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LABORATORY METHODS

Rodent models of osteoporosis

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The aim of this protocol is to provide a detailed description of male and female rodent models of osteoporosis. In addition to indications on the methods of performing the surgical procedures, the choice of reliable and safe anaesthetics is also described. Post-operative care, including analgesia administration for pain management, is also discussed. Ovariectomy in rodents is a procedure where ovaries are surgically excised. Hormonal changes resulting from ovary removal lead to an oestrogen-deprived state, which enhances bone remodelling, causes bone loss and increases bone fracture risk. Therefore, ovariectomy has been considered as the most common preclinical model for understanding the pathophysiology of menopause-associated events and for developing new treatment strategies for tackling post-menopausal osteoporosis. This protocol also provides a detailed description of orchidectomy, a model for androgen-deficient osteoporosis in rodents. Endocrine changes following testes removal lead to hypogonadism, which results in accelerated bone loss, increasing osteoporosis risk. Orchidectomised rodent models have been proposed to mimic male osteoporosis and therefore remain a valuable tool for understanding androgen deficiency in aged men. Although it would have been particularly difficult to assemble an internationally acceptable description of surgical procedures, here we have attempted to provide a comprehensive guide for best practice in performing ovariectomy and orchidectomy in laboratory rodents. Research scientists are reminded that they should follow their own institution's interpretation of such guidelines. Ultimately, however, all animal procedures must be overseen by the local Animal Welfare and Ethical Review Body and conducted under licences approved by a regulatory ethics committee.

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Introduction

Sex hormones have an important role in the regulation of bone mass and turnover in adult and ageing skeleton. Oestrogen belongs to the gonadocorticoid class of steroid hormones and is responsible for the development of female secondary sexual characteristics, the regulation of menstrual cycle, the timing of ovulation in pre-menopausal women and maintenance of pregnancy.[1] The skeleton is one of the main targets of oestrogen, as it regulates bone growth and remodelling in both men and women. Decreased levels of oestrogen in post-menopausal women is one of the major causes of osteoporosis, alongside ageing and inactivity. Post-menopausal osteoporosis is mainly due to enhanced bone turnover with resorption exceeding formation, increased cancellous bone loss and trabecular thinning and reduced intestinal calcium absorption.[1,2]

Although clear 'menopause' is absent in rodents, such as mice and rats, they do undergo reproductive senescence with age and acyclicity is reached by the age of 11–16 months in mice[3] and 15–18 months in rats.[4] Ageing alone and in combination with age-related oestrogen deficiency results in trabecular and cortical bone loss over life,[5] resembling patterns of osteoporosis in humans.[6,7]

Oestrogen deficiency, however, could be reached at an earlier stage either by chemically inducing ovarian failure, using the chemical 4-vinylcyclohexene diepoxide (VCD), by the suppression of the hypothalamus-pituitary-ovarian axis through the administration of the gonadotropin-releasing hormone agonist buserelin or by surgically removing the ovaries, a procedure referred to as ovariectomy. VCD treatment in rodents causes gradual onset of ovarian failure, with

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hormonal and cyclic changes, which mimic the perimenopausal transition in women.[8,9] Ovariectomy, on the other hand, has been criticized that it interrupts ovarian function rather markedly, leading to an abrupt hormonal loss instead of a gradual decline, which naturally happens in women.[10] Buserelin administration causes 'chemical ovariectomy' in the sense that although ovaries remain intact, there is decreased ovarian oestrogen secretion attributed to the hypothalamic and pituitary suppression of gonadotropin production.[11] Oestrogen loss in all three models leads to a decline in bone mass, which essentially results in an osteoporotic phenotype again resembling the one seen in osteoporotic women. Nonetheless, the skeletal changes in the VCD model are of lower magnitude and develop at a slower rate than those in the ovariectomy model.[10] In addition, VCD has been classified as an irritant to tissues[12] and its clearance from systemic circulation varies in different species, strains and ages,[13,14] posing potential health risks with chronic exposure. On the other hand, although buserelin-mediated oestrogen-deficiency bone loss is reversible,[11,15] it is more suitable for studying mechanisms for repairing osteopaenia or the effects of intermittent oestrogen deficiency on bone.[11] Ovariectomy may not perfectly reproduce the peri- and post-menopausal effects in women and causes a permanent cessation of oestrogen production, but it does remain a valuable and most widely used tool for preclinical studies, investigating potential drugs as therapeutic agents for conditions of accelerated bone loss.[10]

Androgen deficiency is the major risk factor for osteoporosis in men.[16] Although the mechanisms of bone loss caused by androgen deficiency are still unclear, the skeletal effects of androgen withdrawal in aged male rodents are remarkably similar to those induced by oestrogen withdrawal in female rodents.[17,18]

The scope of this protocol is to provide a detailed description of the most common practices when performing ovariectomy and orchidectomy in rodents, including choice of anaesthetics, selection of incision site and current guidelines for post-operative pain management.

Materials and Methods

This section starts with an explanation of the pre-operative care, followed by a detailed description of the surgical procedure and intra-operative care and finally closes with an explanation of the post-operative care, including guidelines on the use of analgesia.

Pre-operative management

Animals should be left to acclimatise for at least 1 week if transported from elsewhere. They should be kept in pathogen-free rooms of a designated animal facility, under a 12 h dark-to-light cycle, with water and pelleted standard commercial diet made available *ad libitum*. Only mice passing a health check prior to surgery should be used for the experiment.

Surgical procedure

Preparation. Prior to commencing the surgical procedure, all areas on the operating table are wiped with disinfectant. Only then, sterile drapes should be laid down and sterile instruments placed on it. On a separate working station, the mouse is weighed and anaesthesia is then induced.

Induction of anaesthesia. There are two kinds of general anaesthetics, inhalable and injectable. If inhalable anaesthesia is preferred, the most common anaesthetic inhalant agents are halothane and isoflurane, both administered in conjunction with air and/or pure oxygen.[19] Halothane is delivered to effect in concentrations of 3–4% for initial induction and 1–2% for maintenance of anaesthesia, whereas the effective concentrations for isoflurane are 3.5–4.5% for induction and 1.5–3% for maintenance in both mice[20] and rats.[21,22] Other volatile inhalable anaesthetics belonging to the 'flurane' family can also be used, such as methoxyflurane, sevoflurane and desflurane, each with its own advantages and disadvantages.[23] Ether has also been used as an inhalable anaesthetic in rodent ovariectomy,[24–27] but its use is rather discouraged, or illegalised in some countries, because of its irritant properties and flammability.

NOTE 1: If inhalation anaesthesia is used, a transparent induction chamber, an anaesthesia machine and a precision vaporiser are required.

NOTE 2: Scavenging of anaesthetic gases is important to protect laboratory personnel performing the surgical procedure.

Prolonged exposure to inhalant anaesthetic agents has been associated with cardiovascular and respiratory depression, vasodilation and hypotension.[19] Moreover, administration of inhalable anaesthesia requires specialised personnel training and specialised equipment. For these reasons, injectable anaesthetics are commonly preferred over inhalant agents. Injectable anaesthetics in rodents are most commonly administered *via* the intraperitoneal route (i.p.); more rarely, they are also administered intramuscularly (i.m.) or intravenously (i.v.), whereas subcutaneous (s.c.) administration is rather uncommon.[19] The use of combination of anaesthetics, known as 'balanced anaesthesia',[28] is preferred because it maximises the desired effects and minimises the adverse effects of the anaesthetic agents. Most common recommended anaesthetic regimes include ketamine and α2-adrenoceptor agonists such as xylazine or medetomidine. Doses of 90–150 mg kg^{-1} ketamine and 7.5–20 mg kg^{-1} xylazine i.p. have been reported for reliable anaesthesia in mice undergoing ovariectomy.[19,29] In rats undergoing ovariectomy, the recommended dose rate is ketamine 50–80 mg kg^{-1} and xylazine 10 mg kg^{-1} i.p.[21,30] Medetomidine is a newer and more potent α2-adrenoceptor agonist compared with xylazine with fewer notable side effects.[19] A regime of ketamine 75–80 mg kg^{-1}-medetomidine 1 mg kg^{-1} i.p. for mice[23,31] and ketamine 60–75 mg kg^{-1}-medetomidine 0.4–0.5 mg kg^{-1} i.p. for rats[19,32] has been reported for ovariectomy procedures. For a shorter recovery time, xylazine and medetomidine effects can be reversed rapidly by the α2-adrenoceptor antagonist atipamezole (1 mg kg^{-1} i.p., i.m., s.c.).[19,23,31,33,34] A larger list of recommended anaesthetic regimes together with the dosage and route of administration in rodents can be seen in studies by Gaertner *et al.*[19] and Otto and von Thaden.[23] A summary of the recommended regimes for anaesthesia is shown in **Table 1**. It is, however, advisable to consult and get approval from a veterinary surgeon before exposing animals to any anaesthetic agents.

NOTE 3: General anaesthesia is reached once the animal does not respond to the pedal withdrawal reflex caused by firm pressure on the hind paws or pinch on the ear or the skin between digits.

NOTE 4: Following induction of anaesthesia, apply a sterile petrolatum-based ophthalmic lubricant, such as Puralube or Lacri-Lube, to prevent corneal drying and damage.[35]

Ovariectomy: model of post-menopausal osteoporosis. To create access into the abdomen area, a single surgical incision is usually made on the dorsal midline at the caudal edge of the ribcage[22,36,37] (**Figures 1a and b**). Double (bilateral) dorsolateral incisions[36,38–40] (**Figure 1c**) and single ventral transverse incision on the middle part of the abdomen[30] (**Figure 1d**) have also been reported when performing ovariectomy in rodents.

Performing ovariectomy using single dorsal midline skin incision. For performing an ovariectomy using a dorsal midline skin incision, the animal is placed ventral side down (ventral recumbent position) and electric shavers are used to remove the fur over its lumbar spine. The shaved area should then be thoroughly cleaned with alternating washes of a disinfectant solution, such as an iodophor or chlorhexidine, and 70% (v/v) alcohol for disinfecting the skin.[41] The disinfected area is then draped with a paper drape or sterile gauze sponges.[41]

Using sterile small scissors, a midline dorsal incision of 0.5–1 cm is made along the lumbar vertebrae,[22,37] and the skin at each side of the cut is separated from the underlying muscle using forceps with blunt end. The same skin incision can be used to remove both ovaries, because the skin in rodents is so loose that it can be retracted from side to side.[22,37] Ovaries are easy to locate because they are embedded in fat pad, which is visible underneath the muscle. To gain access to one of the ovaries, a 0.5–1 cm retroperitoneal incision is made on the erector spinae muscle below the last rib. While holding the muscle layer with sterile serrated forceps, the ovary together with the associated ovarian fat pad are gently withdrawn using sterile blunt forceps.[22,37] This allows the ovary, oviduct and part of the uterus to be exposed. While taking care not to disturb the ovary, a suture (or ligature) is tied and knotted tightly around the cranial portion of the uterus and uterine vessels,[22] about 0.5 and 1 cm distal to the ovary in mice and rats, respectively.[37] This is performed to prevent excessive haemorrhage following the removal of the ovary. The exposed ovary, oviduct and part of the uterus are carefully removed using sterile spring scissors, whereas the caudal portion of the uterus is replaced in the abdomen.[22,37] Although it has been reported that generally it is not necessary to close the peritoneum when performing ovariectomy in rodents,[22] suturing the associated musculature is recommended in rats but remains optional in mice.[37] A second incision on the retroperitoneal wall is made on the contralateral side, and the same procedure is followed to gain access to the ovary, make a ligature, severe the ovary, oviduct and part of the uterus and finally suture the muscle layer. The preferred method of closing the skin incision is by using metal clips,[22,37] although the usage of nonabsorbable sutures and tissue adhesive has also been reported.[22] For sham operations, the same procedure can be followed, except that the ovaries are identified and then placed back in the abdominal cavity.

Performing ovariectomy using double (bilateral) dorsolateral skin incisions. For performing ovariectomy using double (bilateral) dorsolateral skin incisions, the rodent is placed in a ventrally recumbent position and the back is shaved bilaterally caudal to the last rib.[38,39] The exposed skin is disinfected and draped as described above. A 1 cm skin incision is made on one of the dorsolateral surfaces to expose the dorsolateral abdominal muscles.[38,39] As mentioned above, the ovary can be easily located, as it is surrounded by adipose tissue, which is obvious through the muscle layer. Entrance to the peritoneal cavity, identification of the ovary, ligation and severing of the ovary, ovarian fat pad, oviduct and part of the uterus are performed as described above. The remaining part of the uterus is returned to the abdominal cavity and the incisions in the muscle and the skin are sutured.[38,39] Metal clips can be alternatively used for closing the skin incision.[38] Bilateral incisions are made on the contralateral dorsolateral skin surface and abdominal muscle to gain access to the second ovary. The same procedure is followed for ovary removal and closing of muscle layer and the skin. For sham operations, ovaries are identified but remain intact in the abdominal cavity.

Performing ovariectomy using single ventral transverse skin incision. Although ventral midline approach for performing ovariectomy is more common in mares, cats and pigs, it has also been reported in rats.[30] For a single ventral transverse skin incision, the rat is placed in a dorsally recumbent position and the fur in the abdomen is completely removed.[30] The exposed skin is disinfected and draped as described above. Access to the peritoneal cavity is achieved by a 0.4–0.6 cm peritoneal transversal skin incision using a scalpel blade, made in the middle part of the abdomen and slightly to the right, adjacent to the cranial inguinal nipple.[30] Following muscle dissection, the adipose tissue is then pulled away allowing the right uterine tube with ovary to be identified. The ovary and associated ovarian fat pad are gently retracted using sterile blunt forceps. Through the same incision, the left ovary is identified and withdrawn in the same manner.[30] Ligature and severing of the ovary, ovarian fat pad, oviduct and part of the uterus are performed as described above. The remaining part of the uterus is returned to the abdominal cavity and the incisions in the muscle and the skin are sutured.[30] Sham operations are performed by identifying ovaries using the same procedure described above but leaving them intact in the abdominal cavity.

NOTE 5: The person performing the surgery should be wearing Personal Protective Equipment , clean gown and sterile gloves.

NOTE 6: The use of a heat source like a suitable heating pad would prevent heat loss.

NOTE 7: Animals should be continually monitored for the duration of the procedure.

Post-operative management and analgesia

Reversing anaesthesia. Anaesthesia induced by inhalant agents is rapidly reversed simply by discontinuing the administration of anaesthetics.[19] Injectable anaesthesia, however, requires longer recovery time. If xylazine and medetomidine are used as part of the regime, their effects can be rapidly reversed by the α2-adrenoceptor antagonist atipamezole (1 mg kg^{-1} i.p., i.m., s.c.),[19,34] resulting in reduced recovery time.

Administration of analgesia. Although the α2-adrenoceptor agonists xylazine and medetomidine used as part of the anaesthetic regime have mild sedative and analgesic properties,[23] these are short-lived and additional analgesia is

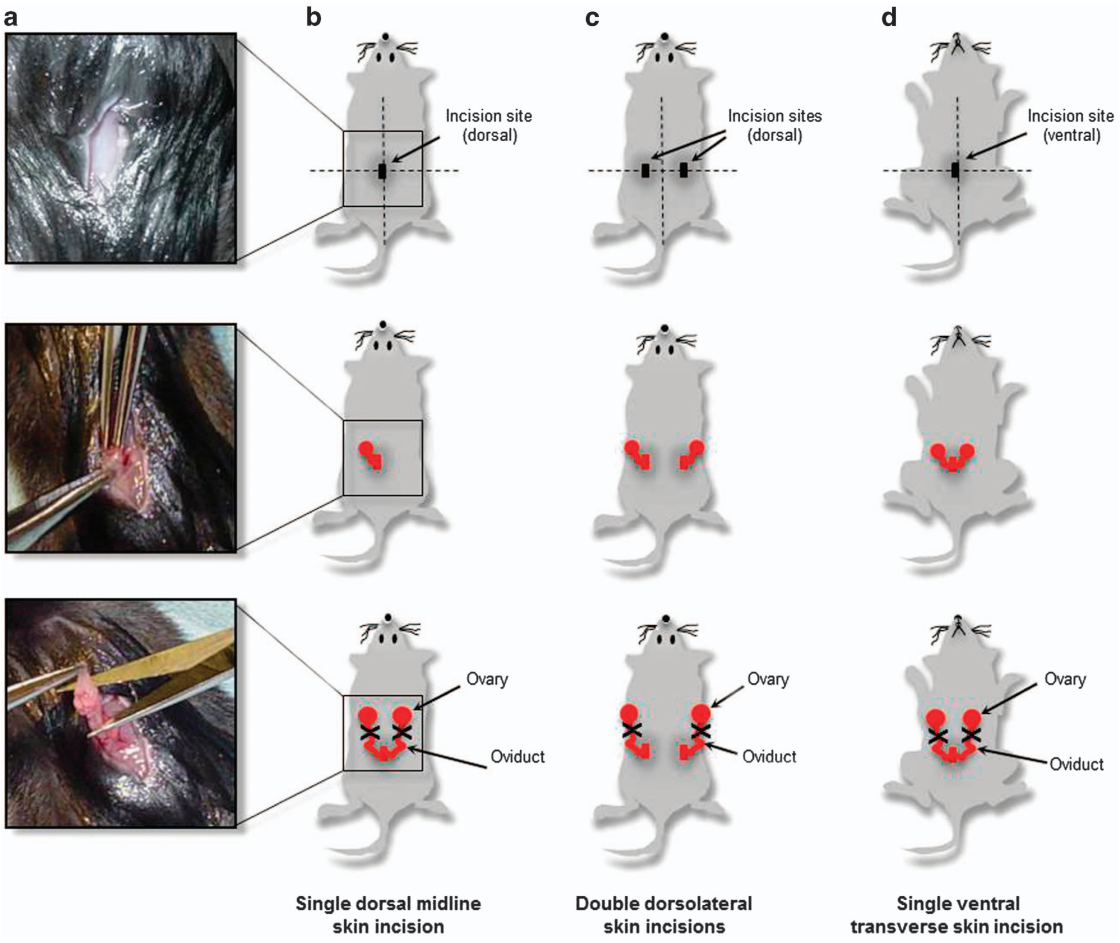

Figure 1 Schematic illustration of ovariectomy using single dorsal midline skin incision (**a** and **b**), double dorsolateral skin incisions (**c**) or single ventral transversal skin incision (**d**). X denotes 'removal'. A visual demonstration of the ovariectomy procedure is available on PubMed online.[64]

required. The most commonly used analgesics in laboratory rodents are nonsteroidal anti-inflammatory drugs and opioids. One of the most common nonsteroidal anti-inflammatory drugs s is carprofen, which can be administered subcutaneously in the loose part of the skin on dorsal neck (5 mg kg^{-1} for rats and mice, once a day, for 2 days).[19,23,42] However, evidence suggests that carprofen should not be used as the sole analgesic for rodent surgery models; instead, it can be combined with the opioid analgesic buprenorphine,[19] the most practical and clinically useful analgesic for relieving moderate post-surgical pain in rodents.[43] Buprenorphine dosages of 0.05 mg kg^{-1} s.c. have been reported in rats and mice undergoing ovariectomy.[44,45] **Table 1** summarises the recommended regime for buprenorphine together with its advantages and disadvantages. A larger list of recommended analgesics, doses and routes in rodents can be seen in studies by Gaertner *et al.*[19] and Otto and von Thaden.[23]

The final decision, however, regarding which analgesic agent to use must always be based on the experimental model that is under study. Ovariectomy is essentially a model of investigating bone loss, but bone resorption could be promoted by the inflammatory cytokine upregulation, which has been observed in ovariectomised animals.[46,47] Therefore, anti-inflammatory drugs like carprofen may not be the best choice of analgesia for the ovariectomised rodent model. Instead, the use of buprenorphine has been mainly recommended for relief of pain in models of inflammation.[19]

Post-operative care. Following analgesic administration, animals should be left undisturbed in a disinfected recovery box at a temperature of 27–30 °C until they are fully conscious. To avoid dehydration in case of prolonged recovery, fluids should be replaced by subcutaneous or intraperitoneal injection of warm sterile saline.[19,22] Once they are fully conscious and active, they are transferred to a clean cage with fresh bedding, and they are monitored at least daily for signs of pain or distress over the following 72 h postoperatively.[19] If the ventral midline approach is chosen for performing ovariectomy, care should be taken to avoid wound infection. For that reason, rodents should be housed individually in polyurethane boxes with bedding made of sterilised cotton fabric for a period of 1 week post-operatively.[30] Nonabsorbable sutures and metal clips can be removed in the second week post surgery.

Orchidectomy: the model of male osteoporosis

Models of post-menopausal osteoporosis in women are well established, but readers should also be informed of the existence of rodent models of male osteoporosis. Osteoporosis is no longer considered the disease of post-menopausal women. It is also prevalent in elderly men, and it is one of the

Table 1 Summary of recommended regimens for anaesthesia and analgesia in mice and rats

Inhalant anaesthesia

Anaesthetic agent	*Effective concentration for initial anaesthesia induction (vol %)*	*Effective concentration for anaesthesia maintenance (vol %)*	*Advantages*	*Disadvantages*
Halothane	Mice: 3–4,[20] 4–5[22] Rats: 4,[21] 4–5[22]	Mice: 1–2,[20] 2–4[22] Rats: 1–2,[21] 2–4[22]	Effective and safe[22] with rapid induction and recovery[23]	Causes depression of the cardiovascular and respiratory system,[23] fatty changes in the liver[19] and may inhibit immune function.[23]
Isoflurane	Mice: 3.5–4.5[20] Rats: 4[21]	Mice: 1.5–3[20] Rats: 1.5–3[21]	Effective and safe,[22] produces stable conditions with low risk and rapid recovery.[23]	Reduces gut motility[19] and may cause post-operative immunosuppression.[23]

Injectable anaesthesia

Anaesthetic agent	*Dosage in mice (mg kg^{-1})*	*Dosage in rats (mg kg^{-1})*	*Advantages*	*Disadvantages*
Ketamine/ xylazine (reversal: atipamezole)	Ketamine: 90–150; xylazine 7.5–20 (i.p.)[19,29] Atipamezole: 1 (i.p.)[19,34]	Ketamine: 50–80; xylazine 10 (i.p.)[21,30] Atipamezole: 1 (i.p.)[19,34]	Excellent muscle relaxation, sedation and analgesia[23]	Adverse effects on cardiovascular function and hypotension.[19,23] In addition, xylazine may cause hyperglycaemia.[19]
Ketamine/ medetomidine (reversal: atipamezole)	Ketamine 75–80; Medetomidine 1 (i.p.)[23,31] Atipamezole: 1 (i.p.)[19,34]	Ketamine 60–75; Medetomidine 0.4–0.5 (i.p.)[19,32] Atipamezole: 1 (i.p.)[19,34]	Sedation, excellent muscle relaxation and analgesia. Medetomidine has much higher affinity for the α2-adrenoceptor than xylazine.[19,23]	May cause cardiovascular and respiratory depression, hypothermia, hyperglycaemia and diuresis.[23]

Analgesia

Analgesic agent	*Dosage in mice (mg kg^{-1})*	*Dosage in rats (mg kg^{-1})*	*Advantages*	*Disadvantages*
Buprenorphine	0.05 (s.c.)[44,45,52,53,65]	0.05 (s.c.)[44,45,52,53]	Provides effective analgesia with minimal adverse side effects,[19] ideal for mild-to-moderate pain.[66]	May be slow in onset[19] and has a rather short duration of action (3–5 h).[66,67]

Abbreviations: i.p., intraperitoneal; s.c., subcutaneous.

main causes of morbidity and mortality in the male ageing population.[48] The orchidectomised rodent model has been proposed to mimic male osteoporosis resulting from androgen deficiency.

Orchidectomy in rodents is most commonly performed through a scrotal incision[37,49,50] (**Figures 2a and b**), but an abdominal approach can be used as well[22,49,51] (**Figure 2c**) because unlike humans, inguinal canals in rodents remain open throughout life.[22]

Performing orchidectomy using vertical scrotal skin incision. Following anaesthesia induction (as described in the section 'Induction of anaesthesia'), the rodent is positioned in dorsal recumbency and the scrotum is aseptically prepared and draped as described in the section 'Performing ovariectomy using single dorsal midline skin incision'. A skin incision is made on the ventral side of the scrotum (0.5 cm for mice and 1.5 cm for rats) using a scalpel.[37,50] The cremaster muscles are incised, and the testicular fat pad is gently pulled through the incision using blunt forceps.[37,50] The testicular content including the testis, cauda epididymis, vas deferens and blood vessels is gently exteriorised.[37,49,50] Vas deferens and blood vessels are ligated with an absorbable suture, and the testis and epididymis are removed using small scissors.[37,49,50] The remaining tissue is placed back in the scrotal sac using blunt forceps. The skin incision is closed with a metal clip, nonabsorbable suture or tissue adhesive,[37,49,50] and the same procedure is followed for the other testis. For sham operations, the same procedure is followed, but the testes are simply identified and left intact. Anaesthesia is reversed as described in the section 'Reversing anaesthesia'.

Performing orchidectomy using ventral suprapubic skin incision. For a ventral skin incision in the suprapubic region, the rodent is anaesthetised as described above and placed in a dorsally recumbent position. The fur over the lower abdomen is removed, and the exposed skin is disinfected and draped as described above. A 0.5–1.0 cm midline skin incision is made using small scissors.[22,49,51] The abdominal cavity is entered through the linea alba by a stab incision.[22] One testis is then manipulated from the scrotal sac into the abdomen and finally exteriorised together with the epididymis, fat, vas deferens and vascular cord, through the abdominal incision using forceps.[22,51] Vas deferens and blood vessels are then ligated by

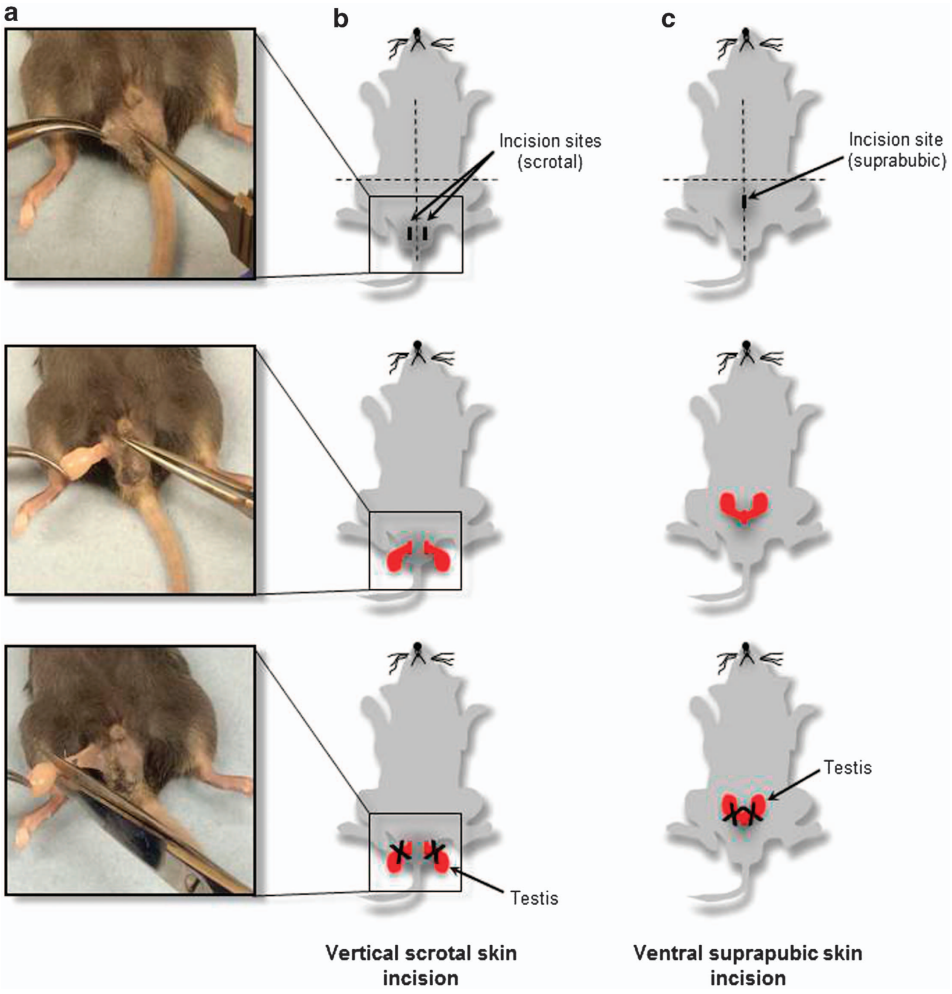

Figure 2 Schematic illustration of orchidectomy using vertical scrotal skin incision (**a** and **b**) or ventral suprapubic skin incision (**c**). X denotes 'removal'.

a single ligature or alternatively ligated first individually and then together with a proximal ligature encircling both.[51] The testis and the epididymis are severed using a small scissor, and the remaining of the tissue is released into the abdomen.[49,51] The same procedure is followed for the adjacent testis through the same skin and body wall incisions. Then, the peritoneal cavity is closed with absorbable sutures, and the skin is closed with nonabsorbable sutures, tissue adhesive[22,49] or metal clip (better tolerated by rats).[51] For sham operations, the same procedure can be followed, except that the testes are identified and then released back in the abdominal cavity. Anaesthesia is reversed as described above.

Post-operative care for orchidectomised rodents. Analgesia is administered postoperatively as described in the section 'Administration of analgesia'. In particular, administration of buprenorphine at 0.05 mg kg^{-1} s.c. has been reported in rats and mice undergoing orchidectomy.[52,53] Animals are monitored and fluids can be replaced as mentioned above. Once fully conscious and active, they are transferred to a clean cage with fresh bedding. Nonabsorbable sutures and metal clips can be removed within 7–10 days, and the incision site should be monitored at least twice daily for 7–10 days postoperatively.[51]

Surgical services

It is worth pointing out that an alternative to performing the operations is to purchase ovariectomised/orchidectomised rodents from animal suppliers who offer surgical services.

Materials

1. Sterile surgical instruments including small scissors, forceps with blunt end, serrated forceps, spring scissors, scalpel blade and needle holder.
2. For closing incisions:
   a. Sutures (absorbable for internal use and nonabsorbable for external use) OR
   b. Metal clips OR
   c. Tissue adhesive
3. Sterile sodium chloride (0.9% w/v)
4. Anaesthesia
   a. Inhalation anaesthesia, that is, isoflurane or halothane OR
   b. Injectable agents, that is, cocktail of ketamine/xylazine or ketamine/medetomidine, followed by atipamezole for reversing anaesthesia

5. A transparent induction chamber, an anaesthesia machine, a precision vaporiser and a gas scavenger if inhalation anaesthesia is used
6. Sterile needles and syringes
7. Analgesic

    a. Opioids, that is, buprenorphine OR
    b. Nonsteroidal anti-inflammatory drugs , that is, carprofen

8. Shavers
9. Skin disinfectant, that is, iodophor or chlorhexidine
10. Sterile cloth and paper drapes
11. Sterile swaps
12. Sterile gloves
13. Sterile cotton buds
14. Weighing scale
15. Heat pad
16. Recovery box

Discussion

This protocol provides a thorough description of the most common tools currently used for mimicking osteoporosis in rodents, the ovariectomy and orchidectomy procedures.

Ovariectomy is known to be sufficient for inducing oestrogen deficiency and uterine atrophy in rodents, unlike in rabbits and other small domestic animals where the removal of the uterus in addition to the ovaries (ovariohysterectomy) is sometimes preferred.[22] This is because rodents are unlikely to develop a uterine disease following ovary removal,[22] making them a more attractive model for this procedure. Bilateral orchidectomy in rodents causes near total testosterone depletion, which eventually leads to a state of hypogonadism. Accumulating evidence suggests that changes in male rodent bone metabolism after orchidectomy are highly reminiscent of the changes induced by ovariectomy in female rodents.[54]

Options for general anaesthesia induction include inhalable or injectable anaesthetics. Both types of anaesthesia have been used in ovariectomy procedures, with the injectable cocktail of ketamine/xylazine or ketamine/medetomidine being the most common and widely used anaesthetic regime. However, care should be taken to avoid anaesthetic overdose or animal recovery by using the minimal required dose, based on the weight of each animal.

Access to the ovaries can be achieved in different ways, depending on the site of skin incision. Three different methods have been described in this protocol. Two of these methods achieve access to the ovaries with dorsal incisions, that is, on the back. A single midline dorsal skin incision followed by two incisions on the underlying muscle would enable access to both ovaries. Alternatively, bilateral dorsolateral skin and muscle incisions could be performed. Apart from the dorsal/dorsolateral approach, a third method has been reported for performing ovariectomy, which is using a ventral midline skin incision. Although the access to the abdomen is excellent with ventral midline incisions, the risk of post-operative complications in rodents is great. This is because, for the ventral midline approach, the incision is subjected to the weight of the viscera, making evisceration and postsurgical hernia occurrence more likely;[55] there is greater loss of abdominal fluid, and for access to the ovaries the gastrointestinal track is somehow manipulated.[55] Moreover, with the ventral approach, the wound is in direct and constant contact with the bedding of the cage, increasing the chances of post-operative infections.[30] For these reasons, a dorsal approach for performing ovariectomy is recommended, with the single midline skin incision technique being preferred and more widely implemented.

Inguinal canals in male rabbits and rodents remain open throughout life providing the possibility of accessing testes either by scrotal skin incisions or by an abdominal incision in the suprapubic area.[22]

An essential part of post-operative management is the effective administration of analgesia. Care should be taken so that the choice of analgesia does not compromise the use of ovariectomy as a model for osteoporosis. However, this decision is best to be mutually agreed between the researchers and the local veterinarians, as well as the Animal Welfare and Ethical Review Body.

Bone loss following ovariectomy in mice reaches about 30% at 3–4 weeks postoperatively,[56,57] but studies have shown that skeletal changes after ovary removal vary among different inbred mouse strains and are site- and compartment-specific.[58] Studies in rats reported that bone loss is about 50% at 5 weeks postoperatively[59] and more than 60% at 13–14 weeks post-operatively.[60,61] Orchidectomy causes about 20% and 50% reduction in trabecular bone volume in rats[62] and mice,[63] respectively, at 4 weeks postoperatively. The decision on whether an investigation should focus on the short-term or the long-term effects of these procedures must depend on the question that is being addressed in each study.

Although the hormonal changes occurring in ovariectomised rodent models are rather abrupt and permanent compared with other models of oestrogen deficiency, that is, following VCD or buserelin treatment, ovariectomy remains the most widely used and valuable tool for mimicking conditions of accelerated bone loss in post-menopausal women. In parallel, the established orchidectomised model of osteoporosis in men is a powerful experimental tool for investigating male osteoporosis due to androgen deficiency.

Recommended further reading

The following sources provide further detail on anaesthesia and analgesia administration:

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Conflict of Interest

The authors declare no conflict of interest.

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