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# THE ASSOCIATION BETWEEN FEMALE AGE, BODY MASS INDEX (BMI) AND TIME-LAPSE PARAMETERS OF SUCCESSFULLY IMPLANTED HUMAN EMBRYOS

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**Abstract:** In this retrospective study we included 260 embryos from 194 patients at different age undergoing ICSI treatment that led to successful pregnancy and live birth. Women were grouped according to body mass index (BMI) categories: <18, 18-25, and >25 kg/m². Embryos were cultured in EmbryoscopeTM. Fifteen morphokinetic parameters were recorded and analyzed (time of pronuclei appearance, time of pronuclei fading, cleavage times, morulae formation time, starting blastulation, full blastocyst stage, expansion and hatching timing). Spearman correlation analysis was done to find the association between the time-lapse variables and female age. The performed correlation analysis showed a significant positive correlation between female age and the initial stages of embryo development of successfully implanted embryos. We observed relatively low, but significant correlations between women age and the time of pronuclei appearance (tPNa) ($R=0.11$; $p<0.05$), time of pronuclei fading (tPNf) ($R=0.15$; $p=0.02$) and time to two cell stage (t2) ($R=0.11$; $p<0.05$). It should be noted that the correlation between patients' age and the described events was highest in underweight patients (BMI<18; N=32) ($R=0.67$; $p=0.01$; $R=0.36$; $p=0.05$ and $R=0.34$; $p=0.05$, respectively) and less significant in the other two BMI categories. In conclusion, embryo morphokinetics differs in the initial stages according to female age, especially in underweight women. Pronuclei emergence and fading, and two cell stage occurred significantly later in older patients. However, the observed change is not crucial for the success of the embryo transfer.

## INTRODUCTION

Embryoscope is a new generation incubator with an integrated time-lapse system that allows a constant monitoring of human embryos and their development in stable conditions (Kirkegaard *et al.,* 2012; Aparicio *et al.,* 2013). The system generates unique parameters that combine the morphological assessment with the timing of all cleavage stages (Sundvall *et al.,* 2013). The obtained morphokinetic

parameters are considered as non-invasive biomarkers for the success of in vitro fertilisation IVF treatment and useful tool for improved embryo selection (Meseguer *et al.,* 2011; Campbell *et al.,* 2013; Conaghan *et al.,* 2013; Herrero and Meseguer, 2013).

The fertility potential of women decreases with aging. The observed decline in female reproductive capacity with age increasing is caused by gradual depletion of the oocytes from the ovary, and a significant decrease in oocyte quality (Tatone *et al.,* 2008). The conventional method of morphological evaluation of human embryo has proved that maternal age affects the human embryo quality (Navot *et al.,* 1991). Moreover, the aneuploidy rates in human embryos increase with increased female age (Fragouli *et al.,* 2013). However, the effect of women age on time-lapse variables has been described by only few authors who did not take into account the confounding effect of additional factors such as the body mass index (BMI) (Grondahl *et al.,* 2012; Akhter and Shahab, 2017).

The negative effects of obesity on women's fertility, natural conception and assisted reproduction are already well known (Rich-Edwards *et al.,* 2002; Bellver *et al.,* 2008; Metwally *et al.,* 2008; van der Steeg *et al.,* 2008; Bellver *et al.,* 2010; Polotsky *et al.,* 2010; Luke *et al.,* 2011). Studies, concerning the effect of BMI on embryo morphokinetics are also still scarce (Bellver *et al.*, 2013; Lammers et al, 2013; Goldman *et al.,* 2014).

To date, it is still not fully clear how important factors, such as female age in combination with BMI could influence embryo time-lapse variables. The purpose of the present study was to find a possible correlation between female age and morphokinetic parameters of successfully implanted human embryos. This is an attempt to search for changes in time-lapse parameters of women with different age and BMI that could exist but are not a limitation for successful implantation and pregnancy.

## MATERIALS AND METHODS

The present retrospective study was carried out at Nadezhda Women's Health Hospital (Sofia, Bulgaria), after approval from local Ethics Committee. All patients signed an informed consent form. The analysis included frozen-thawed embryo transfer cycles performed between May 2016 and December 2017.

A total of 194 women (260 embryos) with successful pregnancy and live birth after ICSI procedure and embryo transfer were included for analysis. The patients who were included were women with recurrent implantation failure (RIF), defined as $\geq 3$ failures of implantation in at least three consecutive IVF attempts. In addition, women were grouped according to pre-pregnancy body mass index (BMI) in three categories: <18 kg/m², 18-25 kg/m², and >25 kg/m².

All embryos were cultured in a single-step culture medium (Global, LifeGlobal) and EmbryoscopeTM time-lapse incubator (Vitrolife, Sweden). Fifteen morphokinetic parameters were recorded and analyzed (time of pronuclei appearance - tPNa, time of pronuclei fading - tPNf, cleavage times (t2, t3, t4, t5, t6, t7, t8, t9), morulae formation time (tM), starting blastulation (tSB), full blastocyst stage (tB), expansion (tEB) and hatching timing (tHB)).

Statistical Package for the Social Sciences (SPSS) software version 21.0 (SPSS, Inc., Chicago, IL, USA) was used to analyze the data. Spearman's correlation coefficient was used to find the association between the time-lapse variables and female age. P<0.05 was considered statistically significant.

## RESULTS AND DISCUSSION

The performed Spearman correlation analysis showed a significant positive correlation between female age and the initial stages of embryo development of successfully implanted embryos. As shown in Figure 1, women age correlated significantly with three time-lapse parameters: the time of pronuclei appearance (tPNa) (R=0.11; p=0.05), time of pronuclei fading (tPNf) (R=0.15; p=0.02) and time to two cell stage (t2) (R=0.11; p=0.05) (**Table 1 and Fig. 1**).

**Table 1.** Spearman correlation coefficients among time-lapse variables and women age.

| Time-lapse parameter | Age, Total n=260 | Age, BMI<18 n=76 | Age, 18<BMI< 25 n=119 | Age, BMI>25 n=65 |
|---|---|---|---|---|
| tPNa | 0.11* | 0.67** | 0,07 | 0,02 |
| tPNf | 0.15* | 0.36* | -0,04 | 0,06 |
| t2 | 0.11* | 0.34* | 0,01 | -0,05 |
| t3 | -0,04 | 0.33* | -0,1 | 0,02 |
| t4 | -0,04 | 0,27 | 0,05 | -0,16 |
| t5 | 0,02 | 0.34* | -0,06 | -0,06 |
| t6 | -0,05 | 0.33* | 0,04 | -0,2 |
| t7 | -0,03 | 0.42* | 0,06 | -0,17 |
| t8 | -0,08 | 0.43* | 0,05 | -0.31** |
| t9 | -0,03 | 0.40* | -0,1 | -0,16 |
| tM | 0,06 | 0.44* | 0,04 | -0,12 |
| tSB | 0,03 | 0,32 | 0,06 | 0,26 |
| tB | 0,01 | -0,05 | 0,02 | 0,15 |
| tEB | -0,02 | 0,13 | -0,14 | -0,13 |
| tHB | -0,05 | -0,95 | 0,05 | -0,62 |

The R values marked with ** or * indicate correlations that are significant at the p<0.01 or p<0.05 levels, respectively.

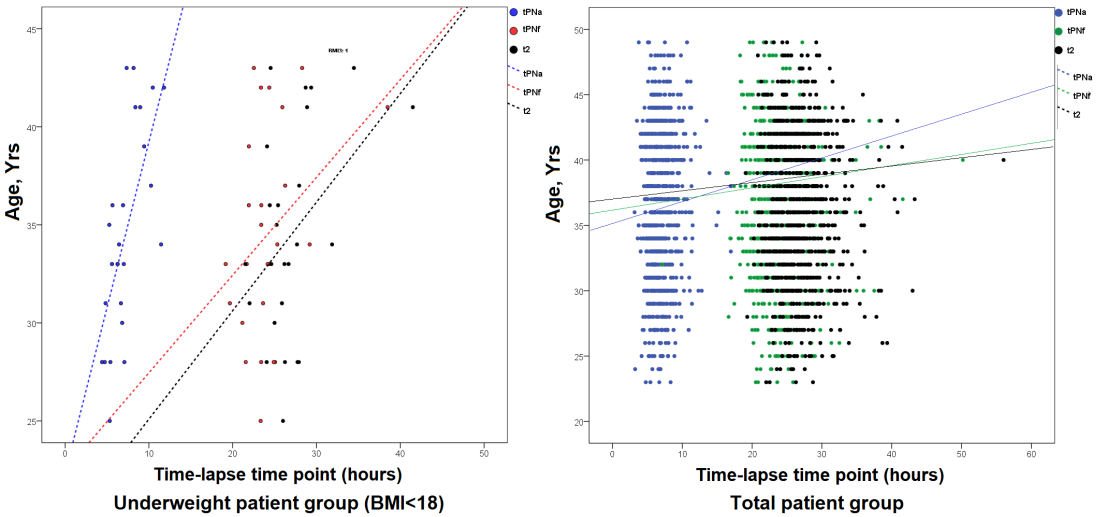


**Fig. 1.** Overlaid scatter plots between patient age and selected morphokinetic variables in the total patient group and in the underweight patient group (BMI < 18.5 kg/m$^2$).

Analyzing the association between women's age and time-lapse variables in the studied three BMI categories ( (1)<18 kg/m², (2) 18-25 kg/m², and (3) >25 kg/m²) we found that the correlation between patient's age and the described events was highest in underweight patients (BMI<18; N=32) (R=0.67; p=0.01; R=0.36; p=0.05 and R=0.34; p=0.05, respectively). In this patients group, we also observed a significant positive correlation with later events – t5, t6, t7, t8, t9 and tM (Table 1). The Spearman correlation coefficients were insignificant for the most of the morphokinetic parameters in the other two BMI categories.

To our knowledge, this study is the first to perform an analysis of the correlation between women age in different BMI groups and time-lapse variables. The influence of age on the morphology of the early human embryo was assessed by several authors by applying different analytical approaches (Grondahl *et al.,* 2012; Akhter and Shahab, 2017). In a study, the authors stated that maternal age had no effect on cleavage parameters or on the morphology of the embryo day 2 post insemination but it has an influence on the 3PN zygote rate (Grondahl *et al.,* 2012). Another recent work, which partly confirms our findings, compared the time-lapse parameters of embryos obtained from women with different age groups. It has been stated that timely cleaved embryos have significant difference in tPNa i.e. time for pronuclear appearance (P≤ 0.001), t4 and t5 i.e. time for 4 and 5-cell cleavage (P≤ 0.05) among different age groups (Akhter and Shahab, 2017).

In other studies, focused on the effect of BMI on embryo morphokinetics, an association between BMI and embryo development and a delay in particular embryo time-lapse variables in overweight women, such as time to five discrete cells (t5) was found (Bellver *et al.,* 2013, Goldman *et al.,* 2014). In addition,

Lammers *et al.* (2013) observed a significant delay of the time from ICSI to 4-cells (t4), five cells (t5) and eight cells (t8) in underweight women compared to the normal weight women. These data give a possible explanation of the observed stronger association between women age and embryo morphokinetics in underweight patient group. It could be suggested that the female age and BMI exert a synergistic effect on the early developmental stages of human embryo.

## CONCLUSION

The morphokinetics differs in the initial stages of human embryo development according to female age. These differences were more noticeable in underweight women. Pronuclear appearance and fading, and two cell stage occurred significantly later in older patients. However, the observed change is not crucial for the success of the embryo transfer.

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### AUTHOR CONTRIBUTIONS

M.I., D.P., G.S. and T.C. conceived the experiment; M.I., D.P., S.N., I.I, I.R. and T.C. conducted the experiment; M.I., D.P., R.G. and T.C. analyzed the results. M.I., D.P. and R.G. wrote the main manuscript text and prepared tables and figures. M.I., D.P., T.C., and G.S. edited the manuscript and made its final revision. All authors critically reviewed and approved the final version of the manuscript.

### CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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