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In Vitro Bovine Embryo Development with Glucose and EDTA in Different Modifications of SOF Medium

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Abstract. This study was carried out to compare effects of glucose and EDTA in different modifications of sequential SOF media on bovine embryo development. In vitro matured bovine cumulus-oocyte complexes (COC) were fertilized and then cultured in different modifications of sequential SOF as below:
SOF A: Basic SOF+EDTA for 3 day, then Basic SOF+10% FCS.
SOF B: Basic SOF for 3 day, then Basic SOF+10% FCS+Glucose
Cleavage rates were recorded on day 2 post fertilization (pi) and embryo development to the blastocyst stage were evaluated at day 7, 8 pi. Total hatching rate was recorded at day 8 and 9 pi. Data were analyzed by means SAS software at $p<0.05$. Results showed that rate of cleavage were significantly higher in SOF A compare to SOF B (57.6 *vs*.46.1%). Rate of 5-8 cells was significantly higher in SOF A than SOF B (83.3 *vs*. 57.5%). Rate of 8-16 cells was significantly higher in SOF A than SOF B (72.2 *vs*. 50%). Compact-Blastocyst rate of SOF B was higher than SOF A (59.7 *vs*. 53.7%) but no significant. Blastocyst rate of SOF A was significantly higher than SOF B group (43 *vs*. 10.6%) at day 8. Hatched Blastocyst rate of SOF A was significantly higher than SOF B group (83.8 *vs*. 27.2%). However, SOF A medium induced significantly better embryo development compared to SOFB groups. The overall results indicated that EDTA and glucose have suitable effect on vitro developmental competency of different sequential modifications of SOF. However it seems that embryos developed in SOF A have better competency for in vitro embryo development and hatching .

Keywords: EDTA, Glucose, SOF, blastocyst, cleavage

1. Introduction

The large–scale production of bovine embryos in vitro is dependent on the production of large number of embryos of high quality (Rizos et al., 2001). Production of bovine embryos in vitro is generally referred to as a three–stage procedure, namely oocyte maturation, fertilization (including in vitro sperm preparation) and culture of the zygote. The post–fertilization culture period is the time during which various important development events including genomic activation which coincides with the developmental block takes place. Overcoming the developmental block is considered to be one of the key determinate of blastocyst quality (Balasubramanian et al., 2007; Marke et al., 1999; Gorodon et al., 1990; Bondioli et al., 1990). During in vitro culture (IVC), embryos are exposed to various stresses that are not normally encountered within the cow´s reproductive tract (Balasubramanian et al., 2007). Moreover, the success rates in terms of blastocyst yield remain modest and range between 30 and 40 %, which is still lower than that obtained from embryos produced *in vivo*. Furthermore, the quality of IVF embryos is inferior to that of embryos produced *in vivo* as judged by morphology, increased susceptibility to cryo–injury and poor implantation and viability (Wright et al., 1995). Furthermore, even with the most efficient IVC systems, it is believed that embryos show change

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in the expression of genes that reflect a stress response to suboptimal conditions (Balasubramanian et al., 2007). A greater understanding of both the physiological requirements of the embryo as it develops from the zygote to the blastocyst stage in vitro and the composition of oviduct and uterine fluids has led to development of second generation of media known as stage–specific or "sequential" complex such as G_1/G_2, SOF, KSOM for extended culture (Gardner et al., 1998; Gardner et al., 1996; Barnes et al., 1995). Synthetic oviductal fluid (SOF) is one medium commonly used for bovine embryo culture *in vitro*. The base of this medium was originally based upon the biochemical analysis of ovine oviductal fluid. Synthetic oviductal fluid has subsequently been modified presence or absence fetal calf serum, glucose and EDTA to the basic SOF during the early and late preimplantation period. Considering these modifications there is no general agreement on a single formulation (Gandhi et al., 2000). Therefore the aim of this study was to assess the bovine embryo developmental competency of the different SOF formulations.

2. Material and Method

Chemical were purchased from Sigma chemical Co. (St. Louis, Mo, USA) unless otherwise indicated.

2.1. Oocyte collection and in vitro maturation:

Ovaries were collected at abattoirs immediately after slaughter and transported to the laboratory in saline solution (0.9% NaCl) supplemented with penicillin G (200 IU/ml), streptomycin (200 µg/ml) at 35˚C, within 2–3 h. At the laboratory, the cumulus–oocyte complexes (COCs) from 2–8 mm follicles were aspirated with a sterile 18 gauge needle attached to a disposable syringe containing H-TCM and FCS (10 µg/ml)+heparin (10 IU/ml) at room temperature. Only oocytes having a homogenous evenly granulated cytoplasm surrounded by a compact cumulus oophorus with more than two layers were selected under stereomicroscope and washed in H-TCM and FCS (10 µg/ml) medium and transferred into maturation medium, TCM199 supplemented FCS (10 µg/ml), HMG (0.1 IU/ml), 17-β-Estradiol (1 µg/ml). Every five COCs were placed in 100 µl maturation droplets over Vero cells (Moulavi et al., 2006). Maturation was carried out in maximum humidity at 38.5˚C and 5% CO2 in air in a C200 incubator (Labotect, Germany).

2.2. Sperm preparation and IVF:

Commercially distributed frozen semen from Holstein bulls with proven fertility was used throughout this study. For IVF, COCs were washed twice and transferred in groups of 25-30 per 200 µl drop of fertilization medium under mineral oil. The IVF medium consisted of NaCl (114 MM), KCl (3.15 MM), NaH2PO4 (0.39 MM), Na-Lactate (13.3 MM), CaCl2 (2 MM), MgCl2 (0.5 MM), Na-Pyrovate (0.2 MM), Penicillin (50 IU/ml), Streptomycin (50 µg/ml), NaHCO3 (25 MM), Heparin (10 µg/ml), Penicillamine (20 µM), Hypotaurine (10 µM), Epinephrine (1 µM), BSA (6 mg/ml). After thawing semen in 37˚C water, motile spermatozoa were obtained by swim up procedure and were added to the fertilization drop in a final concentration of 1 × 106 per ml. Spermatozoa and oocytes were co-incubated for 20 h at 38.6˚C with 5% CO_2 in humidified air.

2.3. In vitro culture:

Following fertilization, presumptive zygotes (note that in bovine embryos the two-pronuclear stage is not observed, so cleavage is presumed to indicate fertilization) were randomly allocated to basic SOF with the following modifications:

SOF A: Basic SOF+EDTA for 3 day, then Basic SOF+10% FCS

SOF B: Basic SOF for 3 day, then Basic SOF+10% FCS+Glucose

Basic SOF was made according to the (Holm et al., 1999). The logics behind the above combination were mainly derived from literature studies and will be discussed in the discussion.

All presumptive zygote were cultured at 90% humidity, 38.5˚C, 5% CO_2 and 5% O_2 for a period of 8-9 days (day 0 is considered as the day of insemination). During the whole period of embryo culture, the embryos were transferred daily to the new culture dish (Nedambale et al., 2004). Also, embryos were evaluated daily and scored as cleaved, 5-8 cells, 8-16 cells, morula, compact, early/expanded and

hatching/hatched blastocyst stage on day 2, 2, 3, 5 and 6 to 9, respectively. The data was presented as percentage cleavage and was calculated by dividing the number of presumptive embryo on day 2 to the number of inseminated oocytes. The percentage of the embryo developmental stage was calculated by dividing the number of embryos in that stage to the total number of presumptive embryos and also, the percentage of hatching was calculated by dividing the number of hatched embryos in this stage to the number of blastocysts on day 9.

2.4. Statistical analysis

The mean values for each stage between different treatment were compared using the Duncan's multiple range tests (DMRT) by SAS software for cases with normal distribution and with chi-squared test by means of SPSS software were distribution were not normal. Differences were considered to be significant at $P \leq 0.05$.

3. Results

Table show the results rates of cleavage on day 2, compaction/morula on day 5, blastocyst on days of 8 and 9 and batching blastocyst on day 9.

Table 1*: Comparison of different developmental stage between Vero+TCM with different modification of SOF.

Treatments	No. Inseminated Oocyte	Stage of Development (%)					
		Cleavage	5-8 Cells	8-16Cells	Compact–Morula	Blastocyst-D8	Hatching-D9
SOF A	156	57.6^{a}	83.3^{a}	72.2^{a}	53.7^{ab}	43^{a}	83.8^{a}
SOF B	184	46.1^{b}	57.5^{b}	50^{b}	59.7^{a}	10.6^{b}	27.2^{b}

*: In each column, data with uncommon letter are considered as significantly different.

These results indicate that the rate of cleavage was high in the SOF A group (57.6%), while the low cleavage was in the SOF B group (46.1%). The cleavage rate was significantly different between SOF A group and SOF B group.

The 5-8 cells was high in the SOF A group (83.3) it was significant with SOF B. The rate of 8-16 cells was high significant ($P \leq 0.05$) in the SOF A group compare to SOF B group (72.2 *vs*. 50%).

Percentages of compaction/morula on day 5 indicate that the highest was in SOF B group (59.7%) but no significant to SOF A group.

Percentages of blastocyst on days 8 indicate significant difference ($P \leq 0.05$) in the SOF A group compared to the SOF B group (43 *vs*. 10.6%).

Percentages of hatching/hatched blastocyst on day 9 evaluated on the basis of blastocyst 9 day and indicate significant difference ($P \leq 0.05$) in the SOF A group compared to the SOF B group (83.8 *vs*. 27.2%).

4. Discussion

The results this study reveal that there is a significant different in the first cleavage rate on day two between SOF A group with SOF B group. This difference may be due to presence of EDTA in SOF A group and its absence in the SOF B group. This suggesting that presence of EDTA to single in the early stage of embryo development may plays a beneficial role in bovine embryo development, especially in the blastocyst rate. On the other, the precise nature of the action of EDTA in embryo culture is unknown but, chelation of heavy metal contaminates in the culture conditions is a likely possibility. Such contaminants could be overcome by EDTA, as shown by its ability to neutralize metal toxicity to culture medium. The notion that the contaminants could be toxic heavy metals is supported by the effectiveness of amino acids themselves as chelating agents, thus increasing the possibility of heavy metal contamination during purification and storage

of amino acid stock solution. Also, EDTA to cause of protein synthesis in embryos developing in culture medium and when embryo development blocked by hypoxanthine, it is reversing induced-block. On the other, another example is the two-cell block produced by oxygen-free radicals, which can be overcome by the addition of apoferritin, transferrin, and EDTA. The use of EDTA after than 3 day, this is shown to inhibit glycolytic activity of the cleavage stage embryo, thereby preventing the premature stimulation of glycolysis and enhancing development. However, EDTA should not be used for the later stage embryo as the inhibition of glycolysis reduces energy production at the blastocyst stage and significantly inhibits inner cell mass development (Gardner et al., 2000, Rizos et al., 2001, Johnson & Nasr-Esfahani 1994, Gomez et al., 2000).

On day 5 the lowest percentage compact/morula embryos were observed in the SOF A group which no contains glucose throughout the culture period. However, SOF B group was contains glucose during the later stage of embryo development has significantly higher percentage of compact/morula on day 5 post insemination. This suggests that glucose may help further embryo development in the second stage of development. On the other, the use of glucose in sequential culture medium before morula stage have been caused to decrease embryo development and quality that because for unable to use glucose as source of energy for metabolism. In additional, the presence of glucose is necessary for blastocyst formation in vitro. Also, this strongly suggest that glucose may act at the cell surface or as an internal signal after transport rather than via its role as an energy substrate within the mouse embryo. A decrease in oxygen uptake was observed when glucose was added and decrease is likely to be related to increase in oxidative phosphorylation. Addition glucose, however, caused of increased release of lactate and produces ATP and thus decreases the need for ATP generation through oxidative pathway but, addition glucose to the culture medium did not affect pyrovate uptake (Donnay et al., 2002 & Gomez et al., 2000).

The rate of hatching was highest in the SOF A group and the difference between SOF A and SOF B groups was significant. Highest rate of hatching in the SOF A group could be due to presence of EDTA which to may cause of better embryo development. EDTA is present in the early part of culture in SOF A group but is absent in SOF B group.

5. Conclusion

Results shown that sequential culture SOF medium have beneficial effects on embryo development and SOF A with composition (basic SOF + EDTA followed with basic SOF+FCS) have had greatest blastocyst development and promoted a higher hatching rate.

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7. References

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