Relationship between serum total cholesterol and progesterone levels and the number of transferable embryos in superovulated Holstein heifers and cows

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Summary. In an effort to find preselection criteria that can be used to show good ovarian response to superovulation treatment, the relationship between serum total cholesterol and progesterone levels and the number of transferable embryos that can be obtained per donor was investigated. Superovulation was induced by either PMSG or FSH in 106 Holstein heifers and cows between days 9 and 13 of the estrous cycle. Serum total cholesterol and progesterone were analyzed. Percentage of animals in which over 3 transferable embryos were obtained was significantly less in animals with serum total cholesterol level of less than 130 mg/dl as compared to the animals with 130 mg/dl and more. It was also found that percentage of animals in which no transferable embryos were obtained was significantly higher in animals with total cholesterol of less than 130 mg/dl. Animals with progesterone level of less than 1.0 ng/ml did not produce over 3 transferable embryos. These results indicate that serum total cholesterol and progesterone levels may be used as a criteria to indicate good ovarian response to superovulation.

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Introduction

Success of embryo transfer in cattle is dependent on the number of transferable embryos that can be obtained from one donor. At least 4 transferable embryos should be obtained per collection, and in addition, 6 to 7 transferable embryos are assumed to be the economic numbers obtainable per collection from a donor¹⁾. Superovulatory treatment in cattle has been conducted since the 1940s²⁾ and is now the method being routinely carried out. Nevertheless, the results of treatment are unpredictable and variable among in-

dividuals. This problem can be solved by applying preselection criteria to determine cows most likely to respond favorably to superovulation treatment. These criteria were investigated in the present study on the relationship between serum total cholesterol (T-CHO) and progesterone levels and the number of transferable embryos that can be obtained per collection.

Materials and Methods

One hundred-six Holstein heifers and cows, aged between 16 months to 12 years, were used as donors from March 1982 to June 1983.

Superovulation was induced in all animals using 2,000–4,000 IU of PMSG (Teikoku-Zoki Co.) or 35–55 mg of FSH (Denka Co.) between days 9 and 13 of the estrous cycle. PGF_{2α} (The Upjohn Co.) was given intramuscularly to induce luteolysis 2 days after gonadotropins injection. The amount of PGF_{2α} used were either 25 to 35 mg one time or 10 to 15 mg twice a day. The animals were then artificially inseminated 2 or 3 times with frozen semen at 12 hours interval following the end of estrus. The embryos recovered were classified as either transferable or non-transferable according to their morphological characteristics and whether the transferable embryos reached the expected developmental stage corresponding to their chronological age³⁾. Blood samples were collected from the coccygeal vein on the day of gonadotropins injection. Serum was separated and stored about -20°C until analysis. T-CHO was analyzed spectrophotometrically by an enzyme method⁴⁾. Serum progesterone was analyzed by the method validated by MAKINO⁵⁾. The recovery rate and the assay sensitivity for progesterone was 91.3% and 0.19 ng/ml, respectively. Intra- and inter-assay coefficients of variation were 3.3 and 3.9%, respectively. Animals were

divided into groups according to serum T-CHO (130 mg/dl >, 130 mg/dl ≤) or serum progesterone level (1.0 ng/ml >, 1.0–1.9 ng/ml, 2.0 ng/ml ≤). Difference of the percentages between groups was evaluated by the χ^2 -test.

Results

The relationship between serum T-CHO level and the number of transferable embryos is shown in Table 1. 13.0% (7/54) of the while that had T-CHO level of less than 130 mg/dl 48.1% (25/52) of the cows that had 130 mg/dl and more produced more than 3 transferable embryos ($P < 0.01$). No embryos were obtained in 48.1% (26/54) and 17.3% (9/52) of animals having less than 130 mg/dl and 130 mg/dl and more, respectively ($P < 0.01$). The relationship between the progesterone level

Table 2. Relationship between serum progesterone level and the number of transferable embryos in superovulated Holstein heifers and cows

Progesterone (ng/ml)	1.0 >	1.0–1.9	2.0 ≤
No. of animals tried	9	29	14
No. of animals with embryos			
≥4	0 (00.0%) ^a	9 (31.0%)	8 (57.1%) ^b
1–3	5 (55.6%)	10 (34.5%)	4 (28.6%)
0	4 (44.4%)	10 (34.5%)	2 (14.3%)

^{a, b} $P < 0.05$.

Table 3. Percentage of animals with more than 4 transferable embryos in relation to progesterone and total cholesterol

T-CHO (mg/dl)	Progesterone (ng/ml)		
	2.0 >	2.0 ≤	Subtotal
130 ≤	25.0% (4/16)	87.5% (7/8)	45.8% (11/24)
130 >	22.7% (5/22)	16.7% (1/6)	21.4% (6/28)
Subtotal	23.7% ^a (9/38)	57.1% ^b (8/14)	

^{a, b} $P < 0.05$.

Table 1. Relationship between serum total cholesterol level and the number of transferable embryos in superovulated Holstein heifers and cows

Cholesterol (mg/dl)	130 >	130 ≤
No. of animals tried	54	52
No. of animals with embryos		
≥4	7 (13.0%) ^a	25 (48.1%) ^b
1–3	21 (38.9%)	18 (34.6%)
0	26 (48.1%) ^a	9 (17.3%) ^b

Percentage with different superscripts in the same cow are significantly different ($P < 0.01$).

and the number of transferable embryos is shown in Table 2. Over 3 transferable embryos were obtained in 0.0% (0/9), 31.0% (9/29) and 57.1% (8/14) in animals with a progesterone level of less than 1.0 ng/ml, between 1.0–1.9 ng/ml and more than 2.0 ng/ml, respectively. This percentage was significantly lower in animals with a progesterone level of less than 1.0 ng/ml as compared to animals with a progesterone level of more than 2.0 ng/ml ($P < 0.05$). 87.5% (7/8) of animals with T-CHO of 130 mg/dl and more, and progesterone of 2.0 ng/ml and more produced more than 3 transferable embryos (Table 3). This percentage was higher than those groups in which T-CHO level was less than 130 mg/dl, with progesterone level of either more or less than 2.0 ng/ml; or more than 130 mg/dl, with progesterone of less than 2.0 ng/ml.

Discussion

The relationship between ovarian response to hormonal treatment and parameters such as hormones^{6,7)}, blood chemical components^{8,9)} and body characteristics¹⁰⁾ was investigated in order to see if any of these components could be used as criteria to predict superovulatory response in the donor. HAHN *et al.*⁸⁾ mentioned that there is a possible relationship between serum T-CHO levels and ovarian response to superovulatory treatment. They reported that a high level of cholesterol (121.4 ± 17.1 mg/dl $<$) was found in heifers with undesirable superovulatory response. On the contrary, the results of present study indicated that the number of transferable embryos was higher in animals with T-CHO level of 130 mg/dl and more. The reason for this difference was not clear and needs further investigation. SOMMER¹¹⁾, LOTTHAMMER *et al.*¹²⁾ and KWON *et al.*¹³⁾ reported that cows with a low

cholesterol level before calving had a greater possibility of suffering diseases after calving as compared to the cows with a normal cholesterol level. It was found that the normal range of T-CHO in dry cows was between 120 mg/dl and 170 mg/dl. However, in the case of cows with peak daily milk of more than 30 kg, the upper levels of the normal range seem to be higher¹³⁾. In the present study the normal range of T-CHO was thought to be 130 mg/dl and more, based on the percentage of cows in which over 3 transferable embryos were obtained. Similar result was observed from the two different situations with respect to the occurrence rate of diseases after calving and the ovarian response to superovulation treatment. T-CHO level in cattle varies according to several factors such as parturition, lactation and occurrence of diseases^{14,15)}. It was observed that cows which suffered diseases within 1 week after calving and showed symptoms such as anorexia, fever and pain had lowered T-CHO up to 10 weeks after calving¹⁵⁾. T-CHO is one of the metabolite which represent the condition of lipid metabolism of cattle¹⁶⁾. It is thought that cows with T-CHO of at least 120 mg/dl or 130 mg/dl and more may be healthy metabolically and that these cows produce good response to external stimulation. ELSAESSER *et al.*⁶⁾ reported that the milk progesterone level on the day of superovulation treatment was significantly correlated with the number of corpus lutea palpated through the rectum at flushing ($r=0.45$). In the present study over a half of animals on luteal phase of the estrous cycle had less than 2.0 ng/ml of progesterone. A significant difference was observed between the animals which had a progesterone level below 1.0 ng/ml and over 2.0 ng/ml which is used to

determine the existence of active corpus luteum¹⁷⁾. It was suggested that active corpus luteum in the ovary during superovulation treatment is an important factor to show good ovarian response¹⁸⁾. ELSAESSER *et al.*⁶⁾ suggested that the relationship between the responsiveness of the ovaries with respect to the stimulation of progesterone secretion and ovulation rate is mediated through some common mechanism. The present results revealed that animals with a T-CHO level of 130 mg/dl and more, and a progesterone level of 2.0 ng/ml and more showed good ovarian response to superovulation treatment. Hence this criterion can be used in preselection of the donor in embryo transfer procedures. Further studies and more observation are needed to confirm this finding.

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ホルスタイン種乳牛における過剰排卵誘起時の血清総コレステロール および黄体ホルモン値と移植可能な胚数との関係

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過剰排卵処置に対する卵巢反応を予測する試みとして、過剰排卵処置時の血清総コレステロールおよび黄体ホルモン値と移植可能な胚数との関係を検討した。供試牛はホルスタイン種乳牛 106 頭で過剰排卵誘起は性周期の 9～13 日の間に PMSG または FSH によって行っ

た。総コレステロール 130 mg/dl 未満の牛がそれ以上の牛より 4 個以上の正常卵を得られる確率が有意に少なかった ($P < 0.01$)。また、正常卵を得られない確率も高かった。黄体ホルモン値 1.0 ng/ml 未満の牛では 4 個以上の正常卵が得られなかった。