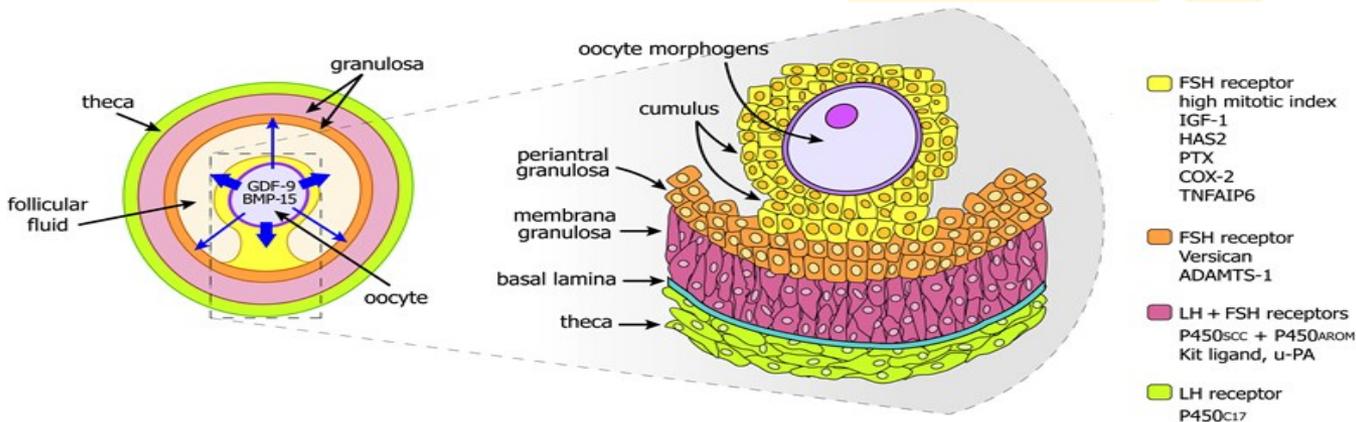
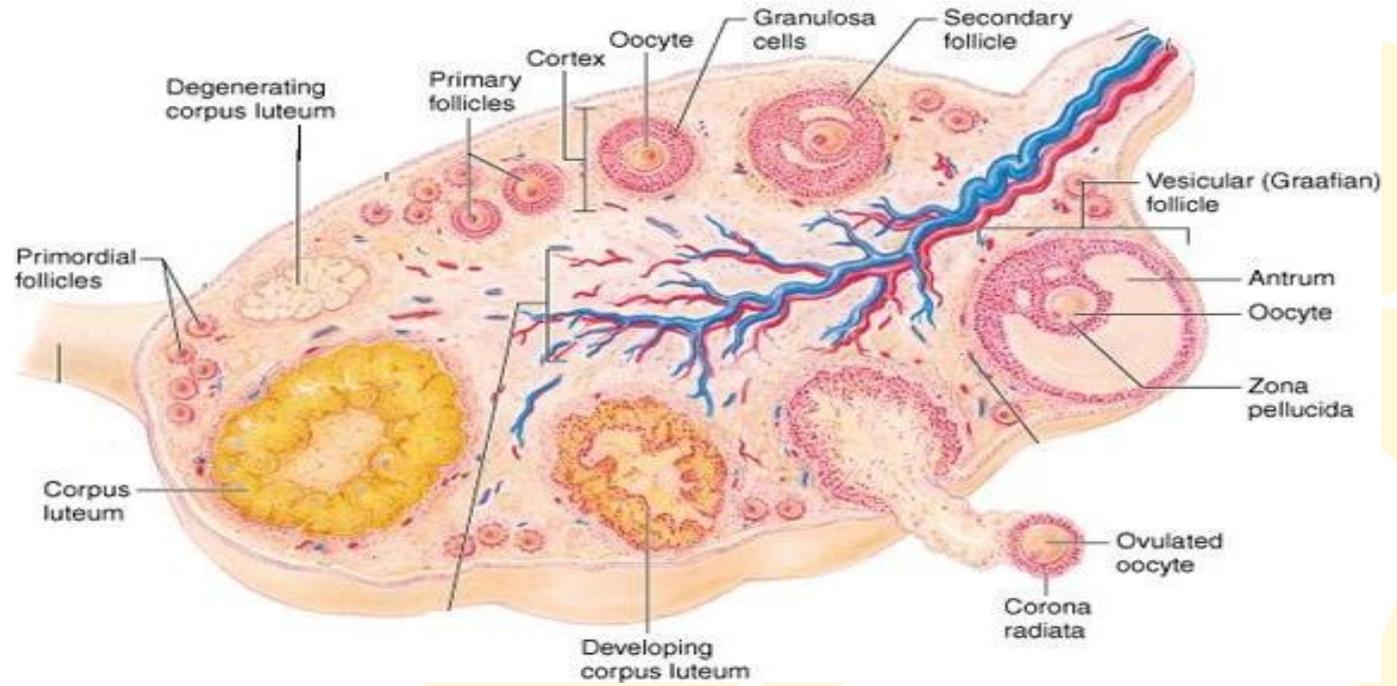


Tests for Ovarian Reserve

Structure of the Ovary



What is ovarian reserve?

- ❑ Capacity of ovary to provide eggs
 - Capable of fertilization
 - Result in healthy / successful pregnancy
- ❑ *Important in the treatment of infertility*
- ❑ As maternal age advances, number of eggs that can be successfully recruited for a possible pregnancy declines
- ❑ Women with poor ovarian reserve are unlikely to conceive with ART (assisted reproductive technology)

Why tests for ovarian reserve are needed?

- ❑ Fecundity (the capacity to bear a child) declines with increase in female age
- ❑ Due to ovarian ageing i.e. a decrease in the size of ovarian follicles & decrease in the quality of the oocytes
- ❑ Each month one ovulatory egg is released; ~99% of follicles undergo atresia

Hormonally controlled granulosa cell apoptotic process

Cannot be assessed using trans vaginal ultrasound (TVS)

Ovarian Reserve Tests (ORTs)

- ❑ ORTs provide an indirect estimate of a woman's remaining follicular pool
- ❑ An ideal ORT should be easy to perform, reproducible, and decisions based on their results should help differentiate women with a normal and poor ovarian reserve
- ❑ This should in turn help identify and counsel couples with negligible chance of conception against any expensive and repeated treatment
- ❑ Largely, these tests have been used in subfertile women prior to first IVF attempt to predict a poor ovarian response

Ovarian Reserve Tests (ORTs)

- ❑ The initial evidence suggested that various ORTs have a good predictive value for pregnancy
- ❑ However, in the recent years it has been understood that these tests are effective in predicting the ovarian response to stimulation and not for the prediction of pregnancy or its outcome
- ❑ Their role in the assessment of ovarian reserve in subfertile women not necessarily undergoing IVF or general population, to identify those at the risk of diminished ovarian reserve, is still poorly understood

Assessment of Ovarian Reserve

Pool (No.) of antral follicles in ovaries capable of growing in presence of gonadotrophins

Biochemical

Follicle Stimulating Hormone (FSH) - early follicular phase

Inhibin B

Anti Mullerian Hormone (AMH)

Estradiol (E2)

Ultrasound techniques

Antral Follicular (2-10mm) Count (AFC) & Ovarian Volume with TVS

Ovarian blood flow

Dynamic tests

Chlomiphene citrate challenge test (CCCT)

Exogenous FSH Ovarian Response Test (EFORT)

Gonadotrophin agonist stimulation test (GAST)

Anatomical test

- Ovarian biopsy

Follicle Stimulating Hormone (FSH)- Physiology

- ❑ Is a hormone synthesized and secreted by the gonadotrophs of the anterior pituitary
- ❑ FSH regulates the development, growth, pubertal maturation, and reproductive processes of the human body
- ❑ In *females*, FSH initiates follicular growth, specifically affecting granulosa cells. With the concomitant rise in inhibin B, FSH levels then decline in the late follicular phase. This seems to be critical in selecting only the most advanced follicle to proceed to ovulation. At the end of the luteal phase, there is a slight rise in FSH that seems to be of importance to start the next ovulatory cycle

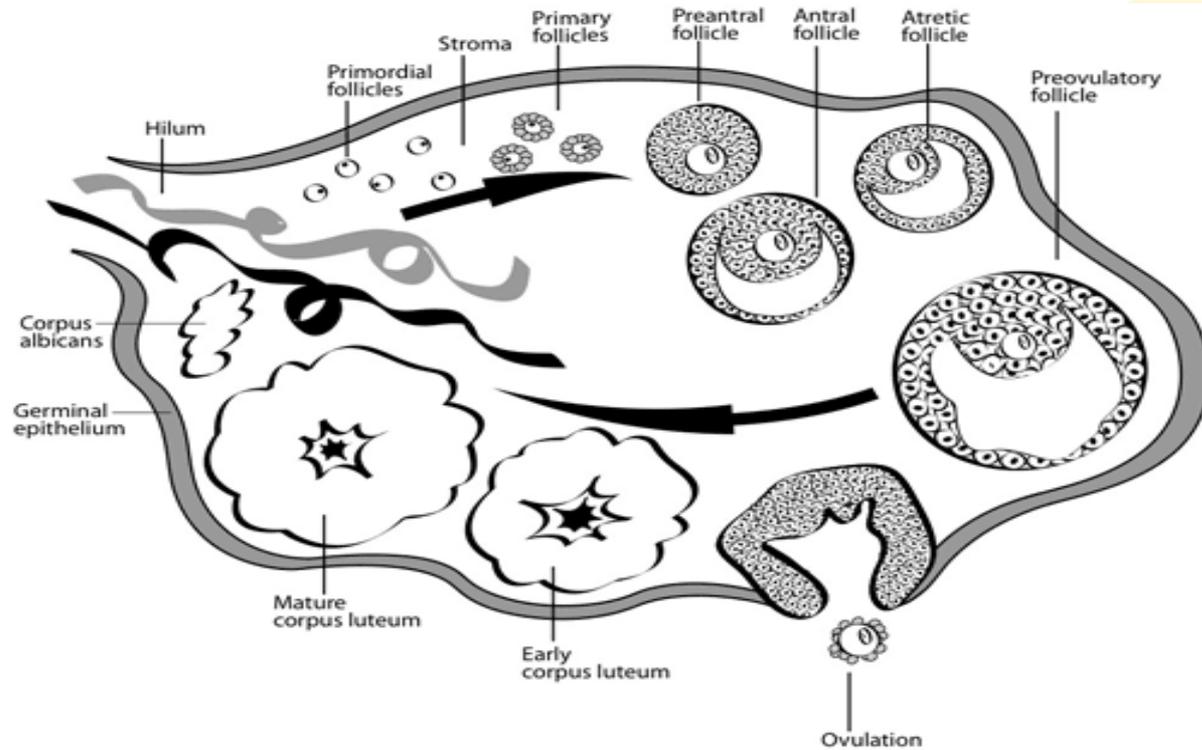
FSH as ORT

- ❑ Basal FSH levels measured on **Day 3** of the menstrual cycle is the most widely used ORT to assess ovarian response to stimulation
- ❑ An **increase** in FSH levels occurs due to follicle depletion
- ❑ FSH is known to have diurnal, intra and intercycle variability
- ❑ A wide range in the threshold values up to 25 IU/L has been used to define abnormal levels of basal FSH
- ❑ In regularly cycling women, FSH can predict a poor response adequately only at very high levels, and hence will be helpful only to a small number of women as a screening test, for counselling purposes

Pitfalls of FSH as ORT

- ❑ Ovarian aging begins several years before any elevation in FSH is noted; hence a **normal test cannot rule out a poor ovarian response** in some patients
- ❑ The usefulness of basal FSH in a general sub fertile population or elevated levels in young, regularly cycling women is unclear
- ❑ Combined with other markers it can be used to counsel couples regarding a poor response but should not be used to exclude regularly cycling women from ART (assisted reproductive technology)

Ovarian follicle stages



Anti Mullerian Hormone (AMH)- Physiology

- ❑ In females, produced by growing primary, preantral & small antral ovarian follicles
- ❑ AMH is exclusively produced by the granulosa cells of ovarian follicles from birth up to the menopause (expression begins post-natally)
- ❑ AMH continues to be expressed in growing follicles in ovary until they reach the size & differentiation state at which they are selected for dominance by action of FSH
- ❑ It also decreases the responsiveness of growing follicles to FSH
- ❑ The **ovary-specific expression pattern makes AMH an ideal marker for the size of the ovarian follicle pool**

Anti Mullerian Hormone (AMH) - As ORT

- ❑ Antral follicles (AFs) decrease in number with age; hence AMH production appears to diminish and become undetectable at
- ❑ AMH levels strongly correlate with basal antral follicle count (AFC) measured by transvaginal ultrasonography
- ❑ Unlike other markers, it can be **measured on any day of the cycle**
- ❑ Can **identify poor responders of ART** with 80-87% sensitivity and 64-93% specificity
- ❑ AMH is the earliest marker to show a decline longitudinally in young women, therefore can be used as a **screening test for women wishing to delay childbirth**
- ❑ At levels 0.5-1.26 ng/ml, AMH indicates perimenopausal transition within 3-5 years; levels within this range still suggest favorable results with ART

Anti Mullerian Hormone (AMH) - Summary

- ❑ Marker for predicting ovarian aging & potential for successful IVF
- ❑ Predicts occurrence of Menopausal transition
- ❑ Elevated levels in patients with PCOS

- *The only marker decreasing continually during the fertile life*
- *The only marker not influenced by gonadotropin feedback mechanism*
- *The only marker stable during the entire cycle*

Inhibin B - Physiology

- ❑ Inhibin B is released by the granulosa cells of the follicle
- ❑ Women with a **low day 3 inhibin B concentration** (<45 pg/ml) have a poor response to superovulation for IVF and are **less likely to conceive a clinical pregnancy**
- ❑ Decrease in inhibin B probably precedes the increase in the FSH concentration
- ❑ At very low threshold levels, the accuracy in the prediction of a poor response and nonpregnancy is only modest and hence its routine use cannot be recommended

Basal Estradiol (E2)

- ❑ Initial studies did show an association between an elevated basal E2 level and a poor ovarian response
- ❑ A large study showed that a poor ovarian response was more commonly seen in those with <20 or >80 pg/ml of estradiol but did not show any correlation to the pregnancy rate
- ❑ Basal E2 does not add to the predictive value of other commonly used ORTs, its routine use in clinical practice is not recommended

Assessment of Ovarian Reserve

Pool (No.) of antral follicles in ovaries capable of growing in presence of gonadotrophins

Biochemical

Follicle Stimulating Hormone (FSH) - early follicular phase

Inhibin B

Anti Mullerian Hormone (AMH)

Estradiol (E2)

Ultrasound techniques

Antral Follicular (2-10mm) Count (AFC) & Ovarian Volume with TVS

Ovarian blood flow

Dynamic tests

Chlomiphene citrate challenge test (CCCT)

Exogenous FSH Ovarian Response Test (EFORT)

Gonadotrophin agonist stimulation test (GAST)

Clomiphene citrate challenge test

- ❑ Clomiphene citrate is used in female infertility, for ovulation induction as well as for ovulation hyper stimulation, as part of IVF procedure
- ❑ Clomiphene citrate challenge test (CCCT) is a dynamic test involving the administration of 100 mg of clomiphene citrate from the fifth day of the cycle for 5 days
- ❑ Basal FSH is estimated on day 3 of the cycle and stimulated FSH levels on day 10. Abnormal values on day 3 or day 10, or on addition of the two, is considered as a predictor of a poor ovarian response

The drawback shared by all dynamic tests in that it is expensive, more invasive, more time consuming, and associated with the possible side effects of administered drugs

Exogenous FSH Ovarian Response Test

- ❑ This dynamic test involves the measurement of basal FSH and estradiol followed by the administration of 300 IU FSH on day 3 of the cycle
- ❑ The serum estradiol concentration is determined 24 h later
- ❑ It is found to be better than CCCT in predicting hyper-responders and inferior to the latter in predicting a poor response

In view of the high rate of false positives, the authors did not recommend this test alone for the identification of hyper-responders

Gonadotrophin agonist stimulation test (GAST)

- ❑ It involves the assessment of serum estradiol on day 2 of the cycle followed by the subcutaneous administration of GnRHa (Gonadotrophin releasing hormone agonist) 100 µg
- ❑ A change in estradiol levels is noted by repeating the test 24 h later on day 3
- ❑ A rise in estradiol is considered to be indicative of good ovarian reserve

SUMMARY OF OVARIAN RESERVE TESTS

- ❑ It is well understood that the ovarian follicular pool and hence fertility declines with age. However, there is a large individual variation in its onset
- ❑ Even though ORTs primarily have been used to identify **poor responders**, and counsel such women to avoid repeated ineffective treatment, it is now known that some of them are able to predict a **hyper-response**. This helps avoid maximal ovarian stimulation in such women and minimize the risk of life-threatening OHSS without compromising the pregnancy rate
- ❑ Majority of the ORTs, including the most widely used basal FSH levels, show abnormal values late in a woman's reproductive life to be of practical help
- ❑ AMH and AFC (antral follicle count by USG) are the basal markers found to predict the ovarian response, both poor and hyper, with a high sensitivity and specificity

SUMMARY OF OVARIAN RESERVE TESTS

- ❑ Serum **AMH** levels show minimal intra- and intercycle fluctuations and thus can be performed at any stage of the menstrual cycle. They show **distinct age-related declines at a very young age**, much earlier than other markers including AFC
- ❑ AMH has the ability to be applied to the general population for identification of diminishing ovarian reserve before it reaches a critical level below which no effective treatment can be offered. This may help women who wish to delay pregnancy to make an informed decision
- ❑ AMH is the **only ORT** found to be useful in **evaluating the residual ovarian reserve in young women treated for malignancies** with chemotherapy or radiotherapy
- ❑ Dynamic tests do not add to the value of baseline tests and hence cannot be recommended as a diagnostic tool with the available evidence

