Egyptian J. Anim. Prod. 48, Suppl. Issue, April (2011):27-35

IN VITRO SURVIVAL OF OPEN-PULLED-STRAW (OPS)-VITRIFIED GOAT BLASTOCYSTS AFTER ONE-STEP DILUTION IN SUCROSE-FREE MEDIUM OR AFTER REPEATED VITRIFICATION

M. El-Gayar

Department of Animal Production, Faculty of Agriculture, Suez Canal University, Ismailia, Egypt. (E-mail: melgayar@yahoo.com).

SUMMARY

In mice, warming of OPS-vitrified embryos in medium devoid of sucrose and repeated vitrification are feasible (El-Gayar et al., 2008 and 2010). If these would apply to other mammalian species, in particular farm animals, these findings will facilitate the applicability of the OPS-vitrification technique in the field. In order to test the suitability of these findings for the OPS-vitrified goat embryos, two separate studies were conducted. In the first study, of 50 OPS-vitrified goat blastocysts, 25 embryos were exposed to the standard warming and dilution regimen involving exposure to 3 solutions of decreasing sucrose content (Control). The remaining 25 embryos were warmed, in a single step, in sucrose-free medium. Embryos were then cultured in vitro. In the second study, of 50 OPS-vitrified goat blastocysts, 25 embryos were exposed to the standard warming and dilution regimen (Control) and cultured in vitro. The remaining 25 embryos were subjected to repeated vitrification. These embryos too were re-warmed and cultured. Survival of embryos was defined as their ability to continue development to the hatched blastocyst stage. After 48 h in culture, OPS-vitrified embryos warmed in sucrose-free dilution medium showed a slightly higher survival rate (84%) than embryos exposed to the standard warming and dilution regimen (80%). After repeated vitrification, hatching rate was reduced (60%). Therefore, it may be concluded that repeated vitrification decreased the viability of goat blastocysts, whereas warming in a dilution medium devoid of sucrose in a single step, a warming mode that will permit direct transfer of OPS-vitrified embryos to recipients, did not.

Keywords: Goat embryos, Vitrification, Sucrose, Repeated vitrification, Open pulled straw

INTRODUCTION

There have been two approaches to the cryopreservation of embryos. The first was slow freezing. The other approach is vitrification, which was first applied in embryology by Rall and Fahy (1985). Vitrification implies freezing without the formation of ice crystals, which are known to cause of cell injury (Berthelot *et al.*, 2000; and Vajta, 2000). An additional advantage of vitrification over conventional slow freezing methods is that no controlled-rate cooling apparatus is required.

Since the implementation of a one step-transfer of conventionally cryopreserved bovine embryos exposed to a sucrose solution (Leibo, 1984), has been successfully applied to warm vitrified embryos in the bovine (Saha *et al.*, 1996), ovine (Baril *et al.*, 2001; Isachenko *et al.*, 2003a), caprine (Guignot *et al.*, 2006), porcine (Cuello *et al.*, 2004) and murine (Kasai *et al.*, 2002; and El-Gayar *et al.*, 2008), the technique is ideally suited for use in the field because no microscope or other laboratory equipment are required, permitting direct transfer of vitrified embryos. Institutions and breeding organizations often have in store cryopreserved embryos collected over time. If it were possible to assess their state of intactness or karyotype and genotype them and refreeze them for later use, it would be of considerable practical advantage (Nowshari and Brem, 2000). In humans, having the option to re-freeze embryos gives the patient additional opportunity for an un-stimulated frozen embryo transfer if more embryos cryopreserved at the pronucleate stage survive the thawing procedure than are required at transfer (Sheehan *et al.*, 2006). Refreezing of embryos has been successfully accomplished with conventional slow freezing in the murine (Leibo *et al.*, 1991; and Vitale *et al.*, 1997), bovine (Vitale *et al.*, 1994) and in man (Farhat *et al.*, 2001; Yokota *et al.*, 2001; and Smith *et al.*, 2005). Repeated vitrification of embryos has been accomplished with appreciable success in human morulae (Kumasako *et al.*, 2009) and blastocysts (Chang *et al.*, 2008; and Kumasako *et al.*, 2009), ovine blastocysts (Leoni *et al.*, 2001) and murine embryos at various stages of development (Isachenko *et al.*, 2003b; Sheehan *et al.*, 2006; and El-Gayar *et al.*, 2010).

Vitrification of embryos by the open pulled straw (OPS) method (Vajta *et al.*, 1998) proved to be a time saving and efficient means, applicable to various mammalian species (El-Gayar and Holtz, 2001; Vajta *et al.*, 1997; Lopez-Bejar and Lopez-Gatius, 2003; Zhou *et al.*, 2005 and 2007). In mice, warming of OPS-vitrified embryos in medium devoid of sucrose and repeated vitrification are feasible (El-Gayar *et al.*, 2008 and 2010). If these would apply to other mammalian species, in particular farm animals, these findings will facilitate the applicability of the OPS-vitrification technique in the field. In this investigation, the suitability of these findings for the OPS-vitrified goat embryos was tested.

MATERIALS AND METHODS

Thirty parous Baladi goat does, aged 3-5 years and weighing 29-36 kg, were involved in this experiment. Does were housed and managed at the experimental farm, Faculty of Agriculture, Suez Canal University, Ismailia, Egypt (30º37´N, 32º16´E). Estrus was synchronized with intravaginal sponges containing 60 mg of Medroxy Progesterone Acetate (MAP, Veramix®, Pharmacia & Upjohn, Orangeville, Canada) for 12 days. Donors were intramuscularly injected with two luteolytic doses of 5 mg prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$, Dinoprost, Lutalyse™, Pharmacia, Puurs, Belgium) at 12 h interval on the day of sponge removal. In order to obtain multiple ovulations, donors were intramuscularly injected at sponge removal with 1000 IU eCG (equine chorionic gonadotropin, Folligon®, Intervet, Boxmeer, Holland) followed, 45 to 48 h later, by 750 IU hCG (human chorionic gonadotropin, Ovogest®, Intervet, Unterschleissheim, Germany). Animals showing estrus were mated daily with fertile bucks as long as females permitted the male to mount. Embryos were

collected transcervically as described by Suyadi *et al.* (2000) seven days after the last mating. Briefly, superovulated does were intramuscularly injected with a luteolytic dose of 5 mg $PGF_{2\alpha}$ at 24 h before embryo collection. Embryo collection was performed in unanesthetized does in a standing position. One person restrained the doe in standing position by its neck. For viewing and grasping of the lip of the os cervix, the vagina must be dilated by insertion of a duck-billed speculum into the vagina and grasping of the lip of the external os of the cervix with the Allis forceps (Surgicon, J-20-290, 25 cm, Pakistan). The flushing catheter (Rüsch® No. 12, 40 cm long, Kernen, Germany) was then inserted into the os cervix and passed through the cervical canal deep into one uterine horn by exertion of gentle pressure. The tip of the catheter may be directed either into the left or right uterine horn with a finger in the vaginal fornix. Once the catheter was introduced into the desired uterine horn, 20 ml PBS flushing medium (Dulbecco's Phosphate Buffered Saline) at 39°C was infused into the free end with a 20-ml syringe. After infusion of 20 ml of medium, the syringe was disconnected from the catheter to permit spontaneous back-flow of the medium. The medium was collected in a 50 ml test tube. This process was repeated 10 times. The catheter was then introduced into the other horn and another 10 flushings were conducted. Thereafter, the catheter was positioned back into the right uterine horn for 5 more flushings and again into the left horn for another 5 flushings. The collected medium was poured into a petri dish (94 x 16 mm) to be examined under a stereo microscope at 20 to 40 x. With the aid of a unopette (20 µl capacity, Becton-Dickinson, Rutherford, New Jersey, USA), embryos were picked out and transferred to a small petri dish (35 x 10 mm) containing 2 ml of M2 medium (Nagy *et al.*, 2003), then examined under a stereo microscope at 40x for stage of development and morphological integrity.

Blastocysts, symmetrical in shape, uniform in color with no visible imperfections and intact zonae pellucidae were pooled, washed three times in drops of M2 medium and vitrified in the OPS-fashion according to the protocol described by Vajta *et al.* (1998) with slight modifications described in El-Gayar and Holtz (2001). Briefly, five of the pooled embryos at a time were equilibrated at 39°C in holding medium (10% TCM 199 [M-0650, Sigma, Steinheim, Germany], 2 mM sodium bicarbonate, 2 mM sodium pyruvate, 25 mM Hepes-sodium salt, 1 mM L-glutamine, adjusted to pH 7.4 and 280 mOsm) supplemented with 20% heat-inactivated goat serum, containing 10% ethylene glycol (EG) and 10% dimethyl-sulfoxide (Me_2SO). After 1 min they were transferred, in 1 to 2 µL of solution, to a 20 µL droplet of holding medium containing 20% EG and 20% Me_2SO. Within 20 sec, the droplet containing the embryos was drawn into the narrow end of the OPS-straw by capillary action. The straw then was immediately submerged in liquid nitrogen in a vertical position, thin end first.

In the first study, of 50 OPS-vitrified goat blastocysts, 25 embryos were exposed to the standard warming and dilution regimen (Control) established by Vajta *et al.* (1998). Briefly, the end of the straw containing the embryo was immersed in 1.2 ml holding medium containing 0.33 M sucrose at 39°C while the opening on the other end was occluded by the tip of a finger. Within 1 sec, the vitrification medium liquefied and the embryo slid out of the straw. After one minute, the embryo was transferred to fresh medium of the same composition. The embryo was then transferred to holding medium containing 0.2 M sucrose for another minute and, eventually, to holding medium devoid of sucrose for 5 min. The remaining 25

embryos were warmed, in a single step, in sucrose-free medium. Embryos of the two groups were then transferred to drops of M16 culture medium (Nagy *et al.*, 2003) supplemented with 4mg/ml of bovine serum albumin (BSA, A-9647, Sigma, Steinheim, Germany) under mineral oil (M-8410, Sigma, Steinheim, Germany) and incubated at 39^{o}C under a moisture-saturated atmosphere of 5% CO_2 in air.

In the second study, of 50 OPS-vitrified goat blastocysts, 25 embryos were exposed to the standard warming and dilution regimen (Control) and cultured in vitro as described above. The remaining 25 embryos were subjected to repeated vitrification. These embryos too were re-warmed and cultured. Survival of embryos was defined as their ability to continue development to the hatched blastocyst stage. Differences in survival rate were tested for significance by Chi-square test (Steel and Torrie, 1960).

RESULTS

All of the vitrified embryos were recovered after warming. In the first study, prior to in vitro culture, more than 90% of embryos showed no morphological defects and virtually all of them continued developing to the expanded and hatched blastocyst stage. After 48 h in culture, as indicated in Table 1, OPS-vitrified embryos warmed in sucrose-free dilution medium showed a slightly higher survival rate (84%) than embryos exposed to the standard warming and dilution regimen (80%). The differences among the two treatment groups were not significant (P > 0.05).

Table 1. In vitro survival of vitrified goat blastocysts after warming in sucrose-free dilution medium

Sucrose concentration (M)	Number of blastocysts cultured	Hatched blastocysts	
		n	%
Control*	25	20	80
0.0	25	21	84

* 3 step-procedure (El-Gayar and Holtz, 2001).

In the second study, after the second OPS-vitrification, in approximately 30 to 40% of the embryos displayed morphological abnormalities and several were fragmented with very dark colored blastomeres prior to in vitro culture. After 48 h in culture, as shown in Table 2, of the blastocysts vitrified only once, 88% proceeded to hatching. The corresponding value for re-vitrified embryos was 60%. The differences between the two groups were statistically significant ($P < 0.01$).

Table 2. In vitro survival of goat blastocysts subjected to repeated vitrification by the OPS-method

OPS-vitrification	Number of blastocysts cultured	Hatched blastocysts	
		n	%
Once (control)	25	22	88[a]
Twice	25	15	60[b]

[a,b] Within columns values with different superscripts differ ($P < 0.01$, χ^2-test).

DISCUSSION

The open pulled straw method is based on ultra-thin straws derived from normal French straws drawn to about half their original diameter and wall thickness. According to Vajta *et al.* (1998) cooling and warming rates of the minute amount of medium (0.5 µl) enveloping the embryo in the thin-walled straw are enhanced to about 20,000°C/min, compared with 2,500 with the original straw. Therefore, the potentially damaging temperature zone is traversed rapidly, minimizing chilling injury. Due to the open ends of the straw, no pressure changes occur during the process of freezing, avoiding fracture damage (Vajta *et al.*, 1997). The rapid cooling process implies minimal de- and rehydration of cells, reducing the degree of strain placed on the cell membrane.

Using the OPS-method, the short exposure time is likely to have insured low intracellular cryoprotectant concentrations with the result that the osmotic differential between the embryos and their environment never became high enough to cause serious osmotic damage, even when diluted directly into sucrose-free handling media. That being the case, conceivably the post-warming de- and rehydration processes may be omitted, saving the cells unnecessary osmotic stress, an idea that has been contemplated before by Isachenko *et al.* (1997). The results presented in the first study confirm this assumption; goat embryos warmed and diluted in a single step in medium devoid of sucrose performed as well as those treated according to the more elaborate regimen followed by Vajta *et al.* (1998) and El-Gayar and Holtz (2001). This simplified regimen of warming and diluting OPS-vitrified embryos offers the possibility to conduct a direct transfer of embryos without the necessity of a microscope and other laboratory equipment.

The second study was conducted with the intention to ascertain whether the OPS method is suited for re-vitrifying goat blastocysts that had been vitrified and warmed before. Repeated cryopreservation has been successfully accomplished in mouse embryos by conventional slow freezing (Vitale *et al.*, 1997) and by vitrification with the CryoLoop method (Sheehan *et al.*, 2006). In the rat a related study was conducted with the "super-fine open pulled straw" (SOPS) method (Isachenko *et al.*, 2003b) and human embryos were re-vitrified in standard French straws (Kumasako *et al.*, 2009). Using the open pulled straw, repeated vitrification embryos has been accomplished with appreciable success in mouse (El-Gayar *et al.*, 2010). The repeated OPS-vitrification of goat embryos, however, was not well tolerated by a significant proportion of embryos in the present study. The outcome of the in vitro embryo culture following the second vitrification suggest that, despite the fact that the potentially damaging temperature zones encompassing the phases of homogenous nucleation and transition to the glass phase are traversed rapidly (Vajta *et al.*, 1998), OPS-vitrification and/or subsequent warming exerts strain upon the embryos. In view of the brief exposure, a noxious effect of the cryoprotectants on the blastomeres appears unlikely. More likely is that, as mentioned by Sheehan *et al.* (2006), zona hardening in response to repeated exposure to the highly concentrated cryoprotectants played a major role. In an early study on re-freezing of mouse embryos by conventional cryopreservation (Vitale *et al.*, 1997) a reduction in cell number by about 40% has been described. In the present study cell numbers were not recorded.

Therefore, it may be concluded that repeated vitrification decreased the viability of goat blastocysts, whereas warming in a dilution medium devoid of sucrose in a single step, a warming mode that will permit direct transfer of OPS-vitrified embryos to recipients, did not.

REFERENCES

Baril G., A.L. Traldi, Y. Cognié, B. Leboeuf, J.F. Beckers and P. Mermillod, 2001. Successful direct transfer of vitrified sheep embryos. Theriogenology, 56**:** 299-305.

Berthelot F., F. Martinat-Botté, A. Locatelli, C. Perreau and M. Terqui, 2000. Piglets born after vitrification of embryos using the open pulled straw method. Cryobiology, 41: 116-124.

Chang C.C., D.B. Shapiro, D.P. Bernal, G. Wright, H.I. Kort and Z.P. Nagy, 2008. Two successful pregnancies obtained following oocyte vitrification and embryo re-vitrification. Reproductive BioMedicine Online, 16: 346-349.

Cuello C., M.A. Gil, I. Parrilla, J. Tornel, J.M. Vázquez, J., F. Roca Berthelot, F. Martinat-Botté and E.A. Martínez, 2004. In vitro development following one-step dilution of OPS-vitrified porcine blastocysts. Theriogenology, 62: 1144-1152.

El-Gayar M. and W. Holtz, 2001. Technical note: Vitrification of goat embryos by the open pulled-straw method. Journal of Animal Science, 79: 2436-2438.

El-Gayar M., M. Gauly and W. Holtz, 2008. One-step dilution of open-pulled-straw (OPS)-vitrified mouse blastocysts in sucrose-free medium. Cryobiology, 57: 191-194.

El-Gayar M., M. Gauly and W. Holtz, 2010. In vitro and in vivo survival of mouse blastocysts after repeated vitrification with the open pulled straw (OPS) method. Cryoletters, 31 (6): 454-459.

Farhat M., B.S. Zentner, F. Lossos, Y. Bdolah, H. Holtzer and A. Hurwitz, 2001. Successful pregnancy following replacement of embryos previously refrozen at the blastocyst stage. Human Reproduction, 16: 337–339.

Guignot F., A. Bouttier, G. Baril, P. Salvetti, P. Pignon, J.F. Beckers, J.L. Touzé, J. Cognié, A.S. Traldi, Y. Cognié and P. Mermillod, 2006. Improved vitrification method allowing direct transfer of goat embryos. Theriogenology, 66: 1004-1011.

Isachenko V.V., E.F. Isachenko, F.I. Ostashko and V.I. Grishchenko, 1997. Ultrarapid Freezing of Rat Embryos with Rapid Dilution of Permeable Cryoprotectants. Cryobiology, 34: 157-164.

Isachenko V., J.L. Alabart, M. Dattena, F. Nawroth, P. Cappai, E. Isachenko, M. Cocero, J. Olivera, A. Roche, C. Accardo, A. Krivokharchenko and J. Folch, 2003a. New technology for vitrification and field (microscope-free) warming and transfer of small ruminant embryos. Theriogenology, 59: 1209-1218.

Isachenko V., J. Folch, E. Isachenko, F. Nawroth, A. Krivokharchenko, G. Vajta, M. Dattena and J.L. Alabart, 2003b. Double vitrification of rat embryos at different developmental stages using an identical protocol. Theriogenology, 60: 445-452.

Kasai M., K. Ito and K. Edashige, 2002. Morphological appearance of the cryopreserved mouse blastocyst as a tool to identify the type of cryoinjury. Human Reproduction, 17: 1863-1874.

Kumasako Y., E. Otsu, T. Utsunomiya and Y. Araki, 2009. The efficacy of the transfer of twice frozen-thawed embryos with the vitrification method. Fertility and Sterility, 91: 383-386.

Leibo S.P., 1984. One-step method for direct non-surgical transfer of frozen-thawed bovine embryos. Theriogenology, 21**:** 767-790.

Leibo S.P., C.R. Christian and L.R. Malinak, 1991. In vitro and in vivo survival of twice-frozen mouse embryos. Cryobiology, 28: 534 (Abst.).

Leoni G., L. Bogliolo, P. Pintus, S. Ledda and S. Naitana, 2001. Sheep embryos derived from FSH/eCG treatment have a lower in vitro viability after vitrification than those derived from FSH treatment. Reproduction Nutrition Development, 41: 239-246.

Lopez-Bejar M. and F. Lopez-Gatius, 2003. Nonequilibrium cryopreservation of rabbit embryos using a modified (sealed) open pulled straw procedure. Theriogenology, 60: 789-790.

Nagy A., K. Vintersten, M. Gertsenstein and R. Behringer, 2003. Manipulating the mouse embryo: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Nowshari M.A. and G. Brem, 2000. Refreezing of murine intact and biopsied embryos by rapid-freezing procedur. Human Reproduction, 15: 2577-2581.

Rall W.F. and G.M. Fahy, 1985. Ice-free cryopreservation of mouse embryos at –196^{o}C by Vitrification. Nature, 313: 573-575.

Saha S., T. Otoi, M. Takagi, A. Boediono, C. Sumantri and T. Suzuki, 1996. Normal calves obtained after direct transfer of vitrified bovine embryos using ethylene glycol, trehalose and polyvinylpyrrolidone. Cryobiology, 33: 291–299.

Sheehan B., M. Lane and D.K. Gardner, 2006. The CryoLoop facilitates re-vitrification of embryos at four successive stages of development without impairing embryo growth. Human Reproduction, 21: 2978–2984.

Smith L.K., E.H. Roots and M. J. O. Dorsett, 2005. Live birth of a normal healthy baby after a frozen embryo transfer with blastocysts that were frozen and thawed twice. Fertility and Sterility, 83: 198-200.

Steel R.G.D. and J.H. Torrie, 1960. Principles and Procedures of Statistics, McGraw-Hill Book Company, New York.

Suyadi B. Sohnrey and W. Holtz, 2000. Transcervical embryo collection in Boar goats. Small Ruminant Research, 36: 195-200.

Vajta G., 2000. Vitrification of the oocytes and embryos of domestic animals. Animal Reproduction Science, 60-61: 357-364.

Vajta G., P.J. Booth, P. Holm, T. Greve and H. Callesen, 1997. Successful vitrification of early stage bovine in vitro produced embryos with the Open Pulled Straw (OPS) method. Cryoletters, 18: 191-195.

Vajta G., P. Holm, M. Kuwayama, P.J. Booth, H. Jacobsen, T. Greve and H. Callesen, 1998. Open Pulled Straw (OPS) vitrification: A new way to reduce cryoinjuries of bovine ova and embryos. Molecular Reproduction and Development, 51: 53-58.

Vitale N.J., D.M. Barry, M. Meintjes and R.A. Godke, 1994. In vitro development of refrozen bovine embryos. Theriogenology, 41: 327 (Abstr.).

Vitale N.J., M.W. Myers, R.S. Denniston, S.P. Leibo and R.A. Godke, 1997. In-vitro development of refrozen mouse embryos. Human Reproduction, 12: 310-316.

Yokota Y., H. Yokota, M. Yokota, S. Sato and Y. Araki, 2001. Birth of healthy twins from in vitro development of human refrozen embryos. Fertility and Sterility, 76: 1063–1065.

Zhou G.B., Y.P. Hou, F. Jin, Q.E. Yang, Z.Q. Yang, G.B. Quan, H.M. Tan and S.E. Zhu, 2005. Vitrification of mouse embryos at various stages by open-pulled straw (OPS) method. Animal Biotechnology, 16: 153-163.

Zhou C., G.B. Zhou, S.E. Zhu, Y.P. Hou, F. Jin, X.M. Zhaoand and Q.H. Hong, 2007. Open-pulled straw (OPS) vitrification of mouse hatched blastocysts. Animal Biotechnology, 18: 45-54.

التطور المعملي لأجنة الماعز المجمدة تزججياً باستخدام القصيبة المسحوبة مفتوحة الطرفين بعد الإذابة أحادية المرحلة في بيئة خالية من السكروز أو بعد تكرار تجميدها تزججياً

معتز الجيار

قسم الإنتاج الحيواني، كلية الزراعة، جامعة قناة السويس، الإسماعيلية، مصر

تساعد تقنية حفظ الأجنة بالتجميد على رفع كفاءة برامج نقل الأجنة في الحيوانات المزرعية. فعلى سبيل المثال، تساعد على نقل الأجنة المتفوقة وراثياً ما بين بلدان العالم بدون الحاجة إلي نقل الحيوانات حية، كما تساعد على حفظ أجنة الأنواع الحيوانية النادرة والمهددة بالانقراض. حالياً يعتبر التجميد التزججي من أكثر طرق تجميد أجنة الثدييات شيوعاً في العالم. تعتبر القصيبة المسحوبة مفتوحة الطرفين هي أحدث ما تم إستخدامه في السنوات الأخيرة لرفع كفاءة عملية التجميد التزججي لأجنة الثدييات. واستمراراً لأبحاثي السابقة علي أجنة الفئران (كنموذج لأجنة الثدييات) والهادفة إلي تبسيط هذه التقنية ثم تطبيقها علي أجنة الحيوانات المزرعية لتوفير الوقت والجهد والتكاليف لبرامج نقل الأجنة، كان الهدف من هذه العمل هو اختبار إمكانية تطبيق النتائج السابقة والمتحصل عليها باستخدام أجنة الفئران علي أجنة الماعز من خلال التجربتين التاليتين:-

١. إذابة أجنة الماعز خلال خطوة واحدة في بيئة لا تحتوي علي مادة حفظ تجميدية (السكروز) ليسمح لنا بالنقل المباشر للأجنة المجمدة تزججياً إلي الأمهات المستقبلة.
٢. تكرار التجميد التزججي لأجنة الماعز المجمدة تزججياً لتعظيم الاستفادة العلمية والعملية من هذه الأجنة.

في التجربة الاولي تم تجميد أجنة الماعز في مرحلة البلاستوسيست تزججياً باستخدام القصيبة المسحوبة مفتوحة الطرفين. وعند الإذابة قسمت الأجنة إلي مجموعتين (٢٥ جنين / مجموعة). في المجموعة الأولي (مجموعة المقارنة) تم إذابة الأجنة المجمدة تبعاً للإسلوب القياسي خلال ثلاثة مراحل في بيئة تحتوي علي تركيزات متدرجة من مادة الحفظ التجميدية (السكروز). أما في المجموعة الثانية فقد تم إذابة الأجنة المجمدة خلال مرحلة واحدة في بيئة لا تحتوي علي سكروز. وبعد إذابة الأجنة تم تقدير حيوية الأجنة بقدرتها علي التطور مرة أخري معملياً. أوضحت نتائج هذه التجربة أن الأجنة المجمدة تزججياً قد واصلت التطور مرة أخرى بعد الإذابة من مرحلة البلاستوسيست إلي مرحلة الفقس (hatching) بعد مرور ٤٨ ساعة في بيئة التطور المعملي بدون أية فروق معنوية ما بين المجموعتين (٨٠%، ٨٤% علي التوالي).

في التجربة الثانية تم تجميد أجنة الماعز في مرحلة البلاستوسيست تزججياً باستخدام القصيبة المسحوبة مفتوحة الطرفين. وعند الإذابة قسمت الأجنة إلي مجموعتين (٢٥ جنين / مجموعة). في المجموعة الأولي (مجموعة المقارنة) تم إذابة الأجنة المجمدة تبعاً للإسلوب القياسي ثم حضنت معملياً. أما في المجموعة الثانية فقد تم إذابة الأجنة المجمدة ثم أعيد تجميدها تزججياً وإزابتها بنفس الإسلوب السابق ثم حضنت لتقدير حيويتها بقدرتها علي التطور مرة أخري معملياً. أوضحت نتائج هذه التجربة أن الأجنة المعاد تجميدها تزججياً قد واصلت التطور مرة أخرى بعد الإذابة من مرحلة البلاستوسيست إلي مرحلة الفقس بعد مرور ٤٨ ساعة في بيئة التطور المعملي بمعدل أقل معنوياً (٦٠%).

ويستنتج من هذه النتائج قابلية إذابة أجنة الماعز المجمدة تزججيا باستخدام القصيبةً المسحوبة مفتوحة الطرفين بنجاح خلال خطوة واحدة في بيئة خالية من مادة الحفظ التجميدية (السكروز)، لتفتح الأفاق لنقل هذه الأجنة المجمدة تزججياً مباشرة إلي الأم المستقبلة بدون الحاجة إلي أية مستلزمات معملية كالميكروسكوب وخلافه، مما سيساعد علي رفع كفاءة برامج نقل الأجنة. في حين أثر تكرار التجميد التزججي بالسلب علي حيوية أجنة الماعز.