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Short communication: Effects of serum obtained from dairy cows with low or high body condition score on in vitro embryo development

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ABSTRACT

The objective of the study was to determine whether the serum obtained from animals differing in body condition score (BCS) affects in vitro embryo development. After in vitro fertilization, serum obtained from dairy cows of either low (L-BCS; 2.1 ± 0.14 on a scale of 1 to 5) or high BCS (H-BCS; 4.0 ± 0.0), or commercially available bovine serum (control) was added at 5% concentration to the in vitro culture medium. Use of serum obtained from H-BCS cows increased the cleavage rates compared with control serum at both 24 and 48 h after in vitro fertilization (78.3 vs. 71.9% and 79.9 vs. 75.1%, respectively), whereas use of serum obtained from L-BCS cows increased the blastocyst rate compared with control serum at 7 d (23.8 vs. 19.1%), but this difference was not evident at 8 or 9 d after in vitro fertilization. As nonesterified fatty acid concentrations were highest in control serum, followed by serum from L-BCS and H-BCS cows (621, 559, and 272 μEq/L, respectively), a high concentration of nonesterified fatty acids might adversely affect the very early stages of embryo development, and its negative effects might be greater immediately after fertilization compared with developmental stages after morula formation. Our findings also indicate that factors promoting early stage embryo development do not necessarily promote blastocyst development. Serum obtained from animals under different physiological conditions may be used for in vitro embryo culture to study the effects of nutritional management of dairy cattle on embryo development.
Key words: dairy cow, in vitro fertilization, body condition score, nonesterified fatty acid

Short Communication

Greater capacity for milk production is associated with a reduction in reproductive efficiency in dairy cows and it is attributed to postpartum negative energy balance (Butler, 2003). Cows that lost more than 1.0 unit of BCS within 5 wk after calving had drastically reduced conception rates at the first AI compared with those that lost less body condition (Butler and Smith, 1989). The period when the oocyte matures and the early embryo develops is a critical time affecting reproductive efficiency, as the early embryo is extremely sensitive to the maternal environment (Ashworth et al., 2009). The specific physiological mechanisms that regulate how negative energy balance affects reproductive performance have not been completely elucidated, and the effects of nutrition and physiology of the dam on early embryo survival is of research interest. However, it is challenging to conduct in vivo studies evaluating the effects of nutrition or physiological state of animals on reproductive performance, because it generally requires a large number of experimental units to detect significant treatment effects on conception rate due to variability in nutrition, management, production, and physiology. The use of serum obtained from animals under differing physiological conditions or nutritional management in the in vitro culture (**IVC**) medium may be an efficient approach to study effects of maternal conditions or nutritional status on early stage embryo development (Adamiak et al., 2004). Therefore, the objective of the study was to determine whether the serum obtained from animals differing in BCS affects in vitro embryo development.

All procedures for the animal study were approved by the Animal Experiment Committee at Rakuno Gakuen University. Serum was obtained from 2 groups of Holstein cows differing in BCS (on a scale of 1 to 5): a group of 3 nonlactating, nonpregnant dairy cows [high BCS (**H-BCS**); BCS = 4.0 ± 0.0] and a group of 3 lactating, nonpregnant dairy cows [low BCS (**L-BCS**); BCS = 2.1 ± 0.14]. Commercially available bovine serum (#16170; Life Technologies Corp., Carlsbad, CA) and the sera obtained from cows in this study were filtered and heat-inactivated at 56°C for 30 min. Sera were analyzed by a local commercial laboratory (SRL Inc., Tokyo, Japan; Table 1) for concentrations of NEFA,

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Table 1. Concentrations of metabolites and hormones of bovine serum (control) and serum obtained from dairy cows differing in BCS

Item	Control	Low BCS	High BCS	*P*-value[1]
BCS	—	2.1	4.0	<0.01
Glucose, mg/dL	85.0	62.7	66.3	<0.01
NEFA, μEq/L	621	559	272	<0.01
BHBA, μmol/L	282	541	282	<0.01
Insulin, μIU/mL	2.7	0.9	4.3	<0.01
IGF-1, ng/mL	169	104	277	<0.01

[1]*P*-values are for the comparison between low BCS and high BCS.

BHBA, and glucose using enzymatic methods, and for concentrations of insulin and IGF-1 by a chemiluminescent enzyme immunoassay and RIA, respectively.

Bovine ovaries were obtained from a local abattoir on 10 different dates over a 6-wk period. Cumulus-oocyte complexes (**COC**) were collected, cultured in the maturation medium for 20 h, and fertilized in vitro by coincubation for 18 h (d 0) in the presence of sperm as described by Takayama et al. (2006). Cumulus cells were removed by vortexing for 150 s, and presumptive zygotes were placed in IVC consisting of CR1aa medium supplemented with 5% serum (commercial bovine serum as a control, serum obtained from L-BCS cows, or serum obtained from H-BCS cows) in 100-μL droplets (20 to 25 zygotes per drop) covered with paraffin oil at 38.5°C under an atmosphere consisting of 5% CO_2, 5% O_2, and 90% N_2. The zygotes were examined for cleavage using an inverted microscope (40× or 100× magnification) at 24 and 48 h, and embryo development to the blastocyst stage was determined at 7, 8, and 9 d after in vitro fertilization (d 0). In addition, blastocysts were evaluated for inner cell mass and trophoblast cell counts on d 7 as described by Fouladi-Nashta et al. (2005). The 100-μL droplet containing 20 to 25 zygotes was considered as an experimental unit (n = 52, 46, and 47, respectively, for control, L-BCS, and H-BCS). Data were analyzed using Fit Y by X procedure of JMP (ver. 10; SAS Institute, Cary, NC).

The use of serum obtained from H-BCS cows in the IVC medium increased ($P < 0.05$) the cleavage rate compared with control serum, both at 24 and 48 h after in vitro fertilization (78.3 vs. 71.9% and 79.9 vs. 75.1%, respectively; Table 2). Additionally, the use of serum obtained from L-BCS cows in the IVC medium increased ($P < 0.05$) blastocyst rate compared with control serum at 7 d (23.8 vs. 19.1%), but not at 8 or 9 d, after in vitro fertilization. The use of either H-BCS or L-BCS serum in the IVC medium decreased ($P < 0.05$) the proportion of inner cell mass cells compared with control serum (41.8 and 41.6 vs. 46.3%). However, no differences were observed between H-BCS and L-BCS sera for all response variables determined in the current study.

Negative energy balance of dairy cows is generally associated with high serum concentrations of NEFA and BHBA, and low serum concentrations of glucose, insulin, and IGF-1. The H-BCS serum used in the current study had lower ($P < 0.05$) concentrations of NEFA and BHBA, and a higher ($P < 0.05$) insulin concentration compared with the L-BCS serum; therefore, we had expected that the L-BCS serum would negatively affect cleavage rate and blastocyst yield. Although we did not observe the expected differences between H-BCS and L-BCS sera treatments, a higher cleavage rate for the H-BCS serum and a greater blastocyst rate for the L-BCS serum were evident compared with control

Table 2. Cleavage rate, blastocyst rate, and cell profile of embryos from in vitro culture using bovine serum (control) or serum obtained from dairy cows differing in BCS

Item[1]	Control	Low BCS	High BCS	SE	*P*-value
Cleavage at 24 h, %	71.9^{b}	75.9^{ab}	78.3^{a}	1.7	0.02
Cleavage at 48 h, %	75.1^{b}	78.9^{ab}	79.9^{a}	1.6	0.05
Blastocyst at 7 d, %	19.1^{b}	23.8^{a}	20.4^{ab}	1.4	0.04
Blastocyst at 8 d, %	27.9	30.5	27.5	1.6	0.30
Blastocyst at 9 d, %	32.8	33.3	31.4	1.6	0.65
Total cells at 7 d, no.	154.8	155.4	155.0	5.3	0.99
ICM cells at 7 d, no.	71.3	63.9	64.7	2.8	0.10
TE cells at 7 d, no.	83.5	91.5	90.3	3.7	0.23
ICM cells at 7 d, %	46.3^{a}	41.6^{b}	41.8^{b}	1.3	<0.01
TE cells at 7 d, %	53.7^{a}	58.4^{b}	58.2^{b}	1.3	<0.01

[a,b]Means with different superscripts differ ($P < 0.05$).

[1]ICM = inner cell mass; TE = trophectoderm; total cells = ICM + TE cells.