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Leucemia inhibitory factor; investigating the time-dependent effect on viability of vitrified bovine embryos

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Contents
Leucemia inhibitory factor (LIF) is involved in various reproductive processes, including sperm development, regulation of ovulation, as well as blastocyst formation, hatching and implantation in embryos. Moreover, LIF has also been shown significantly to enhance the blastocyst formation rates of bovine embryos, a finding that remains controversial. Our purpose was to investigate time-dependent effect of LIF on bovine embryo culture, especially in terms of addition timing. Presumptive zygotes were cultured in five different groups. In this study, 100 ng/ml LIF was added to the culture medium were as follows; control: SOF alone, group A: at day 0 (fertilization day), group B: at day 4 post-insemination (p.i.), group C: at day 4 to 7 (p.i. before vitrification) and group D: at day 8 (p.i. after thawing). Addition of LIF to the culture medium at day 4 significantly increased the percentage of blastocyst rate when compared day 0, day 4 at 6/7 and control group (41.8% versus 24.3%, 19.7%, 34.6%). In conclusion, the addition of LIF only on day 4 (p.i.) to the culture medium was found to be beneficial for bovine embryonic development based on several measures, including blastocysts rate, re-expansion rate and cellular cryotolerance after vitrification.

1 | INTRODUCTION

Cryopreservation of bovine embryos has become a crucial component of embryo transfer technologies. However, cryopreservation procedures may negatively affect viability of embryos as a consequence of damages to the cell (Leibo & Loskutoff, 1993). This may be owing to presence of in vivo regulatory autocrine and paracrine pathways that may be lacking in the in vitro environment (Martal et al., 1998). The successful cryopreservation of bovine embryos depends on the compliance in vitro culture conditions (Pollard & Leibo, 1994). The improvement of in vitro culture systems is important for production of embryos with high developmental competence. It is essential to appreciate that the culture media are only one part of the overall culture system. Culture media formulations can be based on three factors; levels of nutrients in the female reproductive tract, the changing requirements of the embryo, and minimizing in vitro-induced stress as determined by studies on embryo physiology and metabolism (Gardner, Lane, & Watson, 2004). A range of growth factor ligands and their receptors have been found to be expressed and produced in the reproductive tract or the pre-implantation embryo, in particular epidermal growth factor (EGF), transforming growth factors alpha (TGF-a) and insulin-like growth factors I and II (IGF-I, IGF-II). Furthermore, other cytokines such as interleukin 1b (IL- 1b), interleukin-6 (IL 6), granulocyte-macrophage colony-stimulating factor (GM-CSF), leucemia inhibitory factor (LIF) and interferon-tau (IFN-t) have been found to be expressed and produced in the uterus or conceptus, or in both (Martal et al., 1998; Moore, Kramer, Rodriguez-Sallaberry, Yelich, & Drost, 2007; Spanos, Becker, Winston, & Hardy, 2000; Watson, Hogan, Hahnel, Schultz, & Wiemer, 1992).

One of the cytokines is LIF, a pleiotropic member of the interleukin-6 (IL-6) family. LIF is a highly glycosylated 40–50 kDa glycoprotein with a range of biological functions. It is expressed in various embryonic and adult tissues (Hilton, 1992), follicular fluid (Arici et al., 1997) with particularly high levels in the uterus. LIF is involved in various reproductive processes, including sperm enhancement, regulation of ovulation, as well as blastocyst formation, hatching and implantation in embryos (Attar, Ozsait, Bulgurcuoglu, Serdaroglu, & Arici, 2003; Lavranos, Rathjen, & Seamark, 1995;

Stewart et al., 1992). Also LIF is secreted in numerous species including mice, humans and ruminants (Amiri, Parvini, Amini, & Heidarbeigi Kh, 2008; Kimber, 2005; Neira, Tainturier, L'Haridon, & Martal, 2007; Watson et al., 1994). Leucemia inhibitory factor receptors (LIFR) have been detected in embryos of different species that is considered essential for blastocyst development such as hatching rate, cell count and cryotolerance (Fry, Batt, Fairclough, & Parr, 1995; Han, Lee, Mogoe, Lee, & Fukui, 1995; Kocyigit & Cevik, 2015; Seshagiri, Roy, Sireesha, & Rao, 2009; Yamanaka, Amano, & Kudo, 2001). LIF was originally implicated in regulating the early development of the embryo (Nicola & Babon, 2015; Salleh & Giribabu, 2014; Smith & Treutlein, 1998).

Moreover, LIF has also been shown significantly to enhance the blastocyst formation rates of bovine embryos (Han et al., 1995; Sirisathien et al., 2003), a finding that remains controversial (Vejlsted, Avery, Gjorret, & Maddox-Hyttel, 2005). Despite these facts addition of LIF to bovine embryo culture media has led to inconsistent results with addition timing. Supplementation of bovine embryo culture medium with LIF beginning at morula or early blastocysts stages resulted in improvement in further development to expanded blastocysts (Fukui & Matsuyama, 1994). In contrast addition of LIF to culture media for several intervals following in vitro insemination on Day 0, for example Days 1–3, Days 4–7, did not improve development of embryos (Funston, Nauta, & Seidel, 1997). These findings lead to the controversy regarding the necessity of supplementing LIF in the bovine embryo culture medium to imitate the conditions of maternal uterus. Based on these findings, our purpose was to investigate the time-dependent effect of LIF on bovine embryo culture. In our study, we investigated this effect not only in the standard culture period, but also after vitrification. We believe that it will contribute to the standardization of in vitro fertilization (IVF) protocols.

2 | MATERIALS AND METHODS

All chemicals used in this study were purchased from Sigma-Aldrich (Istanbul, TURKEY), except where otherwise indicated.

2.1 | Collection and in vitro maturation of oocytes

Although no animal care and ethics approval were required due to ovaries are derived from slaughterhouse material (post-mortem), the project commenced after informed Ondokuz Mayis University Ethic Committee on Animal Research. Bovine ovaries were collected from a local slaughterhouse and transported to the laboratory at approximately 34 ± 2.0°C in physiological saline solution supplemented with gentamycin sulphate (0.1 μl/ml). Cumulus oocyte complexes (COCs) were recovered from follicles 2–8 mm in diameter by aspiration. The COCs were collected in 3–4 ml Hepes-buffered Medium-199 containing Earle's salts and supplemented with 1% v/v antibiotic–antimycotic solution. Before in vitro maturation, the COCs were assessed morphologically and only oocytes with compact, non-atretic cumulus investment and evenly granulated cytoplasm were selected for maturation. Maturation medium was sodium bicarbonate-buffered Medium-199 with sodium pyruvate (5.5 μl/ml), antibiotic–antimycotic solution (1% v/v), heat-inactivated foetal calf serum (FCS, 10% v/v). The COCs were matured for 22 hr in a humidified atmosphere of 5% CO_2 in air at 38.5°C.

2.2 | Spermatozoa preparation and in vitro fertilization

After a 22 hr maturation period, oocytes were transferred into 44 μl fertilization drops. The fertilization medium was glucose-free-modified Tyrode's albumin lactate pyruvate (TALP) supplemented with bicarbonate (25 mM), Na-lactate (22 mM), Na-pyruvate (1 mM), fatty acid-free bovine serum albumin (BSA) (6 mg/ml), and heparin-sodium salt (184 units/mg heparin, 10 mg/ml) and antibiotic–antimycotic solution (0.5 μl/ml) (pH 7.4 and 280–300 mOsm/kg). Frozen-thawed semen was used for the fertilization of oocytes. We used a commercial bull sperm (0.25 ml straws) with at least 60% motility, which we tested in our past IVF studies. Percoll density gradient system was used for the separation of the motile fraction of the frozen-thawed semen (Parrish, Krogenaes, & Susko-Parrish, 1995). Sperm were then diluted to 50×10^6 spermatozoon/ml in TL-HEPES, including the 2×10^6 spermatozoa/ml as final concentration. The fertilization procedure was completed by adding 2 μl of diluted sperm, 2 μl heparin (5 μg/ml) and 2 μl of PHE solution (20 μM penicillamine, 10 μM hypotaurine and 1 μM epinephrine in final concentration) into the fertilization drops containing oocytes. The oocytes were fertilized with 2 μl diluted semen per fertilization drops for 22 hr in a humidified atmosphere of 5% CO_2 in air at 38.5°C.

2.3 | In vitro culture

Cumulus cells surrounding the oocytes were removed from presumptive zygotes at approximately 22-hr post-insemination by vortexing 3 min. The zygotes were transferred in groups of 20–30 for the culture droplets. Embryos were cultured in the synthetic oviduct fluid (SOF) medium supplemented with pyruvate (0.4 mM), fatty acid free (BSA-FAF) (8 mg/ml), 100× minimum essential medium (MEM) (20 μl/ml), 50× basal medium eagle (BME) (10 μl/ml), penicillin (100 U/ml) and streptomycin (100 μg/ml) on the day of use a humidified atmosphere of 5% CO_2, 5% O_2 and 90% N_2 at 38.5°C.

Presumptive zygotes were cultured in five different groups. Only SOF medium was used without LIF (L-5283, Sigma Chemical Co, St Louis, MO, USA) addition in the control group. A concentration of 100 was used as indicated in the literature which our past work was also included. In this study, 100 ng/ml (5,000 U/ml) LIF was added to the groups as follows; group A: at day 0 (fertilization day), group B: at day 4 post-insemination, group C: daily supplement from day 4 to day 7 and group D: at day 8 (after thawing). Blastocysts were collected at day 7 of in vitro culture and morphologically evaluated by visualizing presence of the blastocoel and distinct ICM. The embryos evaluated to reach the blastocyst stage were vitrified.

2.4 | Vitrification and warming

The vitrification procedure is based on the classical straw method with minor modifications (Ishimori et al., 1993). After two initial washes in SOF medium, blastocysts were placed in a 10% glycerol solution for 1 min, then transferred to 20% ethylene glycol (EG) + 10% glycerol solution for 1 min and finally transferred to %25 EG + %25 glycerol for 25 s. Blastocysts were immediately drawn by capillary action into sterile straws (0.25 ml), along with a maximum of 1–2 ml medium. Blastocysts were vitrified using the classical method of directly submerging embryos into liquid nitrogen after brief exposure to a cryoprotectant solution. For warming (1 day after cryopreservation), straws were thawed in a water bath at 35°C for 15 s, and their contents released into 0.5 M sucrose solution. Thawed blastocysts were recovered and equilibrated in 0.5 and 0.25 M sucrose for 5 min, each and then washed once in SOF medium containing BSA-FAF (8 mg/ml) and cultured in SOF drops for 24 hr. Blastocysts were considered that they survived the vitrification (viable) if they re-expanded or hatched from the zona pellucidae after in vitro cultured.

2.5 | Differential staining

To determine the differential cell count of TE and ICM, re-expanded blastocysts (at day 8 p.i.) from all groups were differentially stained as described by Van Soom, Boerjan, Ysebaert, & de Kruif, 1996. Briefly, zona intact blastocysts were incubated with 10 mM picrylsulphonic acid (Sigma, in cold Ca2þ-free PBS) for 5 min at 4°C. Then blastocysts were washed and incubated for 30 min at 39°C in antidinitrophenyl antibody (Sigma) diluted to 30% (v/v) with Caþ2free PBS. Embryos were then transferred into guinea pig complement (55852, ICN biochemical, Irvine, CA, USA) diluted to 20% (v/v) in Ca^{2+}-free PBS containing 50 mg/ml propidium iodide, 12.5 mg/ml bisbenzemide, and 50 mg/ml RNAse a for 30 min at 39°C. Finally, embryos were fixed in 2% paraformaldehyde in PBS for 1–2 min at room temperature. Before mounting on slides with a 10 ml drop of 0.2 M 1.4 diazabicyclooctane in 50% glycerol (v/v) in Ca^{2+}-free PBS as an antifading solution. Embryos were examined in whole mount under a fluorescence microscope (Nikon Invert Microscope Eclipse Ti-FL, 340–380 nm excitations and 430 nm suppression). Thus, ICM nuclei labelled with bisbenzemide appeared blue and TE nuclei labelled with both bisbenzemide and propidium iodide appeared pink to red. Numbers of ICM and TE nuclei were counted directly under the fluorescence microscope.

2.6 | Statistical analysis

Blastocysts were randomly allocated to each treatment group were replicated at least six times.

Statistical data analysis was performed using the general linear models (GLM) procedure of statistical analysis system (SAS). One-way Analysis of Variance (ANOVA) followed by Duncan's Multiple Comparison Test was used for statistical comparison of the groups. All values reported as least-squares means ± *SEM*, and statistical differences were considered as significant when p values were less than .05.

3 | RESULTS

A total of 393 in vitro blastocysts were obtained; 84 for the control group (SOF alone), 72 for the group A (day 0), 110 for the group B (day 4), 54 for the group C (day 4 at 7) and 73 blastocyts for the group D (day 8, after thawing) were used.

3.1 | Time-dependent effect of LIF on blastocyst development

Addition of LIF to the culture medium at day 4 significantly increased the percentage of blastocyst rate when compared day 0, day 4 at 7 and control group (41.8% versus 24.3%, 19.7%, 34.6%). Moreover, the presence of LIF promoted the blastocyst development only when added on day 4.

Total blastocyst rates did not differ between the group A (24.3%) and group C embryos (19.7%).

Group D was not evaluated because it was composed of blastocyst stage embryos. Results on blastocyst development rates are presented in Table 1.

3.2 | Blastocyst viability and cell allocation following vitrification

The re-expansion ability of blastocysts from group B (27 of 110, 25.2%) was significantly higher than that of other groups (control: 20.3%, group A: 8.1%, group C: 7.6% and group D: 15.4%) ($p < .05$). Additionally the re-expansion ability of blastocysts was not different between group A (6 of 72; 8.1%) and group C (4 of 54; 7.6%) ($p < .05$). Similarly, the re-expansion ability of blastocysts was not different between control group (17 of 84; 20.3%) and group D (11 of 73; 15.4%) ($p < .05$).

The embryos of control group consisted of an average of 67 ± 4 trophectoderm cells in total for 17 blastocysts counted. The embryos of group A consisted of an average of 59 ± 8 trophectoderm cells in total for 6 blastocysts counted. The embryos of group B consisted of an average of 79 ± 1 trophectoderm cells in total for 26 blastocysts

TABLE 1 Time-dependent effects of LIF on developmental competence of bovine embryos

Group	Oocytes *n*	Cleavage rates % Mean + *SE*	Blastocyst rates % Mean + *SE*
Control	241	60.5 ± 8.1	34.6 ± 1.3[b]
A	297	37.7 ± 1.2	24.3 ± 3.4
B	264	54.1 ± 6.6	41.8 ± 3.7[a]
C	289	47.4 ± 4.2	19.7 ± 2.6

[a,b]Values with different superscripts are significantly different; $p < .05$.

TABLE 2 Time-dependent effects of LIF on viability and cell ratio of blastocysts

Group	Blastocysts *n*	Re-expansion rate % Mean + *SE*	Trophectoderm cell number Mean + *SE*	Cell ratio (ICM/total cell)
Control	84	20.3 ± 2.1^{b}	67.24 ± 4.1	0.29
A	72	8.1 ± 6.5	59.32 ± 8.4	0.36
B	110	25.2 ± 4.7^{a}	79.19 ± 1.8^{a}	0.31
C	54	7.6 ± 3.8	50.28 ± 2.1^{b}	0.39^{a}
D	73	15.4 ± 1.3^{b}	61.34 ± 9.7	0.33

[a,b]Values with different superscripts are significantly different; $p < .05$.

counted. The embryos of group C consisted of an average of 50 ± 2 trophectoderm cells in total for 4 blastocysts counted. The embryos of group D consisted of an average of 61 ± 9 trophectoderm cells in total for 10 blastocysts counted.

For ICM/total cell ratio yield, group C blastocysts had significantly higher rate (0.39) compared to the other groups (0.29, 0.36, 0.31 and 0.33, respectively). As shown in Table 2, their allocation to TE and ICM cell numbers and ICM/Total cell ratio in stained blastocysts (Figure 1).

4 | DISCUSSION

Although with the advancements of culture procedures, in vitro produced (IVP) embryos it usually shows low quality and viability than developed in vivo counterparts. In the present study, bovine embryos cultured in the presence of LIF had better rates of blastocysts development and viability, particularly when cryopreserved by vitrification. The role of LIF in bovine embryo culture has been controversial in previous studies, including our work. Addition of LIF has also been shown significantly to enhance the blastocyst formation rates of bovine embryos (Han et al., 1995), a finding that remains inconsistent (Vejlsted et al., 2005). The present study results showed that LIF was significantly effective on in vitro bovine embryo development especially when added on day 4 of culture.

Single concentration of LIF supplementation, that is 100 ng/ml, was studied. This concentration is commonly used in media for culture of bovine embryos (Kocyigit & Cevik, 2015; Neira, Tainturier, Pena, & Martal, 2010). In the present study, we used human recombinant LIF, because bovine LIF (bLIF) is not commercially available. Human LIF (hLIF) is a greater sequence homology has been shown between

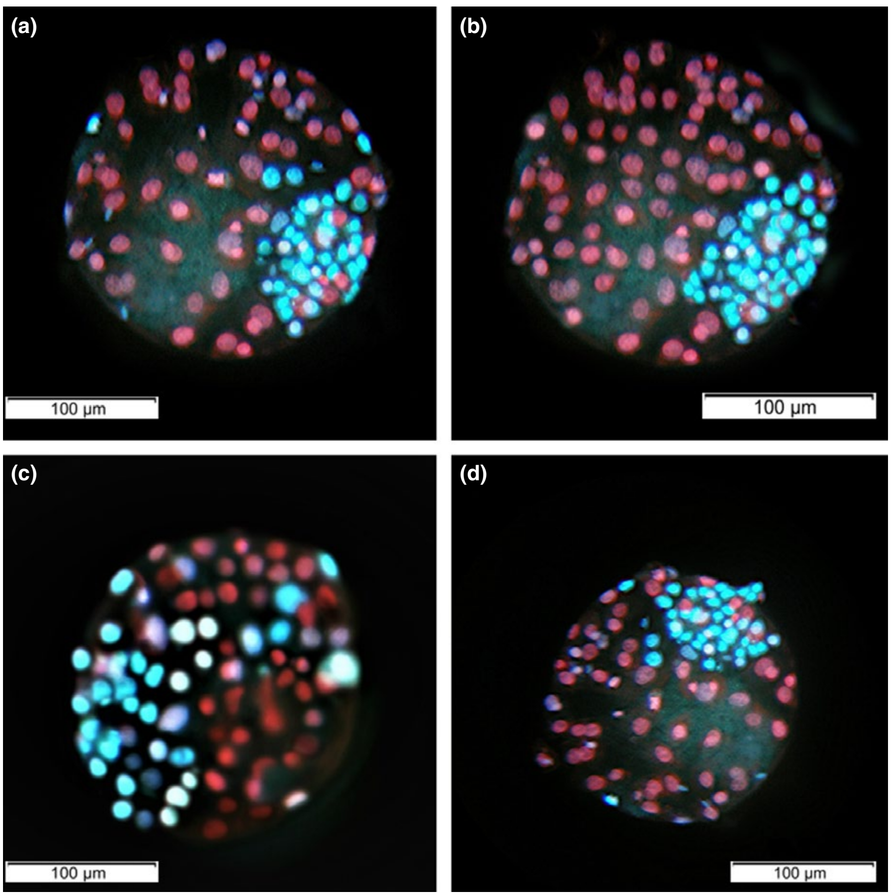

FIGURE 1 Differential cell-stained bovine blastocysts of different groups, a,b,c,d; Experiment group of blastocyst

human and bovine LIF than between mouse and bovine (89.1% and 76.8%, respectively; Piedrahita et al., 1997; Rodriguez et al., 2007).

The present study results showed that addition of LIF at day 4 (p.i.) was significantly effective on in vitro bovine embryo development especially ratio to reach blastocyst. Although total blastocyst rates did not differ between the control group and group C. Group B blastocysts were found to have higher total cell number and ICM nuclei than other treatment group blastocysts. Expression of LIFR has been demonstrated in all pre-embryonic stages from the two-cell stage to the expanded blastocysts. As is known better results have followed addition of LIF to culture media for later stages of development than for earlier cleavage stages (Fukui & Matsuyama, 1994). However, our study findings agree with other reports demonstrating detrimental effect on blastocyst development when LIF was added at the culture media day 0 and day 4 at 7. This detrimental effect which no precedent was found in the literature, might be result of the antimitotic effect of LIF especially when used early cleavage stages. Because the number of cells in this stages is critical for blastocyst development. The negative effect of daily addition LIF between days 4 to 7 on the viability of blastocyst might be result of the manipulation stress. Component adding to the media at daily can cause stress on the embryos (Krisher & Wheeler, 2009).

In assessing the viability of the embryos, morphological observations are most widely used as a gross indicator, but blastocyst cell number and the ICM/total cells ratio are valid indicators for the viability of pre-implantation embryos, while morphological criteria alone are a poor indicator. Proliferation and differentiation of somatic cells are regulated by autocrine and paracrine secretion of mitogenic growth factors by maternal and embryonic tissues at specific stages of development as evidenced by measurements of total cell numbers and ICM:TE ratios (Van Soom et al., 1996). However, the use of hLIF has yielded controversial results as compared to bLIF, and mLIF has not been used for this purpose (Rodriguez et al., 2007). Rodrigues et al. 2007, reported that, embryonic development was impaired by hLIF, while the number of ICM cells was reduced by mLIF, in disagreement with the effects described by Yamanaka et al. for bLIF (Yamanaka, Kudo, Kimura, Amano, & Itagaki, 1999). While bLIF has been described to increase TE cell counts without affecting the ICM (Yamanaka et al., 2001), hLIF has been noted to increase (Kocyigit & Cevik, 2015; Sirisathien et al., 2003), decrease (Vejlsted et al., 2005) or have no effect on the ICM (Rodriguez et al., 2007). In addition, mLIF was found to have no effect on the number of TE cells yet to reduce the ICM.

The ratio (ICM/total cell) is crucial to the developmental competence of the embryo, as it is assumed that a certain minimal number of ICM cells are required to obtain a pregnancy. In the current study, the quality of in vitro produced early and expanded blastocysts, assessed by their cryotolerance, hatchability rate and the number of ICM and TE cells, was affected by culture conditions. It is unknown whether specific traits of ICM and TE could lead to differences in survival to vitrification, although a detrimental effect (such as apoptosis) of vitrification on the numbers of cells on the ICM has been observed (Gómez et al., 2008). Embryo cryopreservation represents a pivotal tool for the long-term storage and exchange of valuable genetic resources of livestock and endangered species. This technology is the best method for the long-term preservation of valuable genetic resources from experimental and livestock animals. The use of cryopreservation is also essential for the widespread use of embryo transfer, which allows the exchange of genetics with reduced transportation cost, avoiding animal welfare problems and with a minimal risk of disease transmission (Alminana & Cuello, 2015). The vitrification procedure usually results in cellular and subcellular damage, most of which are gradually restored over a 24-hr period (Vajta, Hyttel, & Callesen, 1997). A reduction in the inner cell mass and trophectodermal cell number in vitrified embryos might be one of the possible causes of placentary alterations, which may be associated with lower gestation rates after in vitro embryo manipulations (Kaidi et al., 2001).

During the last decade, new strategies to produce embryos with improved ability to resist stressing sources (such as vitrification) have been proposed. The quality of the IVP bovine blastocyst has been classically associated with their sensitivity to cryopreservation (Iwasaki, Yoshikane, Li, Watanabe, & Nakahara, 1994) and to the amount and proper allocation of the embryonic cells (Van Soom et al., 1996). Blastocyst cell numbers are usually reduced in IVP embryos as compared to in vivo-derived embryos (Gómez et al., 2008). Therefore, many efforts have been undertaken for several decades to improve post-thaw quality and viability of mammalian embryos. In our study, re-expanded blastocysts were selected for staining. The re-expansion ability of vitrified group C blastocysts was significantly higher than other groups' blastocysts. These results may indicate that an embryos with cultured LIF only added on day 4, higher cryotolerance of than LIF addition that early (day 0) or later (day 4 at 7) stages. In addition to these results, we have not found that the positive effect of LIF addition after thawing on re-expansion rate of vitrified/warmed embryos (Group D).

The cell ratios of vitrified-thawed were significantly higher than those of group C blastocysts. Although not statistically significant, because of ICM cell deficit, the cell ratios of blastocysts in group A are higher than the average ratio. Culturing bovine IVP embryos in media with LIF added at different times has brought unclear findings with regard to embryo developmental kinetics, cryotolerance and cell count.

In conclusion, the addition of LIF only on day 4 (p.i.) to the culture medium was found to be beneficial for bovine embryonic development based on several measures, including blastocysts rate, re-expansion rate and cellular cryotolerance after vitrification. However, as already mentioned, research on the use, dosing and culture addition timing of LIF in bovine embryo culture has not yet reached the intensity to provide the desired information. It may be useful to carry out advanced studies in this regard.

CONFLICT OF INTEREST

There are no known conflicts of interest associated with this publication, and there has been no significant financial support for this work that could have influenced its outcome.

AUTHOR CONTRIBUTIONS

A. Kocyigit performed embryo culture, vitrification and staining. M. Cevik conceived the original idea and collaborated in the experimental design, statistical analysis and critical reading.

REFERENCES

Alminana, C., & Cuello, C. (2015). What is new in the cryopreservation of embryos? *Animal Reproduction*, *12*, 418–427.

Amiri, I., Parvini, M., Amini, A. & Heidarbeigi Kh, M. N. (2008). Relevance of LIF and EGF on mouse preimplantation embryo development. *Yakhteh Medical Journal*, *10*(3), 213–217.

Arici, A., Oral, E., Bahtiyar, O., Engin, O., Seli, E., & Jones, E. E. (1997). Leukaemia inhibitory factor expression in human follicular fluid and ovarian cells. *Human Reproduction*, *12*(6), 1233–1239.

Attar, E., Ozsait, B., Bulgurcuoglu, S., Serdaroglu, H., & Arici, A. (2003). Effect of leukaemia inhibitory factor on long-term sperm motility and survival. *Reproduction Biomedical Online*, *7*, 71–74.

Fry, R. C., Batt, P. A., Fairclough, R. J., & Parr, R. A. (1995). Human inhibitory factor improves the viability of cultured ovine embryos. *Biology of Reproduction*, *46*, 470–474.

Fukui, Y., & Matsuyama, K. (1994). Development of in vitro matured and fertilized bovine embryos cultured in media containing human leukemia inhibitory factor. *Theriogenology*, *42*(4), 663–673.

Funston, R. N., Nauta, W. J., & Seidel, G. E. (1997). Culture of bovine embryos in buffalo rat liver cell-conditioned media or with leukemia inhibitory factor. *Journal of Animal Science*, *75*(5), 1332–1336.

Gardner, D. K., Lane, M., & Watson, A. J. (2004). *A laboratory guide to the mammalian embryo* (pp. 41–78). Oxford: Oxford University Press.

Gómez, E., Rodríguez, A., Muñoz, M., Caamaño, J. N., Hidalgo, C. O., Morán, E., & Díez, C. (2008). Serum free embryo culture medium improves in vitro survival of bovine blastocysts to vitrification. *Theriogenology*, *69*(8), 1013–1021.

Han, Y. M., Lee, E. S., Mogoe, T., Lee, K. K., & Fukui, Y. (1995). Effect of human leukemia inhibitory factor on in vitro development of IVF-derived bovine morulae and blastocysts. *Theriogenology*, *44*(4), 507–516.

Hilton, D. J. (1992). LIF: Lots of interesting functions. *Trends in Biochemical Sciences*, *17*(2), 72–76.

Ishimori, H., Saeki, K., Inai, M., Nagao, Y., Itasaka, J., Miki, Y., & Kainuma, H. (1993). Vitrification of bovine embryos in a mixture of ethylene glycol and dimethyl sulfoxide. *Theriogenology*, *40*(2), 427–433.

Iwasaki, S., Yoshikane, Y., Li, X., Watanabe, S., & Nakahara, T. (1994). Effects of freezing of bovine preimplantation embryos derived from oocytes fertilized in vitro on survival of their inner cell mass cells. *Molecular Reproduction and Development*, *37*(3), 272–275.

Kaidi, S., Bernard, S., Lambert, P., Massip, A., Dessy, F., & Donnay, I. (2001). Effect of conventional controlled-rate freezing and vitrification on morphology and metabolism of bovine blastocysts produced in vitro. *Biology of Reproduction*, *65*(4), 1127–1134.

Kimber, S. J. (2005). Leukaemia inhibitory factor in implantation and uterine biology. *Reproduction*, *130*(2), 131–145.

Kocyigit, A., & Cevik, M. (2015). Effects of leukemia inhibitory factor and insulin-like growth factor-I on the cell allocation and cryotolerance of bovine blastocysts. *Cryobiology*, *71*(1), 64–69.

Krisher, R. L., & Wheeler, M. B. (2009). Towards the use of microfluidics for individual embryo culture. *Reproduction, Fertility and Development*, *22*(1), 32–39.

Lavranos, T. C., Rathjen, P. D., & Seamark, R. F. (1995). Trophic effects of myeloid leukaemia inhibitory factor (LIF) on mouse embryos. *Journal of Reproduction and Fertility*, *105*(2), 331–338.

Leibo, S. P., & Loskutoff, N. M. (1993). Cryobiology of in vitro-derived bovine embryos. *Theriogenology*, *39*(1), 81–94.

Martal, J. L., Chene, N. M., Huynh, L. P., L'Haridon, R. M., Reinaud, P. B., Guillomot, M. W., & Charpigny, S. Y. (1998). IFN-tau: A novel subtype I IFN1. Structural characteristics, non-ubiquitous expression, structure-function relationships, a pregnancy hormonal embryonic signal and cross-species therapeutic potentialities. *Biochimie*, *80*(8–9), 755–777.

Moore, K., Kramer, J. M., Rodriguez-Sallaberry, C. J., Yelich, J. V., & Drost, M. (2007). Insulin-like growth factor (IGF) family genes are aberrantly expressed in bovine conceptuses produced in vitro or by nuclear transfer. *Theriogenology*, *68*(5), 717–727.

Neira, J. A., Tainturier, D., L'Haridon, R. M., & Martal, J. (2007). Comparative IFN-τ Secretion after Hatching by Bovine Blastocysts Derived Ex Vivo and Completely Produced In Vitro. *Reproduction in Domestic Animals*, *42*(1), 68–75.

Neira, J. A., Tainturier, D., Pena, M. A., & Martal, J. (2010). Effect of the association of IGF-I, IGF-II, bFGF, TGF-β1, GM-CSF, and LIF on the development of bovine embryos produced in vitro. *Theriogenology*, *73*(5), 595–604.

Nicola, N. A., & Babon, J. J. (2015). Leukemia inhibitory factor (LIF). *Cytokine & Growth Factor Reviews*, *26*(5), 533–544.

Parrish, J. J., Krogenaes, A., & Susko-Parrish, J. L. (1995). Effect of bovine sperm separation by either swim-up or Percoll method on success of in vitro fertilization and early embryonic development. *Theriogenology*, *44*(6), 859–869.

Piedrahita, J. A., Weaks, R., Petrescu, A., Shrode, T. W., Derr, J. N., & Womack, J. E. (1997). Genetic characterization of the bovine leukaemia inhibitory factor (LIF) gene: Isolation and sequencing, chromosome assignment and microsatellite analysis. *Animal Genetics*, *28*(1), 14–20.

Pollard, J. W., & Leibo, S. P. (1994). Chilling sensitivity of mammalian embryos. *Theriogenology*, *41*(1), 101–106.

Rodriguez, A., De Frutos, C., Diez, C., Caamaño, J. N., Facal, N., Duque, P., & Gómez, E. (2007). Effects of human versus mouse leukemia inhibitory factor on the in vitro development of bovine embryos. *Theriogenology*, *67*(5), 1092–1095.

Salleh, N., & Giribabu, N. (2014). Leukemia inhibitory factor: Roles in embryo implantation and in nonhormonal contraception. *The Scientific World Journal*, *2014*, 1–11.

Seshagiri, P. B., Roy, S. S., Sireesha, G., & Rao, R. P. (2009). Cellular and molecular regulation of mammalian blastocyst hatching. *Journal of Reproductive Immunology*, *83*(1), 79–84.

Sirisathien, S., Hernandez-Fonseca, H. J., Bosch, P., Hollet, B. R., Lott, J. D., & Brackett, B. G. (2003). Effect of leukemia inhibitory factor on bovine embryos produced in vitro under chemically defined conditions. *Theriogenology*, *59*(8), 1751–1763.

Smith, D. K., & Treutlein, H. R. (1998). LIF receptor-gp130 interaction investigated by homology modeling: Implications for LIF binding. *Protein Science*, *7*(4), 886–896.

Spanos, S., Becker, D. L., Winston, R. M., & Hardy, K. (2000). Anti-apoptotic action of insulin-like growth factor-I during human preimplantation embryo development. *Biology of Reproduction*, *63*(5), 1413–1420.

Stewart, C. L., Kaspar, P., Brunet, L. J., Bhatt, H., Gadi, I., & Abbondanzo, S. J. (1992). Blastocyst implantation depends on maternal expression of leukaemia inhibitory factor. *Nature*, *359*(6390), 76–79.

Vajta, G., Hyttel, P., & Callesen, H. (1997). Morphological changes of in-vitro-produced bovine blastocysts after vitrification, in-straw direct rehydration, and culture. *Molecular Reproduction and Development*, *48*(1), 9–17.

Van Soom, A., Boerjan, M., Ysebaert, M. T., & de Kruif, A. (1996). Cell allocation to the inner cell mass and the trophectoderm in bovine embryos cultured in two different media. *Molecular Reproduction and Development*, *45*(2), 171–182.

Vejlsted, M., Avery, B., Gjorret, J. O., & Maddox-Hyttel, P. (2005). Effect of leukemia inhibitory factor (LIF) on in vitro produced bovine embryos and their outgrowth colonies. *Molecular Reproduction and Development*, *70*(4), 445–454.

Watson, A. J., Hogan, A., Hahnel, A., Schultz, G. A., & Wiemer, K. E. (1992). Expression of growth factor ligand and receptor genes in the preimplantation bovine embryo. *Molecular Reproduction and Development*, *31*(2), 87–95.

Watson, A. J., Watson, P. H., Warnes, D., Walker, S. K., Armstrong, D. T., & Seamark, R. F. (1994). Preimplantation development of in vitro-matured and in vitro-fertilized ovine zygotes: Comparison between coculture on oviduct epithelial cell monolayers and culture under low oxygen atmosphere. *Biology of Reproduction*, *50*(4), 715–724.

Yamanaka, M., Amano, T., & Kudo, T. (2001). Effect of the presence period of bovine leukemia inhibitory factor in culture medium on the development of in vitro fertilized bovine embryos. *Nihon Chikusan Gakkaiho*, *72*(4), 285–290.

Yamanaka, M., Kudo, T., Kimura, N., Amano, T., & Itagaki, Y. (1999). Effect of bovine leukemia inhibitory factor on hatching and numbers of inner cell mass and trophectoderm of bovine intact and biopsied blastocysts. *Nihon Chikusan Gakkaiho*, *70*(6), 444–450.

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