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ORIGINAL ARTICLE

The RPMI-1640 vitamin mixture promotes bovine blastocyst development *in vitro* and downregulates gene expression of *TXNIP* with epigenetic modification of associated histones

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Diverse environmental conditions surrounding preimplantation embryos, including available nutrients, affect their metabolism and development in both short- and long-term manner. Thioredoxin-interacting protein (TXNIP) is a possible marker for preimplantation stress that is implicated in *in vitro* fertilization- (IVF) induced long-term DOHaD effects. B vitamins, as participants in one-carbon metabolism, may affect preimplantation embryos by epigenetic alterations of metabolically and developmentally important genes. *In vitro*-produced bovine embryos were cultured with or without Roswell Park Memorial Institute 1640 vitamin mixture, containing B vitamins and B vitamin-like substances, from day 3 after IVF and we evaluated blastocyst development and *TXNIP* messenger RNA (mRNA) expression in the blastocysts by reverse transcription-quantitative polymerase chain reaction. The degree of trimethylation of histone H3 lysine 27 (H3K27me3) at *TXNIP* promoter was examined semi-quantitatively by chromatin immunoprecipitation polymerase chain reaction. Total H3K27me3 were also compared between the groups by Western blot analysis. The vitamin treatment significantly increased the rates of blastocyst development ($P < 0.05$) and their hatching ($P < 0.001$) from the zona pellucida by day 8. The mRNA expression of *TXNIP* was lower ($P < 0.01$) in blastocysts in the vitamin-mixture-treated group concomitant with higher ($P < 0.05$) level of H3K27me3 of its promoter compared with the control group. The total H3K27me3 in the vitamin-mixture-treated group was also higher ($P < 0.01$) than that in the control group. The epigenetic control of genes related to important metabolic processes during the periconceptional period by nutritional conditions *in utero* and/or *in vitro* may have possible implication for the developmental programming during this period that may impact the welfare and production traits of farm animals.

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Introduction

The periconceptional period of mammalian embryo development is a critical window during which diverse environmental conditions, including available nutrients, have both short-term consequences, for example, effects on cell proliferation and long-term consequences, for example, lasting influences on metabolic and developmental processes throughout gestation and even during postnatal and adult life.[1] During preimplantation development, dynamic epigenetic rearrangements involving substantial changes in DNA methylation and histone modification occur, which epigenetically regulate specific and heritable patterns of gene expression.[1–3]

Thioredoxin-interacting protein (TXNIP) is a protein which binds to and thereby inhibits the activity of thioredoxin (TRX), a major cellular thiol-oxidoreductase.[4] The TRX-mediated reducing system is a fundamental mechanism for cellular redox regulation and TXNIP also exerts various TRX-independent metabolic effects. Therefore, pathophysiological effects induced by TXNIP activity cover wide range including metabolic, cardiovascular, malignant and immune disorders.[5,6] In a mouse model, expression of *TXNIP* in preimplantation embryos depended on the environment in which the embryos developed and, surprisingly, the epigenetic and transcriptional *TXNIP* misregulation seen in the embryos persisted in certain adult tissues concomitant with alterations in the postnatal growth trajectory and metabolic homeostasis.[7] Therefore, TXNIP may be a marker of preimplantation stress that contributes to subsequent postnatal phenotypes. Intriguingly, *TXNIP* gene has been associated with production traits in livestock, for example, growth[8] and responses to mammary infections.[9,10]

Recently, the importance of one-carbon metabolism in mammalian preimplantation development has successively been reported.[11–15] One-carbon metabolism consists of methionine and folate (vitamin B_9) metabolic pathways with the participation of several other B vitamins as cofactors. One-carbon metabolism is involved in epigenetic mechanisms via provision of S-adenosylmethionine, the only donor of methyl groups to DNA and histones.[16] The relationship

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between one-carbon metabolism and epigenetic mechanisms of gene expression is of particular interest in terms of nutritional sensing by organisms that outputs long-lasting phenotypic effects via epigenetic modifications.[17,18]

For the study reported herein, we focused on how addition of Roswell Park Memorial Institute 1640 (RPMI-1640[19]) vitamins (Table 1) into a conventional culture medium affects bovine embryo development. Two previous reports found no beneficial effects of minimum essential medium- (MEM) type vitamins on bovine embryo development.[20,21] We assessed how the mixture of B vitamins and B vitamin-like substances in the RPMI-1640 formulation affected blastocyst development, *TXNIP* gene expression and an epigenetic histone modification including that of *TXNIP* promoter during bovine embryo development *in vitro*.

Methods

In vitro *culture of bovine embryos with or without the RPMI-1640 vitamin mixture*

Bovine oocytes were recovered from commercial abattoir-derived ovaries of Japanese Black or Japanese Black × Holstein F1 cows. Groups of 10 cumulus-enclosed oocytes (CEOs) were *in vitro* matured (IVM) for 22 h in 50-µl drops of Medium 199 with Earle's salts (Life Technologies) supplemented with 5% (v/v) fetal calf serum (FCS) and 0.2 IU/ml follicular-stimulating hormone (Kyoritsu Seiyaku) under a layer of mineral oil. The total number of CEOs per culture was ~200 and allocated to 20 drops in a Petri dish (Falcon 351007; Corning Incorporated). The matured CEOs were subjected to *in vitro* fertilization (IVF) with frozen-thawed sperm from a Japanese Black bull as described elsewhere.[11] The day of IVF and the beginning of insemination were designated day 0 and 0-h post-insemination (hpi), respectively. At 21 hpi, the resulting one-cell embryos were freed from cumulus cells and subsequently *in vitro* cultured (IVC) in 500 µl of modified synthetic oviduct fluid (mSOF[20]) with some modifications (Table 1). At 72 hpi, only the embryos that had developed to the five-cell stage or more ($n = 30$ per group) were further cultured up to 192 hpi in 500 µl of mSOF supplemented with 1% (v/v) of phosphate buffered saline (PBS) (control) or RPMI-1640 Vitamins Solution (Sigma-Aldrich). The compositions of the control and vitamin-supplemented mSOF are shown in Table 1. These media were used under a 500-µl layer of mineral oil in four-well multidishes (Nunc 179820; Thermo Fisher Scientific). The cultures were performed at 38.5°C under 5% CO_2 in air for IVM and IVF or under 5% CO_2, 5% O_2 and 90% N_2 for IVC. The rate of blastocyst development and that of complete hatching from the zona pellucida were evaluated at the end of the culture. To assess the number of cells and their allocation to inner cell mass (ICM) and trophectoderm (TE) in the blastocysts, the blastocysts were differentially stained by CDX2 immunolabeling as previously described.[22]

Table 1. *Compositions of modified synthetic oviduct fluid (mSOF) used in the present study*

Components	mSOF-control[a]	mSOF-vitamins
Basic components[b]		
NaCl (mM)	107.7	
KCl (mM)	7.16	
KH_2PO_4 (mM)	1.19	
$CaCl_2$ (mM)	1.71	
$MgCl_2$ (mM)	0.49	
$NaHCO_3$ (mM)	25.07	
D-glucose (mM)	1.5	
Sodium pyruvate (mM)	0.5	
Sodium lactate (mM)	3.3	
Bovine serum albumin (mg/ml)	3.0	
Antibiotic antimycotic solution[c] (% v/v)	1.0	
BME essential amino acid solution[d] (% v/v)	2.0	
MEM non-essential amino acid solution[e] (% v/v)	1.0	
L-glutamine (mM)	1.0	
Vitamin supplementation[f] (µM)		
Thiamine·HCl (B_1)	–	2.96
Riboflavin (B_2)	–	0.531
Niacinamide (B_3)	–	8.19
D-pantothenic acid calcium salt (B_5)	–	0.525
Pyridoxine·HCl (B_6)	–	4.86
D-biotin (B_7)	–	0.819
Folic acid (B_9)	–	2.27
Vitamin B_{12}	–	0.00369
Choline chloride	–	21.5
Myo-inositol	–	194
p-amino benzoic acid	–	7.29

BME, basal medium eagle; MEM, minimum essential medium.

[a]mSOF-control was supplemented with 1% (v/v) PBS (vehicle of the vitamin solution used).

[b]Identical to[20] except for the concentration of sodium pyruvate, omission of phenol red and the composition of antibiotics.

[c]From Wako Pure Chemical Industries; containing 10,000 units/ml penicillin G, 10 mg/ml streptomycin sulfate and 25 µg/ml amphotericin B.

[d]From Sigma-Aldrich; 50 ×.

[e]From Sigma-Aldrich; 100 ×.

[f]From Sigma-Aldrich; RPMI-1640 Vitamins Solution (100 ×).

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) analysis of TXNIP *gene expression in blastocysts*

Blastocysts in each experimental group were collected ($n = 30$) in a small volume of RNAlater (Sigma-Aldrich). Total RNA was extracted from the samples by using RNeasy Micro kit (Qiagen) and reverse transcribed in a 31.5-µl volume with SuperScript III reverse transcriptase and the oligo $(dT)_{20}$ primer (Life Technologies). The synthesized complementary DNA (2 µl) was used as a template in a qPCR reaction with 0.2 µM of each primer and 1 × SYBR Select Master Mix

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(Life Technologies) in a volume of 20 µl. qPCR was performed using the StepOnePlus Real-time PCR system (Life Technologies) with the following cycle parameters: 50°C for 2 min and 95°C for 2 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. Melting curve analysis and agarose gel electrophoresis were performed after the amplification to confirm the specificity of the PCR products. The data analysis was performed with the StepOne Software V 2.3. Quantification of *TXNIP* transcripts was performed by the ΔΔCt method using *glyceraldehyde-3-phosphate dehydrogenase (GAPDH)* and *succinate dehydrogenase complex flavoprotein subunit A (SDHA)* transcripts as multiple internal controls. The primer pairs were 5'-CTCCGGAACCTGTGCATCAT-3' and 5'-AGGGACG CTAACGTAGATCAGT-3'for *TXNIP* and as a previous report[23] for *GAPDH* and *SDHA*. The experiments were repeated three times.

Semi-quantitative chromatin immunoprecipitation polymerase chain reaction (ChIP-PCR) of trimethylated histone H3 at lysine 27 (H3K27me3) at TXNIP *promoter*

Sample collection for ChIP was performed in wells of a four-well multidish. Blastocysts in each experimental group were collected ($n = 60$) and crosslinked for 10 min with 1% formaldehyde in PBS containing 20 mM Na-butyrate (PBS-NaBu) and then quenched with 125 mM glycine for 10 min. After washing with PBS-NaBu containing protease inhibitor cocktail (PBS-NaBu-PIC), the blastocysts were transferred to a 1.5-ml tubes with a small volume of PBS-NaBu-PIC and stored at −80°C. ChIP was performed with True MicroChIP kit (Diagenode) according to the manufacturer's protocol with some modifications. The frozen blastocysts were suspended in Lysis Buffer to a final volume of 37.5 µl, and the lysate was mixed with 82.5 µl of PBS-NaBu-PIC. The sample was sonicated to shear chromatin using a Bioruptor UCD-250 (Cosmo Bio) for 10 × 30 s with 30-s pauses in ice-water. The sample was centrifuged for 10 min at 14,000 ***g*** and the supernatant (110 µl) was transferred to a new tube and mixed with 110 µl of ChIP Buffer. From each 220-µl sample of sheared chromatin, 20 µl was set aside at 4°C as 'Input' and the rest (200 µl) was mixed with 2.5 µg of anti-H3K27me3 antibody (17–622; Merck Millipore) and incubated for 16 h at 4°C with rotation at 40 rpm. Protein A-coated magnetic beads (10 µl) were added to the sample, which was then rotated at 40 rpm for 2 h at 4°C. The DNA was eluted from the immunoprecipitate, decrosslinked and subsequently purified with MicroChIP DiaPure columns (Diagenode), which resulted in 12 µl of ChIPed DNA. The same DNA purification method was also applied to the Input sample. From the 12 µl of ChIPed or Input DNA, 4 µl was used as a template in a real-time-PCR reaction. The PCR condition was as described above with the exceptions that PowerUp SYBR Green Master Mix (Life Technologies) was used and 45 cycles of PCR were performed. The primer pairs were 5'-TCCTCCGCCCCATCCTAAA-3' and 5'-CCGGCTCACAAATCGCAAG-3'. The amount of ChIPed DNA was calculated as the percentage of the Input (%Input) from Ct values semi-quantitatively. The experiments were repeated four times.

Western blot analysis of H3K27me3

Blastocysts in each experimental group ($n = 38$–58) were lysed in NuPAGE LDS Sample Buffer with Reducing Agent (Invitrogen) in 30 µl volume and boiled for 5 min. The samples were subjected to Sodium dodecyl sulfate polyacrylamide gel electrophoresis through a 12% Bis-Tris gel and transferred to Polyvinylidene difluoride membrane using iBlot Dry Blotting System (Invitrogen). The blotted membrane corresponding to 15–20 kDa was blocked with 10% (v/v) FCS in PBS containing 0.05% (v/v) Tween 20 (PBST) for 30 min. The aforementioned anti-H3K27me3 antibody was diluted 1000 times with PBST containing 5% (v/v) FCS and mounted onto the membrane for 1 h. After extensive washing with PBST, the membrane was treated for 30 min with 2000 times-diluted alkaline phosphatase-conjugated bovine anti-rabbit IgG (sc-2372; Santa Cruz Biotechnology). After washing, the signal was developed for 5 min with Novex AP Chemiluminescent Substrate (Invitrogen) and exposed to Hyperfilm ECL (GE Healthcare). After the detection of H3K27me3 band, the blot was freed from antibodies by washing with Restore PLUS Western Blot Stripping Buffer (Thermo Fisher Scientific). The blot was then re-blocked and reprobed by an anti-H3 antibody (2650S; Cell Signaling Technology). The degree of total H3K27me3 was calculated as a ratio of band intensity of H3K27me3 to that of H3. The experiments were repeated three times.

Statistical analyses

Embryonic development expressed as a percentage, cell number and the RT-qPCR results were analyzed by the *t*-test. Semi-quantitative ChIP-PCR and Western blot data were analyzed by the paired *t*-test which included replicate pairs. The data are presented as means ± standard error of the mean. All analyses were performed using SPSS (SPSS Inc.). Significance was accepted at $P < 0.05$.

Results

Effects of the RPMI-1640 vitamin-mixture treatment on in vitro *development of bovine embryos*

We first examined the effects of the RPMI-1640 vitamin mixture on blastocyst development of bovine preimplantation embryos *in vitro*. Addition of the vitamin mixture significantly ($P < 0.05$) increased the rate of blastocyst development and tripled the rate of complete hatching from the zona pellucida compared with the control (Table 2; Fig. 1). Although the mean number of cells in the blastocysts and their allocation to ICM and TE were not significantly different for the two groups, the vitamin-mixture treatment widened the cell number range toward the upper direction (Table 3).

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Effects of the RPMI-1640 vitamin-mixture treatment on expression of TXNIP *messenger RNA (mRNA)*

We assessed the effects of the vitamin mixture on expression of *TXNIP* mRNA in the blastocysts using RT-qPCR. The vitamin-mixture treatment significantly decreased ($P < 0.01$) *TXNIP* mRNA expression in comparison with the control (Fig. 2).

Effects of the RPMI-1640 vitamin-mixture treatment on H3K27me3 at TXNIP *promoter*

As the vitamin-mixture treatment significantly decreased expression of *TXNIP* gene, we hypothesized that the treatment affected the transcriptionally repressive histone modifications at the gene loci. We focused on repressive H3K27me3[24,25] and carried out semi-quantitative ChIP-PCR to examine H3K27me3 at *TXNIP* promoter (Fig. 3). The vitamin-mixture treatment significantly enhanced ($P < 0.05$) the level of H3K27me3 compared with the control.

Effects of the RPMI-1640 vitamin-mixture treatment on total H3K27me3

In order to compare the degree of total H3K27me3 in blastocysts between the treatments, Western blot analysis was performed (Fig. 4). The signal of H3K27me3 normalized to pan-H3 was significantly higher ($P < 0.01$) in the vitamin-mixture treatment compared with the control.

Table 2. *Effects of Roswell Park Memorial Institute 1640 vitamin mixture on blastocyst development*

Groups	*n* (replicates)	Blastocyst yield (% ± SEM)	Hatched blastocyst (% ± SEM)
Control	234 (8)	56.6 ± 2.4^{A}	4.6 ± 1.0^{C}
Vitamins	232 (8)	64.4 ± 2.6^{B}	15.1 ± 1.8^{D}

A *v.* B, $P < 0.05$ and C *v.* D, $P < 0.001$ by *t*-test.

Discussion

Several studies have reported the effects of adding B vitamins and B vitamin-like substances, for example, choline and myo-inositol, into the culture media of bovine preimplantation embryos in an attempt to improve the culture system.[20,21,26] In contrast to the results for rodent and rabbit embryos for which B vitamins had beneficial effects on preimplantation development *in vitro*,[27,28] MEM-type vitamins had no effect on[20] or tended to decrease bovine blastocyst development.[21] When myo-inositol was added into a bovine serum albumin-supplemented culture medium, it had no effect on blastocyst development and embryo hatching from the zona pellucida, despite it being added at a much greater concentration (2.77 mM) than used in our present study (194 μM).[26] Therefore, the vitamin composition of the RPMI-1640 so far would have been an undiscovered resource to improve the culture system of bovine preimplantation embryos. The major differences in the MEM-type and RPMI-1640-type vitamin compositions are the absence of D-biotin, vitamin B_{12} and p-amino benzoic acid in the MEM vitamin mixture. These differences may cause the distinctive effects on preimplantation development of bovine embryos. In addition, the favorable effects of B vitamins and B vitamin-like substances may partially reflect the importance of folate-mediated one-carbon metabolism in preimplantation development; evidence for which has accumulated recently.[11–15]

The susceptibility of preimplantation embryos to surrounding environment, which can have long-lasting phenotypic effects in

Table 3. *Effects of Roswell Park Memorial Institute 1640 vitamin mixture on the total, inner cell mass (ICM) and trophectoderm (TE) cell numbers of blastocysts*

Treatment	No. of embryos	Cell number (± SEM) Total	TE	ICM	Range of total cell numbers
Control	20	144.0 ± 9.8	88.3 ± 6.4	55.7 ± 4.1	76–237
Vitamins	25	163.8 ± 12.4	103.6 ± 9.1	60.2 ± 4.9	76–339

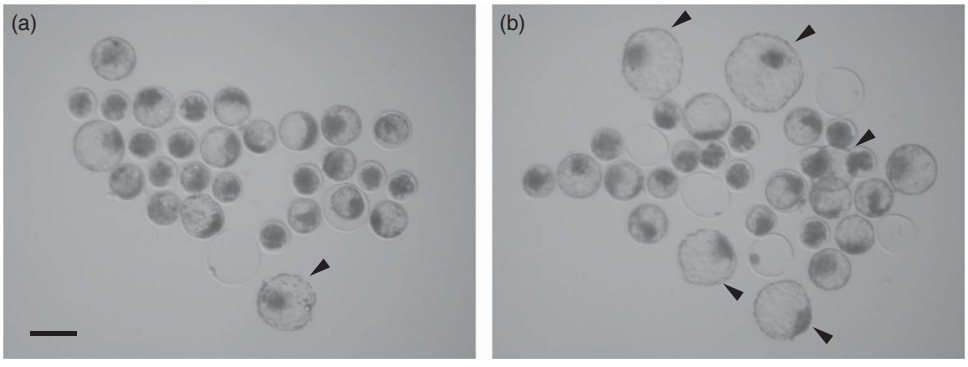

Fig. 1. Representative images of embryos at the end of culture in control (*a*) and vitamin-mixture-treated (*b*) groups. Hatched blastocysts are indicated by arrowheads. Scale bar represents 200 μm.

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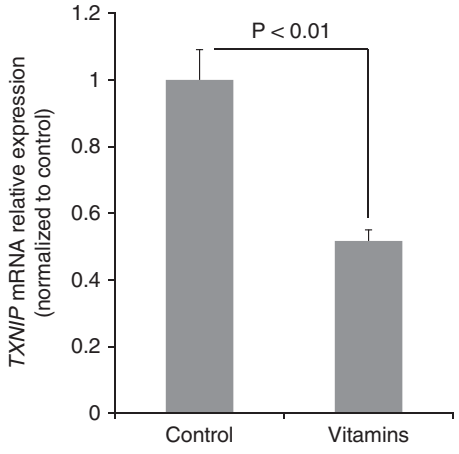

Fig. 2. Effect of Roswell Park Memorial Institute 1640 vitamin mixture on the expression of thioredoxin-interacting protein (*TXNIP*) messenger RNA (mRNA) in blastocysts. $P < 0.01$ by *t*-test.

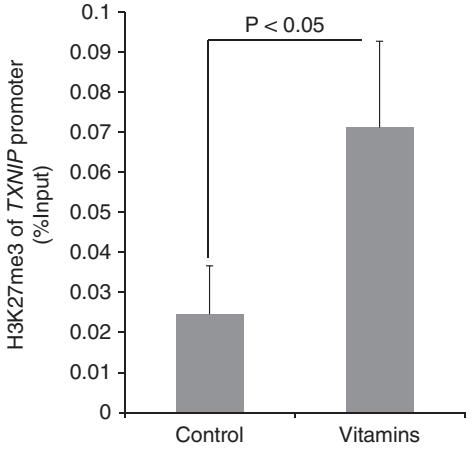

Fig. 3. Effect of Roswell Park Memorial Institute 1640 vitamin mixture on the levels of trimethylated histone H3 at lysine 27 (H3K27me3) at thioredoxin-interacting protein (*TXNIP*) promoter in blastocysts. $P < 0.05$ by paired *t*-test.

later life is an emerging issue in periconceptional maternal/paternal care and assisted reproductive technologies.[1,29] Many studies have reported that exposure of preimplantation embryos to poor nutritional environment can lead to physiological changes in the offspring's pre- and postnatal growth trajectories, can upregulate metabolic processes involving insulin resistance and fat accumulation, and can cause cardiovascular and immune dysfunctions and these phenotypic changes correlate with the symptoms of human metabolic syndrome.[1] The correlation of poor periconceptional nutrition with the phenotypic changes often postulates the existence of (1) 'thrifty genotype' that would have been evolutionally selected during a time of poor food availability and the need for large energy expenditures and (2) 'thrifty phenotype' that might in part reflect epigenetic changes associated with 'thrifty genes' induced by a nutrient

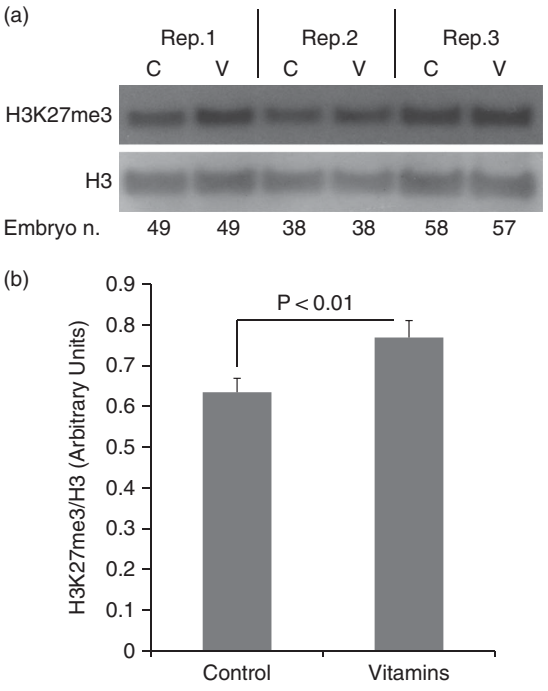

Fig. 4. Effect of Roswell Park Memorial Institute 1640 vitamin mixture on the levels of total trimethylated histone H3 at lysine 27 (H3K27me3) in blastocysts. (*a*) The image of Western blot analysis from three biological replicates (Rep. 1, 2 and 3) are shown. C and V mean control and vitamin-treated groups, respectively. Embryo n. represents the numbers of blastocysts loaded. (*b*) Semi-quantitative values of the band intensity shown in (*a*). $P < 0.01$ by paired *t*-test.

deficient periconceptional environment in order to adapt to predicted deprived environment during subsequent development.[30] In addition, the thriftiness can enhance inflammatory response via adipocyte-derived cytokines and reservation of energy for high cost of the immune system, which, for our ancestors, might have been advantageous for recovery from and survival in infection and trauma.[31] Therefore, the 'thrifty genes' might have been evolutionally co-selected with and/or had overlapping functions with 'proinflammatory genes.' Chronic inflammatory aspects of human metabolic syndrome can be seen as maladaptation of such 'proinflammatory genotype.'[31,32] By binding TRX, TXNIP modulates TRX-binding-dependent oxidative, inflammatory and apoptotic processes, and in addition, TXNIP negatively affects glucose uptake independently of TRX activity.[5,6] Given the close relationship between TXNIP and both glucose homeostasis and inflammatory response, it is tempting to speculate TXNIP as one of the 'thrifty genes.'[33] Indeed, the higher expression of *TXNIP* gene is a candidate marker of preimplantation disturbance in relation to postnatal metabolic programming in a mouse model.[7] In the blastocysts that had been derived from IVF and subsequent culture of the mouse model, *TXNIP* gene expression was upregulated compared with *TXNIP* expression found in their *in vivo*-generated counterparts, and the IVF-conceived

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offspring showed long-term metabolic alterations toward glucose intolerance and fat accumulation compared with their *in vivo*-generated counterparts. Furthermore, the upregulation of *TXNIP* in response to oxidative stress and its attenuation by melatonin, an antioxidative indoleamine, have been shown in bovine preimplantation embryos.[34] We found that the vitamin-mixture treatment can reduce expression of *TXNIP* gene in bovine blastocysts produced *in vitro* (Fig. 2).

Regulation of *TXNIP* gene expression involves epigenetic histone modifications at the promoter region of the gene.[7,35] Therefore, in an attempt to reveal the mechanism of the *TXNIP* downregulation by the RPMI-1640 vitamin mixture, we examined whether the vitamins could affect a repressive histone mark H3K27me3 at *TXNIP* promoter. The results of our ChIP-PCR study demonstrated that the vitamin treatment enhanced the level of H3K27me3 at this region in the blastocysts compared with blastocysts that were not exposed to the vitamin mixture (Fig. 3), which suggests that the vitamin treatment during *in vitro* culture of the bovine embryos downregulated *TXNIP* gene expression via epigenetic modification of histones. This epigenetic modification of *TXNIP* may be heritable during subsequent development and may exert its long-term effects on developmental and metabolic trajectory as demonstrated in the aforementioned mouse model.[7] How the vitamin treatment affects the histone methylation of *TXNIP* gene remains unknown. The RPMI-1640 vitamin mixture includes compounds, that is, folic acid, choline, vitamins B_2, B_6 and B_{12}, involved in folate-mediated one-carbon metabolism. One-carbon metabolism provides the methyl groups for specific methylation of DNA and histones, which are two major epigenetic modifications of the mammalian genome.[17,36] Therefore, it is possible that the vitamin treatment of the bovine preimplantation embryos acts via one-carbon metabolism in a manner similar to that of methionine treatment,[22] where methylation of histones at a specific gene locus is altered. In addition, it is also possible that the decreased *TXNIP* expression in the vitamin-mixture treatment directly promotes the blastocyst development because TXNIP has prooxidative, proapoptotic and anti-proliferative properties.[5,6]

The involvement of TXNIP in metabolic process and cellular redox regulation implicates its association with the welfare and production traits in farm animals. For example, a single-marker and haplotype analytic study showed significant effects of SNPs of *TXNIP* gene on carcass weight and daily gain and *TXNIP* mRNA expression in skeletal muscle was significantly lower in the fast-growth group compared with the slow-growth group in pig.[8] Concerning mastitis, decreased *TXNIP* mRNA expression was found in *Staphylococcus aureus*-stimulated dendritic cells from clinically resistant ewes compared with their susceptible counterparts.[9] *TXNIP* mRNA expression was also altered in bovine mammary epithelial cells cocultured with *Escherichia coli* depending on their acute or persistent strains.[10] These findings suggest that epigenetic control of genes such as *TXNIP* may improve livestock health and valuable production traits.

In the present study, only *TXNIP* was investigated gene specifically. However, the Western blot analysis showed significant increase of total H3K27me3 in the vitamin-treated group compared with the control (Fig. 4), suggesting that the vitamin treatment widely affects histone modifications involved in other gene expressions. For example, we conducted supplemental examination of interferon τ (*IFNT*) gene expression in consideration of the possible association between immunological and metabolic processes and found that *IFNT* was also downregulated by the vitamin treatment (Supplementary Fig. S1). IFNT is well characterized as a ruminant-specific key factor for the maternal recognition of pregnancy and its immunomodulatory properties contribute to the function.[37] The downregulation of *IFNT* by the vitamin treatment suggests the influences of the nutrients preferentially on immune-related genes. In addition, given the correlation of the higher IFNT expression with compromised blastocyst survival,[38,39] vitamin-supplemented embryo culture may be advantageous. Collectively, comprehensive and integrated analyses of gene expressions and histone modifications in response to the vitamin treatment are of interest in the context of investigating the range and specificity of nutrients-associated epigenetic gene regulations.

In summary, we demonstrated that treatment with the RPMI-1640 vitamin mixture during bovine embryo culture promotes blastocyst development and downregulates *TXNIP* gene expression with epigenetic modification of histones in the blastocysts. Addition of the commercially available RPMI-1640 vitamin mixture to culture media appears to be a simple and practical mean of increasing blastocyst yield. Furthermore, optimal control of B vitamins in *in utero* and/or *in vitro* environment may be applicable for the developmental programming[40–42] in preimplantation embryos so as to improve the welfare and production traits in farm animals.

Acknowledgments

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Conflicts of Interest

None.

Ethical Standards

This study was carried out in accordance with the Regulation on Animal Experimentation at Kyoto University. The bovine ovaries were purchased from a commercial abattoir.

Supplementary material

To view supplementary material for this article, please visit https://doi.org/10.1017/S2040174417000563

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