Archives of Gynecology and Obstetrics
https://doi.org/10.1007/s00404-022-06538-9

GYNECOLOGIC ONCOLOGY

Protective effect of edaravone on cisplatin-induced injury in rat ovary

Ozlem Kara[1] · **Emin Kaymak**[2] · **Birkan Yakan**[3]

Received: 31 October 2021 / Accepted: 16 March 2022

Abstract

Purpose This study was aimed to evaluate the protective effect of edaravone on cisplatin-induced ovarian injury.

Methods A total 40 female Wistar–Albino rats were utilized to form four groups: Group 1 (control group) ($n=10$), no procedure was performed. Group 2 (cisplatin group) ($n=10$), single-dose 7.5 mg/kg cisplatin was administered and no procedure was performed. Group 3 (edaravone group) ($n=10$), single-dose 1 mg/kg edaravone was administered and no procedure was performed. Group 4 (cisplatin + edaravone group) ($n=10$), single-dose 7.5 mg/kg cisplatin and 1 mg/kg edaravone were administered. Seventy-two hours later, ovaries were surgically extirpated in all groups. Malondialdehyde (MDA) levels and nitric oxide (NO) levels were studied in blood samples. In ovarian tissue samples, DNA damage and apoptosis were assessed using TUNEL method. Ovarian tissue damage was evaluated by immunohistochemical staining with caspase 3 and caspase 8.

Results According to the findings obtained from the study, edaravone showed protective properties on ovarian damage due to cisplatin. MDA and NO levels were significantly higher in cisplatin group than other groups. Histopathological ovarian tissue damage in the cisplatin group was significantly higher than other groups. Similarly, DNA damage and apoptosis were higher in cisplatin group and this difference was found to be statistically significant. The immunohistochemical staining which was done using caspase 3 and caspase 8 was revealed that immunoreactive cells were statistically higher in cisplatin group than cisplatin + edaravone group.

Conclusion Edaravone seems to be effective in prevention of ovarian damage and short-term treatment.

Keywords Cisplatin · Edaravone · Apoptosis · Ovarium · Rat

Introduction

Cisplatin is one of the platinum-derived antineoplastic agents used alone or in combination with other antineoplastic agents in the treatment of cancer. It is immunosuppressive, antimicrobial and sensitive to the biological effects of radiation [1]. Cisplatin has been utilized in the treatment of solid tumors such as advanced ovarian cancer, cervix, bladder, prostate, esophagus, testicular tumors, non-small cell lung cancers, and osteogenic sarcoma [2]. The increase in the activity of cisplatin on cancer cells is significantly correlated with the increase in dose [3]. However, the use of cisplatin in these high doses creates toxic effects in many tissues and organs besides cancer cells [4]. Therefore, cisplatin-induced toxic state causes dose reduction or early discontinuation of chemotherapy [5].

Many researchers have conducted different studies to prevent toxic and side effects caused by drugs used in cancer treatment. The clinicians who think that oxidative stress has a role in these toxic effects have tried products containing different phytochemicals of natural or herbal origin [6]. Edaravone is a powerful synthetic substance that acts by inhibiting lipid peroxidation and scavenging free radicals. It contains both vitamin C and vitamin E properties and can easily cross the blood–brain barrier. In addition, neuroprotective, antiapoptotic and anti-inflammatory effects have been reported as a result of studies [7].

✉ Ozlem Kara
ozlemozturk34@hotmail.com

[1] Department of Histology and Embryology, Kirsehir Ahi Evran University Medical Faculty, Bagbasi Mah, Şehit Necdet Yagiz Cad. No: 143/E, Merkez, Kirsehir, Turkey

[2] Department of Histology and Embryology, Yozgat Bozok University Medical Faculty, Yozgat, Turkey

[3] Department of Histology and Embryology, Erciyes University Institute of Health Sciences, Kayseri, Turkey

Published online: 31 March 2022

Materials and methods

The planning and execution of this study was carried out in Erciyes University Faculty of Medicine, Department of Histology-Embryology. The ethical consent was approved by Erciyes University Animal Experiments Local Ethics Committee (ERU-HADYEK) with the date of 18.01.2019 and number of 19/001. The project was supported by the Erciyes University Scientific Research Projects Unit with the project code TDK-2019-9118. 8-10-week-old Wistar albino type rats were utilized under veterinary control in accordance with the International Declaration of Animal Rights. A 12-h light/12-h dark photoperiod was applied at a room temperature of 18–22 °C, and standard laboratory diet and water requirements were provided.

Experimental study

Forty female Wistar albino rats aged 8–10 weeks were divided into 4 groups:

Group 1: Control ($n = 10$).
Group 2: Cisplatin group (7.5 mg/kg single-dose cisplatin), ($n = 10$).
Group 3: Edaravone group (1 mg/kg edaravone single dose), ($n = 10$).
Group 4: Cisplatin + edaravone group (7.5 mg/kg single-dose cisplatin + 1 mg/kg edaravone single dose), ($n = 10$).

Ketamine hydrochloride (45 mg/kg, Ketalar®, Eczacibasi, Istanbul, Turkey) and xylazine hydrochloride (5 mg/kg, Rompun®, Bayer, Leverkusen, Germany) were utilized for anesthesia. For biochemical analysis, blood was drawn from the hearts of the rats and the ovaries were removed. Intracardiac blood samples were stored at − 80 °C for measurement of malondialdehyde (MDA) and nitric oxide (NO) levels. Ovarian tissues were embedded in paraffin for immunohistochemical and histopathological evaluation with caspase 3, caspase 8, TUNEL and hematoxylin–eosin.

Ovarian tissue samples in 10% formaldehyde solution were fixed for 72 h for light microscopic examination and immunohistochemical staining. Then dehydration process was performed. After clarification with xylene, the tissue samples were kept in an oven at 60 °C in melted paraffin overnight and then blocked. The tissues were cut at 5 µm thickness and these sections were stained with hematoxylin and eosin dye (H&E). Sections stained with Hematoxylin & Eosin were evaluated under light microscopy (Olympus® Inc. Tokyo, Japan). Microscopic areas were scanned to determine the severity of tissue damage. Histopathological changes were defined in terms of hemorrhage, edema, congestion, leukocyte infiltration, and follicle degeneration. Scoring was made between 0 and 3 according to the severity of the damage. 0; none, 1; pathological findings are < 33%, 2; pathological findings are 33–66%, 3; pathological findings are > 66%. Total score was calculated by summing up of each parameter.

Caspase 3 and caspase 8 determination

Active caspase 3 and caspase 8 primary antibodies were used for immunohistochemical staining. Histopathological assessment was performed according to the severity and extensity of the damage as mild, moderate and severe.

TUNEL

The TUNEL method was used following the manufacturer's manual. The commercial kit utilized for TUNEL obtained from Suarge Biotech Industry and Trade. Ltd. (Roche® Inc. Istanbul, Turkey, In Situ Cell Death Detection Kit, Fluorescein). At 40× objective, ten different area were scanned and apoptotic cells were counted for calculation of apoptotic index.

Biochemical analyses

Blood samples taken from rats were centrifuged and stored in Eppendorf tubes into − 80 °C. The MDA kit (Cat. No: E0156Ra, Bioassay Technology Laboratory) was studied using ELİSA method and their amounts were determined as ng/ml at 450 nm in the ELISA reader. The NO levels were detected using the NO kit (Cat. No: E0703Ra, Bioassay Technology Laboratory) and the measurement was explained as µmol/l at 450 nm in the ELISA reader.

Statistical analysis

Statistical package for the Social Sciences (18.00 SPSS Inc., Chicago, IL) was used for statistical analyses. One-way ANOVA test and post hoc Tukey HSD multiple comparison test were used for levels of MDA and NO. Tissue damage scores were compared by Kruskal–Wallis test. Evaluation of caspases was determined by Fisher's Exact Test as p value. p value < 0.05 was accepted as statistically significant.

Results

The MDA and NO levels were higher in the cisplatin group than the cisplatin + edaravone group, and these differences were found to be statistically significant ($p < 0.05$) (Table 1).

Table 1 Nitric oxide (NO), malondialdehyde (MDA) measurements in serum samples of the groups

	NO (nmol/lt)	MDA (nmol/mg)
Group 1 ($n=10$)	14.53 ± 1.57^{a}	$0,29 \pm 0.04^{a}$
Group 2 ($n=10$)	8.53 ± 0.70	0.38 ± 0.03^{b}
Group 3 ($n=10$)	18.03 ± 1.06^{b}	0.29 ± 0.05^{a}
Group 4 ($n=10$)	14.13 ± 1.98^{a}	0.27 ± 0.05^{a}
p value*	0.019	0.0012

The scores demonstrating histopathologic damage were significantly higher in the cisplatin + edaravone group than the cisplatin group ($p < 0.05$) (Table 2).

When the experimental groups were evaluated macroscopically, in the control and edaravone groups, necrosis and bleeding observed. In the control group, the morphologic appearance of the ovarian tissue was normal. Germinal epithelium was single-layered and cuboidal type. Subsequently it had the appearance of prismatic epithelium. Tunica albuginea, primary and secondary follicles were intact (Fig. 1A). Hemorrhage, edema, and follicle degeneration were more intense in the cisplatin group compared to the other groups (Fig. 1B). These damages were not

Table 2 Distribution of histological damage according to the groups

	Group 1 ($n=10$)	Group 2 ($n=10$)	Group 3 ($n=10$)	Group 4 ($n=10$)
Hemorrhage	0.00	3.00*	0.00	2.20 ± 0.6*
Edema	0.00	3.00*	0.00	1.60 ± 0.6*
Vascular congestion	0.00	3.00*	0.00	2.30 ± 0.7*
Leukocyte infiltration	0.00	3.00*	0.00	1.50 ± 0.5*
Follicle degeneration	0.60 ± 0.5	3.00*	0.60 ± 0.6	1.25 ± 0.5*

*Kruskal–Wallis test, the difference between group 2 and group 4 was statistically significant ($p < 0.001$)

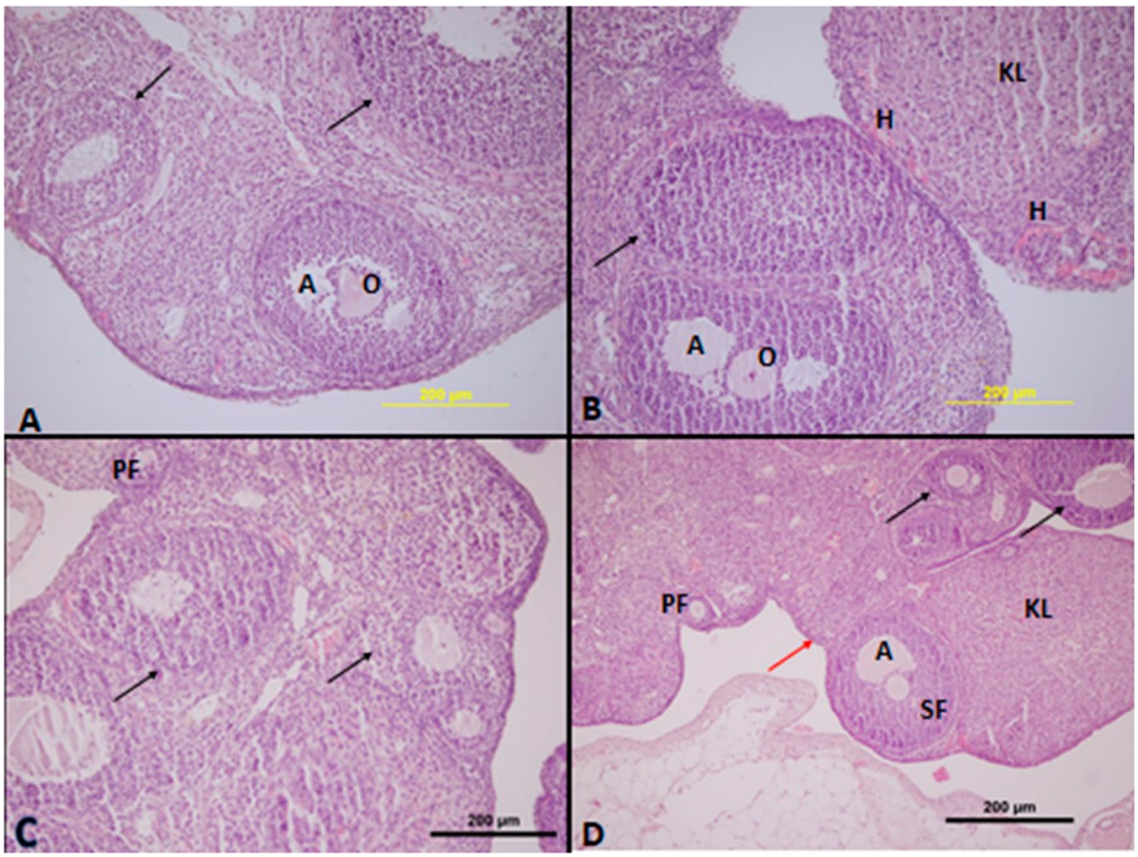

Fig. 1 Hematoxylin and eosin staining of ovarian tissue. **A** Control group, **B** cisplatin group, **C** edaravone group, **D** cisplatin + edaravone group. Corpus luteum (KL), secondary follicle (SF), follicles (black arrow), antrum (A), oocyte (O), hemorrhage (H), germinal epithelium (red arrow)

observed in the edaravone group and the connective tissue was containing normal histological features (Fig. 1C). In the cisplatin + edaravone group, the combined use of edaravone with cisplatin improved the congestion moderately, cellular degeneration and neutrophil infiltration weakly, and abolished hemorrhage and cell damage at a high level (Fig. 1D).

Apoptosis was observed in the cortex and medulla of the ovary with TUNEL staining. Apoptosis was observed not only in follicles but also in corpus luteum, stroma and germinal epithelium (Fig. 2). Ovarian sections, which were examined for the immunoreactivity of caspase 3 and caspase 8, were evaluated under the light microscope. There were no caspase 3 and caspase 8 immunoreactivity in the control and edaravone groups, The cisplatin group had more intense caspase immunoreactivity than cisplatin + edaravone group (Fig. 3).

Discussion

We investigated the effect of edaravone on cisplatin-induced ovarian toxicity, in this prospective randomized controlled trial. Our study has indicated that MDA and NO levels were increased due to cisplatin. Addition of edaravone led to a decrease in MDA and NO levels. In addition, the ovarian damage parameters such as hemorrhage, edema, congestion, leukocyte infiltration, and follicle degeneration were less in cisplatin + edaravone group than the cisplatin group ($p < 0.05$). Our study was demonstrated that edaravone usage prevented the toxic effects of cisplatin on ovarian tissue. We think that, this is the first study related with the protective effect of edaravone on cisplatin-induced ovarian damage.

Cisplatin increases the levels of reactive oxygen species (ROS) and free radicals. The degree of the increase determines the severity of the toxicity. Since cellular anatomy is negatively influenced by oxidative stress [8]. Borovskaya et al. indicated the harmful effects of cisplatin on ovarian tissue. They thought that direct toxicity could be play a role in the pathogenesis of gonadotoxicity [9]. Dixit et al. reported that ovarian failure occurred in up to 40% of patients who were prescribed cisplatin [10].

Vitamin C and E were studied top prevent the damage due to oxidative stress. Ourique et al. reported that the increased lipid peroxidation was responsible for the testicular injury in the rats administered valproic acid. They tried to give vitamin C and E to reverse the harmful effect

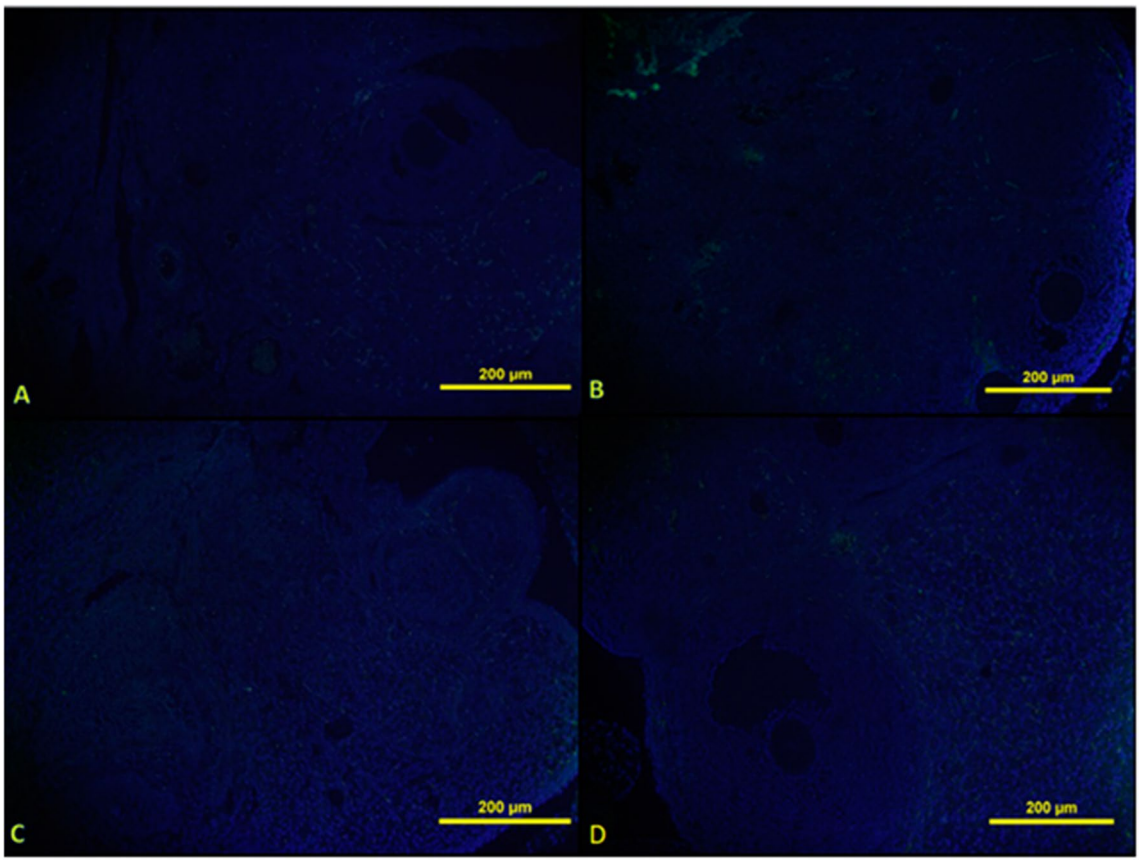

Fig. 2 TUNEL painting of ovarian tissue in all groups. **A** Control group, **B** cisplatin group, **C** edaravone group, **D** cisplatin + edaravone group. TUNEL×20

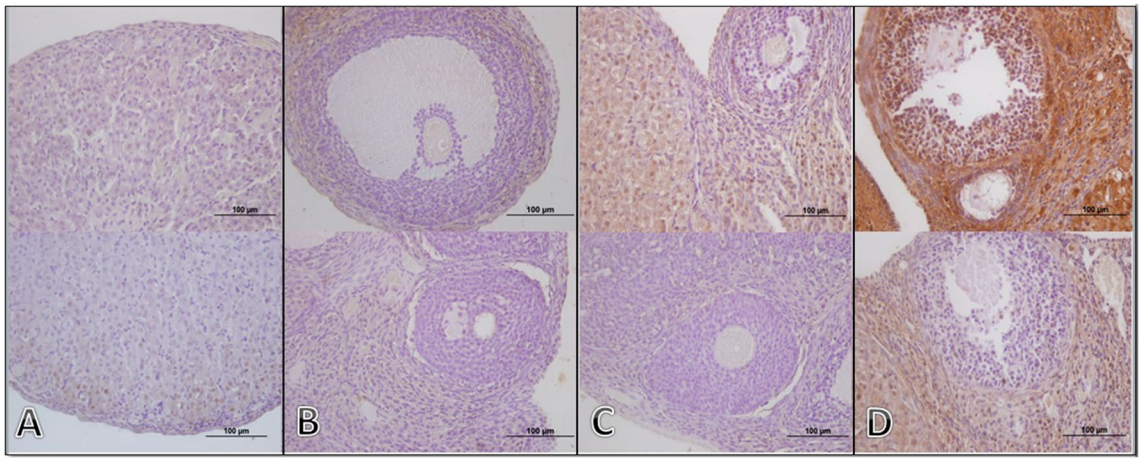

Fig. 3 Immunohistochemical staining with caspase 3–caspase 8 antibody, all groups. Ovarian tissue of **A** control group, **B** edaravone group, **C** cisplatin + edaravone group, **D** cisplatin group (top picture caspase 3, lower picture caspase 8 ×20)

[11]. Since edaravone has vitamin C and vitamin E properties, it inhibits lipid peroxidation and scavenges free radicals against oxidative stress [12]. Therefore, we thought that edaravone could prevent the cisplatin-induced ovarian injury. In our study, serum MDA value was significantly lower in the cisplatin + edaravone group than the cisplatin group ($p < 0.05$). In addition, NO levels were found to be significantly higher in the cisplatin group than the cisplatin + edaravone group ($p < 0.05$). It was also observed that edaravone treatment decreased the cisplatin-induced damage on ovarian tissue. The improvement in the morphology and structural characteristics was prominent in the cisplatin + edaravone group. The histopathological features in TUNEL staining, and caspase 3 and 8 antibodies were compatible with H&E dye.

In conclusion, the treatment of edaravone seems to reduce ovarian injury due to cisplatin. However, large prospective, randomized trials are needed.

Acknowledgements None declared.

Author contributions OK: project development, data collection, and manuscript writing. BY: project development, data management, and manuscript writing. EK: data collection and analysis of data. Surgical and medical practices—OK, EK. Concept—BY. Design—BY, OK. Data collection or processing—OK, EK. Analysis or ınterpretation—OK, EK. Literature search—OK. Writing—OK.

Funding Funding was provided by Erciyes Üniversitesi (Grant number: TDK-2019-9118).

Declarations

Conflict of interest The project was supported by the Erciyes University Scientific Research Projects Unit with the project code TDK-2019-9118.

Ethical approval The planning and execution of this study was carried out in Erciyes University Faculty of Medicine, Department of Histology-Embryology. The ethical consent was approved by Erciyes University Animal Experiments Local Ethics Committee (ERU-HADYEK) with the date of 18.01.2019 and number of 19/001.

Informed consent No informed consent was obtained, because it was an experimental animal study.

Research involving human and animal rights Institutional and national guidelines were used for the animals. All animals were handled in accordance with these criteria.

References

1. Gerald KMcE, Pharm D (2006) AHFS drug information, Bethesda, Maryland
2. Kayaalp O (2005) Türkiye İlaç Kılavuzu TİK-4 2005 Formülleri. Turgut Yayıncılık, Ankara
3. Chtourou Y, Aouey B, Kebieche M, Fetoui H (2015) Protective role of naringin against cisplatin induced oxidative stress, inflammatory response and apoptosis in rat striatum via suppressing ROS-mediated NF-κB and P53 signaling pathways. Chem Biol Interact 239:76–86
4. Kim JH, Jeong SJ, Kwon HY, Park SY, Lee HJ (2010) Decursin prevents cisplatin-induced apoptosis via the enhancement of antioxidant enzymes in human renal epithelial cells. Biol Pharm Bull 33(8):1279–1284
5. Brouwers EE, Huitema AD, Boogerd W, Beijnen JH, Schellens JH (2009) Persistent neuropathy after treatment with cisplatin and oxaliplatin. Acta Oncol 48(6):832–841
6. El Gharras H (2009) Polyphenols: food sources, properties and applications—a review. Int J Food Sci Technol 44(12):2512–2518
7. Masuda T, Shimazawa M, Hara H (2017) Retinal diseases associated with oxidative stress and the effects of a free radical scavenger (Edaravone). Oxid Med Cell Longev 1–14
8. Boulikas T, Vougiouka M (2003) Cisplatin and platinum drugs at the molecular level (review). Oncol Rep 10(6):1663–1682

9. Borovskaya TG, Goldberg VE, Fomina TI, Pakhomova AV, Kseneva SI, Poluektova ME et al (2004) Morphological and functional state of rat ovaries in early and late periods after administration of platinum cytostatics. Bull Exp Biol Med 137:331–335
10. Dixit M, Yang JL, Poirier MC, Price JO, Andrews PA, Arteaga CL (1997) Abrogation of cisplatin induced programmed cell death in human breast cancer cells by epidermal growth factor antisense RNA. J Natl Cancer Inst 89(5):365–373
11. Ourique GM, Saccol EM, Pês TS, Glanzner WG, Schiefelbein SH, Viviane M (2016) Protective effect of vitamin E on sperm motility and oxidative stress in valproic acid treated rats. Food Chem Toxicol 95:159–167
12. Kara M, Daglioglu YK, Kuyucu Y, Tuli A, Tap O (2012) The effect of edaravone on ischemia-reperfusion injury in rat ovary. Eur J Obstet Gynecol Reprod Biol 162(2):197–202