Arch Gynecol Obstet (2016) 293:197–202
DOI 10.1007/s00404-015-3834-7

Measurement of oxidative stress in the follicular fluid of infertility patients with an endometrioma

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Received: 29 March 2015 / Accepted: 27 July 2015 / Published online: 6 August 2015

Abstract
Purpose Follicular fluid (FF) might reflect the environment during follicle and oocyte growth, and an evaluation of oxidative stress in the FF might be useful in predicting oocyte quality. In order to measure the oxidative stress (OS) in the FF from a single follicle of patients with endometrioma (EM), we evaluated whether an EM might affect the environment of follicular growth.
Methods Between December 2011 and July 2013, 26 patients with a unilateral EM (EM group) and 29 without EM (control group) were enrolled in this study. The FF was obtained during the first puncture of follicular aspiration, and was stored at −30 °C until it was assayed. A Free Radical Elective Evaluator (WISMERLL, USA) was used to perform d-ROM and BAP tests to measure oxidative stress (U.CARR) and antioxidant power (μmol/L).
Results The d-ROM values in the EMC and control groups were 328.7 ± 97.8 and 414.9 ± 84.2, respectively, and the BAP values for the two groups were 2474.3 ± 432.0 and 2552.8 ± 435.58, respectively. These values were similar between the two groups (mean ± SD). The number of patients with a modified BAP/d-ROM ratio of <1.0 in the EM group was similar to that for the control group at 16 and 15, respectively (61.5 and 51.7 %).
Conclusions The oxidative stress and antioxidant potential in the FF of the patients with unilateral EM showed values similar to those without an EM. Therefore, we concluded that EMs do not affect the environment for follicle growth during ART treatment.

Keywords Antioxidant potential · BAP · d-ROM · Endometrioma · Follicular fluid · Oxidative stress

Introduction

Oocyte quality is an important factor in the success of assisted reproductive technology (ART) treatment, and the environment of follicle growth has a significant influence on this quality. It is very difficult, however, to directly evaluate oocyte quality. We previously tried to evaluate oocyte quality in order to measure peripheral arterial blood flow resistance, and we demonstrated how the decrease in intraovarian arterial blood flow resistance measured after human chorionic gonadotropin (hCG) administration might be a good indicator to use in the retrieval of mature oocytes, but this result did not indicate oocyte quality [1]. Follicular fluid (FF), however, might reflect the environment during follicle and oocyte growth. Therefore, an evaluation of oxidative stress in the FF might be useful in predicting oocyte quality.

Endometriosis is believed to affect the environment during follicular growth in ART, because endometriosis produces several cytokines that interfere with reproduction [2]. Endometriomas (Ems) are located in the ovaries; hence, both follicular growth and oocytes could be directly affected by this condition. At least one report has analyzed the iron availability in an individual human ovarian follicle [3]; however, this report evaluated neither the oxidative stress and nor the antioxidant ability of FF. Therefore, we attempted to quantify the oxidative stress of FF based on the examination of a single follicle.

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Oxidative stress (OS) refers to an overproduction of reactive oxygen species (ROS) and/or a deficit or depletion of the scavenging capacity of antioxidant defense systems leading to biologic macromolecule oxidative damage. Oxidative injury has been implicated as a causal factor in the poor quality of oocytes. Oocytes, particularly just before ovulation, are at high risk of oxidative stress and are extremely susceptible to oxidative damage from reactive oxygen species (ROS). Numerous markers of oxidative stress have been identified [4–6]. Total hydroperoxidase (TH) represents a group of ROS generated from lipids, peptides and amino acids. Thus, the determination of TH provides information on some of the fundamental mechanisms of oxidative stress involved in critically ill neonates. Conversely, biological antioxidant potential (BAP) is used to represent overall antioxidant activity. Serum BAP provides a reliable measure of the power of an antioxidant barrier to oxidation, by measuring the ability to reduce ferric to ferrous ions [7].

In the present study, in order to measure the oxidative stress in the FF from a single follicle of patients with EMs in ART treatment, we evaluated whether an EM might affect the environment of follicular growth.

Materials and methods

Patients

A total of 55 patients who were undergoing assisted reproductive technology (ART) treatment at the division of Reproductive Medicine in the Sugiyama clinic between December 2011 and July 2013 were enrolled in the present study. Signed informed consent was obtained from all patients, and this study was approved by the Institutional Board of the Sugiyama Clinic. The patients were classified into two groups. Twenty-six patients with a unilateral endometrioma (EM group) and 29 patients who had no EM (control group) received ovarian stimulation via our conventional protocol and underwent oocyte retrieval. None of the patients in the EM group received laparoscopic surgery for endometriosis, and their unilateral endometrioma were diagnosed by both transvaginal ultrasound and MRI. Moreover, no patient was included in this study who presented with recurrent endometrioma during ovarian stimulation. Almost half (12 out of 29) of the patients in the control group received diagnostic laparoscopy to confirm the lack of endometriosis in their peritoneal region and pelvic cavity, but all patients in the control group were checked for an EM by transvaginal ultrasound before the beginning of the ovarian stimulation. Moreover, no patient showed elevated CA125 levels. EMs showing that were less than 10 mm in size were excluded from this study. Our conventional protocol was stimulated with a combination of clomiphene citrate (CC; Cerophen®, Merk Serono, Japan) and recombinant follicle stimulating hormone (rec-FSH; Gonal-F®, Merk Serono, Japan) [8]. All patients were monitored to mark the point at which the dominant follicles reached ≥16 mm in diameter, and their estradiol levels reached ≥500–600 pg/ml, then 300 μg of buserelin acetate (Buserecur, Fuji Pharma, Tokyo) was administered nasally, and oocyte pick-up (OPU) was performed 35 h later.

Anti-Müllerian hormone (AMH) and basal FSH (follicular stimulating hormone) levels were measured before start of ovarian stimulation. Measurement of AMH was not restricted to a particular time of the menstrual cycle, and AMH assays were performed with the use of a Beckman-Coulter generation II assay, and the values were presented as ng/ml. Basal FSH level was measured between the 2nd and 4th day of the menstrual cycle.

IVF procedure and follicular fluid collection

The IVF procedure and follicular fluid (FF) collection has been previously described [8]. Oocytes were retrieved transvaginally using a needle-guided technique, and were aided by ultrasonography. The follicles with a mean diameter of >16 mm were aspirated separately, using an 18-gauge needle connected to a 5-ml syringe for suction. The needle was removed after the aspiration of each follicle. The aspiration was interrupted and a new syringe was used if blood appeared in the tube connected to the syringe, thus avoiding contamination by blood. FF was obtained from a single follicle at the first puncture for oocyte pick-up without blood contamination, and was centrifuged at 1000 g for 10 min, passed through a filter, and stored at −30 °C for assay.

In the control group, the FF was obtained from one follicle in either the right or left ovary, but in the EM group, the FF was obtained from one follicle at the first puncture in both ovaries. About half of the follicles in the ovary with an endometrioma were adjacent to the endometrioma, and the others were apart from an endometrioma.

Measurement of oxidative stress and antioxidant potential

Oxidative stress was measured via a Free Radical Elective Evaluator (F.R.E.E; WISMERLL, Italy), and total hydroperoxide levels were measured using a d-ROM (reactive oxygen metabolite) kit (U.CARR; Diacron Srl, Grosseto, Italy) as previously described [9]. A FF sample was dissolved in an acidic buffer for the d-ROM test. The hydroperoxides reacted with the transition metal ions that were liberated from the protein in the acidic medium, resulting in a conversion to proxy and peroxyl radicals that oxidized *N,N*-diethyl-para-phenylenediamine, which could

eventually be detected spectrophotometrically [10]. Redox potential (RP) levels were determined using a biological antioxidant potential (BAP) test [11]. In the BAP test, a source of ferric (Fe3+) ions adequately bound to a special chromogenic substrate is a colored solution that is discolored when Fe3+ ions are reduced to ferrous ions (Fe2+) following the addition of a reducing/antioxidant system [12]. Measurements obtained in the d-ROMs and BAP tests were expressed as Carr units [13] and μmol/L, respectively. A modified BAP/d-ROM ratio was calculated by dividing a single BAP/d-ROM ratio by mean BAP/d-ROM ratio. The values of d-ROM and BAP for each sample were measured twice, and average values were used for analysis.

We evaluated the characteristics of the patients in both groups and compared the antioxidant power and oxidative stress in the FF between the two groups. We also evaluated the antioxidant power and oxidative stress in the FF obtained from both ovaries in the same patients with unilateral ovarian EMs. The data, expressed as the mean ± SD, were analyzed for statistically significant differences via a parametric test for two independent or related samples (unpaired *t* test).

Results

A total of 55 samples were obtained. The backgrounds of the EM and control groups are summarized in Table 1. The average age of the EM group was 37.4 ± 2.9 years (mean ± SD), which was similar to that for the control group. The EMs in the EM group ranged between 13 and 67 mm in size. Basal FSH level (IU/L) in the EM and control groups were 9.1 ± 3.2 and 8.7 ± 3.5, respectively, with no significant differences. The AMH value (ng/ml) for the EM group was 2.2 ± 1.3, which was similar to that in the control group (2.8 ± 1.3). The totals for the dosage of gonadotropins (IU) for ovarian stimulation and peak estradiol concentration (pg/ml) in the EM group were 653 ± 40 and 1092 ± 415, respectively, which were comparable to those in the control group (622 ± 35 and 1121 ± 466, respectively). The numbers of retrieved oocytes in the EM and control groups were 4.9 ± 3.2 and 4.6 ± 2.0, respectively, and the insemination methods were similar for both groups. The fertilization rates in the EM and control groups were 72.1 and 45.2 %, respectively, and there were no significant differences. In the EM group, 23 patients had received embryo transfer either using fresh or thawed embryos, and 11 patients achieved pregnancy (pregnancy rate per patients was 47.8 %). In the control group, 24 patients received embryo transfer using either fresh or thawed embryos, and 10 achieved pregnancy (pregnancy rate per patients was 41.7 %). The miscarriage rates were similar for both groups.

Table 1 The characteristics of the patients in the EM and control groups

	EM group	Control group
Number of patients	26	29
Number of samples	26	29
Age (years)	37.4 ± 2.9	37.5 ± 2.6
Indications for ART, % (*n*)		
Unexplained	0	25
Male	2	4
Endometriosis	24	0
Basal FSH level (IU/L)	9.1 ± 3.2	8.7 ± 3.5
AMH[b] value (ng/ml)	2.2 ± 1.3	2.8 ± 1.3
Total dosage of gonadotropins (IU)	653 ± 40	622 ± 35
Peak estradiol concentration (pg/ml)	1092 ± 415	1121 ± 466
Number of retrieved oocytes	4.9 ± 3.2	4.6 ± 2.0
Percentage of ICSI (%)	47.8	48.3
Fertilization rate (%)	72.1	75.2
Number of patients who received ET	23	24
Number of pregnancies, *n* (%)	11 (47.8)	10 (41.7)
Miscarriage rate (%)	18.2	20.0

Values are reported as the mean ± standard deviation unless otherwise specified

ART assisted reproductive technology, *bAMH* anti-Müllerian hormone, *ET* embryo transfer

A total of 52 samples were obtained from the EM group. The BAP values (μmol/L) for ovaries with EMs averaged 2487.6 ± 571.6, and this value was similar to that from the ovaries without an EM (2461.0 ± 393.1, Fig. 1a). The d-ROM values (U.CARR) from the ovaries with an EM averaged 326.6 ± 57.7, which also was similar to that from the ovaries without an EM (330.9 ± 74.0, Fig. 1b). The BAP values in the EM group averaged 2474.3 ± 432.0, which was comparable to that for the control group (2552.8 ± 435.5, Fig. 2a). The d-ROM values in the EM group averaged 328.7 ± 97.8, which also was comparable to that in the control group (414.9 ± 84.2, Fig. 2b).

The number of patients whose modified BAP/d-ROM ratio was less than 1.0 in the EM group was 16, and the percentage of the patients showing a lower modified ratio was 61.5 % (16/26). By contrast, the number of the patients in the control group was 15, and the percentage was 51.7 % (15/29). There were no significant differences (Fig. 3).

Discussion

There is evidence that oxidative stress causes deleterious effects in mammalian oocytes in vitro [14, 15], but the relevance of these studies is questionable in light of equivocal conclusions reached in a limited set of clinical

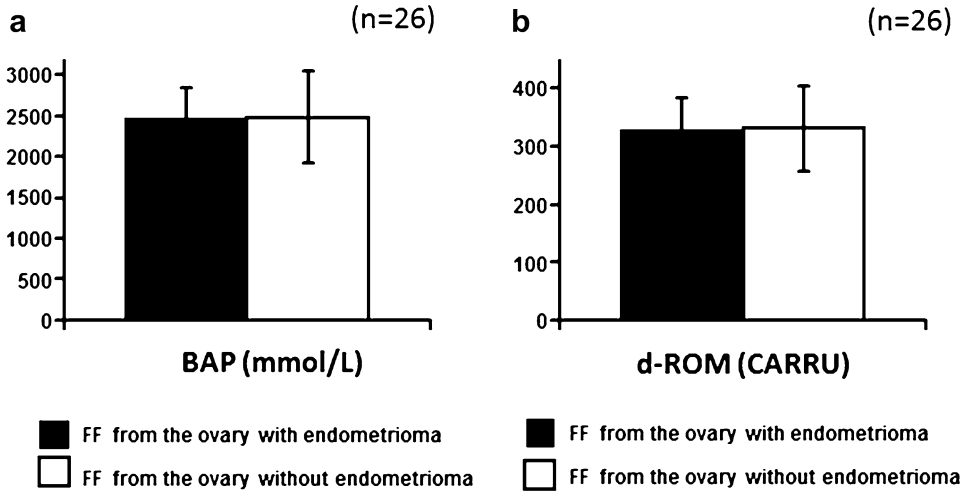

Fig. 1 These figures show the BAP and d-ROM values in the FF from ovaries with or without an EM ($n = 26$). The *black column* in **a** indicates a BAP value (μmol/L) in the FF from an ovary with an EM at 2487.6 ± 571. This value was similar to that from an ovary without an EM (2461.0 ± 393.1, *white column*). *The black column* in **b** indicates the d-ROM value (U.CARR) in the FF from an ovary with an EM, at 326.6 ± 57.7, which also is similar to that from an ovary without an EM (330.9 ± 74.0, *white column*)

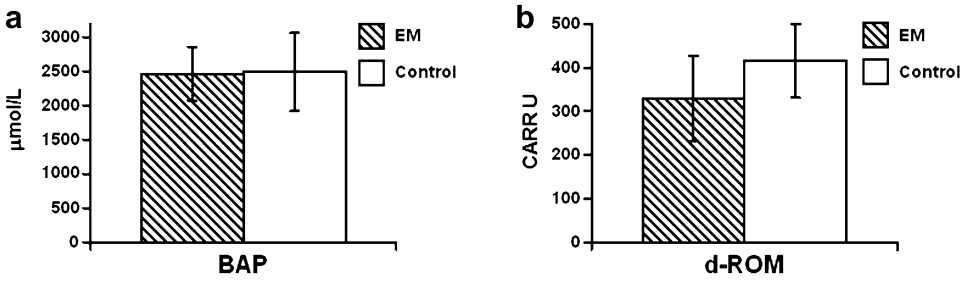

Fig. 2 These figures compare the BAP and d-ROM values between the endometrioma (EM) and the control groups. The *diagonal column* in **a** indicates the BAP values in the EM group at 2474.3 ± 432.0, which is similar to the value in the control group (2552.8 ± 435.5, *white column*). The *diagonal column* in **b** indicates the d-ROM value in the EM group at 328.7 ± 97.8, which also was similar to that in the control group (414.9 ± 84.2, *white column*)

studies. The follicular fluid content makes up the actual environment of a mature oocyte before fertilization has occurred and may influence IVF outcome parameters such as fertilization, embryo cleavage, and pregnancy rates [16–18]. Few published studies have tracked individual follicles and detailed the correlations between FF measurements and oocyte/embryo outcomes. Additionally, there are few studies using individual follicles to track oocytes [19, 20]. Previously, several published reports have measured the oxidative stress and antioxidant potential in FF, and these reports indicated that oxidative stress in FF is positively correlated with embryo developmental ability and ART outcomes [19, 21]. Therefore, oxidative stress in the FF might be a good predictive marker for the outcome of ART.

It is well known that women with peritoneal endometriosis or an ovarian EM have reduced spontaneous pregnancy rates, and that the surgical removal of peritoneal

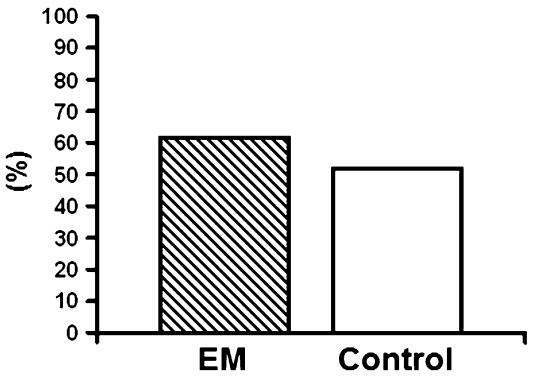

Fig. 3 This figure compares the percentage of patients showing a lower modified BAP/d-ROM ratio in both the EM and control groups. The *diagonal column* indicates the percentage of the patients showing a lower modified BAP/d-ROM ratio (ratio <1.0) at 61.5 % (16/26). By contrast, the percentage in the control group was 51.7 % (15/29, *white column*), but the difference is not significant

endometriosis improves the spontaneous fecundity and success rates of artificial insemination and IVF treatment [22, 23]. Intraovarian endometriosis, or the presence of an EM, generates an unfavorable fluid environment as observed with peritoneal endometriosis [2]. This matter is particularly relevant in IVF, because the developing oocytes would be in contact with the affected follicular fluid. The link between peritoneal endometriosis, inflammation and infertility is well established, but the association between intraovarian inflammation and an EM is unknown. With most endometriomas, there is no evidence of increased cytokine concentration in the ipsilateral leading follicle. Infrequently, the concentration of inflammatory cytokines is increased in the FF [24].

In evaluating the follicular environment using FF, it is questionable as to whether the FF conditions in the right and left follicles are similar. To solve this question, we previously tried to compare the FF from the right and left follicles in the same patients [25]. In that report, no significant differences were found concerning antioxidant power and oxidative stress in the follicular fluid between the right and left ovaries of a single patient based on single-follicle analysis [25]. In the present study, the oxidative stress in the FF from ovaries with an EM was almost equal to that in the FF from ovaries without an EM in the same women who had a unilateral intraovarian EM. The antioxidant potential in the FF was also similar, and there were no differences in the antioxidant potential in the FF from ovaries with or without an EM in the same patient. These data suggest that an EM might not affect the environment of follicle growth. This result agrees with a recent report, which showed no differences in cytokine concentrations in the FF from ovaries with or without an EM [24].

There is concern that the presence of an EM in the ovary might induce oxidative stress during follicular growth, because recent reports have indicated that EMs contain an abundant amount of free iron, and this free iron has been significantly associated with greater oxidative stress and frequent DNA mutations [26]. This report is evidence that the presence of an intraovarian EM might affect the environment of follicular growth in ovaries. In the present study, however, the oxidative stress in the FF of the EM group was comparable to that in the control group. At least one other report, however, has indicated a lower antioxidant capacity in infertile women with endometriosis [27]. A recent report indicated that the presence of an EM increased iron levels in individual follicles proximal to the EM [3]. In the present study, however, the antioxidant potential in the FF of the EM group was also comparable to that in the control group. Therefore, regardless of the presence of a unilateral EM, there was no difference in the intrafollicular environment among the women with or without an EM, and this result was not associated with the distance between a follicle and an EM. A question was raised as to whether the size of an EM might affect the oxidative stress in the FF. We analyzed the BAP and d-ROM values according to the EM size. The average BAP values (μmol/L) for ovaries with smaller EMs (the EM size <30 mm) was 2256, which compares favorably to that for ovaries with larger EMs (EM size ≥ 30 mm; BAP = 2552). The average d-ROM values (U.CARR) from the ovaries with smaller EMs was 328.1, and this was also similar to that for ovaries with larger EMs (EM size ≥ 30 mm; d-ROM = 356.8). Based on our results, the EM size affected neither oxidative stress nor antioxidant potential.

In the present study, we used d-ROM and BAP tests to evaluate oxidative stress and antioxidant potential, respectively. For evaluating oxidative stress, several tests were available, and d-ROM is one of the main tests used. This test is very convenient because it is easy to measure and the measuring time is short (within 2 h). However, this test expresses the earlier phase of oxidative damage, and reflects neither the middle nor the late phases of oxidative stress. On the other hand, the BAP test is also convenient because it also is easy to use and the measuring time is short (within 2 h). This test, however, is easily affected by the concentration of ferric (Fe3+) ions, and this test is used to measure the reduction ability of ferric (Fe3+) ions.

The modified BAP/d-ROM ratio was used as an index for latent antioxidant potential. This ratio was less than 1.0, which indicated that antioxidant potential was decreased in a latent fashion. In the present study, the percentage of patients who showed a low modified BAP/d-ROM ratio in the EM group was 61.5 % (16/26), and this percentage was comparable to that in the control group. According to this result, about 60 % of the patients in the EM group showed low antioxidant potential, but a similar percentage of patients was found in the control group. Therefore, the presence of an EM did not induce an adverse environment for follicular growth.

A recent report indicated that critical endometrioma size was associated with reduced ovarian responsiveness in ART treatment [28]. In patients with endometriomas ≥ 30 mm, endometrioma size was the most influential contributor to the total number of follicles and oocytes retrieved. In our study, 15 out of 26 patients in the EM group had a larger endometrioma (size ≥ 30 mm); however, their responsiveness to ovarian stimulation was similar to those in the control group.

In conclusion, the oxidative stress and antioxidant potential in the FF from a single follicle of the patients who had a unilateral EM showed values similar to those of the patients without an EM. Therefore, we must conclude that EMs do not affect the environment for follicle growth during ART treatment.

Compliance with ethical standards

Conflict of interest The authors have received no funding for this study, and they have no financial interest in any companies.

References

1. Nakagawa K, Ohgi S, Kojima R, Itoh M, Horikawa T, Irahara M, Saito H (2006) Reduction of perifollicular arterial blood flow resistance after hCG administration is a good indicator of the recovery of mature oocytes in ART treatment. J Assist Reprod Genet 23:433–438
2. Harada T, Iwabe T, Terakawa N (2001) Role of cytokines in endometriosis. Fertil Steril 76:1–10
3. Sanchez AM, Papaleo E, Corti L, Santambrogio P, Levi S, Viganò P, Candiani M, Panina-Bordignon P (2014) Iron availability is increased in individual human ovarian follicles in close proximity to an endometrioma compared with distal ones. Hum Reprod 29:577–583
4. Hracsko Z, Safar Z, Orvos H, Ovak Z, Pal A, Varga IS (2007) Evaluation of oxidative stress markers after vaginal delivery or Cesarean section. In Vivi 21:703–706
5. Franco MC, Kawamoto EM, Gorjão R, Rastelli VM, Curi R, Scavone C, Sawaya AL, Fortes ZB, Sesso R (2007) Biomarkers of oxidative stress and antioxidant status in children born small for gestational age: evidence of lipid peroxidation. Pediatr Res 62:204–208
6. Brakke K, Bechensteen AG, Halvorsen BL, Blomhoff R, Haaland K, Staff AC (2007) Oxidative stress markers and antioxidant status after oral iron supplementation to very low birth weight infants. J Pediatr 151:23–28
7. Hussein MH, Daoud GA, Kakita H, Hattori A, Murai H, Yasuda M, Mizuno K, Goto K, Ozaki Y, Ito T, Tanaka T, Fukuda S, Kato I, Fujimoto S, Suzuki S, Sobajima H, Togari H (2007) The sex differences of cerebrospinal fluid levels of interleukin 8 and antioxidants in asphyxiated newborn. Shock 28:154–159
8. Nakagawa K, Nishi Y, Sugiyama R, Jyuen H, Kuribayashi Y, Sugiyama R, Inoue M (2012) A programmed schedule of oocyte retrieval using mild ovarian stimulation (clomiphene citrate and recombinant follicular stimulating hormone). Reprod Med Biol 11:85–89
9. Buonocore G, Perrone S, Longini M, Terzuoli L, Bracci R (2000) Total hydroperoxide and advanced oxidation proteins products in preterm hypoxic babies. Pediatr Res 47:221–224
10. Fukui T, Yamauchi K, Maruyama M, Yasuda T, Kohno M, Abe Y (2011) Significance of measuring oxidative stress in lifestyle-related diseases from the viewpoint of correlation between d-ROMs and BAP in Japanese subjects. Hypertens Res 14:1041–1045
11. Pasquini A, Luchetti E, Marchetti V, Cardini G, Iorio EL (2008) Analytical performances of d-ROMs test and BAP test in canine plasma: definition of the normal range in healthy labrador dogs. Vet Res Commun 14:137–143
12. Dohi K, Satoh K, Ohtaki H, Shioda S, Miyake Y, Shindo M, Aruga T (2005) Elevated plasma levels of bilirubin in patients with neurotrauma reflect its pathophysiological role in free radical scavenging. In Vivo 19:855–860
13. Cornelli U, Terranova R, Luca S, Cornelli M, Alberti A (2001) Bioavailability and antioxidant activity of some food supplements in men and women using the D-Roms test as a marker of oxidative stress. J Nutr 14:3208–3211
14. Tarín JJ, Vendrell FJ, Ten J, Cano A (1996) The oxidizing agent tertiary butyl hydroperoxide induces disturbances in spindle organization, c-meiosis, and aneuploidy in mouse oocytes. Mol Hum Reprod 2:895–901
15. Zhang X, Wu XQ, Lu S, Guo YL, Ma X (2006) Deficit of mitochondria-derived ATP during oxidative stress impairs mouse MII oocyte spindle. Cell Res 16:841–850
16. Barroso G, Barrionuevo M, Rao P, Graham L, Danforth D, Huey S et al (1999) Vascular endothelial growth factor, nitric oxide and leptin follicular fluid levels correlate negatively with embryo quality in IVF patients. Fertil Steril 72:1024–1026
17. Mendoza C, Ruiz-Requena E, Ortega E, Cremades N, Martinez F, Bernabeu R, Greco E, Tesarik J (2002) Follicular fluid markers of oocyte developmental potential. Hum Reprod 17:1017–1022
18. Arnot AM, Vandekerckhove P, DeBono MA, Rutherford AJ (1995) Follicular volume and number during in vitro fertilization: association with oocyte developmental capacity and pregnancy rate. Hum Reprod 10:256–261
19. Pasqualotto EB, Agarwal A, Sharma RK, Izzo VM, Pinotti JA, Joshi NJ, Rose BI (2004) Effect of oxidative stress in follicular fluid on the outcome of assisted reproductive procedure. Fertil Steril 81:973–976
20. Sabatini L, Wilson C, Lower A, Al-Shawaf T, Grudzinskas JG (1999) Superoxide dismutase activity in human follicular fluid after controlled ovarian hyperstimulation in women undergoing in vitro fertilization. Fertil Steril 72:1027–1034
21. Wiener-Megnazi Z, Vardi L, Lissak A, Shnizer S, Reznick AZ, Ishai D, Lahav-Baratz S, Shiloh H, Koifman M, Dirnfeld M (2004) Oxidative stress induces in follicular fluid as measured by the thermochemiluminescence assay correlate with outcome parameters in in vitro fertilization. Fertil Steril 82(Suppl 3):1171–1176
22. Werbrouck E, Spiessens C, Meuleman C, D'Hooghe T (2006) No difference in cycle pregnancy rate and in cumulative love-birth rate between women with surgically treated minimal to mild endometriosis and women with unexplained infertility after controlled ovarian hyperstimulation and intrauterine insemination. Fertil Steril 86:566–571
23. Opoien HK, Fedorcsak P, Byholm T, Tanbo T (2011) Complete surgical removal of minimal or mild endometriosis improves outcome of subsequent IVF/ICSI treatment. Reprod Biomed Online 23:389–395
24. Opøien HK, Fedorcsak P, Polec A, Stensen MH, Åbyhol T, Tanbo T (2013) Do endometriomas induce an inflammatory reaction in nearby follicles? Hum Reprod 28:1837–1845
25. Sugiyama R, Nakagawa K, Hisano M, Mitui M, Yamaguchi K (2014) The ovarian stimulation method can cause oxidative stress in the follicular fluid. Hum Reprod 29(supp 1):i309–1310 (**P-463**)
26. Yamaguchi K, Mandai M, Toyokuni S, Hamanishi J, Higuchi T, Takakura K, Fujii S (2008) Contents of endometriotic cysts, especially the high concentration of free iron, are a possible cause of carcinogenesis in the cysts through the iron-induced persistent oxidative stress. Clin Cancer Res 14:32–40
27. Prieto L, Quesada JF, Cambero O, Pacheco A, PellicerA Codoceo R, Garcia-Velasco JA (2012) Analysis of follicular fluid and serum markers of oxidative stress in women with infertility related to endometriosis. Fertil Steril 98:126–130
28. Coccia ME, Rizzello F, Barone S, Pinelli S, Rapalini E, Parri C, Caracciolo D, Papageorgiou S, Cima G, Gandini L (2014) Is there a critical endometrioma size associated with reduced ovarian responsiveness in assisted reproduction techniques? Reprod Biomed Online 29:259–266