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Expression of Sex-Related Genes in the Fetus of Mouse: 2-Bromopropane and Sex Differentiation

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생쥐 태자의 성 관련 유전자 발현: 2-Bromopropane과 성 분화

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ABSTRACT : The recent reports that endocrine disruptors(EDs) bring about abnormalities in reproductive organs and functions of invertebrates suggest that mammals be affected by the EDs. The present study examined the influence of 2-bromopropane(2-BP) by looking at the sexes of litters in mouse. The expression of sex-related genes during sex differentiation was also investigated in the fetus of mouse. The male and female mice were infused with 2-BP for 3 weeks before mating. The litters were sexed at the weaning time from the 4 different groups. The sex-related genes were identified by RT-PCR from the fetuses at gestation 10 days. The sequences of the genes were analysed by comparing to those of other animals. The mean numbers of litters survived by the weaning time were slightly reduced in the only group of both female and male mice treated with 2-BP. The female litters were greater than male litters in the only group of female treated with 2-BP. The other groups showed male litters greater than female litters. The sex-related genes, SRY, DAX1, SF1, and AMH genes were identified and sequenced, showing 416, 466, 326, 389 base pairs, respectively. All of the genes had the homology of 89~90% with rat and 81~92% with human within the range of bases identified. They were expressed at the time of sex determination. Therefore, it appears that 2-BP somewhat affects the reproductive activity of adult mouse. Influence of 2-BP on the reproductive function is expected to be studied through the expression of the sex-related genes.

Key words : 2-bromopropane, Fetus, SRY, DAX1, SF1, AMH.

요 약 : 환경호르몬(내분비계 장애물질)이 하등동물의 생식기 및 생식 기능 이상을 초래한다는 최근보고는 포유동물도 그 영향하에 있음을 암시한다. 따라서 2-bromopropane(2-BP)이 생쥐 차산자의 성별에 미치는 영향과 성 분화 과정 중에 발현되는 유전자를 조사하였다. 생쥐를 2-BP로 3주일 동안 주입한 암수를 4종류 조합으로 교배시킨 후 태어난 새끼들의 성별을 이유시기에 결정하였다. 성관련 유전자들은 수태 후 10일에 어미 생쥐를 희생시켜 RT-PCR 방법으로 태자들에게서 발현되는 유전자를 탐지하였고, 동정된 범위의 핵산 서열을 기존의 보고된 서열과 비교 분석하였다. 이유시기까지 살아남은 한배 차산자 평균수는 암수를 모두 2-BP로 처리한 군에서만 약간 감소하였다. 차산자의 성비에서 암컷 어미가 2-BP로 처리된 군에서만 차산자 암컷이 수컷보다 많았으며, 그 이외의 군에서는 수컷이 암컷보다 많았다. 성 분화 시기에 발현되는 유전자들인 SRY 유전자는 416 염기, DAX1 유전자는 466 염기, SF1 유전자는 326 염기, AMH 유전자는 389 염기를 동정하였다. 이 유전자들은 흰쥐와는 89~90 %의 상동성을, 그리고 사람과는 81~92 %의 상동성을 보였다. 이 유전자들은 성이 결정되는 시기인 수태 10 일경에 모두 발현됨을 알 수 있었다. 따라서 2-BP는 생식능력에 어느 정도 영향을 미치는 것으로 사료된다. 포유동물의 성 분화에 미치는 내분비계 장애물질의 영향을 성관련 유전자들의 발현과 관련지어 연구할 수 있을 것으로 기대된다.

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INTRODUCTION

Endocrine disruptors(EDs) are natural and synthetic substances that interferes with normal function of hormones in the living organisms. Among the effects of EDs, abnormalities of reproductive organs have been reported in a wide range of animals, regardless of invertebrates and vertebrates(Yoon, 1998). Apart from the reversal of sex in some invertebrates exposed to EDs, deformities of sex organs in some vertebrates have been realized. But little is known about the influence of EDs in mammals. In the case of exposure to EDs, mammals examined up to date showed deformity of sex organs and abnormality of reproductive activity. Specially it is documented that fetal period is vulnerable to EDs. Sex is determined in the period of fetus. Nothing has been known about the impact of EDs on the sex determination in mammals.

Sex is primarily determined by the sex chromosomes in mammal that are inherited from the parent(Rugh, 1990). This genetic sex is established at the moment of fertilization when the sperm introduces either an X or a Y chromosome into the eggs. In mouse, the female is XX and male is XY. Eggs contain one X chromosome; if the sperm introduces another X the embryo will be female, if a Y it will be male. The presence of a Y chromosome leads the embryo's gonads to develope into testes rather than into ovaries. Following fertilization, all mammals start off as embryos along a sexually neutral developmental pathway.

In mouse, the potential germ cells of the male appear at about 8 days gestation, at which time there may be only about hundreds of cells that are the ancestors of the millions of spermatozoa. Paired genital ridges arise independently at 9 days gestation, adherent to the paired mesonephroi, toward which the primordial germ cells migrate. During further development subsequent proliferation and differentiation are medullary in the testis, while they are cortical in the ovary. Sexual differentiation proceeds very rapidly.

The major gene for the testis-determining factor resides on the short arm of the Y chromosome. A male-specific DNA sequence that could encode a peptide, which would act as a transcription factor, was found(Brennan et al., 1998; Sinclair et al., 1990). This gene is named SRY (sex-determining region of Y chromosome) that encodes the testis-determining factor. The Sry gene is expressed in the somatic cells of the indifferent gonad immediately before or during its differentiating into a testis; its expression then disappears(Hacker et al., 1995). DAX1 gene, residing on the short arm of the X chromosome, is also involved in the sex determination(Goodfellow & Camerino, 1999). SF1 (steroidogenic factor 1) is one component of interest because it regulates the expression of steroidogenic enzymes in mammals and is differentially expressed during development of testis and ovary(Birk et al., 2000). The AMH(anti-Müllerian duct hormone) is a glycoprotein made in the process of sex differentiation as well as the Sertoli cells(Tran et al., 1977; Cate et al., 1986). When fragments of fetal testes or isolated Sertoli cells are placed adjacent to cultured segments containing portion of the Wolffian and Müllerian ducts, the Müllerian duct atrophies even though no change occurs in the Wollfian duct. This atrophy is caused by cell death and by the epithelial cells of the duct becoming mesenchymal and migrating away(Trelstad et al., 1982). The development of animals involves many other genes as well, and the resulting determination of sex is a combination of genes expressed during fetal period(Parker et al., 1999; Ottolenghi et al., 2000). In the light of the effects of EDs on genital organs reported recently(Guillette et al.,1994; Yoon, 1998), the process of development is somehow affected by EDs.

Therefore, the effect of 2-BP on the differentiation of sex was investigated by examining the sexes of litters. The expression of sex-related genes, SRY, DAX1, SF1, and AMH genes in the fetus of mouse was also examined during the development of animals and the nucleotide sequences identified were compared to those reported in other animals.

MATERIALS AND METHODS

1. Animals and 2-BP treatment

Adult ICR mice(10 weeks) were kept in a controlled environment consisting of 12 hours of light and 12 hours of dark and a temperature of 22±1℃. They were provided with food and water *ad libitum*. Adult female and male mice were equipped with catheter connected to infusion pump(Kd Scientific) at one end and to the nape of the mice at the other end. 2-bromopropane was mixed with corn oil and continuously infused at the concentration of 1000 mg/day/kg of body weight for 3 weeks.

They were then subjected to mating. The mating groups were divided into 4 groups: 1) intact females and males, 2) 2-BP -treated females and intact males, 3) intact females and 2-BP -treated males, and 4) 2-BP-treated females and males. Following mating for 5 days to ensure fertilization, males were removed. Litters were observed every day but were not counted at birth not to perturb the dams. They were sexed at the weaning time. The ovaries of some intact females and 2-BP-treated females were examined histologically using the routine hematoxylin-eosin staining.

2. Tissue preparation

Adult ICR mice housed as mentioned above were used. After checking the estrous cycle and mating on the proestrous day, plugs were checked on the next morning. Males were removed and females were maintained in the cage. On the day of gestation 10 days, during which the sexual differentiation proceeds, the females were sacrificed by cervical dislocation. Uteri were exposed and fetuses were detected with the naked eyes by looking at the enlarged portions of the uteri. The uteri were longitudinally cut along with the midline of the uteri. Each whole fetus was isolated and subjected to the extraction of total messenger RNA(mRNA).

3. Total mRNA extraction

Total mRNA was extracted with acid guanidinium thiocyanate -phenol-chloroform method(Chomczynski & Sacchi, 1987). Briefly, each fetus was homogenized in 600 μl of denaturing solution containing 4 M guanidinium thiocyanate, 25 mM sodium citrate (pH 7), 0.5% N-lauroyl sarcosine, and 0.1 M 2-mercaptoethanol. Sixty μl of 2 M sodium acetate (pH 4), 600 μl of water-saturated phenol, and 120 μl of chloroform-isoamylalcohol mixture (49:1) were added. After cooling on ice for 15 minutes, the samples were centrifuged at 10,000 g at 4℃ for 20 minutes and precipitated with ethanol. Following washing with 70 % ethanol, the RNA pellet was dried under vacuum and dissolved in 20 μl of sterilized distilled water.

4. Reverse transcription-polymerase chain reaction (RT -PCR)

First strand complementary deoxyribonucleic acid (cDNA) was synthesized from 0.5 μg of total mRNA prepared as above. The mRNA was mixed with transcription buffer using 0.5 μg of random primers and 200 U of M-MLV reverse transcriptase in the presence of 0.8 mM dithiothreitol, 25 U of RNase inhibitor, and 1 mM dNTPs. After incubation at room temperature for 10 minutes, the reaction tubes were allowed for 60 minutes at 37℃ followed by 5 minutes at 95℃. PCR was performed on 4 μl aliquots of the 10 μl of the first strand cDNA reaction using specific primer sets. They were 5'-gccatgtcaagcgccccatg-3' and 5'-cccagtggggatatcgacag-3' for SRY gene, 5'-agcaaacgcacgtgtctcgg -3' and 5'-cacctgcacttcgagatgat-3' for DAX1 gene, 5'-gtccagtgtgtgg-tgacaag-3' and 5'-ccaatggcttcaagctggag-3' for SF1 gene, and 5'-ct-tcctagagaccctcactc-3' and 5'-cggagctgcgggagtcgttg-3' for AMH gene. PCR was performed using 50 μmol of each primer, 2.5 U of Taq polymerase in the presence of 200 μM dNTPs, and 1.5 mM $MgCl_2$. The PCR reaction cycles were composed of 94℃ for 3 minutes at the first stage, 94℃ for 50 seconds, 50℃ for 50 seconds, and 72℃ for 1.5 minutes for 40 cycles at the second stage, and 72℃ for 10 minutes at the last stage. Control reactions were done with 1 μg of total mRNA after either RNase or DNase treatment. To avoid the contamination of the solutions, control reactions were processed without the addition of mRNA.

The PCR products were electrophoresed through 1.5% agarose gels and compared to the expected size. The PCR products were directly sequenced using specific oligonucleotide primers (sense and antisense) by Korea Basic Science Institute to confirm the identity of the PCR products.

5. Data analysis

The sequences of the sex-related genes determined from the fetus of mouse were analyzed for the homology. Homology was, within the limitation of PCR outcome, calculated by the numbers of the known nucleotides that were previously reported in other animals. x

RESULTS

The effects of 2-BP on the litters of mouse were shown in Table 1. The mean numbers of litters survived by the weaning time were as followings: 9.5 litters from both non-treated female and non-treated male mice, 10.0 litters from non-treated male mice and 2-BP-treated female mice, 9.5 litters from non-treated female mice and 2-BP-treated male mice, and 8.0 litters from

Table 1. Effects of 2-bromopropane on the numbers and sexes of litters of mouse

Male \ Female	Non-treatment		2-Bromopropane treatment	
	Litters (dams 8)		Litters (dams 5)	
Non-treatment	Female	Male	Female	Male
	38 (4.8±1.98)	38 (4.8±1.16)	30 (6.0±1.00)	20 (4.0±2.00)
	Litters (dams 11)		Litters (dams 9)	
2-Bromopropane treatment	Female	Male	Female	Male
	50 (4.5±1.69)	55 (5.0±1.61)	32 (3.6±1.94)	40 (4.4±1.88)

The numbers are summation of each sex of litters survived by the time of weaning. The numbers in parenthesis are mean±SD.

both 2-BP-treated female and 2-BP-treated male mice. The sex ratio that is the numbers of male litters based on the numbers of female litters are complicated. The sex ratios are as followings: 1.00 from both non-treated female and non-treated male mice, 0.67 from non-treated male mice and 2-BP-treated female mice, 1.10 from non-treated female mice and 2-BP-treated male mice, and 1.25 litters from both 2-BP-treated female and 2-BP-treated male mice.

The ovaries of mice treated with 2-BP were histologically examined and compared to those of mice that did not receive 2-BP(Fig. 1). The numbers of Graafian, antral, and growing follicles were slightly reduced in the ovaries of 2-BP-treated mice compared to the numbers of those in the ovaries of intact mice. Ovaries of mice treated with 2-BP showed eccentric pyknotic cells scattered around oocytes.

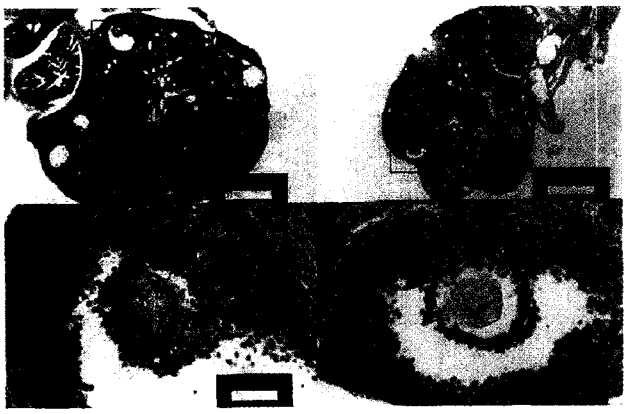

Fig. 1. Histological view of ovaries of mice. The mice were continuously infused with 2-BP at the concentration of 1,000 mg/day/kg of body weight for 3 weeks. Left panels show intact ovary and right panels show ovary treated with 2-BP. The rectangles in the upper panels were enlarged in the lower panels. Magnification: Upper, ×40; lower, ×400.

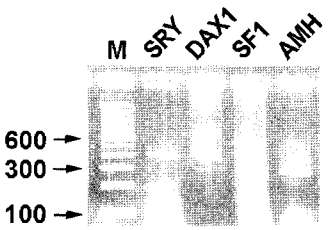

Fig. 2. RT-PCR products of sex-related genes. Dams were sacrificed on the gestation day 10. Each fetus was subjected to the RT-PCR. The sex-related genes (SRY, DAX1, SF1, and AMH) were detected on 1.5% agarose gel electrophoresis. M: 100 base pair marker.

Fig. 2 shows the RT-PCR products of sex-related genes, SRY, DAX1, SF1, and AMH genes. The RT-PCR products were clearly appeared at the reaction cycles of 40. The RT-PCR products were not eliminated in samples treated with DNase but eliminated by the treatment of RNase prior to RT-PCR(data not shown). Contamination was not occurred because any RT-PCR product was not seen in the absence of mRNA. The size of RT-PCR products was determined by the molecular marker(lane 1, 100 base pairs). The sizes of RT-PCR products of SRY, DAX1, SF1, and AMH genes were as expected. The RT-PCR products of the sex-related genes were directly sequenced in both directions of sense and antisense. The nucleotides identified in the electropherogram were 416, 466, 326, and 389 for SRY, DAX1, SF1, and AMH genes, respectively (Figs. 2 and 3).

Fig. 4 through 7 demonstrate a comparison of the mouse sex-related genes examined in this study to the nucleotide sequences reported beforehand in some mammals. The homology was calculated, within the limitation of the nucleotide sequence identified in this study, for the nucleotides which has been known. The nucleotide sequence analysis of mouse SRY gene, which is resided in the Y chromosome, shows a homology of 89% and 81% with rat and human, respectively. But there are a few nucleotides that are different each other in some mice. The sequence analysis of mouse DAX1 gene, which is resided in the X chromosome, shows a homology of 94% and 86% with the same order of the animals mentioned above. The mouse SF1 gene shows a homology of 100% and 92%, and AMH 90% and

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SRY
gccatgtcaa gcgccccatg aatgcattta tggtgtggtc ccgtggtgag  50
aggcacaagt tggcccagca gaatcccagc atgcaaaata cagagatcag 100
caagcagcta ggatgcaggt ggaaaagcct tacagaagcc gaaaaaaggc 150
ccttttttcca ggaggcacag agattgaaga ccctacacag agagaaatac 200
ccaaactata aatatcagcc tcatcggagg gctaaagtgt cacagaggag 250
tggcatttta cagcctgcag ttgcctcaac aaaactgtac aaccttctgc 300
agtgggacag gaacccacat gccatcacat acaggcaaga ctggagtaga 350
gctgcacacc tgtactccaa aaaccagcaa agcttttatt tgcagcctgt 400
cgatatcccc actggg                                      416

DAX1
agcaaacgca cgtgtctcgg gaagcacccg aggcacatcg cagaggcgag  50
tggtggcagc tgtcctactg tacccagagt gtgggtggcc cagaggggct 100
gcagagcaca caggccatgg cgttcctgta ccgcagctat gtgtgcggtg 150
aagagcagcc ccagcagatc agcgttgcct ctggcacgcc cgtgagcgca 200
gaccaaacac cagcgacccc gcaagagcag ccgagggctc cctggtggga 250
cgcctcacct ggtgtgcagc gtctgatcac actcaaggat ccacaggtgg 300
tgtgcgaggc agcgtccgct ggcctgttga agaccctgcg ctttgtcaag 350
tacttgccct gcttccagat cctgccccta gatcagcagc tggtgctggt 400
gcggagctgt tgggcgcccc tactcatgct tgagttggcc caagatcacc 450
tgcacttcga gatgat                                      466

SF1
gtccagtgtg tggtgacaag gtgtcgggct accactacgg gctgctcacg  50
tgcgagagct gcaagggctt cttcaagcgc acagtccaga acaacaagca 100
ttacacgtgc accgagagtc agagctgcaa aatcgacaag acgcagcgta 150
agcgctgtcc cttctgccgc ttccagaagt gcctgacggt gggcatgcgc 200
ctggaagctg tgcgtgctga tcgaatgcgg ggtggccgga acaagtttgg 250
gcccatgtac aagagagacc gggccttgaa gcagcagaag aaagcacaga 300
ttcgggccaa tggcttcaag ctggag                           326

AMH
cttcctagag accctcactc gcttggttcg tgctctgcgg ggacctctga  50
cccaggcttc gaacacgcaa ctggccctgg accctggtgc gctggccagc 100
ttcccacagg gcctggtcaa cctgtcagac cccgcagcac tgggacgcct 150
gctcgactgg gaggaacccc tattactgct gctgtcaccc gctgcggcca 200
cggagaggga acctatgccg ctgcacggcc ccgcttctgc tccctgggca 250
gcgggcctgc aacgcagggt ggcagtggag ctgcaggcgg cagcctcaga 300
gctgcgggac ctcccgggtc tgccacccac agctcccccg ctgctggcgc 350
gcctgctagc gctgtgtccc aacgactccc gcagctccg              389
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Fig. 3. Nucleotide sequences of sex-related genes detected in the fetus of mouse. RT-PCR was performed from dams sacrificed on the gestation day 10. The nucleotides were directly determined.

88% in the nucleotide sequence analysis of the animals mentioned above.

DICUSSION

The present results indicate that 2-BP affects the numbers of litters. As shown in Table 1, The mean numbers of litters survived by the weaning time were reduced to 8.0 in the only group of both female and male mice treated with 2-BP, compared to 9.5～10.0 in the other groups. The reduced num-

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Mouse gccatgtcaa gcgccccatg aatgcattta tggtgtggtc ccgtggtgag  50
Rat   ····a····· ·········· ·········· ·········· ···c··a···
Human --------·· ···a····· ··c·····c· ·c········ t··c·a·c··

Mouse aggcacaagt tggcccagca gaatcccagc atgcaaaata cagagatcag 100
Rat   ·········· ····t··a·· ·········· ·····g···t ··········
Human ····g····a ····t·tag· ·········a ····g···ct ··········

Mouse caagcagcta ggatgcaggt ggaaaagcct tacagaagcc gaaaaaaggc 150
Rat   ·········· ····atca·· ·········· ·········t ··········
Human ·········· ····a·ca·· ·····tg·· ···t····· ·····t···

Mouse cctttttcca ggaggcacag agattgaaga ccctacacag agagaaatac 200
Rat   ·········· ·····g···· ···c····· ·········· ··········
Human ·a··c····· ·········· ·a···ac··g ··a·g····· ··········

Mouse ccaaactata aatatcagcc tcatcggagg gctaaagtgt cacagaggag 250
Rat   ·········· ·········· ·····a···· ·t········ ··········
Human ··g··t···· ·g·····---- ---------- ---------- ----------

Mouse tggcatttta cagcctgcag ttgcctcaac aaaactgtac aaccttctgc 300
Rat   ·tat·c···g ·g··a····· ·········· ·········· ····g····
Human ---------- ---------- ---------- ---------- ----------

Mouse agtgggacag gaacccacat gccatcacat acaggcaaga ctggagtaga 350
Rat   ·a·······a c····t···c a·t··t·t·· ··g·a····· ··········
Human ---------- ---------- ---------- ---------- ----------

Mouse gctgcacacc tgtactccaa aaaccagcaa agcttttatt tgcagcctgt 400
Rat   ·····---- ---------- ---------- ---------- ----------
Human ---------- ---------- ---------- ---------- ----------

Mouse cgatatcccc actggg                                    416
Rat   ---------- ------
Human ---------- ------
```

Fig. 4. A comparison of nucleotides of SRY gene reported in some animals. Mouse: *Mus musculus*, Rat: *Rattus* sp.; 89%, Human: *Homo sapiens*; 81%. · indicates the same nucleotides that expressed in mouse. - represents nucleotides unknown.

bers of litters of mice treated with 2-BP is supported by the previous reports(Sekiguchi & Honma, 1998; Ishikawa et al., 2001; Sekiguchi et al., 2001). When the female F344 rats were treated with 2-BP at intervals of 2 or 3 days for 15～17 days, the number of ovulated ova in spontaneous ovulation was decreased(Sekiguchi et al., 2001). 2-BP also caused the number of ova superovulated by pregnant mare's serum gonadotropin and human chorionic gonadotropin treatment to decrease remarkably in mouse(Sekiguchi & Honma, 1998). Moreover, when 2-BP was administered to pregnant mice intraperitoneally during the early preimplantation period, the embryo cell number were decreased(Ishikawa et al., 2001). Spermatogenesis was impaired by 2-BP(Son et al., 1999; Li et al., 2001). Besides to the reduction of daily sperm production, weights of reproductive organs such as testes, epididymis, seminal vesicle, and prostate were decreased(Li et al., 2001). The oral administration of 2-BP at a high concentration of 3.5g/day/kg of body weight induced

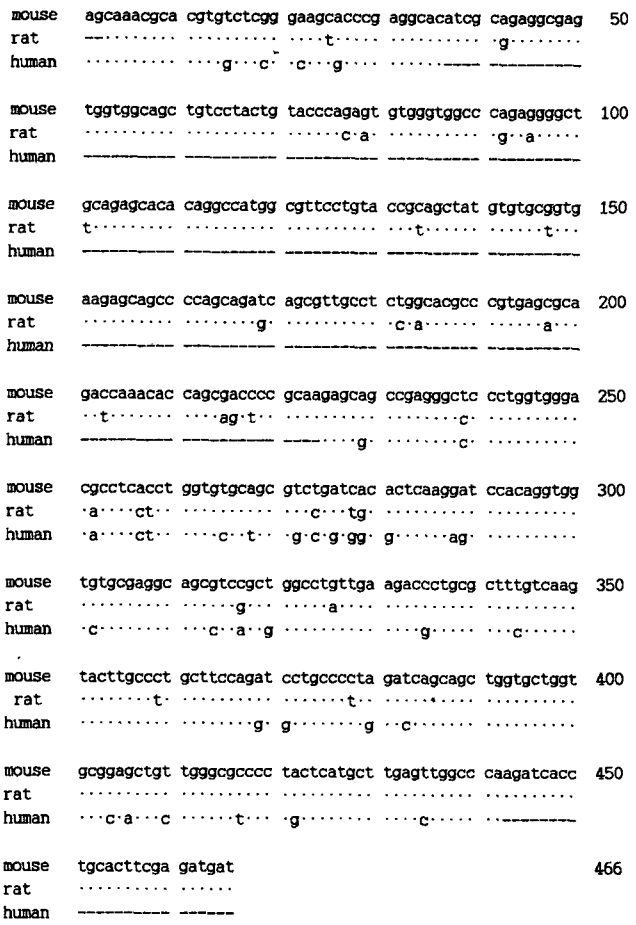

Fig. 5. A comparison of nucleotides of DAX1 gene reported in some animals. Mouse: *Mus musculus*, Rat: *Rattus* sp.; 94%, Human: *Homo sapiens*; 86%. · indicates the same nucleotides that expressed in mouse. - represents nucleotides unknown.

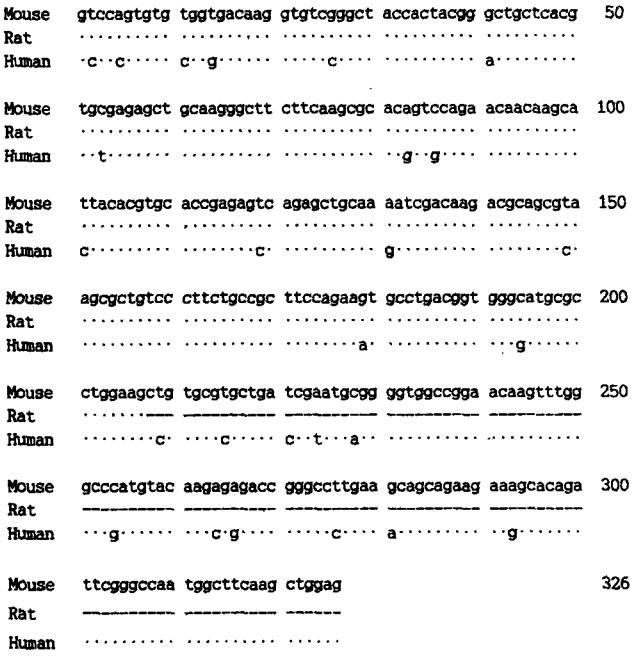

Fig. 6. A comparison of nucleotides of SF1 gene reported in some animals. Mouse: *Mus musculus*, Rat: *Rattus* sp.; 100%, Human: *Homo sapiens*; 92%. · indicates the same nucleotides that expressed in mouse. - represents nucleotides unknown.

testicular atrophy followed by depletion of spermatocytes, spermatids, and spermatozoa(Son et al., 1999). Thus, 2-BP seems to affect reproductive activity of both female and male, resulting in reduced number of litters presented here. The difference was not conspicuous in this study. A main cause may be due to the short period of 2-BP treatment because the formation of mature spermatozoa needs at least 6 weeks. The 3 week period of 2-BP treatment might not be enough to completely deplete the formation of Graafian follicles although the period affected at least 1 full cycle of estrus.

On the other hand, the present results of the sex ratio are confusing. The female litters were greater than male litters in the only group of female treated with 2-BP. The sex ratio was 0.67. The other groups showed male litters greater than female litters. The sex ratios were in the range of 1.00~1.25. The sexes were checked at the weaning time instead of birth time. The sex ratio may be different if sex was examined at birth. But in the light of the ratio of male to female which is somewhat above one, the lowered sex ratio of 0.67 is not likely to occur. It can be thought that the male litters could be sensitive to 2-BP that would be released from their dams. But the litters from 2-BP-treated dams mated with 2-BP-treated males showed greater males than females. Thus the smell sensation might not be exerted at this point. It might be due to small number of dams in the group, resulting in a deviation of general sex ratio pattern. The present results of litters indicate that 2-BP affects somehow reproductive function, leading to reduction of litters and putative change of the sex ratio.

The ovaries of 2-BP-treated female mice seemed not to be remarkably affected by the histological examination, although the numbers of litters were slightly reduced. Graafian, antral, and growing follicles were appeared in the ovaries of both intact and 2-BP-treated female mice. Ovaries of 2-BP-treated mice showed eccentric pyknotic cells scattered around oocytes. The results are consistent with the previous report used rat(Yu et al., 1999). Although deformity of the cells in the follicles was observed, it did not affect the numbers of litters.

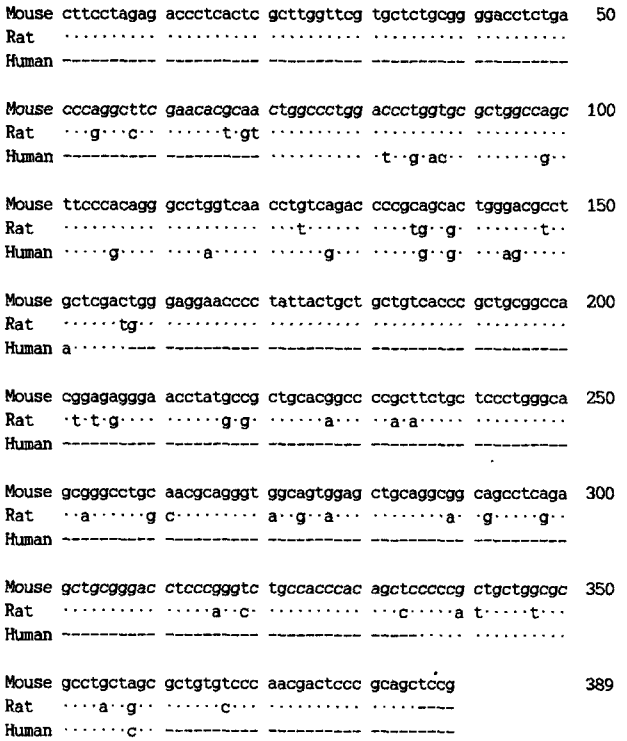

Fig. 7. A comparison of nucleotides of AMH gene reported in some animals. Mouse: *Mus musculus*, Rat: *Rattus* sp.; 90%, Human: *Homo sapiens*; 88%. · indicates the same nucleotides that expressed in mouse. - represents nucleotides unknown.

The RT-PCR products of sex-related genes, SRY, DAX1, SF1, and AMH genes were 416, 466, 326, and 389 for SRY, DAX1, SF1, and AMH genes, respectively. The sequences of the genes were identical to those reported previously. But there are a few nucleotides that are different each other in some mice. The present results demonstrate nucleotide sequences of sex -related genes, SRY, DAX1, SF1, and AMH genes which are expressed during the sex differentiation in mouse. The outcome will contribute to investigate the normal relationship of sex-related gene expression and sex determination at molecular level in mouse.

It has been well known that sex is primarily determined by the sex chromosome in mammal. All mammals starts off as embryos along a sexually neutral developmental pathway. The gonadal rudiment can develop into either an ovary or a testis, which is followed by sexual determination. The presence of a Y chromosome leads the embryo's gonads to develop into testes rather than into ovaries.

In mammals, the major gene for the testis-determining factor resides on the short arm of the Y chromosome. The SRY gene located on the short arm of the Y chromosome acts as a developmental switch, initiating a pathway of gene activity that leads to the differentiation of testis rather than ovary from the indifferent gonad (genital ridge) in mammalian embryo(Sinclair et al., 1990; Hacker et al., 1995; Brennan et al., 1998; Koopnan, 1999). Its expression then disappears(Brennan et al., 1998). DAX1 gene is expressed in the genital ridges of the mouse embryo. If there were two copies of this region on the active X chromosome, the SRY signal that results in male would be reversed, leading to female characteristics(Swainet al., 1998; Goodfellow & Camerino, 1999). SF1 known as a regulator of the steroidogenic enzymes in mammals is differentially expressed during the process of development. In a turtle, SF1 expression is increased in gonads at a male-producing temperature and decreased at a female-producing temperature, suggesting a role for SF1 in the sex differentiation pathway(Fleming et al., 1999). AMH is also involved in the process of sex determination(Tran et al., 1977; Cate et al., 1986). The mouse AMH gene has a promotor sequence that is bound by SRY(Haqq et al., 1993). In the present study SRY, DAX1, SF1, and AMH genes were at the same time detected by applying RT-PCR method in the fetus of mouse at the gestation day 10. This period is critical to fetuses in determining sex, which integrally results from the expression of all other genes involved in the sex differentiation as well as the genes identified in this study. These results can contribute to examine the expression of sex-related genes at molecular level in mouse although the mechanism between them has not been examined in the present study. For instance, the effects of EDs could be investigated with regard to the expression of sex-determining factors because sex differentiation in invertebrate is affected by them.

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