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ORIGINAL ARTICLE

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The relationship between good quality embryo rates and IVF outcomes/embryo transfer policies in extended embryo culture

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ABSTRACT

This study aims to investigate which parameters affect the change in good quality embryo rates during the cleavage stage and whether they have any effect on embryo transfer policies and IVF results. We analysed changes in good quality embryo (grades 1 and 2) rates during the period on days 2, 3 and 5; patients with five or fewer embryos (group 1), 6–10 embryos (group 2) and more than 10 embryos (group 3). The good quality embryo rates decreased in all groups on day 5. When the infertility reasons are studied among all of the groups, ovulatory dysfunction is found to be significantly higher in group 2 compared to group 1 and unexplained infertility was found to be significantly higher in group 2 compared to group 1 and group 3. Total antral follicle, mature oocyte and total oocyte counts were found to be significantly lower in group 1. However, there is no significant difference found among all of the groups for β-HCG levels and clinical pregnancies. Changes in good quality embryo rates at the cleavage stage in extended embryo culture do not have an impact on IVF results.

IMPACT STATEMENT

- **What is already known on this subject?** The number and quality of embryos in the cleavage stage are important parameters affecting the embryo transfer decision on day 5. There is still insufficient knowledge concerning changes in the percentage of increased good quality embryo transfers associated with IVF outcomes during the second to the third day, and the third to the fifth day.
- **What do the results of this study add?** Day 5 embryo transfer is possible in patients with a low number of embryos, according to our results. The good quality embryo rates of patients with a low number of embryos at the cleavage stage are more promising compared to patients having more than five embryos.
- **What are the implications of these findings for clinical practice and/or further research?** An extended embryo culture option can be used on patients with a low number of embryos for clinical practice.

KEYWORDS
Blastocyst; embryo transfer; extended embryo culture; good quality embryo

Introduction

The initial purpose of the assisted reproductive techniques (ARTs) that have been successfully applied since 1978, is to perform a single-embryo transfer to prevent multiple pregnancies and to achieve higher overall pregnancy rates (Machtinger and Racowsky 2013).

However, it is extremely important to choose the embryo with the highest growth potential to maximise the probability of a successful pregnancy. There is a transfer day and embryo development stage that is routinely accepted for each clinic in single embryo transfer (SET). Since the start of IVF applications, the morphological evaluation of embryos by embryologists has been used as the primary method for identification of embryo development, and selection of embryos with the highest implantation potential (Edwards and Hollands 1988; ESHRE Special Interest Group of Embryology and Alpha Scientists in Reproductive Medicine 2017).

Many embryo morphology scoring systems have been developed and many classification schemes have also been used over the years. The most important embryo grading system was first defined by Gardner and Schoolcraft (1999).

The main morphological properties in the embryo morphology scoring process, include the cell count, rate of fragmentation, cell size and multinuclear/mononuclear cell count, and for the blastocyst stage embryos, the degree of expansion, inner cell mass (ICM) and the status of the trophectoderm (ESHRE Special Interest Group of Embryology and

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Alpha Scientists in Reproductive Medicine 2017). A standard time has been determined for the morphological evaluation of embryos. Accordingly, after fertilisation, findings were noted; good, fair and poor quality embryos were identified by looking at the morphological features of the embryo on the fourth and fifth day of cleavage (Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group of Embryology 2011).

In recent years, to select the embryo with the highest implantation rate, invasive and non-invasive novel methods have been applied in IVF laboratories, in addition to the morphological features of the embryo. These non-invasive methods are as follows: selection based on the morphokinetic changes of individual embryos during early developmental stages that are observed by time-lapse photography (ESHRE Special Interest Group of Embryology and Alpha Scientists in Reproductive Medicine 2017); selection based on oxygen consumption by individual embryos (Gardner and Schoolcraft 1999); selection based on various biochemical markers measured in the culture medium of individual embryos (ESHRE Special Interest Group of Embryology and Alpha Scientists in Reproductive Medicine 2017); and OMICS technologies (epigenomic, genomic, transcriptomic, proteomic and metabolomic) selection based on oxidative stress to which individual embryos are subjected. In addition, a combination of the above-mentioned methods may also be used (Sigalos et al. 2016). Additionally, preimplantation genetics diagnosis (PGD-A) technologies are used in a selection of the highest implantation rates of the euploid embryo. However, these new methodologies remain controversial in embryo selection in clinical practice, and therefore, large randomised studies are considered necessary to the validity and reliability of these technologies (Rocha et al. 2016; Barberet et al. 2019). Using novel technologies is the preference of laboratories; however, they are also expensive technologies, so morphological evaluation can only be used to select good quality embryos in laboratories.

Up to now, the number and quality of embryos in the cleavage stage are important parameters affecting the embryo transfer decision on day 5. There is still inadequate knowledge concerning changes in the percentage of increased good quality embryo transfers associated with IVF outcomes during the second to the third day, and the third to the fifth day, following fertilisation.

Analysing or publishing how the rates of good quality embryos can change between day 2 and day 3, and then from these days up to day 5, are not common for the facilities involved. Therefore, we aim to investigate the effect of changes in good quality embryo rates on the outcome of *in vitro* fertilisation between the transition stages of the second, third and fifth days of extended embryo culture in our study. As a result, we aim to contribute to embryo transfer policies.

Methods

Ethical approval

Medical records of 192 treatment cycles from March 2013 to April 2015, at Sağlık Bilimleri University, Etlik Zübeyde Hanım Women's Health Teaching and Research Hospital, and the Center of Assisted Reproduction were reviewed using a computer-based database. The approval of the local ethics committee was obtained at the start of the study (number: 90057706/900/2017).

Experimental procedure

Controlled ovarian hyperstimulation was performed using long GnRH agonist, microdose flare or antagonist protocols. The type of gonadotropin used was either pure recombinant follicle-stimulating hormone (FSH) or human menopausal gonadotropin (hMG). Gonadotropin doses were individualised for each patient. Cycles were monitored by serial transvaginal ultrasound evaluation and serum oestradiol levels. Recombinant human chorionic gonadotropin (hCG) (Ovitrelle, Serono, Istanbul, Turkey) was administered when at least three follicles showed a mean diameter of 17 mm. Oocyte pick up (OPU) procedures were performed by transvaginal ultrasound-guided aspiration 35.5–36 h after the hCG injection. Following retrieval, cumulus oophorus was removed from oocytes by incubation in a solution containing hyaluronidase (Vitrolife, Gothenburg, Sweden). The remaining cells were removed mechanically using commercial denuding pipettes. Denuded oocytes were cultured in G-IVF (Vitrolife, Gothenburg, Sweden) medium at 37 °C in a humidified atmosphere of 5% CO_2–95% air, until used for ICSI.

Fertilisation was checked for signs of fertilisation (the presence of two pronuclei and two polar bodies) 16–18 h after ICSI. Embryos were checked and graded every day using appropriate scoring systems modified from previously defined systems (Fisch et al. 2001; Baczkowski et al. 2004). The embryo quality was evaluated on day 2, day 3 and day 5. Day 2 and day 3 embryo grades were performed 42–44 h and 64–65 h, respectively, after ICSI. Day 2 and day 3 embryos were classified according to size, nucleation and cytoplasmic morphology of blastomeres (percentage of anucleate fragments; cleavage speed and cell number).

On day 5, blastocysts were evaluated based on the degree of blastocoel expansion, visible ICM and continuous trophectoderm with sufficient cells. Five numerical grades (1–5) were given to the blastocysts based on the degree of blastocoel expansion and status hatching. The numeric grading was followed by two alphabetic gradings (from AA to CC), to assess the ICM and trophectoderm cells (TE). Embryos were graded from grades 1 to 5 (best to worst). We accepted grade 1 or 2 embryos as good quality on day 2, day 3 and day 5. Embryos were selected for transfer by standard procedures in the laboratory. Single embryo transfer was performed on the fifth day after ICSI. Luteal phase support was given by vaginal progesterone (Crinone 8% gel, Serono, Welwyn Garden City, UK) twice daily.

Experimental design

There are three groups in our work. The number of embryos was established on the fifth day. Group 1 consists of patients with five or fewer embryos ($n = 65$); group 2 consists of

patients with 6–10 embryos ($n = 97$) and group 3 consists of patients with more than 10 embryos ($n = 30$). Good quality embryo (grade 1 and grade 2) percentages on days 2, 3 5 that we routinely use in our laboratory are used in this research. We calculated the change (increase or decrease) in these percentages from day 2 to day 3 and from day 3 to day 5. We statistically compared the increase or decrease in good quality embryo rates between these three groups in terms of both the demographic characteristics of the patients and the effect on the IVF results.

Pregnancy was determined by β-hCG levels in blood tests performed 12 days after embryo transfer, and clinical pregnancy was defined as the presence of a gestational sac with accompanying foetal heartbeat by ultrasound four weeks following the ET procedure. Patients with β-hCG values of more than 10 were considered as β-hCG positive. We calculated the percentage change in good quality embryo rates between the transition stages of the second and third and fifth days of extended embryo culture in our study.

Age, body mass index (BMI), basal serum FSH luteinising hormone (LH) and oestradiol (E2) values, antral follicle count (AFC), total oocyte count, mature oocyte count, infertility aetiology, IVF outcome, clinical pregnancy rate, biochemical pregnancy rate and live birth rate (LBR) were recorded from the charts.

Patients that applied for IVF treatment, and that had only had day 5 SET, were included in the retrospective study. The number of cycles of patients was ignored. Patients who were observed to have cleavage arrest, or whose embryo transfer was cancelled, were left out of the study.

Statistical analysis

Statistical analysis was performed using R 3.3.2v (open source) (R Foundation for Statistical Computing, Vienna, Austria). Data were presented as a minimum, maximum, median and standard deviation. The distribution pattern of the data was examined through the Kolmogorov–Smirnov test. Independent groups of more than two were examined through the one-way ANOVA or the Kruskal–Wallis test. For paired comparison, the Tukey test or the Mann–Whitney's *U* test with Bonferroni correction were used. Dependent groups of more than two were examined through ANOVA (one-way or two-way) or the Friedman test. For paired comparison, the Bonferroni test or the Wilcoxon test with Bonferroni's correction were used. The correlations between variances were examined using the Pearson correlation coefficient. Statistical significance was determined as $p < .05$.

Results

It was noted that 'Good Quality Embryos' rates decreased in all of the groups on day 5 (Table 1). On the other hand, there was no statistically significant difference observed through a good quality embryo rate on day 5 between the groups (Tables 1 and 2).

If we look at the groups individually, the good quality embryo percentage increased in group 1 while it decreased in group 2 and group 3 from day 2 to day 3. In addition, it was noted that a good quality embryo rate in group 1 on day 5 was found to be higher compared to group 3 (Table 3). These findings are also supported by statistical analysis for day 3.

When the reasons for infertility are studied among these groups, ovulatory dysfunction is found significantly to be higher in group 2 compared to group 1 and unexplained infertility is found to be significantly higher in group 2 compared to group 1 and 3 ($p < .05$). Total antral follicle, mature oocyte and total oocyte counts are found to be significantly lower in group 1. However, there are no significant differences found among all of the groups for β-HCG levels and clinical pregnancies ($p > .05$).

Table 1. Assessment of the percentages 2, 3 and 5 days embryo numbers separately.

Number of embryos		Avg ± SD	*M* (Q1–Q3)	
≤5	D2 percentage	0.88 ± 0.19	1 (0.75–1)	.001*
	D3 percentage	0.9 ± 0.17	1 (0.8–1)	
	D5 percentage	0.65 ± 0.28	0.6 (0.4–1)	
6–10	D2 percentage	0.87 ± 0.16	0.9 (0.78–1)	.001*
	D3 percentage	0.82 ± 0.18	0.84 (0.71–1)	
	D5 percentage	0.54 ± 0.26	0.5 (0.33–0.7)	
>10	D2 percentage	0.87 ± 0.15	0.91 (0.8–1)	.001*
	D3 percentage	0.78 ± 0.18	0.8 (0.63–0.91)	
	D5 percentage	0.5 ± 0.26	0.44 (0.3–0.63)	

*Statistically significant.

Table 2. Comparison of the 2, 3 and 5 days embryo percentages.

Number of embryos		Avg ± SD	*M* (Q1–Q3)	
≤5	D3D2_100	2.4 ± 18.16	0 (0–0)	.019*
6–10		−4.22 ± 15.9	0 (−14 to 0)	
>10		−8.27 ± 16.28	−1 (−17 to 0)	
≤5	D5D3_100	−24.86 ± 27.37	−30 (−50 to 0)	.062
6–10		−28.19 ± 28.12	−25 (−50 to (−8))	
>10		−28.13 ± 33.48	−34 (−50 to (−12))	

*Statistically significant.

Discussion

Although instant morphological evaluation of the embryo, which has a very dynamic nature during the preimplantation period in terms of embryo development and viability, is a quantitative evaluation, the key morphological features of the embryo seem to be related to its developmental potential (Rhenman et al. 2015).

Many evaluations could be made throughout the development process of the embryo. Although methods show variability according to ART laboratories, embryo development is extremely dynamic, and standard embryo evaluation hours have been determined by Alpha/ESHRE consensus, starting with insemination time (Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group of Embryology 2011).

Generally, in the morphological instant evaluation of the embryo, determining the quality of the embryo involves the following criteria: looking at the stage-specific cell size; the number of blastomeres and amount of multi-nucleation; the

Table 3. Demographic characteristics and pregnancy outcome of patients.

	Number of embryos			
	≤5 (n = 65)	6–10 (n = 99)	>10 (n = 30)	p Value
Female age	29.23 ± 5.04	29.21 ± 4.72	28.97 ± 3.39	.963[a]
BMI	25.8 ± 4.86	26.51 ± 4.61	27.55 ± 4	.233[a]
D3_FSH	7 (5–9)	7 (5–7)	6 (5–7)	.193[b]
D3_LH	4 (3–6)	5 (3–7)	6 (4–9)	**.003**[b]
D3_E2	40 (28–57)	37 (25–49)	43.5 (28–55)	.272[b]
Total antral follicle count	12.89 ± 7.08	15.27 ± 7.22	18.87 ± 7.61	**.001**[a]
Total oocyte count	11.8 ± 6.29	14.71 ± 6.34	17.68 ± 7.48	**<.001**[a]
Mature oocyte count	7 (5–11)	11 (9–14)	15 (9–17)	**<.001**[b]
Oocyte quality index	5.15 ± 0.84	5.38 ± 0.67	5.11 ± 0.56	.076[a]
***Semen** analysis category*				
Normal	32 (49.2)	54 (55.1)	19 (65.5)	.321[c]
Oligospermia	30 (46.2)	39 (39.8)	8 (27.6)	
Azoospermia	3 (4.6)	5 (5.1)	1 (3.4)	
Oligoasthenospermia	0 (0)	0 (0)	1 (3.4)	
Indication male factor	28 (43.1)	38 (38.4)	10 (33.3)	.647[c]
Tubal factor	6 (16.2)	11 (18)	3 (15)	.942[c]
Age related infertility	3 (8.1)	5 (8.2)	0 (0)	
Ovulatory dysfunction	15 (56.8)	51 (83.6)	21 (75)	**.014**
Unexplained infertility	17 (45.9)	42 (68.9)	16 (80)	**.023**[c]
Endometriosis	2 (5.4)	3 (4.9)	2 (10)	.759[c]
hCG positively/ET	1 (0–207)	35.5 (1–241)	4 (1–153)	.132[b]
Clinical pregnancy rate/**ET**	25 (39.7)	48 (50)	13 (44.8)	
Live birth rate/**ET**	16 (24.6)	29 (29.3)	9 (30)	.775[b]

[a]One-way ANOVA is used. Descriptive statistics are given as average ± standard deviation.
[b]The Kruskal–Wallis test is used. Descriptive statistics are given as M (IQR).
[c]Ki-Kare test is used. Descriptive statistics are given as n (%).
Bold values represent statistically significant.

in compact embryo, presence and degree of compaction; the degree of expansion in the blastocyst; and the characteristics of the TE and ICM (Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group of Embryology 2011).

Embryo quality, based on morphological parameters, is a major predictor for the success of IVF treatment and the association between the quality of embryos, the implantation rate and pregnancy outcome has been well established (Giorgetti et al. 1995; Ziebe et al. 1997; Van Royen et al. 1999).

Embryos with the best quality for transfer are selected through the cumulative embryo scoring system from zygote to the blastocyst stage in embryo culture in the embryology laboratory. It has been put forward that the embryo qualities at the zygote, day 2–3 (cleavage stage), day 4 and day 5 have a significant impact on IVF outcomes. Pronucleus classification has been performed in the zygote (Tesarik and Greco 1999; Depa-Martynow et al. 2007) and it has been suggested that using this classification is of importance in the selection of embryos with a high rate of implantation (Sakkas et al. 1998). The pronuclear (PN) zygote score is also really important for the best embryo selection. This procedure includes PN morphology, the number and distribution of nuclear precursor bodies (NPBs), and the presence of a clear cortical zone. Zygotes that include halo positive, PNs aligned, a similar number and polarised NPBs, and which have appropriate orientation of polar bodies, show a good PN score, and embryos that develop from this type of zygote show better implantation. Additionally, abnormal findings of PN morphology are correlated to a higher rate of cleavage arrest and lower blastocyst development (Gamiz et al. 2003; Gianaroli et al. 2003). The zygote score is extremely important for predictability of chromosomal abnormalities, since 50–70% of human cleavage embryos are aneuploid, and most aneuploid embryos do not develop to term or result in miscarriages or infertility (Gamiz et al. 2003; Cavazza et al. 2020). Cavazza et al. describe in their studies that an error-prone mechanism of parental genome unification often results in aneuploidy. If the parental genome clustering fails, this case leads to chromosome segregation errors and causes non-healthy embryonal development (Cavazza et al. 2020). Only 25% of aneuploid embryos can reach the blastocyst stage; additionally, a low percentage of monosomies are found at the blastocyst stage, and an important percentage of trisomic embryos achieves the blastocyst stage, which causes pregnancy resulting in miscarriage (Gamiz et al. 2003; Cavazza et al. 2020). According to research, slow-developing or arrested embryos exhibit more polyploidy than normally developing embryos (Gamiz et al. 2003). It has been demonstrated that embryos which are seen to have early division among other embryos, with the same quality during the control performed 25–27 h after insemination, have a higher chance of implantation. The number of blastomeres and division time on day 2 and day 3 is used in the selection of embryos with a higher potential of implantation (Hlinka et al. 2012). While LBRs remain unchanged in embryos with a fragmentation rate up to 20%, this rate in embryos with uneven sized blastomeres and with less than a 20% fragmentation rate is the same as for those with more than 20%. Therefore, it is believed that fragmentation should not be considered as a criterion by itself in morphological evaluation. It has been demonstrated that the implantation potential of embryos, which have a multi-nucleus in their uneven blastomere and more than one blastomere, is low (Van Royen et al. 2003; Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group of Embryology 2011). It has also been

demonstrated that the implantation potential of embryos developing to grade 1, an early blastocyst stage according to the day 4 embryo scoring system or of those fully compact, is high (Feil et al. 2008). For blastocyst grading, the Schoolcraft system is commonly used (Schoolcraft and Gardner 2001). Inner cell mass is important for a blastocyst. However, also studies showing a strong trophoectoderm layer is much more important for implantation (Ahlström et al. 2011; Ebner et al. 2016), it has been shown that the expansion and hatching stage of blastocysts should be taken into consideration in the selection of high-quality blastocysts (Van den Abbeel et al. 2013). There has been research regarding the impact of the number and quality of embryos at the cleavage stage on forming blastocyst and pregnancy outcomes.

Novel techniques have been developed, since the morphological evaluation of the embryo is considered to be insufficient in the estimation of the quality of the embryo. These techniques particularly focus on developing new non-invasive methods using molecular approaches based on small non-coding RNA, including proteomics, metabolomics, and in culture media microRNA. Furthermore, time-lapse microscopy (TLM) is an exciting new technology with great potential to improve embryo selection in the embryology laboratory. Evaluation of embryos according to these non-invasive morphokinetic features has provided a new tool in the estimation of embryo development and implantation potential. A number of authors have analysed the timing of each early event during the development of good-quality blastocysts, depending on whether they were implanted or not. Finally, few parameters, such as time to syngamy (tPNf), times to 2 (t2), 3 (t3), 5 (t5) and eight (t8) cells, t2-tPNF, t5–t2 or cc3 (t5–t3), are significantly different between implanted and non-implanted blastocysts (Chamayou et al. 2013; Desai et al. 2014). Consequently, new studies are needed to determine which morphokinetic criteria are the best embryo selection criteria (Motato et al. 2016; Zaninovic et al. 2017). Since static and morphokinetic parameters alone are not sufficient in the selection of the euploid embryo, PGT-A, which is an invasive procedure, has become a method used to select the euploid embryo these days. It has been shown that using static and morphokinetic methods in embryo selection can reduce the number of embryos applied with PGT-A (Minasi et al. 2016).

It is not always possible to access novel technologies that are invasive or non-invasive. In these cases, the instant morphological evaluation of the embryo in embryology laboratories is made according to previously determined times and algorithms. As a result, the best quality single embryo, which is considered to have the highest implantation rate, is transferred on the second, third or fifth day according to the demographic and cyclical characteristics of the patient. Although the number and quality of embryos obtained to determine the day of transfer, each embryology laboratory has its transfer policy.

Extended embryo culture was first suggested by Edwards and Holland and practiced by Bolton et al. for the first time. Extended embryo culture is an extension of embryo culture time to five days, instead of two or three days, when blastocyst transfer is preferred (Edwards and Hollands 1988; Bolton et al. 1991). In a recent study, it has been observed that there is a correlation between extended embryo culture and an increase in implantation rates and reduced rates of multiple pregnancies. The reduction in the number of transferred embryos and the synchronisation between embryo and endometrium during embryo transfer have been reported as other advantages of extended embryo culture. Additionally, extended embryo culture allows for this, since embryonic genome activation (EGA) will take place at the 4–8 cell stage.

It was observed that embryos were exposed to a high rate of developmental block on the second or third day of the cleavage phase in the extended embryo culture. It has also been observed that these developmental errors frequently correlate with aneuploidy. Therefore, extended culture conditions will increase the probability of selection of euploid embryos for transfer, allowing the embryos to compete chromosomally (Tesarik et al. 1988; Munné et al. 1995; Latham and Schultz 2001; Martins et al. 2017).

However, since embryologists cannot predict at which cleavage stage embryos will develop into a viable blastocyst, assisted reproduction centres do not prefer to adopt extended embryo culture in order that they can avoid embryo transfer cancellation. Furthermore, it has been suggested that repeating implantation failures in blastocyst transfers may be beneficial for patients with a limited number of oocytes and embryos (Schoolcraft and Gardner 2001; Martins et al. 2017). While the outcomes of day 5 and day 3 transfers in patients with one or two eight-cell embryos are controversial, according to a study conducted by Racowsky et al., a number of studies suggest that day 5 embryo transfer is advisable for patients who have three or more eight-cell embryos. The number of eight-cell embryos is a key determinant for selecting day 3 or day 5 transfer (Racowsky et al. 2000). Sasikala et al. found that the quality of embryos is a better indicator than the number of embryos. In addition, their study demonstrates good blastocyst development rates with a sensitivity of 100% for groups with less than three embryos, and specificity of more than 97%, compared to groups with more than three embryos. In conclusion, it was noted that the number of embryos available on day 3 may not act as a deciding factor in the extended culture of embryos and good blastocyst formation. Instead, good pregnancy rates can be achieved only with a single embryo, owing to suitable laboratory conditions and technical expertise (Sasikala et al. 2015). It is possible to identify patients with a high potential of benefiting from further embryo culture thanks to morphological scoring of embryo quality on day 3. Having at least four good embryos on day 3 is an indication that the patient will undergo an embryo transfer (Papanikolaou et al. 2005). In another study, it has been suggested that a single blastocyst transfer policy can be decided before the IVF cycle starts. A day 3 or day 5 embryo transfer policy, based on a day 3 embryo evaluation, does not affect the cycle outcome. Furthermore, it does not divide the patient population based on the ovarian response and reduces the risk of embryo transfer cancellation (Stoop et al. 2011). Day 3 embryos do not have a clear pattern in terms of the morphology at the blastocyst stage, and it has been demonstrated that day 3 and day 5 good quality embryos

(grades 1 and 2) do not increase pregnancy rates compared to low-quality embryos (grades 3 and 4) (Demir et al. 2015). In an observational cohort study in a population with a good prognosis, Guerif et al. confirm the suitability of choosing an embryo culture based on the embryo quality observed on day 2. In fact, on day 2, the number of good quality embryos (four pairs of cells, <20% fragmentation rate) predict the blastocyst rate; when there are more than two top-quality embryos on the second day, a 68% blastocyst rate is obtained, and on the second day, a 50% blastocyst rate is obtained with a non-top embryo. The researchers report similar LBR per blastocyst transfer. However, regardless of the number of top-quality embryos obtained on day 2, once the blastocyst is present, the overall embryo quality on day 2 does not affect subsequent clinical outcomes (Guerif et al. 2009). In another study, it is shown that good quality blastocyst transfer, obtained in the cleavage stage (day 2 or day 3) in a population with a good prognosis, does not change the IVF results (Lundin and Ahlström 2015).

Our research indicated that the quality of embryos during early cleavage stages is related to blastocyst development, but another important point has the number of blastocysts can be expected to affect the cumulative success rate, taking into account both fresh and frozen embryo transfer attempts. Researchers agree that an individual's chance of having a baby following fresh and frozen embryo transfer should be described as the cumulative LBR (Maheshwari et al. 2015). The study of Drakopoulos et al. based on this topic, studied with 1099 eligible consecutive women 18–40 years old undergoing their IVF cycle, and they concluded that women undergoing controlled ovarian hyperstimulation for their first IVF cycle, the number of oocytes, and also good-quality embryos retrieved do not affect the LBR in the fresh cycle but the higher number of the oocyte and also embryo rate is so important for the cryopreservation process considering the cumulative LBRs (Drakopoulos et al. 2016). Wei et al. studied the clinical outcomes of the frozen-thawed cycles of high-quality cleavage embryos with low-quality blastocysts in their research and finally found that transferring two high-quality cleavage embryos and transferring single day 5 low-quality embryos have ideal clinical outcomes (Wei et al. 2020). Zhu et al. researched the association between embryo quality after vitrified cleavage-stage embryos and blastocyst transfer and finally, they found that significantly higher LBR after blastocyst transfer than cleavage embryo transfer (Zhu et al. 2020).

Conclusions

Our study is the first research study regarding the impact of variances in good quality embryo rates when developing from day 2 to day 3, and from day 3 to day 5 on IVF outcomes. Unlike the studies we have mentioned, we evaluated the effect of change in good embryo percentage rates in cleavage-stage on extended embryo culture through IVF results. In our study, in parallel with the studies above, we note that patients with less than five embryos are no different from patients with 5 to 10, and 10 embryos in terms of IVF results. We also conclude that good quality embryo rates on day 2 or day 3 are not decisive for the fifth day of cleavage. Therefore, day 5 transfer may be an option for patients with few embryos.

We also conclude that day 5 embryo transfer on patients with a low number of embryos has no negative impact on IVF outcomes. In addition, the reduction in good quality embryo percentages in all of the groups on day 5 is an expected result of the disadvantages of prolonged culture. Although the increase in good quality embryo rates is found to be higher in group 1 than in groups 2 and 3, and good quality embryo rates are found to be higher in group 1 than in group 3 on day 5, there is no correlation with other characteristics of the cycle and IVF results. Retrospective planning of the study and the number of patients are limitations of this study.

In the extended embryo culture, it was not investigated whether the increase in the number of good quality embryos affects the results of obtaining good quality blastocysts and IVF when going from day 2 to day 3 and day 3 to day 5. Generally, day 5 embryo transfer is avoided in patients with a low number of embryos (five or less) in the embryology laboratory. According to our findings, we can suggest that day 5 embryo transfer is possible in patients with a low number of embryos. Likewise, we observe that the change in good quality embryo rate is not affected by the indication, cycle or demographic characteristics of the patients, and that the number of good quality embryos decreases in all groups regardless of cycle characteristics and culture conditions in the extending embryo culture. Furthermore, the good quality embryo rates of patients with a low number of embryos at the cleavage stage are more promising compared to patients having more than five embryos; that is, an extended embryo culture option can be used on patients with a low number of embryos. According to our research, any increase or decrease in good quality embryo rates in extended embryo culture does not have an impact on IVF results. Therefore, we think that this is not a parameter in predicting IVF outcomes. Additionally, calculating the good quality embryo percentages of the second, third and fifth days is a limitation of our study, because calculating these percentages and differences may not be practical in routine laboratory applications.

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Authors contributions

AT, CMS and ÇE were responsible for the conception and design of the study; AT, İK and SD were responsible for acquisition of data; AT, CMS, AŞİ and İK were performed the data analysis and drafted the manuscript; AT and CMS were revised and commented on the draft. All of the authors have read and approved the final version of the manuscript.

Disclosure statement

None of the contributing authors have any conflict of interest, including specific financial interests, relationships or affiliations relevant to the subject matter or materials discussed in the manuscript.

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