Identification and mathematical prediction of different morphokinetic profiles of in vitro developed bovine embryos

Alline de Paula Reis, Mounya Belghiti, Soundouss Messoudi, Brigitte Marquant-Le Guienne, Ludivine Laffont, Sylvie Ruffini, Eugénie Canon, Pierre Adenot, M Le Brusq, Véronique Duranthon, et al.

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Identification and mathematical prediction of different morphokinetic profiles of *in vitro* developed bovine embryos

A.P. Reis[1], M. Beghiti[1,3], S. Messoudi[3], B. Marquant-Le Guienne[2], L. Laffont[1], S. Ruffini[1], E. Canon[1], P. Adenot[1], N. Le Brusq[1], V. Duranthon[1], A. Trubuil[3]

[1]UMR BDR, INRA, ENVA, Université Paris Saclay, Jouy en Josas, France; [2]Allice, Recherche et Développement, Jouy en Josas, France; [3]MaIAGE, INRA, Jouy en Josas, France.

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The current method of embryo classification (IETS, 2013) is based on a static observation of *in vivo* derived embryos at day 7 post insemination (7 dpi). *In vitro* produced embryos (PIV) features impair their classification with this method. Morphokinetics is a powerful source of information to improve the comprehension of PIV embryo developmental behaviour. The objective of this study is to develop a methodology to read and predict different *in vitro* developmental potential of bovine PIV embryos by combinating morphokinetical parameters. Holstein embryos produced from oocytes recovered from slaughterhouse ovaries, *in vitro* fertilized with the semen of 4 different bulls and cultured for 8 days post insemination (8 dpi). Time lapse pictures were taken every 15 minutes throughout the culture period (672 pictures/embryo; Primovision™). The work was performed in 4 tasks (T): T1) identification of the profiles of *in vitro* development; T2) identification and standardisation of a reading method for bovine embryos morphokinetical parameters; T3) mathematical selection of a parsimonious subset of non-correlated parameters and construction of a predictor through the application of a supervised learning approach combining regression and classification (Random Forest) and creation of a mathematical predictor of the embryo development profiles. A total of 172 embryos were observed. T1: 6 morphokinetical profiles were retained: Arrested Embryos (AE: embryos without mitotic activity, showing signs of life); Dead Embryos (DE: embryos with all cells dead); Anarchic Embryos (ANE: embryos with abnormal morphological and/or kinetical development: some of these embryos can result in a blastocyst); Not Hatched Blastocysts (NHB: blastocysts not hatching by 8 dpi); Hatching Blastocysts (HB - blastocysts hatching *in vitro* from 7.3 dpi to 8 dpi) and Early Hatching Blastocysts (EHB - blastocysts hatching from 6 to 7.2 dpi). T2: a guideline was built to standardise reading of 116 parameters (i.e.: type, timing and duration of cell divisions and embryo cycles, LAG phase, cell degeneration, cytoplasmic particles, fragments, vacuoles,...); T3: a subset of parameters was selected and the mathematical predictor was built. The standardisation of the reading methodology is important to promote scientific exchange and study comparisons on the subject (to our knowledge this work resulted into the first morphokinetics reading guideline for the bovine PIV embryos). In addition, this initial work highlighted a new concept for the *in vitro* bovine embryo assessment and further valorisation: it takes into account the very early embryo's dynamic behaviour to predict its further potential of development. The robustness of the algorythm is satisfactory. The specificity, sensitivity, PPV, NPV of the predictive algorithm range respectively in the intervals [0,944; 0,977], [0,640; 0,962], [0,724; 0,892], [0,931; 0,992] for the different profiles. This predictive method can be useful in the field to select embryos for transfer and for research (groups of embryos sharing potential and morphokinetic similarities).