Uterine and ovarian blood flow in mares treated with silver oxide nanoparticles

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1. Abstract

This study aimed to investigate the influence of treatment with silver oxide nanoparticles intrauterine infusion on ovarian and uterine blood flow, cortisol, estradiol (E2), and interleukin-10 (IL-10). Mares (N=8), diagnosed positive for endometritis, were examined by Doppler ultrasonography. Blood samples were collected before each ultrasound exam. Blood sera were used to assay cortisol, E2, and IL-10. The uterine and ovarian arteries' time average mean velocity (TAMV) and blood flow volume (BFV) were determined. Mares were infused intrauterine with 50 mg/kg body weight of silver oxide nanoparticles on Day 7(from ovulation) and another dose on Day 14 from ovulation. On day 21 from ovulation, there were significant decreases in BFV and TAMV values of the uterine ($P< 0.001$; $P< 0.05$, respectively) and ovarian arteries ($P< 0.0001$). A non-significant change in cortisol and IL-10 was noticed throughout the treatment. In conclusion, silver oxide nanoparticles modulated uterine and ovarian blood flow in endometritis suffered mares on day 21 post-treatment.

Keywords Endometritis; Ovarian hemodynamics; Mare; Nanoparticles; Interleukin 10

2. Introduction

Endometritis is the main equine reproductive infertility problem. Uterine infection occurs shortly after insemination and the majority of mares with intact uterine tone and contractility could resolve this physiological endometritis within 48 hours, but mares with poor uterine contractility and perineal conformation are susceptible to recurrent endometritis [1]. Markers of any inflammation in any organ (including the uterus) are hotness and redness of the site of inflammation due to the engorgement of blood [2]. Uterine blood flow changes in mares are due to infection [3, 4], the site of ovulation, and the estrous phase [5]. Silver is one of the nano-metals that have been widely used for medical purposes [6, 7]. It possesses antibacterial [8], antioxidant [9], and anticancer properties [10, 11]. However, all therapeutic applications of silver nanoparticles have been proved in vitro on cell lines; in vivo, only toxicity studies (acute and chronic) were studied on lab animals with limited studies on farm animals [12] [13]. In vivo therapeutic application of green synthesized silver oxide nanoparticles has been applied on female rats to treat induced endometritis using E-coli [13]. However, its usefulness for the treatment of cases of endometritis in mares was not investigated. Therefore, the current study aimed to study the effect of silver oxide nanoparticles for treating endometritis in mares by investigating the alterations in the uterine and ovarian blood flow and the

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concentrations of estradiol, cortisol, and interleukin-10 before and after treatment.

3. Materials and Methods

Ethical approval

All experimental procedures were conducted under the approval of the institutional animal use committee at the Faculty of Veterinary Medicine, Cairo University with an approval number (VET CU 23052022456).

3.1. Animals, plan, and diagnostic strategy for chronic endometritis

Eight pluriparous Thoroughbred mares suffered chronic endometritis, aged 15-19 years weighed 400-550 kg with a body condition score of 3 were kept at the indoor paddock. They were examined for one estrous cycle (21 days) before any treatment. To confirm estrus symptoms, mares were kept with a stallion at the opposite end of the same stable. In the paddock, artificial lighting was employed at night to keep mares warm and comfortable throughout the day. Mares were maintained on a commercial pelleted diet, hay, and unrestricted access to water. The presence of increased polymorph neutrophils (PMNs > 10%), uterine bacterial culture, and uterine fluid accumulating by ultrasound all supported the diagnosis of chronic endometritis. All females were assessed during the follicular phase, which was demonstrated by the existence of preovulatory follicles (POF) with a large diameter on the second day before ovulation and a lowered level of serum progesterone (1 ng/ml). Mares had a tight cervix, a weak vulvar organization, and poor uterine tone [14]. Blood, bacteriological, and cytological samples were collected on days 2, 7, 14, and 21 (during the follicular phase), and the first and second doses of silver nanoparticles (AgONPs), respectively.

3.2. Physical, uterine cytological, and bacteriological examinations

Each mare physical examination was noted along with clinical symptoms, animal history for senility, poor perineal confirmation, and constricted cervix. Additionally, rectal palpation was performed on all mares with endometritis to determine the tonicity of the uterus. To access the uterus, a mare-specific cytobrush (Minitube; ref-17214/2960; Germany) was inserted into the cervical canal. Once there, it was spun while rubbing the uterine lining, rolled, and examined under a microscope before being stained with the wright dye for cytological evaluation. Bacterial samples were gathered, positive cultures colonies were enumerated (nine isolate/mare via three bacterial sample cultures), and the existence of bacterial pathogens was then determined (*Enterobacteriaceae*, *Pseudomonas aeruginosa* and hemolytic *Escherichia coli*).

3.3. Preparation of AgONPs

Camel grass (Halva Bar, *Cymbopogon schoenanthus*) or thyme species (*Thymus vulgaris* or *Thymus Organo)* in dried and chopped form (5 g) was extended in 50 ml distilled water and kept at 37 °C for 48 h (25 rpm in horizontal shaker incubator (ThermoFisher, USA). The extract was filtrated (Whatman Filter paper 1) and used to prepare the nanoparticles. For every 40 ml of the aqueous plant extract, an amount of 5 g silver nitrate (AgNO3; 169.87 MW) was dissolved in 15 ml distilled water and left at room temperature until the color changed to black silver oxide nanoparticles, and the black precipitate is visualized [15]. With microbiological (minimum inhibitory concentration, MIC) results, a dose of AgONPs was 50 mg /kg body weight, With an average of 5.0 g for horse weighting 500 kg [13]. Two doses of 5.0 g of AgONPs in Ringer lactate or saline 0.9 % were infused intrauterine a week apart. Animals were treated during proestrus (follicular phase;

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day -2), after diagnosis on day 7. The first dose of AgONPs was administrated as an intrauterine infusion with saline at a rate of two administrations a week apart (day 7 and day 14).

3.4. Ultrasonography with B- and colored Doppler modes

The preovulatory follicle presence and diameter were assessed using ultrasound B-mode (EXAGO Doppler ultrasound, IMV, France) with a linear probe frequency of 7.5 MHz. Next, color mode was activated to trace the uterine and ovarian arteries and measure their diameters; and finally, pulsed wave Doppler was activated to assess both ovarian and uterine arteries' hemodynamics [16]. The ovarian artery (OV A) was reached behind the ovaries as it was merged from the abdominal aorta. Spectral Doppler was activated and hemodynamic variables were measured such as [Pulsatility index (PI), resistance index (RI), peak systolic and end-diastolic velocities (PSV) and EDV cm/sec), and OV A. blood flow rate (BFR; bpm; [3]). The time average mean velocity (TAMV) was calculated from the equation PI= PSV-EDV/TAMV. The blood flow volume (BFV ml /min) of each side of the uterine and ovarian arteries was determined using the equation:60 πr^2TAMV, where r is the radius/cm of the artery (diameter/2) and TAMV, is the time average mean velocity of the blood flow. Blood flow volume (BFV; mL/min) was calculated using the equation [17], BFV = TAMV × π × (D × 0.1/2) 2 × 60 .

3.5. Blood sampling and hormone assessment

All mares had their blood drawn from the jugular vein into vacuum tubes, both with and without anticoagulant. After centrifuging all blood samples at 3000 rpm for 10 minutes, the serum samples were collected and kept at -20 °C until hormone analysis. Cortisol (Monocent, Inc. 9025 Eton Ave.bSte C Canoga Park, CA91304, USA) and horse interleukin-10 (IL-10, Chongqing Bioseps Co., Ltd., China) were both measured using commercial ELISA with a sensitivity of 0.35 ng/mL and intra and inter-assay precisions of 2.9 % and 8.65 % for cortisol and 10 and 12 for IL-10. The test sensitivity of the estradiol (E2; pg/mL) ELISA kit was 9.7 pg/ml [18, 19].

3.6. Statistical analysis

Data were presented as mean ±standard error of the mean. ANOVA was performed using SPSS version 20.0. Duncan's multiple range test was performed with a means which is significantly different at $P < 0.5$.

4. Results

As found in figure 1, ovarian arteries on both sides had the lowest ($P<0.0001$) blood flow volumes 21 days after treatment with nano-silver. Though the left ovarian artery blood flow volume (LOA_BFV) was high on day 7 after ovulation and the time of the first dose of silver was infused intrauterine but this increase was not significant compared to Day -2 before ovulation (Day 0) and before nano-silver infusion and Day 14 after ovulation and the time of the second silver infusion. In contrast to the LOA_BFV, the right ovarian artery blood flow volume (ROA_BFV) increased on days -2 and Day 5 and reached the highest value on Day 14 after ovulation and the time of the second nano-silver infusion. Both uterine arteries had the lowest BFV on Day 21 after nano-silver uterine infusion. The uterine arteries' blood flow volume increased linearly ($P<0.001$) from Day -2 before treatment till Day 14 at the time of the second nano-silver infusion (Figure 2).

Cortisol concentrations did not vary from Day -2 before treatment till Day 21 after treatment but showed a slight non-significant decrease on Day 7 at the first

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nano-silver infusion (Figure 3). Interluekin-10 concentrations did not show any significant change throughout the experiment but showed a slight decrease on Day 21 compared to the other days of the treatments (Figure 3). = The ovarian and uterine arteries' time average mean velocities (TAMV cm/sec; Figure 4) showed the lowest velocities on Day 21 and showed the highest one on Day 14. The right ovarian artery TAMV (Table 1) showed significant correlations with the left ovarian TAMV ($r=0.83$; $P<0.0001$), the right uterine TAMV ($r=0.85$; $P<0.0001$), the right ovarian artery BFV ($r=0.93$; $P<0.0001$), and with estradiol (E2) concentrations ($r=0.42$; $P<0.05$). E2 had a positive and significant correlation with Lt Ov TAMV ($r=0.44$; $P<0.05$), but cortisol had a negative and significant correlation with it ($r=- 0.59$; $P<0.001$). E2 had got a positive and significant correlation with the L ut TAMV ($r=0.43$; $P<0.05$) and the L Ut A _BFV ($r=0.45$; $P<0.05$). The right and left uterine arteries'TAMV correlated together ($r=0.41$; $P<0.05$). The right and left ovarian arteries' BFV correlated together ($r=0.468$; $P<0.0001$). The right and left uterine arteries' BFV correlated together ($r=0.63$; $P<0.0001$).

5. Discussion

In the past decade, Doppler ultrasonography has been applied extensively in the field of reproduction raising the capability of the diagnostic tool [20-22]. Similar to the correlation found between the uterine and ovarian arteries TAMV and BFV in mares with endometritis before and after treatment with nano-silver in the current study, cows with endometritis had correlations between the right and left uterine arteries PI and TAMV [23]. Moreover, the decrease in the TAMV 21 days after treatment with nano-silver compared to Day -2 before treatment was also noted in cows 10 days after uterine infusion of policresulen [23]. In agreement with the decrease of ovarian uterine arteries' TAMV and BFV in resistant and susceptible mares to endometritis with time from ovulation and insemination with dead semen, mares of this study showed also a decrease in both TAMV and BFV of both uterine and ovarian arteries after infusion with nano-silver [24]. The increase in both TAMV and BFV of the uterine arteries with the increase of the severity of endometritis in cows [25] can be noticed in mares of this study by comparing the decrease in both TAMV and BFV of both uterine and ovarian arteries 21 days after treatment with nano-silver compared to Day -2 before the treatment, Day 7 at the treatment with the first Dose of silver and Day 14 at the second time treatment with nano-silver. However, the increase in the blood flow to the genital system in response to inflammation [2] has prolonged till after the treatment with the second dose of nano-silver. Nano-silver had induced slight inflammation in addition to its antimicrobial effect and this explained the increase in both uterine and ovarian TAMV and BFV [13]. The insignificant decrease of IL-10 on Day 7 at Dose 1 silver treatment and Day 21 after treatment in mares of the current work was also observed in the gene expression of IL-10 in susceptible mares to endometritis on Day 7 after the infusion of killed semen in comparison to the significant increase in resistant mares on the same day after insemination with killed semen [24]. The insignificant change in the concentrations of cortisol in the current mares after treatment with nano-silver refers to its anti-inflammatory and antioxidant properties which declined stress due to endometritis and also due to resolving the infection and its antimicrobial properties [6].

6. Conclusion

In conclusion, intrauterine infusion of silver oxide nanoparticles modulated the uterine and ovarian blood flow in mares

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suffering from endometritis on day 21 post-treatment.

Conflict of interest

There are no conflicts of interest to declare.

7. References

1. Da Silva-Álvarez E, Gómez-Arrones V, Martín-Cano FE, Gaitskell-Phillips G, Ortiz-Rodríguez JM, Carrasco JJ, et al. Endometrial area of the blood flow as a marker of endometritis in equine. Reproduction in Domestic Animals. 2022;57:98-102.

2. Magata F, Kubota R, Shimizu T. Association among endometrial hyperemia, uterine bacterial infection, and characteristics of large ovarian follicles in dairy cows. Journal of Veterinary Medical Science. 2019: 19-0207.

3. Abdelnaby EA, Emam IA, Salem NY, Ramadan ES, Khattab MS, Farghali HA, et al. Uterine hemodynamic patterns, oxidative stress, and chromoendoscopy in mares with endometritis. Theriogenology. 2020; 158: 112-20.

4. Farghali HA, AbdElKader NA, Fathi M, Emam IA, AbuBakr HO, Alijuaydi SH, et al. The efficiency of intrauterine infusion of platelet-rich plasma in the treatment of acute endometritis as assessed by endoscopic, Doppler, oxidative, immunohistochemical, and gene expression alterations in jennies. Theriogenology. 2022;181:147-60.

5. Abdelnaby EA, El-Maaty AMA, Ragab RS, Seida AA. Assessment of Uterine vascular perfusion during the estrous cycle of mares in connection to circulating leptin and nitric oxide concentrations. Journal of Equine Veterinary Science. 2016;39:25-32.

6. Tyavambiza C, Meyer M, Wusu AD, Madiehe AM, Meyer S. The Antioxidant and In Vitro Wound Healing Activity of Cotyledon orbiculata Aqueous Extract and the Synthesized Biogenic Silver Nanoparticles. International Journal of Molecular Sciences. 2022;23:16094.

7. Zhang X-F, Liu Z-G, Shen W, Gurunathan S. Silver nanoparticles: synthesis, characterization, properties, applications, and therapeutic approaches. International journal of molecular sciences. 2016;17:1534.

8. Li S, Mu B, Zhang H, Kang Y, Wang A. Incorporation of silver nanoparticles/curcumin/clay minerals into chitosan film for enhancing mechanical properties, antioxidant and antibacterial activity. International Journal of Biological Macromolecules. 2022;223:779-89.

9. Chang R, Zhao D, Zhang C, Liu K, He Y, Guan F, et al. Nanocomposite multifunctional hyaluronic acid hydrogel with photothermal antibacterial and antioxidant properties for infected wound healing. International Journal of Biological Macromolecules. 2022.

10. Liu X, Chen J-L, Yang W-Y, Qian Y-C, Pan J-Y, Zhu C-N, et al. Biosynthesis of silver nanoparticles with antimicrobial and anticancer properties using two novel yeasts. Scientific Reports. 2021;11:1-12.

11. Ratan ZA, Haidere MF, Nurunnabi M, Shahriar SM, Ahammad A, Shim YY, et al. Green chemistry synthesis of silver nanoparticles and their potential anticancer effects. Cancers. 2020;12:855.

12. Wen H, Dan M, Yang Y, Lyu J, Shao A, Cheng X, et al. Acute toxicity and genotoxicity of silver nanoparticle in rats. PLoS One. 2017;12:e0185554.

13. Aboelmaaty AM, Sayed MA, Elgabry MA, Kotp MS, Fouad GI, El-Shamarka ME, et al. Therapeutic effects of silver nanoparticles on Escherichia coli-induced endometritis in rats. Egyptian Pharmaceutical Journal. 2022; 21: 456.

Online ISSN: 2537-1045
Print ISSN: 1110-1423

14. Watson ED. Post-breeding endometritis in the mare. Animal reproduction science. 2000; 60: 221-32.

15. Chandrasekharan S, Chinnasamy G, Bhatnagar S. Sustainable phyto-fabrication of silver nanoparticles using Gmelina arborea exhibit antimicrobial and biofilm inhibition activity. Scientific Reports. 2022;12:1-16.

16. Abdelnaby EA, Abo El-Maaty AM. Melatonin and CIDR improved the follicular and luteal haemodynamics, uterine and ovarian arteries vascular perfusion, ovarian hormones and nitric oxide in cyclic cows. Reproduction in Domestic Animals. 2021;56:498-510.

17. Bollwein H, Meyer H, Maierl J, Weber F, Baumgartner U, Stolla R. Transrectal Doppler sonography of uterine blood flow in cows during the estrous cycle. Theriogenology. 2000;53:1541-52.

18. Hashem NM, El-Sherbiny HR, Fathi M, Abdelnaby EA. Nanodelivery System for Ovsynch Protocol Improves Ovarian Response, Ovarian Blood Flow Doppler Velocities, and Hormonal Profile of Goats. Animals. 2022;12:1442.

19. El-Sherbiny HR, Samir H, El-Shalofy AS, Abdelnaby EA. Exogenous L-arginine administration improves uterine vascular perfusion, uteroplacental thickness, steroid concentrations and nitric oxide levels in pregnant buffaloes under subtropical conditions. Reproduction in Domestic Animals. 2022; 57, 1493-1504.

20. Abdelnaby EA, Yasin NA, Abouelela YS, Rashad E, Daghash SM, El-Sherbiny HR. Ovarian, uterine, and luteal vascular perfusions during follicular and luteal phases in the adult cyclic female rabbits with special orientation to their histological detection of hormone receptor. BMC Veterinary Research. 2022;18:1-18.

21. El-Sherbiny H, Shahat A, Hedia M, El-Shalofy A. Effect of sexual maturation on testicular morphometry and echotexture and their association with intratesticular blood flow in ossimi rams. Indian Journal of Small Ruminants (The). 2022;28:85-90.

22. El-Sherbiny HR, Abdelnaby EA, El-Shahat K, Salem NY, Ramadan ES, Yehia SG, et al. Coenzyme Q10 Supplementation enhances testicular volume and hemodynamics, reproductive hormones, sperm quality, and seminal antioxidant capacity in goat bucks under summer hot humid conditions. Veterinary Research Communications. 2022; 46: 1245-57.

23. Debertolis L, Mari G, Merlo B, Merbach S, Schoon H-A, Iacono E, et al. Effects of induced endometritis on uterine blood flow in cows as evaluated by transrectal Doppler sonography. Journal of Veterinary Science. 2016;17:189-97.

24. Lüttgenau J, Imboden I, Wellnitz O, Romer R, Scaravaggi I, Neves A, et al. Intrauterine infusion of killed semen adversely affects uterine blood flow and endometrial gene expression of inflammatory cytokines in mares susceptible to persistent breeding-induced endometritis. Theriogenology. 2021; 163:18-30.

25. Sharma A, Singh M, Abrol A, Soni T. Doppler sonography of uterine blood flow at mid-oestrus during different degree of clinical endometritis in dairy cows. Reproduction in Domestic Animals. 2019; 54:1274-8

Table 1: Correlation coefficients between the ovarian and uterine arteries TAMV and BFV with the cortisol, and E2 hormones

	Lt Ov TAMV	Rt Ut TAMV	Lt Ut TAMV	ROA_BFV	LOA_BFV	RUtA_BFV	LUtA_BFV	Cortisol ng/ml	E2 pg/ml
R Ov TAMV	.827***	.848***	.428*	.938***	.753***	.887***	.602***	-.298	.415*
Lt Ov TAMV		.784***	.531**	.739***	.941***	.832***	.700***	-.585**	.438*
Rt Ut TAMV			.407*	.748***	.781***	.966***	.591***	-.113	.330
Lt Ut TAMV				.370*	.528**	.433**	.864***	-.174	.433*
ROA_BFV					.685***	.814***	.540**	-.251	.328
LOA_BFV						.803***	.669***	-.523**	.362
RUtA_BFV							.628***	-.146	.351
LUtA_BFV								-.282	.452*

***Means significant at P<0.05, ** means significant at P<0.001; *** means significant at P<0.0001**

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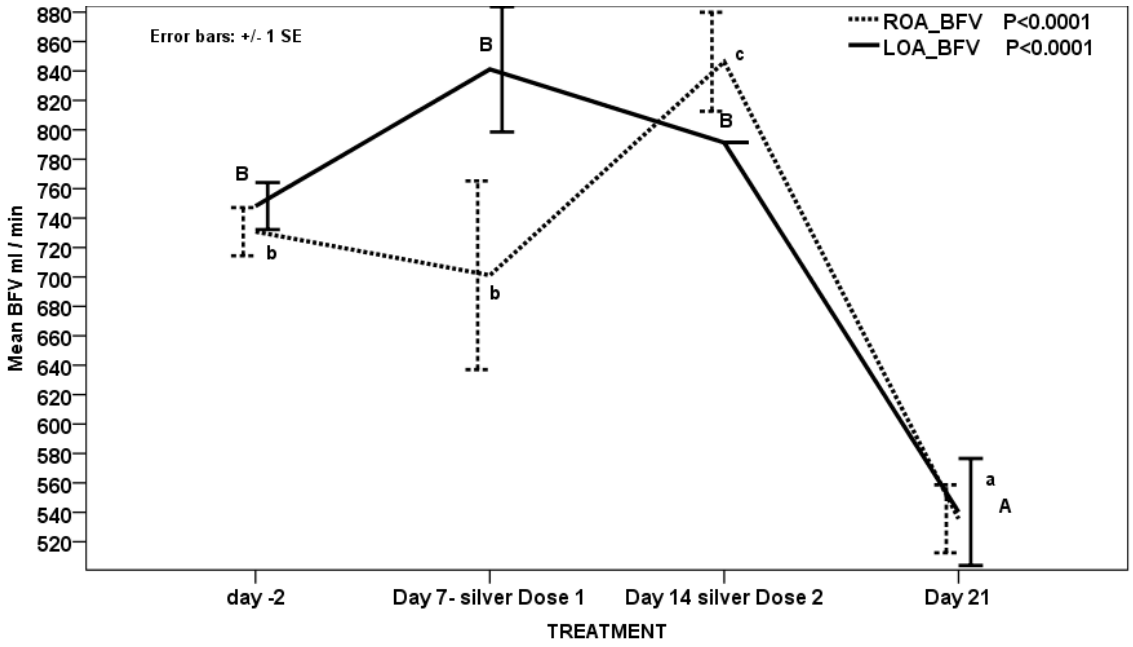

Fig. 1. The blood flow volume of the right (ROA BFV) and left (LOA BFV) ovarian arteries

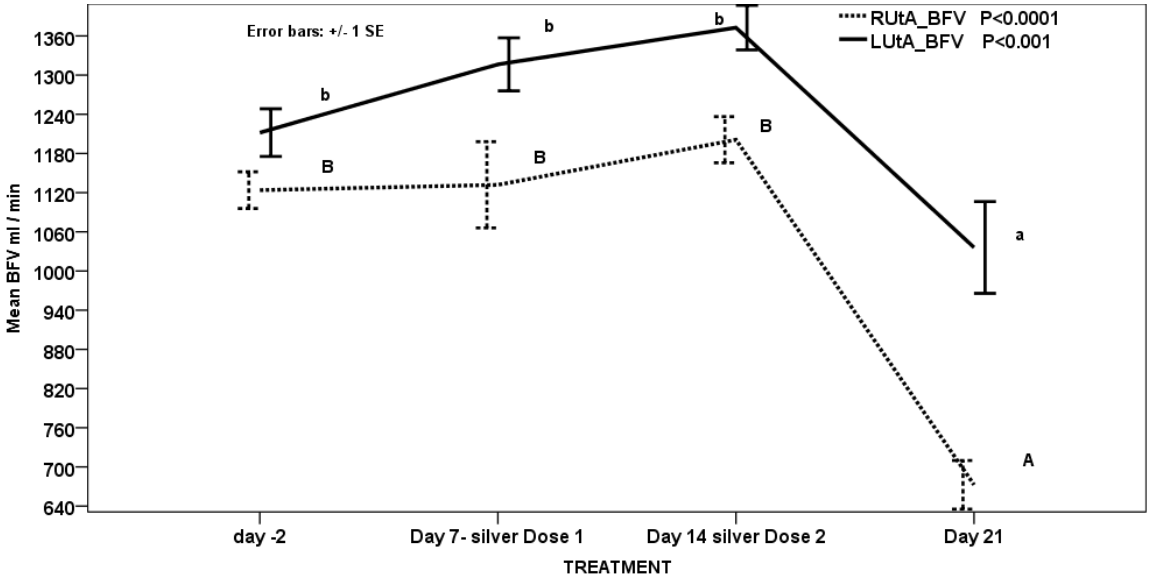

Fig. 2. The Blood flow volume of the right (ROA BFV) and left (LOA BFV) uterine arteries

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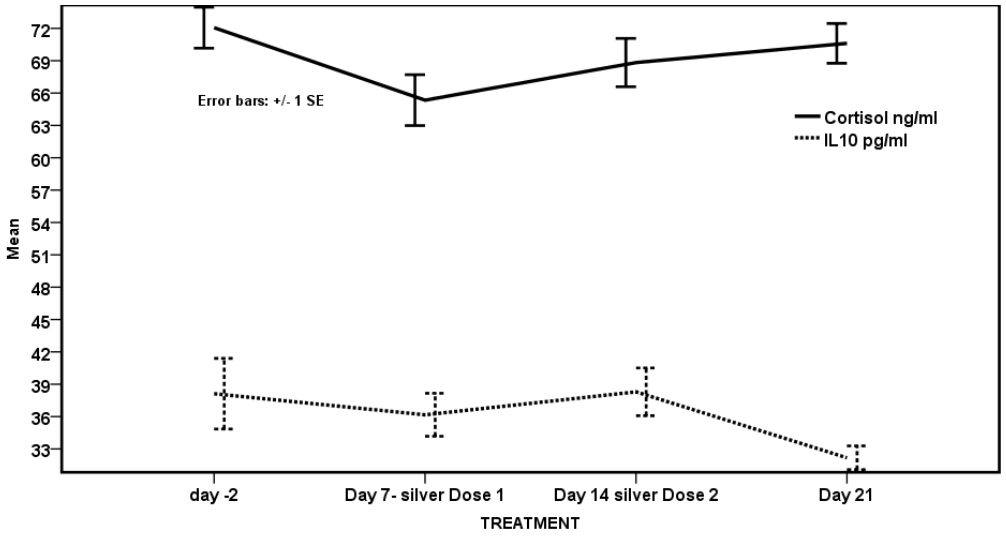

Fig. 3. concentrations of cortisol and interleukin-10 in mares treated with silver nanoparticle

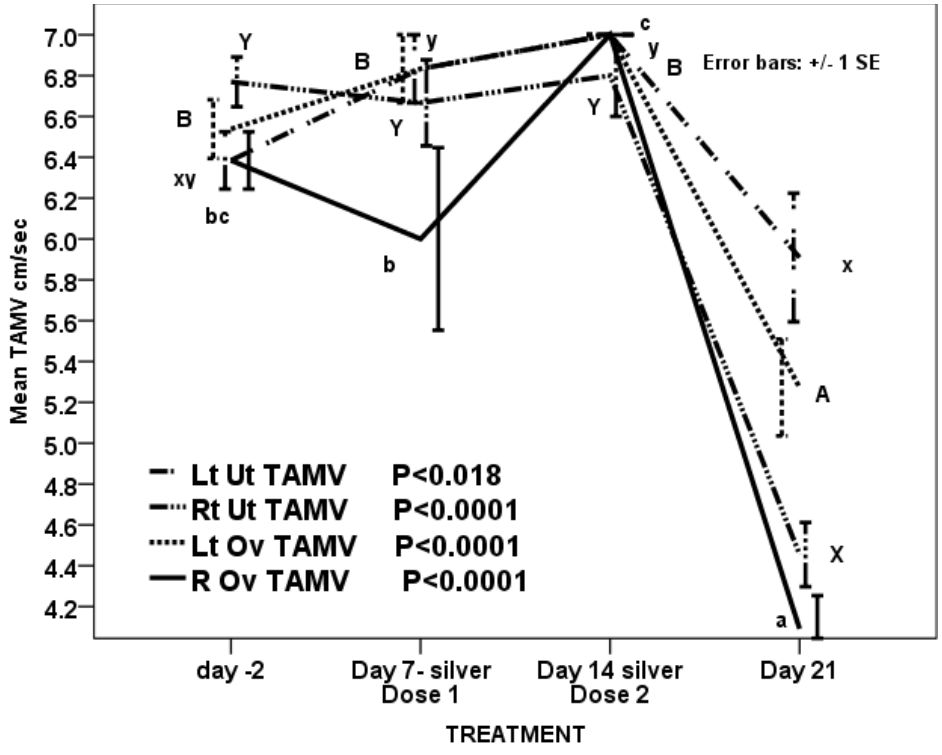

Fig. 4. The ovarian and uterine arteries' time average mean velocity cm/sec

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