Effect of cysteine, insulin-like growth factor-1 and epidermis growth factor during in vitro oocyte maturation and in vitro culture of yak-cattle crossbred embryos

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ABSTRACT

Some antioxidants and growth factors play an important role in promoting oocyte maturation and embryo development in many mammalian species, but there is little information about the yak (Bos grunniens). Therefore, the objective of this study was to evaluate Cys, insulin-like growth factor-1 (IGF-1) and epidermis growth factor (EGF) on yak oocyte maturation, cleavage and blastocyst rates after in vitro fertilized with Jersey sperm. A single or different combination of Cys, EGF and IGF-1 was added to in vitro maturation (IVM) and in vitro culture (IVC) media. The results showed that a single addition of Cys and IGF-1 increased oocyte maturation and blastocyst rates (p < .05), but did not increase cleavage rate; EGF or IGF-1 + EGF increased oocyte maturation, cleavage and blastocyst rates (p < .05) compared with the control. A combination of IGF-1 + EGF + Cys could have a beneficial effect (p < .05). These results indicated that supplementation of IVM and IVC media with Cys, IGF-1, EGF and their combinations could improve in vitro production efficiency of yak-cattle crossbred embryos.

1. Introduction

The yak (Bos grunniens) is one of the world’s most remarkable domestic animals – an herbivore living in and around the Himalayas and further north at altitudes ranging from 2500 to 5500 m. They are very important to local herders by providing milk and meat, as few other domestic animals can survive in such cold, hypoxic ecological conditions, but their production performance is much inferior to the improved cattle breeds. The meat and milk performance of hybrids derived from that yaks crossbred with the improved bovine breeds were greatly improved, however, sterility of F1 males prevents successful inter-se matings (Wiener et al. 2003). With the current development of in vitro production (IVP) and embryo transfer, the F1 females are possible not only give additional milk, but also produce valuable offspring (F1) if they are used as recipients of yak-cattle crossbred embryos. The desirable IVP efficiency of crossbred embryos is prerequisite for F1 producing F1, but because this is the only marketing season for yaks) and transported, within 3 h, to the laboratory in DPBS (29–33°C). IVM and IVF were performed as previously described by Zi et al. (2018). Briefly, groups of 50 yak COCs were matured in a four

2. Materials and methods

2.1. Ethics statement

All animal procedures were approved by the Institutional Animal Care and Use Committee of the Southwest Minzu University and all methods were performed in accordance with the relevant guidelines and regulations.

2.2. Materials

G-IVF™ PLUS, SpermRinse™ and mineral oil were purchased from Vitrolife Sweden AB. Synthetic oviductal fluid (SOF) was purchased from Caission Labs (UT, USA). FSH and LH were purchased from Bioniche Life Sciences Inc. (Belleville, Ontario, Canada). Fetal calf serum (FCS) and Pen Strep were purchased from Gibco (Grand Island, NY, USA). Medium199 (10×), L-cysteine (Cys) and β-estradiol were obtained from Sigma-Aldrich (St. Louis, MO, USA). IGE-1 and EGF were purchased from PeproTech (Rocky Hill, NJ, USA).

2.3. In vitro maturation (IVM), fertilization (IVF), and culture (IVC)

Yak ovaries were collected at local slaughterhouses from October to December (at the end of breeding season, because this is the only marketing season for yaks) and transported, within 3 h, to the laboratory in DPBS (29–33°C). IVM and IVF were performed as previously described by Zi et al. (2018). Briefly, groups of 50 yak COCs were matured in a four
well dish (500 μl/well) containing TCM 199 supplemented with 20% (v/v) FCS, 5 μg/ml FSH, 5 μg/ml LH, 1 μg/ml estradiol-17β, and 100 U/ml penicillin and 100 μg/ml streptomycin (IVM medium) at 38.5°C in a humidified incubator with 5% CO2. After 24 h of IVM, COCs with 61–100% cumulus cells expended were classified as matured oocytes (Hensleigh and Hunter 1985; Gliedt et al. 1996).

Jersey frozen semen was thawed and incubated in Sperm-Rinse™ at 38.5°C for 50 min allowed the motile sperm to swim up. Groups of 30 COCs were inseminated with sperm that had been prepared by swim-up procedure at a final concentration of 2 × 106 sperm/ml in 70-μl drops of the IVF™, and after a period of 24 h post-insemination (hpi), presumptive zygotes were cultured in a four-well dish containing 500 μl of SOF medium (IVC medium) consisting of different concentrations of IGF-1, IGF-2 and EGF with an overlay of mineral oil at 38.5°C. The culture medium was changed at 96 hpi. Cleavage and blastocyst formation were assessed on days 2 and 7 of culture, respectively.

2.4. Experimental design

IVM and IVC media were supplemented with Cys, IGF-1 and EGF to final concentrations of 0.6 mM, 100 ng ml–1 and 10 ng ml–1, respectively, and supplemented with none of them in the control. The experiment contained eight groups: Cys, IGF-1, EGF, IGF-1 + EGF, IGF-1 + Cys, EGF + Cys, IGF-1 + EGF + Cys, and control. These concentrations were chosen because they had previously been shown to be the most effective dosage for IVM and IVC in some studies (Shabankareh and Zandi 2010; Lott et al. 2011; Chen et al. 2017).

2.5. Statistical analysis

All data were subjected to ANOVA followed by Tukey–Kramer test. Analyses were carried out using the GLM procedure of Statistical Analysis System (SAS; SAS institute, Cary, NC, USA).

3. Results and discussion

The effects of supplementation of Cys, IGF-1, EGF and their combinations in IVM and IVC media on maturation cleavage, and blastocyst rates of yak oocytes are listed in Table 1. The results showed that a single addition of Cys or IGF-1 increased oocyte maturation and blastocyst rates of yak-cattle crossbred embryos (p < .05), but did not increase cleavage rate compared to control. EGF increased oocyte maturation, cleavage and blastocyst rates (p < .05) compared to control. No additive effect of combining EGF and IGF-I was seen when results were compared to those following supplementation of the media with EGF alone, but the cleavage rate was greater than those supplemented with IGF-I alone (p < .05). IGF-1 + Cys and EGF + Cys did not give a significantly more beneficial effect compared to Cys, IGF-1 or EGF alone, however, the combination of IGF-1 + EGF + Cys could greatly improve oocyte maturation (84.44%), cleavage (80.45%) and blastocyst rates (38.67%).

The excessive production of reactive oxygen species (ROS) leads to oxidative stress and impedes oocyte maturation and embryonic development (Takahashi 2012). Cys is a critical component amino acid of glutathione (GSH), which plays an important role in protecting from the toxic effect of oxidative damage (Meister 1983). EGF and IGF-1 are involved in regulating cell proliferation and apoptosis (Paria and Dey 1990; Wasielak and Bogacki 2007; Chen et al. 2017). Positive effects on oocyte maturation and/or embryonic development have been observed if a single or different combination of Cys, EGF and IGF-1 concentrations in IVM, IVF and IVC media is optimal in cattle (Ali et al. 2003; Sirisathien et al. 2003; Neira et al. 2010; Nabenishi et al. 2012), pig (Choe et al. 2010; Lott et al. 2011), sheep (Shabankareh and Zandi 2010), buffalo (Pawshe et al. 1998), human (Yu et al. 2012), mouse (Toori et al. 2014); cat (Thongkittidilok et al. 2015), goat (Conceiccao et al. 2016; Zhou et al. 2016) canine (Sato et al. 2018), and yak (Pan et al. 2015; Chen et al. 2017). Our results are in agreement with other reports showing that significant improvements in the proportion of oocytes undergoing cleavage and blastocyst development were achieved when cysteine (0.6 mM) was added to the bovine maturation medium as compared to control medium without antioxidant supplementation (Ali et al. 2003; Lott et al. 2011), however, this concentration did not have favourable effects in porcine oocytes under low oxygen tension (Viet Linh et al. 2009) and bovine oocytes exposed to heat stress. The addition of 1.2 mM cysteine during IVM could alleviate the influence of heat stress for oocyte developmental competence by increasing GSH content and inhibiting the production of oocyte ROS followed by apoptosis of cumulus cells (Nabenishi et al. 2012).

There are studies showing that the positive effect of growth factors on embryo development may vary depending on the maturation and culture medium component like granulosa cell co-culture (Herrler et al. 1992), bovine serum (Palma et al.

| Table 1. Effects of combined cysteine, IGF-1 and EGF on yak oocyte maturation and embryo development. |
|------------------------------------------------------------|
| **No. of COCs** | **Matured** | **Cleaved** | **Blastocysts** |
| Cys | 238 | 178 (75.20 ± 0.08) a | 168 (70.86 ± 0.12) a,b | 51 (30.42 ± 0.08) a |
| IGF-1 | 233 | 172 (73.88 ± 0.06) a | 145 (62.23 ± 0.07) a,b | 44 (30.06 ± 0.05) a |
| EGF | 233 | 173 (74.25 ± 0.10) a | 171 (73.03 ± 0.11) a,d | 52 (30.39 ± 0.04) a |
| IGF-1 + EGF | 253 | 193 (76.44 ± 0.08) a | 198 (78.32 ± 0.10) a,b,c,d | 65 (32.71 ± 0.03) a |
| IGF-1 + Cys | 247 | 187 (75.75 ± 0.07) a | 168 (68.25 ± 0.11) a,b | 51 (30.13 ± 0.04) a |
| EGF + Cys | 255 | 200 (78.75 ± 0.05) a,b | 182 (71.61 ± 0.08) a,b | 59 (32.38 ± 0.07) a |
| IGF-1 + EGF + Cys | 249 | 210 (84.44 ± 0.02) a,b | 200 (80.45 ± 0.12) a,b | 77 (38.67 ± 0.06) a |
| Control | 250 | 166 (66.50 ± 0.04) c | 162 (64.77 ± 0.10) a,b | 34 (21.16 ± 0.08) a |

Means ± SEM presented. Five replicated trials were carried out, and blastocysts were harvested on Day 7 (Day 0: day of insemination). Blastocyst formation rate was calculated from the cleaved zygotes. a,b,c,d Means in a column with superscript are significantly different (p < .05).
were some di...appears that the optimal concentrations of EGF and IGF-1 were suggested by Sakagami et al. (2012), but 50 ng/ml EGF and 100 ng/ml IGF-1 were suggested by Arat et al. (2016). It appears that the optimal concentrations of EGF and IGF-1 were some different among different animal species (Sirisathien et al. 2003; Choe et al. 2010; Shabankareh and Zandi 2010; Thongkittidiok et al. 2015; Zhou et al. 2016; Sato et al. 2018).

In this study, IVM and IVC media of yak were supplemented with only one dose for each Cys (0.6 mM), IGF-1 (100 ng ml\(^{-1}\)) and EGF (10 ng ml\(^{-1}\)).

There was no difference between the rate of embryo development obtained by the addition of similar growth factors to the maturation medium and the rate of blastocyst growth obtained by the addition of growth factors to both the maturation medium and culture medium in cattle (Arat et al. 2016). Since Cys, IGF-1 and EGF were added to both the maturation medium and the culture medium in this study, it was not clear at which stage they contribute to the development of yak embryo. This should be investigated in the future studies.

4. Conclusion

Cys, IGF-1, EGF or their combinations can improve yak oocyte maturation and/or development to blastocyst competence after in vitro fertilized with cattle sperm. This provides important information to improve IVP efficiency of yak-cattle crossbred embryos. However, there is a need to study the optimal concentrations of Cys, IGF-1 and EGF in IVM and IVC media that are the most effective for IVP of yak-cattle crossbred embryos.

Disclosure statement

No potential conflict of interest was reported by the authors.

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Author contributions

F.R.Y. performed experiment and analyzed data. X.R.X. participated in the experimental design. X.D.Z. performed the experimental design and wrote the manuscript.

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