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Full Length Article

The "vanishing follicle" in women with low number of developing follicles during assisted reproduction

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ABSTRACT

Objective: To investigate the occurrence of the "vanishing follicle" phenomenon in women with low number of developing follicles in assisted reproduction.
Study design: Women with ≤ 6 follicles on the day of hCG administration with ≥ 14 mm diameter were prospectively studied. Primary outcome measures were disappearance of ≥14 mm and all-diameter follicles on the day of oocyte pick-up compared to the day of hCG administration.
Results: Among the 120 women recruited, 95 were found eligible and completed the study. The "vanishing follicle" phenomenon occurred in 3.1% (95% confidence level: 0.7%–9.0%) and 18.9% (95% confidence level: 11.6%–28.3%) of cases affecting ≥14 mm and all-diameter follicles, respectively. In all cases, mid-late follicular serum LH and P levels remained within normal follicular phase range and transvaginal scan did not show signs of ovulation. Markedly, the main significant difference between the study and control groups in the ≥14 mm follicle group was serum E_2 level on the day of hCG administration; median (Interquartile range), corresponding to 395 (382.0–405.5) versus 823.0 (544.5–1291.0) pg/mL, respectively ($P = 0.04$). The same trend was encountered in all-diameter vanishing follicles group but it did not reach significance. Interestingly, in all-diameter vanishing group, chronic smoking and the P/E_2 ratio on the hCG day were significantly higher than controls. Post hoc multiple logistic regression analysis of data in accordance with the Bologna criteria reveled that antral follicle count was found to significantly affect the development of the "vanishing follicle" phenomenon.
Conclusions: The "vanishing follicle" phenomenon occasionally occurs in women with low number of developing follicles during assisted reproduction with no signs of ovulation. Our preliminary findings suggest that this phenomenon may be related to exhausted ovarian reserve however, an early-unrecognized LH elevation could not be ruled out.

Introduction

Counterpart to time trend delay in childbirth, age-related infertility has increased considerably and the demand for assisted reproductive technologies (ART) has inflated. Furthermore, ovarian ageing and poor ovarian response to controlled ovarian hyperstimulation (COH) have become a popular theme for basic and clinical research [1]. Since oocyte quality is the prominent limiting factor in female fertility, ovarian ageing has become a key issue of pregnancy achievement and maintenance. The prevailing notion today maintains that oocyte quality has a crucial role for normal and adequate folliculogenesis, mediated primarily through granulosa and cumulus cells by paracrine and autocrine mechanisms, aiming to achieve adequate growth, development and competence. As the ovarian reserve declines and oocyte quality diminishes, the endocrine, paracrine, and autocrine regulations responsible for the ovarian follicular milieu start to adapt to a changing environment that may impend on follicular maturation and development ultimately determining the oocyte own fate [2,3].

Few intriguing clinical manifestations during COH for IVF-ET treatment related to altered folliculogenesis in women with declining ovarian reserve have been described. The first is the empty follicle syndrome when "apparently" adequate folliculogenesis and steroidogenesis is encountered following COH, while no oocyte is retrieved on pick-up [4,5]. The second is "premature luteinization" when late follicular serum progesterone rise is encountered without an evident LH surge increase [6–8]. While the

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mechanisms of both these phenomena are still debated, there is evidence to link them with low ovarian reserve and poor ovarian response [4–8]. The "vanishing follicle", i.e. follicular disappearance with no signs of premature ovulation, was described in a similar setting [9]. Nevertheless, this phenomenon has not been investigated in a targeted study and is yet to be explored.

Gonadotropin releasing hormone analogues (GnRH-a) have been successfully brought into IVF-ET treatment protocols to prevent unintended ovulation and cycle cancelation. This has been accomplished by distinctive pharmacologic hypothalamic-pituitary mechanisms targeting to eliminate premature endogenous LH surge, paving the way for precise control and planning for oocyte retrieval. To the best of our knowledge, the "vanishing follicle" phenomenon has not yet been explored in the ART setting. Disappearance of the leading follicle towards the end of COH just before oocyte retrieval planning may have important clinical implications as well as basic research significance for understanding the basic patho-physiology of the maturing follicle in women with declining ovarian reserve.

Therefore, the aim of our present study was to investigate the occurrence of the "vanishing follicle" phenomenon in women with low number of developing follicles undergoing conventional COH for IVF-ET treatment. Our objectives were to explore whether this phenomenon exists, its incidence among ≥14 mm and all-diameter (≥14 mm ad <14 mm) follicles and to characterize women that may have such a complication.

Materials and Methods

Between January 2011 and Jun 2015, infertile women qualified for IVF/ICSI-ET treatment that turned to Poriya Reproductive Medicine Unit were examined for eligibility and those that consented for participating in this study were prospectively recruited. Women enrolled were regularly menstruating with two intact ovaries and no history of previous ovarian surgery. Women with hypogonadotropic hypogonadism, uncontrolled diabetes mellitus, hyperprolactinemia or thyroid disease were excluded. As well, women with PCOS or severe endometriosis were excluded. Women enrolled could participate in the study only once.

We have chosen COH on a case-to-case basis according to standard clinical practice. Recombinant human chorionic gonadotropin (hCG) 250–500 μg, was administered when the trans-vaginal scan showed ≥2 follicles with a diameter of ≥18 mm each. Oocyte pick-up (OPU) was planned 34–35 h. following hCG administration.

The Institutional Review Board (IRB) approved the study. No additional interventions were employed to the routine clinical and laboratory standards for IVF/ICSI preparation and treatment in our unit. Informed written consent was obtained from all participating women.

Study conduct and objectives

The study was prospective and observational in design. Since the study focused on cases with low number of developing follicles, only women with ≤6 follicles on the day of hCG administration with ≥14 mm diameter were eligible to participate in this study. This was a priori determined before initiating the study. The primary objective of this study was to track disappearance of ≥14 mm and <14 mm follicles on the OPU day as compared to the hCG day and study their endocrine and ultra-sonographic data. The secondary objective was to compare women's basic characteristics, ovarian reserve tests, ovarian response to COH and IVF results between women that had vanishing follicles with others that did not to explore this phenomenon further.

The first two investigators JSY and SY examined eligibility and recruited women for this study. Enrollment was performed following initiation of the COH treatment.

The cut-off of six ≥14 mm follicles on the day of hCG administration was chosen to facilitate a meticulous trans-vaginal ovarian scanning to include all follicles within each ovary, verify their number, diameter and place within the ovary, so an accurate comparison could be made between the scan on the hCG day and the scan on the OPU day. To track accurately each follicle within the ovary, we have employed several measures. A detailed schematic diagram has been drown for both ovaries of each enrolled case so a precise and distinctive comparison could be made between the hCG and OPU days. Moreover, both ovarian scans had to be performed in the ultrasound unit of our department by one skilled and experienced obstetrician and gynecologist with a sub-specialty in ultrasound having 25 years' experience in this field (SH). Furthermore, to exclude any potential bias, all clinical and endocrine measurements were blinded to the ultrasound performer.

Endocrine and ultra-sonographic tests

Early follicular serum basal FSH, LH, and E_2 levels as well as FSH/LH ratio evaluation were performed in a natural cycle in all women before recruitment to the study. During COH treatment, endocrine evaluations were performed every 2–3 days and until the day of hCG administration. Sera obtained for FSH and LH measurements, were analyzed by microparticle enzyme immunoassay (AxSYM®, Abbott, Abbott Park, IL, USA). The intra-assay and inter-assay coefficients of variation were <5% and <11%, respectively for FSH and <7% and <8%, respectively for LH. Serum E_2 and P levels were assayed by solid-phase, competitive chemiluminescent enzyme immunoassay (Immulite 2000, DPC, Los Angeles, CA, USA). The intra-assay and inter-assay coefficients of variation were, <10% and <16%, respectively for E_2 and <18% and <22%, respectively for P.

Early follicular basal antral follicle count (AFC, 2–10 mm) evaluation was performed in all women before recruitment in a natural cycle. During COH treatment, follicular development was performed throughout the follicular phase, every 2–3 days and until the OPU day. Ovarian ultra-sonography was performed employing a trans-vaginal probe (5–9 MHz) (Voluson E-8; General Electric Medical System, Milwaukee, WI).

Statistical analysis

We analyzed all data using the Software Package for Social Sciences (SPSS) for windows version 15.0 (SPSS Inc. 2006, Chicago, IL, USA). Descriptive procedure was used to evaluate patients' characteristics, each variable is presented as median and interquartile range employing Tukey's hinges method. We have applied independent two-sample Mann-Whitney test, Z-test for two proportions and logistic regression analysis wherever appropriate. P value of < 0.05 was considered as statistically significant.

Results

During the study period, 120 women were prospectively recruited. Among these women 25 were eventually excluded; four due to cycle cancelation for inadequate ovarian response, 15 had more than six ≥14 mm diameter follicles on the day of hCG administration and six missed to go into the ultrasound unit for trans-vaginal scanning on the OPU day before oocyte retrieval. The 95 women that concluded the study were 37.5 ± 5.2 years of age with infertility duration of 5.0 ± 3.7 years, their basal serum FSH level was 9.1 ± 3.7 mIU/L and their AFC was 6.9 ± 4.6. Infertility