

Presentation, Diagnosis and Management of Incomplete Removal of Ovarian Tissue in the Bitch and Queen

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INTRODUCTION

Incomplete ovariectomy (IO) is the unintentional partial or complete lack of removal of one or both ovaries during an ovariohysterectomy or ovariectomy procedure. The condition is often referred to as 'ovarian remnant syndrome'. IO is not uncommon, being reported in up to 1 in 900 ovariohysterectomy and 1 in 200 ovariectomy procedures in bitches ([Muraro and White, 2014](#); [Pope and Knowles 2014](#)); data are not available for the queen.

Most commonly IO has a clear clinical presentation although there are a number of other conditions that may have similar presenting signs. [In the bitch and queen these include](#); non-oestrous mounting behaviour, non-oestrous vulval discharge, and solely in the bitch; sexual interest from males and iatrogenic pseudopregnancy.

The purpose of this article is to consider the causes, presentation, methods of diagnosis and management of IO in bitches and queens.

BACKGROUND

It is common practice for surgical neutering of bitches and queens to be undertaken via [coeliotomy](#) or laparoscopy, and for either an ovariohysterectomy or ovariectomy to be

performed. The ovary is fully enclosed within a bursa which, together with the ovarian pedicle, may contain a substantial amount of fat making visualisation and palpation of the ovary difficult. Exteriorisation of the ovary at laparotomy may be challenging in deep-chested animals, and traction on the ovarian pedicle is painful and may result in panting which increases the difficulty of the procedure.

In most cases of IO it is the entire right ovary that has not been removed (probably because this is more cranial in position) although less frequently the left ovary or a portion of either or both is not excised. Although a left flank approach remains widely performed in the queen there is no increased frequency for a specific ovary to be incompletely removed when the surgery is performed via this approach. It is theoretically possible for a portion of ovary to fall into the abdominal cavity and become re-vascularised, but this [has never been reported](#) and is unlikely since the ovary is fully enclosed within a bursa. Many females have para-ovarian cysts present along the ovarian pedicle and broad ligament ([McEntee 1990](#)). These are endocrinologically inactive and are not associated with any clinical signs even if they are not removed at the time of surgical neutering.

IO results from inaccurate placement of surgical clamps or ligatures often during a demanding procedure. [This may occur when using the '3 forceps technique' because of limited space to place each forceps.](#) The [most](#) significant error is [failure to closely inspect the pedicle sites after ligation and transection, and](#) not examining all excised tissue at the time of surgery before closure of the incision.

Leaving an ovarian remnant is not necessarily negligent but an owner can reasonably expect not to pay for any investigation or remedial treatment when IO is confirmed. If in doubt about liability, it is wise to contact your Professional Indemnity Insurers for advice.

CASE PRESENTATION

There appear to be three outcomes when ovarian tissue has not been completely removed which relate to the amount of tissue left behind and its location in relation to any ligatures that have been placed.

1. Oestrous behaviour occurs at the normal interval with subsequent normal cyclicity

In these cases [the authors' experience is that](#) often an entire ovary has been left in situ and ligatures have been placed distal to the ovary. The ovary therefore retains its blood supply and functions normally such that there is follicular development, an associated rise in plasma oestrogen concentration, and the female returns to oestrus at the expected time. Oestrous behaviour and vulval swelling is typical of a normal cycle. In bitches [that have undergone](#) ovariectomy a vulval discharge may occur and be sero-sanguineous (erythrocytes are present in the discharge as a result of diapedesis through the remaining uterus), or sero-mucoid in bitches subject to ovariohysterectomy.

In most bitches there is normal ovulation so that progesterone concentrations increase resulting in cessation of the oestrous behaviour. The corpora lutea that develop have a typical 65 day life-span. Towards the middle of the luteal phase as progesterone concentrations start to decrease, plasma prolactin concentrations increase and the bitch may develop signs of pseudopregnancy. Subsequently these bitches continue to cycle at normal intervals.

In queens, ovulation does not normally occur spontaneously, and at the end of oestrus follicles regress and there is a period of inter-oestrus lasting approximately 2 weeks before a subsequent return to oestrus. Most queens with this presentation typically cycle regularly approximately every 3 weeks from spring to autumn and there may be a period of winter anoestrus.

In both bitches and queens if uterine tissue has not been removed it is possible for a later presentation to be associated with uterine disease; commonly pyometra, which has a typical clinical appearance.

2. Oestrous behaviour is absent for several months to years and is then normal, weak or persistent and may or may not be followed by normal cyclicity

These cases are not common and the authors' experience is that in most the ligature has been placed proximal to the ovary, and the ovary or some portion of it has been left during the clamping and transection procedure. As there is an occluded blood supply the ovary becomes ischaemic and inactive and there is no initial return to oestrous behaviour. Ovarian feedback to the hypothalamus and pituitary gland is removed and plasma concentrations of the gonadotrophins LH and FSH increase. At some later time anastomosis of the ovarian vascular supply occurs, and in face of elevated gonadotrophin concentrations follicles develop. The number of follicles will depend upon the volume of tissue that remains and time taken for re-vascularisation. If an entire ovary remains and re-vascularisation is rapid there may be a normal oestrus, ovulation (in the bitch) and subsequent normal cyclicity. If however only a small portion of ovary remains and re-vascularisation occurs later, there may be weak signs of oestrus, or a lack of ovulation resulting in weak persistent oestrous behaviour.

In both bitches and queens if uterine tissue has not been removed later presentation may be associated with uterine disease such as pyometra as noted previously.

3. Oestrous behaviour is absent for many years until persistent weak behaviour associated with a granulosa cell tumour is detected

The aetiology of these rare cases is unknown however the authors presume that ligation has occurred proximal to the non-removed tissue, such that the ovary becomes inactive and plasma concentrations of FSH and LH increase as described above. Although there is some re-vascularisation the ovary remains inactive but ultimately develops a granulosa cell tumour which produces oestrogen resulting in weak signs of oestrus and then other clinical signs such as a mass effect. It is plausible that the persistent elevation of FSH and LH are responsible for the development of ovarian neoplasia as has been proposed in women (Choi and others 2007).

In clinical practice regardless of the aetiology in the particular case, the features reported in both bitches and queens include; apparent oestrous behaviour, and presence of vulval swelling. In the bitch there may also be a vulval discharge (which in the case of an ovariectomy may be sero-sanguineous), attractiveness of males, and the presence of a pseudopregnancy (which must follow oestrus that may, or may not, have been observed by the owner).

DIAGNOSTIC TESTS

There are a number of diagnostic tests which may be employed to investigate the presence of IO. For each it is important to understand the basis of the test so that it can be applied in the most appropriate situation.

1. Observation of oestrous behaviour and swelling of the vulva

Bitches and queens that demonstrate behavioural changes typical of oestrus combined with vulval swelling usually have IO. Diagnosis is simple in the bitch when this is associated with a sero-sanguineous vulval discharge.

Differential diagnoses that produce true signs of oestrus are few (see below) and can be eliminated by clinical history. Cases where signs of oestrus are less pronounced and in bitches when there is no colour to the vulval discharge are more difficult to interpret and may require additional diagnostic tests, for example, vaginal cytology and vaginoscopy.

2. Vaginal cytology and or vaginoscopy

Under the influence of elevated plasma oestrogen concentrations (at the same time that the bitch and queen show oestrous behaviour) there are increased numbers of vaginal epithelial cell layers. The most superficial cells become large because of increased size of the cytoplasm compared with the size of the nucleus. In the bitch the majority of these cells will become anuclear (Figure 1). Large vaginal epithelial cells can be readily detected by the collection of a vaginal smear and staining of cells with a Rowmanowsky stain such as modified Wright–Giemsa stain or a rapid dunking stain. The great advantage of the vaginal cytology technique is that [the appearance alters even with](#) slightly different stages of the cycle. For example; in proestrus there will be 20 - 40% large epithelial cells and a small number of polymorphonuclear leucocytes, in oestrus there will be 40 - 80+% large epithelial cells and an absence of polymorphonuclear leucocytes, and immediately after oestrus there will 0-5% large epithelial cells and increased numbers of polymorphonuclear leucocytes and bacteria. Importantly, vaginal cytology is only useful for the diagnosis of IO during the period when the female has alleged oestrous behaviour, since at other stages of the cycle, there is no difference in the vaginal smear compared with the female that has no ovaries.

Elevated oestrogen concentrations cause changes to the vaginal epithelial that in the bitch can be observed using a rigid endoscope. Non-sedated bitches tolerate the procedure well. In proestrus or oestrus the epithelium appears moist, swollen and oedematous and is often pale in colour (Figure 2); whereas at all other stages of the cycle and in neutered animals the epithelium is dry, flattened and pink in colour.

3. Ultrasound examination

Identification of the ovaries with ultrasound [may be](#) a difficult procedure, but can be performed reliably by an experienced ultrasonographer [using high quality equipment](#).

Throughout proestrus fluid-filled (anechoic) follicles may be detected and these increase in diameter to approximately 5 - 8 mm during oestrus immediately before ovulation (Figure 3).

In the bitch after ovulation each follicle is replaced by a corpora lutea which also has a central fluid-filled cavity and can be identified until approximately 30 days post-ovulation. In queens, follicle regression occurs over 5 – 7 days after oestrus and so fluid filled follicles can be identified during this time. Occasionally a small portion of the uterine horn may be identified adjacent to the ovarian tissue; sometimes this is distended with fluid. [Doppler ultrasound may be helpful in identifying the origin and nature of the vascular supply to tissue suspected of being an ovarian remnant; the ovarian artery arises caudal to the renal artery and is tortuous in appearance.](#)

Clearly, imaging of an ovary during proestrus, oestrus, or shortly after oestrus is diagnostic of IO, although failure to identify ovarian tissue does not preclude IO as imaging can be difficult in an obese animal and if the remaining tissue is small.

4. Measurement of plasma progesterone concentration

In bitches but not queens there is an increase in plasma progesterone concentration immediately prior to ovulation which then continues to rise for the next 10 days. Progesterone is secreted for approximately 65 days after ovulation and since there is no other source of progesterone except the corpora lutea, detection of an elevated concentration (greater than 2.0 ng/ml / 6.5 nmol/l) will be diagnostic of IO. Importantly, progesterone will only be elevated from immediately before ovulation until 65 days after ovulation, and so testing during signs of alleged oestrous behaviour may not necessarily be diagnostic. In these cases since the bitch may have been presented some time before ovulation it is prudent to delay sample collection until a few weeks later when if ovarian tissue is present and ovulation has occurred progesterone will be elevated.

In queens, ovulation does not occur spontaneously and so there is no increase in progesterone unless mating occurs or ovulation is artificially stimulated. The latter can be achieved by administration of GnRH (2.0 µg/kg intramuscularly) or hCG (44 IU/kg intramuscularly) when the queen is in oestrus. Blood samples taken 1 – 2 weeks later will show increased progesterone (greater than 2.0 ng/ml / 6.5 nmol/l) when there is ovarian tissue as it is only the corpora lutea that produce progesterone.

For bitches or queens, progesterone can be measured by ELISA in the practice laboratory or samples can be sent to a diagnostic laboratory. It is feasible that progesterone concentrations may be lower than expected if only a small amount of ovarian tissue remains.

5. Measurement of plasma oestrogen concentration

Oestrogen concentrations are marginally increased for a few weeks before the onset of proestrus and continue to rise as follicles develop. Oestrogen concentrations measured during behavioural oestrus might therefore be expected to be useful for the diagnosis of IO.

However, oestrogen concentrations decrease substantially several days before ovulation in the bitch, and both the bitch and queen continue to demonstrate oestrous behaviour when oestrogen concentrations are low. There is then every possibility that a sample collected whilst there is overt oestrous behaviour will have low oestrogen concentrations, which could give a false negative diagnosis of IO. Furthermore, oestrogen is difficult to assay, plasma concentrations are quite low (pg/ml rather than ng/ml seen with progesterone) and may be influenced by plasma lipid concentration. Whilst elevated plasma oestrogen concentrations (exceeding 12 pg/ml / 45 pmol/l) are diagnostic of IO, low values are not diagnostic of an absence of ovarian tissue.

6. Stimulation of oestrogen production

Whilst basal oestrogen concentrations are often not useful for investigation of cases of presumed IO, it is possible to stimulate ovarian theca cells to release oestrogen by the administration of hCG (which is LH-like in activity) or GnRH (which stimulates an endogenous rise in LH). These stimulation tests work well for bitches and queens that are in anoestrus and the luteal phase, but do not work well when the female is in proestrus or oestrus (when basal oestrogen is, or has just been, elevated). For either test a blood sample is collected prior to drug administration (44 IU/kg hCG intramuscularly or 2.0 µg/kg GnRH intramuscularly. N.B. these are the same doses as required to stimulate ovulation) and 2 hours later. A 2-3 fold increase in oestrogen concentration is diagnostic of IO, although it is feasible that the total amount of oestrogen produced is dependent upon the volume of ovarian tissue remaining. [The authors have observed two cases where oestrogen increase was only 50% of initial values and a partial remnant was found at surgical exploration.](#)

7. Measurement of plasma anti-Mullerian hormone

Anti-Mullerian Hormone (AMH) is produced by granulosa cells from follicles at all stages of development. In many species AMH is detectable throughout the oestrous cycle but it should not be present in ovariectomised females which makes it a candidate test for detecting IO in bitches. To date, one study showed that AMH concentrations for bitches with IO (4.4 ± 1.1 ng/ml) was significantly higher than for bitches with no ovaries (0.3 ± 0.1 ng/ml). Currently though, different assay methods appear to contradict the cut-off for diagnosis of IO in bitches and queens, and most commercial veterinary laboratories do not offer this assay.

8. Measurement of basal LH and/or FSH

As previously mentioned, once all ovarian tissue has been removed there is an absence of ovarian negative feedback on the hypothalamus and pituitary gland such that FSH and LH concentrations increase. There have been few studies of FSH, but LH concentrations in bitches and queens with no ovaries are usually greater than 6.0 ng/ml / 58 IU/l. Whilst this may be the basis of a diagnostic test, it must be appreciated that LH (and FSH) concentrations are elevated in proestrus, oestrus and the luteal phase. Therefore, to have any diagnostic value, LH concentrations should only be measured in anoestrus; essentially limited to more than 65 days after oestrous behaviour in the bitch, or during winter anoestrus in the queen. At this time females with IO will have LH concentrations less than 2.0 ng/ml / 19 IU/l whilst LH will be high in females that have no ovaries.

WHICH TEST SHOULD BE USED WHEN?

This section considers which tests are most appropriate for diagnosis of IO in the bitch based upon the clinical history and initial findings when presented (Tables 1-3). Since queens are

polyoestrus, it is simplest to request presentation when the owner considers that oestrous behaviour has just begun (Table 4).

1. Owner considers that the bitch is currently in oestrus

When presented during alleged oestrus the aims are to determine whether the bitch is in proestrus / oestrus / has just ovulated, and to differentiate those from the bitch that has no ovaries. [In these cases clinical signs and vaginal cytology are especially helpful.](#)

Table 1. Diagnostic test findings in the bitch presented during alleged oestrus.

Bitch presented during a period of alleged oestrus	
Diagnostic Test	Recommendation and positive diagnostic findings for incomplete ovariectomy (IO) at this stage of presentation
Observation of behaviour and clinical examination	<p>Test is diagnostic at this stage of presentation</p> <p>Bitches that have IO and are in proestrus / oestrus / have just ovulated have behavioural changes that differentiate them from bitches with no ovaries and these include features of standing oestrous behaviour and tail deviation. These bitches may allow mating. Clinical examination will also reveal swelling of the vulval and perineal tissue and a vulval discharge (which may be sero-sanguineous if uterine tissue remains).</p>
Vaginal cytology and or vaginoscopy	<p>Tests are diagnostic at this stage of presentation</p> <p>Bitches that have IO and are in proestrus or oestrus have large anuclear vaginal epithelial cells in the vaginal smear and usually low numbers of polymorphonuclear leucocytes; the percentage of both cell types depends upon the exact time of presentation.</p> <p>Vaginoscopy will reveal a moist, swollen and oedematous epithelium that is pale in colour.</p>
Ultrasound examination	Test may be diagnostic at this stage of

	<p>presentation</p> <p>A whole ovary may be detectable by an experienced ultrasonographer in bitches with IO that are in proestrus / oestrus / have just ovulated because follicles / cavitated CLs will be present. In cases where partial ovarian tissue remains this may not be detectable.</p>
Measurement of plasma progesterone concentration	<p>Test is not diagnostic at this stage of presentation</p> <p>Progesterone will be low in proestrus and oestrus and will only rise at the time of ovulation in late oestrus. Measurement of progesterone will not be able to differentiate a bitch with IO that is in proestrus or oestrus from a bitch that has no ovaries.</p> <p>Testing for progesterone is best performed after signs of alleged oestrus have resolved (i.e. wait and measure progesterone a few weeks later when the bitch will be in the luteal phase).</p>
Measurement of plasma oestrogen concentration	<p>Test is not reliable at this stage of presentation</p> <p>Oestrogen is elevated in proestrus / early oestrus but declines in mid oestrus and thereafter will be low. Diagnostic value of the test will therefore depend upon the exact time of presentation. Only elevated oestrogen concentrations are diagnostic.</p>
Stimulation of oestrogen production with GnRH or hCG	<p>Test is not reliable at this stage of presentation</p> <p>Whilst ovarian tissue in bitches with IO should respond to administration of GnRH or hCG with an increase in oestrogen, the magnitude of response is variable when oestrogen is already elevated or has just been elevated. The response therefore depends upon the exact time of presentation. Only a substantial increase in oestrogen will be diagnostic.</p>
Measurement of plasma anti-Mullerian hormone (AMH)	<p>Test is not widely available</p> <p>At any stage of the cycle AMH concentrations are high in bitches with IO compared with bitches that have no ovaries.</p>
Measurement of resting LH	<p>Test is not reliable at this stage of presentation</p> <p>LH is low in bitches with IO and elevated in bitches with no ovaries, however if a bitch with IO presents in proestrus / oestrus / recently ovulated then LH concentrations may be elevated and so the test is not diagnostic at this stage.</p>

2. Owner considers that the bitch was recently in oestrus

When presented within 2 months after alleged oestrous behaviour the aims are to determine whether the bitch is in the luteal phase and to differentiate that from the bitch that has no ovaries. [In these cases measurement of plasma progesterone is especially helpful.](#)

Table 2. Diagnostic test findings in the bitch presented within 2 months of alleged oestrus.

Bitch presented within 2 months after a period of alleged oestrus	
Diagnostic Test	Recommendation and positive diagnostic findings for incomplete ovariectomy (IO) at this stage of presentation
Observation of behaviour and clinical examination	<p>Test may be diagnostic at this stage of presentation</p> <p>Bitches with IO show no obvious behaviour changes when in the luteal phase.</p> <p>Note that it is common to observe mammary gland enlargement and lactation shortly after complete removal of the ovaries if the surgery has been undertaken within the luteal phase (this is an iatrogenic pseudopregnancy caused by decreased progesterone and increased prolactin and is not diagnostic of IO).</p> <p>If after a subsequent period of apparent oestrous behaviour there is mammary enlargement and lactation and no exogenous hormones have been administered then these features are likely to be diagnostic of IO.</p>
Vaginal cytology and or vaginoscopy	<p>Tests are not diagnostic at this stage of presentation</p> <p>Vaginal cytology and vaginoscopy are not able to differentiate a bitch with IO that is in the luteal phase from a bitch that has no ovaries.</p>
Ultrasound examination	<p>Test may be diagnostic at this stage of presentation</p> <p>During the luteal phase a whole ovary is likely to be detectable by an experienced ultrasonographer because central anechoic fluid will be present within</p>

	each corpora lutea (at least for 30 days post ovulation). In cases where partial ovarian tissue remains this may not be detectable.
Measurement of plasma progesterone concentration	Test is diagnostic at this stage of presentation Progesterone will be elevated for approximately 65 days after ovulation and so can be used to differentiate the bitch with IO from the bitch that has no ovaries.
Measurement of plasma oestrogen concentration	Test is not diagnostic at this stage of presentation Oestrogen concentrations will be similar in both a bitch with IO that is in the luteal phase and a bitch that has no ovaries and therefore measurement of oestrogen is not diagnostic at this stage.
Stimulation of oestrogen production with GnRH or hCG	Test is diagnostic at this stage of presentation Bitches with IO that are in the luteal phase will respond to the administration of GnRH or hCG with a 2-3 fold increase plasma oestrogen whereas bitches that have no ovaries show no change in oestrogen concentration.
Measurement of plasma anti-Mullerian hormone (AMH)	Test is not widely available At any stage of the cycle AMH concentrations are high in bitches with IO compared with bitches that have no ovaries.
Measurement of resting LH	Test is not diagnostic at this stage of presentation LH is low in bitches with IO and elevated in bitches with no ovaries, however if a bitch with IO presents in the luteal phase then LH concentrations may be elevated and so the test is not diagnostic at this stage.

3. Owner considers that the bitch was in oestrus more than 2 months previously

When presented more than 2 months after alleged oestrous behaviour the aims are to determine whether the bitch is in anoestrus and to differentiate that from the bitch that has no ovaries. [In these cases oestrogen stimulation tests or measurement of basal LH are especially helpful.](#)

Table 3. Diagnostic test findings in the bitch presented more than 2 months after alleged oestrus.

Bitch presented more than 2 months after a period of alleged oestrus	
Diagnostic Test	Recommendation and positive diagnostic findings for incomplete ovariectomy (IO) at this stage of presentation
Observation of behaviour and clinical examination	<p>Test is not diagnostic at this stage of presentation</p> <p>Bitches with IO when in anoestrus show no obvious behaviour changes or clinical features that can be used to differentiate them from bitches that have no ovaries.</p>
Vaginal cytology and or vaginoscopy	<p>Tests are not diagnostic at this stage of presentation</p> <p>Vaginal cytology and vaginoscopy are not able to differentiate a bitch with IO that is in anoestrus from a bitch that has no ovaries.</p>
Ultrasound examination	<p>Test unlikely to be diagnostic at this stage of presentation</p> <p>During anoestrus it can be very difficult to reliably detect the ovary and therefore this test is not recommended for differentiation of IO from a bitch with no ovaries.</p>
Measurement of plasma progesterone concentration	<p>Test is not diagnostic at this stage of presentation</p> <p>Progesterone concentrations are basal in both bitches with IO during anoestrus and the bitch that has no ovaries and therefore measurement of progesterone is not diagnostic at this stage.</p>
Measurement of plasma oestrogen concentration	<p>Test is not diagnostic at this stage of presentation</p> <p>Oestrogen concentrations will be basal in both a bitch with IO that is in anoestrus and a bitch that has no ovaries and therefore measurement of oestrogen is not diagnostic at this stage.</p>
Stimulation of oestrogen production with GnRH or hCG	<p>Test is diagnostic at this stage of presentation</p> <p>Bitches with IO that are in anoestrus will respond to the administration of GnRH or hCG with a 2-3 fold increase plasma oestrogen whereas bitches that have no ovaries show no change in oestrogen concentration. This test is therefore diagnostic at this stage of presentation.</p>

Measurement of plasma anti-Mullerian hormone (AMH)	Test is not widely available At any stage of the cycle AMH concentrations are high in bitches with IO compared with bitches that have no ovaries.
Measurement of resting LH	Test is diagnostic at this stage of presentation Bitches with IO that are in anoestrus have low plasma concentrations of LH whereas bitches that have no ovaries have elevated concentrations of LH. This test is therefore diagnostic at this stage of presentation.

4. Queens should be presented when the owner considers they are in oestrus

Queens are polyoestrus and most with IO will cycle approximately every 2-3 weeks. The simplest approach to diagnosis is to request the owner to present the queen at the time of alleged oestrus to enable differentiation from the queen that has no ovaries. [In these cases clinical signs and vaginal cytology are especially helpful.](#)

Table 4. Diagnostic test findings in the queen presented during alleged oestrus.

Queen presented during a period of alleged oestrus	
Diagnostic Test	Recommendation and positive diagnostic findings for incomplete ovariectomy (IO) at this stage of presentation
Observation of behaviour and clinical examination	Test is diagnostic at this stage of presentation Queens that have IO and are in proestrus / oestrus have behavioural changes that differentiate them from queens with no ovaries and these include; vocalisation, treading, rolling, tail deviation and lordosis. These queens may allow mating. Clinical examination may also reveal swelling of the vulva and perineal tissue and a mucoid vulval discharge.
Vaginal cytology	Test is diagnostic at this stage of presentation Queens that have IO and are in proestrus / oestrus have large vaginal epithelial cells in the vaginal smear (although these may not be anuclear as in the

	bitch) and usually low numbers polymorphonuclear leucocytes; the percentage of both cell types depends upon the exact time of presentation.
Ultrasound examination	<p>Test may be diagnostic at this stage of presentation</p> <p>A whole ovary is likely to be detectable by an experienced ultrasonographer in queens with IO that are in proestrus / oestrus because fluid-filled follicles will be present. In cases where partial ovarian tissue remains this may not be detectable.</p>
Measurement of plasma progesterone concentration	<p>Test is not diagnostic at this stage of presentation but can be stimulated to rise by administration of GnRH or hCG</p> <p>Progesterone will be low in proestrus and oestrus and will not rise at the end of oestrus unless the queen is induced to ovulate. This however allows an ideal diagnostic test. Administration of GnRH or hCG will stimulate ovulation and a rise in progesterone which can readily be detected by blood sampling 1 - 2 weeks later when the queen is in the luteal phase.</p>
Measurement of plasma oestrogen concentration	<p>Test is not reliable at this stage of presentation</p> <p>Oestrogen is elevated in proestrus / early oestrus but declines in late oestrus and thereafter will be low. Diagnostic value of the test will then depend upon the exact time of presentation. Only elevated oestrogen concentrations are diagnostic.</p>
Stimulation of oestrogen production with GnRH or hCG	<p>Test is not reliable at this stage of presentation</p> <p>Whilst ovarian tissue in queens with IO should respond to administration of GnRH or hCG with an increase in oestrogen, the magnitude of response is variable when oestrogen is already elevated or has just been elevated. The response therefore depends upon the exact time of presentation. Only a substantial increase in oestrogen will be diagnostic.</p>
Measurement of plasma anti-Mullerian hormone (AMH)	<p>Test is not widely available</p> <p>At any stage of the cycle AMH concentrations are high in queens with IO compared with queens that have no ovaries.</p>
Measurement of resting LH	<p>Test is not reliable at this stage of presentation</p> <p>Although LH is low in queens with IO and elevated in queens with no ovaries, if the queen with IO presents in proestrus / oestrus LH concentrations may</p>

	be elevated and so the test is not diagnostic at this stage.
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DIFFERENTIAL DIAGNOSES

There are a number of differential diagnoses for IO:

1. Presence of a non-oestrus vulval discharge (bitch and queen)

A number of diseases of the vestibule, vagina, urethra, bladder, and kidney may result in a vulval discharge which can have a variety of appearances. **Conditions** associated with a red-coloured discharge include urethral neoplasia and uterine stump granuloma. In none of these cases will there be cornification of the vaginal wall epithelium which occurs during proestrus and oestrus. These differential diagnoses therefore demonstrate parabasal and small intermediate epithelial cells consistent with absence of oestrus.

2. Apparent sexual behaviour (bitch and queen)

It is common and normal for bitches and queens in anoestrus to mount and exhibit thrusting behaviour towards other non-oestrus and oestrus females. This behaviour is also seen after removal of the ovaries. In these cases vaginal cytology will confirm that the behaviour is not associated with oestrus.

3. Sexual interest from **other dogs (bitch)**

It is not uncommon for entire male dogs **as well as female and neutered dogs** to show interest in non-oestrus bitches that have anal gland and skin disease. In neither case will there be a concomitant cornification of the vaginal wall epithelium and so vaginal cytology

demonstrates parabasal and small intermediate epithelial cells consistent with absence of oestrus.

4. Iatrogenically-induced pseudopregnancy (bitch)

Complete removal of the ovaries when the bitch is in the luteal phase results in a rapid decrease in plasma concentrations of progesterone. Decreased progesterone results in increased concentrations of prolactin and the initiation of clinical signs of pseudopregnancy. It is important to recognise that these cases do not have IO and that occurrence of pseudopregnancy alone is not diagnostic for IO. Some cases of iatrogenic pseudopregnancy may be treated by the administration of depot or oral progestogens, which suppresses the release of prolactin and results in a temporary resolution of clinical signs. Prolactin concentrations may increase again at the end of treatment resulting in a recurrence of the pseudopregnancy. Care needs to be taken not to interpret such recurrence of clinical signs after progestogen treatment as suggestive of an ovarian remnant, which it is not.

5. Non-ovarian sources of oestrogen (bitch and queen)

The authors have identified a small number of cases of increased plasma oestrogen concentration associated with male attraction with cornification of the vaginal mucosa (anuclear epithelial cells) in ovariectomised bitches where there is an abnormal appearance of one adrenal gland at ultrasound examination. The exact aetiology is uncertain, but it is known that adrenal oestrogens may increase associated with adrenal disease in other species, especially, for example, ferrets where ovariectomy is commonly associated with the development of adrenocortical neoplasia (de Jong and other, 2014). It is plausible that in ferrets undifferentiated adrenal cells develop gonadal characteristics when they are exposed to high LH and FSH concentrations which occur after neutering; we postulate that such a situation may occur in the neutered bitch although this is rare.

In some circumstances females may ingest human contraceptive or hormone replacement therapy drugs (which are oestrogens) or very rarely oestrogens administered to bitches for the management of urinary incontinence may cause short-term oestrous behaviour with increased numbers of large vaginal epithelial cells. In these circumstances usually the owner is aware that drugs have been ingested or administered.

TREATMENT

Surgical removal of the residual ovarian tissue is the preferred treatment. Any non-removed tissue will be most prominent during either oestrus or the early luteal phase because of the presence of follicles or corpora lutea, respectively. As such, these times should be considered most appropriate for exploratory surgery.

During oestrus, the ovary will have an increased vascularity (Figure 4) produced by the elevated oestrogen concentrations and this is considered by some [inexperienced surgeons](#) to be a contra-indication to surgery at this time. Although the ovary is large during the luteal phase, in the bitch from week 4 onwards progesterone concentrations decrease and prolactin concentrations increase such that surgery from this time onwards risks inducing a prolonged iatrogenic pseudo-pregnancy. In the bitch, therefore, it is most appropriate to perform the surgery approximately two weeks after oestrous behaviour has ceased thus minimising both the problem of haemorrhage and the risk of pseudopregnancy. In the queen, surgery is also best performed during the early luteal phase; in most cases this would be 1 - 2 weeks after induction of ovulation with GnRH / hCG and measurement of progesterone as a diagnostic aid.

Ovariectomy [is](#) performed either using open surgery via a ventral midline coeliotomy, or, laparoscopically (Figure 5) using a [two or three-port technique](#); [the authors prefer the latter](#).

Commonly the right pedicle is examined first but residual tissue may be bilateral so both must be carefully examined (Figure 6). If residual ovarian tissue cannot be found at the sites of previous ovariectomy, a thorough exploration of the peritoneal cavity should be performed, including towards the caudal poles of both kidneys, the omentum and the peritoneal walls, as these are sites where tissue may have retracted or become adhered to. Small islands of residual ovarian tissue might prove difficult to visualise especially if ligature material is still present at the site of the ovarian pedicle. In such cases, it is advisable to remove any remaining vascular pedicle, along with its fat pad and any residual ligature material, as there is the possibility that this will also remove unobserved ovarian material. All excised tissue should be submitted for histopathology to confirm ovarian tissue has been removed.

CONCLUSION

Incomplete ovariectomy is not uncommon in the bitch and queen and is caused by a combination of an inaccurate surgical procedure and failure to inspect excised tissue. After surgery, cases may have a variety of presentations dependent upon the vascular supply to the residual tissue, although in many cases there is normal cyclicity. Whilst these cases may appear daunting to investigate, with an understanding of the normal reproductive physiology and endocrinology a logical diagnostic approach can be achieved. Surgical removal of residual tissue is curative.

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LEGENDS TO FIGURES

Figure 1

Vaginal smear stained with [Diff-Quick \(a rapid dunking stain\)](#) and examined under light microscopy from a 'spayed' bitch that presented with oestrous behaviour. The epithelial cells have a large volume of cytoplasm, are flattened and have an irregular outline. Many of the cells are anuclear. This appearance is typical of oestrus and is diagnostic for incomplete ovariectomy.

Figure 2

Vaginoscopy image from a 'spayed' bitch that presented with oestrous behaviour. The epithelium is swollen into significant rounded contours and the mucosa appears moist, oedematous and pale. This appearance is typical of oestrus and is diagnostic for incomplete ovariectomy.

Figure 3

Ultrasound images of two different 'spayed' bitches that presented in early (left image) and late (right image) oestrus. The left image shows a normal sized ovary (fat arrows) containing two small follicles (thin arrows). The right image shows the ovary (fat arrows) which contains two large follicles (thin arrows). Identification of ovaries is of course diagnostic for incomplete ovariectomy.

Figure 4

Appearance of the entire left ovary of a queen with incomplete ovariectomy [examined at laparotomy](#). The queen was presented with behavioural signs typical of oestrus and surgery was performed at that time. The ovary containing multiple follicles is large and vascular.

Figure 5

Appearance of the entire ovary (thin arrows) of a bitch with incomplete ovariectomy [examined at laparoscopy](#). The surgery was performed 3 weeks after oestrus at which time the ovary retains a large size and contains prominent corpora lutea (fat arrows).

Figure 6

Ovarian tissue removed from a queen with bilateral incomplete ovariectomy. Laparotomy was performed during oestrus and the entire left ovary and a portion of the right ovary were discovered and removed. Both ovaries contain follicles.