FOUR CASES OF FERTILE BOVINE HETEROSEXUAL TWIN FEMALES WITH OR WITHOUT BLOOD CHIMERAS

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The freemartin is congenitally sterile, with vascular anastomosis between heterosexual twin foeti. Freemartin is diagnosed with XX/XY chimerism. Four cases of fertile heterosexual twin females with or without XX/XY chimeras were investigated.

Materials and methods: Holstein heifers (n=4) born as co-twin to a male in commercial farms were enrolled. The vaginal length was measured. Detection of Y specific DNA and chromosomal analyses were carried out, together with a gonadotropin stimulation test. As for the controls, freemartins (n=12) and normal female calves (n=4) were determined.

Genomic DNA in blood samples was extracted and loop-mediated isothermal amplification (LAMP) reaction was carried out (Loopamp Bovine Embryo Sexing Kit, Eiken, Tokyo). Leucocytes were cultured in the medium containing phytohemaggulutinin-M and were analyzed for sex chromosomal chimerism. eCG 1000 IU on d0 and hCG 1500 IU on d2 was administered. Peripheral blood was collected to determine plasma levels of estradiol-17 beta (E2) and progesterone (P4) by ELISA. Data were statistically analyzed by ANOVA for repeated measures.

Results: Vaginal length was greater than 20 cm in 4 cases, whereas freemartins were between 10 to 16 cm. Cases A and B conceived to AI. In case C, the heifer conceived to an unobserved service with a beef full and Case D was inseminated twice, but did not conceive.

Cases A and B possessed the Y specific DNA, whereas Cases C and D lacked Y DNA. Control freemartin possessed Y DNA. Sex chromosomal chimerism was detected, XX/XY: 51/2 in Case A and 44/6 in Case B. In Case C & D, XX/XY: 50/0 was detected. After Gonadotrophin treatment, E2 levels elevated significantly in Case A and normal heifers, whereas it remained low in Case D and freemartins. P4 levels elevated significantly in Case D and normal heifers, whereas it remained low in Case A and freemartins.

Discussions: In fertile cases with XX/XY chimerism, it would be postulated that a choriovascular anastomosis between twins may be established after the sexual differentiation of fetal gonad to ovaries. Fertile case with normal XX chromose, choriovascular anastomosis may not be occurred, albeit they were heterosexual twin females.

Conclusions: The accumulation of data is important to demonstrate some fertile cases of bovine heterosexual twin females. The eCG-hCG stimulation test would confirm the reproductive ability in early stages in the heterosexual twin female.