



Evaluation and classification of bovine embryos

G.A. Bó^{1,2,4}, R.J. Mapletoft³

¹Instituto de Reproducción Animal Córdoba (IRAC), Córdoba, Argentina.

²Instituto A.P. de Ciencias Básicas y Aplicadas, Universidad Nacional de Villa María, Córdoba, Argentina.

³Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, SK, Canada.

Abstract

Bovine Embryo transfer has been used widely to reproduce the most valuable females in the herd, with about 750,000 embryos produced annually from superovulated donors and more than 450,000 embryos produced using in vitro techniques. Furthermore, embryos are the safest and most cost effective alternatives to move genetics internationally because of their low risk of transmitting diseases. One of most important factors associated with the success and widespread application of this technology is evaluation of the embryos before freezing and/or transfer to a recipient. Embryos are usually classified based on a number code system for their stage of development (1 to 9) and for their quality (1 to 4). The basic principles of embryo evaluation are briefly described.

Keywords: classification, embryo, freezable, IETS, transferable.

Introduction

Although the first embryo transfers were performed in rabbits by Walter Heape in 1890, the commercial bovine embryo transfer industry evolved during the early 1970's, with the introduction of continental breeds of cattle into North America (Betteridge, 2003). Since that time, the use of embryo transfer technology in cattle breeding has continued to increase. Nowadays, more than 750,000 embryos are produced annually from superovulated donors and more than 450,000 embryos are produced using in vitro techniques (Stroud, 2012). Embryos are moving around the world because embryos have a relatively low risk of transmitting diseases. In order to do that, embryo transfer teams must adhere to the recommendations of the Manual of the International Embryo Transfer Society "*A procedural guide and general information for the use of embryo transfer technology emphasizing sanitary procedures*" (Stringfellow and Givens, 2010) which has become the reference source for sanitary procedures used in embryo export protocols. Furthermore, one of most important factors associated with the success and widespread application of this technology is the

evaluation of the embryos before freezing and/or transfer to a recipient. In this manuscript the basic principles of embryo evaluation will be briefly described.

Embryo evaluation

Evaluation of bovine embryos is normally done with a stereomicroscope at 50 to 100X magnification, with the embryo in a small holding dish. It is also necessary to "roll" the embryo on the bottom of the dish so as to view the embryo and zona pellucida from different perspectives. The overall diameter of the bovine embryo is 150 to 190 μm , including a zona pellucida thickness of 12 to 15 μm . The overall diameter of the embryo remains virtually unchanged from the one-cell stage until blastocyst stage. The best predictor of an embryo's viability is its stage of development relative to what it should be on a given day after ovulation. An ideal embryo is compact and spherical. The blastomeres should be of similar size with even color and texture. The cytoplasm should not be granular or vesiculated. The perivitelline space should be clear and contain no cellular debris. The zona pellucida should be uniform; neither cracked nor collapsed and contains no debris on its surface.

It is important to be able to recognize the various stages of development and to compare them with the developmental stage that the embryo should be for the day of the estrous cycle that donors are collected (i.e. usually day 7 after standing estrus). The decision as to whether an embryo is worthy of transfer or freezing and whether the embryo is eligible for export will rely on the expertise and experience of the person that evaluates the embryos. Standardized coding systems for use in describing the stage of development and quality of the embryo are described in Chapter 9 and illustrated in Appendix D of the IETS Manual. The code for stage of development is numeric, ranging from "1", an unfertilized oocyte or a 1-cell embryo to "9", expanding hatched blastocyst. Normally, embryos are collected 7 days after estrus for cryopreservation or transfer and the IETS Manual's standards for the stages likely to be encountered at that time are described below.

⁴Corresponding author: gabrielbo@iracbiogen.com.ar.

Phone: +54(935)1683-2151

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Stages

- Morula (Stage code 3): A mass of at least 16 cells. Individual blastomeres are difficult to discern from one another. The cellular mass of the embryo occupies most of the perivitelline space.
- Compact morula (Stage code 4): Individual blastomeres have coalesced, forming a compact mass. The embryo mass occupies 60 to 70 % of the perivitelline space.
- Early blastocyst (Stage code 5): An embryo that has formed a fluid-filled cavity or blastocele and gives a general appearance of a signet ring. The embryo occupies 70 to 80% of the perivitelline space. Early in this stage the embryo may appear of questionable quality because it is difficult to differentiate inner cell mass from trophoblast cells at this time.
- Blastocyst (Stage code 6): Pronounced differentiation of the outer trophoblast layer and of the darker, more compact inner cell mass is evident. The blastocele is highly prominent, with the embryo occupying most of the perivitelline space. Visual differentiation between the trophoblast and the inner cell mass is possible at this stage of development.
- Expanded blastocyst (Stage Code 7): The overall diameter of the embryo dramatically increases, with a concurrent thinning of the zona pellucida to approximately one-third of its original thickness.
- Hatched blastocyst (Stage code 8): Embryos recovered at this developmental stage can be undergoing the process of hatching or may have completely shed the zona pellucida. Hatched blastocysts may be spherical with a well defined blastocele or may be collapsed. Identification of hatched blastocysts can be difficult unless they re-expand when the signet ring appearance is again obvious.

Quality

The codes for embryo quality is also numerical and are based on morphological integrity of embryos. The codes for embryo quality range from “1” to “4” as follows:

- Code 1: Excellent or Good. The embryos have a symmetrical and spherical mass with individual blastomeres that are uniform in size, color, and density. This embryo is consistent with its expected stage of development. Irregularities should be relatively minor, and at least 85% of the cellular material should be an intact, viable embryonic mass. This judgment should be based on the percentage of embryonic cells represented by the extruded material in the perivitelline space. The zona pellucida should be smooth and have no concave or flat surfaces that might cause the

embryo to adhere to a petri dish or a straw. Code 1 embryos survive well to the freezing/thawing procedure and some practitioners call them “Freezable embryos”. Grade 1 embryos are also those recommended for international trade.

- Code 2: Fair. These embryos have moderate irregularities in the overall shape of the embryonic mass or in size, color, and density of individual cells. At least 50% of the embryonic mass should be intact. Survival of these embryos to the freezing/thawing procedure is lower than with Grade 1 embryos, but pregnancy rates are adequate if embryos are transferred as fresh into suitable recipients. Therefore these embryos are often called “transferable” but not “freezable”.
- Code 3: Poor. These embryos have major irregularities in shape of the embryonic mass or in size, color, and density of individual cells. At least 25% of embryo mass must be intact. These embryos do not survive the freezing/thawing procedure and pregnancy rates are lower than those obtained with fair quality embryos if transferred fresh into suitable recipients.
- Code 4: Dead or degenerating. These could be embryos, oocytes or 1-cell embryos. They are non-viable and should be discarded.

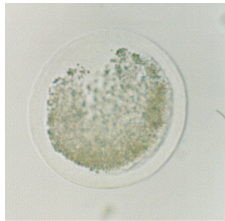
The IETS Manual also states that visual evaluation of embryos is a subjective evaluation of a biological system and is not an exact science. Therefore, pregnancy rates may sometimes be lower than expected due to other factors such as environmental conditions, recipient quality, and technician capability. Generally, unless otherwise agreed, only Code 1 embryos should be utilized in international commerce. Examples of embryos of different stages and quality are indicated in Fig. 1 and 2.

In the superovulated cow, there is likely to be a considerable range of stages of development on any given day after estrus (Mapletoft, 1986). On day 7 after estrus, there may be morula and hatching blastocysts within the same flush. At the same time, there may be embryos of excellent quality and also unfertilized and degenerate embryos. Generally, wide variations in embryo quality and stages of development are signals that the existing embryos are not entirely normal and that pregnancy rates may be disappointing (Mapletoft, 1986). Embryos of excellent and good quality, at the developmental stages of compact morula to blastocyst yield the highest pregnancy rates (Hasler *et al.*, 1987). Other studies have evaluated pregnancy rates according to the stage of embryo development after freezing and thawing. In one study that evaluated 5,287 transfers of embryos cryopreserved in glycerol, pregnancy did not differ between morulae, early blastocysts, blastocysts, and expanded blastocysts (Hasler, 2001). Conversely, other studies found a decrease in pregnancy rates with more developed embryos (blastocysts and expanded



blastocysts; Dochi *et al.*, 1998; Palma *et al.*, 1998; Caccia, 2003; Bó *et al.*, 2012), but in these studies embryos were cryopreserved in ethylene-glycol prior to transfer. Fair and poor quality embryos yield poor pregnancy rates after freezing. It is advisable to select the stage of the embryo

for the synchrony of the recipient. It would also seem that fair and poor quality embryos are most likely to survive transfer if they are placed in the most synchronous recipients (Hasler *et al.*, 1987).



Cycle Day: 7
Stage Code: 1
Quality Code: 4



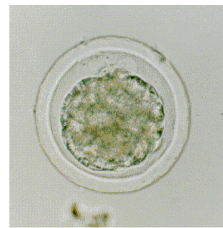
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Stage Code: 1
Quality Code: 4



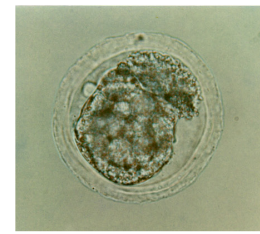
Cycle Day: 7
Stage Code: 1
Quality Code: 4



Cycle Day: 7
Stage Code: 2
Quality Code: 4



Cycle Day: 7
Stage Code: 4
Quality Code: 1



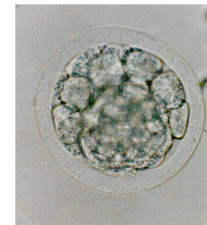
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Stage Code: 4
Quality Code: 2



Cycle Day: 7
Stage Code: 4
Quality Code: 2



Cycle Day: 7
Stage Code: 4
Quality Code: 3



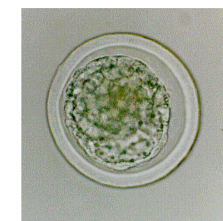
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Stage Code: 4
Quality Code: 3



Cycle Day: 7
Stage Code: 4
Quality Code: 3

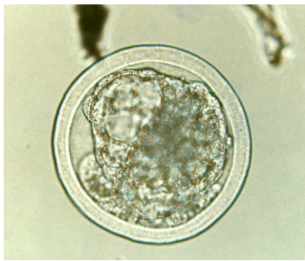


Cycle Day: 7
Stage Code: 4
Quality Code: 3

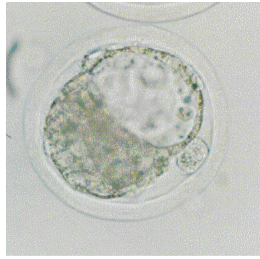


Cycle Day: 7
Stage Code: 5
Quality Code: 1

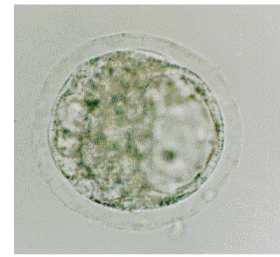
Figure 1. Bovine embryos: examples of developmental stage and quality. Stages 1 to 5.



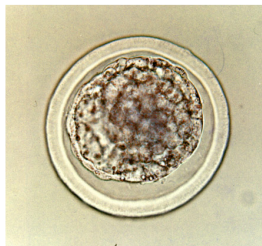
Cycle Day: 7
Stage Code: 5
Quality Code: 2



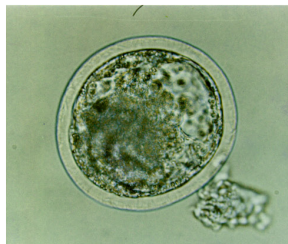
Cycle Day: 7
Stage Code: 5
Quality Code: 1



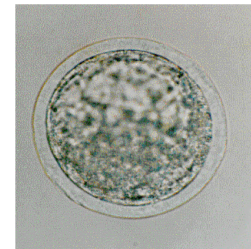
Cycle Day: 7
Stage Code: 5
Quality Code: 2



Cycle Day: 7.5
Stage Code: 5
Quality Code: 1



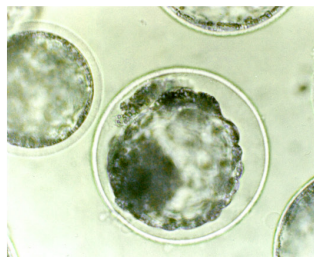
Cycle Day: 7.5
Stage Code: 6
Quality Code: 1



Cycle Day: 7.5
Stage Code: 6
Quality Code: 1



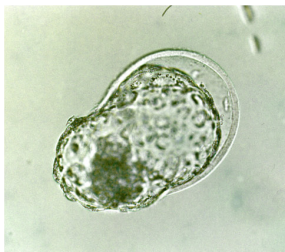
Cycle Day: 7,5
Stage Code: 7
Quality Code: 1



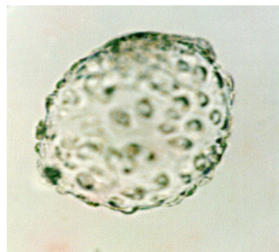
Cycle Day: 7,5
Stage Code: 7
Quality Code: 2



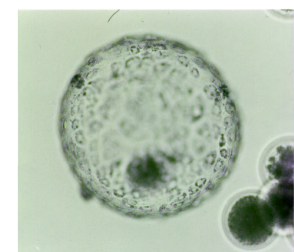
Cycle Day: 7,5
Stage Code: 7
Quality Code: 2



Cycle Day: 8
Stage Code: 8
Quality Code: 1



Cycle Day: 8
Stage Code: 8
Quality Code: 1



Cycle Day: 9
Stage Code: 9
Quality Code: 1

Figure 2. Bovine embryos: examples of developmental stage and quality. Stages 5 to 9.



Summary and final comments

Embryo evaluation is one of the most critical steps of the embryo transfer procedure. The IETS Manual states that embryos must be graded based on a 1 to 9 point system to determine the stage of development and a 1 to 4 system to determine embryo quality. Grade 1 embryos survive well to the freezing/thawing procedure and are recommended for international trade; whereas Grade 2 and 3 must be transferred fresh into suitable recipients. Therefore, the decision as to whether an embryo is worthy of transfer or freezing and whether the embryo is eligible for export will rely on the expertise and experience of the person that evaluates the embryos.

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