Comparison of the development of human embryos cultured in either an EmbryoScope or benchtop incubator

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Received: 2 May 2017 / Accepted: 5 December 2017 / Published online: 15 December 2017
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Abstract

Purpose In this current study, our main goal was to establish that EmbryoScope incubation environment is comparable to standard incubation.

Methods The development of sibling human zygotes was compared after culture in either a benchtop incubator (SI) or an EmbryoScope time-lapse incubator (ES). Between May 2015 to April 2016, a total of 581 normally fertilized 2PN, pronuclear-stage embryos, from 47 patients were allocated to culture in either a benchtop incubator (SI) or an EmbryoScope incubator (ES).

Results The development of embryos to cleavage (up to day 3) and blastocyst stages (day 5/6) was compared between the two different incubators. The proportion of good quality embryos was higher in the ES group compared to the SI on day 2 (66.8 vs. 50.5%, $P = 0.014$) and on day 3 (75.1 vs. 56.0%, $P = 0.006$). Those differences were statistically significant. A higher proportion of embryos developed to good quality blastocysts when cultured in the EmbryoScope compared to the benchtop (49.4 vs. 42.0%, $P = 0.24$), but this was not significant. Finally, no significant differences were noted with the proportion of blastocysts chosen for cryopreservation on day 5/6 in the two incubators.

Conclusions The findings support the view that the EmbryoScope incubator supports at least equivalent in vitro development of human embryos compared to other standard incubation methods and may promote improved development during early cleavage stages.

Keywords Human embryo culture · Morphologic analysis · Time-lapse monitoring

Introduction

Since 1978 when the first baby was born following in vitro fertilization, more than 6 million babies have been born worldwide due to assisted reproductive technologies (ART) [1]. Although technologies for embryo culture and selection have improved significantly since then, in many cases, it is still a major challenge to identify the single embryo with the highest implantation potential most likely to implant and generate a pregnancy. Clinical results achieved using ART are still relatively modest, considering that only 10–30% of the embryos transferred result in a live birth [2]. This could be due to several factors such as the high prevalence of aneuploidy in

embryos or impaired endometrial receptivity following ovarian stimulation [3]. In addition, assisted conception units are increasingly moving towards a policy of elective single-embryo transfer (eSET), to reduce the incidence of multiple pregnancies [4], which is associated with higher risk of adverse perinatal and maternal outcomes [5–7]. Single-embryo replacement has presented a challenge to embryologists, who must try to optimize embryo culture and selection in order to maintain an adequate success rate, whilst reducing the overall number of embryos transferred. Both embryo culture methods and equipment, in the embryology laboratory, are likely to have a major influence on the clinical outcome. Two main approaches to embryo culture are currently used in embryology laboratory: uninterrupted culture using a single medium that can be used continuously from insemination/fertilization until day 5/6 of culture (single-medium approach) [8–10] and secondly, sequential culture where two different culture media are used with a mandatory day 3 changeover (sequential-medium approach) [11]. Gaseous environment is also another important factor in embryo culture, studies have shown that

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low oxygen concentration of 5% plays an important role in reducing the amount of reactive oxygen species within cells, which can have a negative influence on embryonic gene expression. In addition, low oxygen concentration has a positive effect on embryo metabolism of glucose and enhances embryo development to the blastocyst stage and pregnancy outcome [12–15]. Morphology assessment is still one of the most prevalent methods used to select embryos for transfer based on a single daily observation. However, incubators with time-lapse imaging are now available, which allow multiple observations to be made with automated image capture, requiring no embryo disturbance during development. This is likely to improve embryo development due to the relative long-term stability of temperature, gas concentration, pH, and humidity. Time-lapse imaging also has the potential to aid embryo selection by distinguishing dynamic morphology and abnormal patterns of cleavage during the embryo development. Such enhanced selection and deselection may help to identify embryos with high implantation potential and contribute to an increase clinical outcome [16–20]. Furthermore, time-lapse imaging could potentially enhance consistency of embryo assessment and selection from different embryologists in an IVF unit and or within different clinics working in the same region [21, 22]. Concerns have been raised regarding the safety of the time-lapse incubator, considering the exposure to light during the image acquisition. It has been reported that considerable light exposure may be detrimental to embryo development [23, 24]. Furthermore, the presence of a magnetic field, the warmth created by moving parts, and the presence of lubricants may represent an extra risk and might be detrimental to embryo development. Therefore, before introducing time-lapse incubation in a clinical setting, it is recommended to fully validate and document the safety of the instrument. Although some studies have been carried out comparing embryo quality between a time-lapse incubator and traditional culture conditions [20, 25–29], some of these were conducted on mice and bovine models [28, 29] and included human embryos derived from an oocyte donor population which may not representative of embryos from infertile couples [25]. Accordingly, the goal of this study was to compare the development of pronuclear-stage embryos cultured in either a benchtop incubator (SI) or an EmbryoScope incubator with time-lapse facility (ES).

Material and methods

This comparison study was performed between May 2015 and April 2016 at the Edinburgh Reproductive Endocrine Centre, RIE, Edinburgh, NHS Lothian. A total of 581 pronuclear-stage embryos from 47 couples undergoing IVF/ICSI treatment were allocated to culture in either an EmbryoScope incubator or benchtop incubator. The goal was to compare two commercially available types of incubators for the culture of human zygotes: a benchtop incubator (K-MINC-1000, Cook

Medical) and the EmbryoScope time-lapse incubator (EmbryoScope™ Time-lapse System, Unisense FertiTech, Aarhus, Denmark). The EmbryoScope incubator is CE certified and is conformed with the health and safety requirements for equipment in the European Union. The CE certificate confirms the quality of the system from Unisense FertiTech in terms of its manufacture and final inspection. The production, installation, and servicing of this incubator was operated from Unisense FertiTech. The benchtop incubator has been in routine use in our IVF laboratory for the previous 5/6 years. Before zygotes were allocated for culture in the EmbryoScope, it was validated initially using the sperm survival assay [30] and subsequently by assessing division and development of abnormally fertilized human embryos showing 1 or > 2 pronuclei at fertilization check. The nature of the present study is not randomized: the main aim of the current study was to compare the growth of human zygotes cultured in either an EmbryoScope or benchtop incubator.

Ovarian stimulation

All patients received controlled ovarian stimulation with a GnRH agonist (subcutaneous buserelin 0.5 ml daily) or GnRH antagonist (subcutaneous cetrorelix 0.5 mg daily, Merck Serono) treatment. The long protocol was used for downregulation using subcutaneous buserelin or the GnRH antagonist; Cetrotide was administered daily on day 6 of onset of menses. Ovarian stimulation was carried out using either Gonal F (Merck Serono) or Menopur (Ferring) based on individual patient characteristics. Follicular development was monitored by transvaginal ultrasound and ovulation was triggered when three follicles were 18 mm or above. Each patient received hCG (ovitrelle 0.25 mg, Merck Serono) to trigger ovulation. Oocyte recovery was carried out under conscious sedation with transvaginal ultrasound guidance at 35 h after ovitrelle injection.

Oocyte collection, fertilization, and embryo culture

Cumulus-oocyte-complexes (COC) were isolated from follicular fluid and then rinsed and cultured in 0.5-ml equilibrated G-IVF™ medium (Vitrolife Göteborg Sweden) at 37 °C and 6% CO₂ in atmospheric air in a Hera cell 240 incubator (Thermo Scientific).

Sperm used for either routine insemination or the ICSI procedure was collected by masturbation and processed using a standard method as described by Bourne et al. 2004 [31]. All oocyte was cultured in G-IVF™ medium on the day of insemination (day 0). These were inseminated by IVF or ICSI according to patient's etiology and history. Fertilization was identified by the presence of two pronuclei approximately 16–19 h after insemination or microinjection. At this stage, normally fertilized oocytes, 581 pronuclear-stage embryos

from 47 patients, were allocated randomly to either the EmbryoScope time-lapse incubator (ES Group, $n = 322$) or to standard benchtop incubator (SI group, $n = 259$). Patients who had more than 6 pronuclear embryos were assigned to the study. At the beginning, the division was performed equally between the two groups. Subsequently, the allocation was carried out as follows: if the patient had more than 12 pronuclear-stage embryos, the first 12 were allocated to the EmbryoScope time-lapse (using a 12-well EmbryoSlide™) and the remaining were assigned to the benchtop incubator (ES group $n = 322$, SI $n = 259$). The EmbryoSlide™ is a specifically designed device for use with the EmbryoScope™ imaging system, with 12 individual wells for embryo culture, per slide.

Both groups were cultured under the same gaseous conditions (6% CO₂, 5% oxygen and nitrogen balance) at 37 °C, using Vitrolife sequential media G1™-G2™ (G-series). Embryos cultured in the standard incubator were evaluated at magnification $\times 200$ –400 using an inverted microscope (Olympus). Dishes were removed from the benchtop incubator for morphological assessment approximately 42–44 h after insemination on day 2, 66–68 h on day 3, and 114–116 h on day 5. For the EmbryoScope group, images were acquired every 10 min in 7 focal planes and morphological assessment was made by examining a video of development using the Embryoviewer software; therefore, the assessment was carried out without moving embryos for the incubation. Culture in the benchtop incubator was performed as follows: each pronuclear-stage embryo was transferred in 10- μ l dome-shaped microdrop of G-1™ medium, covered with mineral oil (Vitrolife). On the morning of day 3, 66–68 h post-insemination, embryos were moved from G-1™ to a 10- μ l droplet of G-2™ medium, for extended culture to blastocyst stage. For the EmbryoScope, embryo culture was performed using a 12-well EmbryoSlide™; one embryo was allocated in each well, containing 20 μ l of culture medium covered with mineral oil. On day 3, the slide was paused and embryos were moved to another EmbryoSlide™ containing equilibrated G-2™ medium for further culturing to the blastocyst stage. Embryos were assessed morphologically at cleavage stage (day 2/3) on the basis of number and symmetry of blastomeres, degree of fragmentation, and presence of multinucleated cells according to British Fertility Society and Association of Clinical Embryologists guidelines, published by Cutting et al. 2008 [32]. Cleavage-stage embryos were considered good if had 6–10 symmetrical blastomeres ($< 20\%$ size difference) and less than 20% fragmentation. Blastocysts were classified according to degree of expansion of blastocoel cavity (1–6), quality, and cohesiveness of inner cell mass and trophectoderm cells (A–C), using Gardner's score [33]. Blastocysts with a Gardner score of 2BB or better were considered good quality and suitable for embryo replacement on day 5 or cryopreservation on day 5/6. The proportion

of good-quality embryos on day 2, day 3, and day 5 was compared between the two groups. Criteria for extended culture and embryo transfer at day 5 of development, blastocyst stage, were the presence of at least four good-quality embryos on day 3. If the patient had fewer than 4 good-quality embryos on day 3, the best-quality embryo, based on morphology score, was transferred to the uterus on day 3. Following embryo replacement, all surplus embryos were cultured to day 5 or day 6. Good-quality blastocysts were cryopreserved on day 5 or day 6 using a vitrification protocol with a closed device [34].

The primary endpoints of this comparison were proportion of 4 cell embryos on day 2, proportion of top-quality embryos on day 3 (6–10 cells, less than 10% fragmentation), and proportion of blastocysts on day 5/6.

Statistical analysis

A chi-square test was performed to examine the relation between embryo development at cleavage stage day 2/3 and at blastocyst stage on day 5/6 using the two different types of incubators (ES and SI). Differences were considered statistically significance at the level of $P < 0.05$.

Results

A total of 581 zygotes from 47 patients were cultured in either EmbryoScope time-lapse incubator (ES) or benchtop incubator (SI). Embryos cultured in SI were scored manually on day 2, day 3, and at blastocyst stage on day 5. For embryos cultured in ES, morphological assessment was carried out at the same time points using the Embryoviewer software. There was a significant difference in the proportion of 4 cell embryos identified on day 2, approximately 42–44 h after insemination, between the EmbryoScope time-lapse incubator (ES) and benchtop incubator (SI) (66.8 vs. 50.5%, $P = 0.014$). The proportion of embryos classified as top quality on day 3 (6–10 cells, less than 10% fragmentation) was also significantly higher in the ES group compared to the SI group (75.1 vs. 56.0%, $P = 0.006$) (Table 1).

The proportion of good-quality blastocysts developing by day 5 was higher in the EmbryoScope compared to the benchtop incubator (40.7 vs. 32.6%), but this difference was not statistically significant. A similar trend was observed when considering development to day 6 (ES 8.7% vs. SI 9.4%). The overall blastocyst utilization rate, number of blastocysts suitable for transfer, and cryopreservation on day 5/6 were superior in the ES group compared to SI (49.4 vs. 42.0%, $P = 0.24$), but this trend was not statistically significant (Table 2). It is stated that in some patients, a transfer of the best embryo(s) was done on day 3, which means that eventually, the embryo(s) with the best prognosis to develop to

Table 1 Cleavage-stage embryo development: EmbryoScope vs. benchtop incubator

	4-cell embryo on day 2	Top-quality embryo on day 3
EmbryoScope (ES, <i>n</i> = 322)	66.8 (<i>n</i> = 215)	75.1 (<i>n</i> = 242)
Standard incubator (SI, <i>n</i> = 259)	50.5 (<i>n</i> = 131)	56.0 (<i>n</i> = 145)

blastocyst on day 5/6 was taken out of the culture system; therefore, this embryo is not included in the blastocyst utilization rate. The results given in Table 2 are related only to patients, which all available embryos were cultured to blastocyst stage day 5/6.

Discussion

Recently, clinical practice efforts have been directed toward improving embryo selection. The choice and transfer of embryos with a higher implantation potential can reduce the number of embryos to replace without reducing the chances of pregnancy. To this end, different noninvasive embryo selection methods have been proposed to support information on how to distinguish embryos with better prognosis [35]. There are different methods of embryo grading; all of them are based on morphological evaluation under the microscope which can be subjective [36]. One of the noninvasive method is the EmbryoScope time-lapse incubator, which is capable of performing embryo culture in a stable and controlled conditions. Time-lapse monitoring may provide more information of embryonic development compared to culture in standard incubator. Essentially, EmbryoScope allows compression of the entire course of embryo evolution into a short video. This technology can potentially progress in chronological analyses and dynamic embryo developments, which can make a crucial contribution to ART [37]. Preimplantation embryos possess a certain plasticity and are capable of adapting to the environment, although too large deviations may result in embryo stress and decrease the implantation potential [38, 39]. It

is well documented that variations in temperature and pH may impair embryo quality and development potential [40]. The EmbryoScope incubator displays minimal variation of temperature and gas concentrations when doors are opened as well as a fast recovery to optimal conditions compared with the larger incubators [41]. In addition, EmbryoScope purifies the gas in the chamber by constant recirculation through an active carbon filter, a HEPA filter, to efficiently delete volatile organic compounds contaminants, that can negatively affect embryo quality. The aim of this study was to establish the safety of a commercially available time-lapse incubator by analyzing development of human zygotes in two different incubators: the EmbryoScope and the conventional benchtop incubator, which have been already in use in our laboratory for the past 5/6 years. We found significantly better embryo quality at cleavage stage, day 2/3 following culture in the EmbryoScope compared to a benchtop incubator. A higher number of 4-cell embryos on day 2 and top-quality embryo on day 3 (6–10 cells with less than 10% fragmentation) have been found using the advantages of undisturbed embryo culture in a stable incubation such as the EmbryoScope. We have seen no differences in the proportion of blastocyst on day 5 and day 6 between the two groups. A slightly higher blastocyst utilization rate was reported in the ES group compared to SI, but this trend was not statistically significant. Results obtained in our present study, in terms of blastocyst formation, are in line with the study published by Kirkegaard et al. [42], although they have not found a clear difference on embryo quality on day 2 and day 3 as we have reported. A study presented at the ASRM 2013 compared embryo culture (1) in EmbryoScope (6% CO₂ in air), (2) in EmbryoScope (5% O₂, 6% CO₂, 89% N₂), and (3) in Cook MINC Incubator (premixed 5% O₂, 6% CO₂, 89% N₂). Results showed significant differences in favor of both low-oxygen incubators for day 3 embryos and blastocyst formation. All incubators showed high implantation rate but significant differences were found between 1 and 3. Results obtained in our study were comparable with the mentioned abstract, although the limitation in this trial is that we did not know how much of this improvement was due to the low oxygen concentration or to the incubations itself [43]. In a published study, Cruz et al. compare embryo culture between ES and SI and did not find any significant differences in terms of blastocyst development and pregnancy rate [25]. This clinical trial included human embryo derived from oocyte donor treatment, which may not be representative of embryo from infertile couples, which were instead included in our study.

Although the use of conventional incubators has been common for many years and is the routine in many embryology laboratories, the system has limitation due to the need to maximally reduce the frequency of door openings in order to maintain an optimal internal atmosphere, thereby promoting embryo development. On the other hand, a

Table 2 Blastocyst utilization rate: EmbryoScope vs. benchtop incubator

	Blastocyst day 5	Blastocyst day 6	% total blastocyst rate
EmbryoScope (ES, <i>n</i> = 162)	40.7 (<i>n</i> = 66)	8.7 (<i>n</i> = 14)	49.4 (<i>n</i> = 80)
Standard incubator (SI, <i>n</i> = 138)	32.6 (<i>n</i> = 45)	9.4 (<i>n</i> = 13)	42.0 (<i>n</i> = 58)

single observation represents an inaccurate method for embryo assessment in terms of detecting abnormal cleavage events or multinucleation [44].

Furthermore, reducing frequency of incubator openings has been demonstrated to improve the stability of culture conditions and increase embryo viability in mouse model [45]. In human, Zhang et al. demonstrated that reducing the frequency of observation outside the incubator can result in a significant increase in blastocyst formation [46]. The risk is that if we reduce observations to maintain optimal culture conditions, we might miss important events during development, which can be useful to select the most viable embryo. EmbryoScope time-lapse monitoring has become increasingly important in the embryology laboratory and has the potential to be very useful to the embryologist during the process of selecting the most viable embryo to transfer and identifying timing differences between embryos that look the same [16–20, 37]. It can be a valuable tool and enable the most continuous observation of embryo development, by integrating the incubation and image acquisition into one system, avoiding disturbance to the culture environment. In addition, time-lapse can be used to determine a number of anomalous developments which cannot be seen with conventional culture. Such information is of useful value because higher priority for transfer can be given to embryos not showing such anomalies. Abnormal cell division is more and more appreciated as a new method of embryo deselection. Several studies have established how direct cleavage [44], blastomere fusion, and reverse cleavage are negatively associated with embryo implantation [47, 48]. Liu et al. noted that 27.4% embryos showed evidence of reverse cleavage at least once at any stage during the first 3 days of development. Two components were correlated with reverse cleavage, ovarian stimulation with GnRH antagonists compared with agonists, and ICSI compared with IVF. No correlation was found between female age and reverse cleavage. Reverse cleavage can be connected with both oocytes and sperm quality and significantly affect embryo development, resulting in poor implantation potential [49]. In a cohort study published recently, the authors investigated the clinical meaning of the intercellular contact point in human embryo at four-cell stage and found that an abnormal spatial structure at the 4-cell stage is linked to low implantation [50]. Furthermore, a time-lapse deselection model was recently reported, incorporating both the abnormal cleavage parameters and morphokinetic [51]. In addition a large-scale multicenter study comparing 7 time-lapse embryo selection/deselection models was also recently published by Petersen et al. [52].

Worldwide, the majority of IVF clinics are still performing embryo transfer at cleavage stage; therefore, time-lapse

monitoring could be beneficial to select embryos with early cell division profiles that are predictive of implantation potential and embryo viability. The use of morphokinetic variables for embryo selection allowed embryologist to reject embryos with a lower chance of implantation while distinguishing embryos with higher probabilities of implanting based on morphokinetic characteristics. Several published studies have already demonstrated the predictive value of morphokinetic [37, 53–55]. In terms of evaluation of the time-lapse incubator safety, it is important to mention that embryos are repeatedly exposed to light, when digital images are collected. The EmbryoScope time-lapse system used red light centered at 625 nm and with a lower irradiance level as compared to the white light irradiance levels on the dissection and inverted microscopes. Our results indicate that red light used in the EmbryoScope incubation does not decrease the development and quality of human embryo at cleavage and blastocyst stages. Our results are in agreement with several published studies [25–27]. Mio et al. reported no difference in clinical pregnancy rate after time-lapse observation, compared with standard incubation [26]. Li et al. proved that in both mouse zygotes and porcine embryos, red light used in the time-lapse incubator does not decrease blastocyst formation compared to standard incubation [56]. In addition, a study published by Nakahara et al. concluded that there is a little effect on embryos from exposure during time-lapse observation, therefore can be securely used for clinical purposes [27].

In recent years, the proliferation of several culture media has seen significant improvements and optimizations in ART. Two different approaches to the embryo culture are applicable: sequential and single-step media. The present study used Vitrolife media G1™-G2™, which is a specific sequential formulation to facilitate presentation of different components to the embryos during pre- and post-genomic activation. This approach requires a mandatory change of medium on the morning of day 3 from G-1™ to a G-2™ to extend culture up to the blastocyst stage. In single-step culture, embryos are held in the same dish until the blastocyst stage and culture medium is not refreshed at any point. This culture medium is designed to provide all components to embryos at all times, during all stages of post-fertilization in *in vitro* development [9, 10, 39, 57–61]. The movement of embryos to new dishes on day 3 has been considered as a technique to avoid exposure of embryos to the potential risk of loss or introduction of contaminants through handling errors or pipetting of embryos [62]. Single culture media is particularly advantageous when used in conjunction with continuous embryo monitoring systems. It minimizes stress to embryos as a result of changes in pH and temperature fluctuations and though of limited importance, a reduction in the cost of materials used and time [63, 64]. On the other hand, it is critical that single-step media is designed to

limit the buildup of ammonium by replacement of glutamine with a more stable form, as has been suggested and demonstrated by several published studies [10, 39, 65].

The present study had some limitations. This is not a randomized controlled trial; it is a comparative study with the aim to evaluate the development of human zygotes cultured in either an EmbryoScope or benchtop incubator. The allocation between the groups were not perfectly performed as the patient distribution to the two groups would have been expected to be closer to a 50:50. The allocation was carried out as follows: at the start of the study, the pronuclear-stage embryos were equally split between the ES and SI. Afterwards, if the patient had more than 12 pronuclear-stage embryos, the first 12 were allocated to the EmbryoScope incubator (using a 12-well EmbryoSlide™) and the remaining were assigned to the benchtop incubator.

A minor aspect that could be a potential confounder in our study is the different dishes used and disparity in the volumes of media used in the ES and SI. In the ES incubator, each pronuclear-stage embryo was allocated in a well, containing 20 µl of culture media, while in the benchtop incubator, in a 10-µl dome-shaped microdrop. Flat sterile plastic Petri dishes were used for the culture in the benchtop incubator and for EmbryoScope, a 12-well EmbryoSlide™, which is specifically designed for the culture in the EmbryoScope incubator. The concept behind these dishes relies on fabrication of conical wells into the dish design, so that embryos rest at the lowest point in the wells, where putative embryotrophic factors may concentrate. Both dishes for the ES and SI were critically prepared using appropriate techniques to prevent microdrop dehydration and osmolality change which can impact embryo development [66].

The EmbryoScope time-lapse incubator is CE certified equipment. The CE certificate confirms the quality of the system from Unisense FertiTech in terms of its manufacture and final inspection. The production, installation, and servicing of this incubator was operated from Unisense FertiTech. The incubator was validated before the clinical use; before zygotes were cultured in the EmbryoScope, the sperm survival assay was performed first [30] and afterward was evaluated the division and development of abnormally fertilized human embryos showing 1 or > 2 pronuclei at fertilization check. Therefore, the authors consider not necessary to obtain ethics approval nor informed consent required by the patients.

In this study has been investigated the safety of the EmbryoScope time-lapse incubator evaluating embryo development and the number of 4-cell embryos on day 2 and top-quality embryos on day 3 as primary endpoint. We found a significantly improved embryo quality through the use of the EmbryoScope time-lapse incubator.

Although clinical and implantation rates are the ultimate endpoint of clinical trial in ideal research, [67], we do believe the endpoint assessed in this study to be relevant

for the basic research. To perform a powered study using pregnancy rate or live birth rate with pediatric follow-up would necessitate a significantly considerable number of participants. Even though we do understand the utility of such studies, and they must be undertaken in order to constantly assess the safety of time-lapse incubation, we do think performing a smaller study with satisfactory statistical power to identify differences for a relevant parameter is an important step in research. Culture conditions are crucial during in vitro embryo culture and can also have a negative impact on embryonic gene expression [68]. Therefore, it is important to establish optimal incubation conditions in which stress, epigenetics, and gene expression are not compromised in order to improve embryo development and blastocyst formation, which in turn may increase IVF/ICSI outcomes.

Conclusions

Time-lapse technology offers the possibility to monitor embryo development continuously. The present study evaluated whether the culture promoted by an EmbryoScope time-lapse system would have an impact on embryo development and blastocyst formation compared to conventional daily morphology assessment. We have shown that embryo quality at cleavage stage on day 2 and day 3 is significantly better when embryos are cultured in the EmbryoScope compared to a benchtop incubator. Furthermore, blastocyst formation rate is higher in the EmbryoScope. Although this was not statistically significant, it is still crucial to increase the number of blastocyst formed. Better embryo development is an integral part of the concept of culture in this EmbryoScope incubator and therefore represents an important advantage and may eventually lead to an improved cumulative pregnancy rate.

There is a need to recognize viable embryo with highest implantation potential in order to increase success rate in ART. The main goal of ART should be the birth of one single healthy baby in a reasonable amount of time. Many patients without a positive outcome after several treatment attempts are at risk of dropout due to stress or financial reasons. In that respect, increasing the number of utilizable blastocysts for transfer or cryopreservation will reduce embryo wastage and could be important for patients to achieve a successful pregnancy in a short time.

Culture of human embryos in the EmbryoScope time-lapse incubator supports embryo development equally to benchtop incubator. The EmbryoScope provides a suitable culture environment that does not affect embryo quality at cleavage stage and blastocyst development, therefore can be safely introduced in the embryology laboratory.

Acknowledgements We would like to acknowledge the embryology team for their assistance.

Funding Project support was provided by the Edinburgh Reproductive Endocrine Centre, RIE, Edinburgh, UK.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Human and animal rights All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and with the 1964 Helsinki declaration and its later amendments. For this type of study, formal consent is not required.

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