J Assist Reprod Genet (2011) 28:569–573
DOI 10.1007/s10815-011-9549-1

ASSISTED REPRODUCTION TECHNOLOGIES

Embryo quality, blastocyst and ongoing pregnancy rates in oocyte donation patients whose embryos were monitored by time-lapse imaging

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Received: 8 October 2010 / Accepted: 16 February 2011 / Published online: 11 March 2011

Abstract
Purpose In the current study, our aim was to demonstrate that EmbryoScope incubation conditions is comparable to standard laboratory incubation circumstances by comparing embryo quality, development and ongoing pregnancy rates between the EmbryoScope (ES) and a standard incubator (SI). We analyzed 478 embryos from 60 couples undergoing oocyte donation were included in the study.

Methods All embryos retrieved from a patient were randomly distributed in the ES or SI. We calculated blastocyst development rate, blastocyst viability and ongoing pregnancy rate for embryo transfers from ES, SI and mixed (one embryo from the ES and one from the SI). Statistical analysis was conducted by Chi square tests, considering $p<0.05$ significant.
Results No significant differences were found between the ES and SI from all the parameters evaluated.
Conclusions Thus we concluded that time-lapse monitoring in the EmbryoScope does not impair embryo quality while allowing for morphological, spatial and temporal analysis of embryo development.

Keywords Embryoscope · Time-lapse image acquisition · Embryo quality · Dynamic embryo evaluation · Monitoring embryo development

Capsule EmbryScope incubation conditions and time-lapse image acquisition does not affect embryo quality and reproductive outcome. We evaluated the effects of dynamic assessment of embryo quality, a tool that would give more information from the individual embryo in order to improve selection.

This work was presented in the 66th Annual Meeting of the American Society for the Reproductive Medicine in Denver (Colorado, United States)

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Introduction

Assisted reproduction treatments (ARTs) in humans have been highly successful in helping infertile couples. Development of ART technologies allows observation of morphological events during human embryonic development [1]. Information obtained from microscopic observations have contributed significantly to the great success of ART programs in humans and enhanced in vitro fertilization (IVF) success rates. It is critical to identify viable high quality embryos with the highest implantation potential. Consequently, numerous criteria from embryo selection have been proposed [2, 3] and among these, morphological features have been used extensively in selecting excellence embryos. However, embryo choice for transfer should not be based only on cell number and morphological assessment on the day of transfer [4].

IVF technology allows for observations of early actions of human fertilization and embryogenesis. Nevertheless, concern about effects of handling the embryos has limited the frequency of microscopic observations outside the controlled incubation environment, thus the knowledge of growth rates and morphological changes have deduced largely from morphological appearance of embryos at few discrete time points. Such "freeze-frame" images of the active processes of growth and development necessarily limit the information available to the observer and short-lived processes may be missed entirely. In addition, the dynamic and gradual nature of change in cellular morphology can occult events which only become visible when images are condensed into a continuous and coherent movie [5].

Time-lapse image acquisition can minimize disturbance to the culture environment by integrating the incubation and inspection of embryos into one system. On the contrary, culturing with intermittent observations requires the removal of embryos from the incubator to their examination [6]. Time-lapse cinematography has advanced the understanding of fertilization processes and development of early human embryos. The opportunity to follow the dynamic developmental patterns of embryos using time-lapse monitoring will give us useful information for embryo selection. Time-lapse monitoring allows a phenomenon observed on a specific time point to be linked directly to an embryo's developmental capacity and implantation achievement [7].

In essence, micro-cinematography offers unique possibilities for non invasive studies of early embryogenesis in humans. The EmbryoScope provides such an integrated monitoring system consisting of a safe, controlled culture environment including automated time-lapse high resolution image acquisition which allows for retrospectively detection and testing for critical events during embryo development.

The objective of this study was to show that embryo quality, blastocyst rate and reproductive success rates are comparable for embryos cultured in a standard incubator or in the EmbryoScope.

Methods

In a prospective cohort study, we analyzed 478 embryos from 60 couples undergoing IVF treatment; in order to minimize variations in oocyte quality only oocyte donation treatments were included. Ovum donation is a useful tool with which for studying external variables affecting assisted reproduction treatments, since it reduces the variability of oocyte quality associated with female infertility and also reduces endometrial receptivity variability associated with controlled ovarian stimulation protocols [8].

Donors were between 18 and 35 years old without current or past exposure to radiation or hazardous chemical substances, drug use, no family history or hereditary chromosomal diseases, a normal karyotype, and tested negative for fragile X syndrome and sexually transmitted diseases as stated by Spanish law. All donors had normal menstrual cycles of 26–34 days duration, normal weight range (BMI 18–28 Kg/m^2), no endocrine treatment (including gonadotrophins and oral contraception) in the 3 months preceding the study, normal uterus and ovaries at transvaginal ultrasound (no signs of polycystic ovary syndrome), and antral follicle count (AFC) >20 on the first day of gonadotrophin administration, after down regulation with GnRH analogue.

Age of the patients ranged from 32 to 45 years old. The exclusion criteria were pathologies like endometriosis, hydrosalpynx, obesity (BMI>30), uterine pathology (myomas, adenomyiosis, endocrinopaties, trombophylia, chronic pathologies, acquired or congenital uterine abnormalities), recurrent pregnancy loss, or maternal age over 45 years old (aging uterus).

Calculations of the sample size needed to confirm the absence of differences between groups (non-inferiority) were performed using the main endpoint of the study of the blastocyst rates achievement, given that origin of the embryos afterwards transferred did not depend on the incubator. This calculus was based on the comparison of two binomial proportions (blastocyst rate), assuming non-inferiority hypothesis between ES and SI with respect to the blastocyst rate, based on a pre-defined non-inferiority limit of 0.66 for the odds ratio (OR) of Embryoscope versus Standard Incubator (corresponding to limits of −9 to −10% in the difference in proportion scale with an overall Blastocyst rate of 50–60%). The non-inferiority criteria were based on our clinical data and those differences which were considered clinically relevant [9].

To guarantee that the culture conditions in both of incubators are comparable, every day we check temperature and the CO_2 concentration with external sensors in order to confirm that they were in the values we fixed (6% CO_2; 37.0°C); pH is not measured daily but we adjust it between 7.2 and 7.4 when incubators are restarted.

In all donors, ovarian stimulation was achieved by pituitary desensitization using a GnRH analogue followed by stimulation with gonadotrophins [10]. The daily dose of FSH was adjusted according to the donor's ovarian response based on serum estradiol levels and the number and size of ovarian follicles as considered by transvaginal ultrasonography. Oocyte recovery was scheduled 36 h after hCG injection and conventional IVF or ICSI was performed after oocyte aspiration.

For all the recipients who were still cycling, down-regulation was carried out using a GnRH analogue. The day

that the donor announced the onset of her period, the recipient was informed to start with oral estradiol valerate and lately, intravaginally micronized progesterone for luteal-phase supplementation was used.

Successful fertilization was verified by confirmation of two pronuclei 17–19 h after insemination/microinjection. Embryos were randomly selected and evenly distributed in the Embryoscope (n=238) and the standard incubator (n=240); in the EmbryoScope images were acquired every 20 min in 7 focal planes. Embryos in the standard incubator as well as embryos in the Embryoscope were standing apart once a day to assess embryo quality; day 2 and day 3 embryos were categorized in four grades from A (high quality) to D (low quality) depending on the number of blastomeres, fragmentation, multinucleation and symmetry; blastocysts were evaluated according to inner mass cell (ICM), trophoctoderm (TE) and blastocoel expansion degree [11].

Grade A embryos have 7–8 cell, <10% fragmentation, even size and no multinucleation; grade B embryos have 7–8 cell with 11–25 fragmentation, 9 evenly sized cell or more and no multinucleation; grade C embryos have <6 cell, 26–35% fragmentation, uneven cell allowed but no multinucleation; and grade D, if the embryo has 6 cells or less and observation of multinucleation, >35% fragmentation or type IV fragmentation.

Blastocysts were classified as viable or non viable: viable if they were transferred and/or frozen and non viable if they were arrested or they were poor quality embryos. We also calculated ongoing pregnancy rate (OPR) for exclusive transfers from we embryos cultured in the ES, the SI and mixtures where one embryo from the ES and one embryo from the SI were transferred (M).

Transfers were performed on day 3 and day 5, but embryo selection criteria was based solely on the valuation at the same time points (44 and 68 h after fertilization procedure) on a morphology basis, both for the ES and SI groups, hence the additional information provided by time-lapse monitoring system was not used for embryo selection.

The results were compared depending on embryo culture environment by using a Chi squared test and significance level of $p<0.05$; data are presented as proportions and 95% confidence interval (95%CI).

Results

The average number of oocytes that were received per donor was 13.4 (CI95% 12.4–14.4). A total of 478 embryos were analyzed from 60 couples with a median of 8 embryos per couple (range 4–15; average 8.84). Contrast of embryo quality grading at day 3, revealed similar distribution from ES and SI groups respectively: A (n=79) 33.1% (95%CI 26.8–39.4) vs (n=85) 35.6% (95%CI 29.9–41.3); B (n=41) 17.1% (95%CI 12–22.2) vs (n=38) 15.7% (95%CI 11.3–20.1); C (n=48) 20.4% (95%CI 15–25.8) vs 20.6% (n=50) (95%CI 15.8–25.4) and D (n=70) 29.4% (95%CI 23.3–35.5) vs (n=67) 28.1% (95%CI 22.7–33.5), p=0.941.

Blastocyst rate, the percentage of transferred and frozen embryos and non viable discarded embryos were not significantly different between the embryos from the standard incubator and embryos cultivated in the embryo monitoring system (Table 1). Blastocyts OR (Embryoscope vs. Standard Incubator) were 1.158 (CI95% 0.739–1.689), confident intervals were above the limits of the non-inferiority hypothesis and in consequence sample size can be considered adequate. The proportion of viable embryos, resulting from grouping those transferred and frozen were (n=68) 28.5 (95%CI 22.8–34.2) for embryos developed in the time-lapse incubation system and (n=84) 35% (95%CI 29–41) for those developed in the standard incubator, p=0.438.

When we compare how many embryos are transferred in day 3 or day 5 in each incubator we did not find significant differences between the Embryoscope and the Standard Incubator (Table 2). From those embryos transferred in day 3 (22/31) 71% (CI95% 55–87) from the Embryoscope and (18/24) 75% (CI95% 57.7–92.3) from the classical incubator were A or B embryos, as of those blastocyst transferred in day 5 (17/19) 89.4% (CI95% 75.6–103.2) from the time-lapse video system and (30/34) 88.2% (CI95% 77.4–99) from the standard incubator were excellent blastocysts.

No significant variation was found between the ongoing pregnancy rate for embryos incubated in the Embryoscope (6/14) 42.8% (95%CI 16.9–68.7) and in the standard incubator (8/19) 42.1% (95%CI 19.9–64.3) and mixed transfers (8/24) 33.3% (95%CI 14.5–52.1), p=0.399. According to implantation rate, we did not observe any significant difference due to the reduced number of pure

Table 1 Blastocyst rate, proportion of transferred, frozen and discarded embryos incubate in the EmbryoScope vs. the standard incubator

	Blastocyst	CI95%	Frozen	CI95%	Transferred	CI95%	Discarded	CI95%
Embryoscope (n=238)	54.8 (n=130)	47.5–62.1	7.6 (n=18)	2.8–12.4	21.0 (n=50)	15.9–26.9	71.4 (n=170)	65.7–77.1
Standard incubator (n=240)	50.6 (n=121)	44.3–56.9	10.9 (n=26)	7–14.8	24.1 (n=58)	18.7–29.5	65 (n=156)	59–71
p	ns		ns		ns		ns	

Table 2 Proportion of embryos transferred depending on the incubator

	Transferred day 3	CI95%	Transferred day 5	CI95%
Embryoscope	62% (n=31)	48.6–75.4	38% (n=19)	24.6–51.4
Standard incubator	41.3% (n=24)	28.6–54	58.6% (n=34)	45.9–71.3
p	ns		ns	

transfers from each incubator; the values were as follows: for exclusive transfers from Embryoscope were (8/26) 30.8% (CI95% 13.0–48.5) vs (14/34) 41.0% (CI95 24.7–57.7) obtained when we transfer two embryos from the standard incubator.

Discussion

Embryo selection based on discrete routine assessment of embryo morphology is not always associated with a higher implantation or pregnancy rate. Therefore, other criteria should be considered in the recognition of embryos with a good projection for implantation or pregnancy. Time-lapse cinematography imaging has rendered more information of human embryonic development and growth behaviour in comparison with conventional practice or intermittent observation. Basically, this has shed more light on the elementary descriptions of embryo development [12] and enables compression of the entire course of early embryo development into a short movie; hence this technique can potentially progress in morphologic and chronologic analyses of dynamic embryo developmental patterns, making an important contribution to ART [13].

The combination of optimal incubation conditions for early developing embryos and a time-lapse embryo monitoring system are certainly of special meaning in terms of gaining complete information of the development of embryos and assessing their quality for transfer and freezing. In the present study, we illustrate that embryos incubated in the included culture and monitoring system are not affected by the continuous observation associated with the EmbryoScope. Hence, this system offers new openings to get detailed and precise information about morphological characteristics and cleavage status of growing embryos in combination with a safe and controlled incubation environment.

It is important to highlight that embryos cultured in an incubator with an integrated time-lapse system are periodically exposed to light when digital images are acquired and it is known that light exposure is an unnatural stress to embryos and might affect embryo enlargement [6]. Even though the risk of impaired development in these culture surroundings, each embryo were exposed to LED lighting less than 50 s per day; however, we did not find significant differences between embryos cultured with time-lapse cinematography and embryos with discontinuous observations in a conventional incubator.

It should be noticed that extra information, for example timing of early embryo development from the EmbryoScope were not used for selecting high quality embryos for transfer and freezing. Hence, we can conclude that embryo value and reproductive outcomes do not diverge between the series in a classic incubator and the EmbryoScope. The blastocyst development rate and fraction of viable embryos was not notably different between the two groups; even though when we focus in the origin of the embryos (Embryoscope or standard incubator) and in the day of the embryo transfer (day 3 vs day 5), we also did not observe relevant differences, confirming the usefulness of this new system.

These data, along with the similar results in ongoing pregnancy rate in the three different groups (ES, SI and M) confirm that this time-lapse monitoring system is suitable for both research and clinical use. Validating the embryo culture in the EmbryoScope subsequently allows studies aiming at detecting new valuable selection parameters thus optimizing selection of high quality embryos.

This experimental design is closely related to recent publications [14] that assess time-lapse technology effectiveness; not counting the methodological differences between the two studies (we obtained the data from fresh and ongoing cycles while they worked with frozen and supernumerary embryos), both of works can emphasize the time-lapse system as a tool with potential useful in basic research and further clinical practices.

As we have mentioned previously, we analyze embryos from oocyte donation programs in order to avoid introducing bias concerning oocyte quality, so we are likely working with good prognosis females gametes; even though, uterine receptivity is more stable avoiding interference of controlled ovarian stimulation in uterine receptivity [8] However, results presented in this paper could not be necessary as relevant in infertile couples as are in this model (oocyte donation).

It maybe expected that in the future, the employ of the data from time-lapse observation will promote the improvement of non invasive methods for the assessment of embryo viability (15). Non invasive imaging is helpful not only for explaining the morphological proceedings in human embryos but also for evaluating the physiological importance of these events during the early stages of development. The introduction of this new technology is an exciting and

powerful tool for viewing cellular activity and embryogenesis in a coherent, uninterrupted manner, otherwise not available by analysis in real time.

In conclusion, the Embryoscope provides an adequate culture environment for time-lapse imaging that does not affect embryo quality, blastocyst development or viability. It thus is a useful device for monitoring the timing of early cleavages.

Acknowledgements Authors want to thank Sara Fortuño, Noemí Galindo, María Roldán, Niels Ramsing and Karen Marie Hilligsøe for their clinical and technical support in the management of this paper

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