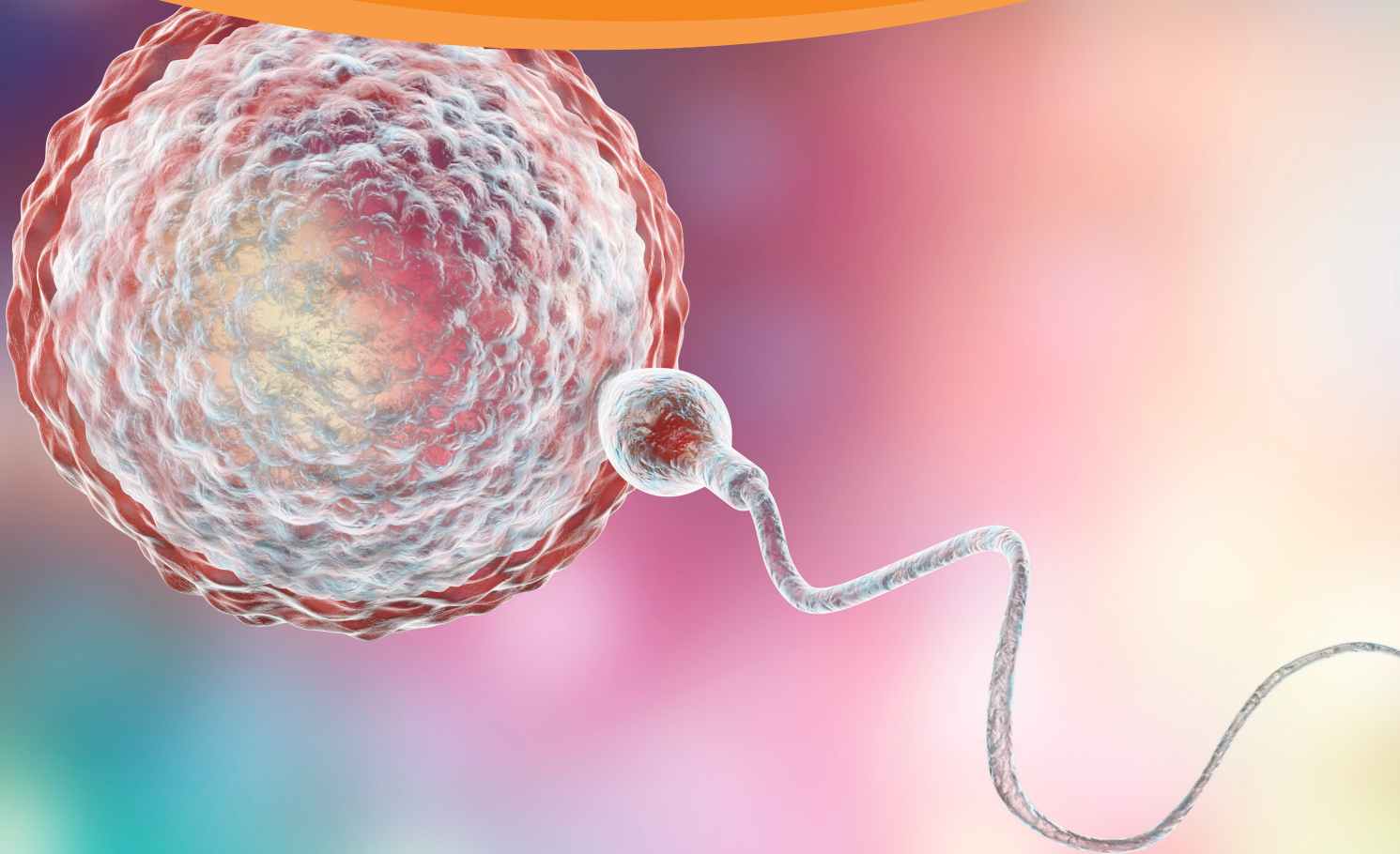


Female Reproductive Hormones and Fertility Testing

By Associate Professor Mirette Saad



Reproductive Hormones

Reproductive endocrinology encompasses the hormones of the hypothalamic-pituitary-gonadal axis and the adrenal glands. These hormones are crucial for proper reproductive function and include Gonadotropin-Releasing Hormone (GnRH), Lutenising Hormone (LH), Follicle Stimulating Hormone (FSH) and a multitude of sex steroids.

LH primarily stimulates the production of hormones by the gonads whereas FSH stimulates the development of the germ cells. The sex steroids are synthesised at the ovaries, testes, and adrenal glands and are responsible for the manifestation of primary and secondary sexual characteristics. The thyroid gland produces hormones that are essential for normal metabolism, hormone production and fertility. This review article discusses female reproductive hormones and their role in fertility testing.

Physiology of Ovarian Cycle

Normal women display considerable variation in cycle length, which varies from 25-30 days, with a normal ovulatory menstruation cycle of 28 days, on average.

Oestrogens, progesterone and androgens are secreted by the ovarian follicles of the ovaries. With a normal ovarian reserve, oestradiol (E2) produced by the ovarian follicles and the corpus luteum increases, reaching a mid-cycle peak then fall off abruptly after ovulation. This exerts negative feedback on the hypothalamic-pituitary axis to stabilise the level of FSH and LH release, such that one dominant follicle is selected each cycle. A mid-cycle surge in LH production stimulates a series of events that culminate in ovulation, with FSH levels falling after this event.

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Any injury to the hypothalamus, or the presence of either psychological or physical stressors, lead to changes in these hormonal cues, resulting in anovulation and amenorrhea.



Ovarian Reserve and Anti-Müllerian Hormone (AMH)

In females, Anti-Müllerian Hormone (AMH) is an established biomarker, produced by antral and pre-antral follicles for assessing ovarian reserve, which is considered an important tool in assessing potential fertility. AMH can help in the assessment of reproductive capacity and in the prediction of oocyte retrieval (egg collection) and ovarian responsiveness to stimulation regimes.

"AMH can help in the assessment of reproductive capacity. Low serum levels of AMH is suggestive of poor ovarian reserve, whereas high levels may indicate a polycystic ovary syndrome (PCOS)."

Menopause and FSH Hormone

Menopause is a normal change that women go through when the ovarian reserve has been depleted. Normally, menopause occurs within a 10-year window, from 45-55 years of age, with an average age in Australia of 51.5 yrs.

Fluctuating levels of FSH and oestradiol can be noticed, during both peri-menopausal and pre-menopausal stages, hence, they are not considered reliable predictors of menopause, as they are sometimes at pre-menopausal levels.

Infertility or Subfertility: Definition and Subtypes

One in six Australian couples experience trouble conceiving. Infertility is defined as a unique medical condition because it involves a couple, rather than a single individual. WHO defines infertility as failure of a couple to conceive after 12 months of regular intercourse without use of contraception in women less than 35 years of age; and after six months of regular intercourse without use of contraception in women 35 years and older.

Primary infertility is infertility in a couple who have never had a child. **Secondary infertility** is failure to conceive following a previous pregnancy and is considered the most common form of female infertility, mainly due to reproductive tract infections. It is estimated that male infertility represents 20-30% of cases, while 20-35% of cases are due to female infertility and the rest are due to combined (~20%) or unexplained reasons (~15%).

Fertility Clinical Assessment in Couples

For conception to occur, viable spermatozoa, ovulation and functional anatomy are required. In cases of infertility, both partners should be investigated.

A couple who is unsuccessful at achieving pregnancy should seek a comprehensive physical and medical assessment by clinicians in order to identify causes that can be medically intervened. In addition to structural and surgical causes, laboratory investigations are essential to identify clues to the underlying aetiology to reach a clinical diagnosis.

Investigations for Male Infertility

- Semen analysis is an essential component of fertility with an utmost predictive value
 - repeat after 6 weeks, if abnormal
- LH, FSH, testosterone
- Prolactin, thyroid function
- If azoospermia present
 - cystic fibrosis screen, karyotyping (for conditions such as Klinefelter's syndrome (47, XXY) and others
 - testicular biopsy

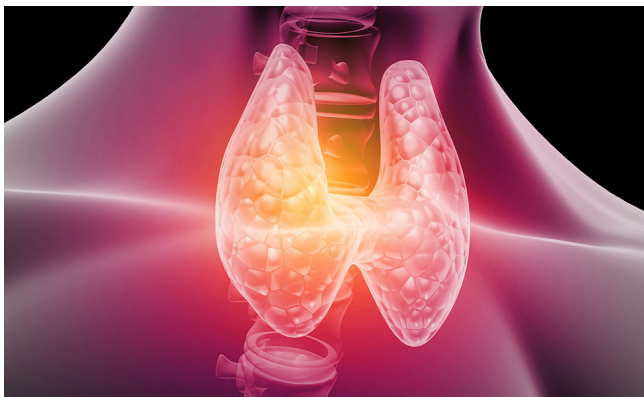
Investigations in Female Infertility (Table 1)

Investigations for anovulation include: FSH, LH, Prolactin, TSH, Testosterone, SHBG, Trans-vaginal U/S, CT/MRI pituitary, Karyotyping.

"A couple who is unsuccessful at achieving pregnancy should seek a comprehensive medical assessment. Laboratory investigations are essential to identify clues to the underlying aetiology to reach a clinical diagnosis."

Pituitary and Ovulatory Biomarkers:

Common causes of anovulation include polycystic ovary syndrome (PCOS), hypothalamic amenorrhoea (HA) and premature ovarian insufficiency (POI).



- **Ovulatory Function:** FSH/LH and Oestradiol (E2) are essential in assessing the ovulatory cycle. They are best evaluated in the early follicular phase (day 2-6). An elevated FSH level (>10mIU/mL) early in the follicular phase is indicative of a low ovarian reserve.
- **Ovulation Predictor:** evaluating the LH surge is the best single assay, while the measurement of LH plus pre-ovulatory oestrogen is the best prediction.
- **Prolactin Testing** is required in order to exclude hyperprolactinaemia. It can be measured anytime during the day or in the menstrual cycle (but not after exercise or stress). Consider a follow up CT/MRI of pituitary if pituitary macroadenoma is suspected, particularly in the context of amenorrhoea, galactorrhoea, visual field abnormality or headaches, which warrant referral to endocrinology.
- **TSH** is also recommended to exclude hypothyroidism.

Ovarian Reserve and AMH Testing:

Low serum levels of AMH is suggestive of poor ovarian reserve, whereas high levels may indicate a polycystic ovary syndrome (PCOS).

Primary Amenorrhea:

- After excluding pregnancy, pituitary and ovarian hormones' testing is warranted (see above).
- Karyotyping chromosomal/genetic analysis should be performed in women with ambiguous genitalia or evidence of primary ovarian insufficiency (hypergonadotropic hypogonadism) or clinical features suggestive of Turner syndrome (45,XO).

Secondary Amenorrhea:

- **Pregnancy:** Human chorionic gonadotrophin (HCG) where a result >25 IU/L (normal <5) is indicative of early pregnancy.
- **Menopause:** where monitoring check is required for both FSH and E2.
- Other medical causes should be investigated.

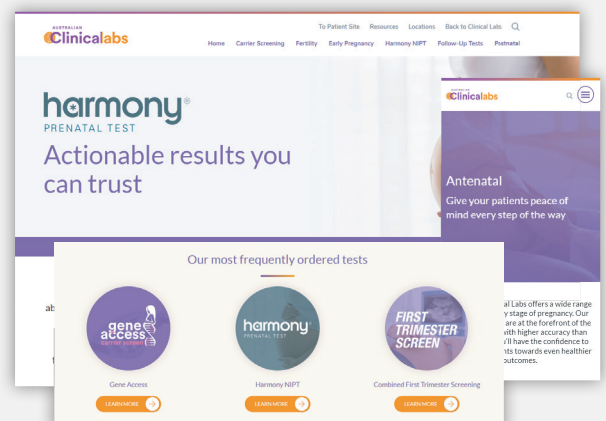
Androgen Testing in Hirsutism:

While hirsutism in females is most commonly idiopathic in aetiology (60% of cases), 40% of cases can be due to elevated androgen levels. Female androgens are produced the ovaries and adrenal glands.

True androgen excess (including total plasma testosterone, DHEA, 17-OH progesterone (17-OHP) and androstendione), can occur in ovarian and adrenal gland disorders. High androgens can be found in PCOS in about 35% of cases, while 5% of cases are due to other metabolic conditions, such as congenital adrenal hyperplasia (CAH), Cushing syndrome, hyperprolactinaemia, and adrenal and ovarian tumours.

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Polycystic Ovary Syndrome (PCOS):

- In PCOS cases, in addition to the above hormonal testing, ovarian ultrasound is usually recommended.
- A follow-up testing for early diagnosis of diabetes mellitus and hyperlipidaemia, including fasting glucose, insulin, HbA1c, oral glucose tolerance tests (OGTT), insulin resistance and lipid levels, is recommended.

Blood Test	When	Results' Interpretation
Progesterone	D21 in a regular cycle or 7 days prior to expected menses if cycle not 28 days	>30 nmol/L confirms ovulation, whereas low values indicate anovulation and a need for follow-up testing
FSH	Day 2-6 (Early Follicular Phase, best on Day 3)	Raised serum FSH (>10 IU/L) during the early follicular phase is reflective of reduced ovarian reserve, albeit it is a relatively late feature
LH	Early Follicular Phase (Day 2-6)	>10 may be seen in PCOS
Prolactin	Anytime of the cycle	>1000 (mIU/L) may indicate pituitary macroadenoma
AMH	Anytime of the cycle; a sensitive hormone marker of fertility potential	Low values may indicate poor ovarian reserve while high values may suggest PCOS
Testosterone	Anytime of the cycle	May suggest PCOS or CAH (17-OHP and DHEA level should be assessed during the follicular phase)

Table 1: Summary of Some Fertility Hormones Testing in Women.

General Workup Investigations

General causes of infertility should be investigated, such as anorexia nervosa, malnutrition, substance abuse, alcoholism, exogenous steroids, tumours/chemotherapy, infections and metabolic conditions (including diabetes mellitus and coeliac disease), and chronic illness such as HIV, hepatitis, sexually transmitted diseases (STDs) and sarcoidosis.

Blood Investigations and Follow-up Management May Include

- General chemistry, including liver function and kidney function tests
- Nutritional Assessment: Vitamins, iron studies and trace elements
- Tumour markers
- Angiotensin-converting enzyme
- Metabolic/endocrine screening: cortisol and IGF-1, haemochromatosis, coeliac autoantibodies and genetic testing
- Drug/alcohol screen
- Chronic infections and STD screen
- Imaging workup in hyperprolactinaemia, PCOS, adrenal and ovarian tumours
- Referral to an endocrinology specialist service for further assessment, if indicated

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