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Use of Open Pulled Straw as a Carrier in Vitrification of Metaphase II Oocyte in Mice

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Abstract: This study taking to account the limitations of studies in this area, aimed to compare the survival rate, fertilization capacity and developmental capacity of oocyte that cryo-preserved in open pulled and conventional straw. Total 90 females NMRI mice were used in this research. First the male mice were scarified then the caudal part of epididymis was isolated. By creating cuts in caudal part of epididymis, sperms were isolated and put in T6 medium + 5 mg mL^{-1} BSA. Later on the sperms were incubated under 37°C and 5% CO_2 for 2 h. Then sperm were added to living control, cryo-preserved in conventional and open pulled straws oocytes. After 4-6 h changing medium, two nuclear and two-cell embryos were evaluated 8 and 24 h after addition of the sperms, respectively. Comparing the two groups shows that the vitrified oocyte of open pulled straw method had better and significant different survival rate than conventional method ($p = 0.001$). Furthermore, between open pulled group and conventional group also there was significant different in term of fertilization rate ($p = 0.001$). The developmental capacity of open pulled straw group was significantly higher that conventional straw group ($p = 0.002$). Oocyte cryo-preservation, if consistent and successful, offers a way to avoid the complications of routine IVF therapy. Oocytes may need to be cryo-stored in the event of unforeseen non-production of sperm during IVF therapy, allowing a more measured consideration of donor sperm use or other means of sperm retrieval.

Key words: Oocyte cryo-preservation, survival rate, fertilization capacity, developmental capacity

INTRODUCTION

During recent decades, many efforts had done to solve the infertility problem and the spectrum of those efforts has been very varied. Some of these efforts include, finding methods appropriate for long-term maintenance of the gamete and embryo as well as cryo-preservation as the best way to keep embryonic cells and embryos has been introduced (Borini and Coticchio, 2009; Varghese *et al*., 2009; Saki and Dezfuly, 2005). However, it should not forget the fact that despite the freezing of sperm and embryos has been very successful, oocyte freezing success rate under certain conditions of the cell and

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its excessive sensitivity to thermal shock and cooling is very low (De Santis *et al*., 2007; Coticchio *et al*., 2007). Few reports are available in relation to the successful cryo-preservation of human oocytes (Borini and Coticchio, 2009; Tucker *et al*., 2004). Low success rate of freezing oocytes can correlate to the lack of information and awareness of oocyte damages (Borini and Coticchio, 2009). Researchers use different freezing methods, including slow method, vitrification and Ultra-rapid to maintain the oocytes (Fadini *et al*., 2009; Dessolle *et al*., 2009). Today by using simple methods such as vitrification and recently the results of this method to specify that it is a better method in comparison to previous methods (Cao *et al*., 2008; Sher *et al*., 2008). The researchers believe that several factors affect the oocyte cryo-preservation include, the method of freezing, concentration and type of the cryo-protectant used, dehydration period before freezing, the number of washing steps and oocyte carrier type (Loutradi *et al*., 2008). Vitrification method commonly used conventional straw freezing, which seems to have many problems. One of those problems is the freezing solution volume that the oocyte is immersed into and the other is too thick wall straw that this thickness can affect the heat exchange while oocyte freezing and thawing and the results can affect ice crystals formed. For solving some of these problems, researchers have tried to make a pulled straw by heating and pulling the straws. Cryo-preservation with open pulled straw, first time was introduced by Vajta and colleagues (Vajta *et al*., 1998). The researchers announced that the developmental capacity and fertility potential of oocyte cryo-preserved in open pulled straw compared with conventional straw is significantly higher (Vajta *et al*., 1997). In contrast, some other studies declared that the survival rate of frozen oocyte in open pulled straw is less (Chen *et al*., 2001). Furthermore, the researchers believe that the direct contact of cryo-protectant solution with liquid nitrogen has a negative effect on cryo-preserved oocyte.

This study taking to account the limitations of studies in this area, aimed to compare the survival rate, fertilization capacity and developmental capacity of oocyte that cryo-preserved in open pulled and conventional straw.

MATERIALS AND METHODS

Preparation of the Mouse Oocyte

This experimental study was performed in physiology research center of Ahwaz Jondishapour University of Medical Sciences (AJUMS) from March 2008 to August 2009. Total 90 females NMRI mice were used in this research. The animals were purchased from Laboratory Animals Care and Breeding Center of Ahwaz Jondishapour University of Medical Sciences, Ahwaz, Iran. For super ovulation 10 IU of HMG (Human Menopausal Gonadotropine) and after 48 h, 10 IU HCG (Human Chorionic Gonadotropine) was injected intraperitoneally. Fourteen hours after the last injection the mice scarified and then distal end of uterine tubes extracted and put in drops of T6 medium containing 5 mg mL^{-1} Bovine Serum Albumin (BSA). Then oocytes were collected after dissection of the fallopian tube using an insulin syringe and were transferred to drops of a medium. After using of Hyaluronidase 0.1%, cumulus mass around the egg was isolated. Then naked oocytes were evaluated by the inverted microscope. Oocyte metaphase II stages with first polar bodies were separated and then randomly divided into three groups randomly. All procedures were approved by international guidelines and by the Institute Research Ethics and Animal Care and Use Committee of Ahwaz Jondishapour University of Medical Sciences. Every effort was made to minimize the number of animals used and their suffering.

Preparation of Open Pulled Straw

Pulled straws were manufactured as described by Vajta *et al*. (1998) and Saki *et al*. (2009). Plugs of 0.25 mL plastic straws (IMV, L'Aigle, France) were removed, then the straws were heat-softened of the midpoint over a hot plate and pulled manually. The pulled straws were cooled in air and then cut at the tapered end with a razor blade. The inner diameter of the tip was ~0.8 mm, with a wall thickness of about 0.07 mm. The thin part of each open pulled straw was approximately 2.5 cm long.

Vitrification Method

For cryo-preservation method Kassai (Edwards and Steptoe, 1977) was used. First the oocytes inserted in one drop of PBI solution by using an oral pipette. Then the oocytes transferred to another dish containing drops of vitrification solution (EFS, containing 20% ethylene glycol, 18% Ficoll and 0.3-M sucrose) and finally were transferred to EFS 40%. Oocyte maximum 2 min exposed to cyto-protectant.

Loading Procedure

Loading a conventional straw includes the use of an insulin syringe that attached to a connector. Thus, the first 1 cm^3 cryo-protectant solution were loaded in straw, then 0.5 cm^3 air, 2 cm^3 cryo-protectant solution containing oocyte, 5 cm^3 of air and finally 3.5 cm^3 cryo-protectant solution were filled. Then blocked with hematocrit pulp and were immersed in liquid nitrogen. For loading an open pulled straw we used the method described former by Vajta *et al*. (1998). In short the loading is done by using Capillary effect and contacts the tip of open pulled straw to cryo-protectant solution containing oocyte.

Thawing Method

In the conventional straw method the straw contains oocytes was removed from the liquid nitrogen. Then the straw was melted for 5 min in room temperature and immersed in 25°C water for 25 sec. The oocytes extracted from the straw and inserted in the sucrose solution for 2 min for rehydration. The rehydrated oocytes were washed in PBI solution. Finally the washed oocytes were transferred to T6 medium +5 mg mL^{-1} BSA. The open pulled straw's thawing method is done as described former by Vajta *et al*. (1998).

***In vitro* Fertilization (IVF) Method**

First the male mice were scarified then the caudal part of epididymis was isolated. By creating cuts in caudal part of epididymis, sperms were isolated and put in T6 medium + 5 mg mL^{-1} BSA. Later on the sperms were incubated under 37°C and 5% CO_2 for 2 h. Then sperm were added to living control, cryo-preserved in conventional and open pulled straws oocytes. After 4-6 h changing medium, two nuclear and two-cell embryos were evaluated 8 and 24 h after addition of the sperms, respectively.

Statistical Analysis

From morphological point of view the oocyte survival rates in each group was calculated. The fertilization rate and developmental capacity for the all three groups were calculated. Analysis results using Chi-square test was performed. The p value <0.05 was considered significant.

RESULTS

The findings in Table 1, post thawing survival rate of the cryo-preserved oocytes in conventional straw was 73.7% and in open pulled straw was 84.8%, respectively. Comparing the two groups shows that the vitrified oocyte of open pulled straw method had better and significant different survival rate than conventional method (p = 0.001).

Two pronuclear developments from the conventional group have a value of 28.6% in comparison to control and open pulled the group were 57.8 and 48.1%, respectively (Table 2). This finding indicates that the higher significant difference between the open pulled group and control comparing to conventional group was evaluated (p = 0.021, open pulled group; p = 0.042, conventional group) (Fig. 1). Furthermore, between open pulled group and conventional group also there was significant different in term of fertilization rate (p = 0.001) (Fig. 1). Considering Table 3, 24.3% of fertilized cryo-preserved oocytes in the conventional straw were developed two-cell embryos stage, while the rate in open pulled straw was 43.8%. This finding indicates that the higher significant difference between the

Table 1: Comparison of oocyte survival rate between conventional straw and open pulled straw methods

Study groups	Total oocytes	Survival rate
Conventional straw group	309 (100)	227 (73.7)
Open pulled straw group	333 (100)	282 (84.8)
p value	----------	0.001

Values in bracket are percentages

Table 2: Comparison of oocyte fertilization rate between conventional straw and open pulled straw methods

Study groups	Total oocytes	Fertilization rate
Control	327 (100)	189 (57.80)
Conventional straw group	309 (100)	88 (28.48)
Open pulled straw group	333(100)	160 (48.05)

Values in bracket are percentages

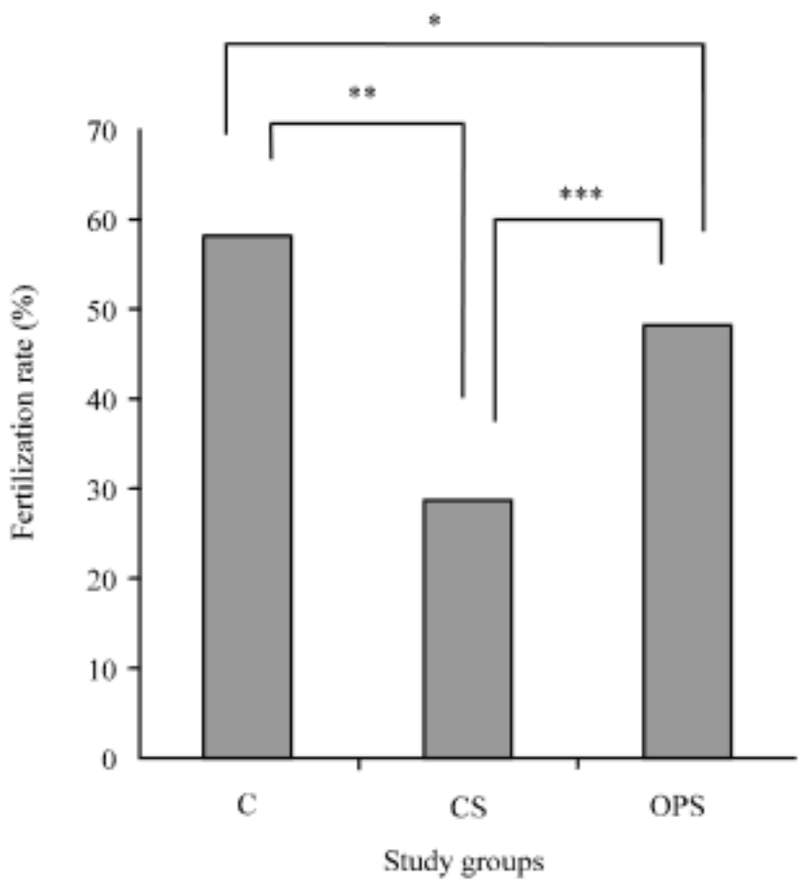

Fig. 1: Between groups comparison of developmental capacity. *p = 0.035; **p = 0.050; ***p = 0.002. C: Control, CS: Conventional straw, OPS: Open pulled straw

Table 3: Comparison of oocyte developmental capacity between conventional straw and open pulled straw methods

Study groups	Total oocytes	Developmental capacity
Control	327 (100)	172 (52.60)
Conventional straw group	309 (100)	79 (25.56)
Open pulled straw group	333 (100)	145 (3.54)

Values in bracket are percentages

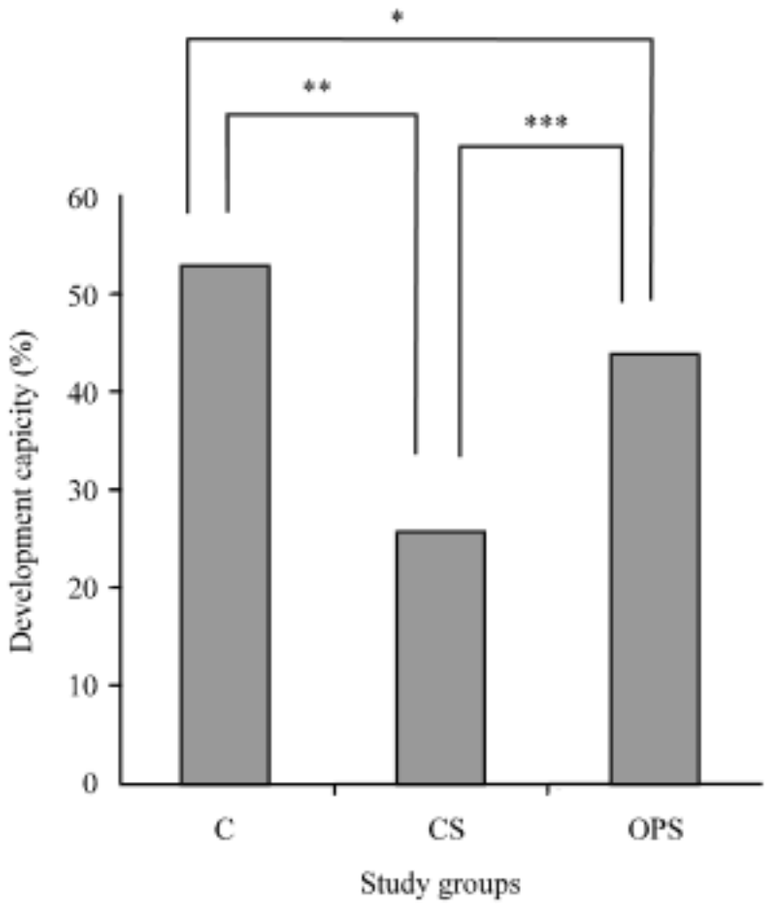

Fig. 2: Between groups comparison of oocyte fertilization rate. *p = 0.021; **p = 0.042; ***p = 0.0001. C: Control, CS: Conventional straw, OPS: Open pulled straw

open pulled group and control comparing to conventional group was evaluated (p = 0.035, open pulled group; p = 0.050, conventional group) (Fig. 2). This mean that developmental capacity of open pulled straw group was significantly higher that conventional straw group (p = 0.002) (Fig. 2).

DISCUSSION

Earlier Study Studies have shown that ethylene glycol due to the high permeability and a small amount of toxicity has low negative effects on fertilization and developmental capacity of embryos obtained from cryo-preserved (Cao *et al*., 2008; Sher *et al*., 2008). Either was used individually (Dela Peña *et al*., 2001) or with other materials such as sucrose and Ficoll (Mullen *et al*., 2007). Sucrose also acts as an important factor in reducing the toxic effects of the ethylene glycol (Ali and Shelton, 1993). Ficoll acts as a stabilizer of glassy property of vitrification solution and a protective coating around the oocyte and embryos (Fadini *et al*., 2009). The reasons described in these studies motivate us to use EFS solution medium.

This study was identified the survival rates, fertilization rate and developmental capacity of fertilized oocyte in the open pulled straw is more than the case of conventional straw. Present study is confirmed by the result of a study that has been done by Vajta *et al*. (1997).

Some researchers have believed that within the use of open pulled straw; the thin wall thickness is caused the heat exchange more rapidly between the solution and liquid nitrogen during freezing. This cause the period of the oocyte shorter is exposed to the freezing solution that in this case is harmful to the oocyte (Arav *et al*., 2002). Because of the inside diameter of open pulled straw is reduced, the volume of freezing solution reaches a minimum asset value. Therefore, the freezing rate in open pulled straw compared with the conventional type is increased.

Accordingly cryo-preserved oocyte in open pulled straw seems to be compared with the conventional method were crossed more quickly the temperature from +15 to -15°C. The oocyte is very sensitive to this temperature and microtubules get hurt in it and passed fleeting possibility of survival at this stage, which leads to reduce the fertilization rate, survival rate and development capacity of oocyte after thawing. This is in agreement with the present study in case of fertilization rate, survival rate and development capacity of oocyte. Controversy, in a study conducted by Chen *et al*. (2001) the oocyte cryo-preservation in open pulled straw after thawing showed fewer survival chances in comparison to the conventional method.

CONCLUSION

Perhaps open pulled straw due to reduced the wall thickness, solution volume used in freezing and the time to fill a straw may provide modern and more advanced technique for oocyte cryo-preservation. Oocyte cryo-preservation, if consistent and successful, offers a way to avoid the complications of routine IVF therapy. Oocytes may need to be cryo-stored in the event of unforeseen non-production of sperm during IVF therapy, allowing a more measured consideration of donor sperm use or other means of sperm retrieval.

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