4. METHODOLOGY

PLACE OF STUDY

This study was conducted at the Sri Ramachandra Medical Centre for Assisted Reproductive Technology (SMART), Department of Reproductive Medicine, Sri Ramachandra University after obtaining approval from the Institutional Ethics Committee of Sri Ramachandra University (IEC-NI/10/JUNE/17/17). [Appendix -1] Sample analysis for AMH assay was carried out at SRL Diagnostics, Mumbai.

STUDY DESIGN

Prospective Cohort study

DURATION OF STUDY

3 years, 8 months (January 2010 to August 2013)

4.1 SELECTION CRITERIA

INCLUSION CRITERIA

1. All patients enrolled for IVF / ICSI during the study period.
3. With bilateral ovaries

EXCLUSION CRITERIA

1. Age < 20 years and > 45 years.
2. Ovarian cyst / follicle ≥ 20mm on day 3 TVS.
3. Hypogonadotropic Hypogonadism.
Methodology

4.2 SAMPLE SIZE

The sample size studied was 246.

With an assured sensitivity of 95% and specificity of 90% based on earlier studies, for a power of 80% and type I error of 5% for one sided study, the total minimum sample size required was 200.

All women enrolled for ICSI during the study period from January 2010 to August 2013 and who satisfied the selection criteria were included in the study. Informed consent form was given to all the participants and their voluntary willingness to participate in the study was obtained from all the participants in the written consent form. [Appendix - 2]

The detailed history and physical examination was done in all these patients enrolled for the study.

First the following observations were noted from the patients.

1. A detailed history, menstrual history, previous obstetric history, past medical & surgical history, family history, personal history and the previous treatment history.
2. A thorough physical examination including bimanual examination.
3. Investigations:
   a) Serum TSH, Prolactin, Testosterone to rule out other endocrine disorders.
   b) Uterine & Tubal factors were ruled out by hysterosalpingography / Sono salpingography / Hystero laparoscopy.
   c) Husband’s semen Analysis.
Methodology

d) On day 3 of the menstrual cycle, serum FSH, LH, E2, were assayed by CLIA method. On the same day with the same venipuncture, 2 ml of blood sample was obtained for serum AMH assay. The serum sample was separated within one hour of venipuncture by centrifugation at 3000 rotations per minute for 10 minutes and stored in aliquots at -20º C till the sample was assayed in batches. On the same day TVS was done using Ultrasound, OP 3.1 -1101.0410 with a trans vaginal probe of 5 -7 MHZ to rule out any pelvic pathology and AFC was measured. AFC was defined as the total number of follicles with a diameter between 2 and 10 mm in both the ovaries on day 3 of the cycle. Serum AMH was assayed by Gen II assay (ELISA). The AMH assay had a sensitivity of 0.35 and 0.08ng/ml. The intra assay co-efficient of variation were found < 7.7% and inter assay variability were found to be ≤ 14.2%. The measurement range is from 0.14ng/ml to 21 ng /ml.

4.3 Controlled Ovarian Stimulation:

All these patients under went COS and ICSI within three months as per the unit protocol. In our study both agonist and antagonist protocols were used based on the ovarian reserve markers, previous response to stimulation. In the agonist protocol, combined oral contraceptive pill (OCP) was started on day 2 or 3 of the cycle. Injection leuprolide acetate depot 3.75mg IM was administered on day 21 of the cycle. On day 3 of subsequent cycle, TVS to exclude the presence of ovarian cysts and endometrial thickness < 5mm and E2 <50pmol/L confirmed complete pituitary desensitisation. In the antagonist
protocol, combined OCP was started on day 2 or 3 of the cycle and continued for 21 days.

On day 3 of subsequent cycle, stimulation is started with gonadotropins (rFSH ± HMG). The initial dose of gonadotropins was decided based on age, ovarian reserve markers and previous response to stimulation. The subsequent dose of gonadotropins is adjusted based on the follicular response. In antagonist protocol, in addition, injection cetrorelix 250 mcg subcutaneous was started from day 6 or when the follicle size had reached 14mm.

When at least 3 follicles exceeded 17mm in diameter, recombinant hCG or GnRH agonist trigger was administered depending on the serum E2 level on the day of trigger and the protocol used. Oocytes were retrieved 34 - 36 hours after hCG administration by trans vaginal ultrasound guidance. Embryo transfer was done on day 2 or 3 post oocyte retrieval if there is no evidence of OHSS or raised progesterone (P4).

4.4 OBSERVATIONS RECORDED

The following data were recorded from the patients undergoing COS for ICSI.

a. Age of the patient.
b. Duration of Infertility.
c. Menstrual pattern
d. Previous treatment details
e. Indication for ICSI.
f. BMI
g. **Investigations**

1) Serum FSH, LH, E2

2) Serum TSH, Prolactin, Testosterone

3) Serum AMH

4) AFC

h. Protocol for stimulation

   i) Agonist protocol

   ii) Antagonist protocol

i. Number of follicles

j. Number of oocytes retrieved

k. Number of mature oocytes retrieved (MII)

l. Total doses of gonadotropins required

m. Number of days of stimulation

n. Number of embryos fertilised

o. Number of embryos transferred

p. Pregnancy rate.

q. Complications encountered

Both the groups were further subdivided into poor, normal, and hyper response as follows:

**Poor response** - defined as retrieval of \( \leq 3 \) oocytes or cycle cancellation

**Normal response** - defined as retrieval of 4 -19 oocytes

**Hyper response** - defined as retrieval of \( \geq 20 \) oocytes.
They were further subdivided into PCOS and Non PCOS groups in each of the three response groups as per the Rotterdam’s criteria\(^9\). These patients were followed up for a period of six weeks from the time of recruitment to the study.

### 4.5 Statistical Analysis

The data obtained were analyzed with SPSS 16.0 version. To describe about the data, descriptive statistics frequency analysis, percentage analysis, means and standard deviation were used. To find significant difference in the multivariate analysis, the one way ANOVA with Tukey’s Post - Hoc test was used, and to assess the relationship between the variables Pearson’s correlation with Scatter Plot was used. To find the significance in the categorical data, Chi square test was used. The Receiver Operating Curve (ROC) was used to find the cut-off value, Sensitivity & Specificity In all the statistical tools, the probability value of \( p < 0.05 \) is considered as significant value.