

HEPES buffer in ovary-transportation medium influences developmental competence of cattle oocytes

Sh. Bohlooli[#], Ş. Bozoğlu & F. Cedden

Department of Animal Science, Faculty of Agriculture, University of Ankara, Ankara, Turkey

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Abstract

This study was conducted to investigate the effects of ovary transportation in a semi-complex medium containing HEPES at different temperatures on the developmental competence and the quality of *in vitro*-produced embryos. The cattle ovaries were transported in normal saline (NS), phosphate buffer saline (PBS), K simplex optimization medium (KSOM), Chatot-Ziomek-Bavister medium (CZB) and Charles Rosenkrans medium (CR1) at various temperatures (38 °C, 25 °C and 4 °C). The developmental competence of retrieved cumulus oocyte complexes (COCs) was evaluated by maturation, fertilization, morula and blastocyst formation and numbers of inner cell mass (ICM) and trophectoderm (TE) cells. The COC maturation rate was affected by medium and temperature. It was found that 4 °C resulted in a higher maturation (81.0 ± 4.75) rate than other transportation temperatures. The CR1 (80.5 ± 6.66) and KSOM (80.2 ± 6.15) gave a better maturation rate than the others. Fertilization rate, which was evaluated by cleavage rate, was not affected by transportation temperature. However, the transporting medium had a significant effect on the fertilization rate. Moreover, CR1 (43.6 ± 4.60), KSOM (43.2 ± 4.86) and CZB (41.1 ± 4.86) media gave higher percentages of cleaved embryos. There was no significant difference in morula and blastocyst formation rate or in ICM and TE cell counts regarding transportation factors. In conclusion, the transport of ovaries in CR1 at 4 °C is effective for maintaining early developmental competence of cattle oocytes.

Keywords: Cattle embryo, *in vitro* embryo production, ovary transportation

[#] Corresponding author: [sbhlooli@ankara.edu.tr](mailto:sbohlooli@ankara.edu.tr)

Introduction:

The oocytes from slaughtered animal ovaries show weakened developmental competence compared with those collected from live animals by ovum pick-up (Baldassarre *et al.*, 2002; Neglia *et al.*, 2003; Manjunatha *et al.*, 2008). The differences in the quality of the oocytes may be associated with the stresses imposed during *in vitro* production (IVP), which begins from ovary removal. Practical problems often encountered are the effects of transportation, preservation medium and temperature (Yuge *et al.*, 2003; Pedersen *et al.*, 2004; Wongsrikeao *et al.*, 2005). The 0.9% saline solution and the PBS are used extensively as ovary, cumulus oocyte complexes (COCs), zygote and embryo transportation media in goats (Silva *et al.*, 2004; Rodriguez-Dorta *et al.*, 2007), sheep (Smith *et al.*, 1994; Wani *et al.*, 2000; Shi *et al.*, 2009), buffaloes (Amer *et al.*, 2008; Ubaidullah *et al.*, 2009), cows (Niemann, 1991; Thibodeaux *et al.*, 1992; Martino *et al.*, 1996; Schernthaner *et al.*, 1997; Yang, 2002), sows (Mattioli *et al.*, 1988; Westhof *et al.*, 1991; Whitaker & Knight, 2004) and other animals (Nureddin *et al.*, 1990; Jewgenow *et al.*, 1998). However, studies have demonstrated that glucose in the presence of phosphate is responsible for the delay or developmental arrest of cleavage stage embryos in cultures (Schini & Bavister, 1988; Chatot *et al.*, 1989; Thompson *et al.*, 1992). It was also shown that increasing phosphate in the medium did not improve embryo development. In contrast, complete elimination of phosphate improved developmental competence (Schini & Bavister, 1988). Nevertheless, it is not known whether phosphate in ovary-transportation media has a negative or positive effect on oocyte maturation and developmental competence. On the other hand, improper transporting and preserving conditions might cause failure in oocyte maturation and developmental competence. A change in the storage temperature and preservation solution during ovary transport has great effect on the developmental competence of oocytes following *in vitro* fertilization (IVF) or somatic cell nuclear transfer (SCNT) (Choi *et al.*, 2006; Kim *et al.*, 2006; Chaves *et al.*, 2008; Lopes *et al.*, 2009). Previous researchers showed that oocyte quality affects the IVP efficiency in most species. Nevertheless, the effects of ovary-transporting media on the developmental competence of the oocyte have not been investigated.

The effects of physiological saline and PBS use on oocyte quality and developmental competence for ovary-transportation medium at different temperatures (4 °C, 15 °C, 25 °C, 33 °C, and 35 °C) have been investigated extensively (Wongsrikeao *et al.*, 2005; Kim *et al.*, 2006; Wang *et al.*, 2011). However, there is little information about partially complex media use, such as KSOM and CZB, on the developmental competence of rescued COCs. In addition, commonly used ovary-transporting media, such as NS and PBS, lack suitable buffers. It is well known that glycolysis is the major anaerobic pathway for ATP production, which should result in increasing lactate levels and decreasing pH under hypoxic conditions. Hypoxic and acidic conditions have adverse effects on cell membrane permeability, net electric charge (Patel *et al.*, 1973) and chromosomal instability (Schomack & Gillies, 2003). Therefore, a lack of buffer systems in ovary-transporting media may be an important factor that influences the subsequent developmental competence of recovered COCs. From the available buffer systems for biological research, HEPES (N-(2-hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid) is one of the best all-purpose buffers. At most biological pHs the molecule is zwitterionic, and is effective as a buffer at pH 6.8 to 8.2. HEPES has been used in a wide variety of applications, including tissue culture, but it has not been studied in ovary transportation media. Thus, the aim of this work was to study the effects of semi-complex transporting media (including HEPES) at various temperatures on the post-transportation characteristics of COCs and pre-implantation embryos.

Material and Methods

Cow ovaries of unknown reproductive status were collected from a local slaughterhouse in Ankara, Turkey. The ovaries were transported to the laboratory within 2 - 3 h in five media, namely NS, PBS, KSOM, CZB and CR1 (Table 1) and three temperatures (4 °C, 25 °C, 38 °C). Then the ovaries were sliced (Das *et al.*, 1996; Bohlooli *et al.*, 2015) and rinsed by maturation medium at 38 °C in order to obtain COCs (Soto *et al.*, 2003). Classification of the recovered COCs was performed according to cumulus corona cell investment and morphology of the ooplasm, as described by Rahman *et al.* (2007). Healthy COCs or naked oocytes with finely granulated and homogeneous ooplasm (Grade A - D) were selected (Rahman *et al.*, 2007) for *in vitro* maturation (IVM).

Table 1 Composition of ovary-transporting media

Component (mM)	NS	PBS	KSOM	CZB	CR1
NaCl	150	130	125	100	135
KCl	-	2.70	2.50	4.86	10
Na ₂ HPO ₄	-	10	-	-	-
KH ₂ PO ₄	-	1.8	0.35	1.17	-
MgSO ₄	-	-	10.2	1.18	-
CaCl ₂ .2H ₂ O	-	-	1.71	1.71	-
Na Lactate	-	-	10	30.10	-
Na Pyruvate	-	-	0.20	0.26	-
HEPES	-	-	10	10	10
Glucose	-	-	0.20	-	-
L-glutamine	-	-	1	1	-
EDTA	-	-	0.01	0.1	-
Gentamicin (µg/mL)	50	50	50	50	50

NS: normal saline; PBS: phosphate buffered saline; KSOM: K simplex optimization medium; CZB: Chatot-Ziomek-Bavister medium; CR1: Charles Rosenkrans medium.

The selected COCs were then washed three times with maturation medium and cultured in groups of 20 under mineral oil in 100 µL TCM-199 medium supplemented with 0.4 mM sodium pyruvate, 1 mM L-glutamine, 100 µM cysteamine, 10% (v/v) foetal bovine serum, 20 µg FSH/mL, 2 µg oestradiol/mL and 50 µg gentamycin/mL. The COCs were incubated at 38.5 °C in a humidified atmosphere of 5% CO₂, 5% O₂ and 90% N₂. Matured oocytes were determined by cumulus expansion. The rates of oocytes exhibiting cumulus

expansion were determined at 24 h after IVM. The degree of cumulus expansion was assessed by the morphology of the cumulus oophorous, which is described by Kobayashi *et al.* (1994). Briefly, category C: no expansion was seen; category B: cumulus cells were non-homogeneously spread and clustered cells were still observed; category A: cumulus cells were homogeneously spread and clustered cells were no longer present.

After maturation, batches of 20 COCs were exposed to co-culture with sperm selected with the Percoll density gradient method with a concentration of approximately 1×10^6 sperm/mL in 200 μ L droplet of fertilization medium, and incubated at 38.5 °C with 5% CO₂, 5% O₂ and 90% N₂. The fertilization medium was Fert-SOF, supplemented with 100 IU penicillin/mL, 100 μ g streptomycin/mL, 2 mM caffeine and 10 μ g heparin/mL. After 18 h co-incubation, the presumptive zygotes were removed from the fertilization medium, denoted, washed three times in synthetic oviduct fluid-BE1 medium (SOF-BE1) and cultured in a humidified atmosphere of 5% CO₂, 5% O₂ until day 7 - 8 after IVF. Half of the embryo culture medium was refreshed every 48 h and 10% foetal bovine serum was added at day 2 of IVC. The cleavage rate was evaluated at day 3 post insemination. The morula and blastocyst rate was recorded at day 8 after IVF.

Differential staining of ICM and TE was done using a method described by Thouas *et al.* (2001) with minor modifications to calculate ICM and TE cells. Briefly, blastocysts were removed from the culture with as little medium as possible, and placed in the 30 μ g propidium iodide solution/mL phosphate buffer saline/polyvinylpyrrolidone (PBS/PVP) containing 1% Triton X-100 (v/v) for 30 seconds. After washing three times in PBS/PVP, the embryos were incubated in Hoechst solution, containing 10 μ g Hoechst 33342/mL in PBS/PVP, supplemented with 4% (w/v) paraformaldehyde for 30 min. After washing, the embryos were transferred onto microscope slides in a small volume of glycerol solution, and covered with the cover slip supported by four small droplets of vaseline/paraffin. The embryos were viewed as soon as possible by UV light under a Leica DMIL LED fluorescence microscope. After imaging, total stained nuclei were counted. In this dyeing method the ICM cells were appeared blue, while TE cells were red.

This study was performed in 3 x 5 factorial design (temperature at three levels and ovary-transporting medium at five levels). Data are expressed as mean \pm SD. The percentages of maturation, cleavage, morula and blastocyst rates, ICM and TE cell numbers from each treatment were used for statistical analysis. After testing normality and arc-sine transformation of proportional data, analysis of variance (ANOVA) was used to assess differences. The Duncan test was used to compare means ($P < 0.05$). The statistical analysis was performed by R program, and the Duncan test was performed with the Agricolae package program (Seefeld & Linder, 2007; de Mendiburu, 2014).

Results and Discussion

This study revealed that ovary-transporting temperature affects maturation rate of COCs ($P < 0.01$). Table 2 summarizes the effects of three transportation temperatures in five media on the maturation rate of bovine oocytes. The NS, CR1, KSOM media at 4 °C provided a higher percentage of category A matured oocytes compared with other treatments. The percentage of non-expanded cumulus complex was higher in PBS (20.7 ± 1.88) at 38 °C and lower in CZB (10.11 ± 2.58), CR1 (10.27 ± 2.74) and KSOM (10.87 ± 1.37) at 4 °C.

As illustrated in Figures 1 and 2, when factors were analysed independently, 4 °C (81.0 ± 6.80) was found better than other transporting temperatures ($P < 0.0008$). Also CR1 (80.5 ± 6.66) and KSOM (80.2 ± 6.15) together were better than other transporting media ($P < 0.0008$) in terms of category A matured oocytes. Regarding category B of expanding COC rate, it was not affected by the temperature ($P = 0.083$), but the transporting medium had a significant effect ($P = 0.024$). As shown in Table 2, the rate of non-matured oocytes of category C varied significantly with transporting temperature ($P < 0.0001$) and medium ($P = 0.0003$).

The IVF of oocytes was affected by transporting media ($P < 0.0001$), but not temperature ($P = 0.17$). The CR1 group at 4 °C (45.0 ± 1.67) and 38 °C (44.2 ± 8.04) provided a higher rate of fertilization compared with PBS (14.2 ± 6.29) at 25 °C (Table 2). Fertilization rate was not affected by transporting temperature ($P = 0.17$). However, transporting medium had a significant effect on the fertilization rate ($P < 0.001$). Figure 3 shows higher cleavage rates in CR1 (43.6 ± 4.60), KSOM (43.2 ± 5.26) and CZB (41.1 ± 4.86), while the lowest cleavage rate was observed in PBS (17.7 ± 6.75).

As shown in Table 3, the morula and blastocyst formation rates were not affected by the ovary-transportation medium or temperature ($P > 0.05$). Also, it can be presumed that the mean of ICM and TE cells was not influenced by ovary-transporting medium or temperature ($P > 0.05$).

This is the first study to compare simple and semi-complex media containing HEPES buffer. It has shown that semi-complex media resulted in higher maturation and cleavage rates (Table 2 and Figures 1 - 3). It was revealed that the ovary-transporting medium and temperature both play important roles in the

Table 2 Mean (\pm SD) maturation rate of cumulus oocyte complexes (COCs) and cleavage in various ovary-transporting media and at different temperatures

Temperature	Medium	N	Category (%)			Cleavage (%)
			A	B	C	
38 °C	NS	180	69.80 ^{bcd} \pm 5.36	9.68 ^{abc} \pm 5.13	20.19 ^{ab} \pm 1.06	31.67 ^{bc} \pm 5.20
	PBS	180	66.84 ^{cd} \pm 3.05	12.43 ^{ab} \pm 3.16	20.72 ^a \pm 1.88	15.83 ^d \pm 8.04
	KSOM	180	80.69 ^{ab} \pm 0.29	4.55 ^c \pm 2.68	14.76 ^{bcde} \pm 2.39	44.17 ^a \pm 6.29
	CZB	180	75.15 ^{abcd} \pm 1.31	7.85 ^{ab} \pm 0.36	17.00 ^{abcd} \pm 0.95	44.17 ^a \pm 3.82
	CR1	180	79.08 ^{ab} \pm 1.29	6.18 ^{ab} \pm 1.99	14.74 ^{bcde} \pm 0.70	44.17 ^a \pm 1.67
25 °C	NS	180	73.08 ^{abcd} \pm 4.14	8.10 ^{bc} \pm 1.52	18.82 ^{abc} \pm 4.33	24.17 ^{cd} \pm 8.78
	PBS	180	65.74 ^d \pm 15.81	15.46 ^a \pm 11.69	18.80 ^{abc} \pm 7.22	14.17 ^d \pm 6.29
	KSOM	180	77.99 ^{ab} \pm 3.83	6.62 ^{bc} \pm 1.54	15.40 ^{bcde} \pm 2.42	41.67 ^{ab} \pm 8.04
	CZB	180	72.78 ^{abcd} \pm 5.85	12.0 ^{abc} \pm 5.24	15.22 ^{bcde} \pm 0.77	38.33 ^{ab} \pm 7.22
	CR1	180	78.70 ^{ab} \pm 3.51	8.31 ^{abc} \pm 2.44	13.00 ^{de} \pm 2.41	41.67 ^{ab} \pm 8.04
4 °C	NS	180	80.95 ^a \pm 7.10	5.35 ^{ab} \pm 3.26	13.70 ^{cde} \pm 3.85	20.00 ^d \pm 3.33
	PBS	180	76.92 ^{abc} \pm 5.14	7.92 ^{bc} \pm 0.69	15.17 ^{bcde} \pm 4.58	23.06 ^{cd} \pm 3.37
	KSOM	180	81.83 ^a \pm 4.13	7.29 ^{ab} \pm 3.11	10.87 ^e \pm 1.37	43.89 ^a \pm 0.96
	CZB	180	81.96 ^a \pm 4.57	7.94 ^{ab} \pm 2.27	10.11 ^e \pm 2.58	40.83 ^{ab} \pm 1.44
	CR1	180	83.57 ^a \pm 2.48	5.49 ^{ab} \pm 0.59	10.27 ^e \pm 2.74	45.00 ^a \pm 1.67

^{a,b,c,d,e} Means in a column with different superscripts differ significantly at $P < 0.05$.

NS: normal saline; PBS: phosphate buffered saline; KSOM: K simplex optimization medium; CZB: Chatot, Ziomek, Bavister medium; CR1: Charles Rosenkrans medium.

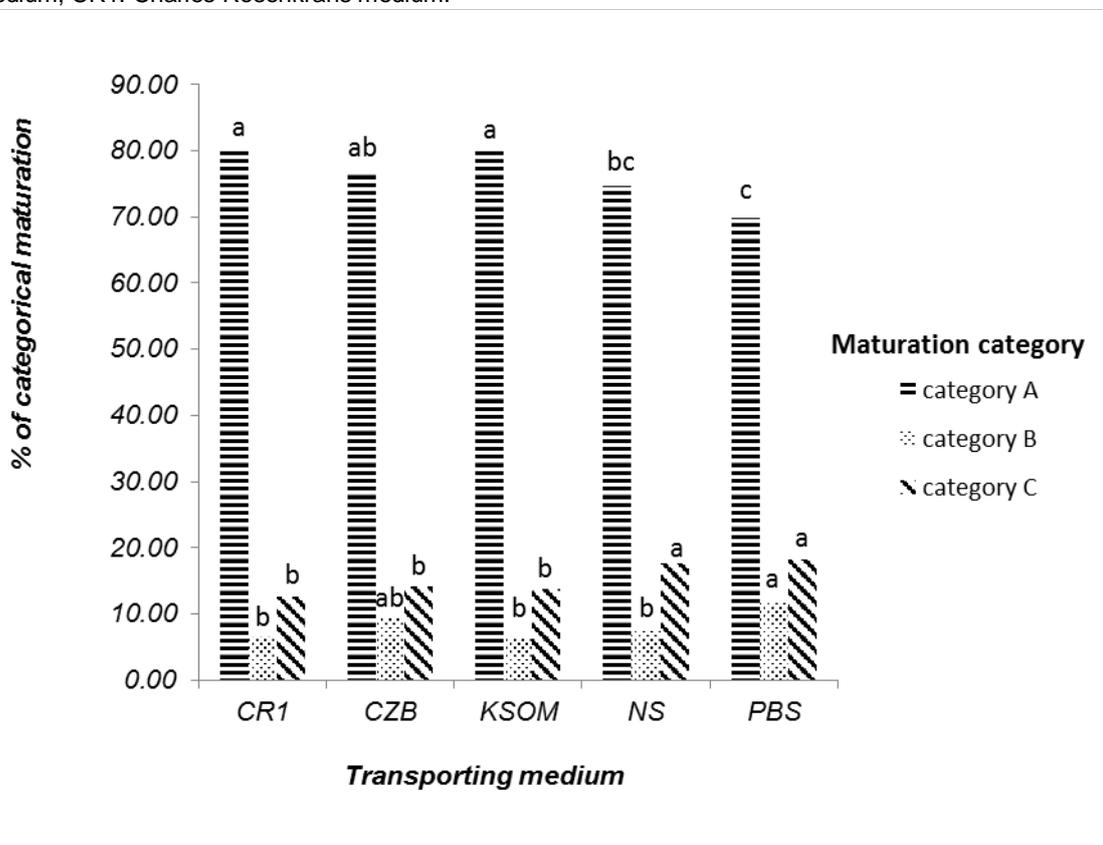


Figure 1 Mean matured (%) cumulus oocyte complexes (COCs) in various ovary-transporting media.

NS: normal saline; PBS: phosphate buffered saline; KSOM: K simplex optimization medium; CZB: Chatot, Ziomek, Bavister medium; CR1: Charles Rosenkrans medium.

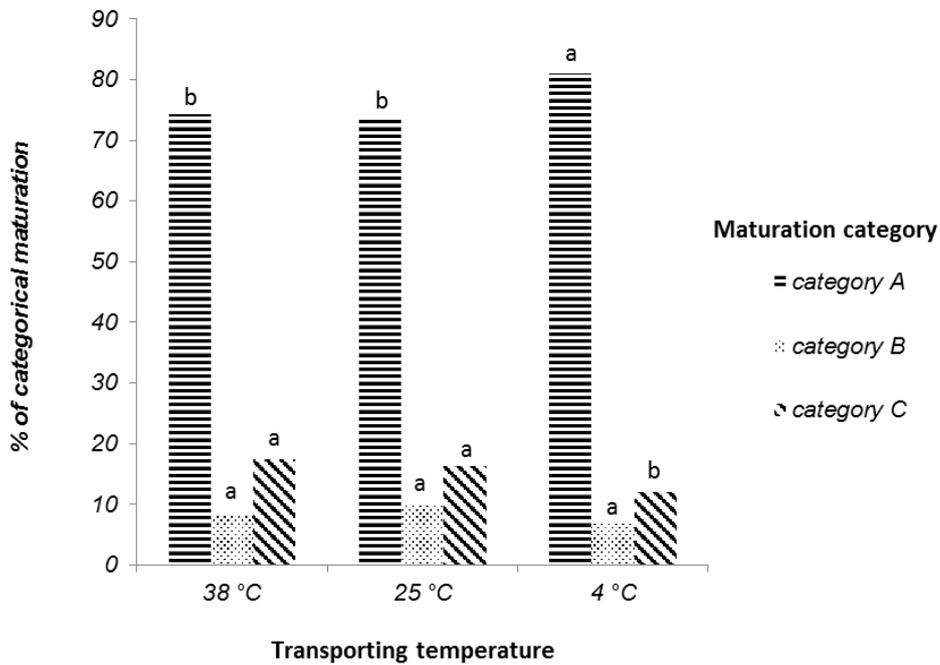


Figure 2 Mean matured (%) cumulus oocyte complexes (COCs) at various ovary-transporting temperatures.

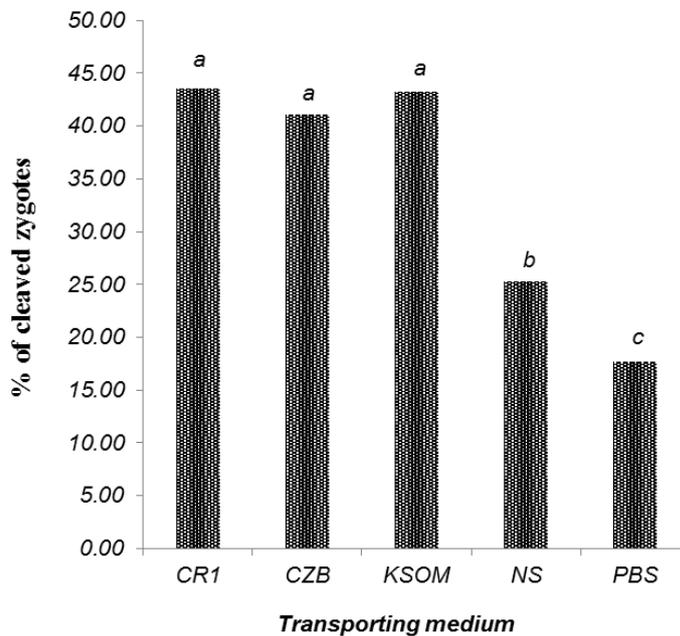


Figure 3 Mean cleavage rate (%) of *in vitro*-matured oocytes in different ovary-transporting media. NS: normal saline; PBS: phosphate buffered saline; KSOM: K simplex optimization medium; CZB: Chatot, Ziomek, Bavister medium; CR1: Charles Rosenkrans medium.

maturation rate of recovered COCs. Transporting media and temperature are both important in early developmental competence, but have no significant effects on morula and blastocyst formation. Neither ICM nor TE cell numbers were influenced by the test factors (Table 3). There were no interactions between ovary-transporting medium and temperature.

As illustrated in Figure 2, ovary transporting at 4 °C resulted in a high ratio of matured oocytes. This result is in accordance with those of Yuge *et al.* (2003), who reported that porcine ovaries being exposed to 25 °C or less before aspiration of oocytes might have an adverse effect on their subsequent IVM. But there is a consonance between Taş *et al.* (2006) and Evecen *et al.* (2010), who investigated the effect of ovary-transporting temperature at 37 °C and 4 °C on canine oocyte maturation, and found that 4 °C should provide more matured oocytes. A study conducted by Matsushita *et al.* (2004) revealed that transporting ovine ovaries at 10 °C had no effect on the developmental competence of recovered COCs. Wang *et al.* (2011) demonstrated that 15 °C was better than 30 °C and 35 °C in terms of reduction of the apoptosis index, but there were no significant differences in cleavage and blastocyst development. The high maturation rate that was achieved at 4 °C may be related to the low temperature, which should reduce cellular metabolism (Chaves *et al.*, 2008), minimize energy consumption, and slow down the tissue autolysis rate (Salehi *et al.*, 2004). In addition, the efficiency of enzymes and incidence of apoptosis decreases at low temperatures (Pedersen *et al.*, 2004). In term of cold injury, COCs may be protected from cold damage because of the intrafollicular environment (Matsushita *et al.*, 2004).

Table 3 Mean (\pm SD) morula and blastocyst (%) formation rate and ratio of inner cell mass to trophectoderm cells of various ovary-transporting media and temperatures

Temp.	Medium	Morula	Blastocyst	Total embryo	ICM	TE	ICM/TE
38 °C	NS	21.1 \pm 3.53	10.3 \pm 2.70	31.1 \pm 3.50	33.3 \pm 2.06	113.85 \pm 8.62	0.29 \pm 0.02
	PBS	25.0 \pm 5.00	10.0 \pm 10.00	35.0 \pm 8.66	31.5 \pm 2.12	113.0 \pm 4.24	0.28 \pm 0.03
	KSOM	22.4 \pm 2.51	13.2 \pm 1.95	35.6 \pm 3.85	33.4 \pm 3.21	111.6 \pm 2.15	0.30 \pm 0.03
	CZB	24.4 \pm 7.47	11.5 \pm 5.77	35.9 \pm 2.24	27.8 \pm 13.97	121.0 \pm 11.42	0.24 \pm 0.12
	CR1	28.2 \pm 4.16	7.6 \pm 3.59	35.8 \pm 0.89	35.0 \pm 2.83	110.8 \pm 9.32	0.32 \pm 0.05
25 °C	NS	23.3 \pm 6.66	14.0 \pm 3.54	37.3 \pm 3.49	31.0 \pm 2.16	120.0 \pm 4.32	0.26 \pm 0.01
	PBS	19.4 \pm 17.35	15.3 \pm 16.84	34.7 \pm 2.41	35.0 \pm 2.83	108.0 \pm 15.56	0.33 \pm 0.07
	KSOM	23.1 \pm 8.13	10.7 \pm 9.27	33.8 \pm 4.47	32.3 \pm 2.22	114.0 \pm 5.72	0.28 \pm 0.03
	CZB	26.0 \pm 3.06	13.4 \pm 6.53	39.4 \pm 3.55	33.7 \pm 3.98	114.7 \pm 12.08	0.30 \pm 0.06
	CR1	26.9 \pm 11.76	9.5 \pm 12.09	36.3 \pm 4.10	31.5 \pm 2.89	116.8 \pm 11.47	0.27 \pm 0.05
4 °C	NS	19.8 \pm 5.36	20.4 \pm 8.04	40.1 \pm 2.68	33.0 \pm 3.56	113.8 \pm 8.34	0.29 \pm 0.05
	PBS	19.0 \pm 12.45	19.4 \pm 17.35	38.4 \pm 5.62	32.4 \pm 4.28	121.0 \pm 8.00	0.27 \pm 0.05
	KSOM	25.8 \pm 11.99	12.8 \pm 11.75	38.6 \pm 0.25	31.80 \pm 2.28	115.4 \pm 5.18	0.28 \pm 0.03
	CZB	13.9 \pm 12.73	17.3 \pm 15.57	31.2 \pm 5.46	31.3 \pm 4.03	116.8 \pm 12.61	0.27 \pm 0.05
	CR1	26.7 \pm 4.77	10.9 \pm 10.72	37.5 \pm 6.16	32.2 \pm 4.09	115.4 \pm 12.56	0.28 \pm 0.06

NS: normal saline; PBS: phosphate buffered saline; KSOM: K simplex optimization medium; CZB: Chatot, Ziomek, Bavister medium; CR1: Charles Rosenkrans medium.

The results have shown that CR1 and KSOM provide superior maturation rates to the others (Figure 1). The effect of transporting medium on maturation rate was investigated, among others, by Lin *et al.* (2011), who focused on the outcomes of pig ovary transportation in PBS, BCS and NS for defining the maturation rate of recovered oocytes. It showed that PBS (72.6 \pm 4.0) and BCS (71.5 \pm 0.5) media resulted in a higher maturation rate than a saline (65.6 \pm 1.1) solution. It was suggested that this difference might be related to other components in PBS and BCS. As shown in Table 1, the components of CR1 are similar to NS, which was complemented by 10 mM HEPES, but KSOM was a relatively more complex media than others. Therefore, these differences may be derived from HEPES buffer. The BCS medium has a bicarbonate buffer (Silva *et al.*, 2000), which may be effective in a CO₂-rich environment. But in these studies the authors replaced bicarbonate with HEPES, which acts independently of environmental CO₂. Moreover, it contains pK_as (4 °C = 7.77, pK_a 25 °C = 7.48 and pK_a 37 °C = 7.31), which may provide a more suitable environment for these trial conditions and temperatures (Anonymous, 2008).

While it has been believed that storage close to body temperature causes cellular autolysis (Pedersen *et al.*, 2004), which decreases the maturation of bovine oocytes and adversely affects the developmental

competence of oocytes (Evecen *et al.*, 2010), the cleavage rate of matured oocytes was high in CR1, CZB and KSOM at 38 °C. These findings are in agreement with those reported by Love *et al.* (2003) and Yuge *et al.* (2003), but contrast those of Wongsrikeao *et al.* (2005) and Evecen *et al.* (2010). Some disparities might be caused by long storage time, which was studied by these investigators. This might be because of the HEPES buffering system in the media used in this study that maintained enzymes, hormones and pH at physiological range. Lack of suitable buffers resulted in pH decreases, which increased fragmented DNA nuclei significantly. It was suggested that the plasma membrane of oocytes was highly permeable to H⁺ ions without any regulatory mechanism (Wongsrikeao *et al.*, 2005). If external pH was maintained by increasing buffering capacity, the expulsion of protons would continue for a prolonged period, thus reducing the drop in intracellular pH buffers (Salehi *et al.*, 2004), which has biological importance in determining the net electric charge of amino acids, which affects cell membrane permeability (Patel *et al.*, 1973).

Table 3 shows that morula and blastocyst formation was not affected by transportation medium or temperature, which is in agreement with Wang *et al.* (2011), who reported that the blastocyst formation rate was not affected by the transporting temperature. However, in terms of total cell numbers, the results of this study was contradictory to these authors (Wang *et al.*, 2011), in which the total cell numbers in SCNT embryos were higher in day 7 blastocysts from ovaries stored at 15 °C than in those from ovaries stored at 35 °C or 25 °C. It seems that ovary-transporting conditions affect the early and late stages of developmental competence (Guignot *et al.*, 1999), but the quality of *in vitro*-produced embryos was not affected by transporting temperature and medium.

Conclusion

This study showed that ovary-transporting media and temperature significantly affected the maturation of bovine oocytes. The result revealed that 4 °C in the transportation temperature showed an optimal maturation rate. CR1 and KSOM media also resulted in a higher maturation rate. But the cleavage rate not affected by transportation temperature and only effected by transporting media which was higher in CR1, CZB and KSOM. Therefore, it was concluded that adding HEPES is beneficial to ovary transportation. The use of media such as CR1, CZB and KSOM that contain HEPES at a temperature of 4 °C could be advised in ovary transportation in order to maintain subsequent IVM and cleavage rate.

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