

The Possibility of Early Estimation for Fertility in Bovine Heterosexual Twin Females

Shinya SATOH, Toh-Ichi HIRATA¹⁾, Yoh-Ichi MIYAKE*, and Yoshihiro KANEDA²⁾

Laboratory of Theriogenology, Department of Veterinary Medicine, Faculty of Agriculture, Iwate University, Morioka 020, ¹⁾Education Farm, Faculty of Agriculture, Iwate University, Shizukuishi 020-05, and ²⁾Laboratory of Theriogenology, Department of Veterinary Medicine, Faculty of Agriculture, Tokyo University of Agriculture and Technology, Fuchu 183, Japan

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ABSTRACT. To diagnose the possibility of early estimation for fertility in bovine heterosexual twin females, we designed a new diagnostic program. The 9 freemartins (FM) and 5 normal females (Normal) were used in this study. All 14 cases, at 4 months of age, were given Pregnant Mare Serum Gonadotrophin (PMSG) and human Chorionic Gonadotrophin (hCG) 1.5-2 days later. Thereafter, the concentration of estradiol-17 β (E₂) was determined by RIA, and that of progesterone (P) was done by RIA and EIA (Ovcheck EIA Kit). The concentration of E₂ in the Group of Normal rapidly increased after administration of PMSG, but in the Group of FM, the concentration of E₂ changed in very low levels over 14 days. The concentration of P in the Group of Normal rapidly increased after administration of PMSG, but in the Group of FM, the concentration of P changed in very low levels over 14 days. — **KEY WORDS:** fertility test, freemartin, sex hormone.

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Many types of congenitally abnormal karyotypes have been reported as a cause of reproductive disorders in cattle [1]. Of these, the freemartin, with absolute sterility and sex chromosome chimerisms, is the most frequently occurring [7], representing 92% of heterosexual twin females. Also, sterile cases similar to the freemartin have been reported in single-born heifers [11, 14] and in heifers born with *Acardius amorphus* [10] with sex chromosome chimerism. Thus, until now it was believed that heifers with sex chromosome chimerism show abnormal sexual organs and are sterile. However, some papers reported cases of fertile heterosexual twin female in bovine, yet showing sex chromosome chimerism similar to the freemartin [2, 5, 8, 9, 13]. Therefore, it becomes clear that the possibility of fertility in heterosexual twin females will not be determined by usual diagnosis of freemartin syndrome. For this reason, we designed a new diagnosis to know the possibility of early estimation for fertility in bovine heterosexual twin females.

The 9 freemartins (FM) and 5 normal females (Normal) which were diagnosed by analyses of sex chromosome chimerism [1] and the Y chromosome-specific PCR test [4, 12] were used in this study. The vaginal length was 4.5-6.5 cm (5.7 cm on average), and the ratio of XY cells was 16.0-96.7% (57.3% on average) in the cases from FM. And the Y specific sequence was detected in all cases from FM. All 14 cases, at 4 months of age, were given intramuscularly 1,000 IU of Pregnant Mare Serum Gonadotrophin (PMSG) and 1,500 IU of human Chorionic Gonadotrophin (hCG) 1.5-2 days later. After the administration of PMSG, blood samples were collected every other day over 14 days from the jugular vein, and the concentration of peripheral estradiol-17 β (E₂) and progesterone (P) was determined by RIA [15], and also the concentration of P was determined by EIA (Ovcheck EIA Kit).

The concentration of E₂ (Fig. 1) rapidly increased the 2nd day after administration of PMSG and reached its peak (808.7 pg/ml on average) on the 8th day in the Group of Normal. On the other hand, in the Group of FM, the concentration of E₂ did not increase and changed in very low levels (under 1.9 pg/ml) over 14 days. The concentration of P (Fig. 1) rapidly increased the 6th day after administration of PMSG and reached its peak (22.4 ng/ml on average) on the 10th day in the Group of Normal. However, in the Group of FM, the concentration of P did not increase and changed in very low levels (under 0.5 ng/ml) over 14 days. There were favorable correlations between the P levels by RIA and those by EIA.

Studies on the freemartin syndrome were well summarized by Marcum [7]. However, several exceptional cases, which do not coincide with Marcum's definition [7],

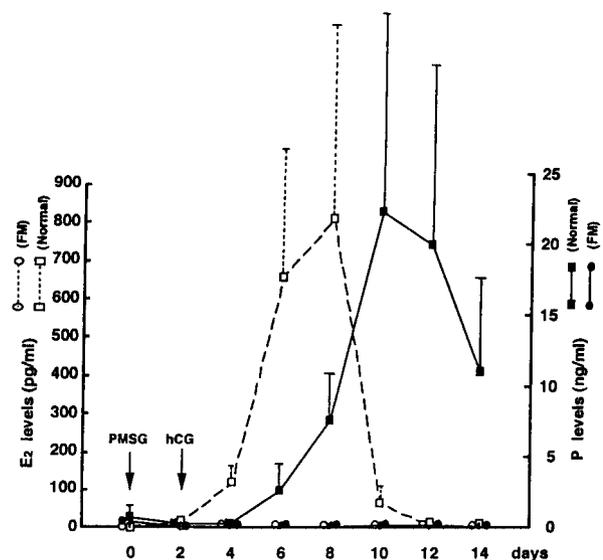


Fig. 1. Changes of peripheral P (solid line) and E₂ (broken line) levels after administration of PMSG and hCG in the Group of Normal (n=5) and FM (n=9).

* CORRESPONDENCE TO: MIYAKE, Y-I., Laboratory of Theriogenology, Department of Veterinary Medicine, Faculty of Agriculture, Iwate University, Morioka 020, Japan.

have been reported more recently [2, 4, 5, 8–11, 13, 14]. From cases studied by many papers, freemartin syndrome has been classified as 1) freemartins which coincided with Marcum's definition [7]; 2) single-born sterile heifers [11, 14]; 3) a heifer born with *Acardius amorphus* with the XX/XY chimerism [10]; 4) a fertile heterosexual twin heifer with XX/XY chimerism; [2, 5, 8, 9, 13] and 5) freemartins with a very low XY cell count [4].

Generally, karyotyping is used to detect the Y chromosome in bovine freemartin syndrome. Recently, it has become possible to diagnose freemartins with a very low XY cell count according to PCR by which the Y specific sequence are easily and rapidly detected [4, 12]. However, PCR is not convenient to diagnose freemartin for almost all veterinarians. Furthermore, it is not possible to detect the fertile heterosexual twin female with sex chromosome chimerism using karyotyping or PCR.

From the result obtained, the new diagnostic program which we designed will be very useful in determining the possibility of early estimation of fertility in bovine heterosexual twin females, because showing the normal sexual cycles in fertile heterosexual twin females [2, 5, 8, 9, 13]. Namely, it appears that the cases with high E_2 levels (> 5 pg/ml after 2nd day) and high P levels (> 1 ng/ml after 6th day) after administration of PMSG and hCG may be estimated to be fertile. Furthermore, this program will become advantageous for veterinarians who have not been able to analyze the sex chromosome chimerism to diagnose freemartins, because of the possibility of determination in peripheral P levels by EIA.

There are some papers in which immature calves, aged 4–24 weeks, react for superovulation with PMSG and hCG [3]. Although we used calves at 4 months of age in this study to get reliable results, it will be possible to estimate fertility in younger calves.

The concentration of P in peak after administration of PMSG and hCG was similar to those at the time of embryo transfer [6]. However, the concentration of E_2 in peak was very high and about 10 times or more compared with those at the time of superovulation [6]. It seems that the amount of hormonal drugs may be too much for ovarian reaction of

young heifers, and/or individual differences may influence the response [3].

We were anxious to find worthwhile effects for future fertility in heifers in the Group of Normal. However, it is ascertained that one normal female used in this study had a calf after the termination of the study.

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