Int J Clin Exp Med 2019;12(1):705-711
www.ijcem.com /ISSN:1940-5901/IJCEM0076653

Original Article

Efficacy level of dimethyl-sulfoxide (DMSO) in the prevention of peritoneal adhesions: an experimental rat model

Emre Gunay, Haci Hasan Abuoglu, Hakan Uzunoglu, Oguzhan Sunamak, Cebrail Akyuz

Department of General Surgery, University of Health Sciences, Haydarpasa Numune Training and Research Hospital, Istanbul, Turkey

Received March 24, 2018; Accepted October 9, 2018; Epub January 15, 2019; Published January 30, 2019

Abstract: Background: The most common causes of adhesions include previous abdominal surgeries, foreign body reactions and ischemia. The efficacy of fibrinolytics, anticoagulants, anti-inflammatory agents, antibiotics, as well as several materials that may serve as a physical barrier to the prevention of these adhesions. Methods: A total of 16 Wistar-Albino rats weighing between 200-250 grams were used for the study. The rats were divided into two groups, as follows: Group 1: Control group and Group 2: Administered with intraperitoneal DMSO. Group 1: The peritoneal cavity was opened and the peritoneal surface on both sides of the abdominal wall was mounted by a hemostat and the base was sutured with 4/0 silk for several times to form peritoneal ischemia and adhesions. Group 2: Same pathway as group 1 has been followed but 1 ml of sterile DMSO solution was infused into the abdomen before the midline incision was closed. Results: The extent and amount of the adhesions was significantly lower in the DMSO group. The blood tPA levels were significantly higher in the DMSO group than the control group. The tPA levels in the peritoneal flush fluid were not significantly different between the two groups. The amount of collagen fibers, the level of vascularization and the fibroblastic activity in the DMSO group were significantly lower than the median value in the control group. Conclusions: The macroscopic, histopathological and hematological findings of the present study demonstrate that the intra-abdominal administration of DMSO reduces intra-abdominal adhesion formation through both local and systemic effects.

Keywords: Adhesion, dimethyl sulfoxide, peritoneal, peritoneum

Introduction

The fast recovery potential and unique characteristics of the peritoneum play a significant role in the formation and prevention of intra-abdominal adhesions [1]. The surface of the peritoneum is covered by a single layer of mesothelial cells, and irrespective of the degree of peritoneal damage, it is repaired by surrounding mesothelial cells within 3-5 days [2].

Peritoneal adhesions generally appear as fibrous bands that form between the serosal surfaces and neighboring organs. The most common causes of adhesions include previous abdominal surgeries (90-95%), foreign body reactions and ischemia [3, 4]. Trauma increases fibrin levels by suppressing fibrinolytic activity, and it also induces the secretion of histamine and vasoactive quinine, thereby increasing capillary permeability. Consequently, the serous-angiosis fluid that is produced plays a role in the formation of fibrous bands between the peritoneal surface and the neighboring organs. The commonly agreed mechanism that underlies the formation of adhesions is associated with post-surgical tissue ischemia and the subsequent development of fibrosis, accompanied by decreased levels of tissue plasminogen activator (tPA) [5].

The ligation of the peritoneal surfaces with silk sutures may result in the formation of adhesions due to foreign body reaction and peritoneal ischemia. The efficacy of fibrinolytics, anticoagulants, anti-inflammatory agents, antibiotics, as well as several materials that may serve

as a physical barrier to the prevention of these adhesions, which may result in intestinal obstructions, volvulus, infertility and abdominal or pelvic pain, has been assessed in several earlier studies [6-8]. Inflammatory response and oxidative stress play significant roles in the formation of peritoneal adhesions, and the majority of currently available agents aimed to prevent adhesions operate by reducing or inhibiting these processes.

Dimethyl-sulfoxide (DMSO) is widely used in medicine due to its anti-inflammatory, anticoagulant, diuretic and analgesic characteristics, as well as its role in the prevention of fibroblast proliferation [9]. In the present study, we investigate the anti-inflammatory, anti-proliferative and fibrinolytic activity of dimethyl-sulfoxide in preventing peritoneal adhesions in an experimental rat model.

Materials and methods

Ethics approval

Ethics committee approval for this experimental study was obtained from the T.R. Istanbul University Animal Experiments Local Ethics Committee (Decision no.: 2016/10). The experimental animals used in this study were provided by the T.R. Marmara University Experimental Animals Application and Research Center, and the study was carried out in the Central Laboratories of the T.R. Marmara University Experimental Animals Application and Research Center.

Experimental groups

A total of 16 Wistar-Albino, mixed-strain rats weighing between 200-250 grams were used for the study. The rats were divided into two groups, each consisted of eight rats as follows: Group 1: Control group and Group 2: Administered with intraperitoneal DMSO.

All of the rats to be used in this study were kept under the same laboratory conditions for one week before the experiment. The rats were kept at room temperature in 12 hours nighttime and 12 hours daytime cycles, and at most in groups of four in standard cages. The rats were fed with standard laboratory meals and water during the preoperative and postoperative periods.

Surgical technique

General anesthesia was achieved in all rats through the intraperitoneal administration of 100 mg/kg cetamine (Ketalar, Parke Davis, Istanbul, Turkey), after which, the rats were placed in the supine position, and their extremities were fixed with wound plasters. The anterior abdominal wall was shaved, and the shaved region was sterilized with a povidone-iodine solution (Betadine, Kurtsan, Istanbul, Turkey). The surgical procedures were carried out using standard tools. Group 1 (Control group): The peritoneal cavity was opened with a midline incision measuring 3 cm. Approximately 0.5 cm^2 of the peritoneal surface on both sides of the abdominal wall was mounted by a hemostat and the base was sutured with 4/0 silk (Ipek, Dogsan, Istanbul, Turkey). This procedure was repeated three times to form peritoneal ischemia and adhesions. The abdominal incision was closed with a continuous suturing technique using a 3/0 polypropylene suture material (Prolene, Dogsan, Istanbul, Turkey). Group 2 (DMSO group): The peritoneal cavity was opened with a midline incision measuring 3 cm. Approximately 0.5 cm^2 of the peritoneal surface on both sides of the abdominal wall was mounted by a hemostat, and the base was sutured with 4/0 silk (Ipek, Dogsan, Istanbul, Turkey). This procedure was repeated three times to form peritoneal ischemia and adhesions. Afterwards, 1 ml of sterile DMSO solution was infused into the abdomen, and the abdominal midline incision was closed with a continuous suturing technique using a 3/0 polypropylene suture material (Prolene, Dogsan, Istanbul, Turkey). All rats were administered with an analgesic after the procedure and left for reanimation. Standard rat feed and tap water were given 6 hours after operation, and the rats were permitted to feed ad libitum.

Sacrification and evaluation

None of the rats died as a result of the surgery or anesthesia during the postoperative follow-up. On the 10th day, general anesthesia was achieved in all rats through the intraperitoneal administration of 100 mg/kg of cetamine. The skin was decollated over the facia to avoid damaging the implantation line. The abdominal wall of all rats was opened in a caudal direction with a “reverse U” incision to preserve adhe-

Table 1. Comparison of adhesion extent and amount based on Nair's macroscopic classification

		Nair's Macroscopic Classification		[a]P
		DMSO (n=8)	Controls (n=8)	
Extent of adhesions	+	1 (12.5)	0	
	++	5 (62.5)	1 (12.5)	
	+++	2 (25.0)	7 (87.5)	
Min-Max (Median)		1-3 (2)	2-3 (3)	0.014*
Amount of adhesions	+	2 (25.0)	0	
	++	6 (75.0)	1 (12.5)	
	+++	0	7 (87.5)	
Min-Max (Median)		1-2 (2)	2-3 (3)	0.001**

[a]Mann-Whitney U Test, *P<0.05.

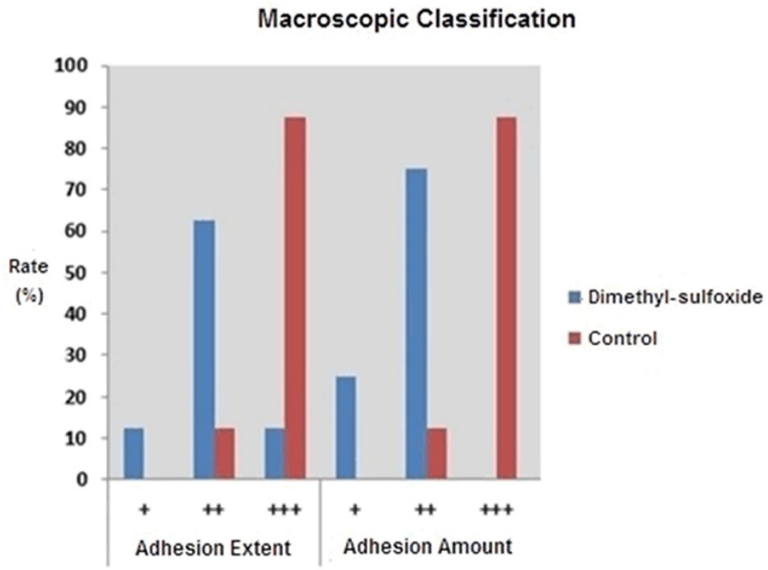

Figure 1. Macroscopic classification.

sion formation. After the peritoneal cavity was flushed with sterile physiological serum, 1 ml of peritoneal flush fluid was obtained for tPA measurement, and intra-abdominal adhesions were quantitatively evaluated according to Nair's macroscopic classification [10]. The evaluations were carried out in a double-blind manner by two different investigators, who used a classification method that was previously explained to them. After the macroscopic evaluations, samples for pathological examination were obtained through excisions of the affected organs, along with the adhesion bands in the rats that developed adhesions and from excision of regions of peritoneal ischemia, including all layers except the skin, from the rats without adhesion formation. Afterwards, pieces obtained for pathological examination were fixed to plates with a 10 percent buffered formaldehyde solution. Then, the anesthetized rats were sacrificed through the drainage of intracardiac blood, and the collected blood samples were centrifuged at 1000 G for 15 minutes. The separated plasma samples were transferred to Eppendorf tubes and stored at -20°C for the measurement of tPA levels. The samples were then transferred in cold-chain to Bezmialem University Biochemistry Laboratories, where tPA measurements were made using the micro-ELISA method (Rat tPA ELISA Kit, Innovative Research, Novi, USA), according to the manufacturer's instructions.

Histopathological examitions were carried out at the Pathology Laboratories of the T.R. Medeniyet University Goztepe Training and Research Hospital. The piees that were treated using conventional laboratory methods were embedded in paraffin blocks, and cross-sections with a thickness of 5 micrometers were placed on a slide and investigated under a light microscope after hematoxylin-eosin staining. The pathologist who carried out the investigations was unaware of the group assignments. The pieces were classified based on Zuhlke's microscopic classification following the histopathological investigations [11].

Statistical analysis

The statistical analyses were carried out using NCSS (Number Cruncher Statistical System) 2007 (NCSS, LLC Kaysville, Utah, USA) software. Descriptive statistics were used to describe the study data(mean, standard deviation, median, frequency and ratio), and the normally-distributed variables were compared between the study groups with a Mann-Whitney U test. Mann-Whitney U test is the nonpa

Table 2. Comparison of tPA (tissue plasminogen activator) levels in peritoneal flush fluid and blood

		Comparison of tPA levels		[a]p
		DMSO (n=8)	Controls (n=8)	
TPA levels in peritoneal flush fluid (ng/ml)	Mean±SD	0.57±0.31	0.65±0.34	0.713
	Min-Max (Median)	0.30-1.26 (0.4)	0.26-1.10 (0.6)	
Blood tPA levels (ng/ml)	Mean±SD	2.06±0.88	0.79±0.42	0.012*
	Min-Max (Median)	0.59-3.02 (2.3)	0.26-1.65 (0.7)	

[a]Mann-Whitney U Test, *P<0.05.

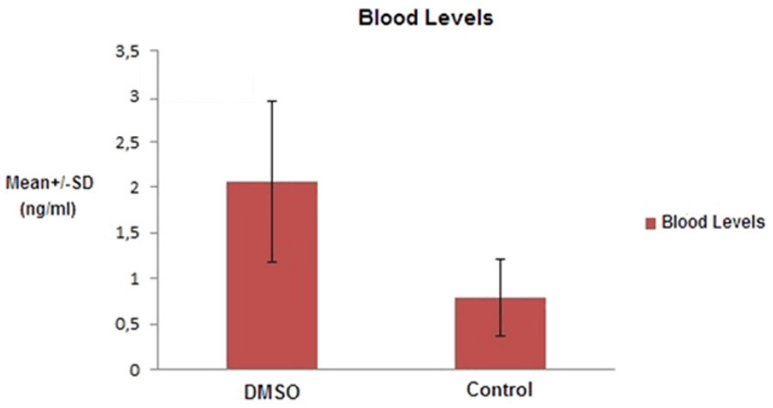

Figure 2. Comparison of blood tPA levels.

rametric alternative test to the independent sample t-test. It is a non-parametric test that is used to compare two sample means that come from the same population, and used to test whether two sample means are equal or not. The results were evaluated within 95 percent confidence intervals and *p*-values <0.05 were considered significant.

Results

The study took place between Feb 1, 2016-May 1, 2016 in the Central Laboratories of the T.R. Marmara University Experimental Animals Application and Research Center, on a total of 16 subjects.

Macroscopic findings

The extent of the adhesions was significantly different between the two groups (*P*=0.014; *P*<0.05), and the median value of the DMSO group was significantly lower than that of the control group. The amount of adhesion was also significantly different between the two groups (*P*=0.001; *P*<0.05), with the median value in the DMSO group being significantly lower than that of the control group (**Table 1**; **Figure 1**).

Hematological findings

There was a significant difference in the blood tPA levels of the two groups (*P*=0.012; *P*<0.015), with the measured levels being significantly higher in the DMSO group than the control group. The mean blood tPA levels in the DMSO and control groups were 2.06 ±0.88 ng/ml and 0.79±0.42 ng/ml, respectively. The tPA levels in the peritoneal flush fluid were not significantly different between the two groups (*P*>0.05)(**Table 2**; **Figure 2**).

Histopathological findings

The amount of collagen fibers differed significantly between the two groups (*P*=0.013; *P*<0.05), with the median value in the DMSO group being significantly lower than the median value in the control group. The level of vascularization was also significantly different between the two groups (*P*=0.014; *P*<0.05), and the median value in the DMSO group was significantly lower than the median value in the control group. There was a significant difference between the two groups regarding fibroblastic activity (*P*=0.025; *P*<0.05), and the median value in the DMSO group was significantly lower than the median value in the control group (**Table 3**; **Figure 3**).

Discussion

Intra-abdominal adhesion formation is a significant cause of postoperative morbidity [12]. Adhesions that form after abdomino-pelvic surgery are among the most challenging complica-

Table 3. Microscopic comparison of the amount of collagen fibers, level of vascularization and fibroblastic activity

		Microscopic Classification		[a]P
		DMSO (n=8)	Controls (n=8)	
Amount of collagen fibers	+	1 (12.5)	0 (0)	
	++	6 (75.0)	2 (25.0)	
	+++	1 (12.5)	6 (75.0)	
Min-Max (Median)		1-3 (1)	2-3 (3)	0.013*
Level of vascularization	+	5 (62.5)	0 (0)	
	++	2 (25.0)	4 (50.0)	
	+++	1 (12.5)	4 (50.0)	
Min-Max (Median)		1-3 (1)	2-3 (2.5)	0.014*
Fibroblastic activity	+	4 (50.0)	0 (0)	
	++	3 (37.5)	4 (50.0)	
	+++	1 (12.5)	4 (50.0)	
Min-Max (Median)		1-3 (1.5)	2-3 (2.5)	0.025*

[a]Mann-Whitney U Test, **P<0.01.

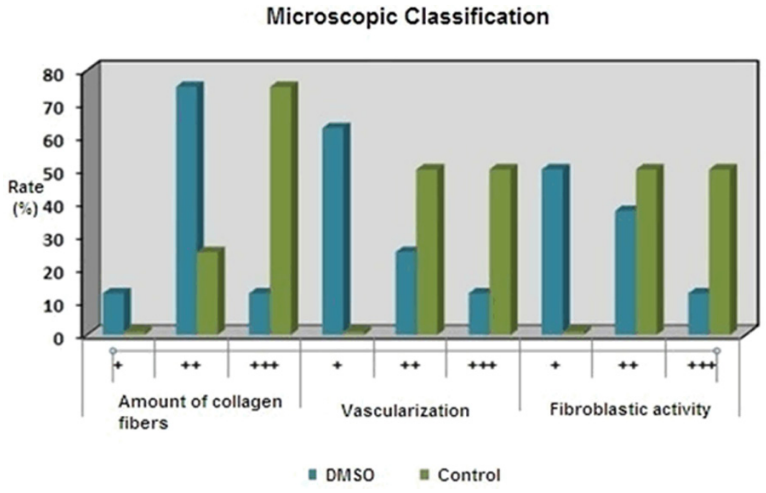

Figure 3. Microscopic classification.

tions for surgeons and gynecologists given that they may result in intestinal obstruction, infertility, chronic pelvic pain and potential complications during future surgeries [13].

Inflammatory response and the associated decrease in tissue oxygenation play significant roles in the formation of adhesions. Inflammation is the first response to peritoneal damage, and the development of this response depends on local eicosanoid secretion(the secretion of prostaglandins in particular) [14, 15].

Previous surgeries, ischemia and foreign body reactions are the leading factors in the formation of adhesions [1-4]. To induce the formation of peritoneal adhesions in this study, approximately 0.5 cm^2 of the peritoneal surface on both sides of the abdominal wall was mounted by a hemostat, and the base was sutured with 4/0 silk, and this procedure was repeated three times. In this study, the formation of adhesions may be a result of foreign body reaction, which can increase the inhibition of the tissue plasminogen activator (tPA). Previous research showed that increased inflammation due to foreign body reaction reduces fibrinolytic activity, as well as the level of tissue plasminogen activator (tPA) by increasing the levels of type-1 and type-2 plasminogen activator inhibitors (PAI). Type-1 and type-2 plasminogen activator inhibitors (PAI) neutralize the activity of tissue plasminogen activators (tPA) [1]. Reduced oxygenation due to increased inflammation may also contribute to the formation of peritoneal adhesions.

Dimethyl sulfoxide (DMSO) is widely used in medicine as a result of its anti-inflammatory, anti-coagulant, diuretic and analgesic characteristics, as well as its role in the prevention of fibroblast proliferation [9].

DMSO is a polar, aprotic solvent that is used as an inactive constituent in several FDA-approved products, which enhances tissue penetration and has a concentration-dependent effect on tissue membranes. Modular dynamic simulations have shown that DMSO can break down the lipid bilayer, can alter membrane viscosity, and can form hydrophilic and hydrophobic water pores at higher concentrations [16].

Weaker fibroblastic activity, as seen in the DMSO group in this study, could be attributed to the high fibrinolytic activity of DMSO. Blood

tPA levels were significantly elevated in the group administered with DMSO in this study, and due to its high viscosity, DMSO may have served as a barrier and prevented the formation of peritoneal adhesions by acting as a lubricant between areas of peritoneal fibrosis and the serosal surfaces of the intra-abdominal organs. In a previous study, Kilic et al. [17] reported relative success in the prevention of adhesion formation despite weak fibrinolytic activity in subjects administered with intraperitoneal physiological serum (PS). This activity of PS could also be attributed to that PS forms a barrier and acts as a lubricant between regions of peritoneal fibrosis and the serosal surfaces of the internal organs.

Experimental and clinical studies have demonstrated that any type of peritoneal damage is repaired by the neighboring mesothelial cells within 3-5 days, and this rapid recovery potential of the peritoneum plays a significant role in the formation and prevention of intra-abdominal adhesions [7]. The findings of the histopathological investigations in this study indicate that damaged peritoneal surfaces are repaired by collagen fibers, capillary vascularization and fibrous tissue.

Dimethyl sulfoxide (DMSO) is also used extensively used as a cryoprotective agent in biological research, being a quite polar organic liquid that consists of one polar sulphonyl group and two apolar methyl groups that is used frequently as a chemical solvent [18, 19]. Its ability to diffuse into biological membranes has led to it being used as a cryoprotective agent for the hypothermal storage of such blood cells as thrombocytes [20, 21]. Its ability to easily diffuse into living cells and modulate membrane permeability makes DMSO an appropriate cryoprotective agent [22], and also reduces the formation of intracellular ice crystals. Accordingly, damage resulting from freezing may be reduced in cell and tissues perfused with DMSO [23]. Some anti-cancer treatment methods may reduce fertility, or even cause infertility, and the preservation of fertility should be recommended to patients before initiating such therapies, as there are many fertility-preserving treatment options available for female patients. Bedaiwy et al. [24] and Martinez-Madrid et al. [25, 26] carried out initial interventions for preservation by perfusing and freezing intact human ovaries in a 10 percent DMSO solution.

Conclusion

The macroscopic, histopathological and hematological findings of the present study demonstrate that the intra-abdominal administration of DMSO reduces intra-abdominal adhesion formation through both local and systemic effects. Further studies are required to investigate the oral use of DMSO, dose adjustments, systemic adverse effects and clinical procedures, such as allergy tests.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Emre Gunay, Department of General Surgery, University of Health Sciences, Haydarpasa Numune Training and Research Hospital, Tibbiye Cad., Selimiye Mah., Haydarpasa Numune Hastanesi Genel Cerrahi Klinigi, Uskudar, Istanbul 34668, Turkey. Tel: +90-532-4982313; E-mail: emregunay2000@yahoo.com

References

[1] Alkan F, Koc Y, Celik I, Erol M, Aydin MF. Researching the effects of methyl-prednisolone (MP) and dimethyl-sulphoxide(DMSO) on the prevention of peritoneal adhesions in rabbits. Vet Bil Derg 2007; 21: 73-79.

[2] Jenkins SD, Klamer TW, Parteka JJ, Condon RE. A Comparison of prosthetic materials used to repair abdominal wall defects. Surgery 1983; 94: 392-398.

[3] Baptista ML, Bonsack ME, Delaney JP. Sepra film reduces adhesions to polypropylene mesh. Surgery 2000; 128: 86-92.

[4] Dinsmore RC, Calton WC, Harvey SB, Blaney MW. Prevention of adhesions to polypropylene mesh in a traumatized bowel model. J Am Coll Surg 2000; 191: 131-136.

[5] Pismensky SV, Kalzhanov ZR, Eliseeva MY, Kosmas IP, Mynbaev OA. Severe inflammatory reaction induced by peritoneal trauma is the key driving mechanism of postoperative adhesion formation. BMC Surg 2011; 11: 30.

[6] Novitsky YW, Harrell AG, Cristiano JA, Paton BL, Norton HJ, Peindl RD, Kercher KW, Heniford BT. Comparitive evaluation of adhesion formation, strenghth of ingrowth, and textile properties of prosthetic meshes after long-term intra-abdominal implantation in a rabbit. J Surg Res 2007; 140: 6-11.

[7] Koehler RH, Begos D, Berger D, Carey S, LeBlanc K, Park A, Ramshaw B, Smoot R, Voeller G. Minimal adhesions to ePTFE mesh after laparoscopic ventral incisional hernia repair:

reoperative findings in 65 cases. JSLS 2003; 7: 335-340.
[8] Leber GE, Garb JL, Alexander AI, Reed WP. Long term complications associated with prostetic repair of incisional hernias. Arc Surg 1998; 133: 378-382.
[9] Cihan M, Baran V, Ozaydın I, Atalan G. Treatment of peritonitis caused by body using intraperitoneal dimethyl-sulfoxide (DMSO). Kafkas Univ Vet Fak Derg 2004; 10: 23-25.
[10] Nair SK, Bhat IK, Aurora AL. Role of proteolytic enzyme in the prevention of postoperative intraperitoneal adhesions. Arch Surg 1974; 108: 849-853.
[11] Zühlke HV, Lorenz EM, Straub EM, Savvas V. Pathophysiology and classification of adhesions. Langenbecks Arch Chir Suppl II Verh Dtsch Ges Chir 1990; 1009-1016.
[12] Ellis H, Moran BJ, Thompson JN, Parker MC, Wilson MS, Menzies D, McGuire A, Lower AM, Hawthorn RJ, O'Brien F, Buchan S, Crowe AM. Adhesion-related hospital readmissions after abdominal and pelvic surgery: a retrospective cohort study. Lancet 1999; 353: 1476-1480.
[13] Liakakos T, Thomakos N, Fine PM, Dervenis C, Young RL. Peritoneal adhesions: etiology, pathophysiology, and clinical significance. Recent advances in prevention and management. Dig Surg 2001; 18: 260-273.
[14] Saed GH, Munkarah AR, Diamond MP. Cyclooxygenase-2 expressed in human fibroblasts isolated from intraperitoneal adhesions but not from normal peritoneal tissues. Fertil Steril 2003; 79: 1404-1408.
[15] Gilroy DW, Tomlinson A, Willoughby DA. Differential effects of inhibitors of cyclooxygenase (cyclooxygenase 1 and cyclooxygenase 2) in acute inflammation. Eur J Pharmacol 1998; 355: 211-217.
[16] Notman R, Noro M, O'Malley B, Anwar J. Molecular basis for dimethyl-sulfoxide (DMSO) action on lipid membranes. J Am Chem Soc 2006; 128: 13982-13983.
[17] Kilic K, Kilic N, Kilic E, Yayla S. A comparison of the efficacy of dimethyl-sulfoxide (DMSO) and synovial fluid in the prevention of peritoneal adhesions: experimental rabbit model. Kafkas Univ Vet Fak Derg 2013; 19: 27-32.
[18] Hollebeeck S, Raas T, Piront N, Schneider YJ, Toussaint O, Larondelle Y, During A. Dimethylsulfoxide (DMSO) attenuates the inflammatory response in the in vitro intestinal Caco-2 cell model. Toxicol Lett 2011; 206: 268-275.
[19] Cavas M, Beltran D, Navarro JF. Behavioural effects of dimethyl-sulfoxide (DMSO): changes in sleep architecture in rats. Toxicol Lett 2005; 157: 221-232.
[20] Dumont LJ, Cancelas JA, Dumont DF, Siegel AH, Szczepiorkowski ZM, Rugg N, Pratt PG, Worsham DN, Hartman EL, Dunn SK, O'Leary M, Ransom JH, Michael RA, Macdonald VW. A randomized controlled trial evaluating recovery and survival of 6% dimethyl-sulfoxide-frozen autologous platelets in healthy volunteers. Transfusion 2013; 53: 128-137.
[21] Djerassi I, Roy A. A method for preservation of viable platelets combined effects of sugars and DMSO. Blood 1963; 22: 703-717.
[22] Asmis L, Tanner FC, Sudano I, Lüscher TF, Camici GG. DMSO inhibits human platelet activation through cyclooxygenase-1 inhibition. A novel agent for drug eluting stents? Biochem Biophys Res Commun 2010; 391: 1629-1633.
[23] Yi X, Wang Y, Han Y. Research advance on frozen injury and protective mechanisms of cryopreservated platelets. J Exp Hematol 2014; 22: 1761-1765.
[24] Bedaiwy MA, Hussein MR, Biscotti C, Falcone T. Cryopreservation of intact human ovary with its vascular pedicle. Hum Reprod 2006; 21: 3258-3269.
[25] Martinez-Madrid B, Camboni A, Dolmans MM, Nottola S, Van Langendonckt A, Donnez J. Apoptosis and ultrastructural assessment after cryopreservation of whole human ovaries with their vascular pedicle. Fertil Steril 2007; 87: 1153-1165.
[26] Martinez-Madrid B, Dolmans MM, Van Langendonckt A, Defrere S, Donnez J. Freeze-thawing intact human ovary with its vascular pedicle with a passive cooling device. Fertil Steril 2004; 82: 1390-1394.