the level of the aortic artery in pigs was done with good results, the patch didn't produce any modification of caliber of the vessel in the implant area. The suture was sealed, without bleeding on the suture line after the removing of the clamp. Sealing presumes having knowledge of some particular rules, the vessel has to be manipulated by clamping only the peri-arterial tissue or the tissue of the adventitia. In the case that direct manipulation is inevitable, the arterial wall must not be caught between the branches of the forceps to not produce any lesions at the intimate level or to the whole vessel wall with appearance of local necrosis aspects that would compromise the sealing of the vessel. Another aspect is that of the arterial wall suture that is recommended for the needle to go from the inside-out to prevernt the formation of fringes that can produce embolism or thrombosis. The inner line of the suture must be smooth, otherwise facilitating the aggregation of plaques and compromising the anastomosis. The suture wire are non-resorbable, monofilament which will not be tightened with the surgical forceps to avoid braking them and compromising the anastomosis. To avoid adherences at the surgery spot we covered the patch with the vessel's adventitia and pieces of the retro-peritoneum. The postoperative care is very important especially vascular collapse, cardiac arrhythmia, ventricular tachycardia, thrombosis prevention, inflammations or collections of serohemoragic liquid. In this sense there have been administered Dextran40 5ml/kg, Ringer lactate 50ml/kg, baking soda 0,6mEq/kg.

For the attenuation of the inflammatory phenomenon we administered Flumixin meglumine 2,0mg/kg i.v. twice daily, once every 12 hours for 3 days. For preventing infections we administered antibiotics 7 days. Prevention of thrombosis was done with heparin serum 1ml i.v. every 6 hours for 7 days and continued with Trombostop oral 1tb/day for 10 days.

They were monitored the whole time: internal temperature, mucosa aspects, capillary refill time, cardiac frequency, arterial pulse, breathing frequency, bleeding time, coagulation time and other modifications.

In the following days, the cardiac and respiratory frequency were at the high limit of the normal value range while the temperature was oscillating being feverish in the first 3 days after surgery and becoming normal after.

The patches we used behaved functional, without reactions from the animals, being well tolerated. The experimental model is compatible and sustainable under biological and functional aspects, and it can very well be applied in pathological situations.

A remark must be made that starting with the second day the operated pigs preferred prelonged decubitus, and movement was made with difficulty, presenting a transitory paresis. Also s.c. edemas were observed at the hind limbs accusing moderate pain that disappeared in the next 5 days. Feeding has decreased for 5 days after surgery and regained in the next 10 days. We have not observed other local or general modifications or complications. Concerning the two synthetic materials used Dacron and EPTFE we can say that the EPTFE patch was easier to manipulate because of the higher elasticity and flexibility properties.

Conclusions

1. The synthetic patch can be used in pigs with parietal defects and can represent a very good experimental model in surgery.
2. Biointegration and tolerance of the prosthesis fron Dacron and EPTFE is of very high quality, durable and without complications.
3. Implementation of patches pre and intra-operatory has to be the same as a complete prosthesis respecting all the conveniences of a bio-prosthesis.
4. The short time required for the procedure contributes to the quick recovery of the operated animals and to the good evolution.

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ULTRASONOGRAPHIC EXAMINATION OF PREGNANCY AT *PYTHON BIVITTATUS*

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Abstract

Increased interest in snakes as pets caused an increase in interest in breeding them in captivity. Therefore it is necessary to know the detailed characteristics and common anatomical aspects of these reptiles by veterinarians and breeders to identify the diseases that may be present in them. Ultrasound examination is an important non-invasive method in identifying individuals who have reached sexual maturity and the stage of development of the egg in *Python bivittatus* (oviparous snakes).

Keyword: *Python bivittatus*, ultrasound, oviparus, snake pregnancy.

Introduction

Being relatively easy to breed, snakes are increasingly common as pets. Therefore, for successful reproduction of snakes in captivity is needed a detailed knowledge of reproduction behavior and physiology of these animals. Although the snake breeding occurs naturally, biological and anatomical knowledge of reproduction help diagnose clinical aspects quite common in captivity. The easiest way to determine pregnancy stage in *Python bivittatus* is by using the ultrasound technique. This technique provides a dynamic view of pregnancy stage and the evolution of the eggs.

Python bivittatus sexual maturity is determined by body size, age playing second criteria (Porter, 1972). In snakes breed in captivity, body mass can evolve quickly, beeing influenced by the environment and feeding pattern, that resulting in individulad that could reach sexual maturity at 18 month, while thouse breed in natural condition reach sexual maturity at the age of 2 or 3 yeras (Tinkle and Gibbons, 1977; Blackburn, 1992; Mader, 2006).

Ultrasound examination play an important role in snake diagnostics, beeing easy to use, radiographic examination is not so common for pregnancy diagnostics because the thin eggs shell (Mader, 2006).

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Material and method

The ultrasound examination was performed on a total of 3 females of Python bivittatus species. The length of specimens was between 1.8 and 2.2 m and body weight was between 14 kg - 30 kg.

Restraint can be done manually or by sedation. For large specimens, that present aggressivity is recommended to performed anesthesia or sedation. Sedation can be done with Ketamine 22-44 mg / kg sc, im (Bennett, 1996), and the anesthesia with ketamine 10-30 mg / kg + butorphanol 0.5-1.5 mg / kg im (Schumacher, 1996). The restraining of examined specimens was done manually without anesthesia or sedation. The restraint of large individuals was performed by two or three persons. When restraining the snake it is necessary to immobilize the head to avoid bites (fig. 1)

Fig. 1. Snake restraining for ultrasonographic examination

The ultrasound examination was performed on a Mindray DC-6 devices, equipped with linear and convex probes.

Examination of coelomic cavity was performed by lateral intercostal approach, using ultrasound linear probe with 7.5 to 10 MHz. B mode ultrasound was used to assess two-dimensional aspects of the ovary and Doppler mode has been used to assess hemodynamics.

Correct application of ultrasound gel prevents artifact formation. Ultrasound gel had to be applied 10-15 minutes prior ultrasonographic investigation, to be absorbed by the scales. Ultrasound gel is indicated for use in high volume because it will remove the air that is present under the scales.

Results and discussions

For ovary examination gallbladder will be use as a benchmark, the gallblader is easy to be identify in larger individuals, but present high mobility. Large individuals are hardly to be restrained; body is rich in muscles, arches continuously, which cause the gallbladder to change its position.

In sexualy imature female, the ovary is small and is not fully visible by ultrasound examination. In sexual mature female the ovarian follicles are visible caudal to gallbladder. Ultrasound examination may evidentiate different stages of follicular development. In Python bivittatus species, half the egg is occupied by hypoechoic albumin, and the other half is occupied by echogenic yolk (Mannion, 2006). Time clutch may be determined by the disappearance in the egg yolk, which took place approximately one week after the yolk is no longer visible by ultrasound (Denardo, 2006).

In Python bivittatus species during pregnancy, the ovary present a previtelogenic stage and vitelogenic stage (Stetter, 2006). In previtelorgenic stage the ovary is organized as a cluster of follicles, anechoic, size below 1 mm.

In vitelogenic preovulatory stage follicles are organized in a linear maner, having dimensions of about 1 cm, with central area presenting a higher echogenity than the peripheral area (Fig. 2, Fig. 3).

In the postovulatory stage ovas are arranged linearly, have a sizes of 1-2 cm, the center area is hypoechoic or anechoic, present a peripheral echogenic area and begins to form crust (shell) (Fig. 4). In more advanced stages content is organized, the egg have an elongated appearance (Fig. 5, Fig. 6). Before time clutch, eggs migrate into the caudal portion of the oviducts, having hyperechoic margins (Fig. 7).

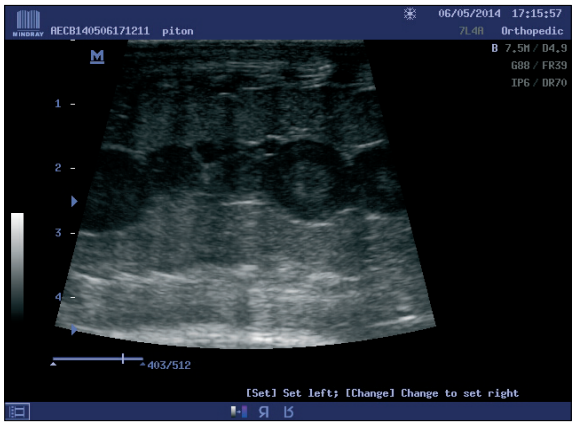

Fig. 2 Previtelogenic follicles (white arrow), vitelogenic preovulatory follicles (blue arrow), *Python bivittatus,* 7,5 MHz frequency, intercostals approach

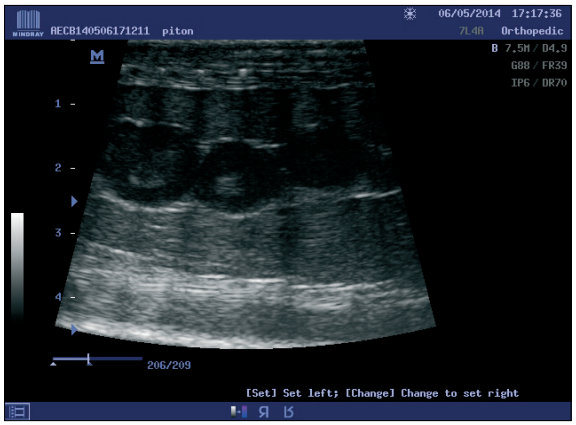

Fig. 3. Vitelogenic preovulatory follicles, *Python bivittatus,* 7,5 MHz frequency, intercostals approach

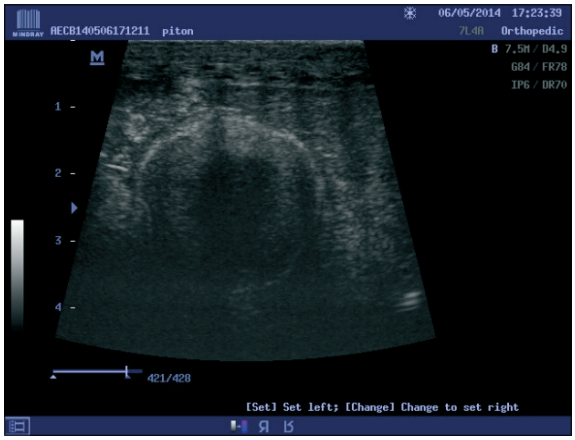

Fig. 4. Postovulatory stage, *Python bivittatus,* 7,5 MHz frequency, intercostals approach

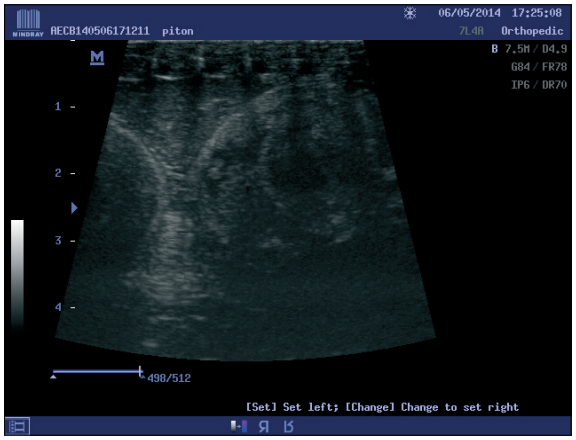

Fig. 5. Postovulatory stage, *Python bivittatus,* 7,5 MHz frequency, intercostals approach

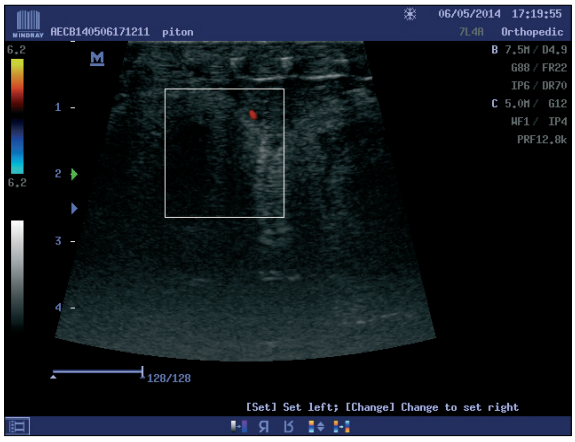

Fig. 6. Ova aspects in the postovulatory stage, *Python bivittatus,* 7,5 MHz frequency, intercostals approach

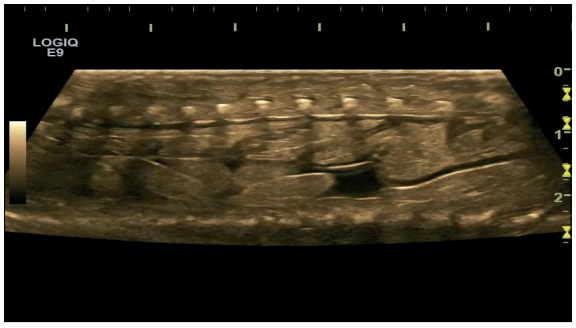

Fig. 7. Eggs in the oviduct, 10 MHz frequency, intercostals approach

Conclusions

Ultrasound is a non-invasive diagnostic method and can be routinely used for monitoring the reproductive status of *Python bivittatus*, the evaluation of ovarian activity, the ovulation and the stages of ovarian follicles, as well as determining the presence of eggs in the oviduct, playing an important role in the management of reproduction in this species. Doppler technique is not relevant for assessing egg vitality.

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EFFECT OF USE CONEFLOWER (*ECHINACEA PURPUREA*) AND VIRGINIAMYCINE ON PERFORMANCE, SOME BLOOD PARAMETERS AND ANTIBODY TITER AGAINST NEW CASTLE VACCINE ON BROILER CHICKS

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Sayeed Masoud DAVOODI[2]

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Abstract

This study was conducted to investigation the effect of use coneflower and virginiamycine on performance of broiler chicks a total 240 one day broilers chicks (Ross 308) were divided into 4 groups of 20 birds each and assigned to 3 treatment diets. Chicks were fed by basal diet as control diet, 2% cornflower (T_1), 2% virginiamycine (T_2). At the end of trial for carcass evaluation 4 birds form each group were slaughtered. In addition some parameters such as feed intake (FI), body weight gain (BW), and feed conversion ratio (FCR) were calculated and compared together. Some blood parameters such as Cholesterol and Triglyceride of blood were determined. Sample of blood were taken for antibody titer against new castle vaccine evaluation on 28, 36 and 42 days old chicks. Data showed no significant difference for feed intake in experimental groups. Chicks were fed with T_2 diet was higher weight gain compared to others. This result showed that all treatments have better final result in compared to control. Liver percentage was significantly decreased ($p<0.05$) were broilers fed with T_1, T2 There were no significant differences in for Heart percentage between treatments. The use of T_1, T2 lead to reduce abdominal fat percentage statistically ($p<0.05$). Drumstick percentage was increased were broilers fed with T_1,T_2 ($p<0.05$).As result relevant form Table 3, breast meat percentage was higher for $T_{2\ \text{than others,}}$ but there were no significant effects observed. Triglyceride, cholesterol and LDL were induced when chicks used $T_1$and T_2. In turn increasing of HDL levels was observed. Data showed that by using coneflower and virginiamycine on broilers diet antibody titers against New Castle diseases virus (NDV) were significantly increased($p<0.05$).In conclusion it seem that inclusion of coneflower And virginiamycine in broiler chicks diet at level of 2% can be useful and have significantly benefits on performance, blood biochemical and immunity parameters.

Keywords: Coneflower, Virginiamycine, Performance, Blood parameters, Broilers.

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Introduction:

There is increasing interest in using alternatives to antibiotics for poultry husbandry and using probiotics is an approach that has potential to reduce enteric disease in poultry and subsequent contamination of poultry products (Vahdatpour *et al.*, 2011; Sarica *et al.*, 2005). Antibiotic growth promoters were supposed to increase growth rate as a result of improved gut health, resulting in better nutrients utilization and improved feed conversion (Demir *et al.*,2003; Toghyani *et al.*, 2011). Virginiamycine was also used in agriculture, specifically in livestock, to accelerate the growth of the animals and to prevent and treat infections (Teymourizadeh *et al.*, 2009). Today, the non prescription use of antibiotics in poultry feeds has been eliminated or severely limited in many countries because of the potential risks associated with their use and development of resistant strains of bacteria, mainly in humans. A complete ban on antibiotics in poultry feeds was brought into force on January 1st by European Union ; thus, all of the antibiotics used at sub-therapeutic doses for growth promotion were withdrawn (Nollet, 2005). Phytogenic feed additives are plant derived products that used in animal feeding to improve performance of animals through amelioration of feed properties, promotion of production performance, and improving the quality of their food (Gill., 1999: Great head., 2003; Windich *et al.*, 2008). Coneflower *(Echinacea purpurea)* is belonging to the group of phytogenic immune stimulants that help in establishment and strengthening of paraimmunity and is reported to possess a number of pharmacologically active substances (Nasir *et al.*, 2010; Weiner. 1994). *Echinacea purpurea* contain a variety of active substances like alkamides, glycoproteins, polysaccharides, phenolic compounds, cichoric acid, cinnamic acids, essential oils and flavonoids. *Echinacea purpurea* was effective treatment in human acute respiratory infection (Narimaniana *et al.*, 2005).The objective of this study was to explore the potential uses of coneflower as growth promoters in comparison to virginiamycine antibiotic on broilers performance.

Materials and Methods:

For determine the effect of use coneflower and virginiamycine on performance of broiler chicks a total 240 one day broilers chicks (Ross 308) were divided into 3 groups of 20 birds each and assigned to 4 replicate. The experiment was carried out at the poultry farm of Veterinary College, Islamic Azad University, Shahrekord branch, Iran for 42 days. Coneflower was purchased from local market and grounded separately to a fine powder and then mixed with the basal diet (Table 1). In addition feed and fresh water were providing adlibitum during the experiment. Treatments were basal diet as control diet, 2% coneflower (T_1), 2% virginiamycine (T_2), that they were balanced according to their requirement as shown in (NRC, 1994) for poultry. The live body weight gains of birds were measured individually and feed consumption and feed conversion efficiency were measured weekly. At the end of experimental plan 2 birds form each groups (totally 24 birds) were slaughtered and to compare body parts were separated and weighted. Blood samples from each bird were collected and stored at refrigerator at +4° C for 24 h, the blood samples were subjected to biochemical for determine their cholesterol, triglycerides by Pars Azmoon commercial kits. Serum antibody titer against Newcastle Disease Virus (NDV) was determined by the hemagglutination inhibition test (HI). HI antibodies were then converted into log2 (Cunningham, 1971).Then data were collected and analyzed by using the general, linear model procedure of (SAS, 2001) and different means Duncan's multiple ranges test was used to detect the differences at level ($p<0.05$).

Table 1. Composition of the experimental diets for broiler chicks

Ingredients %	0-14 (days old)	15-29 (days old)	29-42 (days old)
Corn grain	51.64	56.61	60.37
Soybean meal	37.74	32.30	27.81
Wheat grain	5	5	5
Oil	1.40	2.03	2.84
DCP	1.56	1.47	1.39
Oyster shells	1.17	1.13	1.08
Methionine D-L	0.30	0.29	0.27
Lysine-L	0.13	0.13	0.30
Nacl	0.26	0.24	0.14
Vitamin Premix*	0.3	0.3	0.3
Mineral Premix*	0.3	0.3	0.3
Coneflower/Virginiamycine	0.2	0.2	0.2
Calculated nutrient content			
ME(Kcal/Kgr)	2.850	2.950	3.050
CP (%)	22	20	18.5
Ca (%)	0.90	0.85	0.80
Available Phosphorus (%)	0.45	0.42	0.40
Lysine (%)	1.35	1.20	1.16
Na (%)	0.16	0.15	0.15
Methionine+ Cystine (%)	0.97	0.87	0.85

Supplied per kilogram of feed: 7.500 IU of vitamin A, 2000IU vitamin D3, 30 Mg vitamin E,1.5 µg vitamin B12,2Mg B6,5 Mg Vitamin K,5 Mg vitamin B2,1 Mg vitamin B1,40 Mg nicotinic acide,160µg vitamin Biothine,12 Mg Calcium pantothenate,1MgFolic acid 20 Mg Fe,71 Mg Mn,100µg Se,37Mg Zn,6 Mg Cu,1.14 Mg I,400 µg Cu.

Result

Data of feed intake, broiler weight and feed conversion ration are in (Table 2). Data showed no significant difference for feed intake in experimental groups. Chicks were fed with T_2 diet was higher weight gain compared to others. This result showed that all treatments have better final result in compared to control.

Table 2. The effect of coneflower and virginiamycine on broilers performance

Treatments*	FI (g/d)	BW (g/d)	FCR	FI (kg)	Pre-slaughter weigh (g)
Control	87.43^{b**}	40.12^{b}	2.27	3654.30^{b}	1710.17^{b}
T_1	89.49^{a}	41.23^{a}	2.12	3735.26^{b}	1734.45^{a}
T_2	90.10^{a}	41.14^{a}	2.10	3782.10^{a}	1743.62^{a}
MSE	0.071	0.42	0.07	275.4	11.44

* Control = basal diet, T1= Basal diet with 2% coneflower, T2= Basal diet with 2% Virginiamycine.
** Means within row with no common on letter are significantly different (p<0.05).

Data from Table 3 showed that liver percentage was significantly decreased ($p<0.05$) were broilers fed with T_1, T2. There were no significant differences in for Heart percentage between treatments. The use of T_1, T_2 lead to reduce abdominal fat percentage statistically ($p<0.05$). Also drumstick percentage was increased were broilers fed with T_1, T_2 ($p<0.05$).As result relevant form Table 3, breast meat percentage was higher for $T_{2\ \text{than others,}}$ but there were no significant effects observed. Data showed that percentage of gizzard was higher in the $T_{1\ \text{groups and it was at the lowest in}}$ control groups ($p<0.05$).

Table 3. The effect of coneflower and virginiamycine on percentage some part of chicks' bodies

Treatments*	Liver (%)	Abdominal Fat (%)	Drumstick (%)	Breast Meat (%)	Gizzard (%)	Heart (%)
Control	2.83^{a**}	3.26^{a}	20.31^{b}	24.74	2.13^{b}	0.20
T_1	2.67^{b}	2.88^{b}	23.92^{a}	25.52	2.86^{a}	0.21
T_2	2.59^{b}	2.76^{b}	24.04^{a}	25.76	2.76^{a}	0.21
MSE	0.45	0.19	0.724	1.67	0.011	0.194

* Control = basal diet, T1= Basal diet with 2% coneflower, T2= Basal diet with 2% Virginiamycine.
** Means within row with no common on letter are significantly different (p<0.05).

Data from this study showed that the triglyceride, HDL and LDL were changed with experimental diets (Table 4). Triglyceride, cholesterol and LDL were induced when chicks used T_1 and T_2, In turn increasing of HDL levels was observed.

Table 4. The effect of coneflower and virginiamycine on some blood parameters

Treatments*	Triglyceride (Mg/dl)	Cholesterol (Mg/dl)	HDL (Mg/dl)	LDL (Mg/dl)
Control	82.43^{a**}	125.44^{a}	57.21^{a}	73.85^{a}
T_1	76.54^{b}	120.23^{b}	59.48^{b}	71.29^{b}
T_2	74.21^{b}	118.47^{b}	60.18^{b}	70.42^{b}
MSE	4.17	5.45	0.792	0.847

* Control = basal diet, T1= Basal diet with 2% coneflower, T2= Basal diet with 2% Virginiamycine.
** Means within row with no common on letter are significantly different (p<0.05).

Data from antibody titers against New Castle diseases virus (NDV) as shown on Table 6 showed that antibody titers were significantly increased($p<0.05$) when broilers were fed by T_1, T_2.

Table 5. The effect of coneflower and virginiamycine on antibody titers against New Castle disease virus

Treatments*	28 days (log2)	35 days (log2)	42 days (log2)
Control	2.51^{c**}	3.10^{c}	3.99^{c}
T_1	2.81^{b}	3.65^{b}	4.16^{b}
T_2	2.93^{b}	3.87^{ab}	4.46^{b}
MSE	0.065	0.31	0.87*

* Control = basal diet, T1= Basal diet with 2% Coneflower, T2= Basal diet with 2% Virginiamycine.
** Means within row with no common on letter are significantly different (p<0.05).

Discussion

In the present study, coneflower and virginiamycine supplementation had beneficial effects ($P<0.05$) on the measured values in growing broiler chicks. Although the usage of the coneflower and virginiamycine wasn't significant influences on feed conversion ratio in broiler chicks but also decreased it. Result of Lee *et al.* (2012), studies about coneflower on broilers showed that the body weight gain and feed intake of the broilers were not significantly different by treatment in the starter or grower period .In the grower period, the feed conversion of the coneflower groups was significantly decreased compared with that of the control group. Some scientists showed that beneficial effects of herbal or active substances in animal nutrition may include the stimulation of appetite and feed intake, the improvement of endogenous digestive enzyme secretion, activation of immune response and antibacterial, antiviral, antioxidant and anti helminthes actions (Janssen, 1989; Manzanilla *et al.*, 2001; Jamroz *et al.*, 2003). Landy *et al.* (2011), showed that the body weight obtained in broilers fed diet containing 5g/kg diet coneflower continuously was highest than all treatments in the 14 and 28 days, but the height was not significant. The result of their study showed that the use of 10 g/kg diet dried aerial part powder of coneflower intermittently had significant effect on daily weight gain and feed conversion ratio. The herb coneflower is commonly known as an immune stimulating

substance, So the application of immune stimulating substances to increase the immune status can result in increased performance (Iren, 2000; Ahmadi, 2011).Some studies showed that treatment with virginiamycine at suggested dosages has a beneficial effect on body weight gain and feed conversion efficiency in poultry (Dumonceaux *et al.*, 2006; Rahimi *et al.*, 2009; Mayahi *et al.*, 2011). In the present study a positive effect of coneflower on cholesterol concentration in the blood plasma of broiler chicks was observed. Similar results were reported by (Ghalamkari *et al.*, 2011) who demonstrated that inclusion in the diet of coneflower powder from the dried aerial part (10 g/kg) could improve the total antioxidant activity in the serum of broiler chicks. Feizi *et al.* (2012), showed that use of E. purpurea in each of the foregoing doses had increasing effects on antibody titers, and this fact is significant between the control group and treatment groups. Ghaedi *et al.* (2014), showed that serum cholesterol profile and antibody titer against (NDV) improved in groups that they used herbal and virginiamycine ($P<0.05$). Faghani *et al.* (2014), showed that the use of virginiamycine on broilers diet could increase feed intake and body weight gain and decreased the level of cholesterol and triglyceride in their serum. Ghaedi *et al.* (2014), also suggested that virginiamycine controls microbial growth by acting on the mircoflora's biochemical processes such as protein synthesis or inhibiting the elongation of Methono bacterium and Echerchiacoli it can improve the anti body titer. Allen (2003) showed that coneflower may potentiate the immune response to live vaccination and may provide protective immune stimulation in the presence of natural coccidia population in the litter. In other studies conducted in Swiss mice. Frieier *et al.* (2002), showed that coneflower may increase the humoral immune response.

Conclusion

We could be explained some benefit acts by using coneflower and virginiamycine on performance for broilers chicks. This improvement on growth and health may be due to the biological functions of coneflower to improve growth or that may be due to its role as stimulant, enhanced digestibility, antioxidant, anti-microbial and anti-fungal activities and properties and the prevention of gastric toxicity. The herb coneflower is commonly known as an immune stimulating substance, Whilst the performance of animals is influenced mainly by the health and immune status, a stressed or weak immune system with a load of infectious diseases causes low body weight gain. On the other hand, an enhanced immune system allows maximum performance. Also Further tests are needed to explore and more detail explanation.

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