Predictive Value Of Glucose And β-Hcg Concentration In The Embryo Culture Medium Of Patients Undergoing Intracytoplasmic Sperm Injection

Diagnostic Study

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ABSTRACT

Objective: The aim of this study was to evaluate 3 methods of embryo selection in predicting the embryos with the best method of achieving pregnancy in single embryo transfer cycles, namely embryo morphology, glucose concentration and β-HCG concentration in the embryo culture medium.

Patients and Methods: A total of 66 embryos in 66 ICSI cycles were studied: 33 pregnant and 33 non-pregnant cycles. Each embryo was scored using the Veeck's scoring method and the spent culture medium of each embryo was analyzed for its content of glucose and β-HCG using previously validated methods.

Results: The results showed that the Veeck's embryo score, the measurement of glucose levels and the measurement of β-HCG levels in the spent culture medium are all reliable and practical methods to be used for this purpose. The Veeck's embryo score had the best predictability followed by β-HCG levels, followed by glucose levels in the culture medium. Combining the 3 methods was superior to each methods studied alone as well as any combination of 2 methods.

Conclusion: The measurement of glucose and β-HCG in the spent culture medium of individual embryos is an effective and practical method for embryo selection in patients treated with ICSI. Combining both methods with the Veeck's embryo score gives the best predictability. However, these findings should be confirmed by a prospective randomized study, preferably in single embryo transfer cycles.

Key Words: ICSI, pregnancy, culture media, Glucose , B-HCG

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INTRODUCTION

Despite continuous developments in the field of assisted reproduction, the live birth rate following IVF and ICS remains low. The most recent statistics of the Society for Assisted Reproductive Technologies (SART) of the American Society for Reproductive Medicine (ASRM) show that the live birth rate in women undergoing these procedures in 2011 was 29.2% per retrieval [1]. In order to maximize the success rates, infertility specialists are always faced with the dilemma of transferring too many embryos and risking the occurrence of multiple pregnancies with the resulting complications, or transferring too few embryos and risking failure of the procedure [2]. There is, therefore, an urgent need for a simple and practical method for embryo selection in order to maximize the chances of success and minimize the problems of multiple pregnancies in couples treated with assisted reproduction.

Various methods for embryo selection have been suggested and practiced. These include invasive and non-invasive methods. Invasive methods are used to screen the embryos for genetic abnormalities (PGS). They require the performance of an embryo biopsy: a blastomere biopsy at the 8-cell stage or a trophectoderm biopsy at the blastocyst stage. The procedures are expensive and require advanced skills. More importantly, they are not full proof due to the possibility of embryo injury, undetected mosaicism and segmental aneuploidy [3].This means that some normal embryos may be discarded and some abnormal embryos may be falsely diagnosed as normal. In addition, a meta-analysis of randomized studies conducted in 2011 showed that PGS did not improve the live birth rate compared to morphological selection [4], although a more recent meta-analysis showed that, in good prognosis patients, comprehensive chromosome screening after blastocyst biopsy was associated with higher clinical

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implantation and ongoing pregnancy rates (but not higher live birth rates) when the same number of embryos is transferred [5]. Consequently, more infertility specialists are now considering and using non-invasive methods for embryo selection.

Non-invasive methods for embryo selection include [1] selection on the basis of the morphology of individual embryos at the time of transfer, [2] selection on the basis of the morpho-kinetic changes of individual embryos during early developmental stages observed by time-lapse photography, [3] selection on the basis of the oxygen consumption by individual embryos, [4] selection on the basis of various biochemical markers measured in the culture medium of individual embryos, [5] selection on the basis of oxidative stress to which individual embryos are subjected, or [6] combination of some of the above. The aim of this work was to evaluate two of these methods of embryo selection as predictors of clinical pregnancy taking embryo morphology as the reference method.

PATIENTS AND METHODS

After obtaining an informed consent, embryos from 30 female patients aged 20 to 38 years and undergoing IVF treatment were studied. Patients with repeated implantation failure (3 or more failed IVF attempts) as well as those with pathological causes affecting implantation, such as endometriosis and fibromyomata were excluded. Standard IVF stimulation protocols were used and oocytes were retrieved by the transvaginal ultrasound-guided route. Insemination of the retrieved oocytes was then be performed by standard methods. Each embryo was cultured in an individual well containing 0.6 ml of Menezo's B2 culture medium exactly and in an atmosphere of 5% CO2 in air. Single embryo transfers were performed.

The culture medium of each individual embryo was kept at -80 degrees Celsius until the time of the assay. The concentration of glucose in the culture medium was measured using the automated analyzer (Dimensions, Siemens), while the concentration of β-HCG in the culture medium was measured using the automated immunochemistry analyzer (Advia systems, Siemens). The relationship between the concentration of the stated markers and the clinical outcome was determined using appropriate statistical methods.

RESULTS

Predictive value of embryo morphology. The predictive value of the embryo morphology for achieving clinical pregnancy was studied by comparing the mean embryo score in pregnant and non-pregnant subjects and then by constructing a receiver operating characteristics (ROC) curve to calculate the cut-off score. The mean (+/-SD) embryo score in the pregnant and non-pregnant subjects was 7 (+/- 1.83) and 5 (+/-1.79), respectively. Statistical analysis showed that this difference is highly significant *(<0.0001)*. The ROC curve of the ES for predicting the occurrence of clinical pregnancy showed that the mean area under the curve (AUC) was 0.799 (95% C.I. 0.6860.913-) giving a highly significant predictive value *(P <0.001)*. The cut-off score at the best sensitivity and specificity was >5.

Predictive value of glucose levels in the culture medium. The predictive value of glucose levels in the culture medium for achieving clinical pregnancy was studied by comparing the mean glucose level in pregnant and non-pregnant subjects and then by constructing a receiver operating characteristics (ROC) curve to calculate the cut-off level. The mean (+/-SD) glucose level in the spent culture medium in the pregnant and non-pregnant subjects was 104 (+/- 18.70) and 120 (+/-29.62) mg/dL, respectively. Statistical analysis showed that this difference is also significant *(<0.01)*. The ROC curve of the glucose level in the spent culture medium for predicting the occurrence of clinical pregnancy showed that the mean area under the curve (AUC) is 0.669 (95% C.I. 0.5360.802-) giving a significant predictive value *(P <0.020)*. The cut-off point at the best sensitivity and specificity was ≤110 mg/ml.

Predictive value of β-HCG levels in the culture medium. The predictive value of β-HCG levels in the culture medium for achieving clinical pregnancy was studied by comparing the mean β-HCG levels in pregnant and non-pregnant subjects and then by constructing a receiver operating characteristics (ROC) curve to calculate the cut-off level. The mean (+/-SD) β-HCG level in the spent culture medium in the pregnant and non-pregnant subjects was 9.61 (+/-1.55) and 8.68 (+/-1.97) mIU/mL, respectively. Statistical analysis showed that this difference is also significant *(<0.05)*. The ROC curve of the β-HCG level in the spent culture medium for predicting the occurrence of clinical pregnancy showed that the mean area under the curve (AUC) is 0.681 (95% C.I. 0.5470.816-) giving a significant predictive value *(P <0.013)*. The cut-off point at the best sensitivity and specificity was >9.1 IU/L.

Comparison of the predictive value of the 3 markers (ROC curve). Figure 4 shows the comparison between the 3 ROC curves for the embryo score, glucose level and β-HCG in the spent culture medium as predictors of clinical pregnancy. It may be seen from the figure that the best predictor was the embryo score with the highest AUC (0.799) followed by the β-HCG (AUC = 0.681) while the glucose level in the spent culture medium had the lowest predictive value (AUC =0.669).

Predictive value of combining the 3 markers. Figure 5 shows the combined predictive value of the embryo score, as well as the glucose and β-HCG levels in

the spent culture medium. The AUC for this combination was 0.861 which is higher than their individual values (AUC = 0.799 for the embryo score, 0.681 for the β-HCG level and 0.669 for the glucose level).

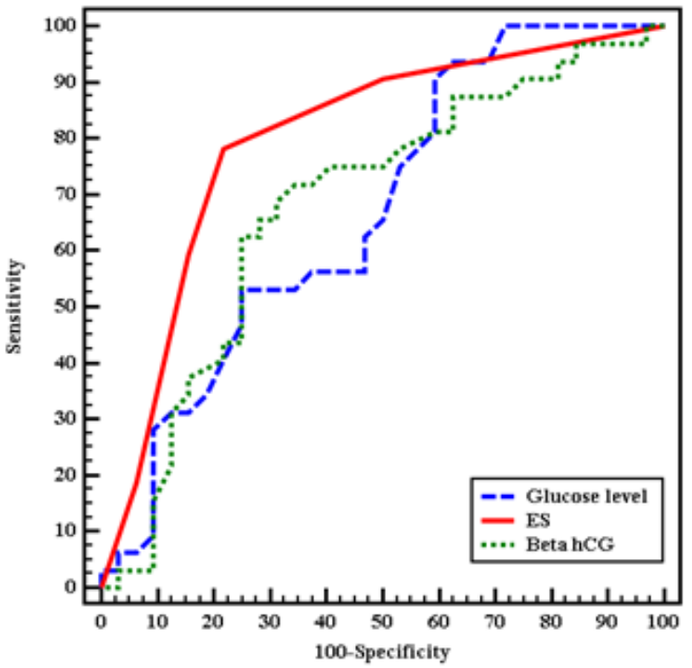

Fig. 1: Comparison between the 3 ROC curves for the embryo score, glucose level and β-HCG in the spent culture medium as predictors of clinical pregnancy

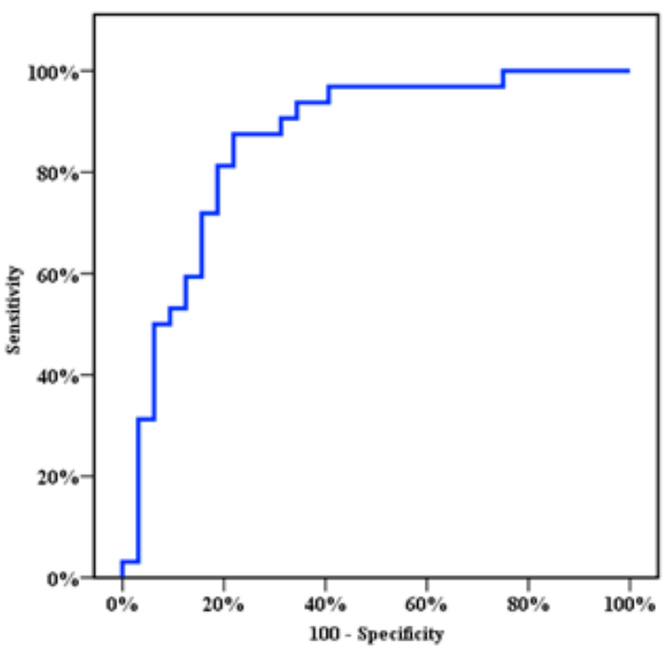

Fig. 2: Combined predictive value of the embryo score as well as the glucose and β-HCG levels in the spent culture medium.

DISCUSSION

The results of this work show that embryos resulting in pregnancy had a very significantly higher score compared to those that did not result in pregnancy *(P< 0.0001)*. The ROC curve showed an AUC of 0.799 (95% C.I. = 0.686 – 0.913) and the cut-off point was >5

As regards glucose consumption by individually cultured embryos, it has been previously shown by Gott *et al,* that human embryos consume more pyruvate than glucose during the first 2 days of their growth, while they consume more glucose than pyruvate starting from day 3 until the blastocyst stage and beyond [6], while during the whole period of their early growth, embryos secrete lactates. The results of this study show that human embryos indeed consume glucose on day 3 of their growth and that embryos resulting in implantation consume significantly more glucose compared to those which did not implant *(P <0.01)*. The ROC curve showed that this method has a good predictability for clinical pregnancy and the AUC was 0.669 while the cut-off level at the best sensitivity and specificity was <110. The results are also in line with those of Gardner *et al* who found that embryos resulting in pregnancy consumed more glucose than those that did not [7]. Interestingly, they also found that embryos resulting in female offspring consumed more glucose compared to those resulting in male babies. The measurement of glucose level in the spent culture medium is therefore a simple and practical and reliable method for embryo selection.

As regards the measurement of β-HCG in the spent culture medium of in-vitro cultured embryos, Ramu *et al* had shown in 2011 that these embryos secrete this hormone as early as day 2 post-fertilization [8]. Similar results were reported in 2013 by Butler *et al* who showed that embryos secrete β-HCG from the 2-pronuclear stage throughout the blastocyst stage [9]. Similarly Xiao-Yan et al found that there was a significantly higher β-HCG concentration in the spent culture medium of embryos resulting in pregnancy compared to those that did not. This significant difference was present in day 3 embryos (P= 0.041) but was more pronounced at the blastocyst (day 5) stage (0.004) [10]. The results of this study are totally in line with these earlier findings. Results also found a significantly higher β-HCG levels in the spent culture medium of embryos resulting in pregnancy compared to those that did not *(P <0.05)*. The ROC curve showed that the method had a good predictability for clinical pregnancy and the AUC was 0.681 and the cut-off at the best sensitivity and specificity was >9.2

Comparing the 3 methods of embryo selection studied, the embryo score remains the best predictive method (AUC = 0.799), followed by the β-HCG (AUC = 0.681) followed by glucose levels in the spent culture medium (AUC = 0.661). However, in an attempt to improve the predictability of our methods we have tried to combine any 2 or all 3 methods of embryo selection studied, as done by previous workers looking at other markers for embryo selection. For example, Kotze *et al* studied the predictability of combining soluble human leukocytic antigen (sHLA) and the graduated embryo score and found that the odds (95% C.I.) of predicting chemical, clinical and on-going pregnancies were 2.62 (95% C.I. =2.1495%) 2.72 ,(3.22- C.I. = 2.223.33-) an d 3.56 (95% C.I. = 2.8811) (4.40-). Similarly, El-Kaffash *et al* found that combining the measurement of sHLA in the spent culture medium and embryo evaluation using the Veeck's score improved the predictability of this combination over each marker on its own. The AUC was 0.968 for the combination compared to 0.794 for the sHLA-G and 0.925 for the Veeck's embryo score [12]. In a different study, Tejera *et al* combined the oxygen consumption of individual embryos with time lapse photography and found that this combination improved the predictability over each individual method [13]. In this study, the results showed that combining the Veeck's embryo score and the glucose level improved the predictability of this combination (AUC = 0.831, 95% C.I. = 0.7270.935-) over each method alone (AUC = 0.799, 95% = 0.6860.913-) and AUC = 0.669, 95% C.I. = 0.5360.802-), respectively). This combination was better than combining the Veeck's embryo score and β-HCG in the culture medium which gave a better predictability (AUC = 0.817, 95% C.I. = 0.7100.924-) over each method alone (AUC = 0.799, 95% = 0.686-0.913 and AUC = 0.681, 95% C.I. = 0.5470.816-), respectively. Both combinations were better than combining the predictability of glucose and β-HCG in the spent culture medium (AUC = 0.757, 95% C.I. = 0.6530.879-) was again better than each method alone (AUC = 0669, 95% = 0.5360.802-) and AUC = 0.681, 95% C.I. = 0.547- 0.816), respectively. However, the best predictability was found by combining the 3 methods, namely the Veeck's embryo score with glucose and β-HCG levels in the spent culture medium. This triple combination was associated with an AUC equal to 0.861 (95% C.I. = 0.7670.956-).

CONCLUSION

In conclusion, this study showed that the measurement of glucose and β-HCG in the spent culture medium of individual embryos is an effective and practical method for embryo selection in patients

treated with ICSI and that combining both methods with the Veeck's embryo score gives the best predictability. However, these findings should be confirmed by a prospective randomized study, preferably in single embryo transfer cycles.

CONFLICT OF INTEREST

There is no conflict of interest.

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