Polish Journal of Veterinary Sciences Vol. 20, No. 2 (2017), 307–312

DOI 10.1515/pjvs-2017-0037

Original article

Improvement of dairy cow embryo yield with low level laser irradiation

G. Palubinskas[1], V. Žilaitis[2], R. Antanaitis[2]

[1] The Centre for Veterinary Continuing Education and Consulting
Large Animal Clinic
[2] Veterinary Academy, Lithuanian University of Health Sciences, Tilzls Str. 18, Kaunas, Lithuania

Abstract

The goal of this study is to estimate the effects of low-level laser irradiation (LLLI) on the superovulatory response according to the number of corpora lutea (CL), follicles (F) and the embryo yield. In recent years, while searching for new, more efficient and organic methods to improve superovulatory response and embryo yield with respect to the conventional methods, low-level laser irradiation (LLLI) is a more sensitive and less costly technology that can be used to improve animal reproduction, namely, artificial insemination and the embryo production system. The dairy-cow donors were treated for superovulation with Pluset®, at any time during the oestrus cycle, and the total dose per donor was 700 IU. The first group of the donors (n=25), test group (TG), was irradiated on the sacroiliac area for 180 seconds per day, from the 1st to 11th superovulatory treatment (ST) days in a row, with LLLI in the 870-970-nm wavelength, 65.93 J/cm dose, frequencies in the 20-2000 Hz range and pulse durations commonly in the range of about 1 second. For the second control group (CG) (n=25), the ST was performed without LLLI. After the ST, The mean number of CL in the right side ovaries in the TG was 25.43% ($p<0.05$) greater than in those of the CG. The number of total recovered and transferable embryos was greater in the TG compared with the CG by 28.97% ($p<0.05$) and 15.8% ($p>0.05$), respectively. With respect to conventional methods, LLLI can be used to improve the superovulatory response and embryo yield as a supplementary environment and animal-friendly method of treatment.

Key words: cows, laser irradiation, superovulatory treatment, embryo yield

Introduction

According to Tonhati et al. (1999), the superovulatory response is not a heritable trait, thus the future response cannot be predicted from the previous response, and environmental factors play a large role regarding variability in the superovulatory response. Extreme variability in the superovulatory response is a major limitation in the profitable and efficient implementation of embryo technology in cattle (Keller et al. 1990). According to Looney (1986), in a study conducted on 2048 donor cows, 70% of the embryos were collected from only 30% of the donors. Similar variability was reported for a group of more than 900 dairy cows (Lerner et al. 1986).

Correspondence to: R. Antanaitis, e-mail: ramunas.antanaitis@lsmuni.lt, tel.: +370 67349 064

In recent years, while searching for new, more efficient and organic methods of treatment and prophylaxis, there have been profound discussions about light therapy and a promising method, namely low-level laser irradiation (LLLI). Cell proliferation is a very important physiological effect for the low-level laser irradiation used in clinical practice. Increased proliferation after LLLI has been shown in many cell types in vitro, including fibroblasts from different systems (Kreisler et al. 2002), keratinocytes (Grossman et al. 1998), human osteoblasts (Stein et al. 2005), calvaria osteoblast-like cells, mesenchymal stem cells and cardiac stem cells (Tuby et al. 2007), rat Schwann cells (Van Breugel et al. 2003), aortic smooth muscle cells (Gavish et al. 2006), endothelial cells from veins (Schindl et al. 2003) and arteries (Kipshidze et al. 2001), quiescent satellite cells (Ben-Dov et al. 1999), human lung adenocarcinoma cells (Shefer et al. 2002) and HeLa cells (Zhang et al. 2008). Various mechanisms for the mitogenic effects of low power laser irradiation have been proposed, including ligand-free dimerization and activation of specific receptors that are in the "right energetic state" to accept the laser energy, leading to their autophosphorylation and downstream effects (Zhang et al. 2008), activation of calcium channels resulting in increased intracellular calcium concentration and cell proliferation (Breitbart et al. 1996). Red to near infrared light is thought to be absorbed by mitochondrial respiratory chain components, resulting in the increase of reactive oxygen species and adenosine triphosphat, and initiating a signalling cascade which promotes cellular proliferation and cytoprotection (Tuby et al. 2007). Following increased ATP and protein synthesis after LLLI, the expressions of growth factors and cytokines increase and ultimately lead to cell proliferation (Hu W-P et al. 2007). However, the mechanisms of cell proliferation induced by LLLI are poorly understood (Gao and Da Xing 2009).

Some investigators have claimed to find "systemic" rather than simply "local" effects, but many studies fail to show either local or systemic benefit. Currently, no universally accepted theory has explained the mechanism of either "laser analgesia" or "laser biostimulation". (Basford 1986) Based on a special mechanism of electromagnetic emission, LLLI affects cell metabolism, stimulates regeneration, reduces pain, inflammation and improves structural characteristics, and has been proven effective in different cell types (Karu et al. 2010). LLLI is characterized by its ability to induce athermic, non-destructive photobiological processes. The mechanisms of LLLI action have been studied and identified with regard to the reduction of pain and inflammation and the healing of tissue (Pryor and Millis 2015). The exploitation of LLLI to improve the reproductive efficiency of sperm cells and the maturation rate in different livestock has been demonstrated by various researchers. The first studies investigating the fact that the LLLI of spermatozoa can increase sperm motility date back to 1984 (Karu 2012). According to Jaffaldano et al. (2010), LLLI increased the sperm motility index, viability, and cell energy charge, and could be useful for improving the quality of semen.

The laser irradiation introduces photonic energy into a biological system, which is converted to ATP and may be then available for cellular metabolism. In this context, the dose of irradiation is an essential parameter for the success of laser therapy, since if insufficient energy is applied, no effect will be observed. On the other hand, if more energy is applied, the biostimulation may disappear and be replaced by bioinhibition (AlGhamdi et al. 2012). LLLI was also able to increase the amount of ATP produced by cell lines maintained in vitro, demonstrating the intense action in cellular metabolism (Magrini et al. 2012).

It has been shown that lasers show up in the field of livestock reproduction as an easy, time saving, less costly and effective technique in addition to having the possibility of its use *in situ*, namely in cattle farms and veterinary clinics (Abden-Salam et al. 2015). Generally, the mechanism of laser beam impact on living tissue is unclear. For the increment of production systems, LLLI, also known as photobiomodulation, arises as a new alternative (AlGhamdi et al. 2012). In new approaches, three physical effects exist in laser-tissue interactions: reflection, absorption and scattering (Niemz 2013).

In earlier studies, we positively evaluated the action of laser irradiation on the health of dairy cows; the efficiency of LLLI is comparable to that of antibiotic therapy in the prophylaxis and the treatment of endometritis in cows (Žilaitis et al. 2013).

Therefore, in this study we wanted to extend the research and determine the possibility of using LLLI to increase the superovulatory response and embryo yield of dairy-cow donors. The goal of the study was to estimate the effects of LLLI on the superovulatory response according to the ovary surface area, the number of corpora lutea (CL), follicles (F) and the embryo yield.

Materials and Methods

Our research was carried out on 50 dairy cows: clinically healthy holsteinizated Lithuanian Black and White cows held in a loose-housing system that

Table 1. Superovulatory treatment of donors.

No	Days of superovulation		Procedures performed
1.	Day 1		Introduction of intravaginal insert of progesterone containing 1.38 g of progesterone
2.	Day 6	Morning	I.m. injection of 4 ml of Pluset 140 IU
		Evening	I.m. injection of 4 ml of Pluset 140 IU
3.	Day 7	Morning	I.m. injection of 3 ml of Pluset 105 IU
		Evening	I.m. injection of 3 ml of Pluset 105 IU
4.	Day 8	Morning	I.m. injection of: – 2 ml of Cloprostenol – 2 ml of Pluset 70 IU
		Evening	I.m. injection of: – 2 ml of Cloprostenol – 2 ml of Pluset 70 IU
5.	Day 9	Morning	– removal of intravaginal insert of progesterone – i.m. injection of 1 ml of Pluset 35 IU
		Evening	I.m. injection of 1 ml of Pluset 35 IU
6.	Days 10-11		Artificial insemination
7.	Day 18		Embryo recovery

had calved 75-90 days before with a productivity of 9.500-10.500 kg during the previous lactation. The donors were fed with feed rationally adequate for their physiological needs.

For the purposes of the study, only cows with corpus lutea (CL) larger than 10 mm and no follicle (F) larger than 10 mm in the right or left ovary were selected before starting the superovulatory treatment.

The donors were treated for superovulation with Pluset® (follicle stimulating hormone (FSH) and luteinizing hormone (LH) in the ratio 1:1; Laboratorios Calier, S.A., Barselona, Spain) at any time during the oestrus cycle. As for the solution, 1 mL of Pluset® contained 35 IU FSH and 35 IU LH, and the total dose per donor was 700 IU.

The superovulatory treatment was induced according to the following scheme (Table 1) (Lafri et al. 2002, Seidel et al. 2003).

The cows were divided into two groups: A test group (TG) of 25 cows (was subjected to LLLI) and a control group (CG) of 25 cows was treated for superovulation with a conventional method without LLLI. The sacroiliac area of each TG cow was irradiated for 180 sec per day (by recommendation of the producer) one hour after each insemination in a row with LLLI in the 870-970-nm wavelength range, 65.93 J/cm dose, frequencies in the 20-2000 Hz range and pulse durations commonly in the range of about one second. (Other main parameters: maximal irradiation power 1.5 W, enlargement of bean (degree) 10x50, spot area at a distance of 100 mm from the surface is 15x90 mm. Weight of device – 780 g, measurements – 720x40x20 mm). The insemination was performed on days 10-11 according to the oestrus signs (twice with an interval of 12 hours). The embryos were collected after 7 days, using a nonsurgical embryo recovery method with a Rusch catheter. The embryos were flushed with a BoviFlush medium with BSA and antibiotics manufactured by Minitube, Germany. For each flushing we used 1000 mL of medium per donor.

On day 1 (the day of the initiation of the superovulatory treatment), before inserting the implant Pride of Progesterone (CIDR®, Pfizer, Inc.) and on day 18 when the embryos were recovered, the ovaries were scanned and the number and size of ovarian structures (F and CL) was determined using Digital Diagnostic Ultrasound Devices (HG 9300, Caresono Technology Co., Ltd) with a 7 MHz rectal transducer.

On day 18, the response to the superovulatory treatment was determined according to the number of CL (> 10 mm), F (> 10 mm), the ovary surface area, and the total and transferable embryo yield. The ovaries were measured at their largest diameter and the ovarian surface area (ovary size) was calculated by multiplying the length and the width.

The descriptive statistic of the sample (arithmetic mean ± standard error) was calculated using an SPSS statistical package (SPSS for Windows 15.0, SPSS Inc., Chicago, IL, USA, 2006). Statistical analysis of the data was carried out using the T test. The data were considered statistically significant when $p<0.05$.

Table 2. The number of follicles after superovulatory treatment.

Dairy cows donors groups	Left ovary	Right ovary	Average of left and right ovaries
TG	2.96 ± 0.4[a]	3.88 ± 0.37[c]	3.42 ± 0.27[e]
CG	3.2 ± 0.41[b]	4.48 ± 0.39[d]	3.84 ± 0,29[f]

c:d columns with different superscript differ significantly ($p<0.05$)
a:b; e:f columns with different superscript differ not significantly ($p>0.05$)

Table 3. The number of corpora lutea after superovulatory treatment.

Dairy cows donors groups	Left ovary	Right ovary	Average of left and right ovaries
TG	5.03 ± 0.49[a]	7.01 ± 0.8[c]	6.02 ± 0.48[e]
CG	4.44 ± 0.39[b]	5.6 ± 1.01[d]	5,02 ± 0.54[f]

c:d columns with different superscript differ significantly ($p<0.05$)
a:b; e:f columns with different superscript differ not significantly ($p>0.05$)

Results

For the TG cows, after superovulation it was estimated that in the ovaries, the mean number of F (10 mm) was lower in comparison with the CG. The average total number of F in the TG was 10.8% less than the mean total number of F in the ovaries of the CG donors (Table 2).

According to the number of CL after superovulation, the TG donors showed a stronger response to the superovulatory treatment in comparison with the CG donors. In the TG, the number of CL was 20.44% ($p<0.05$) greater than the number of CL in the ovaries of the CG donors. The number of CL in the right side ovaries in the TG was 25.4% ($p<0.05$) greater than in those of the CG, and in the left side ovaries of the TG, it was 13.25% ($p>0.05$) greater than in the CG (Table 3).

The response to the superovulatory treatment according to the mean number of total recovered embryos in the TG donors was 28.92% ($p<0.05$) larger compared to the CG donors (6.81±3.14 and 5.28±2.96). On the average, the TG donors yielded 15.8% ($p>0.05$) more transferable embryos as compared with the CG donors (4.31±2.4 and 3.72±2.38), but the ratio of transferable embryos as compared with the total recovered embryos in the TG was 0.63, and was 10.0% less than in the CG. This means that in the TG, the average total and transferable number of embryos washed was bigger; however, the percentage of embryos suitable for transplantation was smaller in comparison with the CG.

Discussion

The implementation of embryo technologies is primarily driven by the need to increase the number of offspring from genetically valuable animals. For this reason, the number of embryos per session is an important parameter reflecting the success of a production procedure (Merton et al. 2003). The major limitation on the development of embryo production in cattle is the wide variability between animals in the ovulatory response to FSH-induced superovulation (Rico et al. 2009).

In the ovaries on both sides of the TG donors, we estimated a 12.28% smaller average total number of F (>10 mm) in comparison with the CG. We have found a link between this fact and a positive LLLI effect on the oocytes and ovulation process.

The biological mechanisms of the LLLI interaction are not totally known, but it can be said that at the molecular level, the activation of certain receptors and messengers determine universal biological responses (Corral – Baques et al. 2009). Depending on the wavelength, dosage, and condition of the irradiated tissue, the laser can induce an anti-inflammatory effect, reducing pain, and accelerating cell proliferation (Manchini et al. 2014). Based on a special mechanism of electromagnetic emission, LLLI may increase cellular metabolism and improve structural characteristics, which has been proven effective in different cell types (AlGhamdi et al. 2012). Several modifications occur during oocyte maturation, necessary for the acquisition of the ability to undergo

fertilization and embryo development. Most of these changes are driven by the granulosa cells, which present a direct communication to the oocyte via gap junctions (Canipari 2000).

There are a few studies concerning the effects of lasers on oocyte maturation. Soares et al. (2014) used lasers to irradiate bovine oocytes. According to these authors, the laser is capable of modulating events in granulosa cells that may lead to changes in oocytes. Research indicates that the mechanisms involved in laser interaction with cells are due to photon absorption by cellular photoreceptors that trigger chemical reactions such as glycolysis and oxidative phosphorylation. This could accelerate RNA transcription and DNA replication (Abdel-Salam et al. 2015). LLLI is an efficient tool to modulate the granulosa cells and oocyte metabolism (Soares et al 20104).

Laser light can penetrate about 3.5 cm through bovine tissue samples (Hudson, 2013). The ovaries of the treatment cows were deeper than 3.5 cm in the body, but a direct laser beam is unlikely to influence an ovary. Scientific data doesn't show evidence of the systemic benefit of a laser beam on living tissue.

The positive effect of LLLI for oocyte maturity and the ovulation process could lead to the ovulation of a larger number of F. Consequently, more CL was obtained in the TG compared with the CG. It should be noted that the positive LLLI effect applied not only to ovulation, but also to fertilization and early embryonic development, because in the TG, a larger number of oocytes were fertilized and more embryos recovered in comparison with the CG. Gavish et al. (2004) reported that photons from the laser irradiation are absorbed by proteins of the mitochondrial respiratory chain that convert this energy into chemical energy. They also described that in isolated mitochondria, the LLLI increased the mitochondrial membrane potential, the proton gradient, and the rate of ADP/ATP replacement. Gao and Xing (2009) reported that the LLLI can induce increased intracellular Ca28, as well as an increase in reactive oxygen species and cyclic-adenosine monophosphate (cAMP) resulting from an increase in mitochondrial activity. This increase of mitochondrial activity, in addition to the stimulatory effect of FSH (supplemented during IVM) in granulosa cells, seems to increase the cAMP levels, leading to a higher number of cells in the S-phase, G2, and M (committed to the cycle). The increased levels of cyclin B during maturation in this group could confirm this result, since cyclin is responsible for entry and progression to the M phase (Gavet and Pines 2010).

Successful fertilization could be based on the fact that many studies have focused on a positive LLLI effect for bovine sperm motility parameters and a significant increase in the percentage of live sperm cells (Henrique et al. 2015).

Conclusion

After superovulation, LLLI reduced the mean number of F, but increased the response to the superovulatory treatment with regard to the mean number of CL and total and transferable embryo yield. Without any doubt, the therapeutic and stimulating effect of LLLI seems promising, as LLLI has shown a positive effect on increasing the response to superovulatory treatment.

In summary, LLLI can be used for improving the superovulatory response and embryo yield as a supplementary environmental and animal-friendly method of treatment.

References

Abdel-Salam Z, Hadith MA (**2015**) Laser researches on livestock semen and oocytes. A brief review. J Adv Res 6: 311-317.

AlGhamdi KM, Kumar A, Moussa NA (**2012**) Low-level laser therapy: a useful technique for enhancing the proliferation of various cultured cells. Lasers Med Sci 27: 237-49.

Basford JR (**1986**) Low-Energy Laser Treatment of Pain and Wounds: Hype, Hope, or Hokum? Mayo Clin proc 61: 671-675.

Ben-Dov N, Shefer G, Irinitchev A, Wernig A, Oron U, Halevy O (**1999**) Low-energy laser irradiation affects satellite cell proliferation and differentiation in vitro. Biochim Biophys Acta 14: 108-116.

Breitbart H, Levinshal T, Cohen N, Friedmann H, Lubart R (**1996**) Changes in calcium transport in mammalian sperm mitochondria and plasma membrane irradiated at 633 nm (HeNe laser). J Photochem Photobiol B 34: 117-121.

Canipari R (**2000**) Oocyte-granulosa cell interaction. Hum Reprod 6: 279-289.

Corral-Baqués MI, Rivera MM, Rigau T, Rodrtguez-Gil JE, Rigau J (**2009**) The effect of low-level laser irradiation on dog spermatozoa motility is dependent on laser output power. Lasers Med Sci 24: 703-713.

Fernandes, GHC, Carvalho PTC, Serra AJ, Crespilho AM, Peron JPS, Rossato C, Leal-Junior ECP, Albertini R (**2015**) The effect of low-level laser irradiation on sperm motility, and integrity of the plasma membrane and acrosome in cryopreserved bovine sperm. Plos one, DOI: 10.1371/journal.pone.0121487.

Gao X, Xing D (**2009**) Molecular mechanisms of cell proliferation induced by low power laser irradiation J Biomed Sci 16: 4.

Gavet O, Pines J (**2010**) Progressive activation of cyclinB1-Cdk1 coordinates entry to mitosis Dev Cell 18: 533-543.

Gavish L (**2004**) Low level laser irradiation stimulates mitochondrial membrane potential and disperses

subnuclear promyelocytic leukemia protein Lasers Surg 35: 369-376.

Gavish L, Perez L, Gertz SD (**2006**) Low-level laser irradiation modulates matrix metalloproteinase activity and gene expression in porcine aortic smooth muscle cells. Lasers Surg Med 38: 779-786.

Grossman N, Schneid N, Reuveni H, Halevy S, Lubart R (**1998**) 780 nm low power diode laser irradiation stimulates proliferation of keratinocyte cultures: involvement of reactive oxygen species. Lasers Surg Med 22: 212-218.

Hu WP, Wang JJ, Yu CL, Lan CC, Chen GS, Yu H-S (**2007**) Laser Irradiation Stimulates Cell Proliferation through Photostimulatory Effects in Mitochondria. J Invest Dermatol 127: 2048-2057.

Hudson DE, Hudson DO, Wininger JM, Richardson BD (**2013**) Penetration of Laser Light at 808 and 980 nm in Bovine Tissue Samples. Photomed Laser Surg 31: 163-168.

Jaffaldano N, Rosato MP, Paventi G (**2010**) The irradiation of rabbit sperm cells with He-Ne laser prevents their in vitro liquid storage dependent damage. Anim Reprod Sci 119: 123-129.

Karu T (**2010**) Mitochondrial mechanisms of photobiomodulation in context of new data about multiple roles of ATP. Photomed Laser Surg 28:159-160.

Karu TI (**2012**) Lasers in infertility treatment: irradiation of oocytes and spermatozoa. Photomed Laser Surg 30: 239-241.

Keller DS, Teepker G (**1990**) Effect of variability in response to superovulation on donor cow selection differentials in nucleus breeding schemes. J Dairy Sci 73: 549-554.

Kipshidze N, Nikolaychik V, Keelan MH, Shankar LR, Khanna A, Kornowski R, Leon M, Moses J (**2001**) Neon laser irradiation enhances production of vascular endothelial growth factor and promotes growth of endothelial cells in vitro. Lasers Surg Med. 28: 355-359.

Kreisler M, Christoffers AB, AlHaj H, Willershausen B, d'Hoedt B (**2002**) Low level 809-nm diode laser-induced in vitro stimulation of the proliferation of human gingival fibroblasts. Lasers Surg Med 30: 365-369.

Lafri M, Ponsart C, Nibart M (**2002**) Influence of CIDR treatment during superovulation on embryo production and hormonal patterns in cattle. Theriogenelogy 58: 1141-1151.

Lerner SP, Thayne WV, Baker RD (**1986**) Age, dose of FSH and other factors affecting superovulation in Holstein cows. J Anim Sci 63: 176-183.

Looney CR (**1986**) Superovulation in beef females. In: Proceedings of 5th Annual Convention of AETA, Fort Worth, Texas. 1986: 16-29.

Magrini TD (**2012**) Low-level laser therapy on MCF-7 cells: a micro- Fourier transform infrared spectroscopy study, J Biomed 17: 101-516.

Manchini MT, Serra AJ, Feliciano R dos S (**2014**) Amelioration of cardiac function and activation of anti-inflammatory vasoactive peptides expression in the rat myocardium by low level laser therapy. PLS One 9: 101-270.

Merton JS, De Roos AP, Mullaart E (**2003**) Factors affecting oocyte quality and quantity in commercial application of embryo technologies in the cattle breeding industry. Theriogenology 59: 651-674.

Niemz MH (**2013**) Laser-Tissue Interactions – Fundamentals and Applications. Springer, Germany, p 307.

Pryor B, Millis DL (**2015**) Therapeutic Laser in Veterinary Medicine. the Vet Clin North Am Small Anim Pract 45: 45-56.

Rico C, Fabre S, Medigue C (**2009**) Anti-mullerian hormone is an endocrine marker of ovarian gonadotropin-responsive follicles and can help to predict superovulatory responses in the cow. Biol Reprod 80: 50-59.

Schindl A, Merwald H, Schindl L, Kaun C, Wojta J (**2003**) Direct stimulatory effect of low-intensity 670 nm laser irradiation on human endothelial cell proliferation. Br J Dermatol 148: 334-336.

Seidel GE, Elsden JR RP, Hasler JF (**2003**) Embryo transfer in dairy cattle. Hoard's Dairyman Book Fort Atkinson USA 96.

Shefer G, Partridge TA, Heslop L, Gross JG, Oron U, Halevy O (**2002**) Low-energy laser irradiation promotes the survival and cell cycle entry of skeletal muscle satellite cells. J Cell Sci 115: 1461-1469.

Soares CA, Annes K, Dreyer TR, Magrini T, Sonoda MT, Martinho H, (**2014**) Photobiological effect of low-level laser irradiation in bovine embryo production system. J Biomed 19: 35006.

Stein A, Benayahu D, Maltz L, Oron U (**2005**) Low-level laser irradiation promotes proliferation and differentiation of human osteoblasts in vitro. Photomed Laser Surg 23: 161-166.

Tonhati H, Lobo RB, Oliveira HN (**1999**) Repeatability and heritability of response to superovulation in Holstein cows. Theriogenology 51: 1151-1156.

Tuby H, Maltz L, Oron U (**2007**) Low-Level Laser Irradiation (LLLI) Promotes Proliferation of Mesenchymal and Cardiac Stem Cells in Culture. Lasers Surg Med 39: 373-378.

Van Breugel HH, Bar PR (**1993**) He-Ne laser irradiation affects proliferation of cultured rat Schwann cells in a dose dependent manner. J Neurocytol 22: 185-190.

Zhang J, Xing D, Gao X (**2008**) Low-Power Laser Irradiation Activates Src Tyrosine Kinase Through Reactive Oxygen Species-Mediated Signaling Pathway. J Cell Physiol 217: 518-528.

Žilaitis V, Rudejeviene J, Juozaitiene V, Krištaponis V, Palubinskas G, Malinowski E (**2013**) The prevalence of subclinical metritis and fertility indices in cows treated with low-intensity laser irradiation and antibiotics in the postpartum period. Veterinarski Archiv. 83: 1-9.