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Original Article

Impact of oxygen concentrations on fertilization, cleavage, implantation, and pregnancy rates of in vitro generated human embryos

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Abstract: The aim of the present study was to determine the impact of oxygen concentration during in vitro culture of human oocytes and embryos on fertilization, cleavage, implantation, pregnancy, multiple gestation and abortion rates. Women 20-48 years old presenting for infertility treatment and accounting for 3484 in vitro fertilization/intracytoplasmic sperm injection cycles were included in the study. Oocytes/embryos were randomly allocated to be incubated under three different oxygen tension environments: (1) 20% O_2 in air; (2) initially 20% O_2 in air, followed on day 2 (2-4 cells stage) by 5% CO_2, 5% O_2 and 90% N_2; and (3) 5% CO_2, 5% O_2 and 90% N_2 throughout. Interestingly, IVF-derived embryos cultured in 5% O_2 yielded higher rates of fertilization and implantation as compared to those incubated in 20% O_2 ($P < 0.05$). Conversely, embryos in 20% O_2 yielded higher rates of fertilization, high quality embryo and implantation than those in the 20%-5% O_2 group ($P < 0.05$). Moreover, ICSI-derived embryos cultured in 20% O_2 resulted in lower rates of cleavage as compared to those from the 20%-5% O_2 group ($P < 0.05$). These results are consistent with in vitro and subsequent in vivo embryo development being more susceptible to O_2 tension fluctuations rather than the degree of O_2 tension itself during culture.

Keywords: In vitro embryo culture, IVF, ICSI, oxygen tension

Introduction

In vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) represent more than 99 percent of all assisted reproductive technology (ART) procedures worldwide, and account for about 48,000 babies born in the United States each year alone [1]. Therefore, optimizing the success rates of these techniques is an important objective of current reproductive medicine research. Given the fact that for IVF/ICSI human embryos are exposed to an in vitro environment for several days prior to transfer into the maternal uterus, optimizing the culture conditions is a main area of research focus [2, 3].

Despite dramatic advances in the in vitro culture conditions that support development of human embryos, in vitro embryo development rates, in particular to preimplantation stages, are still suboptimal. While the genetic makeup of the oocyte weighs heavily on the success rates of ART procedures, one potential factor worthy of consideration is that of damage by free oxygen radicals which relates to the atmospheric oxygen (O_2) tension conditions to which the embryos are exposed during in vitro culture [2-6]. Interestingly, while under physiological in vivo conditions of the human uterus the oxygen tension ranges between 2-8% [7-9], for in vitro culture human embryos are typically placed in incubators with a 5% CO_2 and 20% O_2 atmosphere in the vast majority of assisted reproduction laboratories. Hence these conditions may compromise embryo viability by enhancing the generation of reactive oxygen species (ROS) [7-9] and negatively impacting on the outcome of assisted reproductive techniques. Therefore, to more closely mimic conditions in the natural maternal environment, some laboratories have started to culture embryos at a lower oxygen tension (~5%), despite the higher costs associ-

ated [10, 11]. However, the evidence for an effect of low oxygen concentration on IVF/ICSI success rates remains controversial, with some studies reporting improved pregnancy rates while others report no differences [12-15].

The stage of embryo transfer yielded higher pregnancy rates is also a controversial issue. For instance, some studies have suggested that cleavage stage (day 3) embryo transfers may further stress already compromised embryos and reduce their implantation potential regardless of oxygen concentration during in vitro culture [16]. Since no improvements in terms of pregnancy and implantation rates were observed with day 3 embryo transfers [17] it has been proposed that blastocyst-stage transfers result in higher pregnancy rates [18].

Therefore, in this study we compared the effects of culturing human embryos in three different atmospheric oxygen tension conditions upon the clinical outcomes of IVF or ICSI cycles when performing day-3 embryo transfers.

Materials and methods

Patients

Women with ages ranging 20-48 seeking treatment for infertility at the Center of Reproduction in the First Affiliated Hospital of Zhengzhou University between January 2010 and February 2012 were enrolled in this study. Protocols were approved by the Biomedical Ethical Committee at the same hospital and informed consent was signed by all patients. The study comprised 3484 IVF and ICSI cycles that were randomly allocated in groups differing upon the incubation conditions of the corresponding gametes and embryos from the time of OPU until transfer on day 3 of culture, as follows: (1) gametes and embryos incubated throughout in a 5% CO_2 in air (20% O_2) atmosphere (1131 cycles); (2) gametes and embryos initially cultured in a 5% CO_2 in air (20% O_2) atmosphere, with day 2 cleavage-stage embryos transferred to a 5% CO_2, 5% O_2 and 90% N_2 atmosphere until day 3 of culture (1258 cycles); (3) gametes and embryos cultured in a5% CO_2, 5% O_2 and 90% N_2 atmosphere throughout (1095 cycles). The SANYO (MCO-5AC, Tokyo, Japan) and COOK (K-MINC-1000, Indiana, USA) incubators were used for the bi-gas and tri-gas systems, respectively. All incubations were performed at 37°C.

Ovarian stimulation and oocyte collection

Ovarian stimulations were carried out with recombinant follicle-stimulating hormone/human menopausal gonadotropin (FSH/HMG) after pituitary down-regulation with GnRH agonist. Pituitary suppression was achieved with the administration of daily 0.1 mg triptorelin (Decapeptyl; Ferring Pharma ceuticals, Kiel, Germany) beginning on the mid-luteal phase of the cycle preceding the treatment cycle. Once ovarian suppression was achieved, the dose of Decapeptyl was reduced to 0.05 mg until the day of hCG administration. When the absence of a dominant follicle was confirmed sonographically and by hormone level analysis (E_2 < 30 mIU/mL, LH < 3 mIU/mL), recombinant FSH (Gonal-F, Serono, Switzerl) administration was initiated at daily dosages ranging from 150 to 300 IU or in combination with hMG (hMG, Lizhu Company, Guangdong, China) according to established protocols.

Follicular growth was monitored by ultrasound. Ovulation was induced with human chorionic gonadotropin (hCG; 10000 IU, Lizhu Company) after the average diameter of dominant follicles reached 18 mm. Follicular aspiration and fertilization procedures were performed 34-36 hours and 39-40 hours following hCG, respectively.

Fertilization

IVF: Semen samples were obtained in aseptic conditions. Following incubation at 37°C for 15 min to allow for liquefaction of the sample, motile spermatozoa were recovered from seminal plasma through 45% and 90% gradient centrifugation (SpermGrad, Vitrolife, Goteborg, Sweden) at 350 g for 15 min following the manufacturer's instructions. Recovered spermatozoa were washed (G-IVF, Vitrolife, Goteborg, Sweden) and evaluated. Approximately 15,000-20,000 sperm were suspended in a 50-μl drop of G-IVF under mineral oil (Vitrolife, Goteborg, Sweden). One oocyte was placed in the drop. Sperm and oocytes were co-cultured under the corresponding incubation conditions for 4-6 hours. Following presumed fertilization, oocytes were partially denuded from the cumulus cell mass using a micro-pipette pulled from a Pasteur pipette, and then washed 2-3 times in fresh G-1™ medium (Vitrolife, Goteborg, Sweden) medium. Then oocytes were placed indi-

Table 1. Patient demographics, gonadotropin dosages, number of oocytes retrieved and endometrial thickness at the time of oocyte retrieval in the three treatment groups included in this study

	20%*	20%-5%	5%	*P* value
# of Cycles	1131	1258	1095	
Age (Years)	31.36 ± 4.96	31.38 ± 4.87	31.29 ± 4.45	> 0.05
Infertility duration (Years)	4.79 ± 4.02	4.69 ± 4.31	4.76 ± 4.15	> 0.05
BMI (kg/m^2)	21.82 ± 2.42	21.63 ± 2.34	21.79 ± 2.00	> 0.05
Initial dose of Gn[a] (bolus)	2.55 ± 0.42	2.52 ± 0.44	2.53 ± 0.41	> 0.05
Total dose of Gn (bolus)	33.45 ± 13.61	34.01 ± 13.48	33.76 ± 3.77	> 0.05
Oocytes retrieved	11.56 ± 6.78	11.88 ± 6.54	11.72 ± 5.99	> 0.05
Endometrial thickness (mm)	9.86 ± 3.44	10.02 ± 3.02	9.91 ± 3.59	> 0.05
Number of embryos transferred	2.10 ± 0.19	2.10 ± 0.15	2.11 ± 0.21	> 0.05

*20% = gametes and embryos incubated throughout in a 5% CO_2 in air (20% O_2) atmosphere; 20%-5% = same for gametes and zygotes but day embryos transferred to a 5% CO_2, 5% O_2 and 90% N_2 atmosphere; 5% gametes and embryos cultured in a 5% CO_2, 5% O_2 and 90% N_2 atmosphere. There were no differences among treatment groups (P > 0.05). [a]Gn = gonadotropin.

vidually in fresh G-1 medium culture droplets for the preliminary observation of second polar body release. If second polar body release rate was less than 60%, oocytes were cultured further and observed for up to 1-2 more hours. If the release of the second polar body had not occurred by then, rescue insemination via ICSI was performed on any unfertilized oocytes.

ICSI: The ICSI procedure was used for patients with sperm density lower than 0.2 × 10^6/ml. The corona-cumulus complex was removed from oocytes at around 38 hours following hCG injection. Then, oocytes were placed in culture at 37°C in a 5% CO_2 in air (20% O_2) atmosphere for 1-2 hours prior to ICSI insemination. ICSI was performed in a micro-injection dish prepared with 5-μL droplets of buffered medium (MPOS, Vitrolife, Goteborg, Sweden) and covered with mineral oil (Vitrolife) on a heated stage at 37 ± 0.5°C using Eppendorf (AG 2331 Hamburg) micromanipulators and an Nikon (ECLIPSE Yi-U) inverted microscope.

Embryo culture: Fertilization rates were evaluated 16-18 hours following IVF or ICSI, as assessed by the presence of two pronuclei and two polar bodies, with the day of OPU and fertilization being set as Day 0. Zygotes with two pronuclei were cultured individually in G-1 medium under the incubation conditions corresponding to the randomly allocated treatment. In the morning of day 1, at 26-28 hours following fertilization, embryos were moved to a fresh culture G-1 medium droplet and evaluated for the presence and appearance of 2 pronuclei (PN). On days 2 and 3, embryos were evaluated for cleavage and graded, and their suitability for transfer and cryopreservation was determined.

Day 3 embryo grading

Grading of embryos was performed as follows according to previously published studies [19]. Grade I embryos were those with uniform and symmetrical blastomeres, and displaying a moderate refraction and intact zona pellucida. Grade II embryos were either characterized by slightly irregular in shape or uneven blastomeres, with a slight change in refraction, but with less than 10% debris; or, by displaying asynchronous blastomere cleavage yielding 3, 5, 6 or 7-cell embryos; all embryos in this category had an intact zona pellucida. Grade III embryos contained less than 50% debris, with the blastomeres showing an appearance similar to that described in Grade II embryos, displaying some refraction, and an intact zona pellucida. Grade IV embryos contained greater than 50% fragments, with viable blastomere outside the fragments; Grade V embryos were at the 2 PN stage on Day 2, or due to late or rescue fertilization were still on Day 1; Grade VI embryos were non-viable with dissolved or shrunk blastomeres. Grade I and Grade II were labeled as high quality embryos.

Embryo transfer and pregnancy diagnosis

Superior embryos were transferred at Day 3 cleavage (6-10 cells) stage according to standardized clinical procedures. The embryos that were not transferred were cryopreserved or discarded, depending upon their morphology. Biochemical pregnancy was diagnosed 14 to 18 days following embryo transfer by measuring serum concentrations of human chorionic gonadotropin beta subunit (hCG-b). Clinical

Table 2. Fertilization, cleavage, high quality embryo, implantation, pregnancy, multiple gestation and abortion rates in IVF cycles with oocytes and embryos cultured in different O_2 tension atmospheres

	20%*	20%-5%	5%
Fertilization rate (%)	80.06 (7628/9528)[a]	85.13 (8323/9777)	84.22 (8447/10030)[c]
Cleavage rate (%)	96.01 (7324/7628)	97.57 (8121/8323)	97.30 (8219/8447)
High quality embryo rate (%)	76.66 (4697/6127)[a]	71.0 (5031/7086)[b]	76.14 (5261/6910)
Implantation rate (%)	82.11 (5031/6127)[a]	79.49 (5633/7086)[b]	85.31 (5895/6910)[c]
Pregnancy rate (%)	47.63 (371/779)	46.73 (407/871)[b]	52.18 (371/711)
Multiple gestation rate (%)	19.90 (155/779)	21.93 (191/871)	20.96 (149/711)
Abortion rate (%)	3.59 (28/779)	4.02 (35/871)	3.80 (27/711)

*20% = gametes and embryos incubated throughout in a 5% CO_2 in air (20% O_2) atmosphere; 20%-5% = same for gametes and zygotes but day embryos transferred to a 5% CO_2, 5% O_2 and 90% N_2 atmosphere; 5% gametes and embryos cultured in a 5% CO_2, 5% O_2 and 90% N_2 atmosphere. [a]Significant differences between 20% and 20%-5% oxygen groups ($P < 0.05$); [b]Significant differences between 20%-5% and 5% oxygen groups ($P < 0.05$); [c]Significant differences between 5% and 20% oxygen groups ($P < 0.05$).

Table 3. Fertilization, cleavage, high quality embryo, implantation, pregnancy, multiple gestation and abortion rates in ICSI cycles with oocytes and embryos cultured in O_2 tension atmospheres

	20%	20%-5%	5%
Fertilization rate (%)	77.03 (3212/4170)	76.69 (3036/3959)	78.63 (3903/4989)
Cleavage rate (%)	96.23 (3091/3212)[a]	97.17 (2950/3036)	97.05 (3788/3903)
High quality embryo rate (%)	68.12 (1998/2933)	66.98 (1937/2892)	69.13 (2495/3609)
Implantation rate (%)	74.43 (2183/2933)	74.82 (2164/2892)	75.48 (2724/3609)
Pregnancy rate (%)	47.16 (166/352)	46.77 (181/387)	52.08 (200/384)
Multiple gestation rate (%)	21.02 (74/352)	23.26 (90/387)	22.74 (87/384)
Abortion rate (%)	3.41 (12/352)	4.91 (19/387)	3.91 (15/384)

Note: 20% = gametes and embryos incubated throughout in a 5% CO_2 in air (20% O_2) atmosphere; 20%-5% = same for gametes and zygotes but day embryos transferred to a 5% CO_2, 5% O_2 and 90% N_2 atmosphere; 5% gametes and embryos cultured in a 5% CO_2, 5% O_2 and 90% N_2 atmosphere. [a]Significant differences between 20% and 20%-5% oxygen groups ($P < 0.05$).

pregnancy was determined by the presence of a gestational sac and a heartbeat as evaluated by ultrasound on days 21 and 28 following transfer, respectively.

Outcome measures

The IVF fertilization rate was defined as the number of fertilized oocytes (as assessed by PN formation on day 0) divided by the number of oocytes inseminated. Similarly, the ICSI fertilization rate was defined as the number of fertilized oocytes divided by the number of MII oocytes injected with one sperm. Cleavage rate was defined as the number of blastomeres divided by the number of fertilized oocytes. High quality embryo rate was defined as the number of Grade I and II embryos divided by the number of 2 PN zygotes. Embryo development was defined as the number of high quality Grade I and II embryos per randomized treatment cycle. The percentage of embryos used was defined as the number of transferred and cryopreserved embryos divided by the number of 2PN embryos. Clinical pregnancy rate was defined as the number of ultrasonographically detected pregnancies divided by the number of transfers. Polygestational rate was defined as the number of multiple pregnancies divided by the number of transfers. Abortion rate was defined as the number of abortions/miscarriages divided by the number transfers. Implantation rate, was defined as the number of gestational sacs as assessed via ultrasound divided by the number of transferred embryos. Cryopreservation rate was defined as the number of embryos that were cryopreserved divided by the total number of embryos.

Statistical analyses

All data for the above outcomes were analyzed using a SPSS 13.0 software package for Windows (SPSS, Chicago, USA). Data are pre-

sented as Mean ± S. E. M. or ratio. Measurement data using t test, counting data by chi-square test. Significance was accepted at a $P < 0.05$ level.

Results

Demographics and general characteristics of the study groups

Results are based on a total of 3484 cycles with the corresponding gametes and embryos cultured under three different oxygen tension atmospheres, as detailed in the previous section. While cycles were randomly allocated to each of the three treatment groups, patient ages, infertility duration, and BMI, as well as the gonadotropin doses used, number of oocytes retrieved, endometrial thickness at the time of hCG injection, and number of embryos transferred was similar across groups (**Table 1**). No difference in oocyte quality was detected among groups based upon initial morphological evaluation following OPU.

Impact of oxygen concentrations on IVF results

Embryos from the 5% O_2 group yielded higher rates of fertilization and implantation compared to those from the 20% of O_2 group ($P < 0.05$, **Table 2**), which is consistent with the data published before. Conversely, the 20% O_2 group yielded higher rates of fertilization, good embryo quality and implantation than the 20%-5% O_2 group ($P < 0.05$). Moreover, the latter group resulted in lower high quality embryo, implantation and pregnancy rates as compared to the 20% oxygen group ($P < 0.05$). There were no differences in multiple gestation or abortion rates among the three groups ($P > 0.05$).

Impact of oxygen concentrations on ICSI results

Embryos resulting from the 20% O_2 tension group yielded lower cleavage rates as compared to those from the 20%-5% of O_2 group ($P < 0.05$, **Table 3**). There were no differences in any other parameters evaluated among the three treatment groups.

Discussion

Since the first birth of an IVF produced baby in 1978 [3] the field of human assisted reproduction has been revolutionized with many technical advances. Given that following IVF/ICSI the zygote must be cultured for 2-6 days in defined medium, this long time-period spent within an incubator is undoubtedly an important determinant of its ability to yield a successful pregnancy following transfer. For decades, gametes and embryos have been cultured in an air atmosphere (20% O_2) supplemented with 5% CO_2. However, in the female reproductive tract oxygen tension ranges from 2 to 8% [9, 20]. Moreover, atmospheric oxygen concentrations can be detrimental for development and gene expression as evidenced in recent studies using mice and cattle embryos as models [21]. Similarly, culture conditions with a high oxygen tension atmosphere negatively impacted upon the viability of human embryonic stem cells and embryoid bodies [22]. However, evidence for a detrimental effect of high oxygen concentration on the success of human IVF and ICSI is controversial [12, 13]. Therefore we designed a study that would prospectively include a large number of IVF/ICSI cycles to evaluate the effect of O_2 tension during in vitro human oocyte and embryo incubation by comparing not only low (5%) vs. high (20%) O_2, but also the effect of varying O_2 (20 followed by 5%) tension during culture.

Interestingly, the most striking finding of this study was that embryo quality, implantation and pregnancy rates seemed not to be as much affected by the O_2 tension itself but rather by the fluctuation of such tension, as evidence by the poorer results attained in the 20%-5% O_2 concentration group. These results suggest that changes in O_2 tension during early embryo culture (i.e. 2-cell stage) have not only immediate effects upon embryo cleavage but also impact on later in vivo development. Notably, the 5% O_2 group did yield significantly higher rates of high quality embryos and implantation, and tended to result in relatively higher (albeit not significant) rates of pregnancy than those in the 20% O_2 group, consistent with an hypoxic culture environment being more conducive to initial embryonic development and to fostering further in vivo development. In fact, a favorable effect of low O_2 tension upon late stages of in vitro embryonic (blastocyst stage) and even upon fetal development has been reported in mice [23]. Similarly, recent studies also report

better pregnancy and live birth rates when transferring human blastocysts following in vitro culture in a low O_2 tension atmosphere. Our study provides further evidence to support a crucial effect of O_2 tension early in the cleavage process of in vitro development. Worth noting is the fact that varying O_2 tension during in vitro embryo culture did not appear to affect multiple birth or abortion rates.

Given the mild differences in outcome observed between embryos incubated in low or high O_2 tension conditions it may be premature to recommend the use of 5% O_2 for oocyte and/or embryo culture. Moreover, given that to achieve a low oxygen concentration, an incubator requires both CO_2 and O_2 sensors and the ability to supply three gases (N_2, CO_2 and O_2) simultaneously [6, 24], this increases the risk of fluctuations during embryo culture. For instance, opening the incubator door to handle oocytes/embryos requires re-equilibration of the inside atmosphere with a balanced mixture of gases to maintain a low O_2 concentration, which may take longer than when only CO_2 is used [6, 24]. Nonetheless, there may be instances in which the use of low O_2 tension could be highly advantageous and possibly overcome these drawbacks. In this regard, one study suggested that patients with poor prognosis (e.g. repeated implantation failures and poor responders with a low number of oocytes), as well as patients with advanced age (> 40 years old), benefited from transferring day 3 embryos cultured in a low O_2 tension atmosphere [25]. Therefore, the benefits and disadvantages of both systems may be outweighed in a case-to-case basis potentially driven by patient demographics in a particular clinical setting.

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Disclosure of conflict of interest

None.

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References

[1] Allersma T, Farquhar C, Cantineau AE. Natural cycle in vitro fertilisation (IVF) for subfertile couples. Cochrane Database Syst Rev 2013; 8: D10550.

[2] Johnson MH, Nasr-Esfahani MH. Radical solutions and cultural problems: could free oxygen radicals be responsible for the impaired development of preimplantation mammalian embryos in vitro? Bioessays 1994; 16: 31-38.

[3] Catt JW, Henman M. Toxic effects of oxygen on human embryo development Hum. Reprod 2000; 15 Suppl 2: 199-206.

[4] Bavister BD. Culture of preimplantation embryos: facts and artifacts. Hum Reprod Update 1995; 1: 91-148.

[5] Harvey AJ, Navarrete SA, Kirstein M, Kind KL, Fischer B, Thompson JG. Differential expression of oxygen-regulated genes in bovine blastocysts. Mol Reprod Dev 2007; 74: 290-299.

[6] Karagenc L, Sertkaya Z, Ciray N, Ulug U, Bahçeci M. Impact of oxygen concentration on embryonic development of mouse zygotes. Reprod Biomed Online 2004; 9: 409-417.

[7] Byatt-Smith JG, Leese HJ, Gosden RG. An investigation by mathematical modelling of whether mouse and human preimplantation embryos in static culture can satisfy their demands for oxygen by diffusion. Hum Reprod 1991; 6: 52-57.

[8] Fischer B, Bavister BD. Oxygen tension in the oviduct and uterus of rhesus monkeys, hamsters and rabbits J. Reprod Fertil 1993; 99: 673-679.

[9] Yedwab GA, Paz G, Homonnai TZ, David MP, Kraicer PF. The temperature, pH, and partial pressure of oxygen in the cervix and uterus of women and uterus of rats during the cycle. Fertil Steril 1976; 27: 304-309.

[10] Kang JT, Atikuzzaman M, Kwon DK, Park SJ, Kim SJ, Moon JH, Koo OJ, Jang G, Lee BC. Developmental competence of porcine oocytes after in vitro maturation and in vitro culture under different oxygen concentrations. Zygote 2012; 20: 1-8.

[11] Gomes SD, Oliveira JB, Petersen CG, Mauri AL, Silva LF, Massaro FC, Baruffi RL, Cavagna M, Franco JG Jr. IVF/ICSI outcomes after culture of human embryos at low oxygen tension: a meta-analysis. Reprod Biol Endocrinol 2011; 9: 143.

[12] Meintjes M, Chantilis SJ, Douglas JD, Rodriguez AJ, Guerami AR, Bookout DM, Barnett BD, Madden JD. A controlled randomized trial evaluating the effect of lowered incubator oxygen

tension on live births in a predominantly blastocyst transfer program. Hum Reprod 2009; 24: 300-307.
[13] Kovacic B, Sajko MC, Vlaisavljevic V. A prospective, randomized trial on the effect of atmospheric versus reduced oxygen concentration on the outcome of intracytoplasmic sperm injection cycles. Fertil Steril 2010; 94: 511-519.
[14] Calzi F, Papaleo E, Rabellotti E, Ottolina J, Vailati S, Viganò P, Candiani M. Exposure of embryos to oxygen at low concentration in a cleavage stage transfer program: reproductive outcomes in a time-series analysis. Clin Lab 2012; 58: 997-1003.
[15] de Los SM, Gamiz P, Albert C, Galán A, Viloria T, Pérez S, Romero JL, Remohí J. Reduced oxygen tension improves embryo quality but not clinical pregnancy rates: a randomized clinical study into ovum donation cycles. Fertil Steril 2013; 100: 402-407.
[16] McKiernan SH, Bavister BD. Environmental variables influencing in vitro development of hamster 2-cell embryos to the blastocyst stage. Biol Reprod 1990; 43: 404-413.
[17] Batt PA, Gardner DK, Cameron AW. Oxygen concentration and protein source affect the development of preimplantation goat embryos in vitro. Reprod Fertil Dev 1991; 3: 601-607.
[18] Thompson JG, Simpson AC, Pugh PA. Effect of oxygen concentration on in-vitro development of preimplantation sheep and cattle embryos. J Reprod Fertil 1990; 89: 573-578.
[19] Brinsden PR. A textbook of in vitro fertilization and assisted reproduction: the Bourn Hall guide to clinical and laboratory practice. 2nd ed. New York: Parthenon Pub. Group, 1999.
[20] Fischer B, Bavister BD. Oxygen tension in the oviduct and uterus of rhesus monkeys, hamsters and rabbits. J Reprod Fertil 1993; 99: 673-679.
[21] Takahashi Y, Kanagawa H. Effect of oxygen concentration in the gas atmosphere during in vitro insemination of bovine oocytes on the subsequent embryonic development in vitro. J Vet Med Sci 1998; 60: 365-367.
[22] Lim H, Song H, Paria BC. Molecules in blastocyst implantation: uterine and embryonic perspectives. Vitam Horm 2002; 64: 43-76.
[23] Cooke S, Tyler JP, Driscoll G. Objective assessments of temperature maintenance using in vitro culture techniques. J Assist Reprod Genet 2002; 19: 368-375.
[24] Trokoudes KM, Minbattiwalla MB, Kalogirou L. Controlled natural cycle IVF with antagonist use and blastocyst transfer. Reprod Biomed Online 2005; 11: 685-689.
[25] Bavister B. Oxygen concentration and preimplantation development. Reprod Biomed Online 2004; 9: 484-486.