# Can day 0 of an ICSI cycle be indicative of laboratory outcome

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## INTRODUCTION

In Vitro Fertilization (IVF), is a field of dynamic research focusing on continuous improvement. Numerous studies from embryo morphology to novel biomarkers have been introduced as the next holy grail in efficient embryo selection. The majority of the studies focus principally on the events that follow successful fertilization of the oocyte both in terms of morphology and morphokinetics [1,2]. It appears that in the maze of data aligned towards establishing a model to predict the embryo of the highest implantation potential, perhaps certain aspects have remained understudied or underrepresented in literature. In light of that, it may be time for studies to focus on connecting the dots between the oocyte, its behavior during ICSI, embryo quality and live-birth potential. These early cycle data are not yet encompassed in any of the recent advancements in the field of IVF. It is this rationale that fueled design of the present study. Hereby the authors proceed with providing data aiming to associate these aspects namely quality of the oocyte retrieved, subsequent ICSI application, the respective embryo quality and the cycle outcome. These studied aspects correspond to different time-points in clinical practice. Overall, the present study investigates possible associations between oocyte morphology and behavior during ICSI, with the fertilization outcome, the resulting zygote, embryo quality, and the implantation and live birth rates. Delineating on the associations between different features regarding oocyte morphology and relating them to the oocyte's behavior during ICSI, the zygote, along with the IVF cycle outcome, may subsequently assist practitioners in understanding factors entailed in securing creation of a potent zygote and embryo.

## RESULTS

A total of 477 couples who underwent 477 ICSI cycles, yielding a total of 3452 oocytes, participated in this prospective observational study from 2015 to 2018. All couples included presented solely with male factor infertility, serving as the indication for performing ICSI. To study a possible negative effect of oocyte characteristics and their behavior during ICSI on the cycle outcome, an analysis was performed regarding the aforementioned parameters and the post-ICSI oocytes and embryos that were discarded and not selected for embryo transfer or cryopreservation. Multinomial logistic regression was also employed regarding the associations between oocyte morphology and the respective ICSI behavior, with embryo grading on day 3, as well as with post-ICSI oocytes and embryos selected to be discarded.

No association was observed between the levels of AMH, E2, FSH, LH or progesterone and any of the oocyte characteristics. Age was negatively associated with the observation frequency for normal ooplasm (p<0.001) and normal PVS (p=0.001). The observation frequency regarding granular oocytes and granular PVS was positively associated with maternal age (p<0.001). No other statistically significant association was presented. Z1 score being associated with the best prognosis should be employed as the reference point in the multinomial regression analysis. A dark ooplasm (p<0.001) was positively associated with fertilization failure. Oocytes that failed to be fertilized presented with a lack of ooplasm translucency (p<0.001). A thick ZP was similarly positively associated with non-fertilized oocytes (p<0.001). Grade 1 score being associated with the best prognosis should be employed as the reference point in the multinomial regression analysis. Uncleaved embryos were correlated with lack of ooplasm translucency (p=0.001). Embryos graded as 3,4 and 5 were associated only with ooplasm granularity (p<0.001). A high resistance during ICSI penetration (HR) was associated with lack of ooplasm translucency (p<0.001), an extensively granular PVS (p<0.001) and abnormalities of zona pellucida, namely thick, dark or granular ZP (p<0.001). The large oocyte size (p<0.001), the vacuolated (p<0.001) ooplasm, the extensively granular PB (p<0.001) or the thin, dark, granular or uneven ZP (p<0.001) were statistically significantly associated with a lack of resistance during ICSI (NR). A difficult ooplasm aspiration was positively associated with a dark, granular or vacuolated ooplasm (p<0.001), a granular or large PVS (p<0.001) or a large PB (p<0.001). A sudden ooplasm aspiration was positively associated with a granular, vacuolated or dark ooplasm (p<0.001), as well as, a granular (p<0.001) or large (p<0.001) PVS and a thick (p<0.001) or thin (p<0.001) ZP. Oocytes presenting with No Resistance (NR) were more likely to be graded as Z4 (p<0.001) or fail to be fertilized (p<0.001) when compared to normal resistance. Oocytes presenting with High resistance (HR) were more likely to fail to be fertilized or be categorized as degenerated oocytes post-ICSI, when compared to normal resistance (p<0.001) or to be graded as Z2 or Z4 (p<0.001). In cases where ooplasm aspiration was difficult the oocytes were more likely to fail to be fertilized or to be graded as Z2 or Z3 (p<0.001). In cases where the ooplasm aspiration was sudden the oocytes were more likely to fail to be fertilized (p<0.001) be categorized as degenerated oocytes post-ICSI (p<0.001) or to be graded as Z3 or Z4 (p<0.001). Uncleaved embryos were correlated with NR (p<0.001) as well as sudden ooplasm aspiration (p=0.001). A total of 1200 post-ICSI oocytes and embryos were selected to be discarded during the 477 IVF cycles. The post-ICSI oocytes and embryos selected to be discarded correspond to 83 1PN embryos, 86 3PN embryos, 181 unfertilized and 87 degenerated post-ICSI oocytes, 199 uncleaved, 35 arrested and 549 grade 5 embryos. Regarding the oocyte's characteristics, oocytes with granular ooplasm were associated with higher probabilities of resulting to post-ICSI oocytes and embryos selected to be discarded (p=0.001). Similarly, oocytes presenting with a fragmented polar body were associated with higher probabilities of resulting to an embryo selected to be discarded (p<0.001). Both sudden and difficult ooplasm aspiration were associated with higher probabilities of resulting to post-ICSI oocytes and embryos selected to be discarded (p<0.001). Similarly, either high or no resistance during ICSI were associated with higher probabilities of developing to post-ICSI oocytes and embryos selected to be discarded (p<0.001). A graphical representation of the above-mentioned associations are presented in Figure 1.



Figure 1: All-inclusive associations between oocyte characteristics, behavior during ICSI, fertilization, Z-Score, embryo grading and post-ICSI oocytes and embryos selected to be discarded.
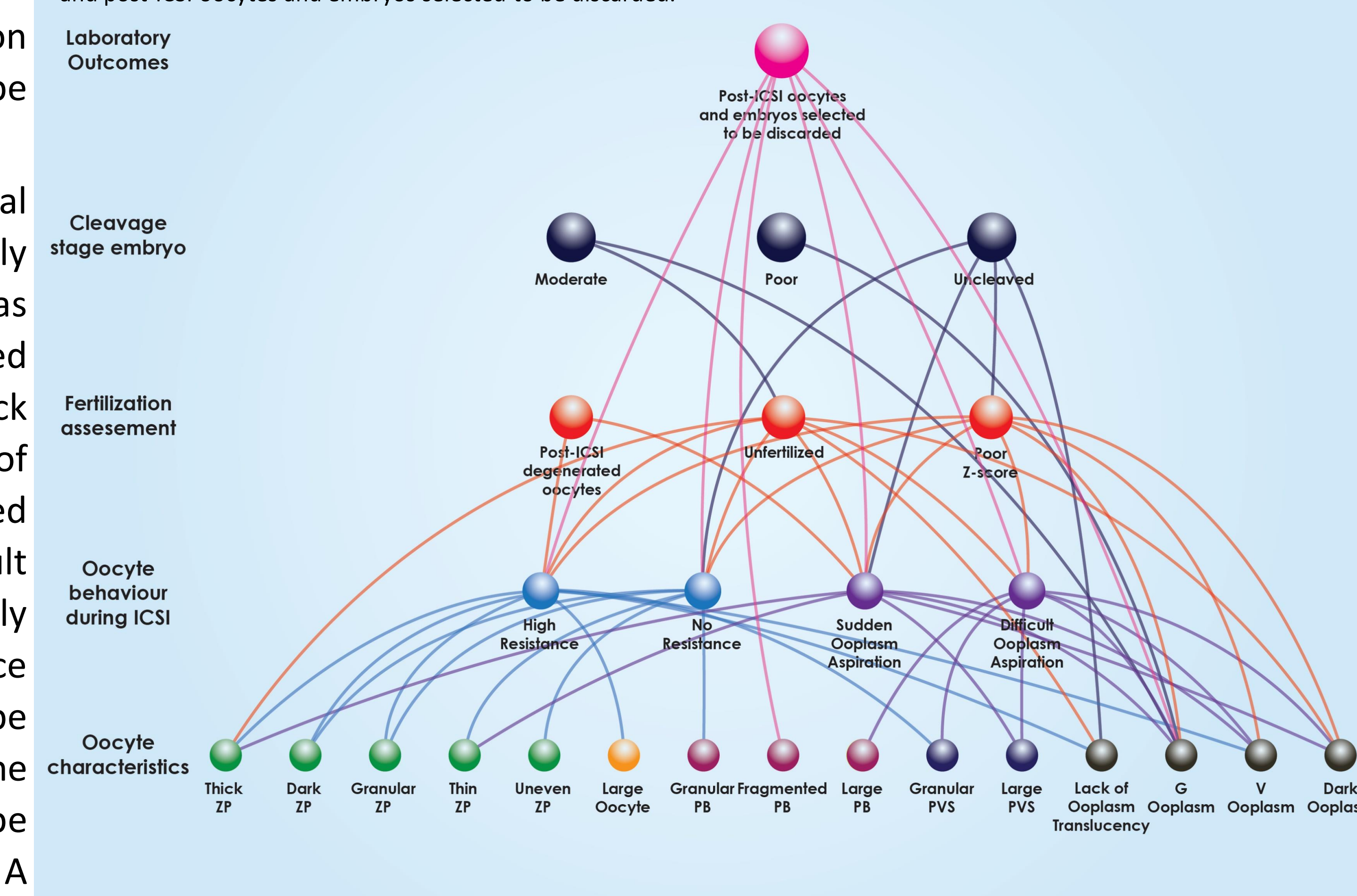
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## CONCLUSION

Identifying oocyte characteristics that may be related to the oocyte's behavior during ICSI, and subsequent z-score classification at fertilization evaluation is undoubtably of value. This may in turn enable the possibility that the outcome of ICSI procedure, fertilization rate and zygote potential may be predicted by an early oocyte evaluation. It is this incentive that served as the driver for this study. Deciphering on "how" these characteristics lead to specific outcomes should be the focus of further studies employing basic research in unveiling the molecular pathways involved. Being able to predict the oocytes' attitudes during ICSI and subsequent zygote dynamic and embryo quality, may prove particularly beneficial especially for countries where the law permits insemination of a certain restricted number of oocytes. For these cases, a preliminary selection of oocytes based on characteristics that have predictive value in regards to an improved performance of the oocyte within an ICSI cycle is a prerequisite. Is it possible that information we collect on the oocyte on Day 0 prior to submitting it to insemination represents a key factor in embryo selection [3]? This scenario has been revisited throughout the years [4]. According to results of our study, it appears that various abnormal oocyte characteristics and abnormal behavior during ICSI negatively influence the laboratory outcomes of an ICSI cycle. It may be possible that several characteristics that have not presented with a direct association may exert an indirect influence on the laboratory outcomes. This indirect influence may hint that the developmental potential of the pre-implantation embryo should not be represented by a linear route with specific parameters influencing the endpoint, but should be rather described as a multi-layered network with the developmental checkpoints remaining undefined. It is identifying these checkpoints that signifies different developmental events and connecting them to either morphological or morphokinetical parameters that may be the missing piece of the puzzle. The message this study aims to convey is that oocyte granularity, as well as polar body fragmentation, along with type of ooplasm aspiration appear to exert a negative direct effect, as evidenced by the increase in the number of post-ICSI oocytes and embryos selected to be discarded. If these observations are validated by other studies, it may be possible that future prediction models may rely on assessing more time-points in order to improve ICSI cycle outcome prediction.

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