Int. J. Dev. Biol. 42: 621-624 (1998)

Short Contribution

Differentiated aspect of female and male mouse mesonephroi

MARIE-DANIELLE VAZQUEZ[1]*, PIERRE BOUCHET[2], BERNARD FOLIGUET[1], HUBERT GÉRARD[1], JEAN-LAURENT MALLET[3] and BRUNO LEHEUP[1]

[1]*Département de Cytologie, Histologie et Embryologie, Faculté de Médecine,* [2]*Centre de Recherche en Informatique de Nancy and* [3]*École Nationale Supérieure de Géologie de Nancy, Vandœuvre-lès-Nancy, France*

ABSTRACT In the mouse, *Sry* is expressed by somatic cells in the genital ridge and leads to initiate the transformation of the indifferent gonad into a testis. Here, we have examined the segmented female and male mesonephroi over the developmental period when the initiation of seminiferous cord formation takes place. We have generated three-dimensional models which reveal structural differences between male and female mesonephric tubules at 11.5, 13.5 and 14.5 days *post coitum*. We evaluate, following structural parameters, that form and orientation of the tubules could evolve differently in both sexes. We propose that the structural organization of the mesonephric tubules presents an early dimorphism.

KEY WORDS: *mesonephros, 3-D reconstruction, sex determination, histological sections*

There is now abundant evidence that *Sry* is the testis-determining gene on the mammalian Y chromosome (Gubbay *et al.*, 1990; Sinclair *et al.*, 1990). *Sry* is expressed at 10.5 and 11.5 days *post coitum (pc)* by somatic cells in the genital ridge, when gonads of both male and female mouse embryos are morphologically indistinguishable (Koopman *et al.*, 1990).

There are two major gonadal somatic cell lineages: the supporting cell lineage (Sertoli cells in the testis and granulosa cells in the ovary) and the steroidogenic lineage (Leydig cells in the testis and theca cells in the ovary). The Sertoli cell lineage has been shown to be critical in sex determination (Palmer and Burgoyne, 1991). It has been shown in XX/XY chimaeric mouse testes, that the Sertoli cell population is predominantly XY (Burgoyne *et al.*, 1988; Patek *et al.*, 1991). *Sry* is believed to act in the pre-Sertoli cells based on genetic analysis of XX/XY chimeras (Burgoyne and Palmer, 1993).

On the basis of histological analysis, several studies indicate that these cells may be derived in whole or in part from the mesonephros, suggesting that pre-Sertoli cells are contributed to the mesonephric tubule cells (Upadhyay *et al.*, 1981; Wartenberg, 1981; Merchant-Larios and Taketo, 1991; Wartenberg *et al.*, 1991).

In the mouse, the mesonephros appears to be the provision of some of the stromal cells (Buehr *et al.*, 1993). Moreover, the attached mesonephros is thought to be required in cord formation in the testis differentiation after 11.5 days *pc* as shown by organ culture experiments (Buehr *et al.*, 1993; Merchant-Larios *et al.*, 1993).

Our present knowledge of the structural components of the mesonephros in the mouse is mostly based on the reconstruction produced by graphical methods (Bovy, 1929).

We are interested in characterizing mesonephric tubules development in the male and female mouse mesonephroi during testis organogenesis. By three-dimensional reconstruction, we have been able to examine anatomical differences of the mesonephric region. In this study, we have compared the morphological characteristics of the mesonephric tubules at three stages: 11.5, 13.5 and 14.5 days *pc* (Table. 1).

By 11.5 embryonic days, the 3-D models of mesonephroi present numerous convoluted tubules which are directly connected to the Wolffian duct (Figs. 1 and 2). In both male and female embryos, the tubules occupy the cranial two-thirds of the mesonephros. Only in female embryo, the more cranial tubule presents several connections with the Wolffian duct, when gonads of both XX and XY embryos are morphologically alike (Fig. 2).

By 13.5 embryonic days, tubules in the mesonephros appear to be degenerating and fragmented. The female mesonephric region reveals the same structural particularity than at 11.5 days *pc* (Fig. 4). This structural particularity can help us to distinguish

Abbreviations used in this paper: a, angle; Sry, sex determining region of Y; A, area; wd, Wolffian duct; DSI, discrete smoothing interpolation; GOCAD, geological computer aided-design; L, length; md, Müllerian duct; mt, mesonephric tubules; pc, post coitum.

***Address for reprints:** Département de Cytologie, Histologie et Embryologie, Faculté de Médecine, 9 avenue de la Forêt de Haye, BP184, 54505 Vandœuvre-lès-Nancy, France. FAX: 33 (0) 3 83 59 28 83. e-mail:vasquez@loria.fr

0214-6282/98/$10.00

Printed in Spain

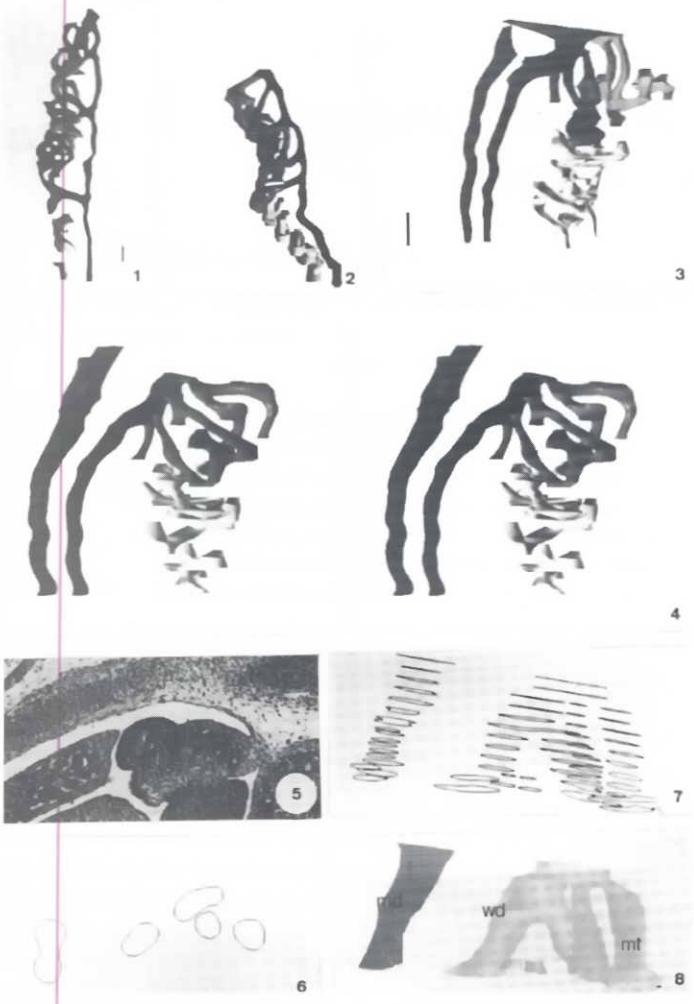


Fig. 1. The 3-D model of male mesonephric region at E11.5. *Bar, 90μm.*
Fig. 2. The 3-D model of female mesonephric region at E11.5. *Bar, 90μm.*
Fig. 3. The 3-D model of male mesonephric region at E13.5. *Bar, 70μm.*
Fig. 4. Stereo view of the female mesonephric region at E13.5, viewed along a fivefold axis. *Viewpoints are displaced by ± 3° relative to the perceived line of sight. Bar, 70 μm.*
Fig. 5. Histological section of a tubular zone at the level of the rostral part of the ovary.
Fig. 6. The external contours of distinct tubules of the previous sections were manually drawn and scanned. *Then, each contour line was converted into polygonal lines, as we can see here.*
Fig. 7. Serially stacked outlines of the rostral female mesonephric region at E13.5.
Fig. 8. After gathering several contours, the surfaces were rebuilt by juxtaposition of layers of triangles. *This figure shows the Müllerian duct (md) and the mesonephric tubules (mt) connected to the Wolffian duct (wd) with bifurcations.*

a female mesonephros from a male one and shows that the mesonephros has a sex differentiated aspect (Figs. 4 and 3).

After rebuilding 3-D models, at each following stage E11.5, E13.5 and E14.5, four parameters have been taken on three male and three female mesonephroi. These parameters are the volume (V), the area (A) and the length (L) of each tubule; and the angle (a) formed between the main axis of the Wolffian duct and the straight line passing through the emergence of the tubule. In the case of abortive tubule, the major axis is chosen. As indicated in Figure. 9, the values are expressed as means ± SE. Fischer's F test by the System SAS was used to analyze statistical differences. The measurements reveal significant differences between male and female tubules ($p<0.05$):

- at E11.5, on the volume of the connected tubules (Pr>F=.0144),
- at E13.5, on the area of the connected tubules (Pr>F=.0372) and of the abortive, isolated tubules (Pr>F=.0236), and on the angle of the connected tubules (Pr>F=.0149),
- at E14.5, on the area of the connected tubules (Pr>F=.0196) and on the angle of the connected tubules (Pr>F=.0021).

These numerical results emphasize the 3-D modeling results and show anatomical differences of the mesonephric region. Although the length of the tubules remains statistically not significant between male and female mesonephros, different forms (volume or area) of the tubule are demonstrated from E11.5 to E14.5. Only, at E13.5 and E14.5, when the gonad differentiation is complete, the orientation of the connected tubules is significantly different between male and female mesonephric region. This result suggests that the tubules which participate to the rete testis or the rete ovarii are recognized by a differentiated organization. The observations of the 3-D study at E11.5 suggest that it should be regarded probably as an early mesonephric dimorphism.

Experimental procedures

Specimens

SPF outbred mice, OF1 Ico: OF1 (IOPS Caw), (stock maintained by the Department, colony of R. Janvier, 53680 Le Genest, France) were used. The embryos were obtained from natural mating of mice. For embryo collections the time point of vaginal plug observation was designated as 0.5 days *post coitum*. (E followed by the number of the day).

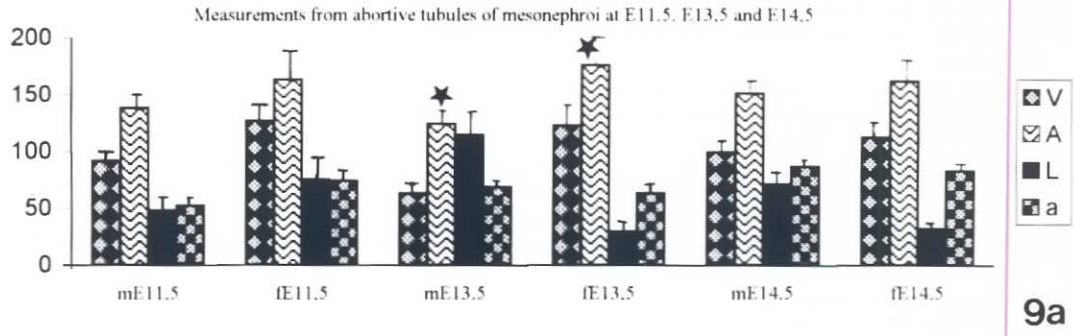
Three embryos with the same sex were collected per female in pregnancy for each stage of the development of the mesonephros. The uterine horns were dissected. Embryos enclosed within their membrane were also sexed by the chromatin test of Farias, Kajii and Gartner (1967). This test was taken up again by other authors (Jost 1972). A piece of amnion was stained with 1% acetic orcein and examined for the presence of Barr bodies in female nuclei.

For histology, the mouse embryos were fixed in Bouin's fluid for 5 to 12 h depending on age and size of embryo, transferred to graded ethanol, and finally embedded in paraffin. Embedded specimens were serially sectioned at 5 μm and stained with hematoxylin and eosin.

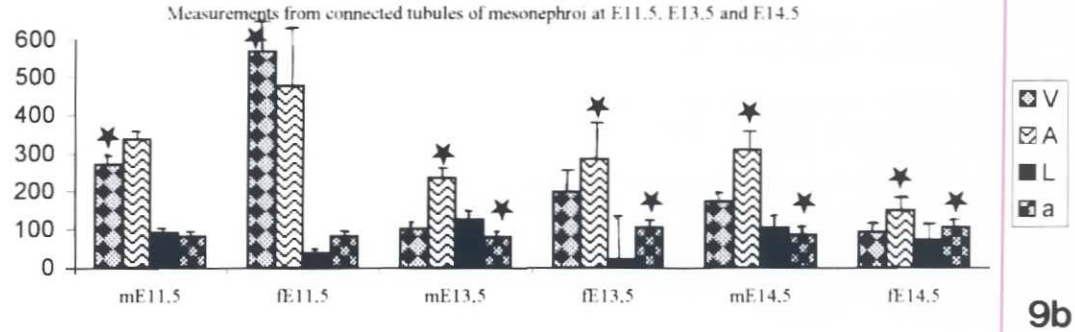
The light microscopical analysis of the prepared specimens was restricted to the rostral part of the mesonephros including mesonephric duct(s) and its tubules. Overall, each mesonephros yielded an average of 100 sections, that was performed in E11.5, E13.5 and E14.5 stages (Table 1).

Reconstruction method

As shown in Figure. 5, histological section of the mesonephric tubular zone allows to realize 3-D reconstructions. Here, from twelve successive histological sections, we have rebuilt a figure of bifurcation (Fig. 7). The external contours of distinct tubules were manually drawn and scanned. Then, each contour line was converted into polygonal line (Fig. 6). The surfaces are rebuilt thanks to the GOCAD (GeOlogical Computer Aided-Design) software. They were defined by juxtaposition of layers of triangles whose vertices are elements of the different polygonal lines obtained previously. An advantage of the use GOCAD software is based on the



9a



9b

Fig. 9. Measurements on abortive tubules (a) and connected tubules to the Wolffian duct (b) at E11.5, E13.5 and E14.5. *(V, volume x 10^3 mm^3; A, area x 10^2 mm^2; L, length x 10^2 mm; a, angle in degree; m, male; f, female).*

Discrete Smoothing Interpolation (DSI) method (Mallet, 1992). This efficient method permits the reduction of small irregularities of microtomy as shown in Figure. 8. It computes the x, y and z coordinates of the free vertices, and produces an adjusted smoothing by a spatial redistribution of the free vertices of the triangles.

Once the various parts of the mesonephros are reconstructed, several parameters were measured with GOCAD program.

The reconstructed surfaces have been gathered for recreating the different parts of the mesonephros.

Statistical Analysis

The values were expressed as means ± SE (Standard Error). The comparison of variance was used to analyze statistical differences (significantly different with Fischer's F test).

Acknowledgments

We are grateful to Dr. S. Magre (Laboratoire de Physiologie de la Reproduction, Université Pierre et Marie Curie, Paris) for valuable discussions and precious advices in dissection. We thank especially B. Cunin and M. Simonetti for their technical contribution in the work. We also thank P. Etienne, A. Filbois, T. Gartiser and L. Pierron for their computer assistance.

References

BOVY, J. (1929). Recherches sur le corps de Wolff et l'origine des connexions urogénitales chez la souris. *Arch. Biol. 39*: 139-174.

BUEHR, M. SUBIN, G.U. and MCLAREN, A. (1993). Mesonephric contribution to testis differentiation in the fetal mouse. *Development 117*: 273-281.

TABLE 1

THE MAJOR CHARACTERISTICS OF MESONEPHROI AT E11.5, E13.5 AND E14.5.

	Major characteristics											
	E11.5 (A and B)		E13.5 (C and D)		E14.5 (E and F)							
sex	nmtb	nmtu	ns	sa	nmtb	nmtu	ns	sa	nmtb	nmtu	ns	sa
male	6	2	175	3b	3	3	69	1ic	2	7	84	1b
male	7	6	134	3b	3	4	71	2b	5	4	99	-
male	5	6	91	-	4	5	72	1ic	4	5	96	-
female	2	5	115	1sc	4	5	78	2ic	7	5	114	-
female	1	10	147	1sc	2	3	63	1sc	5	3	116	-
female	6	2	140	-	2	4	85	1sc	2	5	116	-

Abbreviations:
E11.5 (A and B): litter A for male and litter B for female mesonephoi at 11.5 embryonic days, E13.5 (C and D): litter C for male and litter D for female mesonephoi at 13.5 embryonic days, E14.5 (E and F): litter E for male and litter F for female mesonephoi at 14.5 embryonic days, nmtb: number of mesonephric tubules bound or connected to the Wolffian duct, nmtu: number of mesonephric tubules unbound to the Wolffian duct, lonely or abortive, ns: number of sections, sa: structural aspect, sc: tubule with several connections to the wolffian duct, b: tubule with a branching, ic: tubule with an incomplete second connection.

BURGOYNE, P.S. and PALMER, S. (1993). Cellular basis of sex determination and sex reversal in Mammals. In *Gonadal development and function*. New York: Raven Press.

BURGOYNE, P.S. BUEHR, M. KOOPMAN, P. ROSSANT, J. and MCLAREN, A. (1988). Cell autonomous action of the testis-determining gene : Sertoli cells are exclusively XY in XX/XY chimaeric mouse testes. *Development 102* : 443-450.

FARIAS, E. KAJII, T. and GARDNER, L.I. (1967). Technique for investigation of sex chromatin in amniotic membrane of rat fetuses. *Nature 214*: 499-500.

GUBBAY, J. COLLIGNON, J. and KOOPMAN, P. (1990). A gene mapping to the sex-determining region of the mouse Y chromosome is a member of a novel family of embryonically expressed genes. *Nature 346* : 245-250.

JOST, A. (1972). Données préliminaires sur les stades initiaux de la différenciation du testicule chez le rat. *Arch. Anat. Microsc. Morphol. Exp. 61*: 415-438.

MALLET, J.L. (1992). Discrete smooth interpolation in geometric modelling. *Comput. Aided. Des. 24*: 178-191.

MERCHANT-LARIOS, H. and TAKETO, T. (1991). Testicular differentiation in Mammals under normal and experimental conditions. *J. Electron. Microsc. Tech. 19*: 158-171.

MERCHANT-LARIOS, H. MORENO-MENDOZA, N. and BUEHR, M. (1993). The role of the mesonephros in cell differentiation and morphogenesis of the mouse fetal testis. *Int. J. Dev. Biol. 37*: 407-415.

KOOPMAN, P. MUNSTERBERG, A. CAPEL, B. VIVIAN, N. and LOVELL-BADGE, R. (1990). Expression of a candidate sex-determining gene during mouse testis differentiation. *Nature 348* : 450-452.

PALMER, S. and BURGOYNE, P. (1991). *In situ* analysis of fetal prepuberal and adult XX-XY chimearic mouse testis: Sertoli cells are predominantly, but not exclusively, XY. *Development 112* : 265-268.

PATEK, C.E., KERR, J.B., GOSDEN, R.G., JONES, K.W., HARDY, K., MUGGLETON-HARRIS, A.L., HANDYSIDE, A.H., WHITTINGHAM, D.G. and HOOPER, M.L. (1991). Sex chimaerism fertility and sex determination in the mouse. *Development 113:* 311-325.

SINCLAIR, A.H. BERTA, P. PALMER, M.S. HAWKINS, J.R. GRIFFITHS, B.L. SMITH, M., FOSTER, J.W. FRISCHAUF, A.M. LOVELL-BADGE, R. and GOODFELLOW, P.N. (1990). A gene from the human sex-determining region encodes a protein with homology to a conserved DNA-binding motif. *Nature 346* : 240-244.

UPADAHYAY, S. LUCIANI, J.M. and ZAMBONI, L. (1981). The role of the mesonephros in the development of the mouse testis and its excurrent pathways. In *Development and function of reproductive organs*. (Eds A.G. Byskov., H. Peters.) Int Congr Ser N°559. Excerpta Medica, Amsterdam Oxford Princeton, pp. 18-27.

WARTENBERG, H. (1981). Differentiation and development of the testes. In *The Testis Comprehensive endocrinology*. (Eds. H. Burger., D. de Kretser). New York: Raven Press, pp. 39-80.

WARTENBERG, H. KINSKY, I. VIEBAHN, C. and SCHMOLKE, C. (1991). Fine structural characteristics of testicular cord formation in the developing rabbit gonad. *J. Electron. Microsc. Tech. 19* : 133-157.

Received: August 1997
Accepted for publication: October 1997