Role of endocrine biochemistry laboratories in the investigation of infertility

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Introduction

Infertility is a descriptive term that embraces many specific diagnoses. For practical purposes, infertility is defined as the failure of a couple to achieve a pregnancy despite one year of regular unprotected sexual intercourse. Infertility is common: estimates suggest that as many as one in six couples will need specialist help because of infertility at some time.

Infertility may result from abnormalities in either or both partners or from their incompatibility. Of the many specific causes of infertility, some have a genetic or anatomical basis, some result from a previous disease or treatment, and some are endocrine in origin.¹ Regrettably, it is not always possible to reach a specific diagnosis. The challenge to the investigating physician is great, and it is heightened still further by the pressure to reach a specific diagnosis and start treatment quickly despite a failure of central funding to match the clinical demand.

It is now generally accepted that agreed strategies are required to maximise the efficiency of investigation of infertility.^{2,3} Details of such strategies will vary from centre to centre, but in all cases the services and staff of the pathology laboratory will be vital. This article seeks to identify the role of the endocrine biochemistry service in the investigation of infertility and to recommend ways in which that service may best be provided.

Strategy of investigation of infertility

The perfect strategy for the investigation of the infertile couple would achieve the ideals of reaching a specific diagnosis in the shortest time, with minimum inconvenience to the patient and at least cost to the NHS. The perfect strategy that is applicable to all infertile couples has yet to be designed, but with experience, teamwork, and new technology the best centres are achieving an ever better balance between these ideals. These centres have each adopted a strategy of investigation that can be considered in a number of stages.

STAGE 1 THE INITIAL CLINICAL INVESTIGATION
It is highly desirable that both partners from

the infertile couple attend the initial investigation, which comprises a detailed clinical history and physical examination.

As part of the clinical history, both partners should be asked about congenital abnormalities, previous pregnancies, serious illnesses and infections, past chemotherapy or radiotherapy, and current habits in relation to smoking and the use of alcohol or recreational drugs. Sensitive areas such as psychological disorders, venereal disease, and the frequency and satisfaction of intercourse should be addressed. The male partner should be questioned about the descent of the testes at puberty, penile erections and ejaculation, and specifically asked about mumps orchitis, correction of varicocele, or exposure to radiation, toxins or heat. The female partner will be required to give a full menstrual history, details of all contraceptive preparations used, and of any changes in body weight. Specifically, she should be questioned about abortions, pelvic inflammatory disease, or pelvic surgery.

Physical examination of both partners should include a careful documentation of secondary sex characteristics and any features that could be consistent with hypothalamic or pituitary disease, thyroid disease, or Cushing's syndrome. The male genitalia should be examined, noting the size and consistency of the testes, epididymis, vas deferens and evidence of varicocele. In the female partner it is important to examine the breasts for galactorrhoea, and the face and trunk for hirsutism. A pelvic examination is mandatory in a woman with primary amenorrhoea and may help to identify tenderness behind the uterus that could be explained by endometriosis.

STAGE 2 SELECTION OF INITIAL LABORATORY TESTS

The selection of appropriate initial laboratory tests will be determined by the outcome of the initial clinical investigation (table 1). Over-requesting of laboratory tests should be strongly discouraged. No endocrine investigations are indicated in a eugonadal male. Measurement of serum progesterone is the only valid request in a woman with normal

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Table 1 Selection of initial laboratory tests for the investigation of infertility

Initial clinical findings	Appropriate hormone tests	Other laboratory tests
Male—eugonadal	Nil	Semen analysis
Male—hypogonadal	FSH, LH, prolactin testosterone	Semen analysis karyotype
Female—regular periods	Luteal progesterone	Nil
Female—primary amenorrhoea	Pregnancy test FSH, LH, prolactin, oestradiol	Karyotype
Female—secondary amenorrhoea	Pregnancy test FSH, LH, prolactin, oestradiol	Nil
Female—hirsutism	Add testosterone, sex hormone binding globulin	Nil

periods; paradoxically, assessment of progesterone is unlikely to be helpful in a woman with amenorrhoea.

STAGE 3 INTERPRETATION OF INITIAL LABORATORY TESTS

Table 2 summarises the relevant reference intervals used by the laboratory at Glasgow Royal Infirmary; other laboratories may quote slightly different figures, and it is important that physicians are acquainted with the reference intervals appropriate to the laboratories that they use.

In both men and women clinically important hyperprolactinaemia is unlikely unless the serum prolactin concentration is more than 700 mU/l on two consecutive occasions. If the prolactin is more than 4000 mU/l the patient is either already pregnant or has a pituitary prolactin-secreting tumour. Results between 700–4000 mU/l may be due to a microprolactinoma or the result of a hypothalamic disorder, polycystic ovarian disease, primary hypothyroidism or drug treatment.⁴

The interpretation of initial gonadal hormone results in men is straightforward. An increased serum follicle stimulating hormone concentration (FSH), accompanied by a low or low-normal testosterone value is diagnostic of primary testicular disease. A subnormal

testosterone value in the presence of subnormal or “normal” gonadotrophin concentrations is strongly suggestive of hypothalamic-pituitary disease, although tumours secreting steroids other than testosterone may give the same pattern.⁵

In a woman with normal periods a “day 21” progesterone result of more than 30 nmol/l is indicative of an ovulatory cycle; a result of less than 10 nmol/l indicates anovulation.³ A result of 10–30 nmol/l from an appropriately timed specimen suggests a deficient luteal phase. The amenorrhoeic woman with an FSH of more than 30 IU/l and an oestradiol concentration of less than 200 pmol/l almost certainly has primary ovarian failure. In cases of amenorrhoea or oligomenorrhoea a luteinising hormone (LH) value of more than 15 IU/l in the presence of a normal FSH result is suggestive of polycystic ovarian disease; assessment of the oestradiol concentration will probably be unhelpful in such a patient,⁶ but the free androgen index will be raised if hirsutism is also present.⁷ All too often, however, the hormone results are essentially normal, pointing towards a subtle hypothalamic-pituitary defect.³

STAGE 4 SECOND LINE INVESTIGATIONS

The range of possible second-line investigations is extremely wide, and, most conveniently, may be recorded in a series of algorithms.² Because many infertile couples will not require further endocrine biochemistry tests, it is inappropriate to list all of the options in this review. Instead, the text will deal with the limited number of situations in which the specific diagnosis may have an endocrine basis.

Hyperprolactinaemia

An initial finding of hyperprolactinaemia in either sex requires dual follow up. First, the observation should be confirmed on a repeat specimen. At the same time the clinical history should be re-examined and simple tests performed to eliminate causes other than pituitary tumour. Hyperprolactinaemia induced by drugs is common and can result from many therapeutic pharmaceuticals, including oestrogen, phenothiazines, sulpiride, metoclopramide, α -methyldopa and reserpine.⁴ A pregnancy test should already have been performed; serum creatinine should be checked to exclude renal insufficiency.

Primary hypothyroidism is a cause of hyperprolactinaemia, and amenorrhoea and infertility have been presenting symptoms.⁸ By such means the diagnosis may be reduced to either prolactinoma or other hypothalamic or pituitary disorder, and at this stage detailed radiographic pituitary evidence is essential. The higher the serum prolactin result, the greater the likelihood of a prolactinoma: this is associated with abnormal pituitary radiological findings. Non-prolactin secreting tumours (acromegaly, Nelson's syndrome, chromophobe adenoma) may be the cause of modest hyperprolactinaemia (< 4000

Table 2 Reference intervals for hormones used in the investigation of infertility

Hormone analyte	Normal subjects	Reference interval
Adrenocorticotrophic hormone (ACTH)—plasma	Adults 0700–0900 h	<20 mU/l (<80 ng/l)
Androstenedione	Men 18–40 y 41–65 y Women 18–40 y 41–65 y	1.6–8.4 nmol/l 1.3–6.6 nmol/l 0.6–8.8 nmol/l 0.9–6.8 nmol/l
Cortisol	Adults 0700–0900 h 2200–2400 h	280–720 nmol/l 60–340 nmol/l
Dehydroepiandrosterone sulphate	Men <50 y Women <50 y	2.0–9.0 μ mol/l 2.0–11 μ mol/l
Follicle stimulating hormone (FSH)	Men <50 y Women follicular mid-cycle luteal postmenopausal	1–12 IU/l 3–13 IU/l 9–18 IU/l 1–10 IU/l 18–150 IU/l
17-Hydroxyprogesterone	Women follicular	<13 nmol/l
Luteinising hormone (LH)	Men <50 y Women follicular mid-cycle luteal postmenopausal	2–12 IU/l 2–15 IU/l 22–90 IU/l 1–19 IU/l 16–64 IU/l
Oestradiol	Men <50 y Women follicular mid-cycle luteal postmenopausal	<300 pmol/l 180–1000 pmol/l 500–1500 pmol/l 440–800 pmol/l <200 pmol/l
Prolactin	Adults 50–500 mU/l	50–500 mU/l
Progesterone	Women follicular luteal	<5 nmol/l 18–72 nmol/l
Sex hormone binding globulin (SHBG)	Men <50 y Women <50 y	6–45 nmol/l 30–120 nmol/l
Testosterone	Men <50 y Women <50 y	11–36 nmol/l 1.0–3.2 nmol/l
Free androgen index (testosterone $\times$ 100 $\div$ SHBG)	Men <50 y Women <50 y	36–156 <7.0

mU/l). Ultimately, the differential diagnosis is between microprolactinoma (normal radiological findings) and idiopathic hyperprolactinaemia (deficient supply of dopamine); dynamic tests of prolactin secretion may help to differentiate.⁹

Cushing's syndrome

This is a rare cause of infertility in either sex. Clinical features are usually present and the hypercortisolaemia may be confirmed by following a standard protocol based on cortisol and adrenocorticotrophic hormone (ACTH) measurement.¹⁰

Primary testicular failure

Primary testicular failure is readily identified by the initial endocrine tests. Damage to both the interstitial and tubular elements results in increases in both FSH and luteinising hormone; when tubular function is selectively impaired only FSH is increased. Testosterone concentrations are low in primary testicular failure, in anorchia, and usually in Klinefelter's syndrome. Repeat analysis is required to confirm the pattern. Thereafter, the endocrine laboratory is restricted to checking the adequacy of androgen replacement treatment, for such patients are almost invariably sterile.

Hypogonadotrophic hypogonadism in the male partner

This condition is characterised biochemically by subnormal testosterone concentrations in the presence of low or normal gonadotrophins. The testes are capable of increasing testosterone concentration by more than 100% in response to five days of stimulation with human chorionic gonadotrophin.³ The biochemical pattern may result from either hypothalamic or pituitary disorder, and although the gonadotrophin releasing hormone (GnRH) test may discriminate, it is not infallible.³ Clomiphene (200 mg) can be effective in hypothalamic disease, and in a formal test serum luteinising hormone, FSH, and testosterone will rise by at least 70%, 45%, and 40%, respectively.¹¹ Treatment with either pulsatile GnRH or exogenous gonadotrophin demand laboratory support, but such treatment is lengthy and expensive, and artificial insemination by donor is an effective alternative that requires no laboratory backup for the donor.

Women with regular periods

Once ovulation has been confirmed, there is no requirement for further biochemical monitoring of the female partner; other causes of infertility should be sought. Women with anovular cycles are likely to be given Clomiphene, and endocrine follow up should be restricted to one or two progesterone measurements in the luteal phase of each cycle.

Primary ovarian failure

The biochemical pattern of raised gonadotrophins with subnormal oestradiol values requires confirmation. In all but a very few of these women fertility is no longer an issue,

but hormone replacement therapy is required to assist libido and prevent osteoporosis. No consensus exists as to the extent of endocrine monitoring of patients receiving oestrogen.

Hirsutism

The detailed investigation of hirsutism is complex, and readers are referred elsewhere for a comprehensive treatise.¹² Polycystic ovarian disease is a common cause of infertility that is associated with mild to moderate hirsutism and a variable menstrual pattern. The serum luteinising hormone is usually increased relative to the FSH. The total testosterone concentration may be raised (rarely more than 10 nmol/l), but it may be within normal limits. The sex hormone binding globulin (SHBG) binding capacity is usually reduced, such that the free androgen index is increased. The biochemical pattern should be confirmed by repeat sampling, and the finding of modestly increased androstenedione with or without dehydroepiandrosterone sulphate results substantiates the diagnosis which can be confirmed by ovarian ultrasound examinations. Patients complaining of infertility are likely to be treated with Clomiphene or Dexamethasone rather than aggressive antiandrogen treatment, and hormone measurement should be targeted at monitoring for ovulation.

Severe hirsutism of short duration strongly suggests an androgen secreting tumour, usually adrenal or ovarian. Serum androgens, especially testosterone, are often grossly increased. Such tumours can vary considerably in the pattern of steroids that they secrete, and the laboratory should ensure that all possible androgens are measured in serum and that a urinary steroid profile is performed.¹³ Corticosteroid secretion may also be deranged and ACTH secretion suppressed. Treatment may involve surgery, chemotherapy, or radiotherapy; the laboratory should discuss which of the steroid measurements is the most appropriate tumour marker.

So-called late onset congenital adrenal hyperplasia is increasingly being recognised as a cause of hirsutism and infertility. It is important to discriminate this partial steroid 21-hydroxylase deficiency from polycystic ovarian disease, because corticosteroid replacement is very effective in the former. The measurement of serum 17-hydroxyprogesterone is the key analysis, an exaggerated response 1 hour after synthetic ACTH is the definitive test.¹⁴

Hypogonadotrophic hypogonadism in the female partner

A few women, often with primary amenorrhoea, will have subnormal FSH or luteinising hormone and oestradiol results. They have hypopituitarism resulting either from a tumour in the hypothalamus or pituitary, or from failure of the hypothalamic-pituitary axis to mature. Detailed investigation of the anterior pituitary, including hormone testing, is indicated.

A large proportion of women with amenorrhoea or oligomenorrhoea show no obvious abnormality in their initial hormone results. Repeated baseline hormone measurement is of no value in these patients. A proportion of the women will have polycystic ovarian disease despite the absence of hirsutism—a relative increase in the luteinising hormone: FSH ratio may be a pointer, and limited endocrine follow up is justified as a complement to ovarian ultrasound examination. Many women in this group, however, will have a subtle failure of the hypothalamic-pituitary axis, most notably of the positive feedback mechanism responsible for the mid-cycle surge.

Clomiphene is commonly used to induce ovulation in the latter patients; progesterone measurements during treatment are indicated. The dose of Clomiphene will be lower and the success rate will be higher in patients who have good oestrogen concentrations. Although the initial serum oestradiol result is of some value in this judgment, the progesterone challenge test to induce withdrawal bleeding is still widely used.

A proportion of patients will fail to respond to Clomiphene. These women are candidates for treatment with exogenous gonadotrophins, perhaps following down-regulation of endogenous pituitary FSH or luteinising hormone secretion with a GnRH agonist. Laboratory monitoring of the oestradiol response to gonadotrophin treatment will help to minimise ovarian hyperstimulation.

Laboratory support for programmes of assisted conception

Gamete intrafallopian transfer (GIFT) and in vitro fertilisation (IVF) are being used increasingly to treat carefully selected infertile couples. Follicular growth and oocyte maturation are closely monitored both by ultrasound examination and measurement of either luteinising hormone or oestradiol. Timing is critical to the success of these procedures and laboratories must provide a rapid (2–4 hours) turnaround of results. In practical terms this usually means that special assays are required on a daily basis, including weekends. The laboratory analysis is often best performed adjacent to the patient treatment area.

Range of endocrine biochemistry analyses

A wide range of hormone assays is needed if all possible causes of infertility are to be investigated (table 2). Two major factors will determine which assays will be offered locally and which will be sent to a reputable reference laboratory. Firstly, the clinical demand for each analyte will influence the assay frequency and result turnaround time. The laboratory should seek to return results from non-urgent investigations within the following timescales: 7 days, FSH, luteinising hormone, prolactin, oestradiol, progesterone, testosterone, SHBG; 21 days, ACTH, 17-

hydroxyprogesterone, androstenedione, dehydroepiandrosterone sulphate. Results for patients entered in assisted conception programmes must be returned much more rapidly (see previous section).

Secondly, not all laboratories have the facilities and expertise necessary to perform and interpret the results of all the analyses listed in table 2. Modern immunoassay kits are technically simple to perform but they often have compromises built into their design which can create pitfalls for the unwary. Circumstances will vary considerably among laboratories, but as a guideline it is suggested that FSH, luteinising hormone, prolactin and progesterone assays be provided locally. Once direct immunoassay kits for oestradiol and testosterone are shown to be valid for the investigation of infertility, it is likely that they too should be provided locally. Other analyses are probably best provided by a reputable reference centre.

Specimen requirement

Most hormone measurements are made in blood. A 10 ml specimen is collected without venous stasis and with minimum stress. Of the analytes listed in table 2, all but ACTH are stable in serum kept at room temperature for 24 hours. Longer term storage of serum should be at -20°C .

The timing of blood collection is crucial to the successful investigation of infertility. Several of the hormones listed in table 2 are secreted in pulses or exhibit a circadian rhythm, but in practical terms any specimen collected between 0900–1700 h will suffice, with the exception of cortisol and ACTH specimens which should be collected at 0700–0900 h and 2200–2400 h.

In the male partner blood may be collected on any day for basal investigation. In the female partner the day of collection in relation to the last menstrual period is crucial; where periods do occur the date of the last menstrual period should appear on the request form. Women with regular periods generally only require progesterone measurement to monitor ovulation—sampling should be between 19–24 days after the test menstrual period for a 28 day cycle, and correspondingly later for a longer cycle.

Amenorrhoeic women may be investigated on any day before treatment, but specimens should be timed to coincide with treatment.

Fluids other than blood may be used to investigate infertility. Pregnancy tests are usually performed on urine, and there are now several commercial systems for urine testing at home, both for pregnancy and ovulation. There is likely to be a resurgence of urine analysis in the laboratory with the refinement of non-isotopic assays for the simultaneous measurement of oestrogen and progestogen glucuronides. Measurement of salivary progesterone has been advocated as a simple and effective non-invasive method of monitoring ovulation¹⁵; it is perhaps surprising that the application has not been more widely used.

Potential pitfalls of hormone measurement

Modern isotopic and non-isotopic immuno-metric assays are the methods of choice for the measurement of FSH, luteinising hormone, and prolactin. Individual assays show excellent precision and may be automated. There is method dependent bias arising from a combination of impure calibrants and antibody pairs, however, that recognise different antigenic determinants.¹⁶ Some normal individuals also secrete an isoform of luteinising hormone that may not be recognised by all dual monoclonal antibody assays.¹⁷

Problems persist with direct (non-extraction) immunoassays for serum steroids. Valid assays are available for cortisol and progesterone (which bind to cortisol binding globulin) but most, if not all, direct assays are invalid for the measurement of testosterone in female sera and oestradiol at concentrations below 300 pmol/l. The problem is caused by the endogenous SHBG which compromises the steroid-antibody binding reaction. Until this problem can be resolved prior solvent extraction is recommended.

Traditionally, the measurement of ACTH, SHBG, 17-hydroxyprogesterone, androstenedione and dehydroepiandrosterone sulphate has been confined to a few reference centres. Although methodology is now improving for these analytes, the small workload and lack of experience of the average laboratory suggest that there will be no major expansion in the number of centres offering these tests.

The role of clinical biochemists

If effective strategies for the investigation of infertility are to be formulated and audited it is essential for senior staff from the endocrine biochemistry laboratory to interact directly and regularly with the physicians in charge. The role of the clinical biochemist (chemical pathologist) may be summarised as follows:

- (a) Ensure direct supply or access to a full range of hormone assays of appropriate turnaround time and be able to vouch for the validity of the results.
- (b) Agree with the physicians a detailed strategy for the investigation of infertility.
- (c) Monitor the progress of patients being investigated according to the strategy and, where appropriate, offer specific interpretative comment.
- (d) Attend regular audit meetings with physicians to discuss the success of the strategy, workload trends and the outcome for individual patients.
- (e) Wherever possible, agree with physicians joint research projects that aim to improve the effectiveness of the investigation or management of infertility.

Summary

The staff and services of the endocrine biochemistry laboratory are essential to the efficient investigation of infertility. Each centre should adopt a detailed strategy for the investigation of the infertile couple which specifies the hormone analyses required at each stage. Appropriate first-line hormone tests should be selected after a thorough clinical history and physical examination of both partners. Second-line hormone testing should be determined from the results of the initial investigation and should be restricted to requests that either confirm or clarify an endocrine basis to infertility or monitor the response to treatment. The clinical biochemist should advise on specimen timing and collection, have responsibility for guaranteeing time and valid hormone results, and be part of the team that audits the overall strategy and the outcome for individual patients.

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