**RESEARCH PROPOSAL**

## 1. General Information

Title:
A randomised trial to compare antral follicle count and serum anti-Mullerian hormone level for determination of gonadotrophin dosing in in-vitro fertilisation

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Principle Investigator:
Dr. LI Hang Wun Raymond
Associate Professor, Department of Obstetrics and Gynaecology, The University of Hong Kong, Queen Mary Hospital

Co-Investigators:
Dr. LEE Chi Yan Vivian
Associate Consultant, Department of Obstetrics and Gynaecology, The University of Hong Kong, Queen Mary Hospital

Dr. LAU Yee Lan Estella
Scientific Officer, Department of Obstetrics and Gynaecology, The University of Hong Kong, Queen Mary Hospital

Professor YEUNG Shu Biu William
Professor, Department of Obstetrics and Gynaecology, The University of Hong Kong, Queen Mary Hospital

Professor HO Pak Chung
Chair Professor, Department of Obstetrics and Gynaecology, The University of Hong Kong, Queen Mary Hospital

Professor NG Hung Yu Ernest
Clinical Professor, Department of Obstetrics and Gynaecology, The University of Hong Kong, Queen Mary Hospital

Correspondence to:
Dr. LI Hang Wun Raymond
Department of Obstetrics and Gynaecology, Queen Mary Hospital, 102 Pokfulam Road, Hong Kong.
Tel: (852) 22553111
Fax: (852) 25173278
Email: raymondli@hku.hk

## 2. Background

A number of parameters known as ovarian reserve markers, such as serum anti-Mullerian hormone (AMH) concentration and antral follicle count (AFC) have been shown to predict ovarian responses to gonadotrophin during in-vitro fertilization (IVF) treatment, although it was generally revealed that these markers were not good predictors of pregnancy outcome (Broekmans et al, 2006).

AMH, also known as Mullerian-inhibiting substance, is a dimeric glycoprotein that belongs to the transforming growth factor-beta family. It acts on tissue growth and differentiation, and is involved in the regression of the Mullerian ducts during male fetal development (Lee et al, 1996). In the female, AMH is exclusively produced by the granulosa cells of preantral and small antral follicles, and regulates ovarian activity and follicular steriodogenesis. There have been increasing reports on the use of AMH as an ovarian function marker (Ledger, 2010, Anderson et al, 2012; Nelson et al, 2012).

In IVF cycles, basal serum AMH level was significantly correlated with AFC, the number of follicles obtained after stimulation and the number of retrieved oocytes, and has been found useful in prediction of suboptimal and excessive ovarian responses upon ovarian stimulation (Ficicioglu et al, 2006; Lekamge et al, 2007; Kwee et al, 2008; Broer et al, 2009; Ledger, 2010; Broer et al, 2011). It has been suggested that measurement of serum AMH can be used for patient-tailored individualization of the dosage of gonadotrophin used in ovarian stimulation during IVF treatment (Olivennes et al, 2009; Nelson et al, 2009; Yates et al, 2011). Compared to AFC, AMH has the advantage of having less intra- and inter-cycle variation (van Disseldorp et al, 2010) and is not operator-dependent, despite its additional cost. However, there is yet no prospective randomized trial to compare the performance of AFC and AMH in this regard.

Therefore, we propose this randomized trial to evaluate the role of baseline serum AMH and AFC for determination of gonadotrophin dosing in in-vitro fertilization treatment.

## 3. Objectives:

This is a prospective randomized trial to compare the use of AFC and serum AMH as the basis for gonadotrophin dosing in in-vitro fertilization treatment.

The hypothesis is that the use of serum AMH as the criterion for determination of gonadotrophin dosing in IVF treatment results in more optimal ovarian response than AFC.

## 4. Study design:

Patients undergoing the first-time IVF treatment cycle using either conventional insemination technique or intracytoplasmic sperm injection at Queen Mary Hospital will be invited to participate in this study. Participating subjects will be randomized into either (i) AFC or (ii) AMH group, where the gonadotrophin dosing will be determined based on the baseline AFC and serum AMH respectively as assessed one month before the IVF treatment.

Randomisation will be performed according to a computer-generated list which will be read by an independent research nurse. That research nurse will assign the initial gonadotrophin dose according to the study protocol. The clinician and patient will both be blinded to the randomization throughout the course of treatment. The proportion of subjects having appropriate ovarian response, defined as the number of oocytes retrieved being 6 to 14 inclusive (Broer et al, 2010), will be compared between the two arms.

## 5. Subject selection and exclusion

5.1 Inclusion criteria:

- Subjects undergoing the first IVF cycle during the study period.

5.2 Exclusion criteria:

- Body mass index >=30 kg/$m^2$
- Subjects in repeated IVF cycles
- Subjects undergoing IVF treatment using donor oocytes
- Subjects undergoing pre-implantation genetic diagnosis

## 6. Treatment of subjects

Subjects will attend the Assisted Reproduction Clinic on day 2-5 of the menstrual cycle preceding their scheduled IVF treatment. They will undergo transvaginal ultrasound examination to determine the AFC and assess for other pelvic abnormalities as what would be routinely done before IVF according to our usual protocol. **Blood (10 c.c.)** will also be taken for measurement of reproductive hormones including FSH, LH, E2 and AMH. The subjects will be asked for separate consent to archive the residual serum samples for future studies on reproductive endocrinology as appropriate (such future studies will be subject to approval by the HKU/HA HKWC IRB).

Subjects will be interviewed by a dedicated research nurse to explain on this study. Those who give written informed consent will be randomized into either the AMH or AFC groups according to a computer-generated randomization list.

(All participants will be blinded to AFC during ultrasound examination. A research nurse will keep the ultrasound sheets of all patients separately, which will be filed back to the patients' medical record upon completion of the ovarian stimulation.)

In their IVF treatment cycle, the ovarian stimulation regimen will be determined according to the randomization result as per the following table:

| **Gonadotrophin (FSH or HMG) regimen** | **AMH Group** | **AFC Group** |
|---|---|---|
| 300 IU daily | AMH <= 1.0 ng/ml | AFC <=5 |
| 225 IU daily | AMH >1.0 and <=3.3 ng/ml | AFC >5 and <=15 |
| 150 IU daily | AMH >3.3 ng/ml | AFC >15 |

The AMH cut-off values are based on review of 214 patients previously treated in our centre who had AMH and AFC determined before ovarian stimulation. By regression

analysis, an AMH value of 1.0 ng/ml and 3.3 ng/ml corresponded to AFC of 5 and 15 respectively. Both linear and quadratic regression models gave roughly the same results.

The dose will be charted by the research nurse, and both the clinician and the patient will be blinded to the assigned group.

All subjects will be assessed by transvaginal ultrasound scan on the 7th day of ovarian stimulation, with the dose of gonadotrophin adjusted according to the ovarian response as detailed in the following table:

| **Response category** | **Criteria based on the number of follicles >10 mm (F10)** | **Action on gonadotrophin dose** |
|---|---|---|
| Under-response | F10 <=5 | Step-up the dose:<br>150 → 225 IU daily, or<br>225 → 300 IU daily |
| Normal response | F10 >5 and <=15 | Keep at same dose |
| Excessive response | F10 >15 | Step-down the dose:<br>225 → 150 IU daily<br>150 → 112.5 IU daily |

The dose adjustment will be made for **once only** throughout the treatment cycle.

All subjects will be treated on a GnRH antagonist protocol for pituitary down-regulation. They will receive **cetrorelix or ganirelix** 250 microgram subcutaneous injection starting from the 6th day of ovarian stimulation until the day of hCG trigger, according to our current clinic protocol.

Human chorionic gonadotrophin (hCG) will be administered for ovulatory trigger when the leading follicle reaches 18 mm, with preferably two or more other follicles reaching 16 mm in mean diameter. Oocyte retrieval will be carried out 34-36 hours post-hCG, where all follicles >10 mm will be aspirated.

All subjects will proceed to hCG trigger and oocyte retrieval except for those with zero follicle developing to beyond 10 mm after 12 days of stimulation, or when these are contraindicated for other medical reasons. Any events of ovarian hyperstimulation syndrome (OHSS) or anticipated risk of OHSS will be managed according to current practice.

## 7. Study outcomes

Primary outcome:

- Percentage of subjects having appropriate ovarian response (defined as number of oocytes retrieved being between 6 and 14).

Secondary outcome:

- Percentage of subjects requiring step-up or step-down of gonadotrophin dose upon first ultrasound tracking.

**Hormone assay:**
AMH concentration will be determined with ELISA using a commercial automated method (Access AMH Assay, Beckman-Coulter, Texas, USA), which has a limit of detection of 0.02 ng/ml.

## 8. Statistics

8.1 Statistical tests:

The percentage of subjects having appropriate ovarian response, defined as number of oocytes retrieved between 6 and 14 (Broer et al, 2010), as well as the percentage requiring step-up and step-down of gonadotrophin dose, will be compared between the AMH and AFC groups using $\chi^2$ test or Fisher's Exact test as appropriate. Statistical analyses will be performed using the SPSS software.

8.2 Sample size estimation:

According to review of our Centre's data (unpublished), 50% of our patients have appropriate ovarian response when using AFC to determine gonadotrophin dosing as in current practice. Assuming that an increase to 70% of appropriate response in the AMH group will be clinically significant, we require a minimum of 93 subjects to demonstrate a significant difference between the AMH and AFC groups with 80% power and type I error of 0.05. To allow for drop-outs, a total of 200 subjects (i.e. 100 per group) will be recruited.

## 9. Assessment of safety

There is no safety concern as the study does not involve any extra medical intervention to the patients concerned. It only involves the comparison of two assessment tools for determination of gonadotrophin dosing. The use of AFC for this purpose is the current practice in our Centre, whereas the use of AMH for this purpose is well supported in recent literature.

## 10. Direct access to source data / documents:

Trial-related monitoring, audits and regulatory inspections are allowed.

## 11. Quality control and quality assurance

This shall follow the general quality control policy established for our clinic as well as for our hormone assay laboratory.

## 12. Ethics:

Ethics approval will be applied for before commencement of study.

## 13. Data Handling and record keeping

All data will be stored and analysed using SPSS. All the investigators will be responsible for data management including data coding, monitoring and verification.

## 14. Financing and insurance

The study will be funded by the Department of Obstetrics and Gynaecology, University of Hong Kong.

## 15. Publication policy

The findings of this study will be submitted for consideration for publication in peer-reviewed scientific journal.

## 16. Supplements

Nil

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