Int J Clin Exp Med 2016;9(8):16459-16467
www.ijcem.com /ISSN:1940-5901/IJCEM0027959

Original Article

Woman age limits the improvable effect of reduced oxygen concentration (5% O_2) on human embryo quality and pregnancy potential

Hsiu-Hui Chen[4,5*], Tung-Chuan Yang[1*], Tsung-Hsien Lee[2,3], Chun-Chia Huang[5], Maw-Sheng Lee[1,2,3,5*], Lee-Feng Chien[4]

[1]Department of Obstetrics and Gynecology, School of Chinese Medicine, China Medical University and Hospital, Taichung, Taiwan; [2]Insititute of Medicine, Chung Shan Medical University, Taichung, Taiwan; [3]Department of Obstetrics and Gynecology, Chung Shan Medical University Hospital, Taichung, Taiwan; [4]Department of Life Sciences, College of Life Sciences, National Chung Hsing University, Taichung, Taiwan; [5]Division of Infertility Clinic, Lee Women's Hospital, Taichung, Taiwan. [*]Equal contributors.

Received March 11, 2016; Accepted May 26, 2016; Epub August 15, 2016; Published August 30, 2016

Abstract: This study was to assess the effect of women age on embryo quality from early cleavage to day 3, and pregnancy potential under a 5% or 20% oxygen tension. One hundred seventy-four patients undergoing controlled ovarian stimulation, IVF/ICSI, and day 3 embryo transfer. Fertilized oocytes were cultured to day 3 stage under a 5% or 20% oxygen concentration. In younger women, the early cleavage (2-cell stage) rate in 20% O_2 tension was significantly higher (68.3%) than that in 5% O_2 (60.1%). However, the rate of day 3 good embryo developed from good early cleavage (79.1%), pregnancy (58.0%) and implantation rates (30.7%) in 5% O_2 were significantly higher than those in 20% O_2 (66.8%, 40.0% and 18.8%, respectively) for women <38 years of age. In older women, no matter embryo quality or pregnancy rates between 5% and 20% O_2 groups were no significant difference. It is suggested that embryos from younger but not older women, cultured in 5% O_2 tension, will increase the embryo quality and pregnancy rate and implantation rate.

Keywords: Age, IVF, oxygen concentration

Introduction

The human embryos are commonly cultured in vitro utilizing two different gas phases containing atmospheric (20%) or low (5%) oxygen concentrations, however, the clinical results of in vitro fertilization (IVF) had no definite answer for which one gas phase is more appropriate for human embryo development. The impact of reactive oxygen species (ROS) in embryo development has been noted for years [1, 2], and the atmospheric oxygen culture system in vitro had a distinct higher oxygen tension than that (<5% O_2) within the uterine and oviduct [3]. Furthermore, reduced oxygen concentration (5% O_2) showed a slightly decreased ROS formation [4] and it was suggested that 20% O_2 had a deleterious effect on embryo development [5].

In addition, it seems reasonable that the reduced oxygen tension is appropriate for embryo development, but different opinions of the oxygen tension effects on embryo in vitro have been reported. Several studies suggested that embryo development was improved under reduced oxygen tension [6], e.g. mouse [7], rat [8] and pig [9], but it was thought that 5% O_2 had no improvement on the blastocyst formation rate in the mouse [10]. On human embryos, it was reported that the blastocyst formation rate showed a little but significantly higher on 5% O_2 than 20% O_2, but day 2, 3 embryo quality and pregnancy rate were not affected [11].

Adam et al. [12] also reported that O_2 tension had no remarkable effect on the subsequent embryonic development but suggest that 20% O_2 may delay oocyte maturation and/or the acquisition of fertilizability and impair the developmental competence of oocytes. These results suggested that no beneficial effect of lower O_2 concentration during the first 3 days of

in vitro culture in terms of pregnancy and/or implantation rates per embryo transfer.

On the other hand, it was reported that an age-related decline in the developmental competence of oocytes was noted [13], and the number of follicles declined exponentially, with a marked increase in the rate of disappearance from age 37-38 years onwards, when numbers had fallen to the critical figure of 25,000 at the age of 37.5 years [14, 15]. Moreover, human aging may be related to deleterious attacks of free radicals on cell constituents [16]. The most relevant idea about oocyte aging is related to the accumulation of damage generated by the increased levels of ROS [17-19], which lead the nuclear and cytoplasmic failure of the aged oocyte. The ROS are involved in the modulation of oocyte maturation, ovarian steroidogenesis, corpus luteal functions and luteolysis. Moreover, they play a role during fertilization, embryo development and pregnancy [2].

In this study, we examine the effect of two different O_2 concentrations (5% and 20%) during the first 3 days of culture on embryo quality, and clinical outcomes such as implantation and pregnancy rate. In addition, we analyzed the subgroups of patients, stratified for patient age, who could be benefited the most from the reduced oxygen concentration.

Materials and methods

Patient selection and oocyte retrieval

This study was approved by the Institutional Review Board of Chung Shan Medical University Hospital, Taichung, Taiwan. Written informed consent was obtained from these women included. Data were retrospectively analyzed from infertility patients who underwent IVF treatment using a program with a long protocol for GnRH agonist administration.

Participating women were administered leuprolide acetate (Lupron, Takeda Chemical Industries, Ltd., Osaka, Japan), commencing during the midluteal phase, for the purpose of down-regulation. A serum estradiol (E_2) level of <50 pg/ml was used on day 2 to confirm pituitary suppression and followed by treatment with recombinant follicular stimulation hormone (rFSH; Gonal-F, Serono, Bari, Italy). The participants' ovarian responses were monitored with serial serum E_2 levels and ultrasound examinations. When the leading two follicles reached approximately 18 mm in diameter with an appropriate serum E_2 level, 10,000 IU of HCG (Profasi, Serono) were administered. Transvaginal oocyte retrieval was performed 34-36 hours later [20].

Oocyte fertilization and embryo culture

After oocytes retrieval, the mature oocytes (MII) were utilized for insemination or intracytoplasmic sperm injection (ICSI). Gametes and embryos were alternately assigned to one of two treatment groups (5% O_2 and 20% O_2 concentration culture systems). All oocyte inseminations/ICSI were performed using microdrops of HEPES-buffered human tubal fluid medium (mHTF; Irvine Scientific, Santa Ana, CA, USA) containing 5% (v/v) serum substitute supplement (Irvine Scientific). Immediately prior to ICSI, cumulus cells were removed by pipetting the oocytes in mHTF containing 80 IU/ml hyaluronidase (type 8, H-3757, Sigma Chemical, St Louis, MO, USA). Following insemination or ICSI, all embryos were furthered cultured in microdrops of G3.1/G3.2 (Vitrolife, Copenhagen, Denmark) medium. The appearance of embryonic pronuclei (PN) was observed by microscopy 17-20 hours after insemination/ICSI. Embryonic division and morphology was respectively observed and recorded at 25-27 hours (the 2-cell stage or early cleavage), 45-48 hours (4-cell stage) and 69-72 hours (8-cell stage) after insemination/ICSI.

Assessment of embryo quality

In this study, embryo quality was assessed by the morphological appearance in the 2-cell, day 2, and day 3 stages. Fertilized embryos that had cleaved to the 2-cell stage 25 h post-insemination were designated as 'early cleavage' embryos [21]. An early cleavage embryo contained both 2 equal sized blastomeres and fragmentation <10% was defined as "good early cleavage (GEC)" embryo. The day 3 "good" embryos showed both ≥7 blastomeres and less than 20% fragmentation [22]. To assess the effective good embryos with regular development, the development of GEC embryos form 2-cell to day 3 stage was traced. The day 3 good embryos developed from GEC embryo were respectively defined as "top" day 3 embryos. The mature oocytes and fertilized zygotes development were summarized in **Figure 1**.

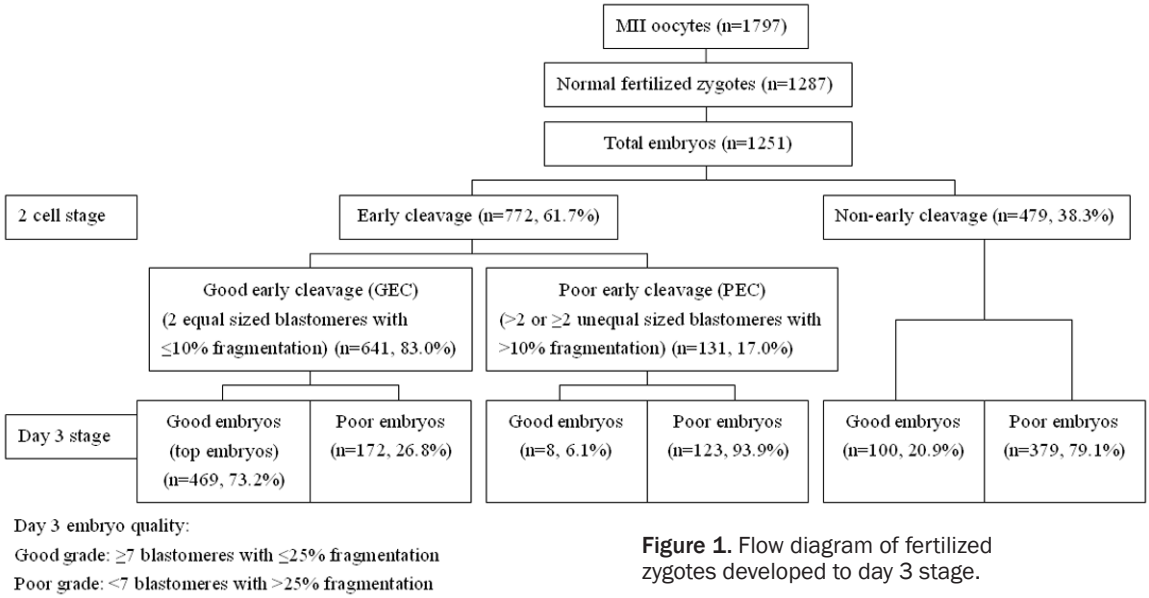

Figure 1. Flow diagram of fertilized zygotes developed to day 3 stage.

Table 1. Infertility factors in different groups

	5% O_2 (<38)	20% O_2 (<38)	5% O_2 (≥38)	20% O_2 (≥38)
Cycles	69	60	24	21
Age	33.4±0.07	33.3±0.07	40.8±0.12	39.9±0.09
Infertility factors				
Male factor (%)	25 (36.2)	27 (45.0)	9 (37.5)	9 (42.9)
Female factor (%)	23 (33.3)	24 (40.0)	10 (41.7)	7 (33.3)
Multiple factor (%)	7 (10.1)	3 (5.0)	2 (8.3)	1 (4.8)
Unexplained (%)	14 (20.3)	6 (10.0)	3 (12.5)	4 (19.0)

Assessment of clinical outcomes under different oxygen tension and female age groups

Our embryo transfer policy was that high-quality embryos were transferred as much as possible, but a maximum of 4, and performed on day 3. After embryo transfer, the clinical outcomes including pregnancy, implantation, and abortion rate were assessed between two groups. Moreover, the patients were further stratified for their age into old (≥38 years) and young (<38 years) age subgroups, resulting in a four-group allocation table (≥38 year with 20% or 5% O_2. and <38 year with 20% or 5% O_2), and comparison of outcomes between these groups was performed.

Statistical analysis

The various biological parameters germane to IVF/ICSI cycles of the data were compared by Student's t-test, Fisher's exact test, or chi-square test as the condition determined. All analyses were performed using the Statistical Package for the Social Sciences (version 14.0; SPSS Inc., Chicago, IL). A confidence level of P<0.01 or P<0.05 was considered to constitute the statistical significance limit for comparison.

Results

In total, one hundred seventy-four cycles were performed in this study. There were 93 cases in the 5% O_2 group and 81 cases in the 20% O_2 group, and 1075 and 722 mature oocytes retrieved respectively. **Table 1** showed the baseline characteristics of patients who completed the study. No significant differences were observed in mean age of the patients, type of infertility between the two treatment groups. The outcomes of embryos cultured in 5% and 20% O_2 tensions were summarized in **Table 2**.

Low oxygen (5% O_2) system improved embryo quality

After insemination/ICSI, the fertilization rates between the 5% (72.8%, 783/1075) and 20% O_2 (69.8%, 504/722) groups were no significant difference. The early cleavage rate at 2-cell stage (ECR) on 5% O_2 group (58.7%, 449/765) was significantly lower than that on 20% O_2 group (66.5%, 323/486; P<0.01), however, the rates of good early cleavage (GEC)

Table 2. Outcomes of embryos cultured in 5% and 20% O_2 tensions

	5% O_2	20% O_2
Cycles	93	81
Average age (years)	35.3±0.13	35.0±0.08
Oocytes retrieved		
Total No.	1314	886
No. of MII	1075	722
Fertilization rate, n/N (%)	783/1075 (72.8)	504/722 (69.8)
Rate of cleavage embryos, n/N (%)	765/783 (97.7)	486/504 (96.4)
Embryo development		
Rate of early cleavage embryos, n/N (%)	449/765 (58.7)	323/486 (66.5[a])
Ratio of GEC/EC embryos, n/N (%)	377/449 (84.0)	264/323 (81.7)
Rate of day 3 top embryo /EC, n/N (%)	293/449 (65.3)	176/323 (54.5[b])
Transferred embryos		
Total No.	358	302
Implantation rate, n/N (%)	95/358 (26.5)	53/302 (17.5[c])
Pregnancy rate, n/N (%)	48/93 (51.6)	31/81 (38.3)
Abortion rate, n/N (%)	5/48 (10.4)	5/31 (16.1)
Live birth rate, n/N (%)	42/93 (45.2)	26/81 (32.1[*])

a, compared with 5% O_2 group, P = 0.0058; b, compared with 5% O_2 group, P = 0.0025; c, compared with 5% O_2 group, P = 0.0057; *, compared with 5% O_2 group, P = 0.0773. Early cleavage (EC) embryo: embryo possessed two cells at 25-27 h post insemination or ICSI. Good early cleavage (GEC) embryo: 2 equal sized blastomeres and less than <10% fragmentation. Day 3 top embryo: embryo with good early cleavage developed to day 3 good embryo.

embryo between 5% and 20% O_2 were similar (49.3% and 54.3%).

In 5% O_2 group, there was a total of 366 good embryos on day 3, and 80.1% (293 embryos) of them were derived from GEC. That is 77.7% of GEC embryos developed to top day 3 good embryos. In contrast, in 20% O_2 group, there were a total of 211 day 3 good embryos, and 83.4% (176 embryos) of them derived from GEC. That is 66.7% of GEC embryos developed to top day 3 good embryos. The rates of total day 3 good embryos between 5% and 20% O_2 groups were no significantly difference (47.8% vs. 43.4%). Following good early cleavage, the top day embryo rate in 5% O_2 tension was significantly higher than in 20% O_2 tension (77.7% and 66.7% respectively, P<0.01).

The embryo transfer was performed on day 3, and there were 358 (mean = 3.85) vs. 302 (mean = 3.73) embryos transferred to patients in 5% and 20% O_2 groups, respectively. The mean of embryo transfer number was no significant difference. A higher pregnancy rate but no significant difference was shown in 5% O_2 group than that in 20% O_2 group (51.6% and 38.3%, P = 0.064). However, the implantation rate in 5% O_2 group was significantly higher than in 20% O_2 group (26.5% and 17.5%, P<0.05). The abortion rate between two groups was no significant difference. The live birth rate in 5% O_2 was higher than 20% O_2 group but no significant difference (45.2% and 32.1, P = 0.0773).

Low oxygen (5% O_2) system improved embryo quality in young age women but not in old age women

The age is an important factor for embryo quality and all patients were further divided into young (<38 years of age, n = 129) and old (≥38 years of age, n = 45) age groups. There were 69 and 60 young age women underwent 5% and 20% O_2 culture protocols. In the meanwhile, there were 24 and 21 old age women underwent 5% and 20% O_2 culture protocols. The outcomes of embryos cultured in different oxygen tensions between two age groups were summarized in **Table 3**.

In young age group, the fertilization rate, total day 3 good embryo rates were not different between 5% and 20% O_2 groups. The rate of early cleavage in 5% O_2 group was significantly lower than 20% O_2 group (60.1% and 68.3%, respectively, P<0.05), but the rates of GEC embryos between two groups were similar (50.0% and 55.5%). The rate of top day 3 embryo in 5% O_2 group was significantly higher than 20% O_2 group (79.1% and 66.8%, P<0.05). Furthermore, the pregnancy and implantation rate in 5% O_2 group (58.0% and 30.7%) were also significantly higher than 20% O_2 group (40.0% and 18.8%, P<0.05). The live birth rate in 5% O_2 was higher than 20% O_2 group but no significantly different (49.3% and 33.3, P = 0.0662).

Table 3. Outcomes of embryos cultured in different oxygen tensions between young (age <38) and old age women (age ≥38)

	5% O_2 (<38)	20% O_2 (<38)	5% O_2 (≥38)	20% O_2 (≥38)
Cycles	69	60	24	21
Average age (years)	33.4±0.07	33.3±0.07	40.8±0.12	39.9±0.09
Oocytes retrieved				
Total No.	1102	721	212	165
No. of MII	899	593	176	129
Fertilization rate, n/N (%)	658/899 (73.9)	423/593 (71.3)	125/176 (71.0)	81/129 (62.8)
Rate of cleavage embryos, n/N (%)	642/658 (98.6)	407/423 (96.2)	123/125 (98.4)	79/81 (97.5)
Cleavage embryos				
Rate of early cleavage embryos, n/N (%)	386/642 (60.1)	278/407 (68.3[a])	63/123 (51.2)	45/79 (57.0)
Ratio of GEC/EC embryos, n/N (%)	321/386 (83.2)	226/278 (81.3)	56/63 (88.9)	38/45 (84.4)
Rate of top day 3 embryo/EC, n/N (%)	254/386 (65.8)	151/278 (54.3[b])	39/63 (61.9)	25/45 (55.6)
Transferred embryos				
Total No.	270	237	88	65
Implantation rate, n/N (%)	83/270 (30.7)	45/237 (18.8[c])	12/88 (13.6)	8/65 (12.3)
Pregnancy rate, n/N (%)	40/69 (58.0)	24/60 (40.0[d])	8/24 (33.3)	7/21 (33.3)
Abortion rate, n/N (%)	5/40 (12.5)	4/24 (20.0)	0	1/7 (14.3)
Live birth rate, n/N (%)	34/69 (49.3)	20/60 (33.3*)	8/24 (33.3)	6/21 (28.6)

a, compared with 5% O_2 (age <38) group, P = 0.0073; b, compared with 5% O_2 (age <38) group, P = 0.0027; c, compared with 5% O_2 (age <38) group, P = 0.0021; d, compared with 5% O_2 (age <38) group, P = 0.0414; *, compared with 5% O_2 (age <38) group, P = 0.0662. Early cleavage (EC) embryo: embryo possessed two cells at 25-27 h post insemination or ICSI. Good early cleavage (GEC) embryo: 2 equal sized blastomeres and less than <10% fragmentation. Day 3 top embryo: embryo with good early cleavage developed to day 3 good embryo.

However, in old age women, the embryo quality and outcomes between 5% and 20% O_2 groups were not significantly different. The early cleavage rate in 20% O_2 group showed a slightly higher than 5% O_2 group but there was no significant difference. The pregnancy, implantation and live birth rates between 5% O_2 and 20% O_2 in old age group were no significant difference.

Discussion

It had been reported that significant impact was not shown on day 3 embryo development under 5% O_2 condition [11, 23]. Our results (**Table 2**) were similar to previous study that 5% O_2 failed to improve embryo quality on day 3. The total day 3 good embryo rate between 5% and 20% O_2 groups was also no significant difference in this study. However, if we traced the development of more "effective" embryos which showed early cleavage at 2-cell stage, the results of embryo development and quality between two oxygen tensions were different.

Several studies have suggested that the appearance of a 2-cell embryo at 25-27 post insemination/ICSI showed better quality of embryos with higher implantation potential [24-26] and our data also showed that most day 3 good embryos developed from early cleavage at 2-cell stage. Our data showed similar "total good embryo rates" on day 3 stages between two groups (47.8% vs. 43.4%), even though the early cleavage rate (ECR) in 20% O_2 tension (66.5%) was significantly higher than that in 5% O_2 tension (58.7%). Our results suggested that embryos under 20% O_2 showed higher potential to entry the first division of cell cycle with faster kinetics of cleavage, but a part of them showed poor development with higher fragmentation after cleavage; on the contrary, 5% O_2 tension contributed a higher potential of embryo quality but expressed a lower percentage of the first division than 20% O_2.

It is well known that the role of oxygen in embryonic metabolism and development is crucial and represents a balance between beneficial and harmful effect. Oxygen is consumed in oxidative phosphorylation and free radicals are generated from 'leakage' of high-energy electrons as they proceed down the electron transport chain [27]. Hence, ROS generated under

hyperoxic conditions, will cause cell injury and death [28].

Several studies also indicate that early human embryos which develop in high oxygen culture conditions undergo cytoplasmic fragmentation due to the elevation of ROS and eventually undergo apoptosis [29]. Moreover, the high incidence of apoptotic bodies in fragmented human embryos strongly suggest that programmed cell death is triggered at a stage prior to blastocyst formation [30], and embryos exhibiting a severe degree of fragmentation were shown to display a significant increase in mRNA associated with apoptosis [31]. Therefore, higher early cleavage rate, but poor ratios of GEC/EC embryos are observed in higher O_2 tension groups.

We found that embryos with early cleavage, but not all, have the higher potential for better development, in this way, early cleavage only is not an appropriate and efficient indicator for embryo quality assessment between 5% and 20% O_2 tension. Ciray et al. in 2006 [32] reported that the morphology of early cleavage correlates to day 3 embryo quality and implantation, and the fragmented cleavage at 2-cell stage should not be considered as early cleavage for good quality prediction of embryo.

In the present study, after assessing the morphologic characteristics of early cleavage, we further defined the "good early cleavage (GEC)", which contained 2 equal sized blastomeres and <10% fragmentation, accounted for 83%, and there were about 17% embryos of early cleavage, which had >2 or ≥2 unequal sized blastomeres with >10% fragmentation, considered as poor early cleavage, and in the meanwhile the GEC rates or ratios of GEC/EC embryos are similar between 5% and 20% O_2 tensions in 2-cell stage. Moreover, most of GEC embryos developed to top embryos on day 3 (67-78%, **Table 2**), but the early cleavage embryos developed to day 3 top embryos was only 56-66% (data not showed). In addition, the significant improvement in the top day 3 embryos and implantation rates was observed, although the pregnancy rates were comparable between two groups.

Fragmentation was significantly lower in the embryos cultured at 5% O_2 as compared with those embryos cultured at 20% O_2. The results of our analysis indicated that the GEC was a more "effective" and "functional" indicator for embryo quality rather than early cleavage only, and they contributed the primary good embryos for embryo transfer (5% O_2: 293/358, 81.8%; 20%: 176/302, 58.2%). Consequently, the reduced oxygen tension really increased embryo quality but the effect was significantly limited in GEC embryos with a better potential to develop more effectively on day 3 stage. Despite the differences between 5% and 20% O_2 in the day 2 embryos could not appear in this study, we thought the deleterious effect of 20% O_2 tension on embryo development may be initiated before or begin from 2-cell stage.

It is well established that oxidative stress influences the spermatozoa, oocytes, thus the fertilization rates, embryos and their environment. Many animal and human studies have elucidated a role for ROS in oocyte development, maturation, follicular atresia, corpus luteum function and luteolysis. ROS appears to play a significant role in the modulation of gamete interaction and also for successful fertilization to take place. ROS in culture media may impact post-fertilization development, i.e. cleavage rate, blastocyst yield and quality (1, 2). Catt et al. in 2000 [27] revealed that there were three methods of potentially minimizing oxidative damage to human embryos were tested using gametes, zygotes, and embryos from a clinical IVF program: (i) decreasing the oxygen tension in the gas phase used for culture during insemination, fertilization, and embryo growth; (ii) changing the formulation of culture media to include some components designed to protect against oxidative damage; and (iii) reducing the duration of insemination to minimize the effect of oxidative damage caused by spermatozoal metabolism. Although all three methods gave an increase in success rates, there was still a dramatic decrease in success with patient age. They suggest that, although the system of handling and culturing embryos can be optimized, there are inherent age-related defects in oocytes and embryos that are still more fundamental than the environmental conditions of the embryo.

Our patients were further stratified into age subgroups (**Table 3**); we analyzed which subgroups of patients most benefited from culture at a lower oxygen tension. In young age women, we noted that higher rate of top day 3 embryos

transferred in 5% O_2 group rather than 20% O_2 was responsible for the significantly higher pregnancy and implantation rates. By contrast, there was no significant difference of outcomes between 5% and 20% O_2 groups in old age women. Our results suggest that reduced oxygen tension may affect the GEC embryo development by different mechanism in young versus old age women, and the balance of redox (pro-oxidant and antioxidant) may play one of important factors to affect the outcome of in vitro culture.

Tarín et al., in 2000 [19] showed that the aging of oocytes had deleterious effects on embryo implantation. The decline of follicle quality with age is reflected by ultra structural changes, which involve the mitochondria, the SER, and the Golgi complex, and suggest a role for oxidative damage to oocyte and granulosa cell structures [33]. It was reported that oocytes from older women (≥38 years of age) were 3.3 folds more to contain the 5 kb mtDNA deletion than oocytes from younger women [34] and the mtDNA deletion may be a potential indicator of failure rate consistent with aging [34]. Annimal and human studies [36-38] showed that genes expressed differentially in aged oocytes or granulosa cells, included oxidative stress genes, encoding for the superoxide dismutases, Cu/ZnSOD (SOD1), MnSOD (SOD2) and catalase.

In addition, oocytes may also be damaged by those produced in the follicular microenvironment. It was reported that a change in the antioxidant enzymatic pattern, impairing ROS scavenging efficiency had been found in the follicular environment in advanced reproductive age [39]. Moreover, ROS enhanced ZP hardening, ooplasmic microtubule dynamics, cortical granule loss [40] and decreased fertilization [41] in old but not young oocytes, and the antioxidant mechanisms controlling the production or metabolism of ROS could be compromised in older oocytes [40].

In present study, young age women had higher rate of top day 3 embryos under 5% O_2 tension, thus oxidative stress may be suppressed by a reducing oxygen tension in these women, whose oocytes and embryos contain higher or enough antioxidants and potency of redox balance. Whereas, if under high oxidative stress, which induced by higher oxygen tension in vitro, lower percentage of embryos developed to good quality following antioxidants depletion. On the other hand, it was not affected in old age women who may have poor quality of aging oocytes with lower total antioxidants, mtDNA deletion, mutation or genes expressed differentially, which induce unbalance of redox in cell biology, even they were under a lower oxidative stress condition.

In conclusion, our study finds that the age of women limits the improvable effect on embryo quality and pregnancy potential of human embryo at reduced oxygen concentrations. Embryos from young age women should be cultured in 5% O_2 tension rather than 20% O_2 to increase good embryo, pregnancy and implantation rates. Whether under 5% or 20% O_2 tension, the old age women may contain more poor oocytes, which have less antioxidants or imbalance of redox, leading to poor embryo development, and further fail to implant. However, this should be tested in a large randomized control study.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Lee-Feng Chien, Department of Life Sciences, College of Life Sciences, National Chung Hsing University, 402, Kuo Kuang Road, Taichung, Taiwan. Tel: 886-4-22347057; Fax: 886-4-22384602; E-mail: msleephd@gmail.com

References

[1] Agarwal A, Gupta S, Sharma RK. Role of oxidative stress in female reproduction. Reprod Biol Endocrinol 2005; 14: 3-28.

[2] Agarwal A, Gupta S, Sharma R. Oxidative stress and its implications in female infertility-a clinician's perspective. Reprod Biomed Online 2005; 11: 641-650.

[3] Leese HJ. Metabolism of the preimplantation mammalian embryo. Oxf Rev Reprod Biol 1991; 13: 35-72.

[4] Goto Y, Noda Y, Mori T, Nakano M. Increased generation of reactive oxygen species in embryos cultured in vitro. Free Radic Biol Med 1993; 15: 69-75.

[5] Orsi NM, Leese HJ. Protection against reactive oxygen species during mouse preimplantation embryo development: role of EDTA, oxygen tension, catalase, superoxide dismutase and pyruvate. Mol Reprod Dev 2001; 59: 44-53.

[6] Bavister BD. Culture of preimplantation embryos: facts and artifacts. Hum Reprod Update 1995; 1: 91-148.
[7] Gardner DK, Lane M. Alleviation of the '2-cell block' and development to the blastocyst of CF1 mouse embryos: role of amino acids, EDTA and physical parameters. Hum Reprod 1996; 11: 2703-2712.
[8] Kishi J, Noda Y, Narimoto K, Umaoka Y, Mori T. Block to development in cultured rat 1-cell embryos is overcome using medium HECM-1. Hum Reprod 1991; 6: 1445-1448.
[9] Berthelot F, Terqui M. Effects of oxygen, CO2/pH and medium on the in vitro development of individually cultured porcine one- and two-cell embryos. Reprod Nutr 1996; 36: 241-251.
[10] Nasr-Esfahani MH, Winston NJ, Johnson MH. Effects of glucose, glutamine, ethylenediaminetetraacetic acid and oxygen tension on the concentration of reactive oxygen species and on development of the mouse preimplantation embryo in vitro. J Reprod Fertil 1992; 96: 219-231.
[11] Dumoulin JC, Meijers CJ, Bras M, Coonen E, Geraedts JP, Evers JL. Effect of oxygen concentration on human in-vitro fertilization and embryo culture. Hum Reprod 1999; 14: 465-469.
[12] Adam AA, Takahashi Y, Katagiri S, Nagano M. Effects of oxygen tension in the gas atmosphere during in vitro maturation, in vitro fertilization and in vitro culture on the efficiency of in vitro production of mouse embryos. Jpn J Vet Res 2004; 52: 77-84.
[13] Volarcik K, Sheean L, Goldfarb J, Woods L, Abdul-Karim FW, Hunt P. The meiotic competence of in-vitro matured human oocytes is influenced by donor age: evidence that folliculogenesis is compromised in the reproductively aged ovary. Hum Reprod 1998; 13: 154-160.
[14] Faddy MJ, Gosden RG, Gougeon A, Richardson SJ, Nelson JF. Accelerated disappearance of ovarian follicles in mid-life: implications for forecasting menopause. Hum Reprod 1992; 7: 1342-1346.
[15] Faddy MJ, Gosden RG. A mathematical model of follicle dynamics in the human ovary. Hum Reprod 1995; 10: 770-775.
[16] Harman D. Aging: a theory based on free radical and radiation chemistry. J Gerontol 1956; 11: 298-300.
[17] Tarín JJ. Aetiology of age-associated aneuploidy: a mechanism based on the 'free radical theory of ageing'. Hum Reprod 1995; 10: 1563-1565.
[18] Tarín JJ. Potential effects of age-associated oxidative stress on mammalian oocytes/embryos. Mol Hum Reprod 1996; 2: 717-724.
[19] Tarín JJ, Pérez-Albalá S, Cano A. Consequences on offspring of abnormal function in ageing gametes. Hum Reprod Update 2000; 6: 532-549.
[20] Lee TH, Liu CH, Huang CC, Wu YL, Shih YT, Ho HN, Yang YS, and Lee MS. Serum anti-müllerian hormone and estradiol levels as predictors of ovarian hyperstimulation syndrome in assisted reproduction technology cycles Hum. Reprod 2008; 23: 160-167.
[21] Shoukir Y, Campana A, Farley T, Sakkas D. Early cleavage of in-vitro fertilized human embryos to the 2-cell stage: a novel indicator of embryo quality and viability. Hum Reprod 1997; 12: 1531-1536.
[22] Van Royen E, Mangelschots K, De Neubourg D, Valkenburg M, Van de MM, Ryckaert G, Eestermans W and Gerris J. Characterization of a top quality embryo, a step towards single-embryo transfer. Hum Reprod 1999; 14: 2345-2349.
[23] Dumoulin J, Vanvuchelen R, Land J, Pieters MH, Geraedts JP, Evers JL. Effect of oxygen concentration on in-vitro fertilization and embryo culture in the human and the mouse. Fertil Steril 1995; 63: 115-119.
[24] Sakkas D, Shoukir Y, Chardonnens D, Bianchi PG, Campana A. Early cleavage of human embryos to the two-cell stage after intracytoplasmic sperm injection as an indicator of embryo viability. Hum Reprod 1998; 13: 182-187.
[25] Salumets A, Hydén-Granskog C, Mäkinen S, Suikkari AM, Tiitinen A, Tuuri T. Early cleavage predicts the viability of human embryos in elective single embryo transfer procedures. Hum Reprod 2003; 18: 821-825.
[26] Lundin K, Bergh C, Hardarson T. Early embryo cleavage is a strong indicator of embryo quality in human IVF. Hum Reprod 2001; 16: 2652-2657.
[27] Catt JW, Henman M. Toxic effects of oxygen on human embryo development. Hum Reprod 2000; 15 Suppl 2: 199-206.
[28] Li J, Gao X, Qian M, Eaton JW. Mitochondrial metabolism underlies hyperoxic cell damage. Free Radic Biol Med 2004; 36: 1460-1470.
[29] Yang HW, Hwang KJ, Kwon HC, Kim HS, Choi KW, Oh KS. Detection of reactive oxygen species (ROS) and apoptosis in human fragmented embryos. Hum Reprod 1998; 13: 998-1002.
[30] Jurisicova A, Varmuza S, Casper RF. Programmed cell death and human embryo fragmentation. Mol Hum Reprod 1996; 2: 93-98.
[31] Jurisicova A, Antenos M, Varmuza S, Tilly JL, Casper RF. Expression of apoptosis-related genes during human preimplantation embryo development: potential roles for the Harakiri gene product and Caspase-3 in blastomere fragmentation. Mol Hum Reprod 2003; 9: 133-141.

[32] Ciray HN, Karagenç L, Ulug U, Bener F, Bahçeci M. Early cleavage morphology affects the quality and implantation potential of day 3 embryos. Fertil Steril 2006; 85: 358-365.
[33] de Bruin JP, Dorland M, Spek ER, Posthuma G, van Haaften M, Looman CW, te Velde ER. Age-related changes in the ultrastructure of the resting follicle pool in human ovaries. Biol Reprod 2004; 70: 419-424.
[34] Keefe DL, Niven-Fairchild T, Powell S, Buradagunta S. Mitochondrial deoxyribonucleic acid deletions in oocytes and reproductive aging in women. Fertil Steril 1995; 64: 577-583.
[35] Yesodi V, Yaron Y, Lessing JB, Amit A, Ben-Yosef D. The mitochondrial DNA mutation (deltamtDNA5286) in human oocytes: correlation with age and IVF outcome. J Assist Reprod Genet 2002; 19: 60-66.
[36] Hamatani T, Falco G, Carter MG, Akutsu H, Stagg CA, Sharov AA, Dudekula DB, VanBuren V, Ko MS. Age-associated alteration of gene expression patterns in mouse oocytes. Hum Mol Genet 2004; 13: 2263-2278.
[37] Tatone C, Carbone MC, Falone S, Aimola P, Giardinelli A, Caserta D, Marci R, Pandolfi A, Ragnelli AM, Amicarelli F. Age-dependent changes in the expression of superoxide dismutases and catalase are associated with ultrastructural modifications in human granulosa cells. Mol Hum Reprod 2006; 12: 655-660.
[38] Steuerwald NM, Bermúdez MG, Wells D, Munné S, Cohen J. Maternal age-related differential global expression profiles observed in human oocytes. Reprod Biomed Online 2007; 14: 700-708.
[39] Carbone MC, Tatone C, Delle Monache S, Marci R, Caserta D, Colonna R, Amicarelli F. Antioxidant enzymatic defenses in human follicular fluid: characterization and age dependent changes. Mol Hum Reprod 2003; 9: 639-643.
[40] Goud PT, Goud AP, Abu-Soud HM and Diamond MP. Role of NADPH and hydrogen peroxide in modulating oxidative stress and oocyte aging. Fertil Steril 2004; 82: S111-S111.
[41] Takahashi T, Takahashi E, Igarashi H, Tezuka N and Kurachi H. Impact of oxidative stress in aged mouse oocytes on calcium oscillations at fertilization. Mol Reprod Dev 2003; 66: 143-152.