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Original article

Elimination of stress factors by continuous embryo culture and its influence on *in vitro* fertilization outcomes

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ABSTRACT

Recently, infertility has become one of the most important endemic conditions, affecting approximately 15–20 % of couples worldwide. Among others, the careerist lifestyle, the increasing maternal age and the parallel increment in the aneuploidy rate of embryos play a crucial role in this phenomenon. In this study, embryological parameters and pregnancy outcomes were investigated in IVF cycles using either sequential embryo culture or a single step culture system. By sequential media, oocytes/embryos are needlessly exposed to the potentially negative effects of light exposure, temperature decrement and altered oxygen tension. In comparison with sequential media, single step media induced 1.28, 1.21 and 1.21-fold increments in implantation, biochemical pregnancy and clinical pregnancy rates, respectively. Pregnancy outcomes showed strong maternal age-dependency, so the difference between the two investigated culture systems was equalized by the increasing maternal ages (35–44 years) and the supposed incidence of embryo aneuploidy. Nevertheless, the significant enlargements in the outcomes of the younger ages (25–34) induced by the single step cultures suggest that, beside the resultant maternal aneuploidy, aneuploidy (reduced pregnancy rates) may evolve from exposure to the mentioned environmental stress factors.

1. Introduction

In vitro production (IVP) of mammalian embryos is comprised of three inevitable steps that involve: (i) *in vitro* maturation (IVM) of oocytes; (ii) their *in vitro* fertilisation (IVF) or reconstruction with the use of cloning by somatic cell nuclear transfer (SCNT); and (iii) *in vitro* culture (IVC) of fertilised or cloned embryos [1–4]. Extracorporeal fertilisation can be achieved by conventional gamete co-incubation or can be performed microsurgically by intracytoplasmic sperm injection (ICSI) into metaphase II-stage oocytes [5,6]. The outcome of IVP is highly biased by extrinsic stimulation of embryonic developmental program in meiotically matured mammalian oocytes. This latter can be triggered by either intrinsic spermatozoon-inherited molecular factors or non-biological (i.e., artificial) stimuli applied to activating ICSI-fertilised or SCNT-reconstructed oocytes [7–11]. Furthermore, the overall IVP effectiveness results largely from physical and chemical conditions of embryo culture microenvironment that have impact on perpetuating capabilities of parental genomes or somatic cell-descended nuclear genome to be epigenetically reprogrammed in IVF- and SCNT-generated embryos, respectively [12–16]. It is also beyond any doubt that intergenomic cross-talk between nuclear and mitochondrial compartments as well as onset and progression of apoptotic or autophagy-dependent cell death play indispensable roles in determining not only *in vitro* developmental competences, but also cytological and molecular quality parameters noticed for mammalian fertilised or cloned embryos [17–21]. To the best of our knowledge, taking into account clinical protocols of IVP intended for humans, SCNT-based approaches are completely excluded from the medical and ethical points of view [22,23]. Moreover, it is noteworthy to point out that only post-ovulatory oocytes that have been recovered from oviducts of super-stimulated female patients provide a source of *in vitro*-fertilised zygotes for a wide spectrum of clinical IVP-mediated systems [24,25].

Because of the changing metabolism of embryos during their development, from oocyte to blastocyst various compositions of necessary nutrients are required. On the first 3 days of embryonic development the main source of sugars is basically piruvate and lactate, but in the later phases glucose plays an enormous role and is the precursor for triacylglycerols, phospholipids, glycoproteins and complex sugars

Abbreviations: AFC, antral follicle counts; AMH, anti-Müllerian hormone; β-hCG, beta subunit of human chorionic gonadotropin; BMI, body mass index; CO_2, carbon dioxide; FSH, follicle-stimulating hormone; ICSI, intracytoplasmic sperm injection; IVF, *in vitro* fertilization; IVM, *in vitro* maturation; IVP, *in vitro* production; LH, luteinizing hormone; O_2, oxygen; PVP, polyvinylpyrrolidone; ROS, reactive oxygen species; SCNT, somatic cell nuclear transfer

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[26–28]. From the 20 different, common and naturally occurring amino acids, the non-essential amino acids positively influence the growth of early cleavage embryos, but the presence of the other essential amino acids during this stage have adverse effects on embryo development and viability [29,30]. On the other hand, the time-dependent proteolysis of amino acids (especially L-glutamine) can be a source of embryotoxic ammonium, which may accumulate in the absence of culture media renewal or replacement [31]. Beside these nutrients, different salts (sodium chloride, potassium chloride, calcium chloride sodium citrate, monopotassium phosphate magnesium sulphate and sodium dihydrogen phosphate) and buffers are important parts of culture media and regulators of their pH. Most of the obtainable sequential or single step media are in the 7.2–7.4 pH range, but in some cases this value is even more alkaline. The intracellular pH of embryos also changes during their development. An early cleavage embryo has its pH homeostasis around 7.2 [32], but from the compactation due to tight junctions between cells, a more sophisticated pH control is available and enables higher pH of media [33,34]. During the laboratory work, the applied CO_2 content (in both the incubators and the environment) highly influences the pH value. Complicating the situation, the presence or absence of chelators, macromolecules, vitamins, antibiotics or indicators can also be different between sequential and single step media, which highly affect their efficiency and this phenomena can also be applied to the different manufacturers [35–37].

On the other hand, the contradiction derives not just from the changing metabolic requirements of cells, but also from the discrepancies of pregnancy outcomes after the usage of sequential or single step media. In the study Sfontouris et al. [38], oocytes were equally randomized into a sequential (ISM1/BlastAssist) or a single step medium (Global). On day 3, the culture media were changed with fresh media and on day 5 blastocyst transfer was performed. Culturing in the single step medium induced 27.7 % gain in the blastocyst formation rate as well as in the rate of good quality blastocysts (30.9 %). Both the increased number and quality of embryos enable freezing of more cells and more frozen cycles. Similar results were published in several cases [39–42]. This tendency was also reported on day 2 or 3 [43]. Interestingly, Nanassy and coworkers [44] published an opposing result with the single step culture system. On day 5, no differences could be observed in the numbers of blastocysts and scores of embryos were significantly lower. Moreover, the elevated embryological parameters manifested neither in the ongoing pregnancies nor in the live births [40,43–45]. In a recent study, Peña et al. [46] investigated the effects of culture media renewal performed on day 3 on pregnancy rates. Without renewal, a nonsignificant ascendant trend was observed both in β-hCG tests and clinical pregnancies. This raises a question: aside from the different chemical compositions of sequential and single step media, with a single step media the unnecessary disturbance of cells can be avoided [47]. During embryo development, the occurrence of stress factors is very high. These possible stress factors are the sub-optimal temperature, pH, light exposure and oxygen [48].

The aim of the present analysis was to compare the embryological and pregnancy results of ICSI cycles used Origio Cleav-Blast sequential media with disturbance on day 3 or Origio Sage 1-Step media without disturbance.

2. Material and methods

2.1. Study design

A retrospective analysis was carried out on the data of 488 ICSI cycles closed with blastocyst transfers during 2017 and 2018. At the end of 2017, our Institute started to use a single step medium for embryo cultures. The objective of this present analysis was to compare the 243 ICSI cycles from 2017 using sequential media with the 245 cycles of 2018 (and the end of 2017) using single step medium (Fig. 1). The only exclusion criteria was the lack of own oocyte, cycles with donor eggs were excluded. We intended to correlate their clinical, embryological and pregnancy parameters.

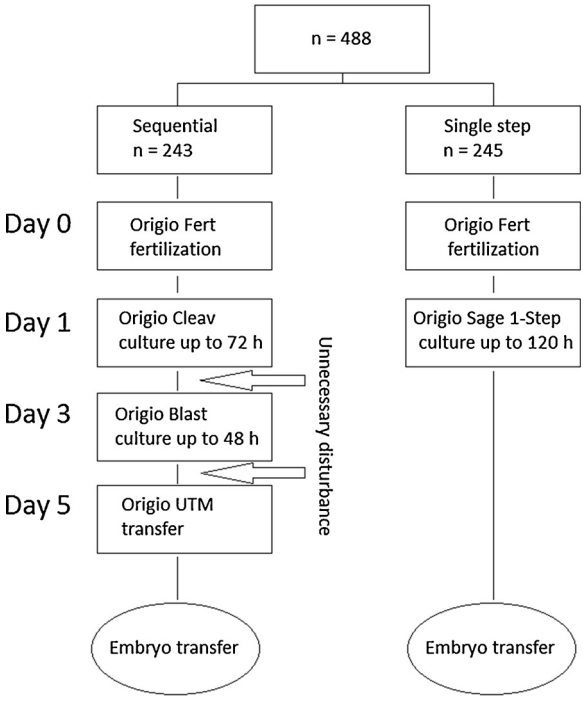

Fig. 1. Flow chart of patients screened for the retrospective analysis and the applied embryological cultivation processes.

2.2. Stimulation

For all patients controlled ovarian hyperstimulation was performed using long protocol desensitization and gonadotropin stimulation. For desensitization daily 0.5 mL (0.525 mg) buserelin (Sanofi-Aventis, France) was administered subcutaneously (s.c.) starting in the mid-luteal phase of a previous cycle. When desensitization was achieved daily buserelin was reduced to 0.25 mL (0.2625 mg) and stimulations were performed either using follitropin alpha (Gonal-F, Merck, Germany), or follitropin alpha - lutropin alpha (Pergoveris, Merck, Germany) s.c. in individual doses depending on the age, AMH level and antral follicle count (AFC) of the patient varying from 150 IU to 450 IU. Follicular growth was controlled by regular vaginal ultrasound folliculometry usually 2–4 times in a cycle. When the dominant follicle reached 18 mm in diameter chorionic gonadotropin alpha (Ovitrelle, Merck, Germany) was administered s.c. in 500 µg dose to trigger the ovulation. 36 h later follicle aspirations were performed in general anesthesia with ultrasound guided needle using a vaginal probe. Luteal phase support was started on the day of follicle aspiration using progesterone gel intravaginally (Crinone, Merck, Germany) in 90 mg daily dose. Pregnancy tests were performed 12–16 days after follicular puncture.

2.3. Embryo culture and transfer

pH-stabilized Origio® media were used for fertilization, culture and transfer. Namely, Origio® Fert™ was used for oocyte preparation and fertilization. (i) In case of sequential media, after the fertilization, the cells were transferred into Origio® Cleav™ and cultured up to 72 h; then transferred into Origio® Blast™ for blastocyst formation up to 120 h; Origio® UTM™ was used for embryo transfer. (ii) In case of single step medium, after the fertilization, the cells were placed into Origio® Sage 1-Step™ HSA medium and cultured until the embryo transfers. The same medium was used for blastocyst transfers (Fig. 1). Cells were

Table 1
The compositions of used media given by the manufacturer.

Component	Sequential Cleav %	Sequential Blast %	1-Step %
Amino acids	< 0.01	< 0.01	< 0.01
Calcium lactate pentahydrate	< 0.01	< 0.01	< 0.1
Calcium pantothenate	< 0.01	–	–
EDTA	< 0.01	< 0.01	< 0.01
Folic acid	< 0.01	–	–
Gentamicin sulphate	< 0.01	< 0.01	< 0.01
Glucose	< 0.01	< 0.01	< 0.1
Human serum albumin	< 1	< 1	< 1
Hydrogen chloride	< 0.01	< 0.01	< 0.01
Magnesium sulfate pentahydrate	< 0.01	< 0.01	< 0.1
Phenol red	< 0.01	< 0.01	< 0.01
Potassium chloride	< 0.01	< 0.01	< 0.1
Potassium phosphate monobasic	–	–	< 0.1
Pyruvic acid, sodium salt	< 0.01	< 0.01	< 0.1
Sodium bicarbonate	< 0.01	< 0.1	< 1
Sodium chloride	< 1	< 1	< 1
Sodium dihydrogen phosphate	< 0.01	< 0.01	–
Sodium hyaluronate	< 0.01	< 0.01	< 0.1
Sodium hydroxide	< 0.01	< 0.01	–
Synthetic serum replacement	–	< 0.01	–
Trisodium citrate dihydrate	< 0.01	< 0.01	–
Vitamins	–	< 0.01	–
Water	> 90	> 90	> 90

incubated at 37 °C, supplemented with carbogen gas containing 5% oxygen, 6% carbon dioxide and 89 % nitrogen. For ICSI, 10 % polyvinylpyrrolidone (PVP) was used and sperm cells were placed into PVP 30–40 min before ICSI. The criteria of ICSI were male partner infertility (37–38 % of cases), severe endometriosis, known immunological factor, advanced maternal age, previous extracorporeal fertilisation failure or at least two unsuccessful cycles. Embryo transfers were performed on day 5 and 1 or 2 embryos were transferred.

2.4. Statistical analysis

Data were given as mean and SD. The Shapiro-Wilks test was used to evaluate the distribution of the data. Non-normally distributed variables were examined using the non-parametric test of Mann–Whitney or Kruskal–Vallis with Dunn's test in case of more groups. Differences between proportions or rates were evaluated with the Fischer exact test. GraphpadInStat 7.0 software was used for statistical analysis.

3. Results

Most of the components are the same is the used three media (Table 1). The main difference in the sugar composition can be found. Namely, the amount of calcium lactate pentahydrate, glucose and sodium pyruvate are higher in the single stem medium, because of the longer cultivation time.

The mean age, the mean of recorded clinical parameters (BMI, AFC, AMH, FSH and LH) and the mean of embryological parameters, such as retrieved oocyte number, fertilizable oocyte number, number of fertilized oocytes, total number of cultured viable embryos per patient, the number of transferred embryos and the number of embryos frozen did not differ between the two groups (Table 2). Based on these results, it is reasonable to say that there were no significant differences between pregnancy chances of women enrolled into the analyzed categories, and the possible outcomes are similar.

Despite similar demographical, clinical and embryological backgrounds, significant alterations were observed between sequential media and single step medium (Table 3). 1.28-fold increment was found in the implantation rate of transferred embryos. In comparison to sequential media, the biochemical pregnancy rate and the clinical pregnancy rate increased by 11.41 % (p = 0.0068) and 9.04 % (p = 0.0279), respectively. The miscarriage rate did not differ significantly.

Table 2
Demographics, clinical characteristics and embryological parameters in case of sequential media and single step medium.

	Sequential		Single step		Single step % of sequential	
	Mean	SD	Mean	SD	%	p
Number of patients	243		245			
Age, years	34.32	4.33	34.46	4.34	100.40 %	0.2963
BMI, kg/m^2	23.74	4.88	23.94	4.83	100.85 %	0.2214
AFC, #	13.45	6.13	13.87	6.05	103.14 %	0.1765
AMH, ng/mL	3.06	2.83	3.19	3.54	104.14 %	0.4959
FSH, IU/L	7.63	2.85	7.39	2.33	96.99 %	0.3457
LH, IU/L	6.06	4.81	5.74	2.84	94.70 %	0.3618
# Oocytes retrieved/ patient	11.91	5.29	12.48	5.72	104.80 %	0.1739
# Fertilizable oocytes/ patient	10.25	4.46	10.82	4.97	105.55 %	0.1359
#Fertilized oocytes/ patient	7.18	3.62	7.60	4.21	105.89 %	0.2760
Total # embryos that grew *in vitro*/patient	2.79	1.72	3.07	2.27	110.30 %	0.2047
# Embryos transferred / patient	1.68	0.47	1.73	0.45	102.83 %	0.1257
# Embryos freezed/ patient	1.09	1.66	1.32	2.17	121.64 %	0.2045

AFC, antral follicle counts; AMH, anti-Müllerian hormone; BMI, body mass index; FSH, follicle-stimulating hormone; LH, luteinizing hormone.

Table 3
Pregnancy outcomes comparing sequential media vs. single step medium.

	Sequential	Single step	p
Implantation rate	29.62 %	37.90 %	**0.0135**
Biochemical pregnancy rate	52.67 %	64.08 %	**0.0068**
Clinical pregnancy rate	41.98 %	51.02 %	**0.0279**
Miscarriage rate	15.69 %	17.60 %	0.4203

With similar number of transferred blastocysts, in comparison to sequential media the usage of single step medium resulted in 30.7 % increment in the biochemical pregnancy rate in the 25–29 age intervals (p = 0.0292) (Fig. 2). In case of older categories, the biochemical pregnancy rates were equalized. The same phenomena was observed in the clinical pregnancy data, 31.1 % was recorded in the 25–29 age group (p = 0.0289) and no differences in 30–34, 35–39 and 40–44 age groups. In parallel with these, based on the data of Franasiak et al. [49], the aneuploidy rates of embryos increase continuously, 27.9 %, 29.5 %, 42.7 % and 74.8 %.

After the division of patients by the number of transferred embryos, when single embryo transfer was performed, the mean ages were 33.9 and 34.8 with sequential and single step media, respectively (Fig. 3). In case of double embryo transfers, these values were 34.5 and 34.3, respectively. With regards to the cycle outcomes, single step embryo transfers resulted in only non-significant improving both in biochemical and clinical pregnancies (because of the low sample size).

4. Discussion

The success of an IVF cycle depends upon several factors: (i) properly performed controlled ovarian hyperstimulation, (ii) technique of oocyte fertilization, (iii) composition of culture media, (iv) appropriate environmental conditions during IVC, (v) proper micromanipulation and embryo transfer, and finally (vi) proper embryo selection [50]. The main subjects of this study are the culture media-dependent alterations of environmental factors and their effects on pregnancy outcomes.

The composition of different culture media is highly influenced by the manufacturer and which stage of oocyte/zygote/embryo

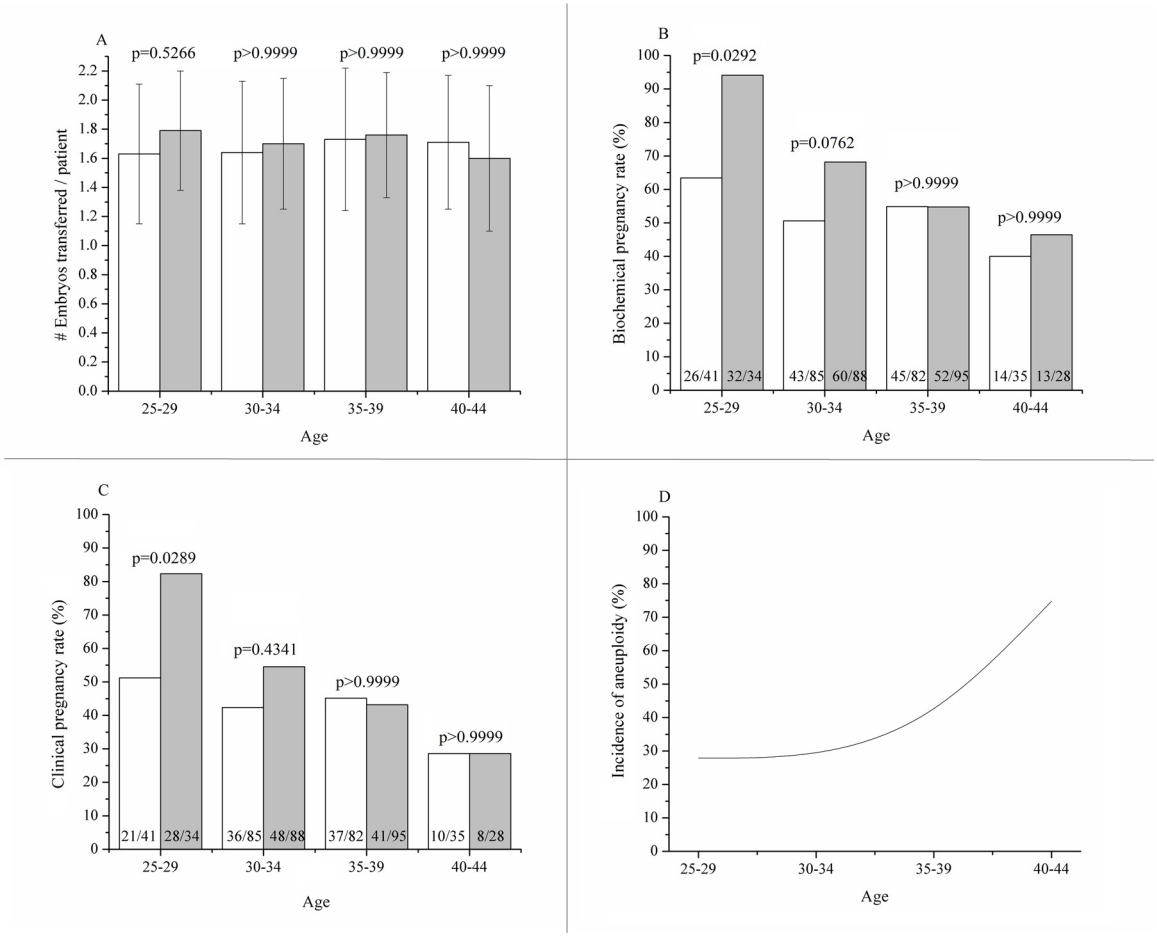

Fig. 2. (A) Mean numbers of transferred blastocysts by age and applied media (white bars: sequential media; grey bars: single step medium); effects of maternal age on (B) biochemical and (C) clinical pregnancy outcomes; and (D) on the incidence of blastocyst aneuploidy (data adapted from the paper of Franasiak et al. [49]).

development is targeted [36,47]. The differing opinions about sequential and single step media spring from the above mentions. In our study, the composition of the used culture media was roughly similar, but also showed some major differences, the sugar ingredients were present in higher concentrations (based on the declared documents of the manufacturer, Table 1). Lactate supports the first cleavage of zygotes [51] and glucose plays a crucial role as sugar substrate during the blastocyst stage of embryo development [26]. Does not matter how different the subject of a research, in case of comparison of several media, the little differences in their compositions cannot be ignore, however unfortunately it cannot be avoided it will always be in the background. This hypothesis has been tried to investigate by the comparison of results of day 3 embryo transfers between the two culture systems. Unfortunately, our day 3 data are not suitable for comparison because the application of sequential medium is “pre-planned”, but the application of single step medium finished with day 3 embryo transfers is a consequence of less retrieved oocytes or worse fertilization.

Nevertheless, the differences between the sequential and single step culture systems come not only from the potentially differing compositions, there is another distinctive reason. The usage of sequential media involves “unnecessary” disturbances. For example, in our lab the transfer of cells from one culture medium to another and their checking takes 3–3.5 min and the translocation of embryos pending transfer into a transfer medium is an additional 0.5 min (depends on the structure of the lab and habits; data not presented). During this time several disturbances can occur: e.g. light exposure, higher O_2 tension and room temperature [48].

4.1. Light

In the paper of Fischer et al. [52] thymidine incorporation as an indicator of rabbit embryo’s cell proliferation was investigated after different exposures to visible light. A time-dependent decrease in the thymidine incorporation was observed and the co-exposure with room temperature enhanced this effect. However, we have to emphasize that for significant results a few hours of exposure were needed. Beside the exposure time, the light intensity (lux) and the wavelength (nm) of light during embryo manipulation are also important factors. Blastocyst formations of hamster embryos showed sensitivity to the increasing light intensity; 200 lux, 500 lux and 900 lux induced 38 %, 49 % and 56 % decrements, respectively. In addition, compared to the visible ray without filtering (390 − 750 nm), blue ray induced 51 % decrease in the blastocyst formation (35 % vs. 51 %) and the red ray was the less harmful (16 %). In parallel with the above findings, both the intracytoplasmic concentration of reactive oxygen species (ROS) and the incidence of embryonic cell apoptosis were increased [53]. Similar findings were reported in several cases [54–58].

4.2. Temperature

Every biochemical reaction, living cell and organism has an optimal temperature range to function or live. This is the case with the oocytes and embryos as well. Movement of oocyte containing dishes to room temperature for only 2 − 3 min can reduce the temperature of culture media by 5 − 8 °C and may induce a time-dependent increase in the incidence of spindle abnormalities and chromosomal dispersion. These

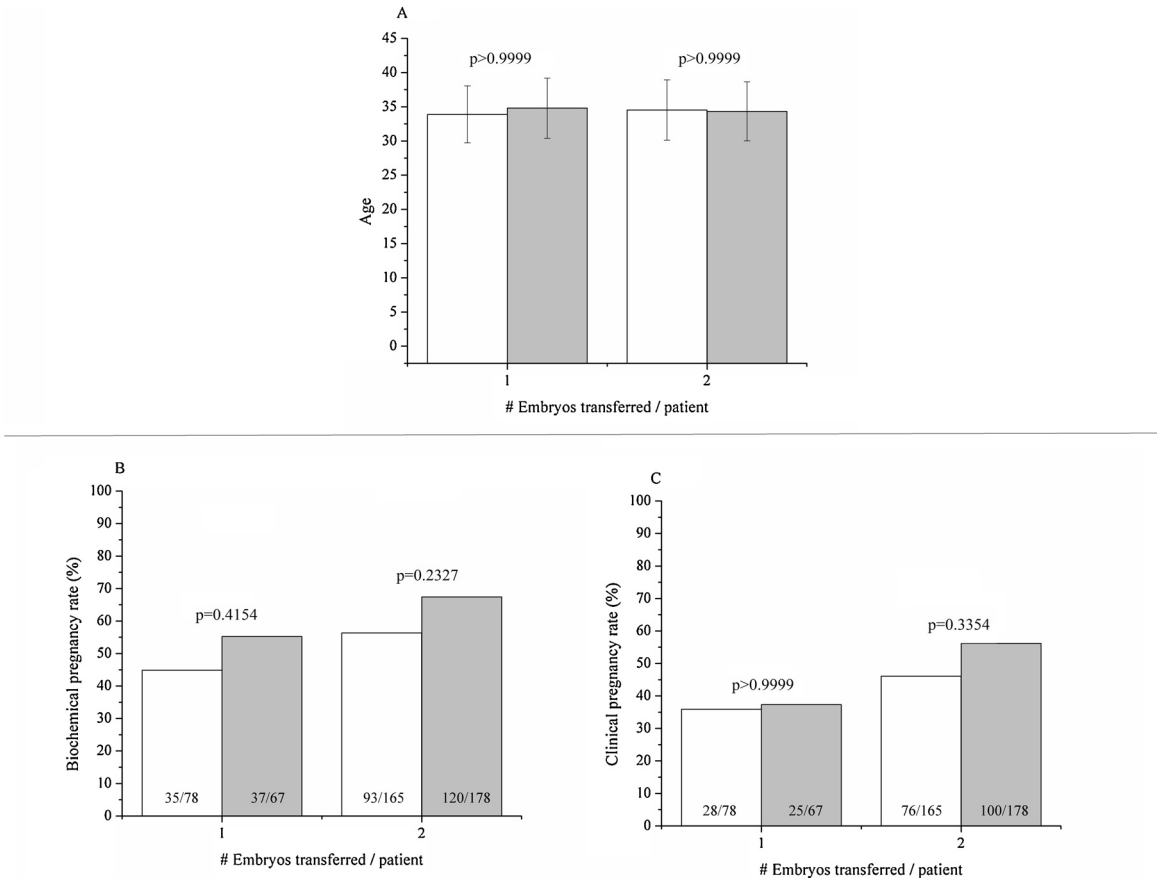

Fig. 3. (A) Mean ages of women by the number of transferred blastocysts (white bars: sequential media; grey bars: single step medium); effects of the number of transferred blastocysts on (B) biochemical and (C) clinical pregnancy outcomes.

negative effects are partially reversible by the replacement of dishes into 37 °C; however, 10 min caused irreversible changes [59–61]. During ICSI, the strictly controlled thermal control of microscopes is indispensable. The length of the ICSI procedure may increase as a function of retrieved number of oocytes. With inadequate systems the temperature of culture medium can decrease by 3 – 4 °C and may result in increased spindle abnormalities, decreased fertilization and pregnancy rates [62]. The altered temperature also has significant impact during *in vitro* development. In comparison to control (37 °C), incubation at 36 °C showed 9.1 %, 8.2 % and 7.2 % decreases on mean number of cells on day 3, mean blastulation rate and mean rate of usable blastocysts, respectively [63].

4.3. Oxygen

The average concentration of O_2 in the oviduct-uterus tract fluctuates within 2–8 % in mammalians [48], and the applied incubators roughly simulate this value range with a generally applied O_2 concentration of 5 %. In contrast, atmospheric concentration of O_2 is ~20 %. During mitochondrial respiration, approximately 1–5 % of molecular O_2 is converted into superoxide anion through the mitochondrial complexes and induces oxidative stress processes [64]. Oxidative stress occurs when the concentration of ROS exceeds the regulator-ability of antioxidant molecules [65], but during the early stages of embryo development genes of these antioxidants are not expressed [66] and ROS may interfere with almost every component of living cells (sugars, proteins, lipids and nucleic acids).In comparison to 5%, incubation in 20 % O_2 caused time-dependent increase in the hydrogen peroxide production of embryos [54]. This manifested in a decreased cell number and blastocyst formation [67,68] and pregnancy rate [69].

As it has been mentioned above, PVP was used for sperm immobilization. Unfortunately, some side effects of PVP have already been published earlier. In the review of Kato and Nagao [70] damage of sperm membranes, induction of acrosome reaction, reduced fertilization, cleavage and blastocysts cell number were published in ICSI cycles using PVP in time-dependent manner. Significantly reduced clinical pregnancy rates were observed which explains our lower outcomes in comparison with the literature. In addition, the indication of ICSI was not only male-factor infertility but severe endometriosis, known immunological factor, advanced maternal age, previous extracorporeal fertilisation failure or at least two unsuccessful cycles. It has been already proved that in case of non-male-factor infertility the usage of ICSI does not improve the outcomes, IVF is recommended [71], but in our study, the ratio of male-factor and non-male factor infertility between the two groups did not differ (37 %–63 % and 38 %–62 %). Nevertheless, PVP and ICSI were used uniformly in the two investigated groups. They had similar effects on both groups. It is assumed they did not affect the comparison of sequential and single step culture systems. Putting these aside, our results clearly demonstrate the positive effects of undisturbed embryo culture by a single step medium (Tables 2 and 3, Figs. 2 and 3). Consistent with earlier findings, a positive tendency (nonsignificant) was observed in the number of embryos developed *in vitro* but this difference was not as conspicuous as published [38–42]. In contrast to the literature [40,43–45], an important novel finding of this study is that the undisturbed environment resulted in increased biochemical and clinical pregnancy rates which depended both on the maternal age and the number of transferred embryos. Namely, 30.7 % and 17.6 % increments in the biochemical pregnancy rates, and 31.1 % and 12.2 % increments in the clinical pregnancy rates, were noticed in the 25–29 ($p = 0.0292$)and 30–34 (ns) age groups, respectively. This improvement disappeared in the 35–39 and 40–44 age groups, and can be explained by the rising incidence of embryo aneuploidy [49].

Embryo aneuploidy is generally accompanied by increasing maternal age, however, based on the lesson of Fig. 2 we have to distinguish a resultant aneuploidy caused by maternal age and an evolved aneuploidy, probably caused by the environment. The above-reviewed stress factors and alterations in the environment are capable of inducing oxidative stress processes, spindle aberrations, abnormal chromosome segregation and aneuploidy. Previously, several research groups confirmed this phenomenon on oocytes after some types of ROS exposure [72–77]. This assumption needs further investigation in connection with sequential vs. single step media, although in the study of Peña et al. [46] the renewal of single step medium on day 3 resulted in decreased pregnancy values. Unfortunately, the low number of patients did not enable significant differences. A recent study of Deng et al. [78] slightly contradicts our results, namely, no differences in the pregnancy rates between the two culture system was observed, in addition the aneuploidy rate was significantly higher after the continuous cultivation in the < 38 years old group. Totally confusing results were published by Vermilya et al. [79] where 53.9 % and 44.3 % euploidy rates were identified in case of continuous and sequential embryo culture, respectively. In that study, this phenomena was explained by the lower lactate content of the investigated single step medium (CSCM-NX, Irvine Scientific™), compared to the sequential (G-Series™, Vitrolife). Fortunately, based on the list components given by the manufacturer, the amount of lactate was not lower in our single step medium (Table 1). Finally, in some cases, no differences were found in the aneuploidy rate [40,80]. These contradictions highlight another important thing. Unfortunately, it is almost impossible to standardize the methods or media because the applying labs differ a lot, each lab has its own habit and these environmental stress factors may partially derive from these habits and they can easily influence the outcomes. So, based on the contradictions of the literature regarding the blastulation, pregnancy and aneuploidy rates, further investigations are needed on this field, but if we consider the hypothesis 'leave nothing undone that might help', single step media may have important role in the embryo culture in the future.

Finally, very important to mention briefly that the source of embryo aneuploidy can be not only (i) the advanced maternal age (egg) and/or (ii) the unfavourable environment of germ cells or embryos, but also (iii) the chromosomal abnormalities of sperm cells [81]. Neither in our study, nor in the literature no assessment of sperm abnormalities has happened. By this way it is very difficult to standardize the circumstances and compare different techniques. In addition, it would make the investigation too complex; it is much more a matter of system biology.

5. Conclusions

In conclusion, the data of the literature suggest that from oocyte retrieval to embryo transfer there are several risks for embryonic development, e.g. temperature decrease, light exposure and the impact of higher oxygen. All of these factors have the ability to induce genetic failures (aneuploidy) via oxidative stress-induced spindle and chromosome aberrations. As a potential result of the reduction of stress factors, culture in a single step medium may cause significantly higher biochemical and clinical pregnancy rates. The increased outcomes showed maternal age dependency, which decreased by the increasing possibility of embryo aneuploidy. In case of the younger subjects where the probability of maternal embryo aneuploidy is low, a sub-optimal environment (sequential media and embryo replace) can provoke an evolved aneuploidy resulting in decreased pregnancy rates. Single step media with disturbance abatement have the ability to improve pregnancy rates.

Disclosure statement

The authors declare that they have no conflict of interest.

Statement of ethics

The study protocol has been approved by the National Public Health and Medical Officer and the study was conducted in accordance with Helsinki Declaration.

Founding source

No external funding declared.

Author contributions

G.M.: Embryological work, Study design, Data collection, Manuscript writing, Final approval of the version to be submitted. A.T.: Gynecological work, Manuscript writing, Final approval of the version to be submitted.

Conflicts of interest

Gábor Máté and Attila Török declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.repbio.2020.08.004.

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