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Effects of oxygen tension and humidity on the preimplantation development of mouse embryos produced by *in vitro* fertilization: analysis using a non-humidifying incubator with time-lapse cinematography

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Abstract: To examine the effects of oxygen tension and humidity on early embryonic development, the preimplantation development of mouse embryos produced by *in vitro* fertilization was assessed by time-lapse cinematography to evaluate morphokinetic development with higher precision. Zygotes were produced from spermatozoa and oocytes from ICR mice and cultured in KSOM under low or high oxygen tension in a non-humidified incubator with time-lapse cinematography (CCM-iBIS). The developmental rates of embryos to the 4-cell and blastocyst stages under lower oxygen tension in CCM-iBIS were significantly higher than those under higher oxygen tension in CCM-iBIS. Ninety-six hours after insemination, a large number of embryos cultured under low oxygen tension developed to the hatching blastocyst stage. Embryonic development was more synchronized under lower oxygen tension. Non-humidified cultures did not affect embryonic development. On average, mouse embryos cultured at lower oxygen tension reached 2-cell at 18 h, 3-cell at 39 h, 4-cell at 40 h, initiation of compaction at 58 h, morula at 69 h, and blastocyst at 82 h after insemination. In conclusion, lower oxygen tension better supports preimplantation development of mouse embryos fertilized *in vitro*, and non-humidified culture conditions do not influence the embryonic development *in vitro*.
Key words: embryo, *in vitro* fertilization, mice, preimplantation development, time-lapse cinematography

Introduction

Since the first successful *in vitro* fertilization (IVF) of mice in 1968 [1], the components of culture medium, culture conditions, and IVF procedures have been substantially improved. Culture conditions, such as temperature and atmosphere, are important factors for the successful preimplantation development of embryos *in vitro*. To culture mouse embryos *in vitro*, an atmosphere of 5% CO_2 in air with a saturated humidity at 37°C in an incubator was typically used. Furthermore, a small droplet of medium, containing embryos on a dish, is covered with paraffin oil to avoid releasing CO_2 from the medium, and evaporation and contamination of the medium, which would affect manipulation or observation of embryos outside the CO_2 incubator [2]. Several laboratories have used 5% oxygen tension as an optimum level for culturing mammalian embryos; however, many IVF laboratories still use 20% [3].

Morphological observation of developing embryos is essential for managing and improving culture conditions in experimental and clinical studies. However, frequent opening and shutting of the incubator for microscopic observation of cultured embryos seems to disturb culture conditions. Therefore, it is necessary to reduce the frequency of embryo culturing for successful preimplantation development in an incubator. However, such fragmentary information may provide limited analysis of

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developing embryos. Fortunately, it has become possible to observe cultured embryos *in vitro* via time-lapse video cinematography developed by Payne *et al.* [4]. An incubator equipped with a time-lapse monitoring system is a more closed system for culturing and observing embryos without exposure to the atmosphere and the associated stress or disturbance. Time-lapse observations have also advanced our understanding of the morphological mechanisms of fertilization, development, and behavior of early embryos during the preimplantation period. Previous studies with time-lapse monitoring have investigated pronuclear formation [4, 5], timing of cleavage [5–9], fragmentation [10–13], compaction [14–19], blastocoel formation [18, 20], and shape of the inner cell mass [21]. Furthermore, culturing human embryos in an incubator with a time-lapse monitoring system may improve development up to the blastocyst stage and pregnancy outcomes, compared to the use of a conventional incubator [22, 23].

Initially, this system was only applied for assisted reproductive technology in human infertility treatment due to its high cost. Recently, a time-lapse monitoring system has been applied to evaluate mouse embryonic development *in vitro*. The first and second cleavages of mouse embryos significantly influence the probability of reaching the blastocyst stage [24]. When the time of cleavage and compaction of 2-cell embryos were evaluated by time-lapse monitoring, it was suggested that the time of the third cleavage and compaction might be a useful parameter for predicting developmental potential [25]. Prior to these studies, by using time-lapse film cinematography, Sutherland *et al.* [26] succeeded, for the first time, to directly observe the division plane of 8-cell blastomeres during the fourth cleavage of mouse embryos cultured from the late 2-cell stage. However, these studies in mice used embryos that were fertilized *in vivo*. Little information is available regarding the morphokinetic development of mouse embryos produced *in vitro*.

In the present study, the effects of oxygen tension and humidity on preimplantation development of mouse embryos produced by *in vitro* fertilization were assessed using a non-humidified incubator with time-lapse cinematography. Sperm penetration time was accurately controlled in the IVF system [27–29] to evaluate preimplantation development with higher precision.

Materials and Methods

Animals

All the animals were purchased from CLEA (Tokyo, Japan). The animals were kept in a barrier unit at 24 ± 1°C with a relative humidity of 50 ± 10% under a lighting regimen of 12 h light/day (lights on 07:00 to 19:00). They were allowed free access to a standard laboratory mouse diet (CA-1; CLEA, Tokyo, Japan) and tap water. All experiments were performed in accordance with the guidelines for the care and use of animals approved by the Obihiro University of Agriculture and Veterinary Medicine. The authors confirmed that this experiment complied with ARRIVE guidelines. All animal experimental protocols were approved by the Institutional Animal Ethics Committee of Obihiro University of Agriculture and Veterinary Medicine.

Preparation of gametes and *in vitro* fertilization

Female ICR mice (CLEA) at 8–14 weeks of age were superovulated by intraperitoneal injections of 175 μl of CARD Hyper Ova (Kyudo Co., Ltd., Saga, Japan) and 5 IU of human chorionic gonadotropin (hCG) (ASKA Pharmaceutical Co., Tokyo, Japan), 48 h apart. The mice were then euthanized by cervical dislocation 16 h after hCG injection, and the oviducts were removed and placed in a culture dish (35 mm, Falcon, Corning Inc., Corning, NY, USA) containing 400 μl TYH (LSI Medience Corp., Tokyo, Japan) covered with paraffin oil (Nacalai Tesque, Kyoto, Japan). The ampullar region of the oviduct was dissected with a needle, and eggs surrounded by cumulus cells were introduced into the medium. Spermatozoa were collected from the cauda epididymis of 18–26-week-old ICR males and cultured in 400 μl of TYH under paraffin oil for 2 h in an incubator (CPO_2-2301, HIRASAWA WORKS Inc., Tokyo, Japan) at 37°C in humidified 5% CO_2 in air.

After pre-incubation of the sperm suspension, a small volume was added to the medium containing the eggs. The final concentration of the spermatozoa was adjusted to 150 cells/μl [27]. Seven hours after insemination, the eggs were transferred in 80 μl of KSOM medium supplemented with amino acids (KSOM, Ark Resource, Kumamoto, Japan) and covered with paraffin oil to collect fertilized eggs with the second polar body and both male and female pronuclei.

Embryo culture

The fertilized eggs were cultured for 113 h in a non-humidifying incubator with time-lapse cinematography (CCM-iBIS, ASTEC Co., Ltd., Fukuoka, Japan) or a conventional CO_2 incubator (CPO_2-2301).

For cultivation using CCM-iBIS, the fertilized eggs were placed in a culture dish (LinKID micro DH-004PG, DNP, Tokyo, Japan) containing 60 μl of KSOM covered with paraffin oil at 37°C in 5% CO_2 in air (higher oxygen tension) or 5% CO_2, 5% O_2, and 90% N_2 (lower oxygen

tension). The CCM-iBIS had a non-humidifying system and was equipped with a 1.3 million-pixel CCD camera with a 10× objective lens, red LED lighting, silicone rubber heater with digital PID control, duty-controlled gas pressure, and the NAS image storage method. The CCD camera for the time-lapse microscope was set up to acquire a single image every 15 min. Parameters regarding embryonic development were annotated as t2, t3, t4, and t5 which represented the time of cleavage to the 2-, 3-, 4-, and 5-cell stage respectively, followed by tOC, time of the onset of compaction after insemination; tM, time of formation of fully compacted morula after insemination; tB, time of blastocyst formation with blastocoel at half volume of embryo after insemination; tExB, time of formation of expanded blastocyst developed to over 120 μm in diameter after insemination; and tHB, time of the onset of hatching from zona pellucida after insemination.

In a part of experiments, the fertilized eggs were cultured in a dish containing 50 μl of KSOM covered with paraffin oil at 37°C in higher oxygen tension under a humidified atmosphere in 5% CO_2 in air in CPO_2-2301 without opening or closing the incubator door from the start of culture to the time of observation. The developmental stages of the embryos were observed under an inverted microscope 48 and 96 h after insemination in this experimental group.

Statistical analysis

The experiments were repeated at least three times. Statistical analyses were performed using the JMP software (SAS Institute, Cary, NC, USA). All data were analyzed using the Mann-Whitney *U* test. The correlation between the attainment times at each developmental stage was assessed using Spearman's rank correlation coefficient. The significance of the correlation coefficient was analyzed using a test for no correlation. Differences were considered statistically significant at $P<0.05$. All graphs were drawn using DataGraph software (Visual Data Tools, Inc., Chapel Hill, NC, USA).

Results

Effect of oxygen tension on *in vitro* development of mouse embryos

The results of cultivation of mouse embryos fertilized *in vitro* in a non-humidifying incubator with time-lapse cinematography (CCM-iBIS) are shown in Table 1. The developmental percentages of embryos to the 4-cell stage 48 h after insemination cultured in 5% CO_2, 5% O_2, and 90% N_2, and 5% CO_2 in air in CCM-iBIS were 96.2 ± 3.0% (288/297) and 88.0 ± 8.4% (488/556), respectively. There were significant differences between the experimental groups ($P<0.05$). Developmental percentages to the blastocyst stage at 96 h (95.1 ± 3.0%, 280/297) and 120 h (96.5 ± 2.6%, 288/297) after insemination under lower oxygen tension in CCM-iBIS were significantly higher ($P<0.05$) than those in higher oxygen tension in CCM-iBIS (78.9 ± 13.3%, 428/556 and 86.3 ± 7.7%, 478/556, respectively).

Table 2 shows the developmental stages of embryos every 24 h after insemination in CCM-iBIS. At 48 h after insemination, 34.6 ± 31.7% of embryos developed beyond the 4-cell stage to the 5-cell stage in CCM-iBIS with lower oxygen tension. Only 13.9 ± 16.8% of the embryos showed the 5-cell stage in CCM-iBIS with higher oxygen tension. After 96 h of insemination, the proportion of hatching blastocysts in CCM-iBIS with lower oxygen tension (74.7 ± 8.6%) was significantly higher ($P<0.05$) than that in higher oxygen tension (48.2 ± 20.8%).

There was no difference in average attainment time after insemination between higher and lower oxygen tensions in each developmental stage using time-lapse cinematography (Table 3). Figure 1 shows a histogram of the number of IVF embryos at each developmental stage determined by time lapse after insemination. Despite oxygen tension, the time of onset of cleavage was similar at each developmental stage. However, it took a considerable amount of time to cleave after the second cleavage (t3) in some embryos cultured under higher oxygen tension. Cleavage and development of IVF embryos were more synchronized at lower oxygen tension than at higher oxygen tension in CCM-iBIS.

Table 1. Results of *in vitro* cultivation of mouse embryos fertilized *in vitro* under high and low oxygen tension in a non-humidifying incubator with time-lapse cinematography (CCM-iBIS)

Atmosphere	No. of fertilized eggs cultured	No. (%) of development to:				
		2-cell (24 h)[1]	4-cell (48 h)[1]	Morula (72 h)[1]	Blastocyst	
					(96 h)[1]	(120 h)[1]
5% CO_2 in air	556	551 (99.3 ± 0.8)[a]	488 (88.0 ± 8.4)[a]	362 (67.9 ± 17.0)[a]	428 (78.9 ± 13.3)[a]	478 (86.3 ± 7.7)[a]
5% CO_2, 5% O_2, 90% N_2	297	292 (97.7 ± 2.0)[a]	288 (96.2 ± 3.0)[b]	215 (74.3 ± 21.4)[a]	280 (95.1 ± 3.0)[b]	288 (96.5 ± 2.6)[b]

Data are shown as mean ± SD calculated from 7 and 6 replicates in the high and low oxygen tension groups, respectively. [1] Hours after insemination. a–b: Values with different superscripts are significantly different in the same column at $P<0.05$ by Mann-Whitney *U* test.

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Effect of humidity on *in vitro* development of mouse embryos

To assess the influence of non-humidified culture conditions on embryonic development *in vitro*, embryos were cultured under high oxygen tension in a conventional incubator (CPO_2-2301) without opening or closing the incubator door from the start of culture to the time of observation. Developmental rates of IVF embryos

Table 2. Developmental stage of *in vitro* fertilized mouse embryos at every 24 h after insemination

Atmosphere	24 h [1]		48 h [1]		72 h [1]	
	2-cell	3-cell	4-cell	5-cell	Com	Morula
5% CO_2 in air	551 (99.3 ± 0.8) [a]	0 (0.0 ± 0.0) [a]	436 (74.2 ± 15.1) [a]	52 (13.9 ± 16.8) [a]	150 (24.9 ± 16.4) [a]	362 (67.9 ± 17.0) [a]
5% CO_2, 5% O_2, 90% N_2	291 (97.5 ± 1.8) [a]	1 (0.1 ± 0.3) [a]	178 (61.6 ± 28.8) [a]	110 (34.6 ± 31.7) [a]	75 (22.7 ± 19.7) [a]	215 (74.3 ± 21.4) [a]
Atmosphere	96 h [1]			120 h [1]		
	BL	ExBL	H-BL	BL	ExBL	H-BL
5% CO_2 in air	143 (26.8 ± 13.6) [a]	18 (4.0 ± 4.9) [a]	267 (48.2 ± 20.8) [a]	87 (19.5 ± 19.1) [a]	35 (6.2 ± 5.9) [a]	356 (60.6 ± 23.3) [a]
5% CO_2, 5% O_2, 90% N_2	45 (13.1 ± 8.2) [b]	14 (8.0 ± 5.6) [a]	221 (74.7 ± 8.6) [b]	6 (1.2 ± 1.5) [b]	1 (0.2 ± 0.4) [a]	281 (96.0 ± 3.0) [b]

Data are shown as mean ± SD calculated from 7 and 6 replicates in the high and low oxygen tension groups, respectively. [1] Hours after insemination. Com: Initiation of compaction, BL: Blastocyst, ExBL: Expanded Blastocyst, H-BL: Hatching Blastocyst. a–b: Values with different superscripts are significantly different in the same column at $P<0.05$ by Mann-Whitney *U* test.

Table 3. Results of attainment time after insemination to each developmental stage of *in vitro* fertilized mouse embryos cultured under high and low oxygen tension in a non-humidifying incubator with time-lapse cinematography (CCM-iBIS)

Atmosphere	Hours after insemination						
	2-cell	3-cell	4-cell	5-cell	Onset of compaction	Morula	Blastocyst
5% CO_2 in air	17.76 ± 0.61 [a]	40.14 ± 1.64 [a]	41.47 ± 1.87 [a]	51.79 ± 2.17 [a]	58.79 ± 1.98 [a]	68.60 ± 2.54 [a]	84.25 ± 4.48 [a]
5% CO_2, 5% O_2, 90% N_2	17.63 ± 1.15 [a]	38.95 ± 1.94 [a]	39.96 ± 2.19 [a]	49.31 ± 2.18 [a]	57.58 ± 2.17 [a]	69.41 ± 3.43 [a]	82.39 ± 2.23 [a]

Data are shown as mean ± SD in decimal from 7 and 6 replicates in the high and low oxygen tension groups, respectively. The embryos developed to the blastocyst stage within 120 h after insemination were used for calculation (range: n=477–478 and n=280–289 in the high and low oxygen tension groups, respectively). Values with different superscripts are significantly different in the same column at $P<0.05$ by Mann-Whitney *U* test.

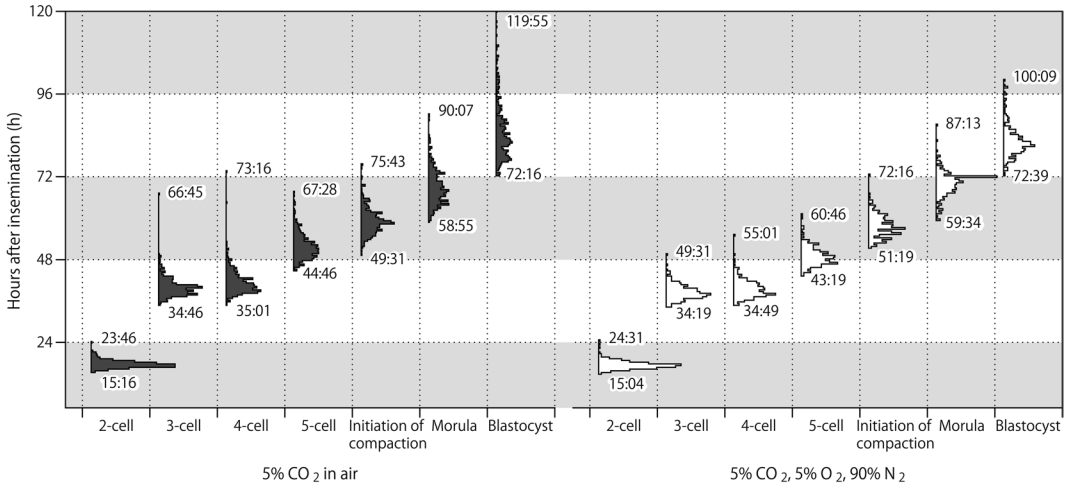

Fig. 1. Histogram of the number of embryos fertilized *in vitro* at each developmental stage with the lapse of time after insemination. Embryos cultured in higher oxygen tension and lower oxygen tension in CCM-iBIS are indicated in black and white, respectively. Values show the range of time to cleavage in each developmental stage. Cleavage and development of embryos fertilized *in vitro* were much more synchronized in lower oxygen tension than in higher oxygen tension in CCM-iBIS.

cultured under high oxygen tension in a conventional incubator (CPO_2-2301) without opening the incubator door for the 4-cell stage 48 h after insemination and blastocyst stage 96 h after insemination were 83.7 ± 21.0% (73/84) and 84.1 ± 12.3% (89/102), respectively (Table 4). These values were not significantly different from those under higher oxygen tension in CCM-iBIS.

Morphokinetic development of mouse embryos cultured under lower oxygen tension

Figure 2 illustrates the progression of cleavage of IVF eggs cultured under lower oxygen tension in CCM-iBIS. The first cleavage occurred 17 h 37 min after insemination. The second cleavage was initiated 38 h 57 min after insemination. The time between the first and second cleavages was 21 h 20 min. The 3-cell stage was performed for 1 h 0 min. The second cleavage was completed 39 h 57 min after insemination. At 9 h 21 min after the second cleavage, the third cleavage was initiated, specifically at 49 h 18 min after insemination. The compaction of embryos was initiated 57 h 34 min after insemination and reached the morula stage 69 h 24 min after insemination. Compacting embryos during development to the morula stage showed a phenomenon in which compaction was loosened due to an increase in the number of blastomeres accompanying cell division. Morula developed into blastocysts at 12 h 59 min, specifically 82 h 23 min after insemination.

When the relationship between attainment time to 2-, 3-, 4-, and 5-cell as well as initiation of compaction and

Table 4. Results of *in vitro* cultivation of mouse embryos fertilized *in vitro* in a non-humidifying incubator with time-lapse cinematography (CCM-iBIS) and a humidifying conventional CO_2 incubator (CPO_2-2301) without handling of culturing embryos inside and outside the incubator

Incubator	No. of fertilized eggs cultured	No. (%) of development to:	
		4-cell (48 h)[1]	Blastocyst (96 h)[1]
CPO_2-2301[2]	84	73 (83.7 ± 21.0)[a]	-
	102	-	89 (84.1 ± 12.3)[a]
CCM-iBIS[3, 4]	556	488 (88.0 ± 8.4)[a]	428 (78.9 ± 13.3)[a]

Data are shown as mean ± SD calculated from 3 and 7 replicates in the CPO_2-2301 and CCM-iBIS groups, respectively. [1] Hours after insemination. [2] Humidifying conventional CO_2 incubator at 37°C, 5% CO_2 in air. [3] Non-humidifying incubator with time-lapse cinematography at 37°C, 5% CO_2 in air. [4] Data are from Table 1. Values with the same superscripts are not significantly different in the same column at $P<0.05$ by Mann-Whitney *U* test.

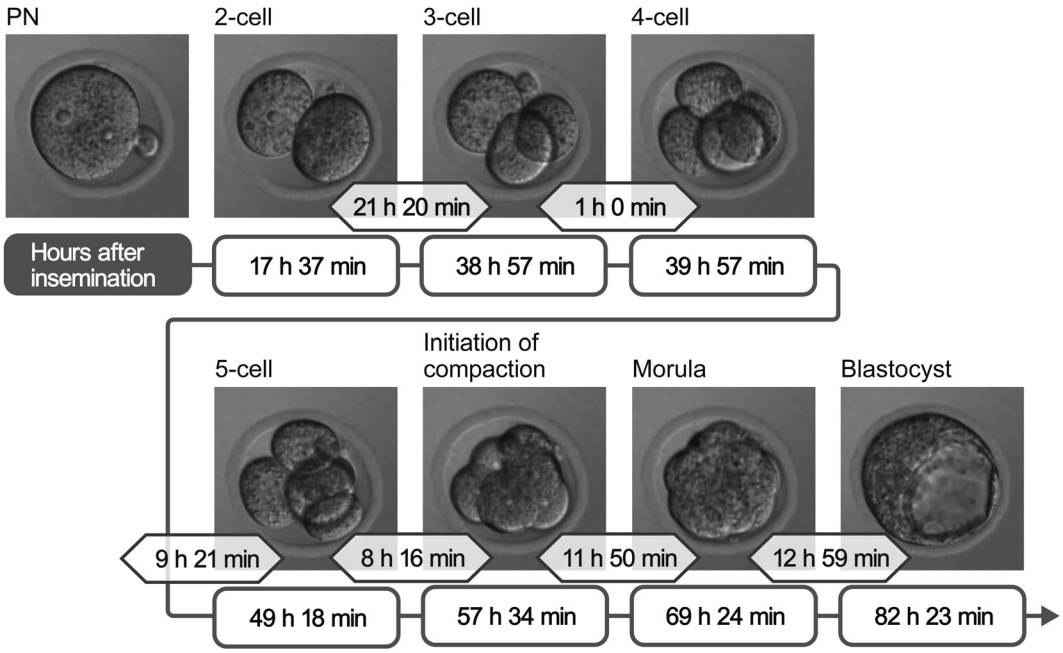

Fig. 2. Timing of cleavage of mouse *in vitro* fertilized embryos during preimplantation development cultured in lower oxygen tension in a non-humidifying incubator with time-lapse cinematography (CCM-iBIS).

morula and attainment time to blastocyst stage under lower oxygen tension was analyzed, the correlation coefficients were identified as ρ=0.5646, 0.6468, 0.6499, 0.6733, 0.4662, and 0.5281, respectively. Therefore, there were significant correlations between the attainment time from the 2-cell stage to the morula stage and the attainment time to the blastocyst stage (P<0.05). The relationship between the attainment time of embryos cultured under higher oxygen tension was similar to that under lower oxygen tension (Fig. 3).

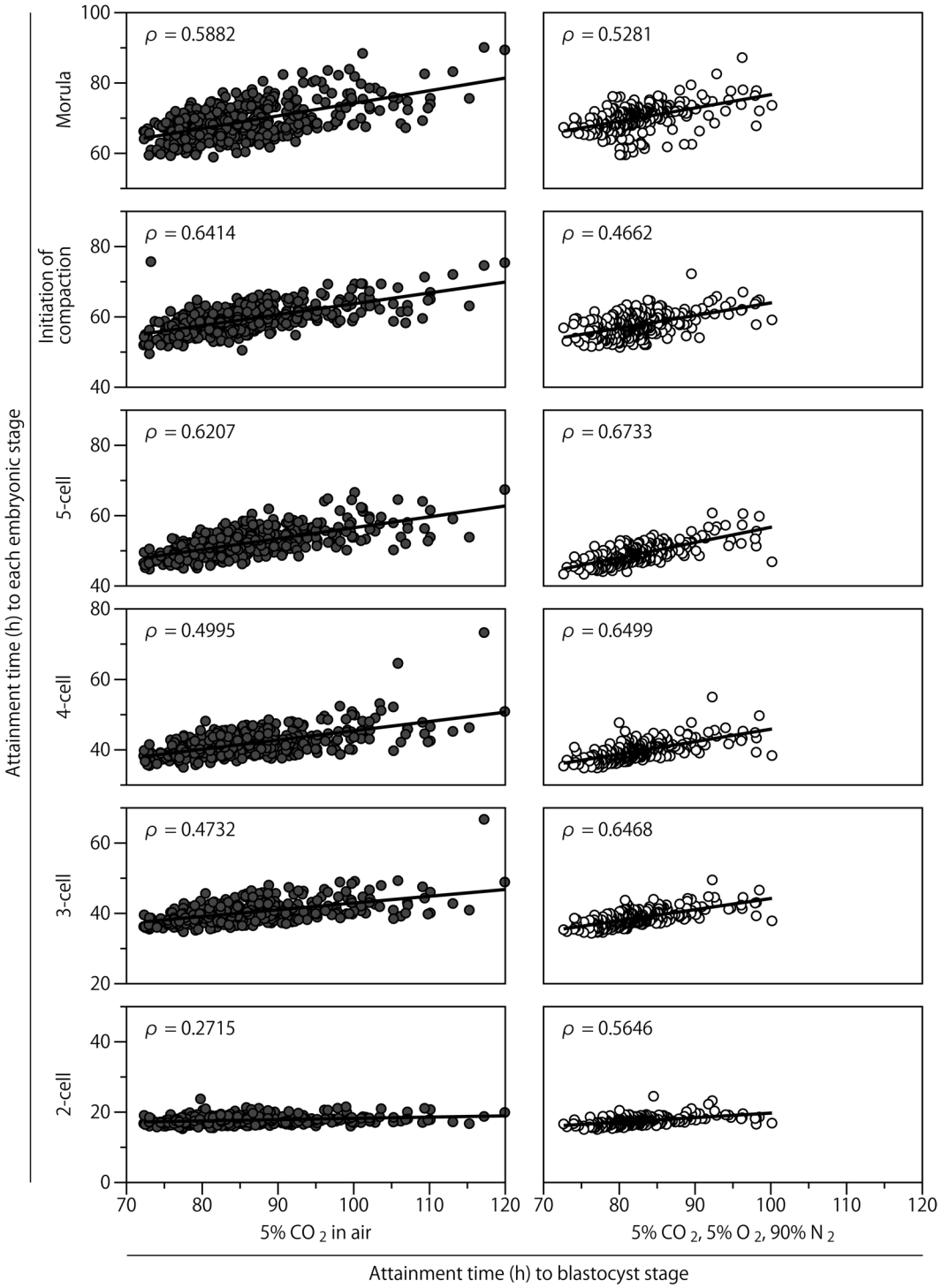

Fig. 3. Relationship between attainment time to 2-cell, 3-cell, 4-cell, 5-cell, initiation of compaction and morula, and attainment time to blastocyst stage. The embryos developed to the blastocyst stage within 120 h after insemination were used for analysis (range: n=477–478 and n=280–289 in the high and low oxygen tension groups, respectively).

Discussion

For infertility treatment in humans, the application of time-lapse monitoring together with an embryo-evaluating algorithm is associated with a significantly higher ongoing pregnancy rate, lower early pregnancy loss, and higher live birth rate [22, 23, 30, 31]. Therefore, an incubator with time-lapse cinematography appears to provide qualitative improvement of embryo *in vitro* culture for humans. There are possible explanations for this improved embryonic development as follows: 1) non-humidifying incubation and 2) minimal handling of cultured embryos inside and outside the incubator. However, some issues regarding non-humidifying incubators have been highlighted. The osmolality of the medium increases gradually during incubation in a non-humidifying incubator [32]. When mouse embryos were cultured in KSOM with higher osmolality, many embryos stopped developing at the 2-cell stage [33]. However, non-humidified culture conditions did not appear to influence the development of *in vitro* fertilized mouse embryos (Table 4). Reportedly, the development rates of mouse embryos from the 2-cell to blastocyst stage after 96 h in culture are not significantly different between humidifying and non-humidifying incubators [34].

Regarding the influence of handling embryos inside and outside the incubator, in our previous study, although there was no difference in the development rate to blastocyst stage between the CCM-iBIS and CPO_2-2301 incubators with higher oxygen tension, embryonic development to the 4-cell stage at higher oxygen tension in CCM-iBIS was significantly higher than that in CPO_2-2301 [35]. In addition, embryos cultured in CCM-iBIS showed a more advanced embryonic stage 96 h after insemination [35]. Thus, the use of embryos outside the incubator adversely affects embryo development. When embryos were cultured in CPO_2-2301 without opening or closing the incubator door from the start of cultivation to the time of microscopic observation, the developmental rates of embryos did not differ from those cultured under higher oxygen tension in CCM-iBIS (Table 4). Therefore, handling of embryos inside and outside the incubator, as well as oxygen tension in the atmosphere in culture, appears to influence the cleavage and development of embryos fertilized *in vitro*.

Although there were no differences in the developmental speed of embryos cultured under lower and higher oxygen tension (Table 3), the development of embryos was better and more synchronized under lower oxygen tension in culture (Tables 1 and 2, and Fig. 1). These results suggest that cultivation under low oxygen tension is suitable for embryonic development in mice. In this study, we assessed the effect of oxygen tension on embryonic development *in vitro* using a time-lapse incubator that does not require the culture dish to be frequently removed to observe the embryos. Low oxygen tension plays a critical role in reducing the high level of detrimental reactive oxygen species within cells, influences embryonic gene expression, and supports embryo metabolism of glucose [3]. Collectively, these findings may help improve embryonic development and implantation.

Although several studies have applied a time-lapse monitoring system to evaluate mouse embryonic development *in vitro*, they have used zygotes or 2-cell stage embryos derived from fertilization *in vivo* [24–26, 36]. For the evaluation of preimplantation development *in vitro* with higher precision, the analysis of *in vitro* fertilized eggs with synchronous ovulation and fertilization is recommended [27,29]. In this study, we precisely showed the timing of cleavage in IVF mouse embryos during preimplantation development using time-lapse cinematography (Tables 2 and 3, Figs. 1 and 2). When the embryonic development of IVF mouse embryos was observed, the development rates to the 2-cell stage 24 h after insemination, 4-cell stage 48 h after insemination, morula 72 h after insemination, and blastocysts 96 and 120 h after insemination were used as indices. Therefore, it is likely that a conventional 24 h interval misjudges the development rate of the morula [35].

High-quality embryos can be successfully selected based on their developmental speed in humans [18] and mice [25]. In the present study, the attainment time of the first, second, and third cleavages and compaction was correlated with the probability of reaching the blastocyst stage (Fig. 3). Delayed embryo development may be due to defects such as DNA lesions. These findings suggest that the fate of embryos could be predicted in the early stages of embryogenesis and remains to be investigated in future studies.

The procedures of oocyte collection and insemination, culture medium, and culture conditions are key to the success or failure of IVF and subsequent embryo transfer. However, accurate monitoring of embryonic development *in vitro* is also an important element. Over time, improvements have been made in the IVF method [1, 27, 37] and culture medium [27, 38–43] making it possible to obtain stable and highly reproducible results. In addition, the advent of culture conditions, particularly the non-humidifying incubator with time-lapse cinematography [4], allows accurate monitoring of embryonic development *in vitro*. There is increasing evidence that early cleavage blastomeres show bias in their development. Blastomeres at the 2-cell stage have an unequal

distribution of mitochondrial ribosomal RNAs [44]. Some epigenetic regulators, such as *Prdm14*, *Dnmt3b*, and *Dnmt3l* [45], and the cell fate regulator *Sox21* [46] are highly heterogeneously expressed between blastomeres of 4-cell embryos. As shown in Table 3, on average, *in vitro* fertilized eggs of mice underwent a first cleavage at 17–18 h and a second cleavage at 39–40 h on average after insemination. However, in the first cleavage, all eggs were in the 2-cell stage 9–11 h after the first egg was in the 2-cell stage. In the second cleavage, it took 15 h under lower oxygen tension and 33 h under higher oxygen tension for all eggs to reach the 3-cell stage after the first egg was in the 3-cell stage (Fig. 1). Single-cell sequencing and live-cell tracking technology, in combination with time-lapse cinematography, provide an opportunity to understand the significance of cleavage speed for developmental competency of the embryo.

In conclusion, the present results clearly indicate that oxygen tension in culture is an important factor in the developmental potential of mouse embryos fertilized *in vitro*. The development of mouse embryos cultured under low oxygen tension was more synchronized. Non-humidified culture conditions had no influence on embryogenesis in mice. Embryo culture with time-lapse monitoring may provide not only a better environment for embryos, but also valuable information for selecting high-quality embryos in mice.

Conflict of Interest

Hiroyuki Watanabe, Haruka Ito, Ayumi Shintome, and Hiroshi Suzuki declare that they have no conflicts of interest.

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