ACADEMIC RESEARCHES IN AGRICULTURE, FORESTRY AND AQUACULTURE SCIENCES

Editor

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OVARIAN TRANSPORT MEDIUM AND TEMPERATURE

Assist Prof. Şeyma AYDEMİR Ph.D.

The knowledge and use of reproductive technologies have been increasing worldwide all along the second part of the 19th century in most domestic mammalian species. First generation technologies (estrous synchronization, semen collection, semen freezing and artificial insemination) are now commonly used in domestic species breeding.

The second generation of reproductive techniques have then been introduced and their use have been promisingly increasing until early nineties. This generation included embryo based techniques, i.e. female superovulation, embryo recovery, freezing and transfer to recipients. At that time, as breeding industry was facing an increasing competition context worldwide, several health crisis, i.e. Bovine Spongiform Encephalopathy (BSE) and foot and mouth disease, negatively impacted the breeding economy and reduced the expansion of assisted reproductive techniques in European countries (Mermillod et al., 2006).

Due to the proliferation of the world population, the use of new technologies in animal science has become mandatory to meet the increasing demands on animal products. The participation of new sciences, inventions and technologies that can be practically used in genetics, breeding and breeding of all livestock is vital to the progress of animal science (Aral et al., 1981).

Although each ovary contains hundreds of thousands of oocytes at birth, most are lost through atresia. This process starts even before birth. This tremendous loss of genetic material could be reduced by harvesting oocytes from the ovary and using in vitro-produced (IVP) techniques. Bovine IVP is now a well-established and reasonably efficient procedure. Moreover, ovum pick-up (OPU) at frequent intervals, in combination with in vitro fertilisation, has proved its worth in improving or increasing the yield of embryos from designated donors. In addition, IVP can be used to salvage irreplaceable genetic material following slaugh-

ter for infectious disease control or culling for other reasons. In vitro fertilisation has also been used to produce the thousands of embryos needed for scientific research, including efforts to produce embryonic stem cells (Mapletoft, 2005).

Compared with conventional superovulation and Embryo Transfer (ET), production of embryos in the laboratory has several advantages. First, In vitro Embryo Production (IVEP) can be used on problem bovines such as females that fail to respond to superovulation treatment. Second, IVEP can be used to salvage the genetic potential of terminally ill females that would not be expected to respond to conventional ET. Third, semen from different bulls can be used to fertilize oocytes harvested from a cow resulting in embryos with different sires being produced at the same time. Fourth, oocytes for IVEP can be obtained from the ovaries of live donor using Transvaginal Oocyte Recovery (TVOR), or from the slaughter ovaries (Suthar, 2009).

Basically, IVP includes three major steps: . in vitro maturation (IVM), in vitro fertilization (IVF and in vitro development (IVD) of the resulting embryos. However, primary oocytes collection should be added upstream of these major steps and embryo management (freezing, transfer) should be added downstream to give a complete overview of the whole process. Since the birth of the first IVP calf in 1982, thanks to intensive research programs worldwide, cattle IVP has done significant progress. However, some residual shortcomings are still limiting the larger commercial use of this promising technique (Mermillod et al., 2006).

After artificial insemination and multiple ovulation and embryo transfer (MOET), in vitro production of embryos (IVP) represents the third generation of techniques aimed at a better control of animal reproduction. This technique involves four major steps.

1) Oocyte collection
2) In Vitro Maturation (IVM)

3) In Vitro Fertilization (IVF)
4) In Vitro Development (IVC)

These different steps are now well established in domestic ruminant species (cattle, sheep and goat) although the variability of the number and quality of the oocytes collected and the low viability of frozen – thawed in vitro produced embryos still limit the large-scale use of this promising technology (Mapletoft, 2005; Mermillod et al., 2006).

The lack, defect or minor change in each step of these methods has a significant effect on the quantity and quality of embryos obtained. Therefore, studies to improve IVP should consider all methods included in this method.

One of the objectives of the study is to determine the new media and temperatures for the ovarian transport medium.

IN VITRO EMBRYO PRODUCTION

Ovaryum Collection

Method

Cattle ovaries were collected from the slaughterhouse to determine the effect of the oocyte transport medium and temperature on the quality and quantity of oocytes and embryos produced from the ovaries. Immediately after cutting, the ovaries are washed with saline and cleaned from blood and wastes and placed in containers containing 500 ml of transport medium (Table 1). Ovaries were transferred to the research laboratory with thermos at three different temperatures (4ºC, 23 ºC, 35 ºC). According to the results of this stage, the most common maturation formation and the CR1 ovary transport medium of transport temperature of 4 ° C were found.

SC: Stock Code

DPBS: Dulbecco’s Phosphate-Buffered Saline
CR1: Culture Media
KSOM: Kalium (K+) Simplex Optimized Medium
CZB: Chatot, Ziomek, and Bavister

Table 1. Content of the media used in washing and transporting the ovaries (mM)

	SC	Saline	DPBS	CR1	KSOM	CZB
NaCl	S5886	150	130	135	125	100
KCl	P5405	-	2.70	10	2.50	4.86
Na_2HPO_4	S5136	-	10	-	-	-
KH_2PO_4	P5655	-	1.80	-	0.35	1.17
MgCl	63068	-	-	-	0.20	1.18
CaCl2	C7902	-	-	-	1.71	1.71
Na Lactate	L4263	-	-	-	10	30.10
Na Pyruvate	P4562	-	-	-	0.2	-
HEPES	H6147	-	-	10	10	100

* 100 iu / mL Penicillin G sodium (P3032) and 100 µg / mL Streptomycin (S1277) were added to the media.

For the set up of the techniques as well as for the large scale production of average genetic merit embryos, large quantities of material can be obtained at low cost by collecting ovaries at slaughterhouses. Ovaries are collected within 10 minutes after the slaughter of the animals, and kept in a sealed container containing physiological saline (0.9% NaCl with 100 µm/ml Streptomycin and 100 IU/ml Penicillin), at a temperature ranging from 28-37°C. These ovaries are shipped to the laboratory (dry or in warmed saline) where the content of the follicles is aspirated. Cattle ovaries can be stored up to 8 hours at 25°C without significant loss of development competence. However, storage at 4°C or 37°C during 4 hours results in decreased oocyte viability.

Cattle, goats, sheep, and buffaloes transported widely in ovarian 0.9% saline and PBS are used (Yang and Rajamahendran, 2002; Rodriguez-Dorta et al., 2007; Amer, 2008, Laurinčík 2012).

A low temperature (15°C) during ovary transport can alleviate the adverse stresses imposed on oocytes and improve the in vitro developmental competence of oocytes after Somatic Cell Nuclear Transfer (SCNT)(Wang et al., 2011). However, it was found that 25-35°C was more suitable when the ovarian transfer was carried out at 4, 15, 25 and 35 °C in the saline environment (Wongsrikeao et al. 2005).

Table 2. The effect of different ovarian transport temperatures and environments on the maturation of COCs

Temperature	Medium	N A	Category (%±SD)		
			B	C	
35 °C PBS KSOM CZB CR1	NS	180	69.80^{bcd}±5.36	9.60±5.13	20.19^{ab}±1.06
	180	66.84^{cd}±3.05	12.43±3.16	20.72^{a}±1.88	
	180	80.69^{ab}±0.29	4.55±2.68	14.76 ^{bcde}±2.39	
	180	75.15^{abcd}±1.31	7.85±0.36	17.00^{abcd}±0.95	
	180	79.08^{ab}±1.29	6.18±1.99	14.74^{bcde}±0.70	
23 °C PBS KSOM CZB CR1	**NS**	180	73.08^{abcd}±4.14	8.10±1.52	18.82^{abc}±4.33
	180	65.74^{d}±15.81	15.46±11.69	18.80^{abc}±7.22	
	180	77.99^{ab}±3.83	6.62±1.54	15.40^{bcde}±2.42	
	180	72.78^{abcd}±5.85	12.0±5.24	15.22^{bcde}±0.77	
	180	78.70^{ab}±3.51	8.31±2.44	13.00^{de}±2.41	
4 °C PBS KSOM CZB CR1	**NS**	180	80.95^{a}±7.10	5.35±3.26	13.70^{cde}±3.85
	180	76.92^{abc}±5.14	7.92±0.69	15.17^{bcde}±4.58	
	180	81.83^{a}±4.13	7.29±3.11	10.87^{e}±1.37	
	180	81.96^{a}±4.57	7.94±2.27	10.11^{e}±2.58	
	180	83.57^{a}±2.48	5.49±0.59	10.27^{e}±2.74	

As it can be seen in Table 2, statistically (P <0.01) differences were found among the research treatments. According to the results obtained, it is determined that the category A ripening is the most in the ovaries carried in CR1 medium at 4 ° C, while it is found in the least amount in the ovaries carried in PBS medium at 23 ° C. In terms of maturing COCs with category B, it was found to be the highest in PBS treatment at 23 ° C and lowest in the ovaries transported in the KSOM environment at 35 ° C, although there were no statistically significant differences between treatments (p = 0.1384). The number of mature COCs in category C was statistically determined (p <0.01) in PBS medium with 35 ° C and lowest in transport ovaries and those with 4 ° C CZB. The interactions between the transport medium and temperature factors were not statistically significant (p> 0.05).

When the factors are analyzed separately, the transport temperature of 4 °C includes A-category COCs (81.05 ± 4.75) more than other temperature levels, while the temperature level of 23 ° C had the lowest rate (73.66 ± 8.37). As can be seen in Table 2, the number of COCs in the B and C category maturation class is highest at 23 ° C (10.10 ± 6.0, 16.25 ± 4.16) and 35 ° C (8.14 ± 3.85, 17.48 ± 2.96) respectively, while 4 ° C (6.79 ± 2.27, 12.02 ± 3.44) temperature was found to be the least.

As seen in Table 2, CR1 (80.45 ± 3.24) transport medium caused mature COCs with category A more than the other medium (p <0.01). PBS medium caused more B category maturation (11.94 ± 6.90) compared to other media, while at least B class maturation appeared in KSOM (6.15 ± 2.52) environment (p <0.05). In terms of C category ripening feature, the ovarian transport environment caused statistically significant differences (p <0.05). While C class ripening was mostly seen in PBS medium (18.23 ± 5.01), the least amount was determined in CR1 medium (12.67 ± 2.69).

The research was carried out in 3 repetitions as 3x5 factorial experiments (temperature factor at 3 levels and ovarian transport environment factor at 5 levels).

These experiments showed that duration of holding time and temperature affect presenceand degree of apoptosis found in equine granulosa cells. Fewer follicles contained apoptotic granulosa cells when the ovaries were held at 20 and 30°C as compared to 35–37°C. Holding temperatures lower than 35–37°C may have delayed the apoptotic process, since enzymes present in warm-blooded animals work most efficiently at body temperature or higher(Pedersen et al., 2004).

Mare ovarian parts were kept in PBS and MEM solutions for 4, 20, or 39 ° C and 4, 12 and 24 hours. Regarding the protocols tested, equine preantral follicles from ovaries during the non-breeding seasonwere best preserved in PBS at 4 °C for 4 h. The lowtemperature contributed to the maintenance of follicularmorphology. In that regard, it was suggested that preservation at 4 °C can reduce the metabolic rate, decreasenutrient and oxygen requirements and thus increasefollicular resistance (Gomes et al., 2012).

According to Garcia-Alvarez et al. (2011) the Iber red deer ovaries, which were transported in saline at 5-8 ºC, caused more cleavage rates than those carried at 20-25 ºC. However, according to Di Francesco (2007), in low-temperature saline medium, ovarian transport improved both the rate of cleavage and the rate of blastocyst.

DISCUSSION AND CONCLUSIONS

IVP is a very promising technique that could find numerous applications in assisted reproduction of ruminant domestic and wild species. It also offers unique occasion to study early mechanisms controlling reproductive physiology and open the way

to advanced biotechnology such as nuclear transfer cloning and transgenesis. The low quality of oocytes recovered from small growing follicles as well as the low resistance of IVP embryos to conventional freezing methods still limit the use of IVP in breeding schemes (Mermillod et al., 2006).

In conclusion, a low temperature (4 ◦C) during ovary transport can alleviate the adverse stresses imposed on oocytes and improve the in vitro developmental competence of oocytes after SCNT. Future research will focus on: (1) studying the effect mechanism of storage temperature on oocytes, for example, to examine the metabolic of oocytes in ovaries stored at different temperatures; and (2) evaluating the long-term effects of storage temperature on the in vivo developmental competence of oocytes after SCNT, and the health status of cloned offspring

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