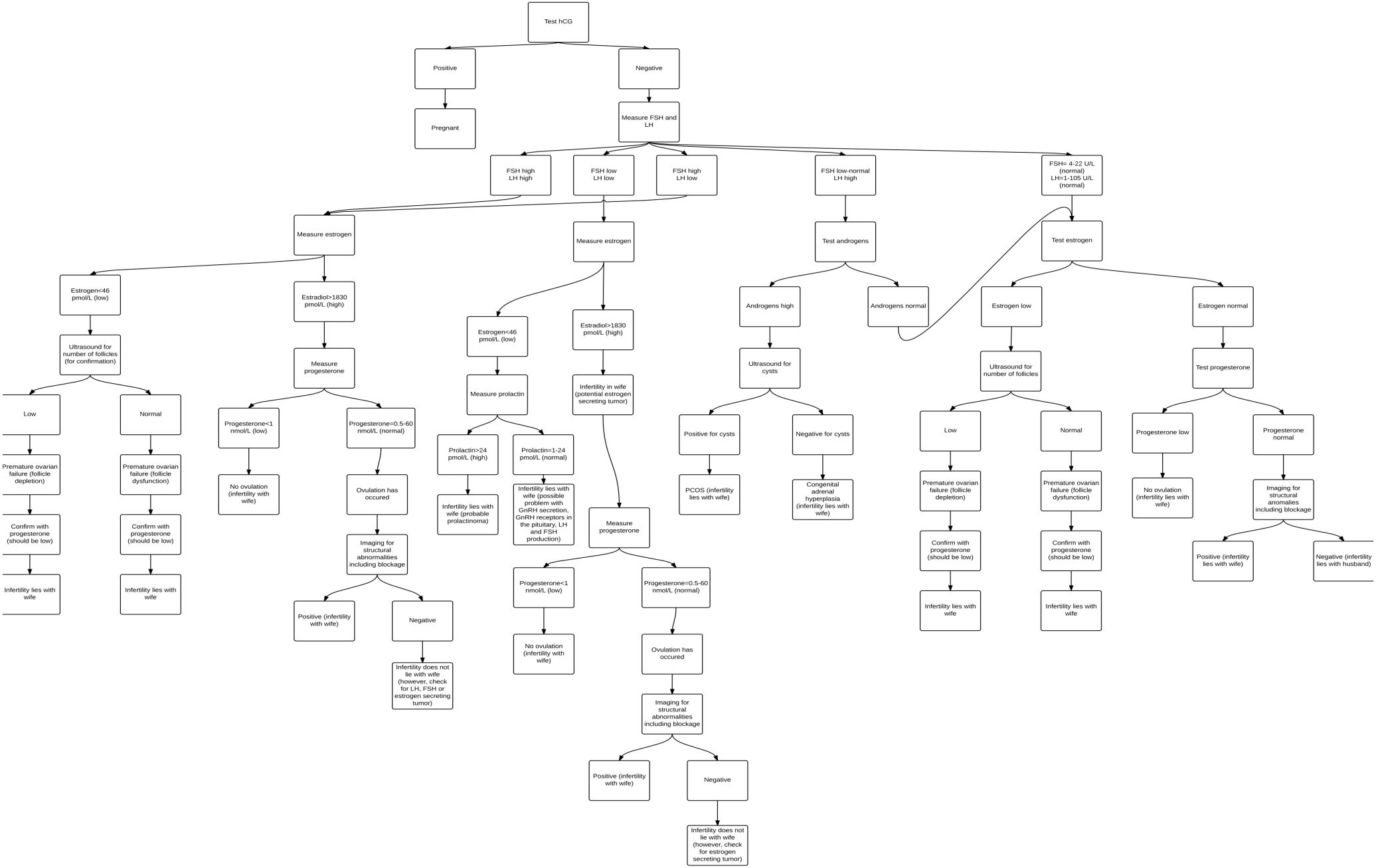
Question 1 [15 marks]

A heterosexual couple, both in their 30's, presents to their family doctor complaining of infertility. They have been having regular, unprotected sexual intercourse for 3 years but have so far been unable to conceive. In taking a detailed history from this couple, the woman states that she experiences what she describes as irregular periods.

Part A) Knowing the hormonal regulation of the menstrual cycle design a biochemical testing strategy which will allow you to determine if the infertility lies with the wife. Describe the tests recommended, how they will be used and what information the possible results would provide. [5 marks]


Test hCG
Positive
Pregnant
Negative
Measure FSH and LH
FSH high LH high
FSH low LH low
FSH high LH low
FSH low-normal LH high
FSH= 4-22 U/L (normal) LH=1-105 U/L (normal)
Measure estrogen
Measure estrogen
Test androgens
Test estrogen
Estrogen<46 pmol/L (low)
Estradiol>1830 pmol/L (high)
Estrogen<46 pmol/L (low)
Estradiol>1830 pmol/L (high)
Androgens high
Androgens normal
Estrogen low
Estrogen normal
Ultrasound for number of follicles (for confirmation)
Measure progesterone
Measure prolactin
Infertility in wife (potential estrogen secreting tumor)
Ultrasound for cysts
Ultrasound for number of follicles
Test progesterone
Low
Normal
Progesterone<1 nmol/L (low)
Progesterone=0.5-60 nmol/L (normal)
Prolactin>24 pmol/L (high)
Prolactin=1-24 pmol/L (normal)
Positive for cysts
Negative for cysts
Low
Normal
Progesterone low
Progesterone normal
Premature ovarian failure (follicle depletion)
Premature ovarian failure (follicle dysfunction)
No ovulation (infertility with wife)
Ovulation has occured
Infertility lies with wife (probable prolactinoma)
Infertility lies with wife (possible problem with GnRH secretion, GnRH receptors in the pituitary, LH and FSH production)
Measure progesterone
PCOS (infertility lies with wife)
Congenital adrenal hyperplasia (infertility lies with wife)
Premature ovarian failure (follicle depletion)
Premature ovarian failure (follicle dysfunction)
No ovulation (infertility lies with wife)
Imaging for structural anomalies including blockage
Confirm with progesterone (should be low)
Confirm with progesterone (should be low)
Confirm with progesterone (should be low)
Confirm with progesterone (should be low)
Infertility lies with wife
Infertility lies with wife
Imaging for structural abnormalities including blockage
Progesterone<1 nmol/L (low)
Progesterone=0.5-60 nmol/L (normal)
Infertility lies with wife
Infertility lies with wife
Positive (infertility lies with wife)
Negative (infertility lies with husband)
Positive (infertility with wife)
Negative
No ovulation (infertility with wife)
Ovulation has occured
Infertility does not lie with wife (however, check for LH, FSH or estrogen secreting tumor)
Imaging for structural abnormalities including blockage
Positive (infertility with wife)
Negative
Infertility does not lie with wife (however, check for estrogen secreting tumor)

The first step in an infertility workup is to rule out pregnancy. This is done through a qualitative hCG test, which is an immuno assay that measures hCG, a hormone produced by an early developing fetus. A positive test indicates that the patient is pregnant, eliminating the need to further investigate infertility.

If the hCG test is negative levels of FSH and LH are then tested. Either a radioimmunoassay, or a chemiluminescence assay is used to look directly at serum FSH and LH levels. FSH and LH are both hormones released by the pituitary gland in response to GnRH from the hypothalamus. They control the female menstrual cycle by controlling follicular maturation and subsequent estrogen release with respect to FSH, and ovulation and subsequent progesterone release with respect to LH. Anomalies in the levels of either hormone can contribute to abnormal menstrual cycles and female infertility.

High FSH and LH

If LH and FSH are high, the cause is usually low estrogen levels. Estrogen normally affects FSH and LH in a negative feedback loop. Therefore, low estrogen levels would cause an increase in FSH and LH in an attempt to increase estrogen. If estrogen, measured through a radioimmuno assay, is low, then the cause of infertility is most likely premature ovarian failure. Premature ovarian failure is caused by either follicular depletion or dysfunction. In follicular depletion, the patient simply does not have enough follicles left for continued menses. Estrogen levels fall due to the lack of mature follicles, causing a subsequent rise in LH and FSH. The irregular periods and infertility are caused by the lack of follicles. To confirm, an ultrasound is performed to look at the number of follicles, which should be low. Follicular dysfunction occurs when follicles do not respond to FSH and therefore do not mature. In this case, an ultrasound would show normal follicle levels, and a progesterone test, which should show a low progesterone level, would be used to confirm premature ovarian failure. In both cases, infertility would lie with the wife.

High LH and FSH normally cause an increase in estrogen levels, therefore a high estrogen is the normal response. In order to further investigate female infertility, progesterone levels would be tested. Progesterone is normally released after ovulation by the ova. This means that low progesterone levels would indicate that ovulation has not occurred. If ovulation does not occur, infertility lies with wife. A normal progesterone level would mean that ovulation has occurred. The next step would be to check for structural anomalies, such as a fallopian tube blockage via ultrasound. If the ultrasound comes back positive, the infertility is caused by the blockage. If there are no structural problems, the patient is most likely fertile, however it would be prudent to check for tumours that may be causing the high FSH, LH and estrogen levels.

Low FSH and LH

Estrogen is still measured in case of low FSH and LH. Increasing levels of estrogen cause a decrease in LH and FSH through a negative feedback mechanism, so the cause of the low FSH and LH may be increased estrogen. If estrogen is high, progesterone would be measured for the same reasons as in the case of high FSH and LH. An ultrasound would also be done if progesterone is normal. In the absence of ultrasound abnormalities, it is most likely that the patient is fertile, though it would be prudent to check for an estrogen secreting tumor.

If estrogen is low, there is a chance that FSH, LH and estrogen production is being suppressed. This may happen in the case of a prolactin secreting pituitary tumor. Prolactin is a hormone that is released during pregnancy, and is used to control milk production. Prolactin also has the ability to suppress FSH, LH and estrogen, potentially causing infertility due to the low FSH and LH levels. The next step would be to measure prolactin levels, and if high, investigate a prolactinoma. If a prolactinoma is found, infertility lies with the wife. In the case of a normal prolactin, it is prudent to investigate GnRH levels, genetic abnormalities in GnRH pituitary receptors, and LH and FSH production. If all investigations return negative, infertility does not lie with the wife.

High FSH and low LH

High FSH and low LH are normally caused by the same factors as high LH and high FSH. Therefore, the same investigations will be used.

Low-normal FSH and high LH

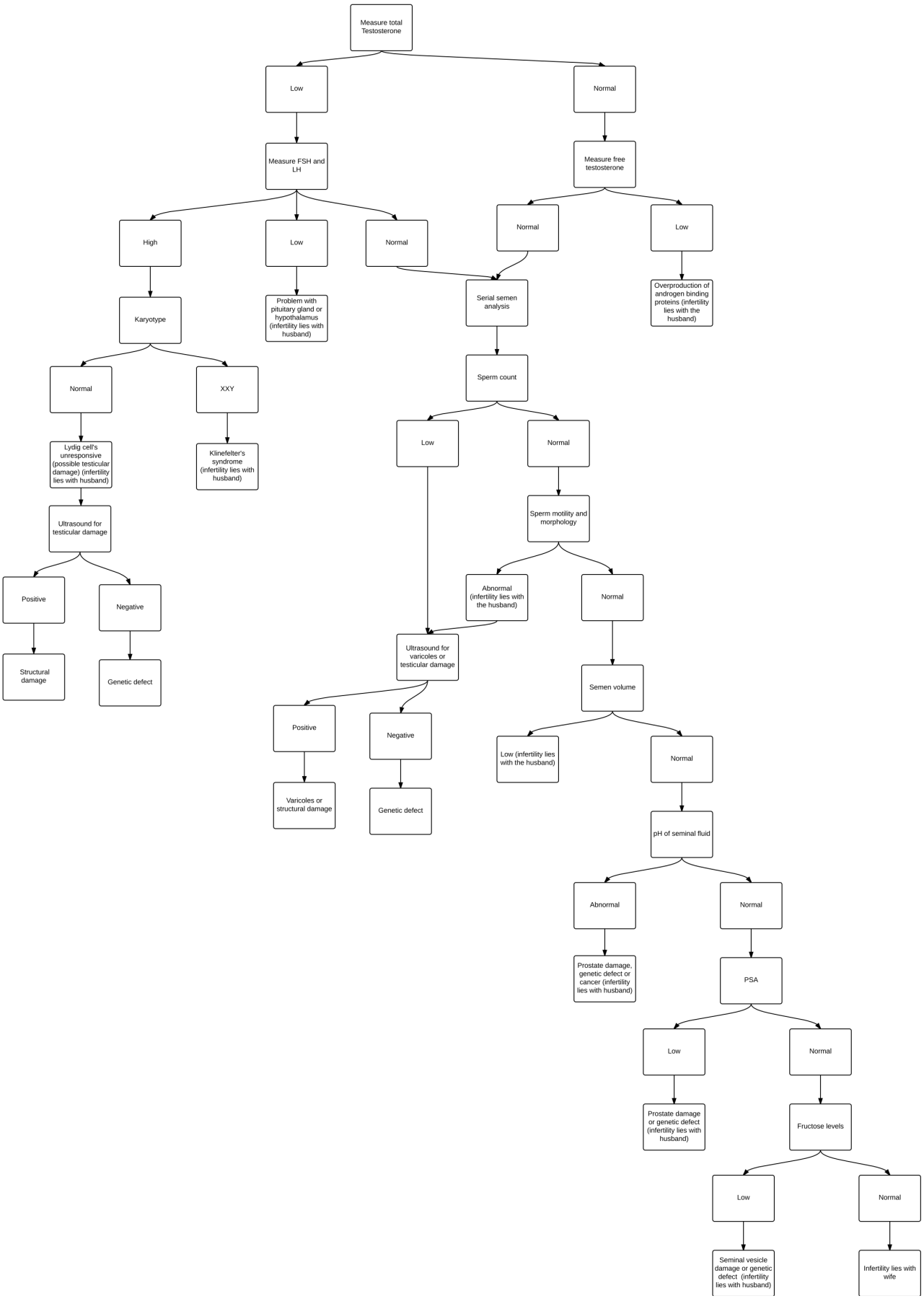
Low-normal FSH and high LH are indicative of an androgen secreting disorder, which may cause infertility. There are two disorders that may cause high androgens, congenital adrenal hyperplasia (CAH), and polycystic ovarian syndrome (PCOS). CAH is caused by a defect in one of two enzymes involved in making corticosteroids. Corticosteroids and androgens use the same precursor. If a corticosteroid producing enzyme is not functioning, precursors will be shifted to the androgen production pathway, causing an over production of androgens that may interfere with female fertility. PCOS is caused by high LH or insulin levels. This causes cysts and an increased production of androgens from the ovaries, resulting in the same interference with female fertility. The first step is to measure androgen levels. If androgen levels are high, an ultrasound should be done to check for possible ovarian cysts. If cysts are found, the patient is diagnosed with PCOS. If there are no cysts and the ovary is normal, CAH is the cause of the increased androgens. In both cases, infertility lies with the wife.

In the case of normal androgen levels, see normal FSH and LH.

Normal FSH and LH

If FSH and LH are normal, it may still be prudent to look at estrogen levels. If estrogen levels are low, there may be a congenital defect in the ovaries resulting in no response to FSH and LH, which would cause infertility. If estrogen levels are normal, progesterone levels should be checked to make sure ovulation occurs. A low progesterone level will indicate that ovulation has not occurred, and there may be an issue in the steps leading up to ovulation. In this case, infertility still lies with the wife. A normal progesterone level would be followed up with an ultrasound to check for structural defects. If structural defects are found, the infertility lies with the wife. Otherwise, the patient is fertile.

Part B) How would you investigate male causes of infertility? What are the major causes of male infertility and what tests would be of use? Design a biochemical testing strategy to investigate the underlying causes of male infertility and provide an interpretive guide for the possible results. [4 marks]



The most important factor in male infertility is testosterone. It is also the first test done in a work up of male infertility. Testosterone is essential to normal sperm and semen development. If low, FSH and LH are measured due to their ability to control testosterone production. FSH and LH levels may be high, normal, or low. A clinical presentation of low testosterone, high FSH and high LH may be indicative of Klinefelter's syndrome. This is caused by an abnormal karyotype, in which the male has two X chromosomes along with a Y chromosome. Klinefelter's syndrome leads to low testosterone levels and the several developmental issues that arise with it. If the karyotype is normal, it may be that the leydig cells of the patient are unresponsive to FSH and LH. Leydig cells are responsible for testosterone production in males, and an unresponsive leydig cell will not produce testosterone. In both cases, the infertility lies with the husband. If the leydig cells are unresponsive however, it is possible that it is caused by either structural damage or a genetic defect. An ultrasound can be done to rule out structural damage.

If total testosterone is normal, there could be several other underlying conditions that could cause male infertility. The first thing done is to measure free testosterone. If free testosterone is low, the infertility is caused by an over production of androgen binding proteins (ABP). ABPs bind androgens and inactivate them, while free androgens are active. An overproduction of ABP reduces the amount of free testosterone available, causing infertility.

If free testosterone is normal, or total testosterone is low but FSH and LH are normal, a serial semen analysis is performed. Multiple semen samples are collected and different characteristics are analyzed. The easiest characteristic of semen to analyze is sperm count. If sperm count is low, this is most likely the cause of infertility. An ultrasound is performed to check for varicoceles (a disorder where dilated blood vessels heat the testicles to a higher than usual temperature) or structural damage. If structural damage or varicoceles is found, this is the cause of infertility. If not, genetic counselling should be sought.

If sperm count is normal, other characteristics of sperm should be taken into consideration, namely motility and morphology. If these are found to be abnormal, an ultrasound should be done to look for the same conclusions considered when looking for the underlying cause of low sperm count. Abnormal motility and morphology would be the cause of male infertility.

If sperm count, motility and morphology are all normal, the next step would be to look at semen volume. A low semen volume may be the cause of male infertility. If semen volume is normal, then the pH of the semen is checked. pH of semen is regulated by the prostate, and is kept at a slightly alkaline level. Abnormal seminal pH may be caused by prostate damage, genetic defects or prostate cancer. An ultrasound should be done to investigate prostate damage or cancer. If the ultrasound comes back normal, the cause of male infertility is likely genetic.

Should the pH be normal as well, the next thing to look at would be prostate specific antigens. This enzyme releases the sperm from their protein prisons upon activation in a neutral pH (caused by the neutralization of semen in the acidic environment of the vagina). Low PSA levels would mean that upon entry to the vagina, the sperm may not be released, contributing to infertility. Again, an ultrasound is done to rule out prostate damage or cancer. If negative, a genetic defect may be contributing to the infertility.

The last thing to look for, if everything else is normal, is the fructose concentration in the semen. Fructose is the molecule of choice for energy production by the sperm. Low fructose levels would mean that the sperm do not have enough energy for sustained movement. As the seminal vesicles secrete fructose into the semen, seminal vesical damage may be the cause of low fructose levels and infertility. An ultrasound is performed to check for damage. Otherwise, genetic counselling should be sought. If fructose levels, and everything else is normal, infertility does not lie with the husband.

Part C) During the consultation, the woman mentions that she has experienced the growth of dark hair on her face. What are the 2 most common conditions that could cause this? Could this be contributing to infertility? Provide the hormone concentrations that would be expected in each case (list all relevant hormones and the expected results for each condition). [6 marks]

The 2 most common conditions that could cause growth of dark hair on a female's face are congenital adrenal hyperplasia (CAH) and polycystic ovarian syndrome (PCOS). Both disorders are characterized by increased androgen levels. Androgens can cause the appearance of masculine features in women, such as growth of dark hair. Both these disorders can contribute to infertility as they are associated with a high LH level. If the LH levels are consistently high, an LH surge cannot happen, meaning that ovulation will not occur. In addition, in CAH, there may be overproduction of progesterones which may also inhibit the ovulation cycle of the female.

Hormone levels

Hormone	Normal values	PCOS values	CAH values
Lutenizing hormone (LH)	Follicular phase: 5-20 IU/L	High	High
Follicle stimulating hormone (FSH)	Follicular phase: 5-20 IU/L	Normal	Normal
LH/FSH ratio	Follicular phase: 1	>1	>1
Testosterone	Total: 0.60-8.6 ng/ml Free: 0.0007-0.0036 ng/mL	Total: elevated Free: elevated	Total: elevated Free: elevated
Dehydroepiandrosterone (DHEA-S)	35-430 ug/dL	High-normal to high	High
Progesterone	If ovulation has occurred: >14 ng/mL If ovulation has not occurred: <14 ng/mL	Normal	High
Estrogen	25-75 pg/mL	Normal	High
Aldosterone	Recumbent: 0.2-0.9 ng/mL	Normal	Low
17-hydroxyprogesterone	<100 ng/mL	Normal	High
Cortisol	Morning: 6-23 ug/dL Evening: 0-9 ug/dL	Normal	Low

Question 2 [10 marks]

A 30 year old woman is visiting you, her family doctor, because she has recently discovered she is pregnant based on the result of a home pregnancy test.

Part A) How does a home pregnancy test work and what are its limitations for detection of pregnancy? How would you confirm this woman is pregnant? [5 mark]

A home pregnancy test works by detecting human chorionic gonadotropin (hCG), a hormone released from the trophoblastic cells in early pregnancy. First, the patient urinates on one side of the home pregnancy test. The urine flows from one side of the stick to the other, bringing with it free floating, gold conjugated anti-hCG antibodies. If there is hCG in the urine, these antibodies will bind to it. As the urine progresses down the stick, it will encounter another set of anti-hCG antibodies, which are immobilized. These antibodies bind a different epitope on hCG than the free floating gold conjugated antibodies. If hCG is present, it will bind to both the free floating antibodies, and the immobilized antibodies. The immobilized antibodies are packed densely enough that when hCG, already bound to the gold conjugated antibody, binds, a coloured band is visible to the naked eye. This band is the test band and only appears if hCG is present. Downstream of the immobilized anti-hCG antibodies are immobilized anti-mouse antibodies, design to bind to free floating anti-hCG. Any anti-hCG not immobilized by the second anti-hCG antibodies will flow down the stick and become immobilized by these anti-mouse antibodies. A second band will form, which is the control band, indicating that the test has functioned properly.

There are a couple of limitations to this test. False positives may be caused by several disorders. If a miscarriage has occurred before the day of the first period, when the test is taken, hCG levels may still be elevated though the woman is no longer pregnant. Hydatidiform moles may also cause a false positive result. They are caused by implantation of a potentially malignant group of trophoblastic cells, with or without fetal material. These trophoblastic cells secret hCG and will appear as a false positive on a home pregnancy test. Ectopic pregnancy may also cause a false positive. In this case the zygote has implanted in the fallopian tube wall instead of the uterus and is not a true pregnancy. The zygote still releases hCG and will trick a home pregnancy test. A false negative may occur if the test is taken too early, when hCG levels are still too low to detect at home.

To confirm pregnancy, a serial quantitative blood hCG test is performed in order to monitor hCG levels. In cases of miscarriage, hCG levels should initially remain constant and then decline. If this isn't the case, and hCG levels double every 48 hours, an ultrasound should be performed once serum hCG is above 2000 IU/L. The ultrasound will rule out both hydatidiform mole and ectopic pregnancy if a fetus is seen. Checking for high levels of serum estrogen and progesterone may also rule out hydatidiform mole and miscarriage. If hCG levels double every hour and the ultrasound shows a fetus, the woman is pregnant.

Part B) She gives birth at 38 weeks to a baby boy. The baby has a yellowish tinge to his skin during the first 3 days of life and the doctor tells the women that her son is jaundiced. What molecule causes jaundice? Describe the screening and treatment of jaundice in neonates and discuss the consequences of untreated jaundice. [5 marks]

- What causes jaundice?
    - Bilirubin
- Screening
    - Use nomogram to determine baby's risk by taking serum bilirubin levels and history (nomogram takes into account baby's bilirubin levels, age and term of birth (baby was born at term which decreases risk). Check for decreasing levels of bilirubin. Depending on the results of the nomogram, initiate treatment as necessary.
- Treatment

    - If jaundice is very mild, no treatment necessary
    - Phototherapy
        - Non invasive
        - Creates hydrophilic isomers of bilirubin. Allows excretion in urine and feces without need for conjugation by liver.
        - Also prevents bilirubin from crossing the blood brain barrier, reducing risk of bilirubin neurotoxicity.
    - Exchange transfusion
        - If phototherapy is unsuccessful or insufficient
        - Baby's blood is replaced by other blood.
        - Clears jaundice in blood avoiding neurotoxicity
- Consequences
    - Acute bilirubin encephalopathy
        - Can cause kernicterus syndrome
            - Involuntary movement
            - Hearing loss
            - Permanent upward gaze

Question 3 [5 marks]

Describe how cut-points are chosen for diagnostic tests. How can this approach also be used to compare the performance of different tests? [5 marks]

Ideal cut off points are determined by analyzing the sensitivity and specificity of the diagnostic test. Sensitivity is defined as the true positive (TP) rate, or the rate at which the test returns a positive result and the person has the disease. Specificity is the true negative (TN) rate, or the rate at which the test returns a negative result and the person does not have the disease.

TP rates are normally calculated by selecting a large cohort of patients that are known to have the disease (through another, already validated, method). The test is administered to each person, and TP is defined as the proportion of this cohort that gave a positive result. Similarly, TN rates are calculated by selecting a large cohort of healthy patients without the disease. The test is administered to each person, and TN is defined as the proportion of this cohort that returned a negative result.

No test is 100% accurate, and unfortunately both TP and TN rates will be less than 1. The ideal cut off point would maximize both TP and TN rates in order to correctly asses whether a person has the disease or not. In order to find a cut off point that maximizes these values, sensitivity is calculated as a function of specificity from 0%-100% and plotted on a graph called a receiver operating characteristic (ROC) curve. Different cut off points give different sensitivity and specificity values, and so a different ROC curve is formed for each potential cut off point chosen. When sensitivity and specificity are maximized, the curve looks like a perfect arc, and the cut off point that formed the curve is chosen as the ideal cut off point.

ROCs can also be used to compare the performance of different tests. The area under the ROC curve (AUC) is proportional to the performance of the test at the set cut point. Therefore, by comparing the AUCs of two different tests at their ideal cut off point, it is possible to determine which test performs better by selecting the one with the higher AUC.

Question 4 [20 marks, 5 marks each]

Describe the mechanism of action, including effect on water, electrolyte and acid-base homeostasis for the following classes of diuretic drugs:

Briefly describe the **indications for use** for each drug and where possible **relate this to the mechanism of action**.

Carbonic Anhydrase Inhibitors

Indications for use
Metabolic alkalosis with or without hypervolemia.
Glaucoma (check)

Mechanism of action
Carbonic anhydrase (CA) inhibition. CA catalyzes the forward and reverse reactions of $H_2O+CO_2 \rightleftarrows H_2CO_3$. Action in the proximal tubule.

Relationship of mechanism to indication of use
Inhibition of CA prevents the reabsorption of HCO_3^-, which causes excess HCO_3^- excretion, lowering the amount of HCO_3^- in blood. HCO_3^- is a base, and so increased excretion reduces blood pH. CA turns H_2O and CO_2 into H_2CO_3 in the tubule cells, which then dissociates into H^+ and HCO_3^-. H^+ is then secreted into the filtrate in exchange for the reabsorption of sodium. If CA is inhibited, there will be less H^+ to exchange for sodium, decreasing sodium reabsorption and increasing its excretion. Increased sodium in the filtrate reduces the osmotic gradient between the filtrate and interstitial fluid, reducing water reabsorption and increasing urine volume.

Effect on water
Increased excretion.

Effect on electrolyte balance
Increased sodium excretion, increased potassium excretion, increased chloride reabsorption

Effect on acid-base homeostasis
Increased HCO_3^- excretion, decreased HCO_3^- concentrations in the blood, which case a decrease in blood pH.

Loop diuretics
Indications for use
Fluid overload

Mechanism of action
Blocks NKCC2 in the thick ascending limb of the loop of Henle which blocks $Na^+K^+2Cl^-$ reabsorption.

Relationship of mechanism to indication of use
NKCC2 normally allows sodium potassium and 2 chloride reabsorption, diluting the filtrating, and increasing the osmotic gradient between the filtrate and the interstitial fluid, so that when the filtrate reaches the collecting duct, which contains aquaporins, enough water will be reabsorbed. Blocking NKCC2 prevents sodium potassium and 2 chlorides from being reabsorbed. The filtrate will be more concentrated than it would be, reducing the concentration gradient between the filtrate and the interstitial fluid, reducing the amount of water reabsorbed in the collecting duct, increasing the amount of water excreted. Increasing water excretion reduces the amount of water in the blood, reducing blood volume and blood pressure, allowing control of hypertension.

Effect on water

Increased excretion.

Effect on electrolyte balance
Increased sodium excretion, increased potassium excretion, increased chloride excretion

Effect on acid base balance
Loop diuretics can cause metabolic alkalosis through 3 different mechanisms. The first is hypokalemia. Loop diuretics block NKCC2, which is involved in potassium reabsorption. Potassium secretion is also increased due to an increased attempt at sodium reabsorption by the collecting duct. The increased potassium excretion can cause hypokalemia. Hypokalemia causes intracellular potassium to shift out of the cell into the interstitial fluid. To balance the loss of positive charges, the cell brings in hydrogen ions. This loss of hydrogen ions from the interstitial fluid to the cell causes intracellular acidosis and metabolic alkalosis due to the decrease in ECF hydrogen concentrations.

Loop diuretics also cause increased hydrogen ion secretion by the collecting duct. The kidney tries to increase sodium reabsorption as a reaction to the loop diuretic. One method to increase sodium reabsorption is to increase the number of sodium hydrogen exchangers in the collecting duct. Increasing the number of sodium hydrogen exchangers increases the amount of hydrogen excretion, causing metabolic alkalosis.

The third method is called contraction alkalosis. Bicarbonate ion amounts stay relatively constant in the body. Therefore, fluid loss, caused by the diuretic, with constant bicarbonate amounts would result in an increased bicarbonate concentration, causing metabolic alkalosis.

Thiazide diuretics
Indications for use
Hypertension, fluid overload

Mechanism of action
Blocks Na^+/Cl^- symporter in distal convoluted tubule.

Relationship of mechanism to indication of use
Na^+/Cl^- normally allows sodium and chloride reabsorption, diluting the filtrating, and increasing the osmotic gradient between the filtrate and the interstitial fluid, so that when the filtrate reaches the collecting duct, which contains aquaporins, enough water will be reabsorbed. Blocking Na^+/Cl^- prevents sodium and chloride from being reabsorbed. The filtrate will be more concentrated than it would be, reducing the concentration gradient between the filtrate and the interstitial fluid, reducing the amount of water reabsorbed in the collecting duct, increasing the amount of water excreted. Increasing water excretion reduces the amount of water in the blood, reducing blood volume and blood pressure, allowing control of hypertension. This diuretic is less potent than loop diuretics as Na^+/H^+ exchanger and Cl^-/HCO_3^- exchanger can also reabsorb NaCl. These transporters are not inhibited and so sodium excretion is less than it could be.

Effect on water
Increased excretion.

Effect on electrolyte balance
Increased sodium excretion, increased chloride excretion and increased potassium excretion

Effect on acid base balance
Thiazide diuretics can cause metabolic alkalosis through 3 different mechanisms. The first is hypokalemia. Thiazide diuretics can cause hypokalemia by increasing attempted sodium reabsorption, and therefore potassium excretion, by the collecting duct. Hypokalemia causes intracellular potassium to shift out of the cell into the interstitial fluid. To balance the loss of positive charges, the cell brings in hydrogen ions. This loss of hydrogen ions from the interstitial fluid to the cell causes intracellular acidosis and metabolic alkalosis due to the decrease in ECF hydrogen concentrations.

Thiazide diuretics also cause increased hydrogen ion secretion by the collecting duct. The kidney tries to increase sodium reabsorption as a reaction to the loop diuretic. One method to increase sodium reabsorption is to increase the number of sodium hydrogen exchangers in the collecting duct. Increasing the number of sodium hydrogen exchangers increases the amount of hydrogen excretion, causing metabolic alkalosis.

The third method is contraction alkalosis. Bicarbonate ion amounts stay relatively constant in the body. Therefore, fluid loss, caused by the diuretic, with constant bicarbonate amounts would result in an increased bicarbonate concentration, causing metabolic alkalosis.

Potassium sparing diuretics

Indications for use

Paired with another type of diuretic when there is a risk of hypokalemia.

Mechanism of action

Amiloride-like potassium sparing diuretics

Blocks epithelial sodium channels (ENaC) on the apical side of the collecting duct.

Spiranolactone-like potassium sparing diuretics

Blocks intracellular mineralocorticoid receptors.

Relationship of mechanism to indication of use

Amiloride-like potassium sparing diuretics

Normally, sodium travels from the filtrate into the collecting duct cells through ENaC. The increased intracellular sodium concentrations increase the activity of Na^+/K^+ ATPase on the basolateral side. This increases sodium excretion into the interstitial fluid. The consequence of this increased Na^+/K^+ ATPase activity is increased potassium influx into the cell from the interstitium, and subsequent loss into the filtrate. Amiloride-like potassium sparing diuretics block ENaC, preventing sodium from entering the collecting duct cell. This prevents the increase in Na^+/K^+ ATPase activity, which prevents the increased K^+ loss.

Spironolactone-like potassium sparing diuretics

Normally, mineralocorticoids function to increase expression of apical levels of ENaC and basolateral levels of Na^+/K^+ ATPase. This increases sodium reabsorption and potassium loss. Spironolactone blocks intracellular mineralocorticoid receptors and prevents the increased ENaC and Na^+/K^+ ATPase expression. This reduces potassium loss and increases sodium excretion.

Effect on water

Slightly increased excretion.

Effect on electrolyte balance

Slightly increased sodium excretion. Reduced potassium excretion.

Effect on acid base balance

Both amiloride-like and spironolactone-like potassium sparing diuretics cause metabolic acidosis. The mechanism by which this occurs is by directly blocking sodium reabsorption in the collecting duct. Sodium is normally reabsorbed through either sodium potassium ATPase or sodium hydrogen exchanger, which reabsorbs a sodium ion and secretes a hydrogen ion. Blocking sodium reabsorption prevents the secretion of these hydrogen ions. This causes a build up of hydrogen ions in the body, causing metabolic acidosis.

Question 5 [10 marks]

Imagine a new hormone is discovered. Patients experience disease both in the case of too much and too little hormone. Provide 5 important considerations for establishing clinically usable laboratory test(s). [10 marks]

- What type of hormone it is?
  There are several types of hormones; protein, peptide, steroid, thyroid and catecholamine. The type of hormone indicates whether it is water soluble or not. Steroid hormones for example, are not water soluble. This affects many characteristics that should be taken into account when making a clinically useable diagnostic test. For example, lipid soluble hormones frequently require a protein chaperone while in the blood stream. The amount of binding protein produced may increase or decrease the amount of measurable hormone. If a hormone is very highly protein bound, it will be much harder to measure. Protein binding may also determine whether or not the test should measure total hormone levels or free hormone levels.
  The type of hormone involved may also determine what it is being measured in. In most cases, a blood test would be fine to measure hormone levels, but they are invasive. Small, water soluble molecules can be excreted into the urine, providing a much less invasive way to measure hormone levels.

- Variability in hormone levels through a period of time
  Some hormones are excreted at different levels during different times of the day. Melatonin levels, for example, are almost undetectable during the day, and are elevated at night. Taking this variability into account is important in designing a clinically usable lab test, as it is necessary to ensure the test is done at the proper time.

- Inter-assay, intra-assay, and inter-operator variability
  Intra-assay variability is the variability in the results obtained when two samples with the same concentration are measured on the same assay. Inter-assay variability is the same, but the samples are measured on two different assays, while inter-operator variability is the variability in results of assays completed by two separate people. Some things that can increase variability are pipetting technique and number of steps involved in the assay. A clinically useable lab test should be designed to minimize these types of variability, as several samples are going to be measured at the same time, on different days, and by several different people. High variability will cause a high error rate and decrease the sensitivity and specificity of the test.

- Sensitivity and specificity
  A good, clinically useable test should have good specificity and sensitivity. Specificity is the rate at which the test positively identifies people with the issue, while sensitivity is the rate at which the test properly identifies people without the issue. Specificity is important in order to reduce the number of false positive results to prevent misdiagnosis and unneeded treatment, while sensitivity is important in order to reduce the number of false negatives to prevent missed diagnosis.

- Cost
  There are several thousand lab tests performed each and every day. All these tests cost money, and if the test employs methods that cost too much, many medical centers will not be able to afford them, and the test will not be used.

Question 6 [6 marks]

A 25-year old female sought treatment for her constant fatigue, lethargy, and depression. She was small in stature and had previously been diagnosed with attention-deficit disorder. On physical examination she was found to have an enlarged thyroid gland (goiter). Blood tests revealed elevated levels of T3, free T4, and TSH, yet she did not exhibit symptoms of hyperthyroidism. What could explain her symptoms?

The patient has generalized thyroid hormone resistance. Generalized thyroid hormone resistance is caused by a mutation in the thyroid hormone nuclear receptor, both in the periphery, the hypothalamus and the pituitary gland. Her symptoms resemble those of hypothyroidism, and are caused by the inability of the thyroid hormones to bind to their peripheral receptors and increase metabolism. This is why she is presenting with depression, fatigue and lethargy. Physiologically, TRH from the hypothalamus stimulates TSH production by the pituitary, which in turn stimulates production of T3 and T4 by the thyroid. T3 and T4 work on the pituitary gland and the hypothalamus to suppress TSH and TRH production in a negative feedback mechanism. In this case, the pituitary and hypothalamic thyroid hormone receptors are non-functional. The negative inhibition doesn't work and both TRH and TSH levels become elevated. The thyroid, responding to the increased TSH, increases the production of thyroid hormones beyond what is physiologically normal, and a goiter forms from overproduction of T3 and T4. While the patient presents with symptoms representative of hypothyroidism, lab results show increased TSH, T3 and T4, which is rather unusual in other thyroid disorders. The ADD was likely misdiagnosed, as generalized thyroid hormone resistance is extremely rare and presents with symptoms similar to ADD. This mechanism explains how elevated TSH, T3 and T4 can present with symptoms of hypothyroidism.

Question 7 [4 marks]

Briefly explain 3 mechanism of goiter formation.

A goiter is an enlarged thyroid gland, which can be caused by many different things.

- Hashimoto's thyroiditis
  Hashimoto's thyroiditis is an autoimmune disease. Auto-antibodies are formed against thyroid tissue. These antibodies act as cytokines and chemokines, recruiting inflammatory cells into the thyroid. These cells end up causing inflammation of the thyroid, called thyroiditis, and it is this inflammation that causes enlargement of the thyroid, and therefore the goiter.
- Grave's disease
  Grave's disease is another autoimmune disease that can cause a goiter. In this case, the antibodies formed are able to stimulate TSH receptors on the thyroid. TSH is normally released by the pituitary and causes thyroid hormone (T3 and T4) production to increase. TSH also changes the structure of the thyroid, and can cause it to enlarge in an effort to produce more thyroid hormones. The autoantibodies constantly stimulate the TSH receptors on the thyroid gland and cause the thyroid to respond as it would to high TSH levels. The thyroid enlarges in an effort to increase thyroid hormone production, producing a goiter.
- Iodine deficiency
  Iodine is absolutely essential in the production of thyroid hormones, and dietary iodine is a major source. Iodine deficiency decreases the amount of T3 and T4 formed. T3 and T4 normally inhibit TSH release in a negative feed back loop, and so low T3 and T4 cause an increase in TSH. An increase in TSH changes the structure of the thyroid gland in an attempt to make more thyroid hormone, producing a goiter.

Question 8 [10 marks]

A 59 yr male presented to the ED with severe eipgastric pain. He has had similar pain on-and-off since he was 18 yrs, but it had worsened significantly in the past few days sending him the ED.

Clinical History: As a boy, he was healthy on a relatively low fat diet, however at 18 yrs he became a merchant sailor and began to experience abdominal pain. He described his diet as high in fat. At a routine check-up at 24yrs, his physician noted that his serum was "milky white" but his cholesterol was only 5.6 mmol/L (reference interval, <5.2 mmol/L). He continued to have epigastric pain. At 48 he was admitted to hospital for abdominal pain. His serum was lipemic with a cholesterol of 15.3 mmol/L and triglycerides of 106 mmol/L (reference interval <1.7 mmol/L). Further testing was done, including a **post-heparin lipolytic activity** measurement which was found to be absent (i.e. very low). The patient is a bachelor. His parents were second cousins. His siblings have normal or slightly elevated lipids, but do not have similar clinical complaints. Over the course of his hospitalization, his hemoglobin dropped and the patient was transfused with 2 units of whole blood. Interestingly, within 2 days of the transfusion, his triglycerides returned to normal and slowly increase back to their previous concentrations over the next 15 days.

1. What is this patient's Fredrickson classification (type I, IIa, IIb, III, IV, or V) and what lipid fraction(s) would you expected to be elevated? [2 marks]
    - Type 1, chylomicrons
2. What is the cause of his epigastric (abdominal) pain and what is the underlying cause? [2 marks]
    - Pancreatitis
    - Chylomicrons obstruct capillaries in the pancreas. This causes ischemia, which exposes triglycerides to pancreatic lipase. Pancreatic lipase is now exposed to triglycerides, converting them to free fatty acids. Free fatty acids can be cytotoxic, causing the release of inflammatory mediators and free radicals. This manifests itself as pancreatitis which is the cause of his epigastric pain.
3. Explain how the post-heparin lipolytic activity assay works and what it is being measuring. [3 marks]
    - Heparin allows the release of LPL from epithelial cells. Heparin is given to the patient, and blood is collected. To do the assay, the patient's plasma sample, previously isolated from the blood collected, is added to an artificial fat emulsion containing triglycerides. Lipoprotein lipase activity is measured as a function of glycerol, a breakdown product of triglyceride. It is important to note that albumin is also added to the assay to bind free fatty acids formed, as unbound FFAs can inhibit LPL activity.
4. Why did the tryglycerides drop after the blood transfusion? Explain the mechanism and identify the cause of his lipid abnormalities. [3 marks]
    - Triglyceride levels dropped after the blood transfusion as the donor's blood had functioning LPL that allowed for the metabolism of the patient's triglycerides. The cause of the patient's lipid abnormalities was a loss of function mutation in his LPL. Non-functioning LPL prevented the triglycerides in the chylomicrons, and therefore the chylomicrons, from being broken down, causing hypertriglyceridemia and subsequent hyperchylomicronemia.