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The effect of oleic and linoleic acids on in vitro bovine embryonic development and embryo quality

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Abstract: The present study was aimed at the investigation of the effects of oleic and linoleic acid on the in vitro development of Anatolian native black crossbred bovine embryos. Following the in vitro maturation and fertilization of oocytes, embryonic development stages were monitored using embryo culture medium supplemented with three different doses of oleic and linoleic acid (10, 100, and 1000 μM). Examination of the culture media 48 h after fertilization demonstrated that linoleic acid had no effect on embryo cleavage rates, while oleic acid produced a significant increase in cleavage rates ($P < 0.01$). It was determined that the highest dose of linoleic acid (1000 μM) and all three doses of oleic acid significantly increased the proportion of cultured oocytes developing to the morula-blastocyst stage ($P < 0.001$). As a result, while oleic acid significantly increased ($P < 0.001$) the proportion of embryos developing to the morula-blastocyst stage, linoleic acid had limited effects on embryonic development and quality. Thus, it was concluded that the supplementation of Anatolian native black crossbred bovine embryo cultures with oleic acid may induce positive effects on embryonic development and quality.

Key words: Oleic acid, linoleic acid, bovine embryo, blastocyst

1. Introduction

It has been proven that the morphological and physiological quality of in vivo-derived embryos is better than that of in vitro-derived embryos (1,2). One of the major reasons for the relative inadequacy of in vitro embryonic development is the generation of reactive oxygen species (ROS) (2). ROS generation occurs as a result of the reduction of free oxygen during the metabolism of molecular oxygen required for embryonic development. The major ROS produced by the embryo itself are the superoxide anion radical, hydrogen peroxide, and the hydroxyl radical. When released at a basal level, ROS have a regulatory effect on embryonic metabolism, embryonic development, and embryo implantation (3).

Oxidative stress-induced damage to the embryo arises from excessively released ROS, and these radicals pass the cell membrane, causing structural changes in cellular molecules such as lipids, proteins, and nucleic acids, which, in turn, result in mitochondrial alterations, blockage of cellular development in the embryo, excessive consumption of ATP, and apoptosis (3–5). Eventually, embryonic development is inhibited and the implantation of the embryo is negatively affected (3).

Against the oxidative stress and damages caused by ROS, in vivo antioxidant defense systems, which protect the embryo, exist in both the embryo and the female reproductive tract that it resides in. These antioxidant systems include, among others, glutathione, taurine, hypotaurine, ascorbate, pyruvate, vitamin E, glutathione peroxidase, superoxide dismutase, and catalase (3). Under in vitro conditions, as the embryo depends only on the antioxidant systems it is itself equipped with against oxidative stress, additional preventive measures need to be taken (6–8). However, the elimination of oxidative stress and oxidative stress factors from the embryo culture medium is both an inevitable and complex problem. The selection of antioxidants and antioxidant doses for the supplementation of culture media can be challenging, as excessive antioxidant doses in the medium may cause negative impacts (6,9).

Previous research has shown that fatty acids incorporated into in vitro culture media exhibit positive effects on oocyte maturation, fertilization, and embryonic development (10,11). The feeding of ruminants on rations of high fat content produces positive impacts on reproductive performance and enables high blastocyst

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rates and the derivation of good quality embryos (12). Research has demonstrated that linoleic acid and oleic acid have antioxidant properties and are thus required for animal growth and animal health (13,14). It has been determined that following the freezing and thawing of bovine embryos, the supplementation of their culture medium with trans-10, cis-12 octadecadienoic acid (t10, c12 CLA), a conjugated linoleic acid derivative, increases the viability of embryos (15). The low survival capability of the embryos results in low rates of gestation and limits the use of in vitro-derived bovine embryos (16). The supplementation of culture media with antioxidant substances is reported to increase oocyte maturation and improve embryo quality (6,8).

The present study was aimed at the investigation of the effects of different doses of oleic acid and linoleic acid, which are unsaturated fatty acids with antioxidant properties, on the in vitro development of Anatolian native black crossbred bovine embryos.

2. Materials and methods

Ovaries belonging to Anatolian native black crossbred cows, obtained from a local slaughterhouse, were transferred to the laboratory in a thermos flask filled with antibiotic-containing (gentamycin 1 ml/L) 0.9% physiological saline, which was maintained at a transfer temperature of 30 °C. In the laboratory, follicles of 2–8 mm in diameter on the surface of the ovaries were aspirated. TCM-199 medium supplemented with 10% fetal calf serum + 0.8 µg/mL follicle-stimulating hormone was used for oocyte maturation. The in vitro maturation procedure of the oocytes was performed in an incubator containing 95% humidified air and 5% CO_2 at 38.5 °C for a period of 22 h. Bull sperm frozen in 0.25 mL-straws, each containing 175×10^5 spermatozoa, was used for in vitro fertilization. Brackett Oliphant medium was used for sperm capacitation and oocyte fertilization. Following in vitro fertilization, the oocytes were denuded from the surrounding cumulus oophorus cells by repeated pipetting and were cultured into CR1aa medium supplemented with different doses of antioxidants (linoleic acid 10, 100, and 1000 µM; oleic acid 10, 100, and 1000 µM) or antioxidant-free CR1aa medium (control). Groups of 15–20 presumptive zygotes (oocytes) were cultured in 100-µL droplets of the culture media.

The cleavage rates 48 h after fertilization of the oocytes cultured into the media containing different antioxidant doses were determined, followed by the monitoring of the morula, blastocyst, and expanded blastocyst rates on day 7 and the assessment of embryo quality. The development rates of the embryos cultured in the antioxidant-containing and antioxidant-free media were compared using the chi-square test. Differences with a value of $P < 0.05$ were considered as statistically significant. The trials consisted of 9 independent replications.

3. Results

The cleavage rates of bovine oocytes on day 2, the rates of oocyte maturation on day 7, and the proportion of fertilized oocytes developing to the morula-blastocyst stage in the media supplemented with different doses of oleic acid and linoleic acid are shown in Table 1.

3.1. Cleavage rates on day 2

Examination of the culture media 48 h after fertilization demonstrated that, when compared to the control group, the cleavage rates had increased in the media supplemented with different antioxidant doses, excluding those of linoleic acid. Furthermore, the increase observed in the cleavage rate in the culture medium supplemented with 1000 µM oleic acid (71.70%), compared to the cleavage rate of the control group (53.64%), was statistically significant ($P < 0.01$).

3.2. Morula-blastocyst rates on day 7

The proportions of cultured oocytes developing to the morula-blastocyst stage (Figure) in culture media supplemented with 1000 µM linoleic acid and 10, 100, and 1000 µM oleic acid were determined as 23.13%, 25.17%, 28.57%, and 34.59%, respectively, and when compared to the control group (13.25%), the increase in the morula-blastocyst rates was found to be statistically significant ($P < 0.001$).

The morula-blastocyst rates determined on day 7 in the culture media supplemented with 10, 100, and 1000 µM oleic acid were 40.43%, 44.44%, and 48.25%, respectively, and comparison with the control group (24.69%) revealed that the increase observed in the morula-blastocyst rates was statistically significant ($P < 0.01$). The supplementation of the culture medium with 1000 µM oleic acid produced the highest rates of morula-blastocyst formation from the oocyte and cleaved embryo stages (34.59% and 48.25%), as compared to the other groups. The comparison of the morula rates in the media on day 7 of culturing demonstrated that medium supplementation with 10 µM linoleic acid (17/147), 100 µM oleic acid (22/147), and 1000 µM oleic acid (25/147) produced morula rates higher than that obtained in the control group (7/147) ($P < 0.001$). Furthermore, it was ascertained that the rate of expanded blastocysts was higher in the group supplemented with 1000 µM oleic acid (12/147) compared to the control group (2/147) ($P < 0.05$). It was found that oleic acid had an obvious effect in terms of quality and size of embryos (Table 2) (embryo size was determined by Nikon Digital Sight system).

4. Discussion

It is known that various factors related to the culture medium have positive and negative effects on embryonic development, embryo morphology, and gene expression. These factors include, among others, the composition,

Table 1. Embryo development rates determined in CR1aa culture media supplemented with different antioxidants following the maturation and fertilization of bovine oocytes.

	Embryo development rates on day 2		Embryo development rates on day 7				
Groups, µM	Number of cultured oocytes, n	Cleavage rate, %	Morula	Blastocyst	Expanded blastocyst	Proportion of cultured oocytes developing to the morula-blastocyst stage, %	Proportion of cleaved embryos developing to the morula-blastocyst stage, %
Linoleic 10	147	62.59 (92/147) ^{ab}	(17/147) ^{ab}	(12/147)	(5/147) ^{abc}	23.13 (34/147) ^{ab}	36.96 (34/92) ^{ab}
Linoleic 100	144	50.00 (72/144) ^{c}	(8/147) ^{bc}	(8/147)	(1/147) ^{c}	11.81 (17/144) ^{d}	23.61 (17/72) ^{b}
Linoleic 1000	152	58.55 (89/152) ^{bc}	(9/147) ^{bc}	(15/147)	(8/147) ^{ab}	21.05 (32/152) ^{bc}	35.96 (32/89) ^{ab}
Oleic 10	151	62.25 (94/151) ^{ab}	(14/147) ^{abc}	(19/147)	(5/147) ^{abc}	25.17 (38/151) ^{ab}	40.43 (38/94) ^{a}
Oleic 100	154	64.29 (99/154) ^{ab}	(22/147) ^{a}	(16/147)	(6/147) ^{abc}	28.57 (44/154) ^{ab}	44.44 (44/99) ^{a}
Oleic 1000	159	71.70 (114/159) ^{a}	(25/147) ^{a}	(18/147)	(12/147) ^{a}	34.59 (55/159) ^{a}	48.25 (55/114) ^{a}
Control	151	53.64 (81/151) ^{bc}	(7/147) ^{c}	(11/147)	(2/147) ^{bc}	13.25 (20/151) ^{cd}	24.69 (20/81) ^{b}
P		**	***	-	*	***	

a, b, c, d: Differences between groups shown with different superscripts in the same column are statistically significant. *: P<0.05, **: P < 0.01, ***: P < 0.001.

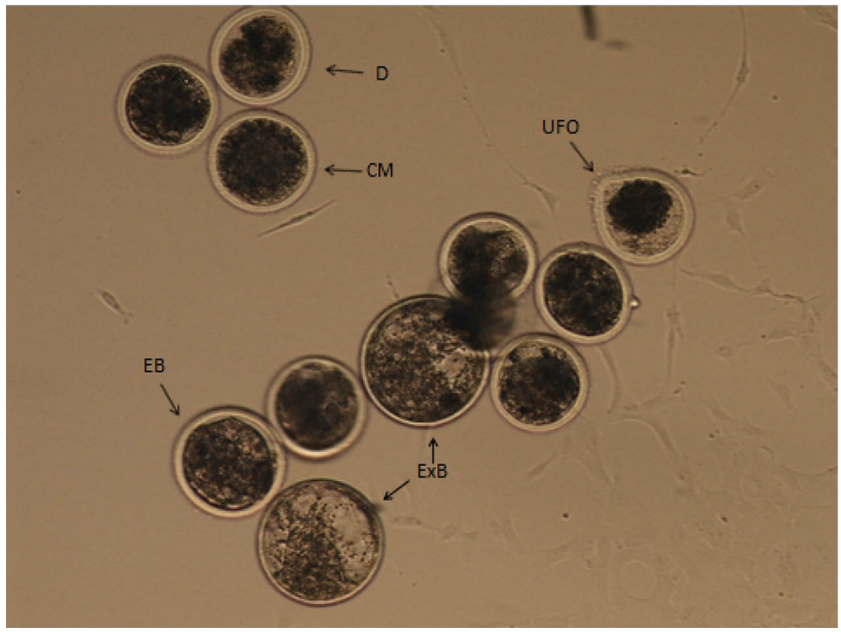

Figure. Different stages (CM: compact morula, D: degenerate embryo, EB: early blastocyst, ExB: expanded blastocyst, UFO: unfertilized oocyte) of embryos after in vitro culture.

Table 2. The average diameter of 7-day embryos (µm).

Linoleic 10	Linoleic 100	Linoleic 1000	Oleic 10	Oleic 100	Oleic 1000	Control
146.59	140.9	152.05	169.04	163.08	164.59	146.27

physicochemical properties, and oxygen concentration of the culture medium and the exposure of oocytes to light (17,18). The generation of free oxygen radicals in the culture medium may have detrimental effects on the functions of embryo cells (3). The effect of culture medium is closely related to the effect of the antioxidant substances added to the culture medium (19). Basic media used for the in vitro maturation, fertilization, and culture of bovine oocytes are supplemented with various additives with an aim to enhance the developmental capability of oocytes and embryos. In the present study, the unsaturated fatty acids, oleic acid, and linoleic acid, with antioxidant properties, were investigated for their possible effects on embryo quality.

It was observed that fertilization and blastocyst rates were higher in the groups supplemented with different doses of oleic acid compared to the groups supplemented with different doses of linoleic acid. In a previous study conducted in rats, it was reported that both oleic acid and linoleic acid increased the proportion of 8-cell embryos developing to the blastocyst stage and that the best results were obtained with culture media containing oleic acid (10). Supplementation of mSOF culture media with linoleic acid had no statistically significant effect on the rates of embryonic cleavage and development in previous research (20), similar to the results obtained in the present study. While some previous studies on the supplementation of in vitro culture media with antioxidants demonstrated a positive effect on embryonic development, some others revealed the absence of any effect (19,21). The proportions of embryos developing to the morula-blastocyst stage obtained with oleic acid and linoleic acid supplementation in the present study were higher than those reported to have been obtained with L-ergothioneine and L-ascorbic acid by Öztürkler et al. (22). They reported that the percentages of morula were 6.5% with L-ergothioneine and 3.0% with ascorbic acid. That is lower than in the present study. The difference between these two studies arises mainly from the antioxidant properties of oleic acid and linoleic acid being stronger than those of L-ergothioneine and L-ascorbic acid and the former two providing better protection of embryos against oxidative stress.

Although it has been previously shown that the supplementation of maturation medium with linoleic

acid improves blastocyst quality (23), it was also indicated that such supplementation reduces the proportion of oocytes developing to the metaphase 2 stage and thus the rate of embryos developing to the blastocyst stage. This adverse effect of linoleic acid is attributed to it increasing the concentration of prostaglandin E2 and the level of intracellular cAMP and reducing the phosphorylation of mitogen-activated protein kinases in the medium (24).

In conclusion, based on the morula-blastocyst rates determined in the culture media on day 7, it was ascertained that higher morula-blastocyst rates were obtained with the three different doses of oleic acid compared to the other groups. Furthermore, supplementation with 100 and 1000 µM oleic acid was observed to improve the morula and expanded blastocyst rates. Thus, it is considered that oleic acid has a stronger effect in eliminating the detrimental effects of oxidative stress and a stronger effect on Anatolian native black crossbred embryonic development. In view of antioxidant additives of unsaturated fatty acid structure having been demonstrated to show effects in in vitro embryo culture systems, there is a need for further research on the molecular properties of such antioxidants as well as a need for the determination of their optimum doses and methods for the increase of the derivation rates of in vitro embryos. With the aid of such data, higher rates of high-quality embryos will be able to be derived from the ovaries of genetically superior cattle.

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