Does large (>24mm) follicle yield a competent oocyte/embryo?

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Abstract

Aim: To evaluate the effect of large follicular size (> 24mm) at day of oocyte retrieval on oocyte/embryo quality.

Patients and Methods: A cohort study conducted in a single tertiary medical center between July 2018 and May 2019. Before ultrasound-guided follicular aspiration, follicles were measured and divided into two groups according to their maximal dimensional size: large: ≥24 mm and normal: <24 mm. Microscopic examination of the follicular aspirates was performed by embryologist. Each follicle aspirated was evaluated for oocyte maturation, oocyte fertilization and embryo quality.

Results: 428 follicles were measured, including 383 (62.81%) in the normal and 45 (14.06%) in the large follicle groups. Oocytes were achieved during aspiration from 297 (75.5%) and 29 (64.4%) of the normal and large follicle groups, respectively (p=0.05). No in-between group differences were observed in mature oocyte (MII), fertilization and top quality embryo (TQE) rates. Nevertheless, once a zygote (2PN) was achieved, a trend toward a higher TQE rate/2PN was found in the large follicle group [16 /19 (84.2%) vs. 115/171 (67.3%); p=0.062].

Conclusion: While a non-significant decrease in oocyte recovery rate was found in follicles ≥24 mm, the zygote and TQE per follicles were comparable.

** Drs. Orvieto and Mohr-Sasson should be considered "similar in author order."

Background

Controlled ovarian hyperstimulation (COH) is a crucial step in assisted reproduction, aiming to increase the number growing follicles that will yield competent oocytes. Ovarian follicles grow at different rates, and COH monitoring is usually guided by follicular size rather than their competence (1). Studies have shown that follicles with greater diameter were most likely to reveal mature oocytes, which are capable of fertilization and best suited for development into a high-quality embryos (2–4).

Data exist, both in human and animals models, on the optimal follicular size on the day of oocyte retrieval, that are most likely to yield a mature oocyte (5). Follicles of 16–22 mm are more likely to yield mature oocytes than smaller follicles, while larger follicles would more likely yield “post-mature” oocytes that are not competent for fertilization (6). However, while Dubey et al. (7) observed a comparable fertilization rates in oocytes from 16–22 mm follicles to those from 22–26 mm follicles, Ectors et al. (6) observed that follicles of 16–23 mm on the day of oocyte retrieval had higher fertilization rates than those > 23 mm. However, the % of good scored oocyte was demonstrated to increase from 55.4% of follicles size of 16–23 mm, to 64.6% of follicle > 23 mm (6).

Recently, we demonstrated in a cohort prospective study (8) evaluating the association between follicle size (maximal dimensional size: large: ≥16 mm, medium: 15 to 13 mm, and small: <13 mm) and

oocyte/embryo quality, that mature oocyte (MII) rate was significantly higher in the large and medium compared to the small follicle size groups. Nevertheless, no in-between group differences were observed in fertilization nor in top quality embryo (TQE) rates among the mature oocytes regardless the follicular diameter they originated.

Prompted by the aforementioned observations we wanted to evaluate the association between large follicle size (≥ 24 mm) and oocyte development and quality. For this purpose, we reanalyzed the data from our previously published cohort prospective study (Mohr-Sasson et al. 2020).

Materials And Methods

The data used in this study were collected in a cohort prospective study that has been reported elsewhere (Mohr-Sasson et al. 2020). Women undergoing COH using the multiple-dose GnRH antagonist protocol between July 2018 and May 2019, in a single university affiliated tertiary medical center, were included. Only those < 43 years old, without a history of endometriosis or Fragile –X gene mutation were included. The study protocol was approved by our IRB (ID 4689-17-SMC) and was registered by the National Institutes of Health (NCT02821702).

Data on patient age, infertility-treatment-related variables, ovarian stimulation characteristics were retrieved from women's medical files. Decision of final follicular maturation triggering was based on physician judgment (9). The timing was based on the lead follicular cohort, usually with at least two leading follicles measuring ≥ 17 mm for maximal diameter. A transvaginal sonography (TVS)- guided follicular aspiration was conducted 36 hours after triggering administration.

At retrieval, up to four leading follicles were measured before aspiration from each women. Follicles were divided into two follicular groups according to their maximal dimensional size: normal: <24 mm and large ≥ 24 mm. Retrieval was done separately for each follicle measured. Microscopic examination of the follicular aspirates was performed by the embryologist. In case where no oocyte was detected, flushing of the system was perform using 0.5-1 cc of medium with Hepes (Quinn's Advantage®, Sage, USA).

Routine IVF or intracytoplasmic sperm injection (ICSI) was then performed, as appropriate. Each embryo was cultured separately until transvaginal ET, which was performed 48 to 72 hours after OPU. All patients received luteal support with progesterone.

Embryos classification was based on the individual embryo scoring parameters, where TQEs were defined as day-2 or 3 embryos with 3–4 or 7–8 cells (respectively) and ≤ 10% fragmentation rate. The information for each oocyte, starting from the follicular size, was followed through all the laboratory procedures including insemination, oocyte stripping for ICSI, ICSI, fertilization and embryo culture.

Primary outcome was defined as the number of oocyte retrieved from each of the follicular groups (oocyte recovery rate). Secondary outcomes included: Oocyte undergone nuclear maturation - (MII); fertilization rate; and TQE rate.

The subgroup analysis which we present here was not planned in theoriginal protocol. The cut-off to be analyzed was chosen, after completion of the original trial (8).

Statistical Analysis

Comparison between unrelated variables was conducted with Student's t-test and Mann–Whitney U test, as appropriate. The chi-square and Fisher's exact tests were used for comparison between categorical variables. Significance was accepted at $p < 0.05$. Statistical analyses were conducted using the IBM Statistical Package for the Social Sciences (IBM SPSS v.19; IBM Corporation Inc, Armonk, NY, USA).

Results

During the study period 199 women met the inclusion criteria, from whom

428 follicles were measured, including 383 (89.5%) in the normal and 45 (10.5%) in the large follicle groups.

No in-between group differences were observed in patients' body mass index or COH characteristics. Oocytes were achieved during aspiration from 297 (77.5%) and 29 (64.4%) of the normal and large follicle groups, respectively ($p = 0.051$). No in-between group differences were observed in fertilization and TQE rates per follicles (Table 1). Nevertheless, once a zygote (2PN) was achieved, a trend toward a higher TQE rate/2PN was found in the large follicle group [16 /19 (84.2%) vs. 115/171 (67.3%); $p = 0.062$].

Table 1
Embryological outcome according to follicular size

	Normal Follicles (n = 383)(%)	Large Follicles (n = 45)(%)	P value
Ovum/follicle	297/383 (77.54)	29/45 (64.44)	0.051
2PN/follicle	171/383 (44.65)	19/45 (42.22)	0.51
TQE/follicle	115/383 (30.02)	16/45 (35.55)	0.46
TQE/2PN	115/171 (67.3)	16/19 (84.21)	0.062

Discussion

In the present analysis, while oocyte recovery rate was non-significant decrease in follicles ≥ 24 mm, the ratios of 2PN and TQE per follicle were comparable. Moreover, zygotes derived from large follicle (≥ 24 mm) yielded a non-significantly higher TQE rate.

The association between follicular size and oocyte maturity has been studied already three decades ago and it dictated the timing of final follicular maturation trigger, whenever several follicles reach diameter of > 17–20 mm (6–7, 10–11). In our previous study (8), we demonstrated higher oocyte recovery rate in

large (≥ 16 mm) and medium (13–15 mm) large compared to the small (< 13 mm) follicle groups, finding which is consistent with previous studies (2, 11, 12, 13). Moreover, MII oocytes were more commonly found in the medium and large follicle groups (8, 11, 14).

In a prospective study conducted by Triwitayakorn et al. (13), including 991 follicles, fertilization rate of mature oocytes, as well as the rate of good quality embryos showed a tendency to increase from the small follicle group to the large follicle group, however, this finding was not significant. Dubey et al. (7) reported that oocytes fertilization rate had a positive linear correlation as follicle diameter increased, while Nogueira et al.(15) demonstrated that matured oocytes retrieved from small follicles generated embryos of lower developmental potential than oocytes derived from larger follicles.

In the majority of studies, large follicles relate to those ≥ 16 mm in diameter. Only few considered larger (≥ 24 mm) diameters. In the present analysis we observed a non-significant decrease in oocyte recovery rate in follicles ≥ 24 mm, with comparable 2PN and TQE per follicle ratios. However, once a zygote was recovered from large follicle (≥ 24 mm), a non-significantly higher TQE rate was observed. In accordance with our observation, Ectors et al. (6) observed that follicles of > 23 mm on the day of oocyte retrieval had higher maturation rate compared to those < 23 mm. Moreover, the % of good scored embryos was demonstrated to increase from 55.4% of follicles size of 16–23 mm, to 64.6% of follicle > 23 mm (Ectors et al. (6).

In most centers, final follicular maturation is triggered once two to three follicles reach at least 17–18 mm in diameter, actually 2 days prior to oocyte retrieval. Sometimes, few follicles reach the required size while others are still small or medium size, and it is common to "scarify" the larger, on behalf of allowing the development of the smaller cohort of follicles. In the present study we could demonstrate, that by letting follicles to develop to large diameter (≥ 24 mm), not only that they are not scarified, but they have good probability to yield MII oocytes, and once recovered, to develop to TQEs.

Strengths and Limitations

The study has several limitations. Women included in the study were treated for infertility due to various reasons. Furthermore, treatment protocols were not homogeneous to all study population, therefore, follicles exposed to different gonadotropins and trigger modes were included. This might have influenced the chance to achieve oocyte during retrieval.

Although various studies exist concerning the association between follicular size to oocyte recovery rate at retrieval, data relating to large (≥ 24 mm) follicles is scarce. This study strength is in its being conducted in a single center by professional consistent team on a large study group.

Conclusions

In summary, the results of this study indicate that while oocyte recovery rate was non-significant decrease in was found in follicles ≥ 24 mm, the zygote and TQE per follicles are comparable. Moreover, zygotes

derived from large follicle (≥ 24 mm) yielded a non- significantly higher TQE rate. This information should be of value to physicians and patients alike. Further investigation is required to strengthen this finding.

Declarations

Ethics approval and consent to participate
The study protocol was approved by the "Sheba Medical Center" Institutional Review Board (ID 4689-17-SMC) on the 21st of December 2017, and was supported by the National Institutes of Health (NCT02821702).
Availability of data and materials
Data will be made available from the corresponding author on request.

Competing interests
The authors have nothing to declare.

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Consent for publication
Not applicable (cohort historical) Adva Aizer[1], Jigal Haas[1,2]
Authors' contributions
RO- Principal investigator, designed the study, wrote the paper and edited it in all its revisions. AMS- Participated in designing the study, retrieved the data, performed the statistical evaluations, proof read the paper and took part in discussions regarding the results. SB- Retrieved the data, proof read the paper and took part in discussions regarding the results. RN- Retrieved the data, proof read the paper and took part in discussions regarding the results. AA- Retrieved the data, proof read the paper and took part in discussions regarding the results.

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