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# ABSTRACT

# Improvement of pregnancy rate in Japanese Black cows by administration of hCG to recipients of transferred frozen-thawed embryos

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Japanese Black primiparous and multiparous beef cows (n = 120) were selected as recipients and randomly divided into three groups (A, B, and C) of 40 recipients each. Group A received an intramuscular (i.m.) treatment of 1500 IU human chorionic gonadotropin (hCG) on day 1 (day 0 = onset of estrus), while Group B received an i.m. treatment of hCG on day 6. Group C received an i.m. treatment of 5 ml saline on day 6 as a control. On day 7, frozen-thawed embryo transfer was conducted in all groups, and pregnancy was diagnosed by palpated per rectum 40-50 days after the transfer. Twelve recipients were randomly selected from each group. Plasma progesterone (P) and estradiol-17beta (E2) concentrations were determined in these recipients on days 6, 7 and 14, and at the time of pregnancy diagnosis, and their ovaries were examined for a corpus luteum and follicles by palpated per rectum. The pregnancy rate in Group B was higher (67.5%. P < 0.05) than the rate in Group C (45.0%) and in Group A (42.5%). The plasma P concentration on day 14 tended to be higher although not significantly in Group B than in Groups C and A. At the time of pregnancy diagnosis, the blood P concentration of pregnant recipients in Group B was higher (P < 0.05) than that of those in Groups C and A. The plasma E2 concentrations on days 7 and 14 were lower (P < 0.05) in Group B than in Groups C and A. These results showed that administration of hCG 6 days after estrus improved the pregnancy rate for non-surgical frozen embryo transfer 7 days after estrus by enhancing luteal function and depressing E2 secretion.

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## Introduction

Some reports have shown that 85–90% of bovine oocytes are fertilized by mating [1], [2], but approximately 30% of embryos are lost within 25 days after fertilization [3]. It has also been reported that early death of embryos occurs at a high frequency during the period from 9 to 17 days after transfer of bovine embryos (16–24 days after estrus) [4].

Previous studies have enumerated the following as factors for early embryonic death in transferred bovine embryos: transfer of an embryo of morphologically poor quality [5], inconsistency of the estrous cycle of donors with that of recipients [6], breeding of cattle in high-temperature environments [7], bacterial infection of the uterine endometrium [8], nutrition of recipients [9], and luteal insufficiency in recipients [10], [11]. It has also been indicated that the decrease in peripheral plasma progesterone (P) concentration due to luteal insufficiency in bovine recipients is a particularly important factor in the reduction in pregnancy rate following embryo transfer [12]. Administration of P [13], [14], human chorionic gonadotropin (hCG) [15], [16], [17], and gonadotropin releasing hormone (GnRH) [18], [19], [20], [21] have been employed to prevent early loss of the transferred embryos, which is caused by luteal insufficiency, and to improve the pregnancy rate following embryo transfer. However, these treatments vary in their effectiveness. There have been no reports showing marked improvement of pregnancy rate by hCG treatment in embryo transfer.

We have previously investigated the correlation of pregnancy rate and ovarian function in Japanese Black beef cattle recipients following the transfer of frozen embryos by the stepwise method, and confirmed that the pregnancy rate was increased in accordance with the increase in plasma P concentration [22]. It has also been reported that the pregnancy rate was decreased when the plasma concentration of estradiol-17 $\beta$  (E2) was increased, and the pregnancy rate increased as the ratio of plasma E2 to plasma P concentration (plasma E2/P ratio) decreased [23]. Furthermore, we showed that administration of hCG at a dose of 1500 IU 5 days after ovulation directly stimulated the development of the corpus luteum (CL), and induced ovulation of the dominant follicle of the first wave of follicular development, resulting in induced CL formation with increased P and decreased E2 concentrations [24]. Therefore, this method is considered applicable for prevention of early loss of the transferred embryo attributed to luteal insufficiency, and is considered to contribute to improvement in pregnancy rates.

In the present experiment, the influence of hCG at an administered dose of 1500 IU on pregnancy rate achieved with frozen-thawed embryo transfer was investigated by administering hCG on day 1 (day 0=onset of estrus) or day 6 to Japanese Black beef cattle recipients at breeding farms.

#### Section snippets

#### Recipients

The bovine recipients used were 120 primiparous and multiparous Japanese Black beef cattle that were maintained at 32 breeding farms. They were divided randomly into three groups: Group A (n=40) received a single intramuscular (i.m.) treatment of hCG at a dose of 1500 IU on day 1; Group B (n=40) received the i.m. treatment of hCG on day 6; and Group C (n=40) received a single i.m. injection of 5 ml saline solution on day 6 as a control. Twelve recipients were randomly selected from each group,

#### Pregnancy rate

Data for pregnancy rates following frozen-thawed embryo transfer are shown in Table 1.

#### Examination of ovaries

Group A showed the original CL on days 6 and 7, and the growth was similar to that in Group C on days 6, 7 and 14. Group B showed the original CL on days 6 and 7, and the growth was similar to that in Group C on these days. In 11 (91.7%) of the 12 recipients monitored in Group B, however, 1 or 2 newly formed induced CL were observed on day 14 and the day of pregnancy diagnosis at the site where follicles

#### Discussion

In the present study of non-surgical transfer of frozen embryos in cows, the pregnancy rate was 67.5% in Group B administered 1500 IU hCG on day 6, significantly higher (P<0.05) than the pregnancy rate in Groups C and A. In this respect, we demonstrated the relationship between pregnancy rate and the luteal function of bovine recipients after frozen embryo transfer, showing that the pregnancy rate was increased with the increasing plasma P concentration on days 6 and 7 [22]. According to another

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