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Research Article

Endometriosis and Oxidative Stress. Do Reactive Oxygen Species Always Lead to Damages of Gametes and Embryos?

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Abstract

Reactive Oxygen Species (ROS) play a crucial role in the pathogenesis of many reproductive disorders, such as endometriosis on the one hand, but on the other hand they participate in different cellular proliferation processes, too. Endometriosis is an apoptotic endometrial, menstrual cells and lysed erythrocytes-induced inflammatory disease outside the uterine cavity, which activates macrophages leading to ROS production and oxidative stress. However, based on the available literature, the reproductive outcomes are still contradictory. In this study, the demographic, embryological and clinical results of 252 patients suffering from tubal infertility (control), ASRM I-II and III-IV endometriosis were analyzed. Endometriosis was associated with decreased anti-Müllerian hormone level and increased gonadotropin doses during stimulation ($p<0.0001$). In ASRM III-IV, reduced embryological parameters were observed, which resulted in 13.73% and 15.21% decrements in the implantation rates, 19.96% and 23.89% in the clinical pregnancy rates of patients suffering from ASRM III-IV endometriosis in comparison with control or ASRM I-II, respectively. In addition, miscarriage rates were 19.04%, 29.03% and 38.46% in control, ASRM I-II and ASRM III-IV, respectively. In our study, the supposed altered oxido-reduction environment of gametes and embryos obviously exerted negative effects on the embryological and clinical parameters, but these effects could not be observed in case of mild endometriosis with low level of stress.

Keywords: Antioxidants; Embryo development; Endometriosis; Oxidative stress; Infertility

Abbreviations

8-OHdG: 8-Hydroxy-2'-Deoxyguanosine; AFC: Antral Follicle Count; AMH: Anti-Müllerian Hormone; ASRM: American Society of Reproductive Medicine; BMI: Body Mass Index; FISH: Fluorescence *In Situ* Hybridization; FSH: Follicle-Stimulating Hormone; H_2O_2: Hydrogen Peroxide; hCG: human Chorionic Gonadotropin; ICSI: Intracytoplasmic Sperm Injection; IU: International Unit; IVF: *In Vitro* Fertilization; LH: Luteinizing Hormone; $O_2^{\bullet-}$: Superoxide Anion Radical; ●OH: Hydroxyl Radical; PCOS: Polycystic Ovary Syndrome; ROS: Reactive Oxygen Species

Introduction

Every aerobic living organism suffers from the harmful effects of oxygen due to their mitochondrial respiration process. Namely, 1-5% of molecular oxygen is converted into the highly reactive and toxic superoxide anion (and further reactive oxygen species, ROS) but normally, it is neutralized by intracellular enzymatic or non-enzymatic antioxidant molecules. Their potential toxicity is determined by the balance between the ROS accumulation and antioxidants. So, oxidative stress can occur when the generation of ROS exceeds the amount of antioxidants or the synthesis of antioxidants is blocked [1]. Nevertheless, not only harmful effects of ROS are known, as they also have important intracellular signal transduction and regulatory functions in folliculogenesis, maturation of oocytes, dissolution of corpora lutea, implantation and embryo development [2,3].

Many human diseases are characterized by oxidative stress (e.g. inflammations, cardiovascular diseases, chronic pulmonary diseases, chronic kidney diseases, neurodegenerative diseases, cancers and infertility) [4]. Almost every type of infertility can be connected to oxidative stress. Among others, polycystic ovary syndrome, male infertility, advanced maternal age or endometriosis are associated with oxidative stress [5-7]. Endometriosis is an inflammatory disease, in which endometrial cells and tissues can be found outside the uterine cavity. Obviously, endometrial implants are hormone-dependent, namely estrogen-dependent tissues [8]. Endometriosis is a very frequent finding among infertility patients. Since the diagnosis of endometriosis is often established only after several years, accurate statistics are not available on the frequency of the disease. However, it is commonly accepted that 10 to 15% of reproductive-age women suffers from endometriosis, but in infertility patients papers mention a frequency between 25-50% [9]. In infertile patients, the exact staging of the disease is very important. The classification recommended by the American Society of Reproductive Medicine (ASRM) [10] is used world-wide. In patients with ASRM I and II endometriosis, there is a good chance for a successful infertility treatment either using surgical- or medical treatment, their combination, or assisted reproductive techniques. Nowadays, there is no unified view on what kind of therapy could be recommended for patients at these stages. On the

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other hand, it is now unanimous that in the cases of stages III and IV, only the assisted reproduction techniques are justified. However, the success rate of assisted reproduction methods in these patients is significantly lower than that in stage I and stage II patients [11]. In a recent paper, Juneau et al. [12] demonstrated that aneuploidy rates of patients with endometriosis are equivalent to their age-matched peers in IVF population without endometriosis. On the other hand, Mansour et al. [13] found that endometriosis-related genetic errors leading to aneuploidy were significantly higher, in comparison to control. In addition, a plenty of indirect data suggest that *via* oxidative stress processes, endometriosis goes hand in hand with aneuploidy.

What make the topic really interesting are the contradictions of published results. In one half of the papers, clear evidence of endometriosis-induced oxidative stress and reduced embryological and clinical outcomes can be read, but in the other half, there is no difference between control and endometriosis patients. The aim of this study was to share our results on this field and show the importance of the exact classification of the disease because, depending on the stage of the disease, it might happen that we detect no differences and significant alterations, too, at the same time.

Materials and Methods

Study design

A retrospective analysis was carried out on the data of 73 women with stage I-II endometriosis, 70 women with stage III-IV endometriosis and 109 women with tubal infertility as control, who referred to Pannon Reproduction Institute, Tapolca, Hungary in 2017. Patients with endometriosis underwent laparoscopy or surgery before the IVF cycle and were scored based on the criteria of ASRM [10]. In control, only the patency of fallopian tube was observed (presence of blockages), laparoscopy of surgery did not happened and pain or pelvic adhesion did not implied to the presence of endometrioma. The objective of this analysis was to compare their clinical, embryological and pregnancy parameters.

Stimulation

For all patients, controlled ovarian hyperstimulation was performed using long protocol desensitization and gonadotropin stimulation. If the ovaries are affected by endometriotic tissues, the incidence of menstrual bleeding in the ovaries is reduced by agonist long protocols. For desensitization, 0.5 ml (0.525 mg) buserelin (Sanofi-Aventis, France) was administered per day subcutaneously (s.c.), starting in the midluteal phase of the previous cycle. When desensitization was achieved, the daily dose of buserelin was reduced to 0.25 ml (0.2625 mg) and stimulations were performed either using follitropin alpha (Gonal-F, Merck, Germany), or follitropin alpha - lutropin alpha (Pergoveris, Merck, Germany) s.c. in individual doses depending on the age, AMH level and antral follicle count (AFC) of the patient varying from 150 IU to 450 IU. Follicular growth was controlled by regular vaginal ultrasound folliculometry usually 2-4 times within a cycle. When the dominant follicle reached 18 mm in diameter, chorionic gonadotropin alpha (Ovitrelle, Merck, Germany) was administered s.c. in 500 μg dose to trigger the ovulation. 36 hours later, follicle aspirations were performed in general anesthesia with ultrasound guided needle using a vaginal probe. Luteal phase support was started on the day of follicle aspiration using progesterone gel intravaginally (Crinone, Merck, Germany) in 90 mg daily dose. Pregnancy tests were performed 12-16 days after the oocyte retrieval.

Embryo culture and transfer

pH-stabilized Origio® sequential media were used for oocyte denudation, fertilization, culture and transfer. Namely, Origio® Fert™ was used for oocyte preparation and fertilization in the first 18-22 hours; Origio® Cleav™ for embryo cleavage up to 72 hours; Origio® Blast™ for blastocyst formation up to 120 hours; and Origio® UTM™ was used for embryo transfer. Cells were incubated at 37°C, supplemented with carbogen gas containing 5% oxygen, 6% carbon dioxide and 89% nitrogen. ICSI was performed both in control and endometriosis groups, as we will discuss below, the residue of follicular fluid affected by endometriomas may exert a negative effect on the sperm cells during IVF. During the ICSI, 10% polyvinylpyrrolidone was used to facilitate the handling and immobilization of sperms. Embryo scoring was performed according to the methods of Bączkowski et al. [14]. Embryos from both test groups underwent evaluation of morphologic quality by grading from 1 to 3, where 1 is good quality, 2 is fair quality, and 3 is poor quality. Embryo transfers were performed with 1-3 embryos (if available) on day 3 or day 5.

Statistical analysis

Data were given as average and deviation. Shapiro-Wilks test was used to evaluate the distribution of the data. Non-normally distributed variables were examined using the non-parametric test of Kruskal-Wallis, while for multiple comparisons, Dunn's test was applied. Differences between proportions or rates were evaluated with Fischer exact test. Graphpad InStat 7.0 software was used for statistical analysis.

Results

The demographic parameters of the three investigated groups were almost the same (Table 1). The average value of age, BMI, AFC and the levels of main basal hormones did not differ significantly, but as a predictor of ovarian reserve, lower serum AMH levels were observed in both endometriosis groups, in comparison with control, 0.23-fold and 0.40-fold decreases were observed in ASRM I-II and III-IV groups, respectively. These alterations induced significant increment in the necessary amount of gonadotropin used for the stimulations, which was increased by 36.81% and 42.18%. In addition, some non-significant trends were observed: the levels of AFC and LH were non-significantly lower; and the level of FSH increased.

ASRM III-IV endometriosis induced significant elevations in the embryological parameters (Table 2). In comparison with the control, in the ASRM III-IV group the number of retrieved oocytes/patients decreased by 33.2%, the number of fertilizable oocytes (MII) decreased by 34.2%, the number of fertilized oocytes (2PN) decreased by 39.2%, the total number of embryos developed *in vitro*/patient decreased by 24.9% and the average grade of transferred embryos showed 19.8% decrement. The average day of embryo transfer or the number of transferred embryos did not differ significantly. This phenomenon resulted in 0.49-fold decrement in the implantation rate. In comparison with ASRM I-II, the number of retrieved oocytes/patients decreased by 28.3%, the number of fertilizable oocytes (MII) decreased by 25.5%, the number of fertilized oocytes (2PN) decreased by 30.9%, the total number of embryos developed *in vitro*/patient decreased by 16.1% and the average grade of transferred embryos

Table 1: Demographic parameters, results of ovarian function's tests and required gonadotropin doses during stimulation in control and patients with ASRM I-II and ASRM III-IV endometriosis.

Parameter	Control	ASRM I-II	ASRM III-IV	*p*-value	Significant Difference
	(A)	(B)	(C)		
Number of patients, #	109	73	70		
Age, years	34.27±4.58	34.78±3.93	34.84±3.91	0.801	-
BMI, kg/m^2	23.91±4.28	23.17±3.52	23.37±4.14	0.4577	-
AFC, #	12.87±4.91	11.42±4.92	11.22±4.85	0.0869	-
LH, IU/L	7.14±9.01	6.09±2.97	5.43±2.87	0.4635	-
FSH, IU/L	7.96±2.79	7.79±2.58	9.53±6.28	0.3123	-
AMH, ng/mL	3.10±2.36	2.37±2.00	1.85±2.07	<0.0001	A-B, A-C
Gonadotropin doses, IU	2526.88±1224.27	3457.04±1693.98	3592.85±1786.07	<0.0001	A-B, A-C

AFC, antral follicle counts; AMH, anti-Müllerian hormone; BMI, body mass index; FSH, follicle-stimulating hormone; LH, luteinizing hormone.

Table 2: Embryological data of patients in the control and endometriosis groups.

Parameter	Control	ASRM I-II	ASRM III-IV	p-value	Significant Difference
	(A)	(B)	(C)		
# Oocytes retrieved/patient	11.03±6.27	10.27±6.77	7.37±5.15	0.0003	A-C, B-C
# Fertilizable oocytes/patient	9.42±5.57	8.32±5.10	6.20±4.35	0.0004	A-C, B-C
#Fertilized oocytes/patient	6.36±4.39	5.60±4.32	3.87±3.29	0.0003	A-C, B-C
Total # embryos that grew *in vitro*/patient	3.34±2.76	3.01±3.21	2.51±2.24	0.0797	-
# Embryos transferred/patient	1.57±0.80	1.60±0.81	1.63±0.98	0.8933	-
Average day of embryo transfers	3.98±1.06	3.77±1.09	3.68±1.04	0.2159	-
Grades of transferred embryos	1.42±0.54	1.53±066	1.77±0.60	0.0011	A-C, B-C
Implantation rates	27.06%	28.54%	13.33%	0.0083	A-C, B-C

Table 3: Comparisons of ICSI success rates in case of control and endometriosis groups.

Parameter	Control	ASRM I-II	ASRM III-IV	*p*-value	Significant Difference
	(A)	(B)	(C)		
Biochemical pregnancy rates, %	44.95%	42.46%	32.85%	0.2607	-
Clinical pregnancy rates, %	38.53%	42.46%	18.57%	0.0047	A-C, B-C
Miscarriage rates, %	19.04%	29.03%	38.46%	0.3216	-

showed 15.7% decrement. The average day of embryo transfer or the number of transferred embryos also did not differ significantly. These values between control and ASRM I-II did not differ significantly.

In comparison to control, diminished rates of biochemical and clinical pregnancy rates were noticed in case of ASRM III-IV (Table 3). Biochemical pregnancy rate decreased from 44.95 to 32.85 % (ns) and clinical pregnancy rate decreased from 38.53% to 18.57% (p=0.0047). Additionally, a 201.9% rise was documented in the rate of miscarriage (ns). The comparison of ASRM I-II and III-IV showed similar results, the biochemical and clinical pregnancy rates decreased from 42.46 to 32.85% and from 42.46 to 18.57%, respectively. The miscarriage rate was 29.03 *vs.* 38.46 %. The control and ASRM I-II groups did not differ this time either.

Discussion

The most common cause of endometriosis is retrograde menstruation, which is delivering different type of endometrial cells and tissue debris. Thus, it can be a source of inflammation processes *via* macrophages and neutrophils activation [15-18]. Increased number of macrophages was published by Capobianco and Rovere-Querini [19]. During their biological function (respiratory burst), high amount of ROS is produced [20]. On the other hand, from the stagnated erythrocytes transition iron ion can be escaped, which also allows ROS generation *via* Fenton's reaction. In serum of women with endometriosis, the concentration of iron was significantly increased by 1.98-fold [21]. Under uncontrolled conditions, when the concentration of ROS exceeds the regulator-ability of antioxidant molecules, oxidative stress occurs. ROS may interfere with almost every component of living cells (sugars, proteins, lipids and nucleic acids) [1,22].

Endometriosis induced elevated levels of oxidative stress markers, including the heat shock protein Hsp70 [23]; (ii) increased concentration of $O_2^{\bullet-}$, H_2O_2 and malondialdehyde in the follicular and peritoneal fluids and in the serum [21,24-29]; (iii) in turn it indicates alterations in the main antioxidant molecules, such as vitamin A, C, E, total superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, glutathione S-transferase and glutathione measured in sera, follicular fluid, peritoneal fluid, or endometrial

and endometriotic cells of patients [28-32]. If the antioxidant defense system cannot regulate, oxidative damages will occur. In case of infertility, maybe the oxidative DNA damage is the most important concomitant. It can act on the DNA directly and indirectly: endometriosis is usually accompanied by (i) direct DNA oxidation (8-OHdG) and fragmentation can be detected from peritoneal fluid [24,33], and (ii) indirectly exert negative effects by the perturbation of mitotic/meiotic spindle system leading to incorrect chromosome segregation on mouse oocytes incubated in peritoneal fluid of patients with endometriosis [13,34-37]. The toxic environment and the presence of highly reactive oxygen species in the peritoneal fluid are thought to damage both gametes and embryos [8,38].

Effects on ovarian functions

Endometriosis is often associated with altered folliculogenesis and ovulatory dysfunctions. In natural cycles, the follicular phase was significantly longer [39], the growth and size of follicles were underdeveloped [40,41], moreover, as a predictor of ovarian reserve, lower serum AMH level (a harbinger of diminished ovarian reserve) was observed [42,43] (Table 1) which is a consequence of the endometrioma [44]. In comparison to controls without endometriosis, reduced responsiveness to gonadotropin stimulation was observed and the number of retrieved oocytes was reduced in case of endometriomas [44,45] (Table 1 and 2). In the study of Opøien et al. [43] which investigated 2245 cycles, stage III-IV endometriosis caused elongated gonadotropin stimulation. As a consequence of the presence of inflammatory cells (and probably ROS) in the peritoneal fluid and the ovaries, altered ovulation and reduced oocyte number were observed [46] (Table 2). On the other hand, in some studies endometriosis did not influence the duration of follicular phase or development [47], no alteration was detected in the follicular fluid AMH level [48], and unvaried number of obtained oocytes was published [49,50].

Effects on oocytes

Recently, it has been a widely accepted hypothesis that endometriosis causes oxidative stress *via* ROS production by the above mentioned immunological inflammation processes [2,33]. The success of fertilization highly depends on the proper nuclear integrity (maturation, spindle formation and chromosome segregation) of oocytes which is extremely sensitive to oxidative stress [30]. ROS-caused (e.g. H_2O_2) meiotic chromosome segregation errors and spindle abnormalities have been confirmed in several cases [34,36,37]. In women with endometriosis, abnormal spindle formation rate in *in vitro* maturated human oocytes was 4.17-fold higher, in comparison to control [35]. Mouse and bovine metaphase II oocytes were incubated in peritoneal fluid obtained from women with or without endometriosis. In comparison to control, endometriosis provoked much higher frequency of abnormal meiotic spindle and chromosomal misalignment, 2.78-fold and 4.29-fold increments in the occurrence of microtubule and chromosome aberrations were measured, respectively [13,51-53]. FISH analysis of oocyte polar bodies performed on control and endometriosis patients showed elevated occurrence of chromosome aneuploidy [54,55]. On bovine and mouse model systems, the supplementation of peritoneal fluid with antioxidants resulted in lower incidence of chromosome and spindle abnormalities [13,52]. In the research of Steinleitner et al. [56], ovarian hyperstimulation was performed on hamsters and on days 2 and 3 of the cycle, peritoneal fluid of endometriosis patient or saline as control were injected intraperitoneally. On day 3, the animals were injected with hCG and mated. 24 h after mating, the animals were killed and zygotes were retrieved from the uterine horns and the fallopian tubes. During the microscopic assessment of fertilization, significantly reduced fertilization (by 88.5%) was observed. In our study, 34.2% decrement was detected (Table 2). The studies of Simón et al. [57] and Díaz et al. [58] are other confirmations of the harmful oxido-reduction microenvironment induced by endometriosis. In the study of Simón et al., IVF outcomes with donor oocytes were investigated and when the donated oocytes derived from women with endometriosis, the lowest pregnancy rates were observed in comparison to tubal infertility, PCOS or idiopathic infertility. Diaz et al. performed the opposite of this study, oocytes were donated from healthy woman and when the recipients were women with endometriosis, the rates of pregnancy were the same as that of healthy recipients.

Effects on sperm cells

Basically, there are two ways how endometriosis affects the sperm quality: (i) in the peritoneal fluid, elevated levels of estradiol, prostaglandin and vascular endothelial growth factors were observed in women suffering from endometriosis, and exposure of sperm cells to these hormones for 135 mins induced significant decrements both in the motility and the progressivity, the acrosomal reaction was also diminished [59]. (ii) On the other hand, the altered inflammatory and oxido-reduction environment have been supposed to be the source of the sperm's changed quality and function [8]. In *in vitro* studies, when human spermatozoa were treated with xanthine-xanthine oxidase (generates $O_2^{\bullet-}$ and H_2O_2), the rate of DNA fragmentation was increased [60]. On Rhesus Macaque sperms, xanthine-xanthine oxidase initiated lipid peroxidation process and reduced motility [61,62]. The plasma membrane of sperm cells contains a high amount of unsaturated fatty acids that allow lipid peroxidation processes [63]. Both *in vitro* and *in vivo* dietary supplementation with antioxidant molecules reduce the negative effects of ROS [60,64-66]. Co-incubation of healthy human sperm with the peritoneal fluid of endometriosis affected patient caused significantly greater DNA fragmentation rate [13]. In this case, contradictory results can be also found, Sharma et al. [67] did not find any effects of peritoneal fluid from endometriosis patients on the sperm function.

Effects on embryos and pregnancy outcomes

The increased activation of macrophages, the increased inflammation and the increased ROS production exert negative effects both on oocyte and sperm qualities, hereby it is inevitable to influence the fertilization, the embryo development and the pregnancy [68]. When bull and macaque sperms were treated with xanthine-xanthine oxidase, reduced fertilization and embryo development were observed, moreover, DNA fragmentation of blastomeres displayed significant increment. If the cells were exposed to antioxidants, the negative effects of ROS were lightened [61,62,69]. H_2O_2 exposure of mouse zygotes resulted in dose-dependent cell cycle arrest, altered pattern of cleavage and increased rates of apoptosis [70,71]. Co-incubation of mouse sperms with peritoneal and follicular fluids of healthy and endometriosis women induced declined fertilization rates, slower

embryo cleavage, less number of blastomeres, DNA fragmentation and apoptosis [72-77]. Similar results have been experienced in our study (Table 2). In the study of Illera et al. [78], mice were injected with the peritoneal fluids of women with endometriosis and with treated endometriosis, as a control saline injection was used. 4 days after the injections, the implants were counted and the endometriosis group showed significant decrement in comparison to control. Interestingly, peritoneal fluid of women with treated endometriosis did not affect the number of implants. Similar results were obtained by others [56]. In a recent study, strong maternal age-dependency has been observed. Namely, ≥35-year-old endometriosis patients show the negative effects on embryological parameters and IVF outcomes even more, in comparison with younger subjects [79]. Not just the implantation failures have greater frequency in case of endometriosis, but the occurrence of early miscarriage is also more common. In the paper of Kohl et al. [80], the rates of miscarriage were 22% and 35.8% for control and endometriosis patients, respectively. The same phenomenon was observed in our lab (19.04 *vs.* 38.46 %), but unfortunately the difference was not significant (Table 3).

Diminished rates of implantation and pregnancy, and increased rates of miscarriage may in part be explained by the decreased quantity and quality of available oocytes and embryos. Patients in the ASRM III-IV group had fewer oocytes that could be retrieved, fewer fertilizable oocytes, fewer oocytes that actually fertilized (2PN), and fewer three- or five-day embryos available for transfer per patient (Table 2). Embryos from all test groups underwent evaluation of morphologic quality by grading from 1 to 3, where 1 is good quality, 2 is fair quality, and 3 is poor quality. Transferred embryos from endometriosis patients had worse average scores for morphological quality than those of control patients (1.42 for control, 1.53 for ASRM I-II and 1.77 for ASRM III-IV patients; p=0.0011). These data demonstrate that patients with Stage III-IV endometriosis have fewer retrievable oocytes, fewer fertilizable and fertilized oocytes, fewer embryos, poorer quality embryos, lower rates of implantation and fewer fetuses than the group of patients without endometriosis or with ASRM I-II endometriosis. In general agreement with our data, other investigators have observed decreases in retrieved oocytes, fertilizable oocytes, fertilization rate, yields of high-quality day 3 embryos, rates of blastocyst formation, and rates of implantation and pregnancy in IVF endometriosis patients compared to control subjects, and also in a mouse model of endometriosis embryotoxicity [29,81-83]. A concomitant increase in the rate of spontaneous abortions was also observed this study [80].

However, in contrast with the above findings, in several cases no alterations were published in the rates of top quality embryos, their implantation and pregnancy outcomes or in the miscarriage rate [45,49,50,84-86]. Nevertheless, this raises the need for a correct classification of endometriosis, because, as we have seen above, embryo yield and score, implantation or pregnancy outcomes of ASRM I-II did not differ significantly from control (Table 3). This kind of low level oxidative stress, which does not affect the ovaries, follicles and gametes may even increase the outcomes. This may even be the reason for different results. However, this assumption needs further investigations.

Treatment horizons

As a counterattack against the ROS accumulation, antioxidant therapies could be successful. In a recent review of Baboo et al. [87], the effects of several antioxidant molecules on endometriosis-related symptoms were summarized. Vitamin C and E, resveratrol, melatonin, curcumin and epigallocatechin-3-gallate have been observed to reduce the size of endometriotic implants and the pain of patients. Several similar studies have been published [88,89]. Moreover, as it was mentioned above, on animal model systems, the supplementation of peritoneal fluid of endometriosis patients with antioxidants resulted in lower incidence of chromosome and spindle abnormalities [13,52]. Unfortunately, no improvements on the pregnancy outcomes have been documented after the antioxidant therapy so far, further investigations are required. Nevertheless, very promising results have been published about the anti-inflammatory and anti-angiogenic dienogest. Three months pretreatment of endometriosis patients with dienogest before IVF resulted in the reduction of the size of endometriomas; increment in the number of oocytes retrieved and embryos developed; improvement in the implantation, clinical pregnancy and live birth rates [90]. On the other hand, these findings are also controversial, because in the study of Tamura et al. [91], negative effects of dienogest treatment on the above parameters were noticed.

Conclusion

In conclusion, our results have revealed the toxic action of endometriosis on IVF outcomes (in agreement with part of the literature) involves (i) the depletion of ovarian reserve manifesting in reduced AMH levels and extended gonadotropin stimulations; (ii) the altered endometriotic, oxido-reduction microenvironment of gametes during maturation, fertilization and development resulted in the decrement in the number of retrieved oocytes, fertilizable oocytes and fertilized oocytes, which contributed to worse grades of available embryos for transfer; (iii) the direct consequence of worse embryos is the reduced implantation and clinical pregnancy rates. An interesting finding of our study is the severity dependence of results. Namely, ASRM I-II and ASRM III-IV showed opposite results in many cases explaining the controversial results of the literature arising the demand for exact classification of endometriosis. Unfortunately, the ASRM classification has several critical limitations. For example, it is an arbitrary scoring system based on subjective score allocation, observer variability may be present, score can be affected by surgical technique and timing of surgery. This kind of ambiguities may contribute to the contradictions in the literature. Finally, although no measurements of antioxidant or anti-inflammatory therapy were carried out in our study, but based on the characteristic of the disease, they may be able to moderate the negative accompanying oxidative effects. However, further investigations are needed.

Author Contributions

Attila Török: Gynecological work, Manuscript writing. Gábor Máté: Embryological work, Study design, Data collection, Manuscript writing, Final approval of the version to be submitted.

Disclosure Statement

The authors declare that they have no conflict of interest.

Statement of Ethics

The study protocol has been approved by the National Public

Health and Medical Officer (23522-5/2020/EÜIG) and the study was conducted in accordance with Helsinki Declaration.

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