JSHC
Japan Society of
Histochemistry and Cytochemistry
Established 1959

Acta Histochem. Cytochem. 56 (6): 127–136, 2023
doi: 10.1267/ahc.23-00052

*Regular Article*

# A Single Administration of Progesterone during the Neonatal Period Shows No Structural Changes in Male Reproductive Tracts in Mice

**Takuya Omotehara[1,2], Hiroki Nakata[3], Kenta Nagahori[2,4], Miyuki Kuramasu[2], Koichiro Ichimura[1] and Masahiro Itoh[2]**

*[1]Department of Anatomy and Life Structure, Juntendo University Graduate School of Medicine, Tokyo, Japan, [2]Department of Anatomy, Tokyo Medical University, Tokyo, Japan, [3]Department of Clinical Engineering, Faculty of Health Sciences, Komatsu University, Ishikawa, Japan and [4]Division of Basic Medical Science, Department of Anatomy, Tokai University School of Medicine, Kanagawa, Japan*

Received August 2, 2023; accepted November 10, 2023; published online December 23, 2023

The concentration of female-dominant steroid hormones, such as progesterone and estrogen, drops after birth in neonates. We have reported that neonatal estrogen treatment results in inflammation in the epididymis after puberty in male mice. Our recent study discovered that progesterone receptor was specifically expressed in efferent ducts just before birth in male mice. Therefore, this study aimed to reveal the impact of neonatal progesterone administration on the efferent ducts after puberty. Progesterone was subcutaneously administered to neonatal mice on their birthday in three groups: high-dose (200 mg/kg), low-dose (8 mg/kg), and control (cottonseed oil). Their testis and epididymis were collected at 12 weeks old. Semi-serial paraffin sections of these tissues were prepared and evaluated through PAS-hematoxylin staining. Efferent ducts were reconstructed into a three-dimensional structure, and their length and volume were analyzed. Spermatogenesis in the testis and epithelium of the tracts appeared normal, even in individuals administered with progesterone. There were no significant differences in the length and volume of the efferent ducts among the three groups. This study suggests that progesterone treatment in neonatal mice does not cause any structural changes in the male reproductive tracts at puberty, unlike the neonatal estrogen treatment.

**Key words:** progesterone, efferent duct, epididymal duct, three-dimensional reconstruction

## I. Introduction

After birth, fetuses are exposed to a new and different endocrinological environment. Female-dominant sex hormones, such as estrogen and progesterone (P4), which can be transferred from mother to fetus, decrease in the offspring after birth. In our previous study, male mice which were exposed to estrogen once in their neonatal stage developed epididymal inflammation and obstruction of the epididymal duct after puberty [28]. This delayed effect may be due to the rapture of the epithelial barrier in the epididymis caused by estrogen exposure, which leads to immune cells recognizing and attacking spermatozoa transported from the testis after the onset of spermatogenesis in the testis.

During pregnancy, P4 levels are high in the mother's body to support gestation. However, in rodents (but not in human), P4 levels decrease before parturition [7, 8, 14, 27, 42], which triggers the onset of labor [25, 41, 42]. P4 in the fetus is mainly derived from the mother [5], and the concentration of P4 is the same in male and female fetuses [44]. Therefore, the P4 levels in the offspring decrease nat-

Correspondence to: Takuya Omotehara, Department of Anatomy and Life Structure, Juntendo University Graduate School of Medicine, 2–1–1 Hongo, Bunkyo-Ku, Tokyo, 113–8421, Japan.
E-mail: t.omotehara.hb@juntendo.ac.jp

urally during the perinatal period.

Our recent study discovered that just before birth, murine efferent ducts express progesterone receptor (PGR) exclusively [31]. Although PGR was enriched in the efferent ducts of matured men [23], it was not detected in the epididymis of neonatal to adult men [26]. PGR may play a crucial role in the development of efferent ducts, but there has been limited research on the role of P4 in the development and function of the male reproductive tracts. This is probably due to the fact that male mice lacking PGR showed normal fertility [24]. According to a previous study [38], male sheep fetuses had low levels of metabolic enzymes for P4 in their liver. Additionally, P4 administration during early pregnancy induced the downregulation of male-specific genes. Another study reported that epididymal cysts were caused in some aged mice which were administered with P4 neonatally for five days [15]. However, the adverse effects of P4 on the pubertal male have not been reported. Therefore, further investigation is required to determine whether an overdose of P4 can affect the male reproductive system in the pubertal male. Furthermore, a comparison of the impact of neonatal treatment with P4 and estrogen will contribute to understanding the importance of these hormones in neonatal males.

The efferent ducts consist of some convoluted ductules that run parallel from the rete testis to the epididymis. These ductules converge into a single common efferent ductule, which then connects to the initial segment of the epididymis [10, 29]. Their main function is to absorb luminal fluid from the testis [2, 9, 12]. If this absorption is disturbed, the increased fluid can cause back pressure, which impairs spermatogenesis in the testis [11, 21, 30, 36]. Furthermore, obstructive lesions in the epididymis, especially in the proximal region, including the efferent ducts are not rare [1]. Patients with obstruction in the proximal region exhibit lower sperm motility and vitality than those with obstruction in the caudal region [34]. Therefore, it is important to understand what causes malformation and malfunction of the proximal region, including the efferent ducts.

For precise evaluation of the efferent ducts, three-dimensional (3-D) analysis is necessary. We have been using a 3-D reconstruction method to investigate the structure and development of the efferent ducts [29, 32]. This technique enables us to explore not only branching patterns but also the length and volume of each segment of the ducts. Although a previous study reported that P4 administered to a mother for prevention of preterm birth showed no evidence of benefit or harm in child development [39], a potential risk of P4 treatment on the development of the male reproductive tracts may be found in a detailed examination using 3-D analysis. Herein, we investigated the impact of neonatal exposure to an overdose of P4 on the development and function of the male reproductive tracts using the 3-D reconstruction method.

## II. Materials and Methods

### *Neonatal progesterone administration (NPA)*

This study was approved by the Institutional Animal Care and Use Committee of Tokyo Medical University (Permission #R3-0060). C57BL/6J mice at pregnancy day 14 were purchased from Japan SLC, Inc. In general, if researchers handle newborn mice, primiparous mother mice may kill their babies. To minimize this risk, female mice which have given birth previously were chosen as mothers in this study. The mice were maintained at a specific pathogen-free facility at Tokyo Medical University. P4 was purchased from Sigma-Aldrich (catalog no. P8783) and dissolved in cottonseed oil (Nacalai Tesque). For a high-dose group (NPA-H), a 15 mg/ml solution was prepared (near-saturated solution). This solution was diluted to 0.6 mg/ml for a low-dose group (NPA-L). P4 administration was performed according to the previous study in which a high dose of estrogen was subcutaneously administered to neonatal mice [28]. A pup was given 20 μl of the administration. Because the weight of the pups was 1 to 1.5 g, the injected concentration was about 200 and 8 mg/kg in the NPA-H and -L groups, respectively. The dose in NPA-H (200 mg/kg) was about twice as high as in a previous study [15]. The low dose was referred to a previous study in which P4 was used as a treatment for brain ischemia in adult mice [4]. As a control group (NPA-Ct), the solvent was administered. P4 solution was subcutaneously injected into the back of the newborn male on the morning of birth. When the delivery was not confirmed in the morning but in the evening, the administration was performed around 6:00 PM. The number of offspring was not controlled among mothers (7 males and 4 females in NPA-Ct, 8 males and 5 females in NPA-L, and 6 males and 2 females in NPA-H from two mother mice in each group). They were weaned on postnatal day 21. These mice were kept at 21–25 degC and 40%–60% relative humidity with a 12-hour light-dark cycle.

### *Tissue collection*

Four male neonates in a different litter from the administered mice were collected for the immunohistochemical investigation of PGR. They were anesthetized by inhalation of isoflurane and euthanized by decapitation. Their testes with adjacent epididymis were collected and fixed in modified Davidson's fluid [20] for 24 hr at ambient temperature. Male mice at 12 weeks old were weighed and anesthetized by intraperitoneal injection with a combination of 0.3 mg/kg of medetomidine (Domitor®, Nippon Zenyaku Kogyo Co., Ltd., Tokyo, Japan), 4.0 mg/kg of midazolam (Dormicum®, Maruishi Pharmaceutical. Co., Ltd., Tokyo, Japan), and 5.0 mg/kg of butorphanol (Vetorphale®, Meiji Animal Health Co., Ltd., Tokyo, Japan) [18]. After euthanization by cervical dislocation, the spleen, left testis, and left epididymis were removed and weighed. The right testis with adjacent epididymis was fixed in modified Davidson's

fluid for 24 hr at ambient temperature. The fixed tissues were dehydrated and embedded in paraffin, as previously reported [33]. The neonatal and adult testes were cut in 5-μm-thick serially for immunohistochemistry and 4-μm-thick at 32-μm-intervals for histology, respectively.

***Immunohistochemistry***

To check the PGR expression in the neonatal efferent ducts, immunohistochemistry was performed according to a previous study [31]. The primary and secondary antibodies were anti-Progesterone Receptor antibody [SP42] (1:50, ab101688, RRID: AB_10715248, abcam) and EnVision rabbit for DAB (Agilent Dako), respectively. To detect their localization, 3-amino-9-ethylcarbazole (AEC substrate kit, Peroxidase (HRP), SK-4200, Vector) was reacted. Gill's hematoxylin V (Muto Pure Chemicals) was used for counterstain. After coverslipped in Aquatex (Merck), the sections were digitized with a virtual slide scanner, Panoramic MIDI II (3DHISTECH), at ×20 magnification.

***Histology***

The sections were deparaffinized and rehydrated, and PAS-hematoxylin staining was performed as follows. The slides were immersed in 1% periodic acid solution for 5 min at ambient temperature. After being washed in flowing tap water and distilled and deionized water (DW), the sections were reacted in cold Schiff solution (Muto Pure Chemicals) for 30 min at ambient temperature. They were washed in DW and flowing tap water for at least 5 min. After being washed in DW, the sections were immersed in Gill's hematoxylin V (Muto Pure Chemicals) for 10 sec as a counterstain and then immersed in tap water at 40 degC. After being washed in DW, the slides were coverslipped in Marinol (Catalog No. 20092, MUTO PURE CHEMICALS). The sections were digitized as above.

***Three-dimensional (3-D) analysis***

The tissues from four neonatal and three adult males in each group were analyzed with Amira software (version 2020.2, Thermo Fisher Scientific). The scanned images of the neonates and adults were exported as jpg-formatted files at 4 and 2.5 magnification in 2833:2125 and 3840:2160 pixels, respectively. The serial sections were aligned in the software, and the efferent ducts were thoroughly segmented. After the segmented area was shrunk four times in the software, the core lines running through the center of the reconstructed ducts were drawn, and their length and volume were calculated in the software.

***Statistical analysis***

Obtained numerical values were analyzed by the Tukey-Kramer test [19] to compare each group using R software (version 4.1.2 [37]). Statistical significance was recorded when the *p*-value was under 0.05.

## III. Results

***Expression patterns of PGR on the day of administration***

To check the site of PGR expression through male reproductive tracts in the neonatal mouse, immunohistochemistry for PGR and 3-D reconstruction were performed (Fig. 1). Arrangement of each compartment in the neonatal testis was similar to that of the pubertal testis (Fig. 1A–C). Each convoluted efferent ductule was separated from a single common ductule, as shown in fetal mesonephric tubules [32]. Epithelial cells of the efferent ducts, especially in the common ductule and each ductule near the common ductule, showed positive for PGR (Fig. 1D, E). On the other hand, PGR was negative in efferent ductules near the rete testis and testis, similar to expression patterns of PGR on embryonic day 18.5 observed in a previous study [31].

***No histological changes were observed in the testis***

The absolute and relative weights of the body, testis, epididymis, and spleen were not significantly different between groups (Fig. 2). The contralateral testis was collected for histology and stained with PAS-hematoxylin. Spermatogenic cells, such as spermatogonia, spermatocytes, spermatids, and spermatozoa, were present in all groups without any obvious abnormality (Fig. 3A–F). There was also no dilation and accumulation of spermatozoa in the rete testis in all groups (Fig. 3G–I). Therefore, spermatogenesis and transfer of the spermatozoa appeared normal in all groups.

***No histological changes were observed in the epididymis***

Even when spermatogenesis in the testis appears normal, inflammation may occur in the epididymis, as in the neonatal estrogen treatment [28]. Therefore, we next checked the histology of the epididymis (Fig. 4A–F). Numerous spermatozoa were observed in the lumina of the cauda epididymis in all groups. None of the groups showed any signs of cysts or inflammation, such as immune cells invading the epithelium or lymphoid cells accumulating in the mesenchyme.

***No structural changes were observed in the histology and 3-D reconstruction of the efferent ducts***

Before birth, PGR is specifically expressed in the epithelium of efferent ducts [31]. It is therefore hypothesized that an overdose of P4 directly affects the development and function of the efferent ducts. The efferent ducts consist of two segments - a vast lacuna (Fig. 4G–L) and a narrow lacuna (Fig. 4M–R) - surrounded by the cuboidal epithelium. In a section, these structures were found to be similar across groups without any sign of inflammation or obstructive lesions.

In a 3-D model (Fig. 5), the efferent ducts were coiled similar to the previous study [29]. It was found that three to five ductules were connected to the rete testis. There were also one to three blind-ending ductules that did not have

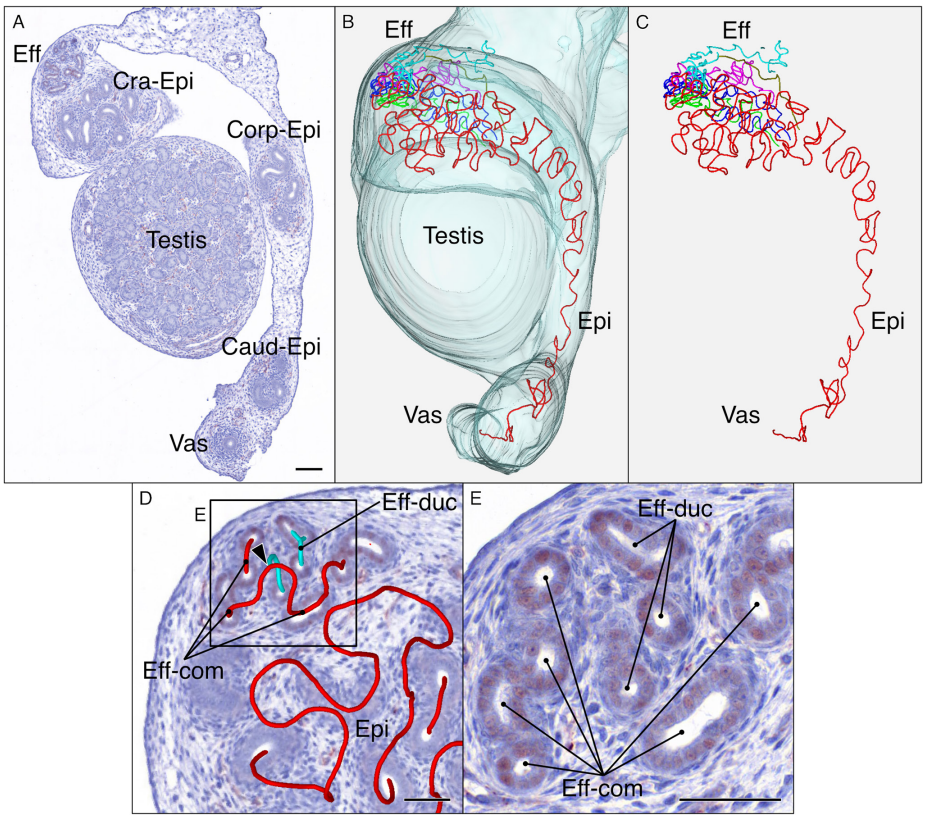


**Fig. 1.** PGR expression in the efferent duct in the neonatal male mouse. (**A**) A representative image of immunohistochemistry for PGR at low magnification. (**B, C**) A ventral view of the reconstructed left testis and epididymis. The pale blue surface represents the reconstructed testis, efferent duct, and epididymis. A red line indicates the common efferent ductule and following epididymal duct, and lines in other colors represent efferent ductules that run parallel from the rete testis to the epididymis. (**D, E**) A representative image of immunohistochemistry for PGR in the efferent and epididymal ducts. An arrowhead indicates the branching point of the efferent ductule (Eff-duc) from the common efferent ductule (Eff-com). A boxed region in panel **D** is magnified in panel **E**. Note that PGR is positive in the epithelium of the efferent ducts. Caud-Epi: Caudal epididymis, Corp-Epi: Corpus epididymis, Cra-Epi: Cranial epididymis, Eff: Efferent duct, Epi: Epididymal duct, Vas: Vas deferens. Bars = 100 μm (**A**), 50 μm (**D, E**).

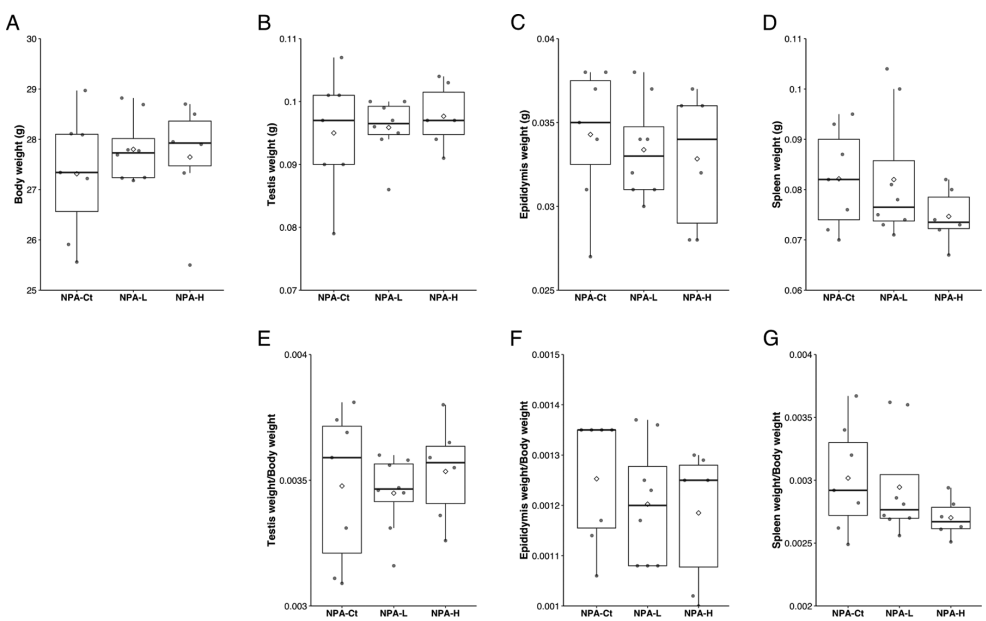


**Fig. 2.** Weight of body, testis, epididymis, and spleen in neonatal progesterone administration (NPA) groups with control (Ct), low (L), and high (H) doses. White diamonds represent the average weight of each group.

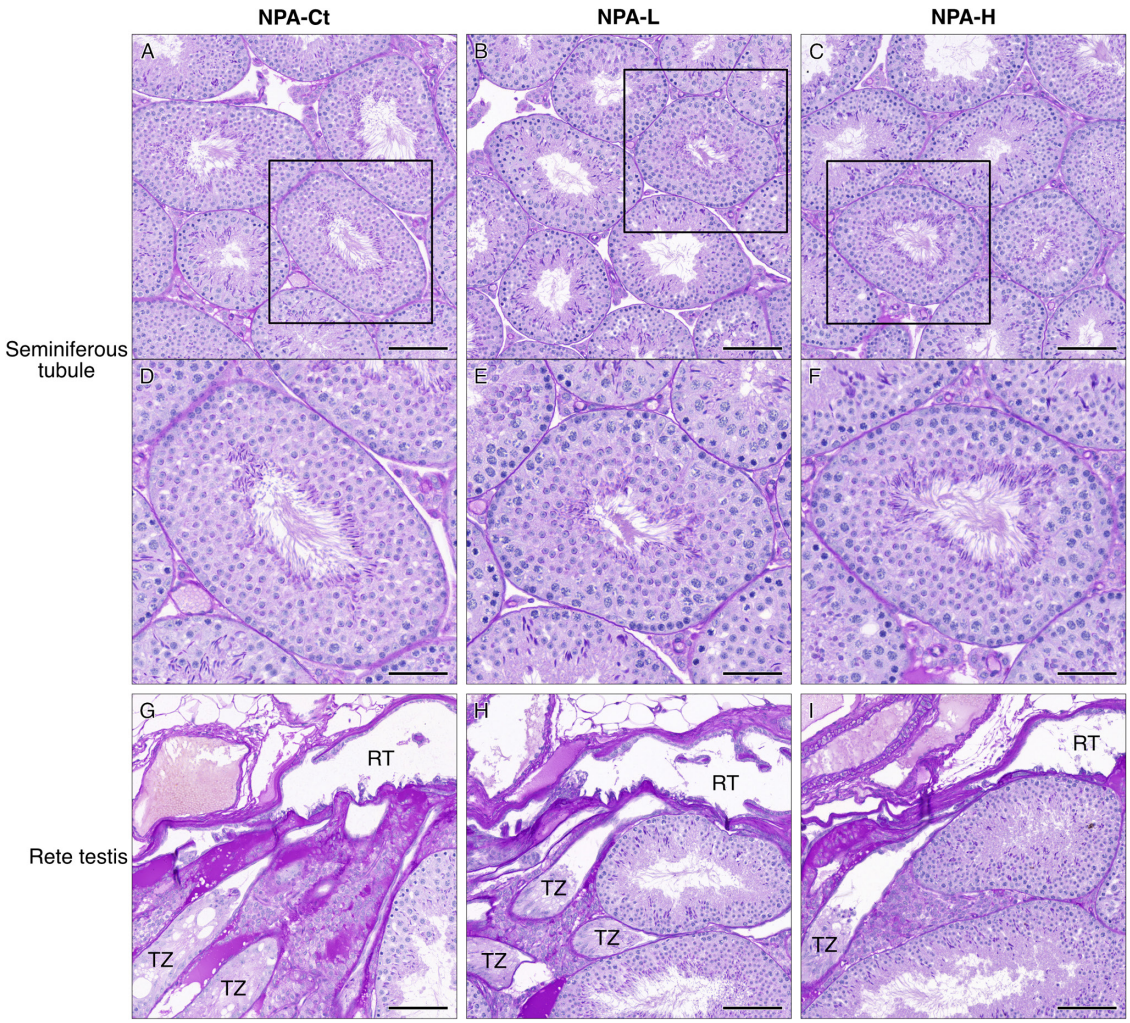


**Fig. 3.** Histology of the testis in neonatal progesterone administration (NPA) groups with control (Ct), low (L), and high (H) doses. (**A–F**) Representative pictures of seminiferous tubules. A boxed region in **A–C** is magnified in **D–F**, respectively. (**G–I**) Representative pictures of the rete testis (RT). TZ: Transitional zone of a seminiferous tubule. Bars = 100 μm (**A–C, G–I**), 50 μm (**D–F**).

contact with the rete testis in all individuals, in contrast to the previous study that reported wild-type male mice rarely had these blind-ending ductules [11, 29]. Some ductules merged into a single common ductule that continued to the initial segment of an epididymal duct with high columnar epithelium (Fig. 4M–R). The common ductule was convoluted in the efferent duct and at the surface of the caput epididymis (Fig. 5).

The reconstructed efferent ducts visually show a complex structure (Fig. 6A), but their branching pattern was similar to that of fetal mesonephric tubules [32]. There were no significant differences in the length and volume of each efferent ductule among the groups (Fig. 6B, C). The length and volume of each segment of the common efferent ductule also showed no significant difference (Fig. 6D, E). Additionally, there was no significant difference in the total length and volume of the efferent ducts between the groups (Fig. 6F, G).

## IV. Discussion

This study investigated the impact of neonatal P4 exposure on the male reproductive tracts after puberty. There were no apparent changes in the testis, epididymis, and efferent ducts between the groups. Additionally, there were no significant differences in the length and volume of the efferent ducts. These findings suggest that administering P4 once during the neonatal period does not impact the structure of male reproductive tracts during puberty, even in the high dose.

We hypothesized that neonatal P4 administration would lead to malfunction of efferent ducts that would impair sperm transport and spermatogenesis in pubertal male mice. Another suspected outcome was the elongation of the efferent ducts in P4-treated mice. However, the results of this study showed no changes in the structure of the male reproductive tracts against these hypotheses.

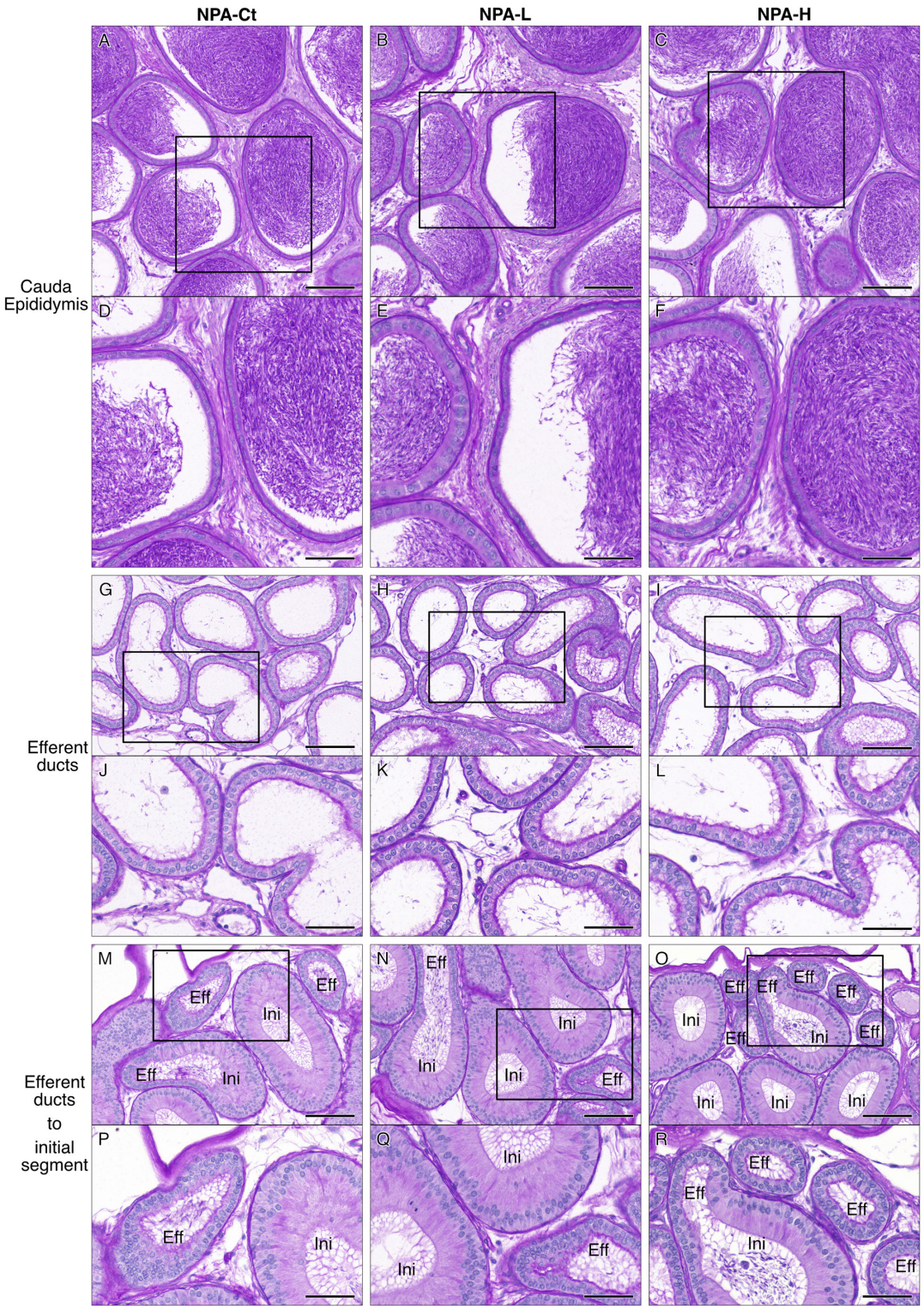


**Fig. 4.** Histology of the cauda epididymis and efferent ducts in neonatal progesterone administration (NPA) groups with control (Ct), low (L), and high (H) doses. (**A–F**) Representative pictures of the cauda epididymis. A boxed region in **A–C** is magnified in **D–F**, respectively. (**G–L**) Representative pictures of the efferent ductules that run parallel from the rete testis to the epididymis. A boxed region in **G–I** is magnified in **J–L**, respectively. (**M–R**) Representative pictures of a transitional region from the common efferent ductule (Eff) to the initial segment (Ini) of the epididymal duct. A boxed region in **M–O** is magnified in **P–R**, respectively. Bars = 100 μm (**A–C, G–I, M–O**), 50 μm (**D–F, J–L, P–R**).

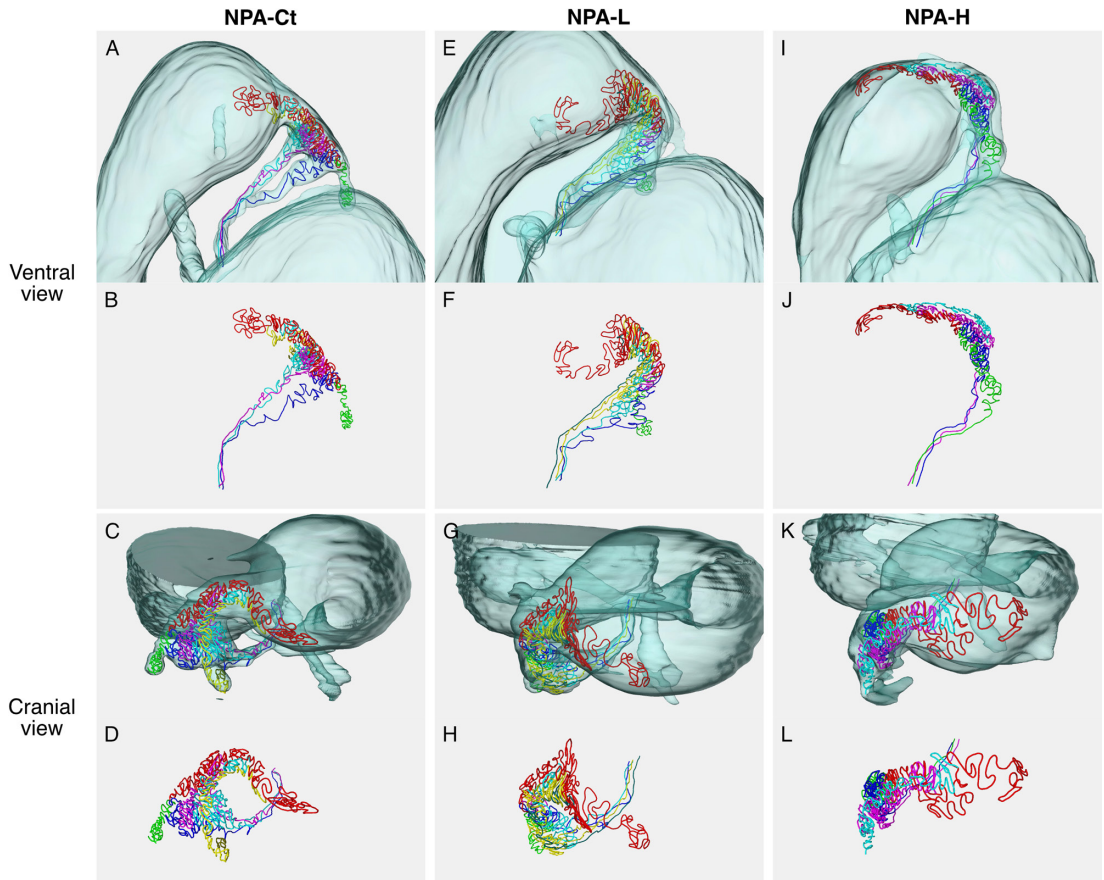


**Fig. 5.** Representative three-dimensional reconstruction images of the efferent ducts in the ventral and cranial views in neonatal progesterone administration (NPA) groups with control (Ct), low (L), and high (H) doses. The pale blue surface represents the reconstructed testis, efferent duct, and epididymis (**A, C, E, G, I, K**). A red line indicates the common efferent ductule, and lines in other colors represent efferent ductules that run parallel from the rete testis to the epididymis.

These results suggest that PGR expressed in the neonatal efferent ducts is not crucial for the absorption of luminal fluid by the epithelium during puberty. Furthermore, PGR expressed in the efferent ducts during the perinatal stage is suggested not to be associated with proliferation of the epithelial cells. Because PGR-knockout male mice showed normal fertility [24], expression of PGR in the efferent ducts during the perinatal period may not be necessary for their proper development and functions.

Compared to neonatal estrogen treatment, by which a delayed effect was observed in the testis and epididymis after puberty [6, 15, 28], pubertal male mice that received the neonatal P4 treatment showed no noticeable change in this study. To explain this difference, there are three possibilities to be considered. First, expression patterns of PGR and estrogen receptor (ESR1) are different. PGR is found only in the epithelium of the efferent ducts, but ESR1 is expressed in the mesenchymal cells around the epididymal duct as well as the efferent ducts just before birth [31]. Because inflammation observed in the epididymis by the neonatal estrogen treatment began from the caudal region, mesenchymal cells expressing ESR1 are suggested to mediate the estrogen effects to the epithelium, resulting in dysregulation of the epithelial barrier at the caudal epididymis [31]. Second, the concentration of estrogen and P4 in a mother mouse just before parturition is 0.1–0.2 ng/ml and 10–50 ng/ml plasma, respectively [7, 8, 13, 35]. Administered solution of estrogen was 3 mg/ml in the previous study [28], and that of P4 was 15 mg/ml in the NPA-H group of the present study. Therefore, the fold change in the amount of estrogen between the physiological condition and the administered dosage was much greater than that of P4. Third, P4 is an intermediate form of steroids, and therefore, it may be easier for the fetus to metabolize P4 into other forms. Taken together, severe effects can be observed in the overdose of estrogen but not P4.

Alternatively, it is hypothesized that PGR suppresses the malfunction of estrogen receptor in the efferent duct. PGR is known to play an antagonistic role against estrogenic actions mediated by estrogen receptor [17]. The efferent duct is the only portion where both PGR and estrogen receptor are expressed among male genital tracts [31]. A previous study reported that there were no positive and negative effects on the reproductive organs in the aged

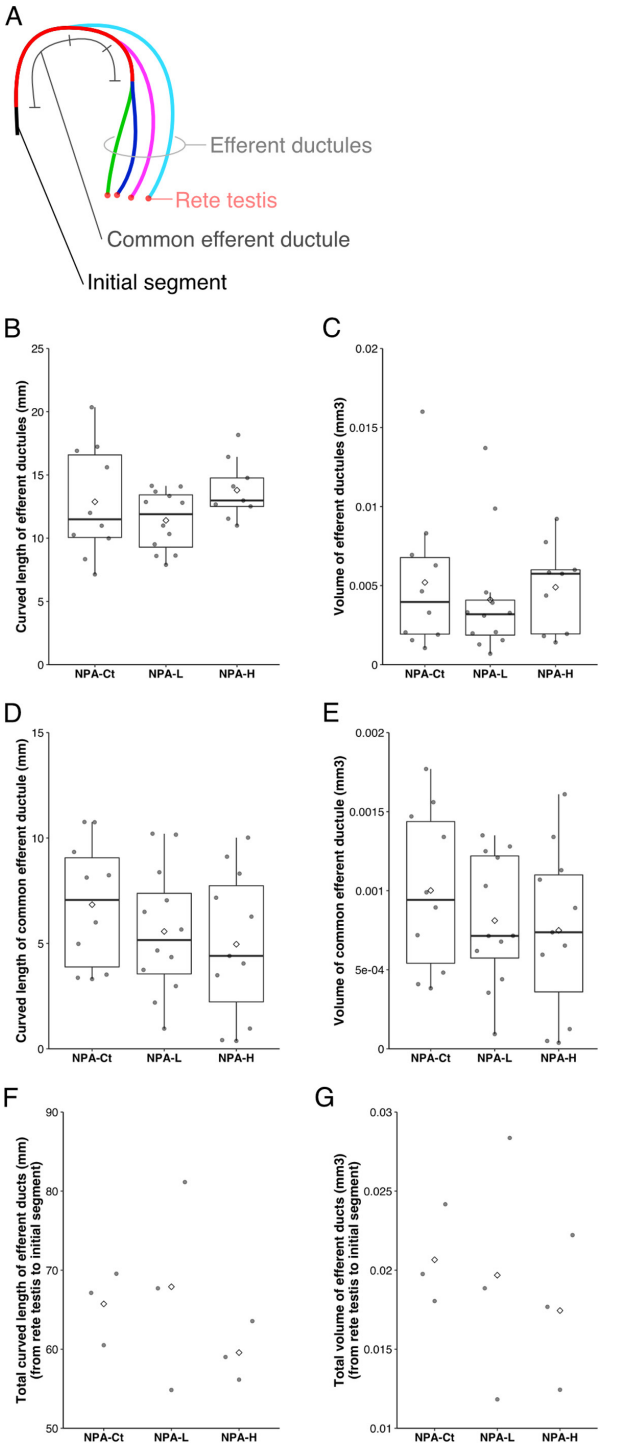


**Fig. 6.** Length and volume of efferent ducts in a 3-D model among neonatal progesterone administration (NPA) groups with control (Ct), low (L), and high (H) doses. (**A**) A scheme of a branching pattern of efferent ducts. Color of each segment corresponds to that of reconstructed ductules in Fig. 5. (**B, C**) Box and dot plot on the length and volume of each efferent ductule, respectively. (**D, E**) Box and dot plot on the length and volume of each segment of the common efferent ductule, respectively. (**F, G**) Dot plot on the total length and volume of the efferent ducts from the rete testis to the initial segment of the epididymis, respectively. White diamonds represent the average length or volume of each group.

male mouse by administration of both estrogen and progesterone during the neonatal period, compared to administration of estrogen alone [15]. On the other hand, because PGR knock-out male mice had fertility [24], more detailed studies have not been performed with a focus on the function of PGR in the male genital tracts. Therefore, to test the hypothesis that PGR suppresses the malfunction of estrogen receptor in the neonatal efferent duct, it would be worth investigating adverse effects of estrogen in the PGR knock-out male in future studies.

P4 treatment is applied to women to reduce the risk of miscarriage and preterm birth [3, 43]. Especially in the latter case, PGR may be expressed in the efferent ducts in the male human fetus like in the mouse [31]. However, the effects of P4 on male reproductive tracts have been less considered. The major function of the efferent ducts is to absorb luminal fluid from the testis, which contributes to concentrating spermatozoa and facilitating the flow of the fluid from the seminiferous tubule to the epididymal duct [2, 16]. Any disturbance to this function can lead to dilation of the rete testis and efferent ducts, resulting in failure of spermatogenesis [10, 22]. The dose in NPA-H (200 mg/kg) was about twice as high as in a previous study [15], and that in NPA-L (8 mg/kg) was as high as a dose used to treat ischemia in the brain [4] and higher than the dose of vaginal P4 treatment (200 mg suppository) used for preventing preterm birth [40] (equivalent to 4 mg/kg in a 50 kg body weight). However, no obvious structural changes were found in the male reproductive tracts, even in the NPA-H groups in this study. These results may support a previous report that P4 administered to a mother for prevention of preterm birth has no evidence of benefit or harm in child development [39]. On the other hand, a previous study reported that some aged mice showed adverse effects of neonatal P4 treatment [15], implying that there are unknown factors that can affect the sensitivity of individuals to P4 exposure depending on their unique conditions. Future studies should investigate how P4 exposure during the perinatal period can affect the male reproductive tracts more precisely to reveal whether P4 treatment for pregnant women can affect their male children.

In conclusion, it is suggested that P4 exposure in newborn male mice does not impact the structure of the testis, epididymis, and efferent ducts at puberty, unlike neonatal estrogen treatment. These results help us understand the importance of female-dominant sex hormones in neonatal males.

## V. Acknowledgments

We appreciate Mr. Shuichi Yamazaki's technical assistance in making serial paraffin sections. We thank Ms. Xi Wu and Ms. Yuki Ogawa for their assistance in routine staining, image processing, and secretary works.

## VI. Conflicts of Interest

The authors declare that there are no conflicts of interest.

## VII. References


1. Ball, R. Y. and Mitchinson, M. J. (1984) Obstructive lesions of the genital tract in men. *J. Reprod. Fertil.* 70; 667–673.
2. Clulow, J., Jones, R. C. and Hansen, L. A. (1994) Micropuncture and cannulation studies of fluid composition and transport in the ductuli efferentes testis of the rat: comparisons with the homologous metanephric proximal tubule. *Exp. Physiol.* 79; 915–928.
3. Di Renzo, G. C., Tosto, V., Tsibizova, V. and Fonseca, E. (2021) Prevention of Preterm Birth with Progesterone. *J. Clin. Med. Res.* 10; 4511.
4. Gibson, C. L. and Murphy, S. P. (2004) Progesterone enhances functional recovery after middle cerebral artery occlusion in male mice. *J. Cereb. Blood Flow Metab.* 24; 805–813.
5. González-Orozco, J. C. and Camacho-Arroyo, I. (2019) Progesterone Actions During Central Nervous System Development. *Front. Neurosci.* 13; 503.
6. Goyal, H. O., Robateau, A., Braden, T. D., Williams, C. S., Srivastava, K. K. and Ali, K. (2003) Neonatal estrogen exposure of male rats alters reproductive functions at adulthood. *Biol. Reprod.* 68; 2081–2091.
7. Hashimoto, H., Eto, T., Endo, K., Itai, G., Kamisako, T., Suemizu, H., *et al.* (2010) Comparative study of doses of exogenous progesterone administration needed to delay parturition in Jcl: MCH(ICR) mice. *Exp. Anim.* 59; 521–524.
8. Hau, J. and Skovgaard Jensen, H. J. (1987) Diagnosis and monitoring of pregnancy in mice: correlations between maternal weight, fetal and placental mass and the maternal serum levels of progesterone, pregnancy-associated murine protein-2 and alpha-fetoprotein. *Lab. Anim.* 21; 306–310.
9. Hess, R. A., Bunick, D., Lee, K. H., Bahr, J., Taylor, J. A., Korach, K. S., *et al.* (1997) A role for oestrogens in the male reproductive system. *Nature* 390; 509–512.
10. Hess, R. A. (2002) The Efferent Ductules: Structure and Functions. In "The Epididymis: from Molecules to Clinical Practice", ed. by B. Robaire and B. T. Hinton, Springer, New York, NY, pp. 49–80.
11. Hess, R. A., Bunick, D., Lubahn, D. B., Zhou, Q. and Bouma, J. (2000) Morphologic Changes in Efferent Ductules and Epididymis in Estrogen Receptor-α Knockout Mice. *J. Androl.* 21; 107–121.
12. Hess, R. A., Sharpe, R. M. and Hinton, B. T. (2021) Estrogens and development of the rete testis, efferent ductules, epididymis and vas deferens. *Differentiation* 118; 41–71.
13. Holinka, C. F., Tseng, Y. C. and Finch, C. E. (1979) Impaired preparturitional rise of plasma estradiol in aging C57BL/6J mice. *Biol. Reprod.* 21; 1009–1013.
14. Johansson, E. D. (1969) Plasma levels of progesterone in pregnancy measured by a rapid competitive protein binding technique. *Acta Endocrinol.* 61; 607–617.
15. Jones, L. A. (1980) Long-term effects of neonatal administration of estrogen and progesterone, alone or in combination, on male BALB/c and BALB/cfC3H mice. *Proc. Soc. Exp. Biol. Med.* 165; 17–25.
16. Kanazawa, Y., Omotehara, T., Nakata, H., Hirashima, T. and Itoh, M. (2022) Three-dimensional analysis and in vivo imaging for sperm release and transport in the murine seminiferous tubule. *Reproduction* 164; 9–18.
17. Katzenellenbogen, B. S. (2000) Mechanisms of action and cross-talk between estrogen receptor and progesterone receptor pathways. *J. Soc. Gynecol. Investig.* 7; S33–7.
18. Kawai, S., Takagi, Y., Kaneko, S. and Kurosawa, T. (2011) Effect of three types of mixed anesthetic agents alternate to ketamine in mice. *Exp. Anim.* 60; 481–487.
19. Kramer, C. Y. (1956) Extension of multiple range tests to group means with unequal numbers of replications. *Biometrics.* 12; 307.
20. Latendresse, J. R., Warbrittion, A. R., Jonassen, H. and Creasy, D. M. (2002) Fixation of testes and eyes using a modified Davidson's fluid: Comparison with Bouin's fluid and conventional Davidson's fluid. *Toxicol. Pathol.* 30; 524–533.
21. Lee, K. H., Hess, R. A., Bahr, J. M., Lubahn, D. B., Taylor, J. and Bunick, D. (2000) Estrogen receptor alpha has a functional role in the mouse rete testis and efferent ductules. *Biol. Reprod.* 63; 1873–1880.
22. Lee, K.-H., Park, J.-H., Bunick, D., Lubahn, D. B. and Bahr, J. M. (2009) Morphological comparison of the testis and efferent ductules between wild-type and estrogen receptor alpha knockout mice during postnatal development. *J. Anat.* 214; 916–925.
23. Légaré, C. and Sullivan, R. (2020) Differential gene expression profiles of human efferent ducts and proximal epididymis. *Andrology.* 8; 625–636.
24. Lydon, J. P., DeMayo, F. J., Funk, C. R., Mani, S. K., Hughes, A. R., Montgomery, C. A., *et al.* (1995) Mice lacking progesterone receptor exhibit pleiotropic reproductive abnormalities. *Genes Dev.* 9; 2266–2278.
25. Mesiano, S. (2004) Myometrial progesterone responsiveness and the control of human parturition. *J. Soc. Gynecol. Investig.* 11; 193–202.
26. Misao, R., Fujimoto, J., Niwa, K., Morishita, S., Nakanishi, Y. and Tamaya, T. (1997) Immunohistochemical expressions of estrogen and progesterone receptors in human epididymis at different ages—a preliminary study. *Int. J. Fertil. Womens. Med.* 42; 39–42.
27. Mitchell, B. F. and Taggart, M. J. (2009) Are animal models relevant to key aspects of human parturition? *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 297; R525–45.
28. Naito, M., Hirai, S., Terayama, H., Qu, N., Hayashi, S., Hatayama, N., *et al.* (2014) Neonatal estrogen treatment with β-estradiol 17-cypionate induces in post-pubertal mice inflammation in the ductuli efferentes, epididymis, and vas deferens, but not in the testis, provoking obstructive azoospermia. *Med. Mol. Morphol.* 47; 21–30.
29. Nakata, H. and Iseki, S. (2019) Three-dimensional structure of efferent and epididymal ducts in mice. *J. Anat.* 235; 271–280.
30. Oliveira, C. A., Zhou, Q., Carnes, K., Nie, R., Kuehl, D. E., Jackson, G. L., *et al.* (2002) ER function in the adult male rat: short- and long-term effects of the antiestrogen ICI 182,780 on the testis and efferent ductules, without changes in testosterone. *Endocrinology* 143; 2399–2409.
31. Omotehara, T., Hess, R. A., Nakata, H., Birch, L. A., Prins, G. S. and Itoh, M. (2023) Expression patterns of sex steroid receptors in developing mesonephros of the male mouse: three-dimensional analysis. *Cell Tissue Res.* 393; 577–593.
32. Omotehara, T., Nakata, H. and Itoh, M. (2022) Three-dimensional analysis of mesonephric tubules remodeling into efferent tubules in the male mouse embryo. *Dev. Dyn.* 251; 513–524.
33. Omotehara, T., Nakata, H., Nagahori, K. and Itoh, M. (2022) Comparative anatomy on the development of sperm transporting pathway between the testis and mesonephros. *Histochem. Cell*

*Biol.* 157; 321–332.
34. Pal, P. C., Manocha, M., Kapur, M. M., Rao, D. N., Sharma, R. S. and Rajalakshmi, M. (2006) Obstructive infertility: changes in the histology of different regions of the epididymis and morphology of spermatozoa. *Andrologia.* 38; 128–136.
35. Parkening, T. A., Lau, I. F., Saksena, S. K. and Chang, M. C. (1978) Circulating plasma levels of pregnenolone, progesterone, estrogen, luteinizing hormone, and follicle stimulating hormone in young and aged C57BL/6 mice during various stages of pregnancy. *J. Gerontol.* 33; 191–196.
36. Pereira, M. F. N., Fernandes, S. A. F., Nascimento, A. R., Siu, E. R., Hess, R. A., Oliveira, C. A., *et al.* (2014) Effects of the oestrogen receptor antagonist Fulvestrant on expression of genes that affect organization of the epididymal epithelium. *Andrology.* 2; 559–571.
37. R Development Core Team (2021) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org.
38. Siemienowicz, K. J., Wang, Y., Marečková, M., Nio-Kobayashi, J., Fowler, P. A., Rae, M. T., *et al.* (2020) Early pregnancy maternal progesterone administration alters pituitary and testis function and steroid profile in male fetuses. *Sci. Rep.* 10; 1–12.
39. Simons, N. E., Leeuw, M., Van't Hooft, J., Limpens, J., Roseboom, T. J., Oudijk, M. A., *et al.* (2021) The long-term effect of prenatal progesterone treatment on child development, behaviour and health: a systematic review. *BJOG*. 128; 964–974.
40. Society for Maternal-Fetal Medicine Publications Committee, with assistance of Vincenzo Berghella (2012) Progesterone and preterm birth prevention: translating clinical trials data into clinical practice. *Am. J. Obstet. Gynecol.* 206; 376–386.
41. Sugimoto, Y., Yamasaki, A., Segi, E., Tsuboi, K., Aze, Y., Nishimura, T., *et al.* (1997) Failure of parturition in mice lacking the prostaglandin F receptor. *Science* 277; 681–683.
42. Thorburn, G. D. and Challis, J. R. (1979) Endocrine control of parturition. *Physiol. Rev.* 59; 863–918.
43. Wahabi, H. A., Fayed, A. A., Esmaeil, S. A. and Bahkali, K. H. (2018) Progestogen for treating threatened miscarriage. *Cochrane Database Syst. Rev.* 8; CD005943.
44. Weisz, J. and Ward, I. L. (1980) Plasma testosterone and progesterone titers of pregnant rats, their male and female fetuses, and neonatal offspring. *Endocrinology* 106; 306–316.