Archives of Gynecology and Obstetrics (2021) 303:1495–1500
https://doi.org/10.1007/s00404-020-05934-3

GENERAL GYNECOLOGY

The effect of folate on ischemia/reperfusion injury in a rat adnexal torsion model

Tuba Çandar[1] · Özlem Uzunlar[2] · Mine Kiseli[3] · Sarp Özcan[4]

Received: 28 June 2020 / Accepted: 1 December 2020 / Published online: 5 January 2021

Abstract

Purpose The ischemia/reperfusion (I/R) injury of ovaries in adnexal torsion may have inadvertent consequences. Many agents have been studied in terms of their ability to prevent reperfusion damage to ovaries in suspected cases. In this study, folic acid, known to have antioxidative properties, was investigated to determine whether it played a role in the prevention of I/R damage in a rat ovarian torsion model.

Methods In this experimental study, 40 female adult Wistar-Albino rats were randomly divided into five groups as control, ischemia, I/R, Fol2 (2 mg/kg folic acid), and Fol4 (4 mg/kg folic acid). In the Fol2 and Fol4 groups, folic acid was intraperitonelly administered 30 min before reperfusion. Blood samples were obtained from the tails of each rat at the second hour of reperfusion.

Results The total oxidant status (TOS), total antioxidant status, cystatin C and folic acid levels of the five groups were investigated. Folic acid in 2 mg/kg dose could moderately increase the serum folic acid concentration (15.75–19.95 ng/ml, $p < 0.05$), reduce the level of cystatin C (0.18–0.12 µg/L, $p < 0.05$), and had a tendency to improve the oxidative stress injury (OSI: 76.05–33.06, $p > 0.05$), although there was no statistical difference in TOS levels ($p = 0.07$). Folic acid in 4 mg/kg dose, could significantly increase the serum folic acid concentration (15.75–37.65 ng/ml). However, it did not significantly reduce the level of cystatin C (0.18–0.19 µg/L, $p > 0.05$), and did not improve oxidative stress injury (76.05–130.58, $p > 0.05$).

Conclusion Folic acid in 2 mg/kg dose might improve the ovarian I/R injury though this was not statistically significant. Further studies are required to reach a definitive conclusion about the protective effect of folic acid in I/R injury.

Keywords Ischemia · Reperfusion · Folic acid · Adnexal torsion · Cystatin C

Introduction

Adnexal torsion, which is the fifth common gynecological emergency [1, 2], should be diagnosed and treated as soon as possible because of the risk of ovarian necrosis and loss of ovarian reserve. Deciding on the right time to intervene is challenging since most of the time diagnosis is not definitive. Although the ultrasonographic evaluation and Doppler studies may help, torsion is not the cause of pain in some cases that have undergone laparoscopy. However, delayed diagnosis and surgical intervention may cause loss of ovarian function in the population of reproductive age.

Previously, the surgical excision of the necrotic adnexa was recommended but recently, a minimally invasive surgical approach is suggested, such as the detorsion and preservation of the adnexa regardless of the appearance of the ovary [3, 4]. The preservation of ovarian function in detorsion cases supports the efficacy of minimally invasive surgery [5]. During adnexal torsion, the twisting of the vascular pedicle results in arterial and venous compromise [6]. Tissue ischemia initiates a cascade of events to maintain oxygenation. After detorsion, elevated levels of oxygen, known as reperfusion, cause increased free radicals, cytokines, neutrophil and thrombocyte activation, nitric oxide, and apoptosis

✉ Tuba Çandar
tubacandar@msn.com

1 Department of Biochemistry, Ufuk University Faculty of Medicine, Mevlana Street No: 86 Balgat, 06520 Ankara, Turkey

2 Department of Obstetrics and Gynecology, Ankara City Hospital, Ankara, Turkey

3 Department of Obstetrics and Gynecology, Ufuk University Faculty of Medicine, Ankara, Turkey

4 Department of Obstetrics and Gynecology, Acıbadem Ankara Hospital, Ankara, Turkey

which damage endothelial cells [7, 8]. As a result, oxidative damage due to reperfusion called ischemia/reperfusion (I/R) injury occurs in ovaries [9], which may have more devastating consequences than ischemic damage [10]. Any injury with the release of free oxygen radicals activates the protective mechanisms of the body, the antioxidants that act by preventing the formation of free radicals, reducing their level, and inhibiting oxidation reactions. Generated reactive oxygen species are practically measured as the total oxidant status (TOS) and the overall antioxidant level is appraised as the total antioxidant status (TAS) [11]. With the aim to preserve long-term ovarian function, many agents, such as iloprost, amlodipine, alpha-lipoic acid, edaravone, selenium, montelukast have been studied for their protective effect against reperfusion damage in ovarian torsion.

Cystatin C, a cysteine protease inhibitor, is a potential alternative marker to creatinine in the assessment of kidney function [12]. Cystatin C is known to be affected by large doses of corticosteroids, inflammation, diabetes, hyperthyroidism, hyperbilirubinemia, and rheumatoid factor [13]. Its antiatherosclerotic action has been previously shown [14], and there is also growing evidence concerning the association of cystatin C with various immune responses [15]. In the light of the data reflecting the sensitivity of cystatin c for renal ischemia [16], we aimed to investigate whether this marker had any relationship with ovarian ischemia.

Antioxidant vitamins, such as vitamins A, E and C (ascorbic acid), detoxify free radicals. In addition, each vitamin B has been shown to participate in the antioxidative response against oxidative stress [17]. Folic acid is a vitamin B which is taken as folate from food sources and metabolized in the body. Since folic acid has been shown to have a relationship with antioxidants [18], we aimed to investigate the effectiveness of folic acid in the prevention of reperfusion damage in an ovarian torsion rat model by measuring TOS and TAS. We also aimed to investigate whether there was any alteration in cystatin C levels in case of adnexal torsion.

Materials and methods

This study was approved by the Animal Research Laboratory Ethical Committee of Ankara Education and Research Hospital (04.06.2013, No: 0014). The experiment was performed in accordance with the recommendations of the Declaration of Helsinki (1964) for animal care and the Principles of Laboratory Animal Care (NIH publication No. 86-23, revised 1985). Forty female adult Wistar-Albino rats in reproductive cycling age weighing 200–250 g were randomly divided into five groups of eight rats: control, ischemia, I/R, and 2 mg/kg folic acid (Fol2) and 4 mg/kg folic acid (Fol4).

The rats were anesthetized with ketamin and xylazine in the dorsal recumbent position. After the skin preparation and cleaning, a midline incision of 2 cm was performed to gain access to the abdominal cavity.

Control group

Only laparotomy (sham operation) was performed. The blood samples were taken after laparotomy.

Ischemia group

In the ischemia group, after laparotomy, bilateral adnexal regions above the uterine horns were ligated using 4.0 vicryl and exposed to ischemia for 2 h [19]. The blood samples were taken 2 h after ischemia.

I/R group

The rats in this group were exposed to ischemia for 2 h as explained before. In the second hour of ischemia, the sutures were removed bilaterally and allowed for reperfusion. After 2 h of reperfusion, the blood samples were taken.

Fol2 group

The rats in the Fol2 group were given 2 mg/kg folic acid intraperitoneally 30 min before reperfusion (suture removal) and then after 2 h of reperfusion the blood samples were taken.

Fol4 group

The rats were given 4 mg/kg folic acid intraperitoneally 30 min before reperfusion (suture removal) and then after 2 h of reperfusion the blood samples were taken.

The blood samples were obtained from the tails of each rat. After the collection of blood, all rats were given high-dose anesthetics. Sample taking time was based on a similar study of Yurtcu [20].

The blood samples were centrifuged at 3000 rpm for 5 min, and the analyses were performed. Cystatin C levels were measured by an autoanalyzer (Abbott® Architect c 8000, Distributed by Abbott, Weisbaden, Germany). The method is principally based on the particle enhanced turbidimetric immunoassay. Folic acid levels were measured by Abbott i2000. The principle of Folate assay is a two-step assay for the quantitative determination of folate using chemiluminescent microparticle immunoassay (CIMA) technology.

The TOS and TAS analyses were carried out according to the full-automatic colorimetric method by Heales mb530 device using Rel Assay Diagnostics assay material [11]. In this process, oxidants oxidize the ferrous ion-o-dionisidin complex into ferric ion. Glycerol increases this reaction time

up to three times of base by activating the reaction, and ferric ions form a colorful complex with xylenol orange in the acidic environment. The spectrophotometric measurement in µmol H2O2 Equiv/L was undertaken according to the concentration of oxidants [11]. Measurements were reported as µmol/L and mmol/L, respectively.

The oxidative stress index (OSI) was also searched for the evaluation of systemic oxidative stress and antioxidant response which can be more comprehensive. It is calculated by dividing TOS by TAS [OSI (Arbitrary Unit) = (TOS/µmol H2O2 Equiv/L)/TAS µmol Trolox Equiv/L) × 100] and includes antioxidants that may be evaluated, as well as those that are not yet defined or measured in the serum.

Statistical analysis

Whether the distributions of continuous variables were normal was determined by the Shapiro–Wilk test. The assumption of the homogeneity of variances was examined by the Levene test. Both normality and homogeneity of variance assumptions were not met according to the results of Shapiro–Wilk and Levene tests, respectively. Therefore, the descriptive statistics were expressed as median values (25–75th percentiles) and the Kruskal–Wallis test was conducted to determine whether the differences in biochemical measurements (i.e., cystatin C, folic acid, TAS, TOS, and OSI) statistically significantly differed between the groups. When the p values from the Kruskal–Wallis test statistics were statistically significant, post hoc Dunn's test was used to identify the group that differed from the others. Data analysis was performed using IBM SPSS Statistics software version 17.0 (IBM Corporation, Armonk, NY, USA). A p value less than 0.05 was considered as statistically significant.

Results

The folic acid level of the groups are shown in Fig. 1. The folic acid was higher in the Fol4 group than in the control and I/R groups ($p < 0.001$ and $p = 0.005$, respectively). It was also statistically significantly higher in the Fol2 group compared to the control and I/R groups ($p < 0.001$ and $p = 0.007$, respectively). There was no statistically significant difference in the folic acid levels between the other groups ($p > 0.05$) (Fig. 1).

The TOS, TAS levels and OSI of the five groups are given in Table 1. The TOS levels did not significantly differ between the groups ($p = 0.07$). However, TAS was statistically different between the groups ($p = 0.003$), being significantly lower in the Fol4 group compared to the ischemia group ($p = 0.003$). OSI was significantly higher in the Fol4 group compared to the Fol2 group ($p = 0.049$), but it did not significantly differ between the remaining groups ($p > 0.05$).

The cystatin C levels are shown in Fig. 2. The cystatin C level was statistically lower in the Fol2 group than in the ischemia and Fol4 groups ($p = 0.030$ and $p = 0.003$, respectively). This parameter was also significantly higher in the Fol4 group compared to the controls ($p = 0.025$). The paired comparisons of the remaining groups did not reveal any other statistically significant difference ($p > 0.05$) (Fig. 2).

Discussion

Adnexal torsion is a frequent cause of gynecological emergencies, and ovarian ischemia can be considered a common consequence due to ovarian insufficiency. Ischemia results in an increase in the levels of reactive oxygen species (ROS)

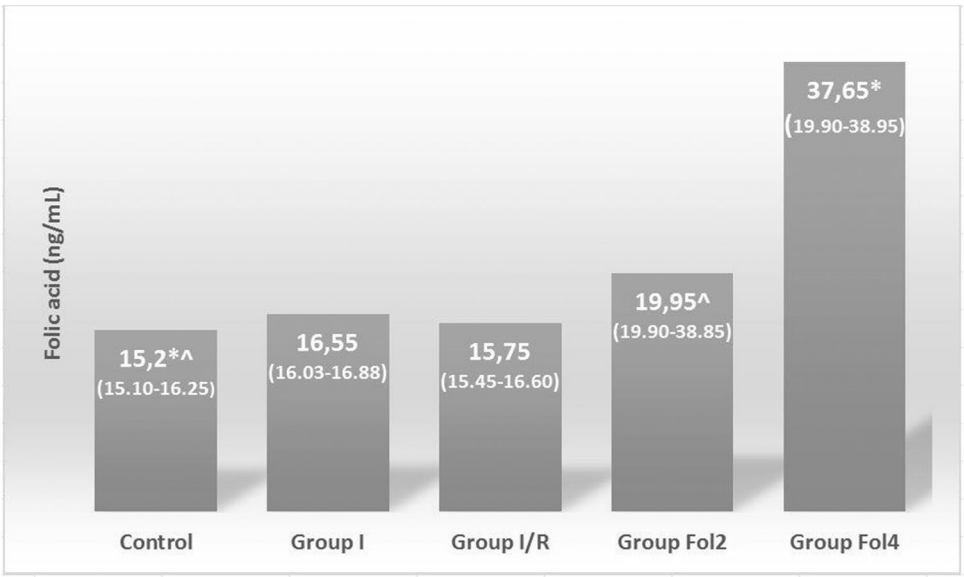

Fig. 1 The folic acid levels in the study groups. *Significant difference between group Fol4 and control, ^significant difference between group Fol2 and control

Table 1 The median values of TOS, TAS and OSI in groups

	Group C	Group I	Group I/R	Group Fol2	Group Fol4	*p* value
TOS (μmol/L)	157.50 (11.25–255.00)	260.00 (103.75–270.00)	207.50 (72.50–612.50)	72.50 (18.75–103.75)	107.50 (43.75–227.50)	0.07
TAS (mmol/L)	3.03 (1.56–4.46)	3.00 (2.41–4.25)[a]	2.65 (2.24–3.82)	1.76 (0.78–2.38)	0.82 (0.41–2.04)[a]	0.003*
OSI	43.59 (8.77–86.22)	75.27 (38.99–94.91)	76.05 (23.72–158.19)	33.06 (20.49–51.15)[b]	130.58 (79.66–166.46)[b]	0.049*

Data were shown as median (25–75th) percentiles

* means statistically significant

[a]Group I versus Group Fol4 ($p = 0.020$)

[b]Group Fol2 versus Group Fol4 ($p < 0.05$)

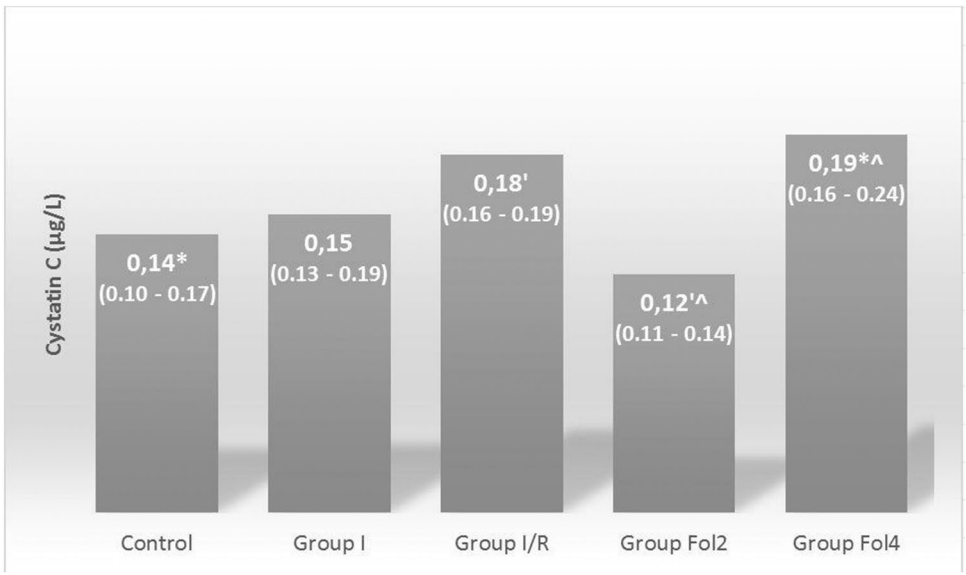

Fig. 2 The cystatin C levels in the study groups. *Significant difference between control and Fol4 group, 'significant difference between groups I/R and Fol2, ^significant difference between groups Fol2 and Fol4

and oxidant status of tissues. After detorsion, resupply of blood may have some adverse effects on tissues. To the best of our knowledge, this is the first study evaluating the protective (antioxidant) effect of folic acid on I/R injury in rat ovaries.

To date, many antioxidants (e.g., tadalafil, sildenafil, and iloprost) have been investigated in terms of their efficacy in preventing oxidative damage in ovarian torsion during reperfusion. Each has been shown to have beneficial effects through different mechanisms. For example, they play a role in the synthesis of methionine via the transfer of one-carbon groups. This transfer is involved in many metabolic reactions, such as the synthesis of purines and thymidine, as well as in the metabolism of homocysteine. 5-methyltetrahydrofolate (5-MTHF) is the primary metabolite of folate that enters the circulation [21]. 5-MTHF has been shown to be associated with nitric oxide synthase (NOS) coupling and nitric oxide (NO) bioavailability [22]. This interaction with NOS coupling and NO synthesis makes folic acid a requirement for a healthy endothelium and protective in cardiovascular diseases. In addition, folic acid is suggested to scavenge superoxide radicals [21]. Furthermore, folic acid supplementation has been reported to be beneficial in many disorders, such as autism spectrum disorders [23] and heart failure [24]. Inadequate folate intake has been shown to increase cancer risk [25]. Folic acid also has a use in male infertility as an antioxidant with its free radical scavenging abilities [26].

The antioxidative effects of folic acid are relatively less conclusive in in vivo studies. Recently, the oral supplementation of 5-MTHF has been shown to decrease oxidative stress in postmenopausal women [27]. In our study, folic acid given to rats at a 2 mg/kg dose decreased the TOS levels, although it was not significant (Fig. 1). The highest levels of TOS are seen in ischemia and I/R. TOS demonstrates the oxidative reaction of tissues to any change, including trauma, ischemia, and necrosis. I/R results in increased oxidative stress in tissues. The lower levels of TOS in the Fol2 group compared to the ischemia and I/R groups, albeit non-significant, is suggestive of the beneficial effect of folic acid.

TAS is a practical way to measure the total level of antioxidants rather than performing the measurement of each agent separately, which is expensive and time-consuming. TAS acts as a defense mechanism in damaged tissues and maintains homeostasis. The Fol4 group had lower levels compared to the ischemia group. The lower TAS levels in the groups given folic acid may be related to its efficacy in reducing TOS. When there is less oxidation, there is no need for the activation of anti-mechanisms. The comparable levels of TAS in the control (sham operation) group with the ischemia group may be due to surgical stress.

In cases where the dose of folic acid was increased to 4 mg/kg, the TAS levels decreased but not significantly. The significantly higher OSI (TOS/TAS) in the Fol4 group compared to the Fol2 group was probably due to the lower levels of TAS and higher levels of TOS. The increase in TOS in the Fol4 group is suggestive of the supraphysiological effects of high-dose folic acid. We are aware that there are some concerns about the safety of high-dose folic acid in individuals with vitamin B12 deficiency [28]. Moreover, Stanger et al. suggested a biphasic effect of folic acid that decreased the homocysteine levels in subjects with high homocysteine levels whereas it directly increased the total antioxidant capacity in subjects with normal homocysteine levels [29]. All these findings indicate that the effect of folic acid depends on many metabolic bases, as well as the individual requirements of the body. Alternatively, there is the possibility of increased oxidative stress with the increase in the synthesis of one-carbon unites via the supplementation of high-dose folic acid. The effects of folic acid at various doses should be further investigated in future research. Our results reveal that OSI shows more beneficial effects of folic acid at the 2 mg/kg dose than the 4 mg/kg dose. The folic acid levels were higher in both groups given folic acid but did not significantly differ between the two groups.

The relationship of cystatin c in renal ischemia has recently been demonstrated [30], and it is generally used as a prognostic marker in different metabolic diseases [31]. It has been shown to better quantify the extent of acute ischemic and/or tubular injury in kidneys but its expression in the female reproductive tract remains unclear [32]. Therefore, we aimed to investigate whether the cystatin C level had any relationship with ovarian ischemia and found that it was lower in the Fol 2 group compared to the ischemia and Fol4 groups. This finding is supportive of the beneficial effect of 2 mg/kg folic acid on ischemia. Additionally, cystatin C may be a potential marker for oxidative damage.

In conclusion, this study found that folic acid at the 2 mg/kg dose rather than 4 mg/kg decreased oxidative stress indicators (TOS and OSI) in a rat I/R model. Further in vivo studies on the antioxidant effects of folic acid should be performed to guide future therapeutic measures in ovarian preservation.

Acknowledgements All authors have accepted responsibility for the entire content of this manuscript and approved submission.

Authors contribution TC: Project development, manuscript writing, interpretation of data, OU: Project development, data analysis, MK: Manuscript writing, editing, SO: Data collection.

Funding This report has no financial support.

Availability of data and materials Applicable.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethics approval This study was approved by the Animal Research Laboratory Ethical Committee of Ankara Education and Research Hospital (04.06.2013, No: 0014).

Consent for publication All authors are in agreement with the content of the manuscript and have given consent for publication.

References

1. Burnett LS (1988) Gynecologic causes of the acute abdomen. Surg Clin North Am 68:385–398
2. Hibbard LT (1985) Adnexal torsion. Am J Obstet Gynecol 152:456–461
3. Adnexal torsion in adolescents (2019) ACOG Committee Opinion No. 783. Am Coll Obstet Obstet Gynecol 134:e56–e63
4. Rody A, Jackisch C, Klockenbusch W, Heinig J, Coenen-Worch V, Schneider HPG (2002) The conservative management of adnexal torsion—a case report and review of the literature. Eur J Obstet Gynecol Reprod Biol 101(1):83–86
5. Oelsner G, Cohen SB, Soriano D, Admon D, Mashiach S, Carp H (2003) Minimal surgery for the twisted ischaemic adnexa can preserve ovarian function. Hum Reprod 18(12):2599–2602
6. Lee EJ, Kwon HC, Joo HJ, Suh JH, Fleischer AC (1998) Diagnosis of ovarian torsion with color doppler sonography: depiction of twisted vascular pedicle. J Ultrasound Med 17:83–89
7. Dokuyucu R, Karateke A, Gokce H, Kurt RK, Ozcan O, Ozturk S et al (2014) Antioxidant effect of erdosteine and lipoic acid in ovarian ischemia-reperfusion injury. Eur J Obstet Gynecol Reprod Biol 183:23–27
8. Li C, Jackson RM (2002) Reactive species mechanisms of cellular hypoxia-reoxygenation injury. Am J Physiol Cell Physiol 282:227–241
9. Taskin O, Birincioglu M, Aydin A et al (1998) The effects of twisted ischaemic adnexa managed by detrosion on ovarian viability and histology: an ischaemia-reperfusion injury of rat ovary. Hum Reprod 13:2823–2827
10. Abramov AY, Scorziello A, Duchen MR (2007) Three distinct mechanisms generate oxygen free radicals in neurons and contribute to cell death during anoxia and reoxygenation. J Neurosci 27(5):1129–1138
11. Erel O (2005) A new automated colorimetric method for measuring total oxidant status. Clin Biochem 38(12):1103–1111
12. Tajima S, Yamamoto N, Masuda S (2019) Clinical prospects of biomarkers for the early detection and/or prediction of organ injury associated with pharmacotherapy. Biochem Pharmacol 170:113664

13. Bokenkamp A, van Wijk JA, Lentze MJ, Stoffel-Wagner B (2002) Effect of corticosteroid therapy on serum cystatin C and beta2-microglobulin concentrations. Clin Chem 48(7):1123–1126
14. Bengtsson E, To F, Hakansson K, Grubb A, Branen L, Nilsson J, Jovinge S (2005) Lack of the cysteine protease inhibitor Cystatin C promotes atherosclerosis in apolipoprotein E deficient mice. Arterioscler Thromb Vasc Biol 25(10):2151–2156
15. Mengting Z, Yuekang X (2018) Involvement of cystatin C in immunity and apoptosis. Immunol Lett 196:80–90
16. Lane BR (2013) Molecular markers of kidney injury. Urol Oncol 31(5):682–685
17. Lindschinger M, Tatzber F, Schimetta W, Schmid I, Lindschinger B, Cvirn G et al (2019) A randomized pilot trial to evaluate the bioavailability of natural versus synthetic vitamin B complexes in healthy humans and their effects on homocysteine, oxidative stress, and antioxidant levels. Oxid Med Cell Longev 2019:6082613
18. Xiao R, Liang J, Li XH, Zhao HF, Niu Q (2004) Effects of folic acid cooperated with soybean isoflavone on the neural tube defects preganat rats. Zhonghua Yu Fang Yi Xue Za Zhi 38(6):400–405
19. Uguralp S, Bay Karabulut A, Mizrak B (2005) Effects of pentoxifylline and vitamin E on the bilateral ovary after experimental ovarian ischemia. Eur J Pediatr Surg 15:107–113
20. Yurtcu E, Togrul C, Ozyer S, Uzunlar O, Karatas YH, Seckin KD et al (2015) Dose dependant protective effects of vardenafil on ischemia-reperfusion injury with biochemical and histopathologic evaluation in rat ovary. J Pediatr Surg 50:1205–1209
21. Stanhewicz AE, Kenney WL (2017) Role of folic acid in nitric oxide bioavailability and vascular endothelial function. Nutr Rev 75(1):61–70
22. Antoniades C, Shirodaria C, Leeson P et al (2009) *MTHFR* 677 C > T polymorphism reveals functional importance for 5-methyltetrahydrofolate, not homocysteine, in regulation of vascular redox state and endothelial function in human atherosclerosis. Circulation 119:2507–2515
23. Pacheva I, Ivanov I (2019) Targeted biomedical treatment for autism spectrum disorders. Curr Pharm Des 25(41):4430–4453
24. Dragan S, Buleu F, Christodorescu R, Cobzariu F, Iurciuc S, Velimirovici D et al (2019) Benefits of multiple micronutrient supplementation in heart failure: a comprehensive review. Crit Rev Food Sci Nutr 59(6):965–981
25. Pieroth R, Paver S, Day S, Lammersfeld C (2018) Folate and its impact on cancer risk. Curr Nutr Rep 7(3):70–84
26. Majzoub A, Agarwal A (2018) Systematic review of antioxidant types and doses in male infertility: benefits on semen parameters, advanced sperm function, assisted reproduction and live-birth rate. Arab J Urol 16(1):113–124
27. Cagnacci A, Cannoletta M, Xholli A, Piacenti I, Palma F, Palmieri B (2015) Folate administration decreases oxidative status and blood pressure in postmenopausal women. Eur J Nutr 54:429–435
28. National Toxicology Program (2015) NTP monograph: identifying research needs for assessing safe use of high intakes of folic acid. National Toxicology Program, Research Triangle Park, NC. Accessed 28 Sept 2017
29. Stanger O, Semmelrock HJ, Wonisch W, Bös U, Pabst E, Wascher TC (2002) Effects of folate treatment and homocysteine lowering on resistance vessel reactivity in atherosclerotic subjects. J Pharmacol Exp Ther 303(1):158–162
30. Lane BR, Babitz SK, Vlasakova K, Wong A, Noyes SL, Boshoven W (2018) Evaluation of urinary renal biomarkers for early prediction of acute kidney injury following partial nephrectomy: a feasibility study. Eur Urol Focus 4569(18):30318-3
31. Zheng J, Xu J, Zhang Y, Zhou N (2012) Effects of insulin combined with metformin on serum cystatin C, homocysteine and maternal and neoanatal outcomes in pregnant women with gestational diabetes mellitus. Exp Ther Med 19(1):467–472
32. Lee RK, Tseng HC, Hwu YM, Fan CC, Lin MH, Yu JJ et al (2018) Expression of cystatin C in the female reproductive tract and its effect on human sperm capacitation. Reprod Biol Endocrinol 16(1):8