Multicenter, prospective study evaluating the first fully automated immunoassay for anti-Müllerian hormone (AMH) for the assessment of ovarian reserve on the cobas e analyzers

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Abstract

Study question
To investigate the value of AMH measurement in the assessment of ovarian reserve as expressed by antral follicle count (AFC) using the first fully automated immunoassay for anti-Müllerian hormone (AMH) on the cobas e analyzers.

Summary answer
500 patients have been enrolled from December 2012 to December 2013 at 7 study sites in Europe (Belgium, France, Germany, Spain, United Kingdom) and Australia. Correlation analysis will be performed for AFC and AMH as well as for follicle stimulation hormone (FSH) and AMH.

What is known already
AMH and AFC are the best biomarkers for assessing ovarian reserve and show good correlation in single center studies but lower correlation in multicenter studies. Both determination of AMH using the available AMH enzyme linked immunosorbent assays (ELISA) and determination of AFC seems to be dependent on the investigating observer/study site.

Study design, size, duration
Multicenter, prospective cohort study enrolling 500 women planned for transvaginal sonography (TVS) and hormone status on day 2-4 of menstrual cycle from December 2012 to December 2013 at 7 study sites in Europe (Belgium, France, Germany, Spain, United Kingdom) and Australia.

Participants/materials, setting, methods
Women planned for TVS and hormone status on day 2-4 of menstrual cycle for AFC determination had AFC determined including antral follicles of 2-10 mm in size using 2-dimensional TVS. AMH and follicle stimulation hormone (FSH) was quantified in serum samples collected on the same day of AFC determination using the fully automated Elecsys AMH assay, Elecsys FSH assay, and Elecsys estradiol (E2) assay respectively.

Main results and the role of chance
AFC was classified in the following groups: AFC low (0-7), AFC medium (8-15), and AFC high (>15). To show the value of fully automated Elecsys® AMH assay for the assessment of ovarian reserve expressed by AFC the following analysis will be done for all patients and separately for each study site: a scatterplot of AFC versus AMH together with Spearman correlation coefficient, an agreement table will be generated using two cutoffs for AFC: 7 and 15, and hence three AFC groups are defined: 0-7, 8-15, >15. According to the prevalences within these groups, quantities on AFC will be computed to define three groups. Receiver Operator Analysis (ROC) curves will be calculated for the prediction of low and high AFC (<7, ≥15) of AMH, FSH and AFC.

Limitations, reason for caution
Although in- and exclusion criteria for the target patients have been the same for all study centers target population may differ somewhat between centers. At all study sites 2-dimensional TVS was used, however the ultrasound equipment was not exactly the same potentially leading to different AFC values between sites.

Wider implications of the findings
Currently, AMH is measured using manual ELISA assays. However, conflicting results were observed in some cases, raising the question concerning the assays’ reliability. The first fully automated immunoassays for anti-Müllerian hormone (AMH) on the cobas e analyzers is a reliable assay for the determination of AMH in serum or plasma. Due to advancement of ultrasound technology AFC values determined nowadays using state of the art 2-dimensional TVS are higher than those published 5 and more years ago.

Study funding/competing interest(s)
Funding by commercial/corporate company(ies)
Roche Diagnostics GmbH, Germany

Trial registration number
NA

Inclusion criteria
• Woman ≥ 18 years <45 years
• Woman planned for transvaginal sonography for AFC determination and hormone status on day 2-4 of menstrual cycle
• Signed written informed consent

Exclusion criteria
• Major ovarian abnormalities detected by transvaginal sonography (i.e. only one ovary; cysts and solid masses > 2 cm)
• AMH determination (last 3 months) known by sonographer
• Positive pregnancy test
• Polycystic Ovary Syndrome (PCOS)
• Endocrine or metabolic abnormalities (i.e. diabetes type I and II, pituitary, adrenal, pancreas, liver or kidney disturbances)
• Ovarian surgery in the past 6 months
• Hormonal contraceptives in the preceding 3 months or hormonal therapy for IVF/ICSI in the cycle immediately preceding (Gonadotropin, Clomifene, Letrozole,GnRH agonists / antagonists)
• Malignancy (documented malignancy, documentation of current radiation therapy or chemotherapy in medical record)
• Alcohol or drug abuse/dependence according to ICD-10 criteria
• Investigational medicinal drug received in the past 3 months (90 days)

Correlation analysis will be performed for AFC and AMH as well as for follicle stimulation hormone (FSH) and AMH.

Trial registration number
NA

Funding by commercial/corporate company(ies)
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3 – Target study population

3 – Documented data

• Date of start of menstrual cycle
• Inclusion criteria and exclusion criteria as listed under target study population
• Patient Characteristics:
  Race, ethnicity, age, weight, height, smoking habits, alcohol consumption, age at menarche, average menstruation cycle length, infertility (cause and duration), any chronic diseases, classification according to ICD-10, gravidity, parity
• Transvaginal Sonography (TVS):
  - Date of sonography
  - Antral follicles classified as those measuring 2-10 mm in size on TVS scan. AFC determined as the overall number of antral follicles counted in both ovaries
  - Initials of person performing the TVS
• Blood Sampling:
  - Date of blood sampling
  - Type of serum collection tube

4 – Sample collection and processing

• Sample collection and processing were done according to local study site procedures and regulations using sample tubes without gel separator.
• A serum sample aliquot ≥ 0.8 mL was provided to Roche Diagnostics (RD).
• Samples were labeled with an unique barcode specific for the subject.
• Samples were frozen and stored at -80°C. Sample storage at -20°C was possible for a maximal duration of 6 months.
• Samples were shipped frozen to RD, Penzberg for further aliquotation and analysis.
• After completion of the sample collection, the samples were sent to a central laboratory to carry out the assessment of AMH and related parameters of fertility such as FSH and E2 using the fully automated immunoassays for AMH, FSH and E2 on the cobas e analyzers.

5 – Reference