Methods

Study design and population

This longitudinal prospective study was conducted at IVFMD, My Duc Hospital, Ho Chi Minh City, Vietnam, from March 2020 to May 2020. The study was approved by the institutional Medical Ethics Committee () on. This study included patients aged of over 18 years old, who underwent their first-time *in vitro* fertilization (IVF) treatment at IVFMD, had ovulation stimulation using GnRH antagonist, and plasma AMH level ≤ 1.25 ng/mL. Patients with *in vitro* oocyte maturation (IVM) and GnRH agonist cycles were excluded from this study.

Patient serum and measurement of AMH level using pico-AMH protocol and fully automatic AMH protocol

Serum samples were initially separated and routinely stored at −80°C until assay for clinical reporting. These Beckman Coulter assays were conducted in duplicate using a robotic platform (Grifols Triturus, Hampshire, UK) using undiluted serum as per the manufacturer's instructions (first thaw). Samples were then re-stored at −80°C. These samples were randomly selected for measurement, in duplicate, in the present study using the Ansh Labs (Webster, Tx, USA) Ultra-Sensitive AMH ELISA (lot 112612) (second thaw). Samples that yielded low results were also subsequently re-assayed using the Ansh Labs pico-AMH assay (lot 062013A) (third thaw). Selected samples were also re-run (third thaw) on another Beckman Gen II assay (lot 329853) following release of the field safety notice to test the impact of complement interference on our original data. These samples were run with a pre-dilution protocol, according to manufacturer instructions. The non-clinical study assays were performed by a single experienced technician who was blinded to the Gen II result. The local ethics committee advised the study did not require formal ethical approval on the basis of it constituting a potential service development, and it utilized samples where we were blinded to any donor information.