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Experimental model of menopausal genitourinary syndrome: Ovariectomy and vaginectomy protocols in rats

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ABSTRACT

Background: The synthesis of estrogen hormones decreases during menopause, leading to various physiological changes that might affect a woman's quality of life. Genitourinary syndrome of menopause (GSM), which occurs because the vaginal and urethral epithelial layers contain a high density of estrogen receptors, is one of the most common clinical manifestations. These problems can be chronic and progressive, consequently affecting menopausal women's sexual and quality of life. Therefore, innovation in novel therapeutic modalities or improvements to current therapies is necessary.

Aim: This study aimed to provide a comprehensive and precise protocol guide for ovariectomy and vaginectomy to establish a suitable animal model for GSM cases.

Method: Forty *Wistar* rats of *Rattus norvegicus* were used in the study and treated according to ethical principles.

Results: The study recommends the double dorsolateral incision technique as the most effective method for ovariectomy. Vaginectomy is essential for the objective evaluation of the vaginal and urethral epithelium in GSM and for the histological assessment of therapeutic response.

Conclusion: The therapeutic modalities that result from a suitable animal model protocol are expected to guarantee safety and efficacy.

Keywords: Genitourinary, Menopause, Ovariectomy, Rats, Vaginectomy.

Introduction

Menopause is a transitional phase between the reproductive and non-reproductive periods in women, usually occurring between the ages of 40 and 50, defined by 12 months of amenorrhea and LH levels >40 IU/l measured at least 4 weeks apart (Thangavel *et al.*, 2019; Meeta *et al.*, 2020; Khapre *et al.*, 2022). Menopause decreases the levels of estrogen and other sex steroid hormones, triggering physiological changes that can affect a woman's quality of life. This condition also negatively affects the clitoris, vaginal vestibule, labia majora and minora, urethra, and bladder. The vagina contains numerous estrogen receptors, particularly in the mucosal epithelium, so a decrease in estrogen hormone production causes the vaginal epithelium to be thinner due to a reduction in the number of epithelial cell layers and the degeneration of collagen and elastin fibers in the underlying connective tissue, causing the

vagina to be less elastic and more fragile. This leads to clinical symptoms, including vaginal burning, dyspareunia, and post-coital bleeding. In addition to the vagina, the urethral epithelium contains various estrogen receptors; therefore, menopause commonly produces urogenital symptoms such as dysuria, urgency, and incontinence due to decreased collagen, urethra wrinkles, and sensitivity of the smooth muscle (Sarmiento *et al.*, 2021). The clinical condition described above is termed genitourinary syndrome of menopause (GSM), which can be chronic and progressive (Vodegel *et al.*, 2023). The incidence of GSM was approximately 5 million in the 20th century, and its prevalence has risen to 13.5 million, constituting nearly one-third of the global female population (Ismail and Bibi, 2024). Consequently, constantly innovating the current therapy strategies is important. Evidence of safety and efficacy from preclinical trials utilizing suitable animal

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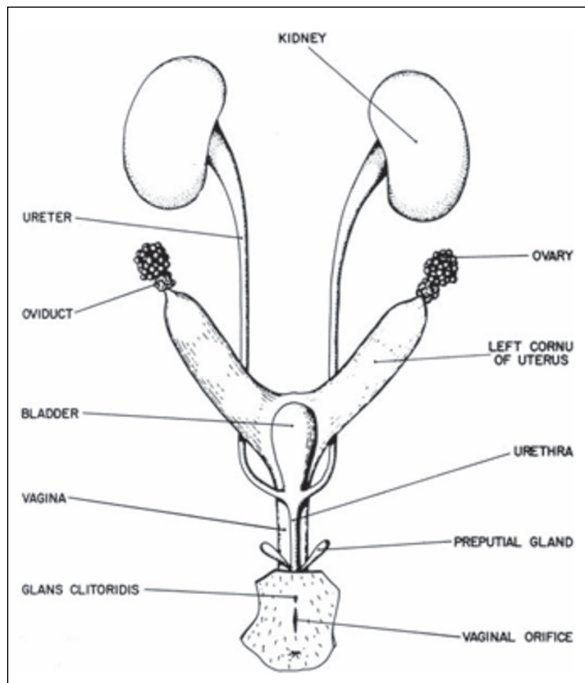


Fig. 1. Female rat urogenital system anatomy (Suckow *et al.*, 2019).

model protocols is necessary before the approval of the new therapeutic modality for human application. Rabbits, rats, mice, guinea pigs, dogs, fish, and non-human primates are all acceptable for use in preclinical studies. Nevertheless, rats (*Rattus norvegicus*) and mice (*Mus musculus*) are the most frequently utilized species due to their numerous morphological, physiological, genetic, and biochemical similarities to humans. Furthermore, these mammals are small, inexpensive to house, easy to obtain, capable of rapid growth, and can be modified at a minimal cost (Nascimento-Gonçalves *et al.*, 2024). Rats undergo natural menopause between 18 and 24 months of age; therefore, ovariectomy is frequently used to induce menopause in experimental animals due to its advantages, including ease of application, reduced cost, and rapid onset of hypoestrogenism. Moreover, ovariectomy-induced menopause shows identical key characteristics to human menopause, including a similar hormonal profile, hypothalamic-pituitary-gonadal (HPG) axis dysregulation, and cognitive and cardiometabolic changes similar to those observed in menopausal women. The predominant rat strains utilized are Sprague-Dawley and *Wistar* rats, which show comparable responses to ovariectomy (Medina-Contreras *et al.*, 2020; Yousefzadeh *et al.*, 2020). Furthermore, vaginal and urethral tissue samples must be objectively analyzed for the histopathological alterations and therapeutic response in rats with GSM (Ho *et al.*, 2009). This study aimed to provide

comprehensive and precise guidance on how to perform ovariectomy and vaginectomy in rats to obtain ovarian, vaginal, and urethral tissue simultaneously while developing suitable animal model protocols.

Materials and Methods

This study used 40 female *Wistar* rats of *R. norvegicus*, each over 200 g, ethically sourced. An anesthetic protocol was administered using ketamine-xylazine at a dose of 40 mg/kg body weight by intraperitoneal injection before the ovariectomy. Subsequently, euthanasia was conducted at the conclusion of the protocol prior to the collection of vaginal and urethral tissue from the rats, utilizing ketamine-xylazine at five times the anesthetic dose (Soewondo *et al.*, 2023).

Ethical approval

The research protocol was approved by the research ethics committee of the Faculty of Medicine, Sebelas Maret University, under the number 207/UN27.06.11/KEP/EC/2024.

Results

Urogenital system anatomy in female rats

In female rats, the ovaries are located within the adipose tissue at the posterolateral pole of the kidneys and connected by the mesovarium to the abdominal cavity's posterior wall. In mature ovaries, they form as a mass of follicles. The open end of the oviduct attaches to the ovary, while the distal end connects to the uterus. Despite the visible fusion of the uterine horns at the distal end, two different uterine ossa exist that lead into the vagina. Each uterus has an internum and an eksternum ostium and a cervical canal. The bladder empties into the urethra, which opens into the perineum at the base of the clitoris through the urethral orifice. The vaginal orifice is on the dorsal aspect of the urethra (Suckow *et al.*, 2019; Herrera-Pérez *et al.*, 2024).

Ovariectomy techniques

There are three ovariectomies techniques (Rejeki *et al.*, 2018; Yousefzadeh *et al.*, 2020; Herrera-Pérez *et al.*, 2024):

1. Double dorsolateral incision ovariectomy
2. Single midline dorsal incision ovariectomy
3. Ventral or transverse incision ovariectomy

A. Pre-operative Stage

1. The rats were anesthetized intraperitoneally with ketamine-xylazine at a dose of 40 mg/kg body weight.
2. The rat's fur is shaved with a razor, specifically in the dorsum area's midline, the dorsolateral areas on both sides, or the ventral abdomen.
3. Disinfection was performed with 70% alcohol followed by povidone iodine.

B. Double dorsolateral incision ovariectomy

1. An incision was made along the midline, approximately 1.5 cm from the right dorsolateral aspect, to access the dorsal muscle and peritoneal cavity.



Fig. 2. Pre-operative stage (A) intraperitoneal anesthesia was administered to rats; (B) shaving the fur of the rat; (C) rats disinfected and ready for surgery.

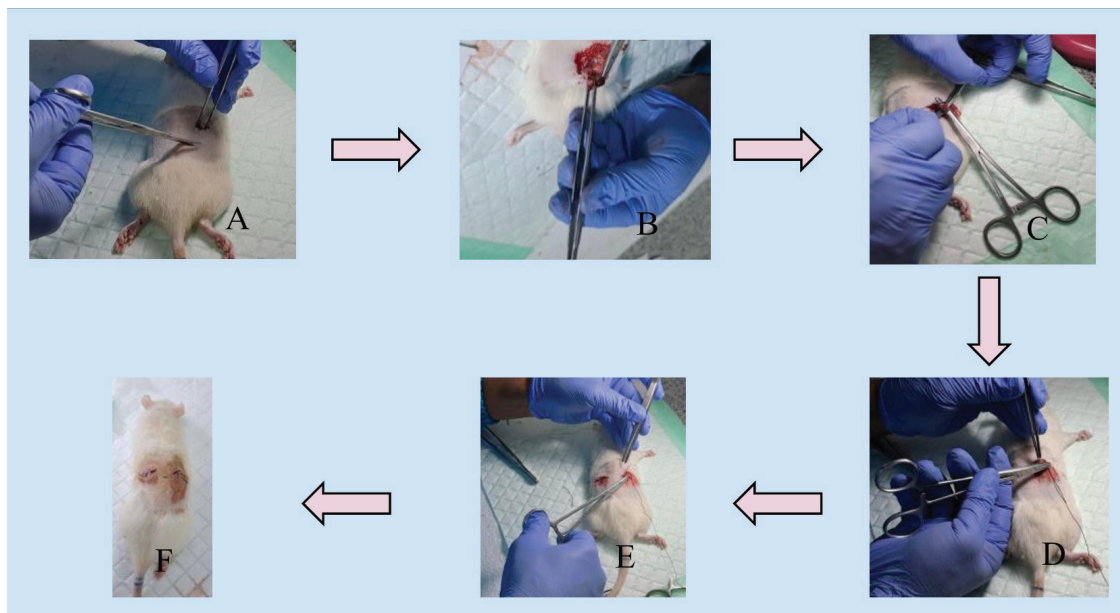


Fig. 3. Double dorsolateral incision ovariectomy (A) incision on the right dorsolateral; (B) visualization of the fat pad and ovary; (C) ligation and ovary removal; (D) suturing the muscle and skin of the rat; (E) the same procedure was performed on the contralateral ovary; (F) ovariectomy completed.

2. The skin and muscle were carefully separated using a cotton bud and blunt surgical instruments.
3. The ovaries can be identified by examining the fat pad underneath the pelvic muscle of the rat.
4. A small incision of approximately 0.5 cm is made on the fat pad.
5. The fat pad was clamped with forceps, then removed and placed on a saline-soaked wet gauze.
6. The ovary and fat pad on the uterine horn are carefully separated using two fine forceps, and any bleeding is then checked.
7. The end of the oviduct and the fat pad are ligated, approximately 3 mm from the ovary.
8. The ovary is cut and appears granular, similar to grapes.
9. The ovarian horn was returned into the abdominal cavity using sterile gauze.
10. A local anesthetic is administered into the muscle at the incision site, then both ends of the muscle are

clamped with two forceps, and a knot is made to close it using absorbable sutures.

11. A single drop of topical anesthetic is applied to the incised skin before suturing with non-absorbable sutures.
12. The suture area is disinfected with iodine-containing povidone.
13. The procedure can be repeated on the left ovary.

C. Single midline dorsal incision ovariectomy

1. An incision is made along the midline, approximately 1 cm in the mid-dorsal region and at the base of the tail.
2. The skin and muscle were carefully separated using a cotton bud and blunt surgical instruments.
3. The ovaries can be identified by examining the fat pad underneath the pelvic muscle of the rat.
4. Blood vessels must be ligated first to prevent bleeding.

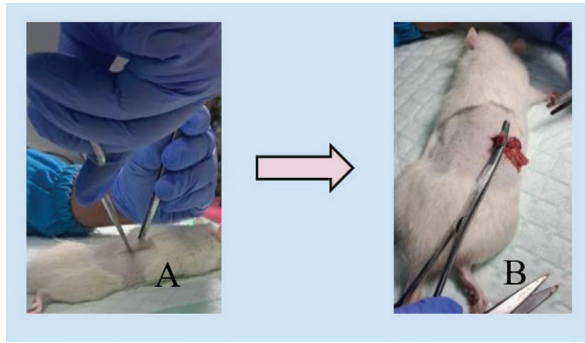


Fig. 4. Single midline dorsal incision ovariectomy (A) incision along the midline in the mid-dorsum area; (B) visualizing and removing the fat pad and ovaries using the previous method without creating additional incisions.

5. A small incision of approximately 0.5 cm is made on the fat pad.
6. The ovary and fat pad on the uterine horn are carefully separated using two fine forceps, and any bleeding is then checked.
7. The end of the oviduct and fat pad are ligated, approximately 3 mm from the ovary.
8. The ovary is cut and appears granular, similar to grapes.
9. The ovarian horn was returned into the abdominal cavity using sterile gauze.
10. The procedure is repeated on the other ovary without creating additional incisions.
11. A local anesthetic is administered into the muscle at the incision site, then both ends of the muscle are clamped with two forceps, and a knot is made to close it using absorbable sutures.
12. A single drop of topical anesthetic is applied to the incised skin before suturing with non-absorbable sutures.
13. The suture area is disinfected with iodine-containing povidone.

D. Ventral or transverse incision ovariectomy

1. A transverse incision is made in the center of the abdomen, slightly to the right, about 0.4–0.6 cm.
2. Dissect the muscle until the peritoneal cavity and the fat pad wrapping the ovary are exposed.
3. The fat pad and ovaries were carefully removed from the incision site.
4. Ligation is performed using absorbable sutures at the distal end of the uterine horn, then the ovary is cut, and bleeding is controlled.
5. The procedure is repeated on the other ovary without creating additional incisions.
6. A local anesthetic is administered into the muscle at the incision site, then both ends of the muscle are clamped with two forceps, and a knot is made to close it using absorbable sutures.
7. A single drop of topical anesthetic is applied to the incised skin before suturing with non-absorbable sutures.



Fig. 5. Transverse incision slightly to the right in the central abdomen, followed by alternating ovaries removal using the same incision site as before.



Fig. 6. Rat's ovary.

8. The suture area is disinfected with iodine-containing povidone.

E. Post-operative Stage

1. After surgery, the rats were placed in individual boxes to prevent contamination and exposed stitches.
2. Two drops of antibiotics and topical anesthetics were applied to the suturing area for 3 days.
3. The rats were provided with adequate food and water according to the standards, along with room temperature regulation and alternating dark-light lighting every 12 hour.

Technique for collecting vaginal and urethral tissue (Ho *et al.*, 2009; Koebele *et al.*, 2023; Soewondo *et al.*, 2023)

1. At the conclusion of the study, the rats were euthanized using ketamine-xylazine at five times the anesthetic dose.

2. The surgical field was sterilized, and the rat was incised along the midline ventral abdomen through skin and muscle.
3. The remaining genitourinary tissues, including the oviduct, uterus, vagina, bladder, and urethra, were isolated and removed from the abdominal fat.
4. A transverse incision of approximately 2–3 mm is made in the vagina and urethra, with the vaginal orifice (dorsal aspect) identified using a cotton bud to differentiate it from the urethral orifice.
5. The tissue was preserved in formalin solution, labeled, and delivered to the anatomical pathology laboratory for histological evaluation.

Discussion

During menopause, estrogen hormone synthesis decreases, leading to various physiological changes that might affect a woman's quality of life. GSM is one of the most common clinical manifestations. The vaginal and urethral epithelial layers contain numerous estrogen receptors; therefore, a decrease in estrogen hormones results in a variety of symptoms of the genitourinary system. These problems may be chronic and progressive, consequently affecting menopausal

women's sexual and quality of life (Sarmiento *et al.*, 2021; Vodegel *et al.*, 2023).

Natural menopause (estropause), ovariectomy, and ovariotoxins, which can accelerate ovarian failure, are all approaches for inducing menopause in rats. Estropause in rats shares similarities to human menopause, despite only 25% of estrous rats demonstrating the same hormonal profile, and this procedure takes a long time to apply. Rats induced into menopause with the ovariotoxin 4-vinylcyclohexene diepoxide (VCD) to accelerate ovarian failure have ovarian and hormonal profiles similar to most menopausal women. However, this method has limitations because VCD is toxic at high doses and requires 15 intraperitoneal injections, increasing concerns about stress on the animals. Consequently, ovariectomy is frequently considered the gold standard procedure due to its numerous advantages over the other two approaches (Medina-Contreras *et al.*, 2020).

Ovariectomy has numerous advantages, including ease of application, reduced cost, and a quick onset of hypoestrogenism. Furthermore, ovariectomy-induced menopause shows comparable main characteristics to human menopause, including a similar hormonal profile, HPG axis dysregulation, and cognitive and cardiometabolic alterations identical to those observed in menopausal women. Three ovariectomy techniques in rats include the double dorsolateral incision, single midline dorsal incision, and ventral or transverse incision.

In our study, ventral incision ovariectomy had a shorter surgical time (<10 minutes), but wound healing took longer (10–15 days), due to the rat's anatomical position, which causes the incision wound on the abdomen to be pressed and constantly in contact with the bedding, making it more vulnerable to breaking and infection (Rejeki *et al.*, 2018; Yousefzadeh *et al.*, 2020; Herrera-Pérez *et al.*, 2024). This contradicts other studies that have reported that ventral incision ovariectomy wounds heal faster, in less than 9 days, and is a painless surgical technique. This technique also involves manipulation of the abdominal organs and is associated with a greater mortality rate of 30%



Fig. 7. Post-operative stage.

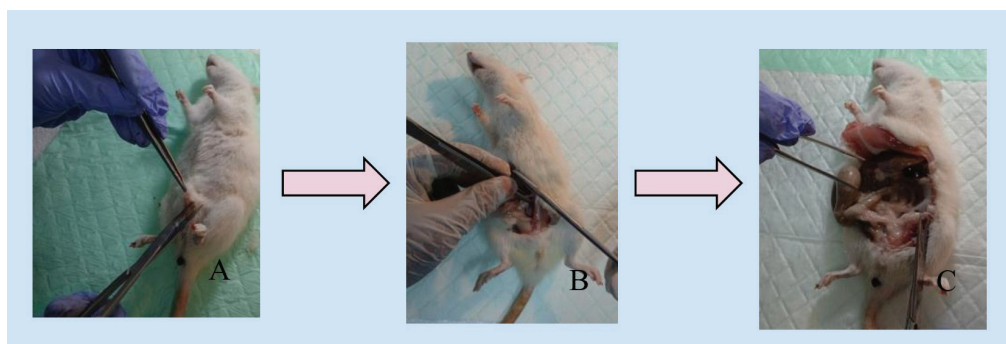


Fig. 8. Vaginectomy in rats (A) rat after anesthesia and disinfection; (B) incision on the midline of the ventral abdomen; (C) separation of the genitourinary tissue from the abdomen.

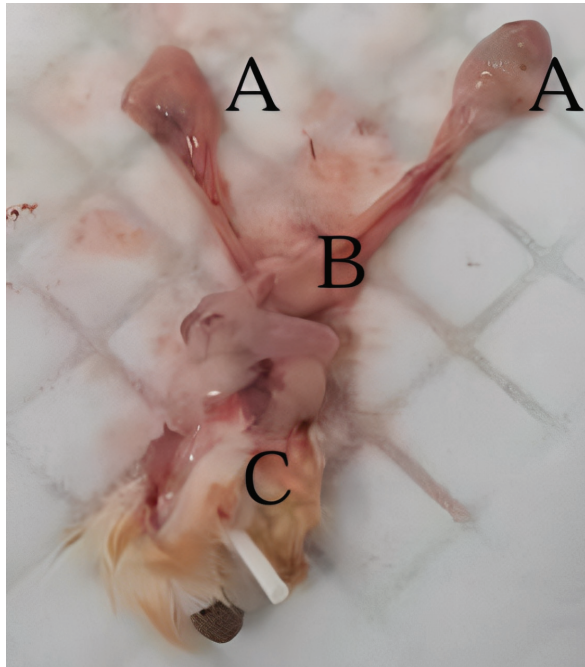


Fig. 9. (A) Oviduct; (B) uterus; (C) vagina (dorsal) and urethra (ventral).

within the first 24 hour, making it less recommended (Khajuria *et al.*, 2012; Yousefzadeh *et al.*, 2020; Herrera-Pérez *et al.*, 2024). The single midline dorsal incision technique requires a larger incision to access the right and left ovary from the dorsal midline, resulting in a longer surgery and wound healing time. In this study, the surgery time for the single midline dorsal incision technique was more than 15 minutes, with a wound healing time of 10–15 days. The double dorsolateral incision has the advantages of accelerated wound healing, a shorter recovery time, and a lower mortality rate with a smaller incision wound. This ovariectomy technique has a surgical time of less than 10 minutes, with a wound healing time of less than 10 days. This incision technique is the most recommended (Yousefzadeh *et al.*, 2020).

Subsequently, to find objective evidence of GSM, vaginal and urethral tissues were collected through vaginectomy when the rats reached menopause after day 21 post-ovariectomy, and histological analysis was performed. At the conclusion of the study, tissue collection through vaginectomy is essential to evaluate the response of vaginal and urethral tissues to a treatment intervention (Rejeki *et al.*, 2018; Ye *et al.*, 2018).

Conclusion

GSM is a condition that significantly impacts the quality of life of menopausal women. Consequently, the development of novel therapeutic modalities or the improvement of current therapies remains essential.

Therefore, comprehensive and precise protocols for the development of suitable animal models are required to guarantee the safety and efficacy of the therapeutic modalities.

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Conflict of interest

The authors declare no conflict of interest.

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Authors' contributions

Conception and design: A.A.; administrative support: A.A and S.; collection and assembly of data: A.A, S, I.N, and U.R.B; data analysis and interpretation: A.A, P.D, E.P.P, and B.W; manuscript writing: all authors; and final approval of the manuscript: all authors.

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