Annual of Sofia University "St. Kliment Ohridski"
Faculty of Biology
Book 4 - Scientific Sessions of the Faculty of Biology
2019, volume 104, pp. 348-354
International Scientific Conference "Kliment's Days", Sofia 2018

INFLUENCE OF BMI AND FSH ON MORPHOKINETICS OF HUMAN EMBRYOS IN WOMEN UNDERGOING IVF TREATMENT

STEFKA NIKOLOVA*, RUMIANA GANEVA, MIRAY IBRYAMOVA, IVKA IVANOVA, KRISTINA NIKOLOVA, DIMITAR PARVANOV, GEORGI STAMENOV, TODOR CHAUSHEV

Nadezhda Women's Health Hospital, 3 "Blaga vest" Street, Sofia, Bulgaria
**Corresponding author: s.nikolova@nadezhda.bg*

Keywords: time-lapse, morphokinetic biomarkers, human embryos, BMI, FSH

Abstract: We have studied 308 embryos, from 123 patients undergoing standard ICSI treatment. Their BMI and basal FSH values were recorded. After ICSI procedure all oocytes were cultured in Embryoscope™. Fifteen morphokinetic parameters were annotated: 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 and hatching timing). Patients were divided by BMI into an underweight group (BMI < 18.5 kg/m²), a normal weight group (18.5 kg/m² < BMI < 25 kg/m²) and overweight/obese group (BMI ≥ 25 kg/m2). Spearman correlation analysis was performed for each group. Analysis revealed positive correlation between FSH and tPNf, t2 and t4 in women with normal weight (R=0.18, p=0.03; R=0.17, p=0.04 and R=0.18; 0.03). In contrast, in the underweight group of patients the serum levels of basal FSH correlated significantly with tM and tSB. Moreover, in the overweight group we found a significant negative correlation between FSH and t7 (R=-0.25, p=0.05), t9 (R=0.38, p=0.01) and tM (R=-0.29, p=0.05). In conclusion, FSH levels have a specific influence on embryo morphokinetics in relation to BMI. It seems that in patients with normal weight FSH has an impact on initial stages of embryo development, while in underweight and overweight women it has a significant effect on later events.

INTRODUCTION

Body mass index (BMI) and follicle stimulating hormone (FSH) levels are important indicators of female fertility. A series of studies demonstrated that BMI and FSH levels can be predictive for the outcome of in vitro fertilisation (IVF)

treatment (Ferlitsch *et al.,* 2004; Sathya *et al.,* 2010). It has been shown that very thin or obese women experience less positive outcomes after assisted reproduction treatment (ART) (Sherbahn, 2011). Many authors have demonstrated that obesity is associated with insufficient follicular development and lower oocyte counts (Fedorcsak *et al.,* 2001; Fedorcsák *et al.,* 2004; Dodson *et al.,* 2006).

It is considered that FSH is the main driver of antral follicle growth and it has an essential role in obtaining developmentally competent oocytes during in vitro culture of follicles (Adriaens *et al.,* 2004). Follicle stimulating hormone triggers granulosa cell proliferation in the preantral phase, prevents atresia, induces the synthesis of luteinizing hormone receptors and activates steroid hormones expression in the animal models and humans (Sasson *et al.,* 2004; Hunzicker-Dunn and Maizels, 2006). However, little is known about the joint influence of the body mass index (BMI) and the basal serum FSH on the embryo morphological development.

Recently the morphokinetic monitoring of human embryos have become a useful tool in embryo selection for IVF. There are many morphokinetic parameters that are correlated with ART outcomes such as blastocyst formation (Cruz *et al.,* 2012; Dal Canto *et al.,* 2012; Chamayou *et al.,* 2013), implantation potential (Meseguer et al. 2011; Basile *et al.* 2015), pregnancy potential (Lemmen *et al.* 2008; Meseguer *et al.,* 2012) and even aneuploidy status (Chawla et al., 2015; Vera-Rodriguez *et al.,* 2015). Furthermore, several predictive algorithms based on morphokinetic parameters and time-lapse evaluations have been proposed (Wong *et al.,* 2010; Meseguer *et al.,* 2012; Polanski *et al.,* 2014).

The aim of this study was to assess the impact of BMI and basal FSH on time-lapse variables of human embryos in women undergoing ART.

MATERIALS AND METHODS

Study design

This was a retrospective study of prospectively acquired data of time-lapse imaging of human embryos. Approval from the institutional ethical board was acquired and ethical guidelines were followed. All patients included in this study signed an informed consent form.

This study included 123 women who underwent standard ICSI treatment between January 2015 and December 2017 year in Nadezhda Woman's Health Hospital. The patients' BMI and basal FSH were recorded and morphokinetic data of the resulted 308 embryos was analysed.

Patients were divided according to their BMI into 3 groups: an underweight group (BMI < 18.5 kg/m²), a normal weight group (18.5 kg/m² < BMI < 25 kg/m²) and an overweight/obese group (BMI ≥ 25 kg/m²).

Ovarian stimulation and oocyte retrieval

The ovarian stimulation was performed by the administration of luteal gonadotropin-releasing hormone analogue (GnRHa), followed by recombinant FSH (Gonal-F) from cycle day 3. Oocyte aspiration of oocyte−cumulus complexes was performed 35 h after human chorionic gonadotrophin administration (Pregnyl 5,000 IU) by vaginal ultrasound-guided procedure. Oocytes were placed in a 4 well dishes containing 0.5 ml Global fertilization medium (LifeGlobal; Guilford, CT), covered with mineral oil, in groups of maximum of 5 oocyte cumulus complex.

Oocyte preparation, ICSI and embryo culture

Oocyte denudation was performed with hyaluronidase 3 h after retrieval. Denuded oocytes were subjected to standard intracytoplasmic sperm injection (ICSI) procedure. The injected oocytes were loaded in the pre-equilibrated EmbryoSlide™.

The EmbryoSlide™ (Fertilitech, Inc., Rockland MA) were prepared on the day before oocyte retrieval. Each of the slide's 12 individual wells were filled with 25 μl Global total medium (LifeGlobal; Guilford, CT) containing 10% SPS and were overlaid with 1.2 ml of washed oil. The slide was equilibrated overnight in incubators with 6 % CO_2, 5 % O_2 at 37 °C.

Cultivation of the fertilized oocytes was performed in incubator fitted with time-lapse image acquisition (Embryoscope, Unisense, Aarhus - Denmark). During incubation in the Embryoscope, seven plane focal images were generated each 20 min and recorded.

Morphokinetics

During embryo cultivation fifteen morphokinetic parameters were annotated: 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 (tHB) timing.

All events were counted from t0 as the time of ICSI. The (tPNa) was defined as the time in which both pronuclei could be observed. The time (tPNf), was the the last observation of both pronuclei, followed by pronuclei membrane disappearing and merging. The times t2, t3, t4, t5, t6, t7, t8 and t9 were annotated as the first times when appearing of corresponding number of cells (t2 for 2 cells, t3 for 3 cells, etc.) as separated by individual membranes. The time tM was defined as the first time in which the embryos were compacted into the morula stage. The tB time was defined at the time point in which a crescent-shaped area began to emerge from the morula. The time tEB of expanded blastocyst, consistent with the increase of the overall volume of the embryo and expansion of the blastocoele cavity.

Statistical analysis

The statistical analysis software IBM SPSS v.21 was used for all the data analysis, with the level of statistical significance being set at $p < 0.05$. Correlation between FSH and BMI was evaluated through Spearman correlation analysis.

RESULTS AND DISCUSSION

Correlation analysis revealed significant positive correlation between FSH and tPNf, t2 and t4 (R=0.18, p=0.03; R=0.17, p=0.04 and R=0.18; 0.03) in normal weight female patients (**Fig. 1**). In contrast, in the underweight group of women the serum levels of basal FSH correlate significantly with tM and tSB. Moreover, in the overweight group we found a significant negative correlation between FSH and t7 (R=-0.25, p=0.05), t9 (R=0.38, p=0.01) and tM (R=-0.29, p=0.05) (**Fig. 1**).

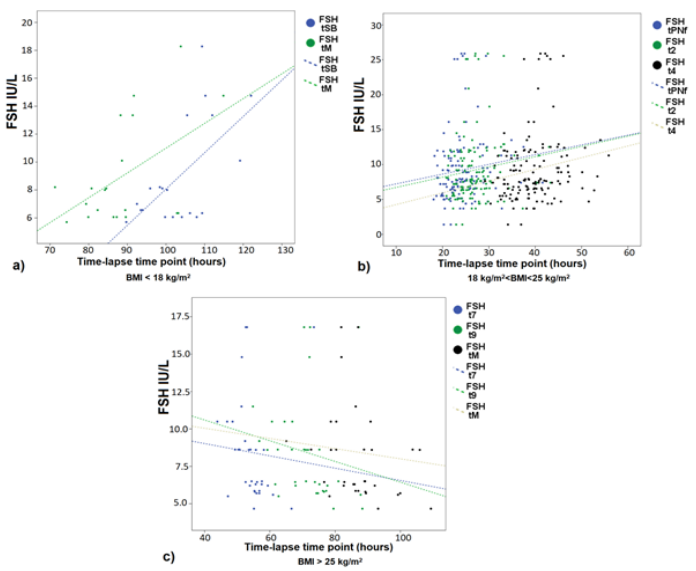

Fig. 1. Overlays scatter plots representing the linear relationship between FSH and the time-lapse time points (in hours) of certain morphokinetic variables in (a) underweight patient group (BMI<18.5 kg/m²) (morphokinetic variables: tSB (blue dots) and tM (green dots)), (b) normal weight patient group (18.5 kg/m²<BMI<25 kg/m²) (morphokinetic variables: tPNf (blue dots), t2 (green dots) and t4 (black dots)) and (c) overweight patient group (BMI≥25 kg/m²) (morphokinetic variables: t7 (blue dots), t9 (green dots) and tM (black dots)). The colored lines indicate the fit lines for the FSH and the corresponding morphokinetic variables.

It was previously suggested that some of the analysed parameters such as t2, t4 and tSB could also have a predictive value for good quality blastocyst formation (Milewski *et al.,* 2015; Storr *et al.,* 2015). We found that the basal FSH level is associated with later events (t7, tM and tSB) in both underweight and overweight females. It was already found by other authors that the BMI could affect not only the hormone levels and the oocyte metabolism (Robker *et al.,* 2009), but also the embryo morphological development. It has been reported that

obese women have embryos of significantly poorer quality than women with normal weight (Carrell *et al.,* 2001; Metwally *et al.,* 2007). However, in contrast to our results, other authors did not found any significant association between morphological embryo quality and BMI (Esinler *et al.,* 2008; Shalom-Paz *et al.,* 2011). Another study suggested that the impact of obesity and overall higher BMI on IVF outcomes was not mediated by alterations in morphokinetic parameters (Dimitriadis *et al.,* 2018). Recently Souter et al. also did not found any significant association among basal FSH levels, BMI and specific morphokinetic parameters (2- to 3-cell and 3- to 4-cell stage) (Souter *et al.,* 2016). However, in contrast to our study, these studies discuss only a restricted group of time-lapse variables. The detailed analysis with more variables gave us an opportunity to discover new relationships between BMI, FSH and embryo morphokinetics unnoticed by previous research.

CONCLUSION

In conclusion, the basal FSH level has a specific influence on embryo morphokinetics, depending on the BMI. FSH has a strong impact on the initial stages of embryo development in normal weight patients, while it has a significant effect on the later events in underweight and overweight women.

AUTHOR CONTRIBUTIONS

S.N., D.P., G.S. and T.C. conceived the experiment; S.N., M.I., I.I., and K.N. conducted the experiment; D.P. and S.N. analyzed the results. S.N., D.P. and R.G. wrote the main manuscript text and prepared tables and figures. S.N., D.P. and R.G. and 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.

REFERENCES

1. Adriaens, I., Cortvrindt, R., Smitz, J. 2004. Differential FSH exposure in preantral follicle culture has marked effects on folliculogenesis and oocyte developmental competence. *Human Reproduction,* 19 (2): 398–408.
2. Basile, N., Vime, P., Florensa, M., Aparicio-Ruiz, B., Velasco, J., Remohi, J., Maseguer, M. 2015. The use of morphokinetics as a predictor of implantation: a multicentric study to define and validate an algorithm for embryo selection. *Human Reproduction*, 30 (2): 276–283.
3. Carrell, D., Jones, K., Peterson, C., Aoki, V., Emery, B., Campbell, B. 2001. Body mass index is inversely related to intrafollicular HCG concentrations, embryo quality and IVF outcome. *Reproductive Biomedicine Online*, 3:109–111.

4. Chamayou, S., Patrizio, P., Storaci, G., Tomaselli, V., Alecci, C., Ragolia, C., Crescenzo, C., Gugliemino, A. 2013. The use of morphokinetic parameters to select all embryos with full capacity to implant. J*ournal of Assisted Reproduction and Genetics,* 30 (5): 703–710.
5. Chawla, M., Fakih, M., Shunnar, A., Bayram, A., Hellani, A., Perumal, V., Divakaran, J., Budak, E. 2015. Morphokinetic analysis of cleavage stage embryos and its relationship to aneuploidy in a retrospective time-lapse imaging study. *Journal of Assisted Reproduction and Genetics,* 32 (1): 69–75.
6. Cruz, M., Garrido, N., Herrero, J., Perez-Cano, I., Munoz, M., Meseguer, M. 2012. Timing of cell division in human cleavage-stage embryos is linked with blastocyst formation and quality. *Reproductive Biomedicine Online*, 25 (4): 371–381.
7. Dal Canto, M., Coticchio, G., Renzini., M., De Ponti, E., Novara, P., Brambillasca, F., Comi, L., Fadini, R. 2012. Cleavage kinetics analysis of human embryos predicts development to blastocyst and implantation. *Reproductive Biomedicine Online*, 25 (5): 474–480.
8. Dimitriadis, I., Arvizu, M., Christou, G., Bormann, C., Chavarro, J., Hsu, J., Souter, I. 2018. The effect of body mass index (BMI) on blastocyst development as assessed by time-lapse (Tl) morphokinetics. *Fertility and Sterility,* 109 (3): e24–e25.
9. Dodson, W., Kunselman, A., Legro, R. 2006. Association of obesity with treatment outcomes in ovulatory infertile women undergoing superovulation and intrauterine insemination. *Fertility and Sterility*, 86: 642–646.
10. Esinler, I., Bozdag, G., Yarali, H. 2008. Impact of isolated obesity on ICSI outcome. *Reproductive Biomedicine Online,* 17: 583–587.
11. Fedorcsák, P., Dale, P., Storeng, R., Ertzeid, G., Bjercke, S., Oldereid, N., Omland, A., Abyholm, T., Tanbo, T. 2004. Impact of overweight and underweight on assisted reproduction treatment. *Human Reproduction,* 19 (11): 2523–2528.
12. Fedorcsak, P., Dale, P., Storeng, R., Tanbo, T., Abyholm, T. 2001. The impact of obesity and insulin resistance on the outcome of IVF or ICSI in women with polycystic ovarian syndrome. *Human Reproduction*, 16 (6): 1086–1091.
13. Ferlitsch, K., Sator, M., Gruber, D., Rucklinger, E., Gruber, C., Huber, J. 2004. Body Mass Index, Follicle-Stimulating Hormone and Their Predictive Value in In Vitro Fertilization. *Journal of Assisted reproduction and Genetics,* 21 (12): 431-436.
14. Hunzicker-Dunn, M., Maizels, E. 2006. FSH signaling pathways in immature granulosa cells that regulate target gene expression: branching out from protein kinase A. *Cellular Signalling*, 18: 1351–1359.
15. Lemmen, J., Agerholm, I., Ziebe, S. 2008. Kinetic markers of human embryo quality using time-lapse recordings of IVF/ICSI-fertilized oocytes. *Reproductive Biomedicine Online,* 17: 385–391.
16. Martin, J., Nisker, J., Tummon, I., Daniel, S., Auckland, J., Feyles, V. 1996. Future in vitro fertilization pregnancy potential of women with variably elevated day 3 follicle-stimulating hormone levels. F*ertility and Sterility*, 65: 1238 –1240.
17. Meseguer, M., Herrero, J., Tejera, A., Hilligsoe, K., Ramsing, N., Remohi, J. 2011. The use of morphokinetics as a predictor of embryo implantation. *Human Reproduction,* 26: 2658–2671.

18. Meseguer, M., Rubio, I., Cruz, M., Basile, N., Marcos, J., Requena, A. 2012. Embryo incubation and selection in a time-lapse monitoring system improves pregnancy outcome compared with a standard incubator: a retrospective cohort study. *Fertility and Sterility,* 98: 1481–1489. pmid:22975113
19. Metwally, M., Tuckerman, E., Laird, S., Ledge,r W., L,i T. 2007. Impact of high body mass index on endometrial morphology and function in the peri-implantation period in women with recurrent miscarriage. *Reproductive Biomedicine Online,* 14: 328–334.
20. Milewski, R., Kue, P., Kuczynska, A., Stankiewiecz, B., Lukaszuk, K., Kaczynski, W. 2015. A predictive model for blastocyst formation based on morphokinetic parameters in time-lapse monitoring of embryo development. *Journal of Assisted Reproduction and Genetics*, 32: 517-579.
21. Polanski, L., Coelho Neto M., Nastri, C., Navarro, P., Ferriani, R., Raine-Fenning, N., Martins, W. 2014. Time-lapse embryo imaging for improving reproductive outcomes: systematic review and meta-analysis. *Ultrasound in Obstetrics and Gynecology,* 44: 394–401. pmid:24890582
22. Robker, R., Akison, L., Bennett, B., Thrupp, P., Chura, L., Russell, D., Lane, M., Norman, R. 2009. Obese women exhibit differences in ovarian metabolites, hormones, and gene expression compared with moderate-weight women. *Journal of Clinical Endocrinology and Metabolism,* 94: 1533–1540.
23. Sasson, R., Rimon, E., Dantes, A., Cohen, T., Shinder, V., Land-Bracha, A., Amsterdam, A. 2004. Gonadotrophin-induced gene regulation in human granulosa cells obtained from IVF patients. Modulation of steroidogenic genes, cytoskeletal genes and genes coding for apoptotic signalling and protein kinases. *Molecular Human Reproduction,* 10: 299–311.
24. Sathya, A., Balasubramanyam, S., Gupta, S., Verma, T. 2010. Effect of body mass index on in vitro fertilization outcomes in women. J*ournal of Human Reproductive Sciences,* 3 (3): 135-138.
25. Shalom-Paz, E., Marzal, A., Wiser, A., Almog, B., Reinblatt, S., Tulandi, T., Holzer, H. 2011. Effects of different body mass indices on in vitro maturation in women with polycystic ovaries. *Fertility and Sterility,* 96: 336–339.
26. Sherbahn, R. 2011. Effect of low and high body mass index (BMI) on embryo development, implantation and live birth rates. *Fertility and sterility*, 96 (3): S190.
27. Souter, I., Dimitriadis, I., Bormann, C., Hauser, R., Messerlian, C. 2016. Maternal predictors of morphokinetic embryo parameters using time-lapse (TL) imaging. *Fertility and Sterility*, 106 (3): e308.
28. Storr, A., Venetis, C., Cooke, S., Susetio, D., Kilani, S., Ledger, W. 2015. Morphokinetic parameters using time-lapse technology and day 5 embryo quality: a prospective cohort study. *Journal of Assisted Reproduction and Genetics*, 32: 1151-1160.
29. Vera-Rodriguez, M., Chavez, S., Rubio, C., Reijo, P., Simon, C. 2015. Prediction model for aneuploidy in early human embryo development revealed by single-cell analysis. *Nature Communications,* 6: 7601. pmid:26151134.
30. Wong, C., Loewke, K., Bossert, N., Behr, B., De Jonge, C., Baer. T. Pera, R. 2010. Non-invasive imaging of human embryos before embryonic genome activation predicts development to the blastocyst stage. *Nature Biotechnology*. 28: 1115–1121. pmid:20890283.