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Morphokinetic characteristics of embryos originating from extremely small follicles: A prospective study

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ABSTRACT

Objective: To investigate the developmental potential of oocytes and embryos derived from extremely small follicles (<10 mm) in comparison to those originated in larger follicles.

Study design: A prospective study, undertaken in a university affiliated single center tertiary hospital. The study included 98 patients undergoing infertility treatments. On the day of ovum pickup (OPU) follicles were counted and measured. Aspiration of follicles larger and smaller than 10 mm was undertaken separately and the development of embryos originating from oocytes from these follicles was followed up using different wells for each embryo. There was no low limit of size for aspiration. Each oocyte retrieved was marked for its origin and numbered for further follow up. We recorded: Oocytes retrieved, maturation stage, fertilization rate, cleavage rate, morphokinetic parameters, embryo transfers, embryo freezing, oocyte freezing and biopsy rate for preimplantation genetic diagnosis (PGD).

Quality was evaluated by the morphokinetic parameters of the embryos developed using time-lapse imaging technology. Day 3 KIDScore was calculated to all embryos.

Results: Small follicles compared to large follicles displayed lower recovery rate (45% vs. 74%, $P < 0.0001$), fewer matured oocytes (37.5% vs. 61.7%, $P < 0.0001$), higher rates of GV oocytes (20.7% vs., 3.7%, $P < 0.0001$), and lower fertilization rate (43.7% vs. 63.3%, $P < 0.0001$. However, morphokinetic variables were similar between embryos that originated from either small or large follicles. Median KIDscores were identical for embryos from small or large follicle origin.

Conclusions: Embryos originated from small follicles were not different than embryos from larger follicles, as assessed by morphokinetic parameters in time lapse system. In view of our findings, physicians should bear in mind that small follicle aspiration might yield good quality embryos.

Introduction

Studies on the right timing of ovulation triggering during ovarian stimulation in IVF cycles have generally showed that larger follicles have a better chance of containing mature oocytes and some even suggested to omit aspiration of small follicles [1–5]. Only few studies have investigated extremely small follicles' potential (<10 mm). They showed lower maturation rates of oocytes retrieved from small follicles compared to that of oocytes retrieved form larger follicles, and disagreed on whether a difference existed regarding to their fertilization potential [2,6,7].

There is inconsistent data in the literature on the quality of embryos developed from oocytes retrieved from small compared to large follicles. While some studies showed a comparable embryo quality regardless to the follicle size, others demonstrated that embryos originated from smaller follicles presented delayed development and lower morphologic scores [7,8–10].

The development of Time-Lapse imaging has enabled analysis of morphokinetic parameters and selection of embryos by their implantation potential [11–13]. To the best of our knowledge, there is no data in the literature on morphokinetic parameters of embryos from extremely small follicles.

Our aim was to explore the developmental potential of oocytes retrieved from extremely small follicles (<10 mm) by

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using Time-Lapse technology, in comparison to oocytes retrieved from follicles measured more than 10 mm.

Materials and methods

This prospective, single center research was undertaken in a university affiliated tertiary hospital and was approved by the local Institutional Review Board. Patients were recruited during their consultation visit to receive a stimulation protocol for various infertility reasons. We included all patients that agreed to participate and signed a consent form. We included all potential patients and infertility causes. Treatment causes were: unexplained infertility (40 patients), PGD (27), fertility preservation (6), and mechanical or male factor (25). we excluded patients that were planned to undergo aspiration with no anesthesia or were expected to present a technically difficult aspiration as was evaluated beforehand by the physician. We recorded demographic data of the patients, infertility cause and stimulation protocol. The protocol was tailored personally according to the patients' history and preferences. Optional protocols and laboratory methods were elaborated previously by our group [14]. On the day of ovum pickup (OPU) follicles were counted and measured. All follicles >10 mm were aspirated from both ovaries. When only extremely small follicles (≤10 mm) were left, the aspiration needle was rinsed with flushing medium and retrieval was continued into a separated well until no follicles left. There was no low limit of size for aspiration. Each follicle was measured again to confirm its diameter was less than 10 mm. Each oocyte retrieved was marked for its origin and numbered for further follow up. We recorded: number of oocytes retrieved, maturation stage, fertilization rate, cleavage rate, morphokinetic parameters, embryo transfers, embryo freezing, oocyte freezing and biopsy rate for PGD. Cleavage stage biopsy for PGD is normally performed in our unit on day 3 embryos with at least 6 blastomeres.

We defined three "quality factors":

freezing rate: number frozen embryos /number of 2PN, in IVF cycles.

Biopsy rate: number of embryos biopsied /number of 2PN, in PGD cycles.

Maturation rate: number of M2 oocytes/number of oocytes aspirated, in oocyte cryopreservation cycles, as a marker of future potential for development of embryos.

Quality was further evaluated by morphokinetic parameters.

Time-lapse monitoring of embryo morphokinetics

All embryos were incubated in the integrated EmbryoScopeTM time-lapse monitoring system (EmbryoScopeTM; UnisenseFertiliTech, Vitrolife Denmark,) from the time of fertilization until transfer. Embryo scoring and selection with time-lapse monitoring were performed by analysis of time-lapse images of each embryo on an external computer by means of software developed specifically for image analysis (EmbryoViewer workstation; Unisense Fertilitech A/S Vitrolife). Embryo morphology and developmental events were recorded to demonstrate the precise timing of the observed cell divisions in correlation to the timing of fertilization: time of pronuclei fading (tPNF); cleavage to a 2-blastomere (t2), 3-blastomere (t3), 4-blastomere (t4) and so forth until reaching an 8-blastomere (t8) embryo. In addition, the synchrony of the second (s2=t4-t3) and third cell cycles (s3=t8-t5) and the duration to the second (cc2=t3-t2) and third cleavages (cc3=t5-t3) were measured. All cleavage times (t2-t8) were standardized with respect to the time of pronuclei fading (tPNf). All the assessments and annotations of the embryos were performed by senior embryologists, ensuring a very low inter-observer variation. Score were allocated to day 3 embryos using the KIDScore algorithm [1].

Statistical analysis

Follicles groups were compared using Pearson Chi-square test and Fisher's exact test for dichotomous variables. Times were presented as medians and compared using Mann-Whitney U test for non-normal distribution.

Results

We recruited 98 patients that completed OPU by the study protocol. Fifty-six patients underwent ICSI, 26 of them due to PGD, 38 conventional IVF and 4 patients cryopreserved oocytes. Average age was 35.9 ± 5.41. We collected 997 oocytes, 836 originated from 1315 follicles that were documented prior to OPU, giving a total recovery rate of 63.57%. Data on the number of follicles prior to aspiration was missing in 17 patients.

Oocyte quality

Small follicles compared to large follicles displayed significantly lower recovery rate (45%vs. 74%, P < 0.0001, respectively). They were also less likely to contain mature oocytes (37.5% vs. 61.7%, P < 0.0001, respectively), had lower fertilization rate (43.7% vs. 63.3%, P < 0.0001, respectively), and higher rates of GV oocytes (20.7% vs. 3.7%, P < 0.0001, respectively).

Small and large follicles derived oocytes were compared according to our predefined "quality factor":

freezing rate was lower in small follicles compared to larger one in IVF cycles (24.28% vs. 37.33%, p = 0.04, respectively).

Maturation rate: small follicles yielded less mature oocytes for cryopreservation (45.5%, 85.2%, p = 0.08, respectively) in oocyte cryopreservation cycles.

On the contrary, there was no difference in biopsy rate of PGD embryos (72.6%, 70.5%). 2PN refers to 2PN in PGD cycles only. Data is presented in Table 1.

Cumulative survival curve showed higher rates of embryos that demonstrated arrest in development among those originated from small follicles (90.79% vs. 74.77%, p < 0.0001, respectively) (Graph 1).

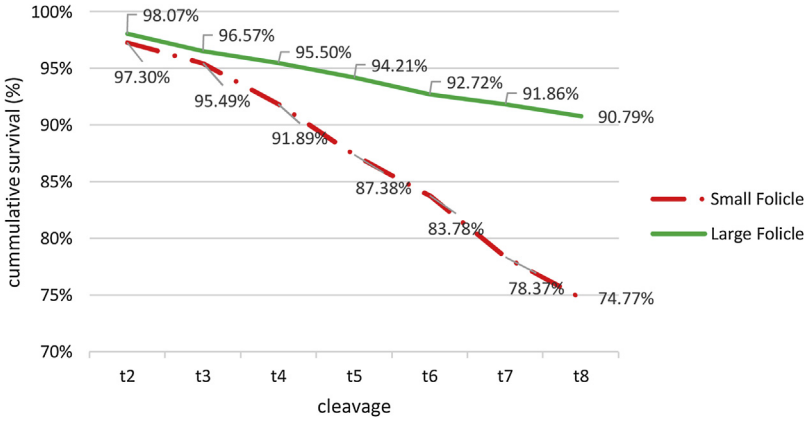
Morphokinetic characteristics

The morphokinetic variables were similar between day 3 embryos that were developed from either small or large follicles. Embryos from large follicles reached t2 faster than embryos from

Table 1
Follicles' aspiration outcomes according to their diameter.

	Large Follicles (>10 mm)	Small follicles (<10 mm)	P-Value
Total oocytes	736	261	
Recovery rate	623 (74%)	213 (45%)	P < 0.0001
M2 / all oocytes	454 (61.7%)	98 (37.5%)	P < 0.0001
GV / all oocytes	27 (3.7%)	54 (20.7%)	P < 0.0001
2PN / all oocytes	466 (63.3%)	114 (43.7%)	P < 0.0001
Abnormal fertilizations/ all oocytes	63 (8.6%)	19 (7.3%)	NS
Unfertilized / all oocytes	69 (9.4%)	58 (22.2%)	P < 0.0001
Quality factor			
IVF(F/2PN, n = 300,70)	112 (37.33%)	17 (24.28%)	P = 0.04
PGD(B/2PN, n = 164,44)	119 (72.6%)	31 (70.5%)	NS
Cryopreservation (M2/all oocytes, n = 61,11)	52 (85.2%)	5(45.5%)	P = 0.008

F- frozen embryos, B- biopsied embryos, M2- in ICSI cycles, 2PN - all cycles.



Graph 1. Survival of embryos originating from small and large follicles from T2-T8. Percentage of cleaved embryos at each time point out of 2PN.

small follicles but then spent more time to complete t3. There was also a trend towards a faster development of small follicles in regard to T6 cleavage time. There was no significant statistical difference in all other cleavage times or intervals.

KIDScore was calculated for 394 embryos that originated from large follicles and 80 embryos from small follicles. Those embryos were present at the embryoscope at 66 h and were able to be graded according to the model. According to this mode each embryo gets a score between 1–5 (1- means the lowest potential for pregnancy and 5 is the highest). Median scores were identical for embryos from small or large follicle origin (4). There was also no statistical difference between the proportion of embryos graded 4 or 5, or 2 or less between both groups. Data is presented in Table 2.

We particularly looked at a group of women with poor ovarian reserve in order to evaluate the added value of aspirating small follicles in that specific group. We found 22 women with less than 6 large follicles (>10 mm). Their average age was 39. Large follicles yielded 33 embryos that were eligible for biopsy, freezing or ET. Importantly, the small follicles from these women contained 9 more embryos, which constitute a 27% addition. Third of these embryos received a KIDScore of 5.

Discussion

We found that embryos originated from very small follicles (<10 mm) were comparable in their morphokinetic development to those derived from large follicles.

Table 2
Morphokinetic variables and scores of day 3 embryos according to their origin.

	Large Follicles (>10 mm) (Q1-Q3) (hours)	Small follicles (<10 mm) (Q1-Q3) (hours)	P-Value
PNf	25.17 (22.8–27.62)	25.88 (23.08–28.88)	NS
T2	2.67 (2.33–3)	2.73 (2.33–3.33)	P = 0.028
T3	14.34 (13.34–16.01)	14.05 (12.34–15.49)	P = 0.02
T4	15.34 (14.07–17.34)	14.72 (13.67–17.18)	NS
T5	27.33 (23.73–30.34)	27.67 (24.47–27.67)	NS
T6	30.02 (27.01–34.34)	28.79 (26.02–32.3)	P = 0.057
T7	31.74 (28.54–37.09)	31.13(28.03–34.74)	NS
T8	33.93 (30.27–41.54)	33.05(29.37–39.47)	NS
CC2	11.7 (10.67–13.01)	11.17 (9.67, 12.34)	P = 0.001
CC3	13.65 (11.55–16.34)	13.4 (11.67–15.34)	NS
S2	0.67 (0.33–1.67)	0.68 (0.33–3.26)	NS
S3	5.34 (2.67–15.88)	5.67 (2.67–15.5)	NS
KIDScore	4 (2–5)	4 (2–5)	NS
Score≥4	251(63.7%)	50 (65%)	NS
score≤2	115 (29.18%)	27 (33.58%)	NS

Values are presented as median (lower quartile-upper quartile).
T2-T8 are presented as hours from tPNf. CC2 (T3-T2), CC3 (T5-T3), S2 (T4-T3), S3 (T8-T5).

To the best of our knowledge, morphokinetics of embryos originating from very small follicles was not investigated before.

Recently, one study has addressed the clinical relevance of follicle size by analyzing the morphokinetics of embryo development using time-lapse technology [15]. The study showed that although small follicles (<17 mm) had significantly lower blastocyst formation and good quality blastocyst rates compared with larger follicles (≥17 mm), once the blastocyst stage was achieved; implantation rates were not significantly different. The time to reach blastocyst was shorter in the small follicles. In this study, the cut off for size was 17 mm, much larger than in our study, and follicles were also differentiated by homogeneity of the follicle's cohort. Additionally, the authors used morphologic criteria for quality assessment of embryos. We used the KIDScore algorithm and found identical median scores for embryos of both large and extremely small follicles. This is in agreement with results by Wirleitner et al., that displayed similar implantation, pregnancy and live birth rate of blastocysts from all follicle sizes. Morphokinetics models, though need further prospective validation, seem to hold great promise in providing clinical assessment of embryo quality, non-invasively [16].

Furthermore, in comparison to large follicles, small follicles displayed significantly lower recovery rate, were less likely to contain mature oocytes and had lower fertilization rate. This congruent with previous studies that demonstrated higher recovery and fertilization rates with larger follicles [2,3,7]. Some studies have declared similar fertilization rates [6,9]. A recent study [17] found, that although the recovery rate was lower in follicles measured 8–12 mm compared to larger follicles, they exhibited similar fertilization and blastocyst rates, and even a trend towards higher live birth rate (LBR).

The differences between the studies might be due to different aspiration criteria or different protocols. We did not have a lower limit for the size of follicle aspirated. Thus, we might have had a relatively higher number of immature oocytes unable to be fertilized. Still, the fact that the "larger follicles" group included relatively small follicles (11–14 mm) and yet exhibited similar embryo quality to that of the small follicles, highlights the potential of oocytes from small follicles.

Our study has a few limitations. First, our study group was heterogenous for the protocols and infertility causes, and this might not be applicable to all patients. We also could not assess further development beyond the 3rd day, due to PGD intervention in a part of the embryos. Yet, we believe that our analysis gave a good perspective of the potential hidden in very small follicles.

We believe that physicians must consider the potential of high-quality embryos in small follicles, and take every effort to aspirate them, especially in poor prognosis patients.

Authors' roles

S.A and B.A were involved in contemplation and study design. S.A, L.Z, L.B, H.A and M.D were responsible for data gathering. R.R and Y.K were leading embryologists. S.A and Y.K contributed substantially to data interpretation. B.A was the primary statistician and contributed to the study design. S.A wrote the manuscript with revisions by B.A and F.A. All authors read and approved the final manuscript.

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Conflict of interest

The authors declare that they have no competing interests.

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