Sex Chromosome Mosaicisms in Five Swine Intersexes

Yoshiro TOYAMA*

Department of Veterinary Anatomy, Faculty of Agriculture,
Tokyo Noko University, Fuchu-shi, 183, Tokyo.

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Abstract Chromosome investigations were carried out on five cases of intersex swine. Four cases showed the XX/XY mosaicism in hemopoietic cells, and one of these showed the normal female karyotype in the skin and the kidney cells. The last one showed the XX/XXY mosaicism in both hemopoietic and non-hemopoietic tissues. This case has not been described in swine previously. The mechanisms of the chromosomal mosaicism were discussed.

Since MAKINO *et al.*¹⁾ have reported the chromosome constitution of an intersex swine, more than eighty cases of intersex swine with or without chromosomal abnormalities have been published²⁻¹⁷⁾. McFEE *et al.*²⁾ have first reported the sex chromosome mosaicism (XX/XY) in an intersex swine, and five additional cases of this type of the mosaicism have been described³⁻⁶⁾. The present paper deals with four additional cases of the XX/XY intersex swine and one case of the XX/XXY intersex swine, not previously described in this species. Anatomical findings of the genital organs as well as cytogenetical findings were described briefly.

Materials and Methods

The five intersex swine were collected at farms or research stations of animal industry near Tokyo in recent six years.

For the leukocyte culture, 1 ml of heparinized peripheral blood was incubated under the sterile condition in 10 ml of the tissue culture medium (TC-199) containing 0.2 ml of phytohemagglutinin-M (Difco) at 38°C for three days. Heparinized bone marrow fluid (0.2 ml) was also incubated under the sterile condition in 10 ml of the medium (TC-199; 8 ml, calf serum; 2 ml) at 38°C for one day.

For chromosome analysis, the cell division was arrested at the metaphase with 0.2 ml of colchicine at the proportion of 50 µg/ml. One percent sodium citrate or 10% calf serum was used for the hypotonic solution. After fixation in Carnoy's solution, cells were mounted on the slide glass and dried according to the air-drying method¹⁸⁾ and stained with Giemsa's solution.

Further, for the chromosomes in non-hemopoietic cells, the skin of the ear tip about the size of 1 cm² and small pieces of the kidney were removed. The blocks were minced with a razor blade into inoculums, which were planted on the surface of the culture bottle directly. After half drying for 10 to 20 minutes, 10 ml of the tissue culture medium (TC-199; 8 ml, calf serum; 2 ml and kanamycine; 1 mg) was overlayed. After the incubation of about seven days at 38°C, growing cells were treated with colchicine in a final concentration of 1 µg/ml for 2.5 hours, and then with 0.3% trypsin for 30 minutes to remove the cells from the wall of the bottle. The

* Present Address: Department of Anatomy, School of Medicine, Chiba University, Chiba-shi, 280

hypotonic treatment, fixation and staining were similar to those described above.

Results

No. 1. Yorkshire. Age of a month. The animal was 40 cm in crown-rump length (CRL). It was the smallest in the littermates. It had the vulva and the scrotal hernia, in which no gonads were located. The vagina, uterus and gonads were not observed in the abdominal cavity. The cranial end of the spindle-shaped urinary bladder was connected to the peritoneal surface of the abdominal wall by the remnant of the alantois duct. The outer orifice of the bladder was dilated forming the vestibulum. The kidneys and the ureters were edematous and about two times as large as normal in size (Fig. 1).

The sex chromosome constitution in hemopoietic cells: XX/XY (16/15).

No. 2. Landrace. Age of two weeks. CRL: 38 cm. The animal was the smallest in the littermates. It showed similar anatomical features to those of No. 1.

The sex chromosome constitution of hemopoietic cells: XX/XY (23/15). (Fig. 7).

No. 3. Landrace. Age of two months. CRL: 85 cm. The animal showed the normal growth rate but had neither the normal vulva nor the penis. The enlarged clitoris was remarkable in the external genital region. The external urethral orifice opened dorsal to the enlarged clitoris. Bilateral testes with epididymides were attached to the cranial ends of the uterine horns by the spermatic cord-like ligaments. The lumen of the poorly developed vagina was constricted at about 2 cm cranial to the supposed vulva, and connected to the urethra (Fig. 2). Histologically, the testis showed the poorly developed seminiferous tubules. The germinal epithelium consisted of only immature sustentacular cells. The interstitial cells seemed to be normal.

The sex chromosome constitution in hemopoietic cells: XX/XY (45/9).

No. 4. Mixed breed. Age of six months. CRL: 125 cm. The growth rate appeared to be normal for the age. The enlarged clitoris-like process and the scrotum of the subnormal size were observed in the external genital region. Bilaterally descended testes with epididymides were palpable in the scrotum (Fig. 3). The lumen of the cranial one fourth of the urethra, which opened dorsal to the enlarged clitoris-like organ, was dilated presenting the poorly developed vagina. The seminal vesicle-like organs were observed (Fig. 4). In histological sections of the testis, the wall of each tubule consisted of a single layer of cuboidal cells, but the interstitial cells seemed to be normal (Fig. 5).

The sex chromosome constitution in the skin and kidney cells: XX (52), (23) respectively. The sex chromosome constitution in hemopoietic cells: XX/XY (107/61).

No. 5. Landrace. Age of four months. CRL: 91 cm. The animal showed the normal growth rate and had the similar external genital features to those of No. 3. It had bilateral ovotestes. Small eight to ten Graafian folliculi were found on the surface of each ovarian part. The lumen of the uterus dilated with a fluid as if it were pregnant. The lumen of the poorly developed vagina was constricted at about 1.5 cm cranial to the supposed vulva, and connected to the urethra (Fig. 6). The connective tissue divided the smaller ovarian part from the larger testicular one, where poorly developed seminiferous tubules were found.

The sex chromosome constitution in the skin and kidney cells: XX/XXY (32/5), (29/7) respectively. The sex chromosome constitution in hemopoietic cells: XX/XXY (38/5). (Fig. 8).

The autosomes in these five cases appeared to be normal both in figure and number.

Discussion

The chromosome number of 38 in the domestic swine has been established^{1,19-21}. Since MAKINO *et al.*¹ have first reported an intersex swine with the normal female karyotype, more than seventy-two cases of intersex swine with the normal female karyotype^{6,7,9-17} and eight cases with the chromosomal abnormality²⁻⁸ have been reported. More cases of the intersex swine with the normal female karyotype were also observed (Y. OMURA, personal communication). On the other hand, in the cases of cattle freemartin, it is widely accepted that the female co-twin shows the XX/XY mosaicism as well as the male co-twin shows. This suggests that the etiology of the most cases in the intersex swine (*i. e.* of the intersex swine with the normal female karyotype) is different from that of the freemartin in cattle. Among the intersex swine with the chromosomal abnormality, McFEE *et al.*² have first reported a case of the XX/XY sex chromosome mosaicism in cultured leukocytes. Since then five cases of the intersex swine showing the XX/XY mosaicism have been reported³⁻⁶. In addition the cases of the intersex swine with the XXY sex chromosome constitution have been reported.^{7,8}

According to previous papers, there are many variations in intersexuality of the swine. Among

Explanation of the figures

c : enlarged clitoris	t : testis with epididymis,
k : kidney,	u : uterus,
ot : ovotestis,	ub : urinary bladder,
r : rectum,	w : abdominal wall,
s : seminal vesicle-like organ.	

Fig. 1. The photograph shows no internal genital organs. The spindle-shaped urinary bladder attaches to the abdominal wall. The kidneys and ureter (arrow) are edematous. (Case No. 1).

Fig. 2. The genital organs of case No. 3. The intra-abdominal testes attach to the cranial ends of the uterine horns.

them, it is interesting to note a report of a phenotypic male swine with normal female karyotype¹⁰⁾ and other reports of true hermaphrodites with female looks and functions to sustain their embryos until the late stage of gestation²²⁻²⁴. Thus, it is generally accepted that the correlation between the organogenesis and the chromosome constitution is obscure in swine intersexes.

Following explanations on the mechanisms of the occurrence of the XX/XY mosaicism in swine are presented.

- 1) The double fertilization or the fusion of XX and XY blastocysts proposed by BASRUR and KANAGAWA⁶⁾.
- 2) The divisional error of the sex chromosomes such as, a normal male zygote (XY)→appearance of an XO cell in the XY zygote by the deletion of the Y chromosome (XO/XY)→appearance of an XX cell line by the non-disjunction of the X chromosome in the XO cell (XX/XY). Such a condition is known in man (OHNO, personal communication).

In the case of either of these mechanisms, the mosaicism can be observed through the whole body of the subject animal.

- 3) The intra-uterine vascular anastomosis by Vogt⁴⁾. That is, in the case of the heterosexual twin embryos, the primitive hemopoietic cells of the co-twin of the opposite sex can invade into the subject animal through the anastomosis. The mosaicism, therefore, appears only in hemopoietic cells and the chromosome complement of other tissues shows its genetic sex. If the anastomosis

Fig. 3. External genitalia of case No. 4. The enlarged clitoris-like process and the small scrotum (arrow) are observed.

Fig. 4. The genital organs of case No. 4. The left testis is out of the figure. The right testis with the epididymis, seminal vesicle-like organs and enlarged clitoris-like organ are seen. The urethra is enlarged in diameter and reveals the vagina-like architecture. The arrow shows the external orifice of the urethra which opens dorsal to the enlarged clitoris.

Fig. 5. Photomicrograph of the left testis in case No. 4. The seminiferous epithelium has no germinal cells. The single layer of the cuboidal cells are observed in each tubule.

Fig. 6. The genital organs of case No. 5. The lumen of the uterus is dilated abnormally. Bilateral ovotestes are shown.

Fig. 7. A karyotype of a cultured leukocyte in case No. 2; 38, XY.

Fig. 8. A karyotype of a cultured skin cell in case No. 5; 39, XXY.

occurs earlier enough than the primordial germ cells reach the genital ridges, the cells of the gonads in the subject animal may also show the XX/XY mosaicism²⁸⁾. The theory of the XX/XY mosaicism in hemopoietic cells has been established in cattle^{27,28)}.

The animal No. 4 had the XX/XY mosaicism in leukocytes and had the normal female chromosome constitution in the skin and the kidney cells. The mosaicism in the hemopoietic tissue of the animal, therefore, might be derived from the intra-uterine vascular anastomosis. This supports the hypothesis proposed by Vogt⁴⁾.

In the case No. 5, the XX/XXY mosaicism, the occurrence of XX cell line by the deletion of the Y chromosome during the development of an abnormal XXY zygote or the fusion of a normal XX zygote and an abnormal XXY zygote may be suggested as the cause of the mosaicism, since the animal showed the mosaicism in the hemopoietic cells, kidney cells and skin cells. This type of mosaicism has been reported in man²⁹⁾ and in a tortoiseshell cat³⁰⁾ but not in swine before.

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References

- 1) MAKINO, S., M. S. SASAKI, T. SOFUNI, and T. ISHIKAWA, *Proc Jap Acad* **38**: 686-689. 1962.
- 2) McFEE, A. F., M. KNIGHT, and M. W. BANNER, *Can J Genet Cytol* **8**: 502-505. 1966.
- 3) BRUERE, A. N., E. D. FIELDEN, and H. HUTCHINGS, *New Zealand vet J* **16**: 31-38. 1968.
- 4) VOGT, D. W., *J Hered* **59**: 166-167. 1968.

- 5) SOMLEV, B., E. HANSEN-MELANDER, Y. MELANDER, and L. HOLM, *Hereditas* **64**: 203-210. 1970.
- 6) BASRUR, P. K., and H. KANAGAWA, *J Reprod Fert* **26**: 369-371. 1971.
- 7) GLUHOVSKI, N., M. BISTRICEAU, M. ROSU, and M. BRATU, *Arch Vet* **3**: 317-333. 1967.
- 8) BREEUWSMA, A. J., *J Reprod Fert* **16**: 119-120. 1968.
- 9) GERNEKE, W. H., *Onderstepoort J vet Res* **34**: 219-300. 1967.
- 10) HARD, W. L., and J. D. EISEN, *J Hered* **56**: 255-258. 1965.
- 11) VOGT, D. W., *J Anim Sci* **25**: 252. 1966.
- 12) HARD, W. L., *Anat Rec* **157**: 255-256. 1967.
- 13) McFEELY, R. A., W. C. D. HARE, and J. D. BIGGERS, *Cytogenetics* **6**: 242-253. 1967.
- 14) SOMLEV, B., Y. MELANDER, E. HANSEN-MELANDER, J. AAMDAL, and K. ANDERSEN, *Hereditas* **64**: 296-297. 1970.
- 15) BACKSTROM, L., and B. HENRICSON, *Acta Vet Scand* **12**: 257-273. 1971.
- 16) MELANDER, Y., *Hereditas* **69**: 51-58. 1971.
- 17) MIYAKE, Y.-I., *Jap J vet Res* **21**: 41-49. 1973.
- 18) ROTHFELS, K. H., and L. SIMINOVITCH, *Stain Technol* **33**: 73-77. 1958.
- 19) GIMENEZ-MARTIN, G., J. F. LOPEZ-SAEZ, and E. G. MONGE, *J Hered* **53**: 281, 290. 1962.
- 20) McFEE, A. F., M. W. BANNER, and J. M. RARY, *Cytogenetics* **5**: 75-81. 1966.
- 21) RITTMANNSPERGER, C., *Wien Tierarztl Monatsschr* **57**: 72-74. 1970.
- 22) FOLGER, A. F., *Acta path microbiol Scand Suppl* **11**: 143-150. 1932.
- 23) HULLAND, T. J., *Can vet J* **5**: 39-41. 1964.
- 24) COX, J. E., *J Reprod Fert* **16**: 321-322. 1968.
- 25) HOADLEY, L., *Anat Rec* **38**: 177-187. 1927.
- 26) HEGHES, W., *Anat Rec* **41**: 213-246. 1929.
- 27) OHNO, S., J. M. TRUJILLO, C. STENIUS, L. C. CHRISTIAN and R. L. TEPLITZ, *Cytogenetics* **1**: 258-265. 1962.
- 28) OHNO, S., *J Reprod Fert Suppl* **7**: 53-61. 1969.
- 29) FORD, C. E., P. E. POLANI, J. G. BRIGGS, and P. S. F. BISHOP, *Nature* **183**: 1030-1032. 1959.
- 30) CHU, E. H. Y., H. C. TULINE, and D. E. NORBY, *Cytogenetics* **3**: 1-18. 1964.

5 例の間性ブタにおける性染色体モザイク

外 山 芳 郎*

東京農工大学農学部, 東京 183

間性ブタ発生機序解明の一助としてその染色体構成を調べた。染色体標本は全例において骨髓細胞および末梢血細胞に由来する。さらに2例において皮膚および腎の細胞の染色体も調べた。例1: 外陰部は雌型を示したが内部生殖器官は全てみられなかった。尿道は腔様であった。例2: 例1とほぼ同様な解剖所見であった。例3: 外陰部には膨大した陰核だけがみられた。管腔の狭い腔, ほぼ正常な子宮, および精巣上体をもつ両側性の腹腔内精巣がみられた。例4: 外陰部は例3と似ているが, さらに小さな陰嚢がみられた。内部生殖器官はほぼ雄型を示し, 両側性の下降した精巣, 精巣上体, 精管, 精嚢様器管がみられた。尿道の頭側部は管腔が広がり, 腔

を思わせた。以上4例は造血細胞においてXX/XYのモザイクであった。例4の非造血細胞ではXXのみであった。例5: 外陰部は例3と同様であった。管腔の狭い腔, 内腔の拡大した子宮, 両側性の卵精巣がみられた。性染色体構成は造血, 非造血細胞ともにXX/XXYであった。例4の造血細胞におけるXX/XYモザイクは非造血細胞が正常雌型のXXを示したので, 子宮内血管吻合の結果と思われる。例5では造血, 非造血細胞ともにXX/XXYモザイクがみられたので, XXY胚の発生過程におけるXX細胞の出現またはXX胚とXXY胚の合一が原因として考えられる。

* 現在千葉大学医学部, 千葉 280