Cytogenetical and Molecularbiological Studies on a Bovine XY Female

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ABSTRACT. A bovine XY female in Holstein-Friesian heifer, which appeared as female with uterus and ovaries but did not show the estrus until 23 months old after the birth, was cytogenetically and molecularbiologically examined. As results of chromosome analysis, leucocyte and fibroblasts from skin, spleen and kidney examined had only metaphase plates with 60, XY. From these results and the clinical characteristics, this case was clearly diagnosed as a pure XY female. It was ascertained that the two genes, ZFY and AMG gene which located on the short arm of the Y chromosome (Yp) were detected in normal bulls and a XY female, but were not detected in normal cow, mother cow and half-sib heifer by Southern blotting.—KEY WORDS: AMG gene, bovine, sterility, XY female, ZFY gene.

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To clarify the role of sex chromosomes in sex anomalies has been a goal of the investigations in the cytogenetical field. Recently, the researchs for detection of testis-determining factor (TDF) on the Y chromosome have been developing by the remarkable progress of molecularbiological techniques [12]. Today, Sinclair *et al.* [19] defined a 35-Kb region of Y-specific sequence immediately adjacent to the pseudoautosomal boundary in which SRY gene was identified. On the basis of these achievements, investigations have been carried out to resolve the cause of sex anomalies in human. Muller *et al.* [13] reported that sex reversal in males with female karyotypes is likely to be caused by the presence of cytogenetically undetectable Y-chromosomal DNA sequences included the testis-determining gene(s). Furthermore, it was suggested that some XY females with gonadal dysgenesis, have lost the sex determining region from the Y chromosome by terminal exchanges between the sex chromosomes [9], by other deletion or mutation [2, 7].

Although many types of sex reversal syndrome have been reported in the domestic animals [4], there are none of researchs by using these molecularbiological methods until now.

Here, we report the results of chromosomal analysis, and detection of 2 genes, Zinc finger protein Y (ZFY) gene [18] and Amelogenin (AMG) gene [16] on the Y chromosome, about a case of bovine XY female which is a female type in appearance, but is not being in heat for 2 years after the birth.

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MATERIALS AND METHODS

A case examined was a Holstein-Friesian heifer which was born on 1987. The case was female type in appearance, but did not show the symptom of estrus and was not inseminated until 23 months old yet.

After moving her to the Veterinary Hospital of Faculty of Agriculture, Iwate University, the detailed examinations concerning about her reproductive function were carried out during 28 days.

The whole internal genital organs collected after slaughter were inspected, and were fixed with 10% formalin solution. The ovarian-like organs were then embedded in paraffin according to the usual manner and sectioned at 5 μm. After being stained with haematoxylin and eosin solution, the sections were examined pathologically.

Blood samples from the case, its mother cow and 2 cases of half-sib heifer were cultured in medium supplemented with PHA-M and FCS. A piece of skin, spleen and kidney derived from the case were also cultured in medium with FCS. After the cultivation of 3 days in blood samples and during optimal periods in fibroblasts, chromosome preparations were made according to the usual manner [5].

Since it was ascertained that this case had the Y chromosome (60, XY) by chromosome analysis as mentioned above, the detection of 2 genes, ZFY gene and AMG gene, both of which are located on the short arm and close to the SRY in human Y

chromosome were performed by the Southern blotting method. DNA samples were extracted from leucocytes of the case, mother cow, half-sib heifer, and cytogenetically normal 2 bulls and a normal cow as a control. DNA extraction, Southern transfer and hybridization were carried out by standard techniques [10, 15, 17]. The probes used were pDP1007 for ZFY gene [18] and 87–4a for AMG gene [16]. In addition, PCR detection of SRY gene was attempted using primers which detected a 270 bp fragment of human SRY gene [14].

RESULTS

By the rectal palpation of this case, the underdeveloped uterus and small ovarian-like organs located in pelvic cavity were palpated. The case had not been showing the external cyclic changes, follicular development, ovulation and the formation of corpus luteum which are usually seen in normal heifers, during the experimental period of 28 days.

At the necropsy, it was found that uterine horns were remarkably short and was 11–13 cm in length (Fig. 1). There were no abnormal findings about vagina and vestibule of vagina, and male genital organs, such as seminal vesicles, were not observed. The bilateral ovarian-like organs were very thin and were 4.0 × 1.5 × 0.6 cm in size. On the surface of organs, there were some folds, and the organs were seen just like the streaked gonad. The follicles or corpus luteum were not observed, even if by incision of the organs. In spite of the detailed examinations of abdominal cavity, the retained testes were not recognized. Microscopically, a few primary oocytes with 1–3 lines of guranulosa cells were observed in the section of ovarian-like organs, but there were not any ovum matured. Furthermore, the structures, such as the seminiferous tubules derived from the testes, were not observed.

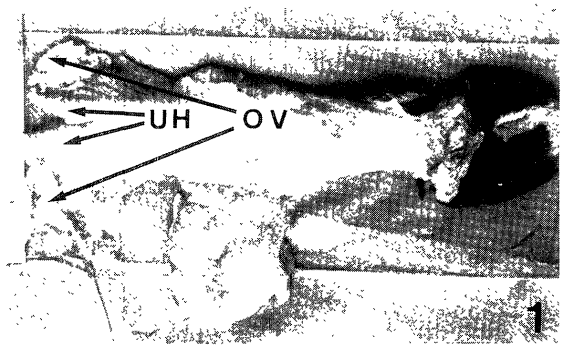

Fig. 1. Internal genital organs in XY female. It was characterized by the underdeveloped uterus and gonadal dysgenesis. UH: uterus horn, OV: ovary.

As the results of chromosome analysis, all tissues examined cytogenetically had only a single type of metaphase plate with 60, XY, that is in the 217 metaphases from blood, 156 metaphases from skin, 31 metaphases from spleen and 12 metaphases from kidney (Fig. 2). The mother cow, and 2 half-sib heifers had a normal 60, XX metaphases only.

The results on the detection of ZFY gene and AMG gene by Southern blotting were shown in Figs. 3 & 4. In the samples from normal bulls and a XY female, ZFY gene was detected at the position

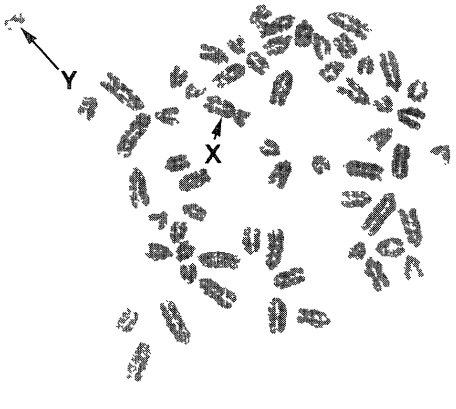

Fig. 2. Metaphase plate showing the 60, XY derived from blood culture. X: X chromosome, Y: Y chromosome.

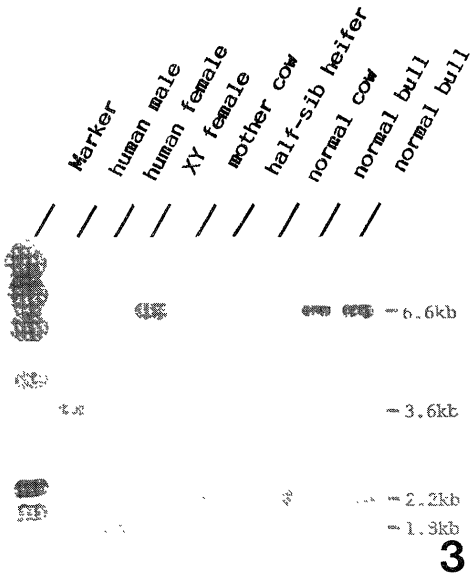

Fig. 3. Detection of ZFY gene (pDP1007) by Southern blotting. ZFY gene is detected at the position of 6.6 Kb in normal bulls and XY female.

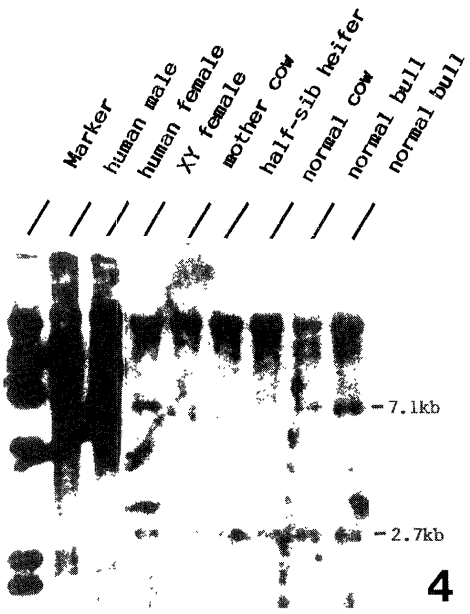

Fig. 4. Detection of AMG gene (87–4a) by Southern blotting. AMG gene is detected at the position of 7.1 Kb in normal bulls and XY female.

of 6.6 Kb by using pDP1007 as DNA probe, but was not detected in the samples from normal cow, mother cow and half-sib heifer. Furthermore, the AMG gene was detected at the position of 7.1 Kb by using 87–4a as DNA probe in normal bulls and a XY female, but was not detected in normal cow, mother cow and half-sib heifer.

By the use of a pair of primers designated to detect a 270 bp fragment of human SRY gene, no signal was detected in any of normal bull, normal cow, the XY female, mother cow and half-sib heifer.

DISCUSSION

Chromosomal aberrations can effect fertility by direct effect, or indirectly by modifying the capability of the animal to react to variable management or environmental factors. Particularly, it is clear that there are strong relationships between sex chromosome aberrations and abnormalities of internal or external reproductive tracts [5], and affected animals, such as the case reported here, may have important influence on the reproductive performances in the farm. The case reported in this paper, was diagnosed as a complete sterility, from its clinical features and chromosome constitution.

For the cytogenetical diagnosis of the XY female syndrome, it is necessary to consider the peculiar freemartins with 60, XY karyotype only [1], which were different from the usual freemartins with XX/XY chimerisms in blood. However, it is known in the freemartins that the cells with 60, XX derived from themselves were in existence dominantly in somatic cells, even if being in dominant existence of 60, XY in blood [11]. Since our case had metaphase plates of 60, XY not only in blood, but also in somatic cells derived from the skin, spleen and kidney, this case was clearly distinguished from the peculiar freemartins as mentioned above, and diagnosed as a pure XY female.

By the molecularbiological analysis using ZFY gene and AMG gene for the Y chromosome, it was recognized that 2 genes existed on the Y chromosome from the case in similar to the normal Y chromosome in bovine. The ZFY gene which was found by Page *et al.* [18] was seemed to be the substance of TDF. But, by finding of SRY (sex-determining region of the Y) gene [6, 8, 19], it becomes clear that SRY/Sry is a gene located in the sex-determing region of the human and mouse Y chromosomes and has many of the properties expected for TDF. However, both genes exsist at about 150 Kb's distance on the short arm of the Y chromosome (Yp) in human. Therefore, it was surmised that the case reported here may have SRY gene on its Yp by the evidence for the detection of ZFY gene. An attempt to detect the SRY gene by the PCR technique was not successful presumably due to sequence diversity between man and bovine.

It comes into a question why the case became a female in appearance and had female genital organs, nevertheless showing XY karyotype. The following 3 hypotheses will be arisen here. First, there is a possibility that TDF exists on Yp in this case, but its expression of gene(s) are suppressed by some causes. As second hypothesis, TDF may be lost from Yp by a point mutation or small deletion. And for last, TDF may be translocated on the X chromosome by crossing over between X and Y chromosome at the meiosis. It is clear that the X and Y chromosome join together end-to-end and forms one chiasma at the meiotic stage of spermatogenesis. Usually, pairing and crossing over occur within the pseudoautosomal segment [3]. But the length of the X-Y pairing segment varies with meiotic stage and can extend well beyond the pseudoautosomal segment into the Y long arm. If, by this crossing over, TDF on Yp can transport on the X chromosome, then sperms without TDF are formed and XY female zygote may be produced by fertilization.

Recently, Berta *et al.* [2] reported in human XY female that a de novo mutation was found in the SRY gene. Furthermore, Jager *et al.* [7] reported that the four-nucleotide deletion occurs in sequence of SRY encoding a conserved DNA-binding motif and results in a frame shift presumably leading to a non-functional protein in sex-inversed XY female.

It is vague that any hypothesis out of 3 ones as mentioned above can successfully explain the case of bovine XY female. Present research will become available for resolving the cause of XY female, and moreover sex determination and differentiation in mammals.

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