Non-invasive embryo assessment in IVF

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SUMMARY

This thesis is focused on non-invasive embryo assessment in IVF. **Chapter 1** presents the background, aim and outline of this thesis. In this chapter we describe the current routine approach for embryo assessment and selection: morphology. We elaborate on some of the shortcomings and concerns of embryo assessment and selection by morphology and the subsequent need for new embryo selection tools that are more accurate in predicting the reproductive potential of an embryo than morphology. Furthermore, we present some background information of a new embryo assessment and selection tool that analyses constituents of spent embryo culture medium (metabolomic profiling of spent culture medium by near-infrared [NIR] spectroscopy). The introduction and validation of this novel assessment and selection technology is the main focus of this thesis.

In **chapter 2** we present a proof of principle study in which we have retrospectively investigated if metabolomic profiling of biomarkers of embryo culture medium by NIR spectroscopy has a correlation with ongoing pregnancy when the transferred embryos were selected by conventional selection criteria. A total of 333 patients scheduled for IVF with a single embryo transfer (SET) were included in the study. Embryos were selected for transfer by routine morphological criteria on days 2 and 3 of in vitro culture, and left over culture media samples were analysed by NIR spectroscopy. The obtained unique spectral profiles were quantified with algorithms into viability scores. Metabolomic profiles from patients without an ongoing pregnancy showed significantly lower mean viability scores compared to those viability scores obtained from profiles observed in ongoing pregnancies. A logistic regression of factors correlated to pregnancy outcomes showed that maternal age, percent fragmentation of the embryo and the viability score all demonstrated a relationship with pregnancy outcomes. The Pearson correlation coefficients between the viability score and number of blastomeres or fragmentation respectively, were both close to 0, indicating no linear correlation between these variables. We concluded that NIR metabolomic profiling of spent embryo culture medium was able to distinguish viable embryos from non-viable embryos. The lack of correlation between embryo morphology and the viability score indicated that we are looking at new aspects of an embryo's intrinsic quality.

In **chapter 3**, the relationship between algorithm generated viability scores, morphology assessment on individual day 2 and 3 embryos selected for single embryo transfer and the implantation rates is further investigated. For this, the data of the first

proof of principle study described in **chapter 2** was supplemented with data of the Kato Ladies Clinic from Japan. A cut-off score for the viability index within different morphological grades of embryos was analysed in relation to implantation. The results indicated that the mean viability scores of embryos that resulted in a positive fetal cardiac activity were significantly higher compared to the viability scores of embryos that did not result in a positive fetal cardiac activity, for both day 2 (n=181) and day 3 (n=304) embryos. Viability scores were found to be independent of morphology for both day 2 and day 3 embryos. Implantation rates were significantly higher among embryos with a viability score above the cut-off value (i.e. viability score≥0.3). We concluded in this chapter that metabolomic profiling of embryo culture medium using near-infrared spectroscopy is independent of morphology and correlates with reproductive potential of embryos.

Chapter 4 presents the results of a retrospective study in which we analysed spent culture media with NIR spectroscopy of 127 frozen-thawed embryos with known implantation potential after SET and related the results to live birth. A viability score was calculated using a predictive multivariate algorithm of fresh day 5 embryos with known pregnancy outcomes. This algorithm generated with fresh day 5 embryos, could help to identify the live birth group from the no live birth group. A series of multivariable regression models that tested the predictive ability of the viability score for live birth, showed before and after adjustment for the variables embryo morphology, resumption of mitosis, elective or non-elective SET, embryo survival rate, IVF or ICSI treatment in fresh cycle, stimulation protocol in fresh cycle, infertility duration, infertility indication and age of patient at oocyte retrieval, an odds ratio (OR) between 1.44 and 1.71 based on a 0.1 difference in viability scores. This means that a 0.1 step increase in the viability score was associated with a 1.44-1.71 times higher odds for live birth. In conclusion, higher viability scores resulted in higher live birth rates. An algorithm generated from fresh embryos might be used to predict the viability of frozen-thawed embryos.

In **chapter 5** we investigated if the selection of a single day 3 embryo by metabolomic profiling of culture media with NIR spectroscopy as an adjunct to morphology was able to improve pregnancy and live birth rates in IVF, compared to embryo selection by morphology alone. For this purpose we conducted a double blind randomized controlled trial (RCT) in which 417 couples undergoing IVF with a single embryo transfer (SET) were included. Patients were randomized in either the control group (embryo selection by routine morphology) or the treatment group (embryo selection by NIR

spectroscopy of spent culture medium in addition to morphology). Main inclusion criterion before SET was the presence of two or more similar best quality embryos. In the treatment group, a viability score for each embryo was generated using NIR spectroscopy and the embryo with the highest viability score was transferred. The embryo with the best morphology was transferred in the control group. In the intention-to-treat analysis as well as in the per protocol analysis, the ongoing pregnancy and live birth rates did not differ significantly between the treatment and the control groups. In 75.4% of the transfers in the treatment group, the embryo with the highest viability score did not have the best morphology. The live birth rate however, was similar to the live birth rate of the control group. This strongly suggests that within a group of good quality embryos, there is more than one embryo able to develop into a live birth. This stresses the need for SET when several good quality embryos are available.

Chapter 6 demonstrates that there is at present no evidence that NIR spectroscopy of spent embryo culture media in its current form can be used in daily practice to improve live birth rates. We performed a meta-analysis with individual patient data and pooled the individual data of all available RCTs that compared embryo selection by morphology with embryo selection by morphology and the use of NIR spectroscopy of spent embryo culture medium by the ViaMetrics-E™. This way, we increased the statistical power considerably which allowed us to provide a more reliable estimate of treatment effect.
The findings of the study underline the necessity of providing evidence-based proof of clinical usefulness before the implementation of new diagnostic tools in routine IVF practice.

In **chapter 7** we describe a retrospective analysis of multilevel images of 659 day 3 single-transferred embryos. The aim of this study was to generate objective variables that resemble embryo quality and relate them to ongoing implantation, by measuring the blastomeres in multilevel images. We introduced three novel, objective variables that quantify, instead of estimate important morphological parameters. The blastomere volume index (BVI) represents the ratio between the total blastomeric volume of an embryo and the mean cytoplasmic volume of an oocyte on day 0 (i.e. the quantification of fragmentation). The blastomere symmetry index (BSI) represents the ratio between the greatest blastomere volume and the smallest blastomere volume within an embryo. The mean ovality (MO) represents the presence of non-spherical blastomeres. The mean BVI was significantly higher for embryos in the ongoing

implantation group compared to the no ongoing implantation group. The mean BSI was associated with ongoing implantation for unevenly cleaved embryos. The MO of blastomeres within an embryo was similar for embryos in the ongoing implantation group compared to the no ongoing implantation group. Multiple logistic regression analyses showed that the association of the BVI and BSI with ongoing implantation was confounded. As a consequence, we concluded that the BVI, BSI and MO were not suitable to use as embryo selection tools.

Chapter 8 describes the evaluation of birthweights of singleton newborns after a fresh (day 3) or frozen-thawed (day 5) SET cycle, who were cultured as an embryo in two different types of commercially available culture media. An immediate switch in our laboratory culture system allowed us to analyse the birthweights of the babies who were cultured as an embryo in either human tubal fluid (HTF) or Sage®, Quinn's advantage protein plus medium within a relatively short period of time. Analysis of 358 singletons born after a fresh SET and 159 singletons born after a frozen–thawed SET showed no significant difference between the HTF and Sage® groups in terms of mean birthweight and mean birthweight adjusted for gestational age and gender or parity (z-scores). Furthermore, we showed that embryo freezing and thawing may lead to a significantly higher mean birthweight.

In **chapter 9**, a general discussion is presented in which we discuss the results of this thesis and their implications for clinical practice. We discuss some novel embryo assessment and selection technologies that have been introduced into IVF clinics over the last few decades to see to what end their (clinical) use is supported by a scientific foundation. This includes the main focus of this thesis: embryo selection by metabolomic profiling of culture medium with near-infrared (NIR) spectroscopy. Furthermore, we discuss the necessity of a proper validation of new embryo assessment and selection technologies in routine IVF and we elaborate on future perspectives with regard to the focus of our research activities.