—Research Note—

Absence of Fetal Cells in Bovine Jugular and Uterine Vein Blood at a Level of 1 in 10,000

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Abstract: The objective of this study was to determine if bovine fetal cell DNA is present in pregnant maternal blood as human fetal cells are present in the blood of pregnant women after they cross the placenta and penetrate into the *v. uterina*. The btDYZ PCR amplification was employed to detect male-specific DNA of bovine male fetal cells. Blood samples were collected from the *v. uterina* and *v. jugularis externa* of 3 pregnant cows (2 with male fetuses and 1 with a female fetus 12 to 20 wks of age) and from only the *v. jugularis externa* of 33 pregnant cows (with 18 male and 15 female fetuses 10 to 40 wks of age). All blood samples were male-specific DNA negative, which indicated that fetal cells were absent in bovine maternal blood at a level of 1 in 10,000 and, therefore, cannot be used for prenatal sexing with PCR.

Key words: Fetal genotype, Polymerase chain reaction, Prenatal diagnosis, btDYZ, *v. uterina*.

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Although the maternal-fetal barrier is generally considered effective in limiting cell transfer from fetus to mother, there have been several reports suggesting that the blood of pregnant women contains nucleated cells such as syncytiotrophoblast [1] and lymphocyte cells [2] derived from the fetus.

For the purpose of prenatal genotyping of the fetus, fetal cells in peripheral maternal blood could be an alternative to obtaining tissue using invasive techniques, such as amniocentesis and chorionic villus biopsy. Furthermore, such a technique could be used at as early as 9 weeks of gestation with fluorescence-activated cell sorting [3] and the polymerase chain reaction (PCR) amplification of Y-specific repeat sequence [4].

However in bovidae, no effort has been made to

determine whether or not the blood of pregnant cows contains fetal nucleated cells. The objectives of this study were to detect bovine male fetal cell DNA in the *v. jugularis externa* of maternal blood and to determine using PCR amplification of btDYZ, bovine Y-specific repeat sequence, if fetal cells cross the placenta and penetrate into the *v. uterina* of maternal blood. This method was successful in detecting male-specific DNA from a mixture of 1 part male to 10,000 parts female+male DNA and this is currently the most advanced test in bovidae [5].

Material and Methods

Two cows pregnant with 2 male fetuses and 1 cow pregnant with a female fetus (each fetus 12 to 20 wks of age) were used for blood collection from

both the *v. uterina* and the *v. jugularis externa*. Surgery was performed following the right flank laparotomy method [6] which has been approved for the care and use of agricultural animals [7]. Blood was collected by means of Medicut Sentinel Line Catheter (14 G, 70 cm, Argyle, Tokyo) via the uterine vein.

Blood samples were collected from only the *v. jugularis externa* of 33 pregnant cows with 18 male and 15 female fetuses 10 to 40 wks of age.

The 200 μ l of sample DNA (approximately 40 μ g) were prepared from 1 ml of blood by Higuchi's [8] or by Wang's [9] method. In the PCR method, the primer pairs were synthesized from the sequence of btDYZ (upstream: CCTTTAGGG-GAAAATGGACTGACA; downstream: ATGTG-ACACTGC-TGGAAGG) reported by Perret [10] and of the bActin (upstream: ACGTCGCCTTG-GACTTCGAGCAGG; downstream: GCTGGAA-GGTGGACAGGG-AGGCCAGGA) reported by Degen [11]. The PCR mixture contained 20 μ l of sample DNA (approximately 4 μ g); 53.5 μ l water; 8 μ l 10 $\times$ PCR buffer (100 mM Tris-HCl, pH 8.3; 500 mM KCl, 15 mM MgCl₂, 0.01% gelatin); 5 μ l of each primer (20 μ M); 2 μ l of 10 mM dNTPs (dATP, dCTP, dTTP, dGTP); and 0.5 μ l of Taq DNA polymerase (5U/ μ l, Perkin Elmer Cetus, Urayasu, Japan). The PCR was carried out for 1 min at 95 C, for 1.5 min at 60 C and for 1.5 min at 72 C in 35 cycles.

The presence of the expected size of the PCR products was confirmed by electrophoresis in ethidium bromide-stained 2.0% agarose gel (Type II, Sigma, St. Louis, MO, USA) in TAE buffer. The DNA bands were photographed under UV light.

As controls, DNA from fertile cows (60 XX karyotype) and male calves (60 XY karyotype) were used. To control for possible contamination, PCR reactions were set up in parallel without DNA and with DNA and without primers. All recommended precautions were followed [12, 13] to prevent a possible carry-over of cells, DNA or PCR products.

Results

With all blood samples from the *v. uterina* or the *v. jugularis externa*, btDYZ amplified products were not detected but bActin products were detected (Table 1 and Fig. 1).

Discussion

The possible explanation for the detection of bActin products was that bActin products were amplified from maternal DNA and not from fetal DNA.

This is the first study ever reported to determine whether or not fetal cells exist in bovine maternal blood. Based on these results, it is reasonable to conclude that fetal cells are very rare, or may be absent in bovine maternal blood and they cannot be used for prenatal sexing with the aforementioned PCR method.

The possible reasons for these results are that the placental barrier between mother and fetus may be stronger in cows than it is in humans, and that bovine fetal cells cannot cross the barrier, since the

Table 1. PCR amplification of btDYZ and bActin from maternal bloods

Maternal bloods	Fetus			Amplification			
	age (months)	sex	n	btDYZ		bActin	
				+	-	+	-
<i>V. uterina</i>	3.5		1	0	1	1	0
	5		1	0	1	1	0
	4		1	0	1	1	0
<i>V. jugularis ext.</i>	3		6	0	6	6	0
	4-6		6	0	6	6	0
	7		6	0	6	6	0
	3		5	0	5	5	0
	4-6		5	0	5	5	0
	7		5	0	5	5	0

Fig. 1. Electrophoresis of btDYZ and bActin amplified products from blood samples. Lanes 2 through 3 and 10 through 11 showed the control products from normal male blood DNA with btDYZ primers (lane 2) and with bActin primers (lane 10), from normal female blood DNA with btDYZ primers (lane 3) and with bActin primers (lane 11). Lanes 4 through 8 and lanes 12 through 16 showed the amplified products from the sample DNA indicated below and btDYZ primers (lanes 4-8) and bActin primers (lanes 12-16). Lanes 1 and 9 showed the ϕ X174/HincII marker. J.: *v. jugularis externa* blood DNA, U.: *v. uterina* blood DNA, or : fetal sex, number: fetal age (months), + or -: contain primers or not.

maternal-fetal barrier is hemochorial in people and epithiochorial in cows [14].

Another possible reason for these results is that the method used in this study might not have had enough sensitivity. Nakagome *et al.* [15] reported the absence of human fetal cells in maternal circulation at a level of 1 part male to 25,000 parts female+male DNA. With the current method, male-specific DNA could be detected only at a level of 1 in 10,000.

Numerous reports have been published on prenatal sexing in domestic animals [16-18]. However, there are no techniques which can be used both for embryo transfer and artificial insemination to save labor. In horses, mules and pigs, fetal erythrocytes cross the placenta and penetrate into the maternal blood [19, 20]. Further work is required to determine the possibility of prenatal sexing using fetal cells in the maternal blood of domestic animals.

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