RESEARCH ARTICLE

Role of Oocyte Quality in Determining Cryosurvival of *In Vitro* Produced Cattle Embryos

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Abstract

The study was conducted over a six-month period at OPU-IVEP-ET Facility of the National Dairy Development Board, Anand. Cumulus-oocyte complexes (COCs) were retrieved from Gir, Sahiwal and Holstein Friesian crossbred donor cows and heifers, with and without ovarian stimulation, using transvaginal ultrasound-guided ovum pick-up (OPU). Recovered oocytes were morphologically graded according to IETS guidelines and subjected to standard *in vitro* maturation, fertilization and culture protocols. Based on oocyte quality and ovarian stimulation status, oocytes were categorized into four groups: Group 1 (Grade 1-2 oocytes from non-stimulated donors), Group 2 (Grade 3-4 oocytes from non-stimulated donors), Group 3 (FSH-stimulated donors with pooled oocytes) and Group D (Pooled non-stimulated-control group). Embryo developmental competence was assessed using cleavage rate, embryo development rate (EDR), freezable embryo percentage and discarded embryo percentage. Morphologically transferable embryos (Code 1 and 2) were cryopreserved using a slow-freezing protocol, and post-thaw survivability was evaluated after 24 h of *in vitro* culture. Data were analyzed using the Kruskal-Wallis test and pair-wise Chi-square test. Cleavage rate and EDR were numerically higher ($p > 0.05$) in all experimental groups compared to group D. Group 3 exhibited the highest proportion of freezable embryos (90.25 ± 6.03%) and the lowest discard rate (9.74 ± 6.03%), indicating superior embryo quality. Post-thaw survivability was also highest in Group 3 (71.43%), followed by Group 1 (68.97%), Group D (60.00%) and Group 2 (54.17%), though no significant differences were observed among groups ($p > 0.05$). The findings indicate that embryo quality parameters, particularly freezable embryo proportion and discard rate, are more sensitive indicators of developmental competence and cryotolerance than cleavage rate or EDR alone. Optimization of oocyte quality and follicular environment, especially through ovarian stimulation, enhances embryo freezing suitability and post-thaw viability in bovine IVEP programs.

Key words: Cattle, Cryopreservation, Cryotolerance, *In vitro* embryo production, Oocyte quality, Ovum pick-up.

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Introduction

The application of Assisted Reproductive Technologies (ARTs), particularly Ovum Pick-Up (OPU), *In Vitro* Embryo Production (IVEP) and Embryo Transfer (ET), has revolutionized livestock breeding by enhancing genetic progress and improving reproductive efficiency in cattle as well as in buffalos. Globally, *in vitro*-produced embryos now dominate, surpassing *in vivo*-derived (MOET) embryos since 2016, due to the repeatability of OPU (every ~2 weeks) on stimulated or non-stimulated donors, enabling multiple cycles annually (Boni, 2012).

In India, the adoption of OPU-IVEP-ET has grown rapidly under the Rashtriya Gokul Mission (RGM) launched in 2014 and revised with enhanced funding (total outlay ₹3,400 crore for 2021-26). As per recent data, 22 IVF laboratories have been established nationwide, producing 26,999 viable embryos, of which 15,005 were transferred, resulting in the birth of 2,366 calves through IVF technology. This demonstrates the mission's practical impact on scaling embryo production, yielding high-genetic-merit calves and supporting overall livestock improvement (DAHD, 2025). Despite these advancements, *in vitro*-produced (IVP) embryos exhibit inconsistent pregnancy rates, largely due to suboptimal embryo quality linked to variability in oocyte quality (Viana, 2022). Oocyte developmental competence defined as the

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intrinsic ability to resume meiosis, fertilize, cleave and support early embryogenesis is acquired during folliculogenesis and depends on the follicular microenvironment, including nutrients, hormones and growth factors (Sirard *et al.,* 2006).

Poor oocyte quality contributes to early embryonic losses, stemming from inadequate cytoplasmic maturation, metabolic imbalances or deficiencies in maternal factors critical until embryonic genome activation (Rizos *et al.,* 2002; Sirard *et al.,* 2006). Oocytes retrieved through OPU or slaughterhouse aspiration show high heterogeneity in morphology and cytoplasmic traits, affecting maturation, fertilization and embryo outcomes (Pontes *et al.,* 2010; Ferreira *et al.,* 2016). Morphological grading of cumulus-oocyte complexes (COCs) per IETS guidelines remains a practical, cost-effective method to assess quality, with higher grades (I and II) yielding better maturation, cleavage and blastocyst rates than lower grades (III and IV) (Blondin and Sirard, 1995). Oocyte quality is modulated by factors including donor breed, age, nutrition, heat stress, follicle size, hormonal milieu and technician skill (Sales *et al.,* 2015; Ferreira *et al.,* 2016; Baruselli *et al.,* 2016).

Oocyte competence directly influences embryo cryotolerance, the ability to survive cryopreservation while retaining viability. IVP embryos generally show lower post-thaw survival than *in vivo*-derived ones, due to higher lipid accumulation, altered membrane composition and metabolic differences that increase susceptibility to chilling injury (Abe *et al.,* 2002; Marsico *et al.,* 2019). High-quality oocytes confer balanced lipid metabolism, mitochondrial function and reserves that enhance embryo cryoresistance (Lonergan *et al.,* 2006; Krisher *et al.,* 2015). Thus, oocyte morphological grade serves as a key predictor of embryo developmental potential and cryotolerance, guiding selection to optimize IVP outcomes, post-thaw viability and overall OPU-IVEP-ET efficiency for genetic improvement and germplasm conservation. Hence, this study was aimed to evaluate the role of oocyte quality in determining cryosurvival of *in vitro* produced cattle embryos.

Materials And Methods

The present study investigated the relationship between oocyte quality (assessed via morphological grading of COCs) and cryotolerance of resulting *in vitro* produced (IVP) embryos. The study was conducted over six months period at the Department of Veterinary Gynaecology and Obstetrics, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Anand, Gujarat, in collaboration with the R&D and Training facility on OPU-IVEP-ET at the National Dairy Development Board (NDDB), Anand, and Department of Veterinary Biotechnology, Kamdhenu University, Anand, Gujrat.

Selection of Animals and Ovum Pick-Up

Genetically superior Gir, Sahiwal, and Holstein Friesian sires were selected, and frozen semen was sourced from Grade-A semen stations. High-genetic-merit donor cows/heifers of the same breeds, maintained at NDDB's OPU-IVEP-ET unit, were disease-screened and selected based on an ovarian follicular count >15 by ultrasonography. Ovum Pick-Up (OPU) was performed on non-stimulated donors at NDDB, Anand, irrespective of estrous cycle stage. Donors then underwent a standardized ovarian stimulation protocol (Patil *et al.,* 2022) and aseptic OPU under epidural anaesthesia (2-5 mL 2% lignocaine) using transvaginal ultrasound-guided aspiration (5-8.5 MHz probe, 18-20G needle, vacuum 65-75 mmHg, 12 mL/min flow). Follicles ≥3 mm were aspirated; contents collected in heparinized media tube (20 µg/mL heparin, 0.3% BSA, 50 µg/mL gentamicin); transported to lab via pass box.

In Vitro Embryo Production (IVEP)

The *in vitro* maturation (IVM) media (Vitrogen) was equilibrated for ≥4 h in CO_2 incubator (5% CO_2, 38.5°C, ≥90% RH). COCs recovered from aspirates were searched under stereo zoom microscope, washed, graded per IETS Manual (5th edition), matured (max 30/drop) for 20-22 h. They were divided into 4 groups: Groups: A (Grades 1-2 non-stimulated), B (Grades 3-4 non-stimulated), C (stimulated pooled), D (non-stimulated control pooled, from routine OPU cycle).

In vitro fertilization (IVF) was carried out using equilibrated media. The frozen semen of selected bulls was processed via Percoll gradient, capacitated (heparin + PHE), and concentration adjusted to 2×10^6/mL. Matured oocytes were then washed and co-incubated with sperm for 18 h in equilibrated drops (5% CO_2, 38.5°C). The presumptive zygotes were denuded, washed, and cultured in equilibrated IVC media (mixed gas incubator: 5% CO_2, 5% O_2, 90% N_2, 38.5°C). Cleavage was checked at 72 h post-IVF; and embryos were evaluated (stage/grade as per IETS Manual, 5th edition) on Days 6-7.

Cryopreservation (Slow Freezing) and Post-Thaw Viability

Code 1-2 blastocysts (early blastocyst, blastocyst, expanded blastocyst, hatched blastocyst) were equilibrated in freezing media (ethylene glycol + sucrose, IMV Technologies), loaded into 0.25 mL straws, frozen using Crysalys bovine standard program (seeding at -6°C, cooling to -32°C at -0.5°C/min), and plunged into LN_2.

Straws were thawed (air 8 s + 32°C water, 30 s), embryos rehydrated, washed, and cultured in IVC media. Post-thaw viability, re-expansion, and hatching was assessed at 24 h via inverted microscopy; survival was defined as >50% viable cells.

Statistical Analysis

Embryo production rates derived from different grades of bovine oocytes were evaluated using descriptive statistical analysis, followed by assessment of statistically significant differences among groups using the Kruskal-Wallis test. The survivability of embryos was compared using the pair wise Chi-square test.

Results and Discussion

Embryo Development Efficiency

The present study evaluated embryo development efficiency across four experimental groups using cleavage rate, embryo development rate (EDR), freezable embryo percentage and discarded embryo percentage. Data are presented as Mean ± SEM and analyzed using the non-parametric Kruskal–Wallis test due to non-normal distribution of variables (Table 1).

Across all experimental groups, cleavage rates were consistently higher than the control group, indicating improved early embryonic competence under experimental conditions. The highest cleavage rate was observed in Group 3 (87.87 ± 2.23%), followed by Group 2 and Group 1, whereas the control group exhibited the lowest cleavage rate (69.66 ± 4.72%). Although these differences were not statistically significant (p=0.076). Thus, the numerical trend suggests that experimental grouping positively influenced early embryonic division. Higher cleavage rates are generally indicative of improved oocyte cytoplasmic maturation and fertilization competence, which are prerequisites for subsequent embryo development, as reported in earlier studies on oocyte morphological quality and developmental competence (Khurana and Niemann, 2000; Ward *et al.,* 2000).

Embryo development rate varied numerically among the groups. Group 2 recorded the highest EDR (56.04 ± 18.85%), followed by Group 1 and Group 3, while the control group showed the lowest EDR (24.40 ± 4.57%) which also did not reveal significant variation (p=0.135, Table 1). The higher EDR observed in Group 2 indicates enhanced progression beyond cleavage; however, this increase did not necessarily translate into superior embryo quality, as reflected by subsequent freezing and discard outcomes. Similar observations have been documented where embryos derived from lower-quality oocytes progressed developmentally but exhibited compromised quality at later stages (Saini *et al.,* 2015).

Assessment of embryo quality through freezable embryo percentage revealed clear group-wise trends. FSH stimulated Group 3 demonstrated the highest proportion of freezable embryos (90.25 ± 6.03%), markedly higher than Group 1 (70.43 ± 13.60%), Group 2 (60.04 ± 8.32%), while the control group had intermediate value (76.12 ± 10.40%). Although the difference was apparent, it remained non-significant (p=0.069). The exceptionally high freezing suitability observed in Group 3 indicates superior embryo structural integrity and developmental synchrony, which are essential determinants of cryotolerance because of homogenous population of oocytes. Earlier reports have demonstrated that improved follicular environment and oocyte homogeneity enhance blastocyst quality and freezing tolerance (Merton *et al.,* 2003; Baruselli *et al.,* 2016).

In contrast, the discarded embryo percentage was highest in Group 2 (39.95 ± 8.32%), followed by Group 1 and the control group. Group 3 exhibited the lowest discard rate (9.74 ± 6.03%) (Table 1), indicating enhanced embryo quality and reduced developmental abnormalities, which is consistent with previous findings that embryos derived from better-quality or synchronized oocyte populations show reduced degeneration rates (Ward *et al.,* 2000; Saini *et al.,* 2015). Similar to freezable embryo percentage, differences in discard rates were not statistically significant (p=0.069); however, the biological relevance of these trends is substantial.

Collectively, the findings indicate that higher embryo development, as reflected by cleavage rate or EDR alone, does not necessarily correspond to improved embryo quality. Group 2, despite exhibiting the highest EDR, showed the lowest freezable embryo percentage and the highest discard rate, suggesting compromised embryo competence. This observation supports previous reports that embryos derived under suboptimal oocyte or follicular conditions may progress to advanced stages but fail to meet quality criteria for cryopreservation (Melka *et al.,* 2009).

It is noteworthy that although some oocytes may be morphologically classified as lower grade because of detachment of cumulus cells from COCs can occur during OPU due to vacuum pressure within the aspiration line or during subsequent searching and handling procedures, according to IETS morphological grading criteria, such cumulus loss does not necessarily reflect intrinsic oocyte quality and may lead to underestimation of oocyte competence. Importantly, the developmental potential of these oocytes may remain intact and can still result in satisfactory EDR. Conversely, Group 3 consistently demonstrated superior embryo quality, characterized by the highest freezable embryo percentage and the lowest discard rate, despite having a moderate EDR. This highlights the importance of oocyte homogeneity and optimal follicular environment in producing embryos with enhanced developmental competence and freezing suitability. The control group, representing routine conditions, exhibited lower cleavage and EDR values, likely due to greater heterogeneity in oocyte quality and follicular dynamics.

Table 1: The COCs grade/group-wise cleavage rate, embryo development rate (EDR), freezable and discarded embryo percentage recorded for cattle

Group	Cleavage rate	EDR	Frozen (%)	Discarded (%)
Group 1	84.04 ± 6.7	48.56±11.11	70.43±13.60	29.56 ±13.60
Group 2	84.71 ± 6.42	56.04 ± 18.85	60.04 ± 8.32	39.95 ± 8.32
Group 3	87.87± 2.23	43.42 ± 7.32	90.25 ± 6.03	9.74 ± 6.03
Control	69.66 ± 4.72	24.40 ±4.57	76.12 ± 10.40	23.27 ± 10.40
Kruskal-Wallis H	7.015	5.565	7.077	7.077
df	3	3	3	3
Asymp. Sig.	0.076	0.135	0.069	0.069

Although statistical significance was not achieved for any parameter, the consistent numerical trends clearly demonstrate that embryo quality indicators-particularly freezable and discard embryo percentages are more sensitive and biologically meaningful measures of developmental competence than cleavage or EDR alone. Similar observations have been reported earlier, indicating that embryo quality and freezing suitability are more reliable indicators of developmental competence than embryo yield alone (Khurana and Niemann, 2000; Ward *et al.,* 2000; Merton *et al.,* 2003; Saini *et al.,* 2015; Baruselli *et al.,* 2016). These findings emphasize that evaluation of IVEP outcomes should prioritize embryo quality alongside quantitative production parameters to optimize cryopreservation efficiency and overall success of embryo transfer programs.

Post-thaw Embryo Survivability

Post-thaw embryo survivability was evaluated across all experimental groups based on the number of embryos surviving after thawing relative to the total number of embryos thawed. Survivability was expressed as a percentage to assess cryotolerance and post-thaw viability.

Group-wise analysis revealed that Group 3 exhibited the highest post-thaw embryo survivability (71.43%; 25/35), followed by Group 1, which showed a survivability of 68.97% (20/29). The control group demonstrated moderate survivability (60.00%; 21/35), whereas Group 2 recorded the lowest survivability rate of 54.17% (13/24) (Table 2).

Pair-wise Chi-square analysis revealed that there were no statistically significant differences ($p > 0.05$) in post-thaw embryo survivability between any of the experimental or control group, although numerical variations were observed among the groups. This indicates that embryos derived from different experimental conditions exhibited comparable resistance to cryopreservation and thawing stress, and that post-thaw survivability was largely independent of group-wise variation. The findings further suggest that standardized embryo selection and cryopreservation protocols may mitigate differences arising from oocyte or embryo source, resulting in uniform post-thaw survival outcomes across groups.

Table 2: Grade/group-wise post-thaw survivability of cattle embryos

Group	No of Embryo	Survivable	Survivability %
Group 1	29	20	68.97
Group 2	24	13	54.17
Group 3	35	25	71.43
Control	35	21	60.00

The variations in survivability among groups suggest that embryos derived from Group 3 possessed superior cryotolerance compared to other groups. This may be attributed to improved embryo quality and structural integrity of embryos, which are critical determinants of resistance to cryo-induced cellular damage. Earlier studies have reported that embryos with better morphological quality and synchronized development exhibit enhanced tolerance to freezing and thawing procedures (Merton *et al.,* 2003; Baruselli *et al.,* 2016).

The comparatively lower survivability observed in Group 2 indicates that, despite reasonable embryo production, embryos from this group were more susceptible to cryo-injury. This finding aligns with previous reports suggesting that embryos derived from lower-quality oocytes or suboptimal developmental conditions may exhibit compromised membrane stability and increased sensitivity to cryopreservation stress (Khurana and Niemann, 2000; Saini *et al.,* 2015). Group 1 showed intermediate survivability, indicating moderate cryotolerance, while the control group demonstrated relatively good post-thaw survival, possibly due to stringent morphological selection of embryos prior to freezing. Similar observations have been reported, where careful embryo grading before cryopreservation minimized differences in post-thaw survivability among experimental groups (Ward *et al.,* 2000).

Conclusion

Overall, the results indicate that post-thaw survivability is influenced not only by embryo quantity but more importantly by intrinsic embryo quality. Although statistical significance was not assessed in the present dataset, the observed trends suggest that embryos from Group 3 possess superior cryotolerance, reinforcing the importance of optimizing oocyte and embryo quality to improve cryopreservation outcomes in IVEP programs.

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References

Abe, H., Matsuzaki, S., & Hoshi, H. (2002). Ultrastructural differences in bovine morulae classified as high and low qualities by morphological evaluation. *Theriogenology*, *57*(4), 1273-1283.

Baruselli, P. S., Batista, E.O.S., Vieira, L.M., Ferreira, R.M., Guerreiro, B.G., Bayeux, B.M., Sales, J.N.S., Souza, A.H., & Gimenes, L.U. (2016). Factors that interfere with oocyte quality for *In vitro* production of cattle embryos: Effects of different developmental & reproductive stages. *Animal Reproduction*, *13*(3), 264-272.

Blondin, P., & Sirard, M.A. (1995). Oocyte and follicular morphology as determining characteristics for developmental competence in bovine oocytes. *Molecular Reproduction and Development*, *41*(1), 54-62.

Boni, R. (2012). Ovum pick-up in cattle-a 25-yr retrospective analysis. *Animal Reproduction*, *9*(3), 362-369.

DAHD - Department of Animal Husbandry and Dairying (2025). *Annual Report 2024-25*. Govt of India, New Delhi. Retrieved from https://www.pib.gov.in/ PressReleasePage.aspx?PRID=2117330®=3&lang=2

Ferreira, R.M., Chiaratti, M.R., Macabelli, C.H., Rodrigues, C.A., Ferraz, M.L., Watanabe, Y. F., Smith, L.C., Meirelles, F.V., & Baruselli, P.S. (2016). The infertility of repeat-breeder cows during summer is associated with decreased mitochondrial DNA and increased expression of mitochondrial and apoptotic genes in oocytes. *Biology of Reproduction*, *94*(3), 66, 1-10.

Khurana, N.K., & Niemann, H. (2000). Effects of oocyte quality, oxygen tension, embryo density, cumulus cells and energy substrates on cleavage and morula/blastocyst formation of bovine embryos. *Theriogenology*, *54*(5), 741-756.

Krisher, R.L., Schoolcraft, W.B., & Katz-Jaffe, M.G. (2015). Omics as a window to view embryo viability. *Fertility and Sterility*, *103*(2), 333-341.

Lonergan, P., Fair, T., Corcoran, D., & Evans, A.C.O. (2006). Effect of culture environment on gene expression and developmental characteristics in IVF-derived embryos. *Theriogenology*, *65*(1), 137-152.

Marsico, T.V., Camargo, J.D., Valente, R.S., & Sudano, M.J. (2019). Embryo competence and cryosurvival: Molecular and cellular features. *Animal Reproduction*, *16*(3), 423-439.

Melka, M., Rings, F., Hölker, M., Tholen, E., Havlicek, V., Besenfelder, U., Schellander, K., & Tesfaye, D. (2009). Expression of apoptosis regulatory genes and incidence of apoptosis in different morphological quality groups of *in vitro*-produced bovine pre-implantation embryos. *Reproduction in Domestic Animals*, *45*(5), 915-921.

Merton, J.S., De Roos, A.P.W., Mullaart, E., De Ruigh, L., Kaal, L., Vos, P.L.A M., & Dieleman, S.J. (2003). Factors affecting oocyte quality and quantity in commercial application of embryo technologies in the cattle breeding industry. *Theriogenology, 59*(2), 651-674.

Patil, S.P., Hadiya, K.K., Layek, S.S., Gorani, S., Raj, S., Karuppanasamy, K., Dhami, A.J., Gupta, R.O. (2022). Ovum pick-up and *in vitro* embryo production from stimulated vs non-stimulated buffaloes. *Indian Journal of Veterinary Science and Biotechnology, 18*(1), 95-100.

Pontes, J., Silva, K., Basso, A., Rigo, A., Ferreira, C., Santos, G., Sanches, B., Porcionato, J., Vieira, P., Faifer, F., Sterza, F., Schenk, J., & Seneda, M. (2010). Large-scale *in vitro* embryo production and pregnancy rates from *Bos taurus*, *Bos indicus*, and *indicus-taurus* dairy cows using sexed sperm. *Theriogenology*, *74*(8), 1349-1355.

Rizos, D., Ward, F., Duffy, P.A.T., Boland, M.P., & Lonergan, P. (2002). Consequences of bovine oocyte maturation, fertilization or early embryo development *in vitro* versus *in vivo*: implications for blastocyst yield and blastocyst quality. *Molecular Reproduction and Development*, *61*(2), 234-248.

Saini, N., Singh, M.K., Shah, S.M., Singh, K.P., Kaushik, R., Manik, R.S., ... & Chauhan, M.S. (2015). Developmental competence of different quality bovine oocytes retrieved through ovum pick-up following *in vitro* maturation and fertilization. *Animal*, *9*(12), 1979-1985.

Sales, J., Iguma, L., Batista, R., Quintão, C., Gama, M., Freitas, C., Pereira, M., Camargo, L., Viana, J., Souza, J., & Baruselli, P. (2015). Effects of a high-energy diet on oocyte quality and *in vitro* embryo production in *Bos indicus* and *Bos taurus* cows. *Journal of Dairy Science*, *98*(5), 3086-3099.

Sirard, M.A., Richard, F., Blondin, P., & Robert, C. (2006). Contribution of the oocyte to embryo quality. *Theriogenology*, *65*(1), 126-136.

Viana, J. (2022). 2021 statistics of embryo production and transfer in domestic farm animals. *Embryo Technology Newsletter*, *40*(4), 22-40.

Ward, F.A., Lonergan, P., Enright, B.P., & Boland, M.P. (2000). Factors affecting recovery and quality of oocytes for bovine embryo production *in vitro* using ovum pick-up technology. *Theriogenology*, *54*(3), 433-446.