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Post-thaw viability of bovine frozen-vitrified embryos

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Abstract
Vitrification is method ultra-rapid cooling method of embryo freezing. In this experiment, we were chosen an embryo produced through *In vitro* embryo production (IVEP) method (n=35). All were produced on day 7 after *In vitro* Fertilization. All were vitrified as per standard protocol and thawed all together to check their survivability and hatchability. All (n=35) embryos were categorized into two grades based on their quality namely, Grade 1 (n=21) and Grade 2 (n= 14). After washing they are kept in *In vitro* Culture medium for observation. Result obtained at the end of experiment was 74.28 percent survivability rate and 34.28 percent hatching rate 48 hours post-thaw.

Keywords: Embryo freezing, vitrification, survivability rate

Introduction
Cryopreservation of germ-plasm has an improvised technique of disseminating and up gradation of germ-plasm throughout the world. Due to freezing technique to bring the live animal from different countries has reduced, for those who are working since a long time in bovine embryo transfer industry understand the percentage of post-thaw embryo viability of frozen embryo because its directly effect on pregnancy rate. Now a days there are mainly two method of freezing embryos- Slow freezing and Vitrification. The advantage of Vitrification over slow freezing is that ice crystal formation should be avoided by achieving a very high cooling temperature and due to that less cryoinjury happened in embryos while freezing.

Materials and Methods
As per the standard protocol of International Embryo technology Society (IETS) the oocyte were collected by Ovum Pick-Up from various donor of different breeds then they are classified based on cumulus mass and cytoplasm density. Further oocytes were proceeded through *In vitro* Maturation (IVM) *In vitro* Fertilization (IVF) and *In vitro* Culture (IVC) subsequently. All media were purchased from Vitrogen. Post seven days after fertilization embryos were evaluated and categorized as per IETS manual (Stringfellow DA *et al*., 2010) [3]. For the vitrification after washing the embryo pass through solution I and solution II for eight minutes and one minute respectively, both solutions were kept at room temperature for the vitrification of embryos and after that embryo were loaded on the self made vitrification device. In the process of thawing all the embryo were thawed through devitrification solution I, solution II, and solution III for one minute, three minutes and five minutes respectively, in the solution I embryo kept at temperature 36-38 °C while solution II and solution III were kept at room temperature for devitrification of embryos, then after giving three to four times washing in IVC medium embryo transfer into pre- equilibrated IVC plate (100mm) for the evaluation of survivability rate. They are evaluated interval of one hour, four hours, twenty-four hours and forty-eight hours.

Result and Discussion
The total numbers of frozen vitrified embryos were 35 selected for devitrification altogether. Among them, twenty-one were 7-1grade and quality and fourteen were 7-2 grade and quality. All were thawed as per standard protocol. In this experiment total number of embryos that were re-expanded after thawing were 26 (74.28 percent). According to stage and grade, out of 21 sixteen were successfully re-expanded (76.19 percent) while out of 14 ten were re-expanded (71.42 percent). Among them, nine were hatched after twenty-four hours (25.71 percent) and twelve were hatched after forty-eight hours (34.28 percent).

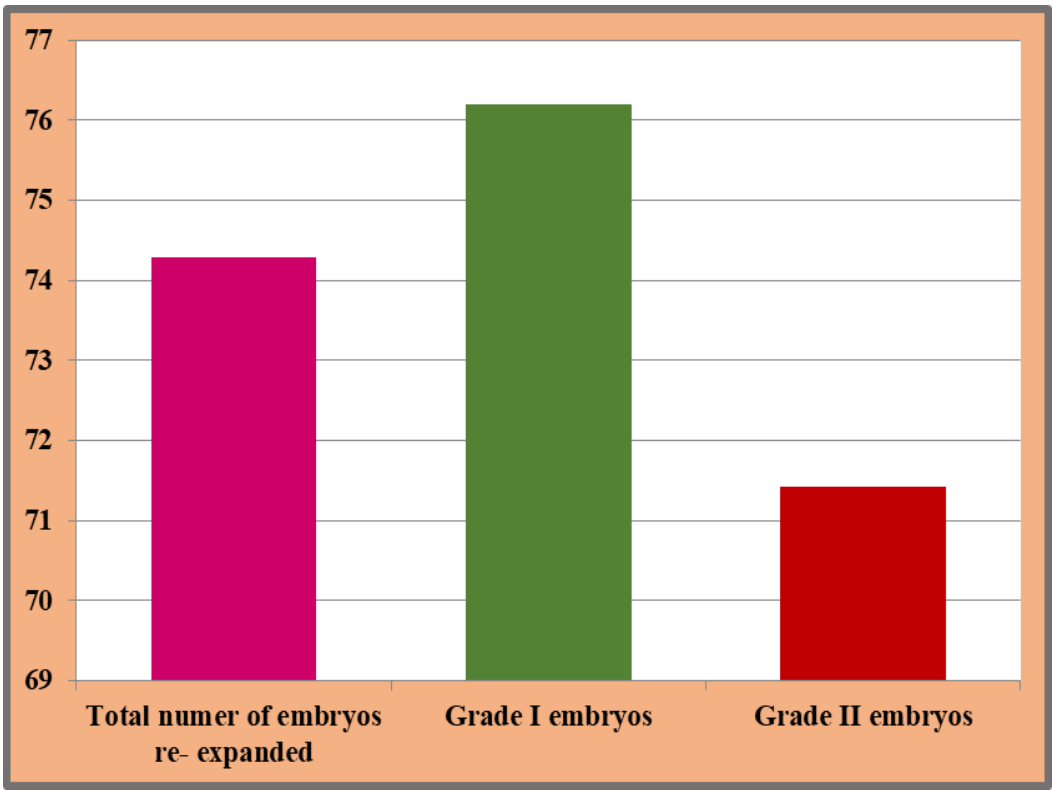

Fig 1: Stage- wise changes in the embryo during IVC

The present study confirmed that the viability of conventional embryos was more in grade 1 (76.19 percent) than in grade 2 (71.42 percent). In a few studies, the result shows a similar pattern, Good and fair- quality embryos had a 100 percent survival rate (Tajimi *et al*., 2018) [4].

In this experiment also observed that embryos were hatched less in the first twenty-four hours of culture and more after up to forty-eight hours. Like these similar studies have also observed. Re-expansion after 2 hours were 95.4 percent, after 24 hours hatching were 20.6 percent and after 48-hour hatching were 53.6 were observed (Camano J N *et al*., 2015) [1]. The survival rate after 24 hours of warming was 79.3 percent and the hatching rate after 48 hours were 51.8 percent (Trigal B *et al*., 2013) [5]. In another study, the post-thaw survival rate was nearly 100 percent in the control group (HA, AN *et al*., 2014) [2]. In the research study, Day 7 expanded blastocysts were re-expanded 67 percent and hatched 63 percent (Vajta G *et al*., 1996) [6].

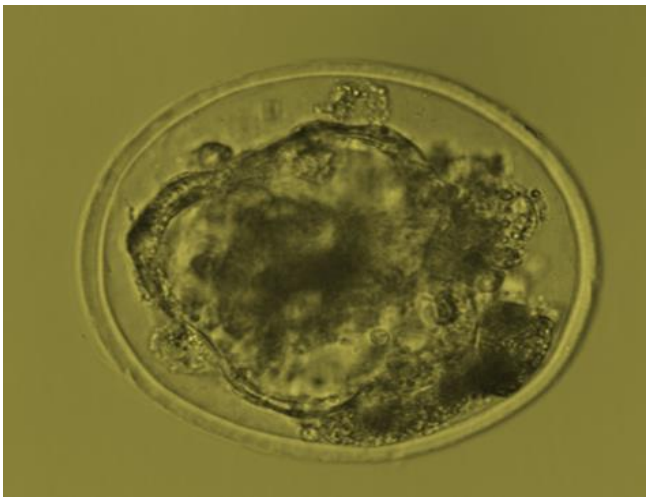

Fig 3: Two Hour after IVC

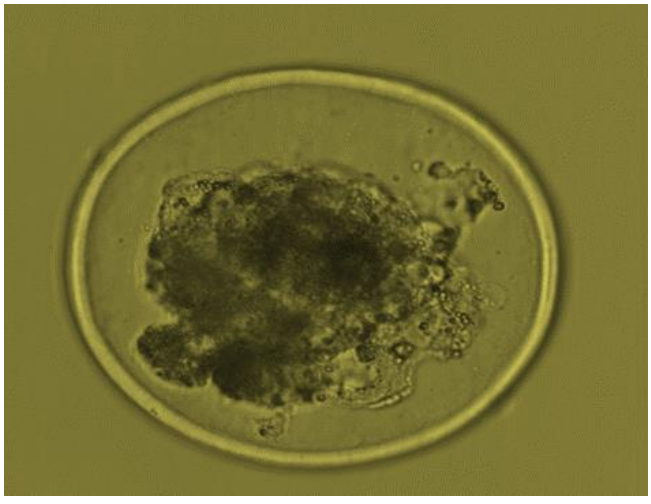

Fig 2: Zero Hour shrinked Inner cell mass embryos

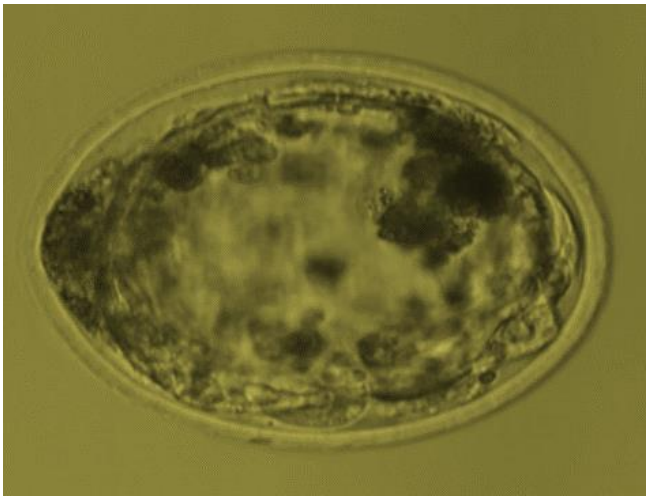

Fig 4: Twenty four hour after IVC

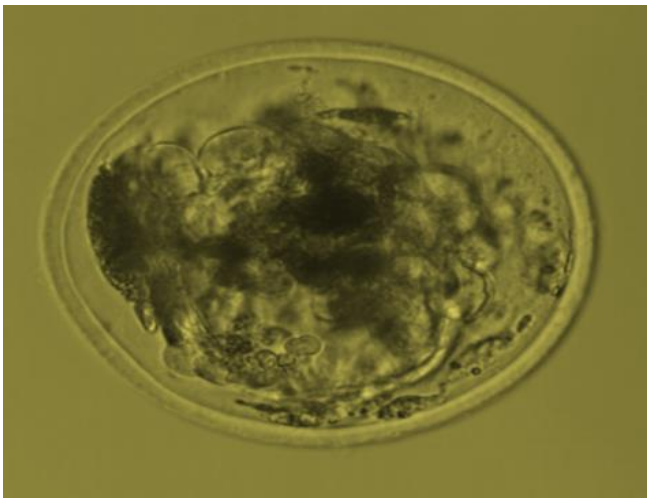

Fig 5: Forty eight hours after IVC

Conclusion

Overall, the survival rate was good for grade 1 embryos than grade 2 embryos. Meanwhile, for 7 days embryos were re-expanded in the first 8 hours but hatching was less after 24 hours as compared to after 48 hours. Overall, the hatching rate was 34.28 percent after 48 hours.

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