Study participants and Collection protocol

Study participants

This case control study recruited a total of 20 participants undergoing in-vitro fertilization and embryo transfer (IVF-ET) at the Reproductive Medicine Department of Nowrosjee Wadia Maternity Hospital and Ankoor Fertility Clinic between March 2023 and January 2024. The participant recruitment criteria included women with infertility undergoing their first or subsequent cycle of IVF treatment and in the age range of 22 - 40 years with a Body mass index (BMI) of 18 - 25 kg/m2 ,and normal ovarian function confirmed through combined assessment such as monitoring regular menstrual cycle (28-35 days, reference range as per American Congress of Obstetricians and Gynaecologists – ACOG) and AMH levels (1.0 – 3ng/mL or > 3ng/mL, reference range as per Society for Assisted Reproductive Technology - SART), and had no history of any significant infections in the past three months. Women with a medical history including conditions such as polycystic ovarian syndrome, endometriosis, damaged fallopian tubes, recurrent pregnancy loss, premature ovarian failure, uterine adenomyosis, aberrant uterine architecture), sexually transmitted diseases (e.g., HBV, HCV, HIV), or other significant medical conditions such as cardiovascular diseases, dyslipidemias, systemic lupus erythematosus, and other rheumatic and autoimmune diseases, were excluded. The recruited women were categorised into two groups: Unexplained Infertility (UI) and control group. The women whose standard evaluations including ovulation assessment confirmed by regular menstrual cycles, mid-luteal progesterone levels, or LH surge, ovarian reserve assessed by Anti-Mullerian Hormone (AMH) levels, fallopian tube patency measured by pelvic ultrasound or hysterosalpingogram and the semen analysis of the male partners performed according to WHO criteria (2021) revealed no identifiable cause for their infertility, were ascribed as the ‘UI’ group. Age- and BMI- matched women with exclusively male-factor infertility formed the 'Control' group. The male partners of women in the control group were confirmed cases of azoospermia, oligozoospermia, asthenozoospermia, necrozoospermia, or any testicular anatomical abnormalities. All participants provided written informed consent to participate in the study. The study received ethics approval from the Ethics Committee of Nowrosjee Wadia Maternity

Hospital where FF from recruited participants was collected (EC-NWMH-AP/2021/072) and the ICMR-NIRRCH Ethics Committee for Human Studies where the samples were processed and analyzed (NIRRCH/422/2020).

FF collection

All participants underwent controlled ovulation stimulation using GnRH antagonist protocols (short-term) and recombinant follicle stimulating hormone (FSH), tailored to their individual requirements. Follicular development and serum sex hormone levels (estradiol, progesterone) were monitored periodically throughout stimulation to assess response and adjust medication dosages as needed. Upon confirmation of at least two follicles exceeding 18 mm in diameter in the bilateral ovaries through ultrasound monitoring, 10,000 IU of human chorionic gonadotropin (HCG) was administered intramuscularly to trigger final oocyte maturation. Oocyte retrieval and FF aspiration were performed transvaginally under ultrasound guidance approximately 34-35 h post HCG injection. During the initial puncture of each follicle exceeding 18mm in diameter, approximately 1 mL of FF was collected in sterile 1.5mL vials (Tarsons, India) under ultrasound guidance and using a sterile aspiration needle. Care was taken to avoid blood contamination of the FF samples and contaminated samples, if any, were excluded. The samples were centrifuged at 12,000 rpm for 20 min at 4°C to eliminate cell debris, aliquoted and cryopreserved at -80°C until analysis.