4. METHODOLOGY

PLACE OF THE STUDY

This study was conducted in the Department of Reproductive Medicine and Surgery, Sri Ramachandra Medical College and Research Institute, Sri Ramachandra University, Chennai. The study was conducted in accordance with the ethical principles and after obtaining approval from the Institutional Ethics committee of Sri Ramachandra University (IEC/10/JULY/83/29). [Appendix -1]

STUDY DESIGN

Prospective observational study.

DURATION OF STUDY

From January 2010 to January 2014.

4.1 SELECTION CRITERIA

Inclusion criteria

1. All patients enrolled for ICSI during this study period as per the department protocol.

Exclusion criteria

1. Patient age <21 and >45 years.

2. Subjects underwent short and ultra short protocol for stimulation as the number of patients were less.

3. Embryos beyond grade III for transfer.
4.2 SAMPLE SIZE

The sample size studied was 403.

The sample size was estimated with an assumed implantation rate of 18% in early cleavage embryo transfer and assumed implantation rate of 5% in late cleavage embryo transfer and with a type I error of 5% and type II error of 10%, then the minimum sample required in each group was 51. As the number of patients enrolled for ICSI were more during the study period, the total sample size included for the study was 403.

All patients enrolled for ICSI during the study period from January 2010 to January 2014 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 in the consent form.

[Appendix - 2]

4.3 METHOD

Two stimulation protocols were used in this study as per the institutional protocol; they were (i) agonist protocol to the patients with young age and good ovarian reserve and (ii) antagonist protocol to the patients with advanced age, poor ovarian reserve and Poly Cystic Ovarian Syndrome (PCOS).

The gonadotropin-releasing hormone (GnRH) agonist protocol - First, pituitary down regulation was done with GnRH agonists. Once the patient was down regulated completely (had menses, E2 <30 pg/ml, endometrium<5mm) gonadotropin injections (recombinant follicle stimulating hormone/human...
menopausal gonadotropin) were given until the day of h CG administration. The initial dose was individualized based on age and ovarian reserve markers. Subsequent doses were adjusted according to the patient's ovarian response.

**The GnRH antagonist protocol** - In this protocol, gonadotropin injections were administered daily from the second day of the menstrual cycle till day of h CG when dominant follicle reaches 14 mm in mean diameter, GnRH antagonist was administered subcutaneously at a dose of 0.25 mgs daily until the day of h CG administration.

In both groups, oocyte maturation was induced by the administration of either recombinant hCG or urinary hCG or GnRH agonist, when at least two follicles reached 18 mm in diameter, and oocyte retrieval was performed 34-36 hours later. Oocytes were retrieved under transvaginal ultrasound guidance. Motile sperms were isolated by a swim-up or gradient centrifugation. Ejaculated, testicular sperm aspiration/ testicular sperm extraction, cryopreserved ejaculated and cryopreserved testicular sperm aspiration/testicular sperm extraction specimens.

The ICSI was performed 3-5 h after oocyte aspiration with the prepared sperm. Normal fertilization was confirmed by the presence of two pronuclear and two polar bodies 16-20 h (day1) after the procedure. Normally fertilized oocytes (zygotes) were spherical and had two polar bodies and two pronuleus (PNs). PNs had approximately the same size, centrally positioned in the cytoplasm with two distinctly clear, visible membranes. The presence of nucleolar precursor bodie their
number and size aligned at the PN junction were assessed. On the same day, early cleavage examination was performed on the zygotes at 27 hours.

Embryos displaying two cells before 27 hours were designated as 'early cleavage' and those not yet cleaved to the 2-cell stage were designated as 'late cleavage'. Two or three embryos were transferred depending on the patient’s age and embryo quality while the excess embryos were cryopreserved. Micronised progesterone was used for luteal phase support as per the Unit protocol. Pregnancy was confirmed by a serum \( \beta \) human Chorionic Gonadotropin (\( \beta \) h CG) test 14 days post transfer. The clinical pregnancy was confirmed by the presence of an intrauterine gestational sac with fetal cardiac activity by ultrasound examination at 4 weeks after embryo transfer.

Based on the type of embryo selected for transfer, the study subjects were divided into three groups. The pregnancy rates were compared between the groups.

- **Group I**: Patients transferred with early cleavage embryos (2 cell stage before 27 hours).
- **Group II**: Patients transferred with late cleavage embryos (2 cell stage after 27 hours).
- **Group III**: Patients transferred with both early and late cleavage embryos.

For analysis purpose group III were excluded as it is difficult to separate EC/LC embryos and transfer. Pregnancy and implantation rates were compared between group I and group II. In patients who have not conceived or have not undergone fresh embryo transfer, were followed up during the frozen embryo transfer and the pregnancy and implantation rates were compared in both the groups.
4.4 OBSERVATIONS RECORDED

The following data’s were recorded and compared among the study groups;

1. Age of the partners
2. Duration of infertility
3. Ovarian reserve markers
4. Semen analysis of male partner
5. Protocol for stimulation
6. Dose of gonadotropins used
7. Number of days of stimulation
8. Number of follicles
9. Number of oocytes retrieved
10. Number of matured oocytes (M II)
11. Number of oocytes injected
12. Number of oocytes fertilized
13. Early cleavage at 27 hours after injection of oocytes
14. Grading of embryos
15. Number of embryos transferred
16. Number of embryos cryopreserved
17. β h CG level after 14 days of transfer
18. Number of sacs seen
19. Implantation rate
20. Pregnancy rate
Methodology

4.5 STATISTICAL ANALYSIS

The collected data was analysed with SPSS 16.0 version. To describe about the data, descriptive statistics frequency analysis, percentage analysis, mean 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. For skewed data bivariate analysis Mann-Whitney U test was used. For multivariate analysis Kruskal Wallis test was used. To find the significance in categorical data Chi-Square test was used. In all the statistical tools, the probability value (p<0.05) was considered as statistically significant.