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

M. Oba,^{*1} S. Miyashita,[†] R. Nishii,[†] M. Koiwa,^{†‡} H. Koyama,[†] D. J. Ambrose,^{*§} and O. Duchi[†]

^{*}Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Canada T6G 2P5

[†]Department of Dairy Science, and

[‡]Department of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido, Japan 069-8501

[§]Alberta Agriculture and Rural Development, Edmonton, Alberta, Canada T6H 5T6

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 $\mu\text{Eq/L}$, 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,

Received June 29, 2012.

Accepted November 28, 2012.

¹Corresponding author: Masahito.Oba@ales.ualberta.ca

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	<i>P</i> -value ¹
BCS	—	2.1	4.0	<0.01
Glucose, mg/dL	85.0	62.7	66.3	<0.01
NEFA, μ Eq/L	621	559	272	<0.01
BHBA, μ mol/L	282	541	282	<0.01
Insulin, μ IU/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- μ L droplets (20 to 25 zygotes per drop) covered with paraffin oil at 38.5°C under an atmosphere consisting of 5% CO₂, 5% O₂, and 90% N₂. The zygotes were examined for cleavage using an inverted microscope (40 $\times$ or 100 $\times$ 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- μ L 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	<i>P</i> -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 ($P < 0.05$).

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

serum. Commercially available bovine serum, which was used as a control for the current study, had the highest concentration of NEFA followed by the L-BCS serum. This was not expected, but the greater cleavage rate at both 24 and 48 h after in vitro fertilization for the H-BCS serum compared with control serum might be partly attributed to the low concentration of NEFA in sera from H-BCS cows.

Negative effects of NEFA on early stage embryo development have been previously reported. Leroy et al. (2005) showed that the addition of palmitic acid and stearic acid to the medium during in vitro oocyte maturation increases apoptotic cumulus cells in COC and decreases cleavage rate and blastocyst yield. More recently, Van Hoeck et al. (2011) reported that oocyte maturation under higher concentrations of NEFA decreases blastocyst cell number, increases apoptotic cell ratio, and alters expression of genes coding for DNA methyltransferase in blastocysts, which is considered to affect early stage embryo development (Ashworth et al., 2009). These previous findings clearly indicate that greater concentrations of NEFA in follicular fluid affect not only oocyte quality, but also its developmental capacity and subsequent embryo quality and viability. Although the current study evaluated effects of addition of serum differing in NEFA concentrations during IVC rather than in vitro maturation, the low cleavage rate observed for control serum, which had the highest concentration of NEFA, is consistent with previous findings (Leroy et al., 2005; Van Hoeck et al., 2011). In the current study, the L-BCS serum had higher concentrations of NEFA compared with H-BCS serum (559 vs. 272 $\mu\text{Eq/L}$), and the cleavage rate was numerically, but not significantly, lower for L-BCS. Greater concentrations of NEFA might have negatively affected cleavage rate in a linear manner, but it is also possible that a threshold exists for NEFA to exert detrimental effects on cleavage rate, as evident from a significantly lower cleavage rate at both 24 and 48 h after in vitro fertilization in the presence of serum with the highest concentrations of NEFA. Clearly, the effects of NEFA on early stage embryo development warrant further investigation.

We expected that other metabolites associated with negative energy balance would also affect early stage embryo development. Leroy et al. (2006) showed that a maturation condition mimicking ketosis—a low glucose concentration and a high BHBA concentration—results in decreased cleavage and blastocyst rates. However, the current results did not suggest that a high concentration of BHBA impaired early stage embryo development; the serum from L-BCS cows had a higher concentration of BHBA than serum from H-BCS or control serum, but neither cleavage nor blastocyst rate

was reduced. The discrepancy might be attributed to the extent of negative energy balance of the L-BCS cows in the current study. Although the L-BCS cows had lower body condition and higher concentrations of BHBA compared with the H-BCS cows, they did not exhibit clinical signs of ketosis and their serum BHBA concentrations remained well within the normal clinical range. In contrast, Leroy et al. (2006) evaluated very low glucose and high BHBA concentrations mimicking clinical ketosis in their in vitro study. Furthermore, the discrepancy can be attributed to the differences in the time frame of exposure to the treatment conditions (i.e., before or after in vitro fertilization), as well as duration and severity of negative energy balance.

Although physiological mechanisms contributing to a greater blastocyst rate in the L-BCS serum treatment compared with control serum at d 7 after in vitro fertilization are unknown, this finding should not be interpreted as greater embryo losses for control serum because cumulative blastocyst rates were similar among treatments at d 8 and 9. Nonetheless, our findings indicate that early embryo development—up to cleavage stage—might be more sensitive to elevated NEFA concentrations or metabolites associated with low energy status than the later development beyond that of the morula stage. In addition, it is noteworthy that overall blastocyst rate (d 9) did not differ among the treatments in spite of the lowest cleavage rate for control serum, which suggests that embryo losses between cleavage and blastocyst stages were reduced in that group. The control serum used in the IVC medium had the highest concentration of glucose, which might have contributed to improved embryo development. Glucose is metabolized for DNA and RNA synthesis via the pentose phosphate pathway and can be an essential substrate supporting embryo development (Sutton et al., 2003). However, our data do not exclude the possibility that unidentified growth factors associated with control serum slowed the embryo developmental rate or reduced embryo losses between cleavage and d 8.

In conclusion, high concentrations of NEFA in serum used in IVC were associated with low cleavage rate but did not affect blastocyst rate, indicating that energy balance is a possible factor affecting early stage embryo development, and that factors adversely affecting early embryo development do not necessarily affect blastocyst development. Nonetheless, the current data need to be interpreted with caution, as the serum used in the IVC medium was characterized only for selected metabolites and hormones related to energy status of animals. Although high concentrations of NEFA likely account for low cleavage rates and early embryonic development, our data do not exclude other possible explanations; it is possible that other unidentified

factors also affected early stage embryo development. The use of serum, obtained from animals under different nutritional management or conditions, in the IVC medium could provide useful preliminary data on the effects of physiological states and metabolic activity on early embryo development.

ACKNOWLEDGMENTS

Financial support of Alberta Milk (Edmonton, AB, Canada), Alberta Livestock and Meat Agency (Edmonton, AB, Canada), and Japan Society for the Promotion of Science (Tokyo, Japan) were gratefully acknowledged.

REFERENCES

- Adamiak, S. J., M. Ewen, K. A. Powell, J. A. Rooke, R. Webb, and K. D. Sinclair. 2004. Inclusion of bovine serum from different dietary background influences embryo viability in vitro. *Reprod. Abstr. Ser.* 31:19. (Abstr.)
- Ashworth, C. J., L. M. Toma, and M. G. Hunter. 2009. Nutritional effects on oocyte and embryo development in mammals: Implications for reproductive efficiency and environmental sustainability. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 364:3351–3361.
- Butler, W. R. 2003. Energy balance relationships with follicular development, ovulation and fertility in postpartum dairy cows. *Livest. Prod. Sci.* 83:211–218.
- Butler, W. R., and R. D. Smith. 1989. Interrelationships between energy balance and postpartum reproductive function in dairy cattle. *J. Dairy Sci.* 72:767–783.
- Fouladi-Nashta, A. A., R. Alberio, M. Kafi, B. Nicholas, K. H. Campbell, and R. Webb. 2005. Differential staining combined with TUNEL labelling to detect apoptosis in preimplantation bovine embryos. *Reprod. Biomed. Online* 10:497–502.
- Leroy, J. L. M. R., T. Vanholder, B. Mateusen, A. Christophe, G. Opsomer, A. de Kruif, G. Genicot, and A. Van Soom. 2005. Non-esterified fatty acids in follicular fluid of dairy cows and their effect on developmental capacity of bovine oocytes in vitro. *Reproduction* 130:485–495.
- Leroy, J. L. M. R., T. Vanholder, G. Opsomer, A. Van Soom, and A. de Kruif. 2006. The in vitro development of bovine oocytes after maturation in glucose and β -hydroxybutyrate concentrations associated with negative energy balance in dairy cows. *Reprod. Domest. Anim.* 41:119–123.
- Sutton, M. L., R. B. Gilchrist, and J. G. Thompson. 2003. Effects of in vivo and in vitro environments on the metabolism of the cumulus-oocyte complex and its influence on oocyte developmental capacity. *Hum. Reprod. Update* 9:35–48.
- Takayama, O., O. Dochi, and H. Koyama. 2006. Effect of meiotic arrest by cycloheximide on the in vitro maturation of bovine oocytes and their subsequent development following in vitro fertilization. *J. Mammal. Ova Res.* 23:67–71.
- Van Hoeck, V., R. G. Sturmey, P. Bermejo-Alvarez, D. Rizos, A. Gutierrez-Adan, H. J. Leese, P. E. J. Bols, and J. L. M. R. Leroy. 2011. Elevated non-esterified fatty acid concentrations during bovine oocyte maturation compromise early embryo physiology. *PLoS ONE* 6:e23183.