Original Research Article

Lipid peroxidation status in embryo culture media: *The impact on fertilization and embryo quality during IVF cycles*

Abstract

Background: The process of preimplantation embryo development in vitro represents a key phase during in vitro fertilization (IVF) cycles. It involves several regulatory signaling pathways as well as an optimized in vitro culture system. The resulting embryo quality helps to determine embryo competence before implantation and pregnancy outcomes. Reactive oxygen species (ROS) are known to play a major role in influencing the process of embryo development. Their role can be reflected in the regulation of signaling pathways as second messengers or ~~in~~ the irreversible cell alterations due to oxidative stress following an excess of ROS levels.

Methods: In this study, we investigated the association between morphological embryo quality (fertilization, cleavage quality, and fragmentation levels) and lipid peroxidation levels (Malondialdehyde) in embryo culture media. After intracytoplasmic sperm injection (ICSI), a total of 103 oocytes were evaluated on day 1 and day 3 of their development, and their corresponding culture media were analyzed by estimating MDA levels using thiobarbituric acid.

Results: The results showed no significant association between MDA levels in culture media and fertilization rate (p=0.3), sperm quality (p=0.99; p= 0.17; p=0.46; p=0.30; p=0.65; p=0.44; p=0.09; p=0.15; p=0.56), embryo fragmentation levels (p=0.79; p=0.40), AMH levels (p=0.31; p=0.36) and female age (p=0.60; p=0.34). However, we revealed a significant association between cleavage quality and MDA levels in the embryo environment (p=0.03).

Conclusion: We conclude that oxidative stress in IVF culture media might be mainly associated with delayed embryonic development

Keywords: Embryo development, reactive oxygen species, malondialdehyde, oxidative stress

INTRODUCTION

Embryo quality is one the most important factors that are assessed during the process of in vitro fertilization (IVF) to increase implantation and pregnancy rates. The evaluation of embryo quality in IVF laboratory mostly involves morphological features such as cleavage kinetics and fragmentation rates and theses process involves a diversity of connected signalization and regulation pathways. Whether occurring *in vivo* or *in vitro*, embryonic development requires several factors that maintain the homeostasis of these signaling pathways at each stage of the pre-implantation process. Among the regulatory factors that have attracted the most interest due to their complexity are reactive oxygen species (ROS). These highly reactive molecules are generated from oxygen reduction by electrons during cellular metabolism and act as second messengers in physiological cell signaling and control pathways [1,2,3,4]. Embryos are known for three free radicals during their normal aerobic metabolism: hydrogen peroxide (H2O2), superoxide anion (O2−), and hydroxyl radical (OH−) [5, 6,1,7]. However, free radicals are more often associated with the principle of oxidative stress, which results from an imbalance in the level of oxidants and antioxidants, in favor of oxidants during cell metabolism [8,9,10,1]. Many studies reported a lot of interesting results about ROS being involved in delayed embryo development [12,13,14,15], playing an essential role in the regulation of some of the most important stages of embryo development such as fertilization, cleavage and embryo hatching [16,15,17,18] or having no association with embryo quality at all [19]. These conflicting findings make ROS involvement in cell signalizations complex and simple at the same time. It has been clearly shown that excess ROS has a negative impact on embryo quality in vitro [20,15,21,22, 23], as we know that any type of excess in our metabolism represents a loss of balance. However, knowing that ROS is implicated in so many regulation pathways that are necessary for embryo quality, it is important to understand to what extent ROS are necessary and impactful during the early stages of embryo development in vitro and whether or not they are impactful.

This study aims to give a better understanding of the dynamics of ROS production during in vitro culture of pre-implantation embryos after intracytoplasmic sperm injection (ICSI) and to explore the relationships between ROS levels in culture media (CM) and embryo quality.

MATERIAL AND METHODS

Patients

A total of 103 women aged between 24 and 50 years old, with primary and secondary infertility and undergoing oocyte retrieval cycles of assisted reproduction, between January 30, 2019, and March 03, 2020, were prospectively included. Couples were not selected for age, sperm parameters, or causes of infertility (Table 1). This work did not involve experiments on humans or animals, 207 spent CM were collected.

Ovarian stimulation

Women underwent controlled ovarian stimulation with the flexible gonadotropin-releasing hormone (GnRH) antagonist protocol. A daily subcutaneous injection of recombinant follicle-stimulating hormone (rFSH; Gonal-F, Merck-Serono) was used alone or in combination with human menopausal gonadotropin (HMG, Menopur; Ferring). The FSH dose was based on the woman's age and AMH concentration in addition to prior history of ovarian stimulation and was adjusted according to usual parameters of follicle growth, determined by serum estradiol (E2) concentration and ultrasound monitoring.

A daily dose of GnRH antagonist (Cetrotide, Merck-Serono, or Orgalutran, MSD) was injected subcutaneously, starting from day 6 of FSH administration. The ovulation trigger was performed with 10 000 IU of human chorionic gonadotrophin (rHCG, Ovitrelle; Merck-Serono) and gonadotrophin-releasing hormone (Decapeptyl, Ferring), after obtaining follicles that reached dimensions of 17mm or greater in diameter and adequate serum E2 levels. Oocytes were retrieved 34-36 hours after hCG administration.

Oocyte and sperm preparation

The retrieved oocytes were isolated from follicular fluid, rinsed, and cultured in CM (SAGE 1-Step, Origio). 2-3 hours after retrieval, the oocyte-corona-cumulus complexes were placed in a HEPES-buffered medium (Ferticult Flushing medium, Fertipro) containing hyaluronidase (Hyaluronidases in Ferticult Flushing medium, 80IU/mL, Fertipro) and were mechanically decoronated using a 20-200µL micropipette. The nuclear maturation grades were classified as metaphase II or non-metaphase II (Metaphase I or Prophase I) oocytes.

Sperm samples were collected from the male partner by masturbation in a sterile container, after 2-3 days of abstinence. At first, semen samples were evaluated for spermatic parameters (concentration, motility, and morphology) based on WHO (2010) recommendations, to be

divided into several groups. For each parameter, sperm samples were divided into two groups. Sperm concentration included samples with normal concentration (> 15 M/mL) and samples with oligospermia (≤ 15 M/mL). The category of motility rate included samples with normal sperm motility rate (>32%) and samples with asthenospermia (≤32%). The category of morphology rate included normal sperm morphology rate (>20%) and teratospermia (≤20%). Motile spermatozoa were then selected using a discontinuous two-layer density gradient technique (Puresperm 80/40; SAGE) as described by Aboulmaouahib et al. (2016).

All mature oocytes underwent ICSI after decoronisation. One micro-injected oocyte per patient was then randomly selected and placed in an oil-covered single drop of 100 µL of culture media (SAGE 1-Step, Origio), in a petri dish.

The medium was renewed on day 1(18-20h after ICSI) and day 3(68-70h after ICSI).

Assessment of fertilization and embryo quality

A total of 103 oocytes were evaluated.

On day 1, fertilization was confirmed by the appearance of a double polar body and/or two pronuclei. At that stage, oocytes were divided into two groups: zygotes and unfertilized oocytes. On day 3, the embryonic quality was evaluated according to the number of blastomeres and the presence or absence of cell fragmentation. Initially, embryos were divided into 2 groups: 7-8 cells embryos and 4-6 cells embryos. Secondly, embryos were divided into 2 groups: fragments-free embryos and embryos with 5-10% fragmentation.

The temperature inside the incubators (IVF-Cube AD3100, ASTEC; Thermo Scientific HeraCell 150) was controlled by a certified thermometer and remained at 37±0.2 °C. The oxygen level inside the incubators was at 5% and the cultivating medium pH was at 7.3 ± 0.02 with CO2 around 5.6%.

Malondialdehyde (MDA) measurement

The spent CM of the corresponding oocytes and embryos (day 1, day 3) were collected for the determination of lipid peroxidation levels.

Microvolume protein concentration was measured using the Nanodrop spectrophotometer as described by Desjardins and colleagues (24). Lipid peroxidation was estimated by thiobarbituric acid reactive substances (TBARS) reaction with malondialdehyde (MDA) as

described by Sanokyszn and Marnett (1990). Briefly, 100 µL of culture media was added to 10% trichloroacetic (TCA) and 0.67% thiobarbituric acid (TBA). The absorbance was measured at 532nm and MDA levels were expressed in µmole/ mg of proteins.

Statistical analysis

The results are expressed as the mean ±Standard deviation or percentage of the total. Data were obtained with the student's t-test using SPSS (Statistical Package for Social Science). Statistical significance was defined as $p < 0.05$.

No. of injected oocytes	103
No. of cycles with no embryos available	18
Age of female partner (years)	37.4 ± 5.4 (25-46)
Age of male partner (years)	42.1 ± 7.2 (32-58)
Primary infertility	92
Secondary infertility	11
Duration of infertility (years)	7.18 ± 5.07 (1-20)
Male infertility	29
Endometriosis	9
OPK	5
Tubal infertility	4
Ovarian insufficiency	4
Combined factors	10
Idiopathic	42

Values are mean ± SD

Table 1: Demographic characteristics of ICSI cycles (103)

RESULTS

Relationship between fertilization and MDA levels in culture media

As shown in Table 2, on day 1, MDA levels were significantly higher in CM of embryos than in the control samples (no oocytes or embryos in CM) (p=<0.0001). However, MDA levels in CM were not associated with fertilization. In fact, there was no significant difference between MDA levels in CM of zygotes and unfertilized oocytes.

Relationship between MDA levels in culture media and embryo quality

All embryos were divided into 2 groups depending on the embryo's cell number (7-8 / 4-6 cells) and fragmentation (with or without fragmentation). On day 1, we noted that MDA levels in CM of 4-6 cells embryos were significantly higher (p=0.03) than in CM of 7-8 cells embryos. While on day 3, there was no significant difference between MDA levels in CM of these two groups.

While comparing fluctuations of MDA levels in CM within the same group, we noted that MDA levels in CM of 4-6 cells embryos were significantly higher on day 1 than on day 3 (p= 0.02). Contrariwise, there was no significant difference between MDA levels on day 1 and day 3 CM of 7-8 cells embryos (Table 2). Likewise, there was no significant difference between MDA levels in CM of fragmented (5-10% fragmentation) embryos and fragments-free embryos, on day 1 as well as on day 3. Also, in control samples, MDA levels significantly increased from day 1 to day 3 (Table 2).

Relationships between cleavage quality in day 3 embryos and fragmentation

The group of fragments-free embryos included 42/ 53 (79.2 %) 7-8 cells embryos and 11/53 (20.7%) 4-6 cells embryos. The group of 5-10% embryo fragmentation included 16/27 (59.2%) 7-8 cells embryos and 11/27 (40.7%) 4-6 cells embryos (Figure 1).

Comparison between MDA levels in culture media, embryo quality, and sperm quality

In our work, we have sought to link the results of MDA levels in CM of embryo quality with sperm quality. The latter was measured by the concentration, mobility, and morphology parameters of each patient. On day 1, there was no significant difference between MDA levels in CM that corresponds to normal sperm conentration and oligospermia, for 7-8 cells embryos or 4-6 cells embryos, either on day 1 or on day 3. The same results were found in MDA levels that correspond to normal sperm motility parameters/asthenospermia and normal sperm morphology parameters and teratospermia (Table 3).

Relationships between cleavage quality in day 3 embryos and sperm parameters

In addition, we have sought to establish relationships between cleavage quality in day 3 embryos and sperm quality in terms of numeration, motility and morphology. In the normal sperm morphology parameters group, 78.8% (41/52) of the embryos were 7-8 cells embryos and 21.1% (11/52) were 4-6 cells embryos. In the teratospermia group, 55.8% (19/34) of embryos were 7-8 cells embryos and 44.1% (15/34) were 4-6 cells embryos. In the normal sperm concentration group, 78.2% (36/46) of embryos were 7-8 cells embryos and 21.7% (10/46) were 4-6 cells embryos. In the oligospermia group, 60% (24/40) of embryos were 7-8 cells embryos and 40% (16/40) were 4-6 cells embryos. In the normal sperm motility parameters group, 79.1% (19/24) of embryos were 7-8 cells embryos and 20.8% (5/24) were 4-6 cells embryos. In the asthenospermia group, 67.7% (40/59) of embryos were 7-8 cells embryos and 32.2% (19/59) were 4-6 cells embryos (Table 4).

Relationships between MDA levels in CM and AMH levels, women's age and type of infertility

Furthermore, no significant difference was noted between MDA levels in CM on day 1 or day 3 and the women's age. In addition, no association was found between MDA levels in CM on day 1/ day 3 and AMH levels as well as the type of infertility (Table 5).

	Control samples (n=36)	**Embryo cultures (n=81)**	**Unfertilized oocytes (n=18)**	**7-8 cells embryos (n=42)**	**4-6 cells embryos (n=22)**	**Fragments free (n=53)**	**5-10% fragments (n=29)**
Day 1	0.0001 ± 0.0002	0.0020 ± 0.0033	0.0035 ± 0.0061	0.0015 ± 0.0033	0.0035 ± 0.0036	0.0021 ±0.0036	0.0019 ±0.0025
p-value		$1.03E^{-06}$*	0.03*	0,03		0.79	
		0.3					
Day 3	0.0007 ± 0.0007	0.0014 ± 0.0020	0.0020 ± 0.0038	0.0013 ± 0.0020	0.0017 ± 0.0017	0.0014 ±0.0020	0.0018 ± 0.0024
p-value		0.009*	0.8*	0.4		0.4	
P value	$2.35E^{-05}$**			0.58**	0.02**	0.20**	0.80**

Table 2: Mean Values, Standard Deviations, and Significant Differences of MDA levels (µg/mg of proteins) at day 1 and day 3 in culture media (CM) of Zygotes, 4-6 cells embryos, 7-8 cells embryos, embryo fragmentation and control samples (no oocytes or embryos in CM)

*Comparison with control

** Comparison between Days1 and 3

The results were evaluated by a non-parametric test: the Mann-Witney U for the comparison of the groups.

	Normal sperm concentration parameters	Oligospermia	Normal sperm motility parameters	Asthenospermia	Normal sperm morphology parameters	Teratospermia
7-8 cells / Day 1	0.0015±0.00025 (n=36)	0.0015±0.0036 (n= 24)	0.0019±0.0031 (n= 19)	0.0013 ± 0.0030 (n = 40)	0.0015±0.0025 (n= 41)	0.0006 ±0.0014 (n= 19)
p-value	0.99		0.46		0.09	
7-8 cells / Day 3	0.0011±0.0016 (n=36)	0.0016±0.0026 (n=24)	0.0015±0.0020 (n=19)	0.0012 ± 0.0021 (n=40)	0.0014±0.0021 (n=41)	0.0014± 0.0021 (n=19)
p-value	0.37		0.65		0.99	
4-6 cells/ Day 1	0.0045±0.0037 (n=10)	0.0024±0.0034 (n=16)	0.0052 ±0.0034 (n=5)	0.0032±0.0036 (n=19)	0.0046±0.0038 (n=11)	0.0025±0.0030 (n=15)
p-value	0.17		0.30		0.15	
4-6 cells/ Day 3	0.0029± 0.0028 (n=10)	0.0013±0.0017 (n=16)	0.0032±0.0036 (n=5)	0.0018± 0.0019 (n=19)	0.0024±0.0028 (n=11)	0.0018±0.0018 (n=15)
p-value	0.13		0.44		0.56	

Table 3: MDA levels (µg/mg of proteins) in CM of 7-8 / 4-6 cells embryos, on day 1/ day 3, according to sperm quality

	7-8 cells embryos	4-6 cells embryos
Normal sperm morphology parameters	78.8% (41/52)	21.1% (11/52)
Teratospermia	55.8% (19/34)	44.1% (15/34)
Normal sperm numeration parameters	78.2% (36/46)	21.7% (10/46)
Oligospermia	60% (24/40)	40% (16/40)
Normal sperm motility parameters	79.1% (19/24)	20.8% (5/24)
Asthenospermia	67.7% (40/59)	32.2% (19/59)

Table 4: Percentage of 7-8 cells embryos and 4-6 cells embryos in each group of sperm parameters.

	Female age (years)		AMH levels		Type of infertility	
	<38 (n=50)	> 38 (n=51)	<1 (n=42)	>1 (n=44)	Male infertility (n=29)	Idiopathic infertility (n=45)
Jour 1	0.0022 ± 0.0035	0.0026 ± 0.0046	0.0026 ± 0.0045	0.0018 ± 0.0032	0.0029 ± 0.0050	0.0019 ± 0.0038
p-value	0.60		0.31		0.38	
Jour 3	0.0014 ± 0.0024	0.0019 ± 0.0026	0.0019 ± 0.0023	0.0014 ± 0.0029	0.0025 ± 0.0037	0.0011 ± 0.0013
p-value	0.34		0.36		0.06	

Table 5: Comparison of MDA levels (µg/mg of proteins) of embryo CM according to female age, AMH levels and type of infertility

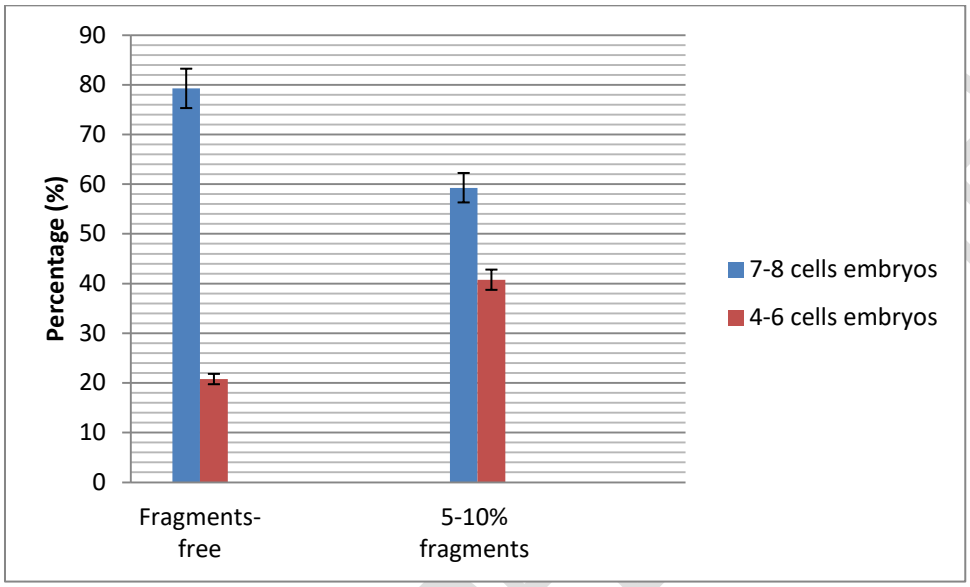

Figure 1: Associations between cleavage quality and fragmentation in embryos on day 3

DISCUSSION

Based on our previous readings [25], research has demonstrated the key role of ROS in the regulation of calcium oscillations for the process of oocyte activation by the sperm; however, the negative impact of oxidative stress on oocyte fertilization *in vitro* is a very well described

outcome in assisted reproductive technology. In a balanced in *vivo* environment, mitochondrial ROS, such as H2O2, O2- and NO- act as second messengers to release internal calcium reserves by acting on the calcium pumps of the endoplasmic reticulum (ER) and the plasma membranes [25,26,27,28,24,29]. During the process of fertilization, as calcium is a key factor in the quality of fertilization, an amount of ROS levels might be also necessary for the regulation of this process. On the other hand, excess intra/ extracellular ROS levels are also known to induce high lipid peroxidation which can disturb calcium oscillations and alter oocyte fertilization [30,31,17,22]. In fact, ROS levels in ICSI and IVF culture media on day 1 have been previously shown to be associated with low fertilization outcomes [33]. All these cellular events show us above all the importance of maintaining extra/ intracellular homeostasis of oocytes and embryos either *in vivo* or *in vitro*.

On day 1, our data showed that MDA levels in CM of fertilized oocytes were significantly higher than MDA levels in the control samples (no oocytes/ embryos in the media). We can speculate that this elevation may be due to the possible production of ROS by oocytes in parallel with the triggering of Ca++ pathways during fertilization, however, it cannot be excluded that it can simply be caused by the production of ROS by culture media components over time [6,33,34]. The radical « singlet oxygen » that is present in the atmosphere can also attack lipidated components of CM and thus produce MDA.

Interestingly, MDA levels in control samples significantly increase from day 1 to day 3. This finding could confirm that in vitro culture conditions alone can cause moderate ROS production and represent a factor that is associated with oxidative stress in embryo culture media.

In this study, since all oocytes have been fertilized by the ICSI procedure, the overproduction of ROS caused by sperm in CM during conventional IVF was widely mitigated [35,36,37] which made MDA levels in CM on day 1 appear to be more associated with the fertilization process. While comparing zygotes and unfertilized oocytes on day 1, we found no association between MDA levels in CM and our fertilization rate. A similar conclusion was reached by a recent study where ROS levels in CM were not associated with fertilization rate as fertilized, polyspermic and unfertilized zygotes showed similar ROS levels [19]. Since zygotes generate different outcomes of embryo quality on day 3, we are tempted to speculate that ROS levels in CM are not a major indicator of fertilization success but some ROS deletions might appear later during the next phases of early development, such as cleavage and compaction.

Successful oocyte fertilization (zygotes) on day 1 does not always result in successful embryo development on day 3 [19,38,39] and early cleavage stage embryos are more sensitive to oxidative stress during the early stages of development (40,41,42,43), thereby, we were interested to study the variations of MDA levels in CM of embryos according to the number of cells and fragmentation on day 3. On day 1, the oocyte is fertilized and ready to initiate cleavage [44,45,46]. At this stage, our results showed that 4-6 cells embryos produced higher MDA levels in CM than 7-8 cells embryos, which can reveal a possible association between cleavage quality and oxidative stress levels in the embryo environment. Overall, this finding is consistent with previous research showing that oxidative stress can be involved in serious embryo damage such as DNA fragmentation and impairment of mitochondrial and enzymes function which can result in delays of embryonic cleavage or embryo arrest [12,32,47,13,20, 14,15,22,23]. By day 3, there was no significant difference between MDA levels of both 4-6 and 7-8 cells embryos which are in concordance with Lan and colleagues [19] who found that ROS levels on day 3 were not significantly associated with cleavage stage and embryo quality. These results may address the fact that by the end of cleavage, embryos might be able to use their antioxidant system to bring ROS levels down to a level that is low enough to trigger the process of compaction, regardless of cleavage quality.While some antioxidants are known to be absent in mature oocytes, such as catalase [42] embryos acquire their antioxidant system once genome activation occurs after the 2-cell stage. Embryos are then able to recover their antioxidant concentrations and use it as an adaptative protection against oxidative stress, as shown in mouse embryos [6,49–52]. In CM of 4-6 cells embryos, we observed a fluctuation of MDA levels between day 1 and day 3, as the levels were higher on day 1 and significantly decreased by day 3. At first, this finding made us suspect that these fluctuations might be due to the initiation of cleavage on day 1, as the need for an amount of ROS by the cells during cleavage for regulation has been largely discussed previously. Several studies have highlighted the complex relationship between intracellular ROS levels and preimplantation embryo development. H2O2 has been shown to increase during the first cell divisions, the 8-cell stage to increase blastulation capacity and hatching in mouse and bovine embryos [25,53–56]. However, in CM of 7-8 cells embryos, no significant difference in MDA levels was found between day 1 and day 3. These results suggest that fluctuations of oxidative stress in CM are more likely to be associated with a delay in embryo development than with positive regulation of the cleavage process. The redox state in CM as in embryos needs to stay stable from day 1 to the end of in vitro culture for better embryo development.

Nevertheless, we are aware of the limitations of our study as there is no following up of embryo development and MDA levels to day 5.

Other reports on the impact of ROS on embryo quality implied that an increase in the rate of ROS in ICSI cycles is associated with an increase in the rate of embryo fragmentation (>10%) [33,57–61]. However, our study showed no association between MDA levels in the embryo surrounding and embryo fragmentation. Embryo fragmentation is defined by the appearance of anucleated fragments in the cytoplasm during the cleavage stage [61,62,59]. Although it is a common feature of IVF-derived embryos [64–67], the mechanisms and causes of embryo fragmentation are still unclear. Many investigators came up with several hypotheses as some reported fragmentation to occurs during the first cell division [65,68,69] and result in the loss of important volumes of blastomere's cytoplasm, reduction of embryo size and depletion of essential organelles, namely mitochondria, mRNA and proteins [70,71]. Plus, due to blastomere's movement during embryo cleavage, fragments in the cleavage cavity may disturb the cell-cell contact and distortion of division planes [21,64,65]. In fact, Our data also showed that 42/ 53 (79,2%) fragment-free embryos had 7-8 cells on day 3, which may suggest that fragment-free embryos have more healthy mitochondria leading to a healthier cleavage, but since we found no significant difference between the rate of 7-8 (16/27; 59.2%) and 4-6 cells (11/27; 40.7%) embryos in the group of fragmented embryos, our results stay controversial. Therefore, we can only deduce that the quality of embryo cleavage can be associated to fragmentation rate and to ROS production; however, there is no significant association between these two factors.

To deepen our research on the impact of oxidative stress levels in embryo surroundings, we also investigated the possible relationships between sperm quality, embryo quality and MDA levels in CM. No significant difference was found in MDA levels between CM of embryos corresponding to normal sperm concentration and oligospermia, and also between CM of embryos corresponding to normal sperm motility rate and asthenospermia, whether it's 7-8 cells or 4-6 cells embryos. This data can be primarily explained by the fact that low sperm concentration and motility factors as well as excess ROS production by conventional IVF are attenuated by ICSI procedures [72,73]. Also, these findings can demonstrate that sperm quality does not impact MDA levels in CM and that sperm quality is more related to embryonic quality than directly to oxidative stress in CM. Our statistics showed that 7-8 cell embryos mainly originate from sperm with normal parameters.

It was also interesting to notice that while in the group of normal sperm morphology parameters, 68.3% (41/52) were 7-8 cells embryos and only 21,1% (11/52) were 4-6 cells embryos, there was no significant difference between the rate of 7-8 cells embryos (55,8%) and 4-6 cells embryos (44,1%) in the teratospermia group. During ICSI, morphology selection of spermatozoa is based on its normal-looking shape under x200 or 400 magnification, which might miss the detection of minor head malformations. Plus, the risk of using spermatozoa with damaged DNA in the case of teratospermia is greater [74–81]. To consolidate our study, we also investigated the possible relationships between MDA levels and AMH, women's age and some types of infertilities (Male infertilities, idiopathic). AMH is produced by granulosa cells from preantral and antral follicles and its concentration is related to the number of small follicles and ovarian reserve [82–84]. As age decreases, AMH gradually decreases which is a sign of a lower ovarian reserve [83]. In fact, It is well established that AMH levels are strongly correlated with age, and age is strongly correlated with oxidative stress and infertility [85,86]. A recent study has shown that starting from age 37, the antioxidant pattern in FF decreases and impairs the ROS scavenging system [87], which can be an indicator of infertility and a greater risk of IVF failure. Yet, our data showed no direct association between CM MDA levels and women's age AMH and idiopathic infertility.

Despite our interesting findings, we are well aware that MDA is not the only compound that can react with thiobarbituric, which can represent a limitation. However, such a limitation in such a complex biological sample can show our results as a door opening to other research using more specific techniques

Conclusion

According to all these findings, although, embryo quality could be associated with other parameters that are not always associated with oxidative stress such as fragmentation levels and sperm quality, or parameters that are related to oxidative stress such as age and AMH levels, oxidative stress in IVF CM might be mainly associated to delayed embryonic development.

Consent: All couples signed an informed consent before the IVF cycle.

Ethical Approval:

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

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