FEMALE FACTORS AFFECTING EMBRYO VIABILITY. EMBRYO CRYOPRESERVATION

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Contents

Summary

In mammals, embryonic development is relatively autonomous and independent of the maternal tract up to the blastocyst stage, the embryo itself being able to regulate cell division and differentiation. However, passage through the genital tract confers improved viability and survival upon cryopreservation to the embryos. Deprivation of factors mediating embryo-maternal communication during *in vitro* culture also exerts medium- and long-term influences that may affect subsequent embryonic development

and viability and lead to pathologies in the offspring. Cryopreservation is an essential tool for embryo and gamete technologies, so that their diffusion, commercial exchange and final pregnancy success with reduced embryonic losses largely depend on accessible and efficient embryo cryopreservation systems. *In vitro* produced (IVP) embryos and cryopreservation need, therefore, to be closely linked. The result of this marriage will be the production of healthy offspring. This would be associated not only with improved cryopreservation procedures, but also with improved rates of IVP embryos and with IVP-embryos more and more similar to their *in vivo* produced counterparts. Nevertheless, inside the genital tract it is difficult to identify the respective female factors (i.e. ovary and oocyte; embryo; oviduct and uterus, etc) that contribute to making a viable embryo. Therefore, in line with progress in yielding embryos of improved quality, we need to define embryo viability as producing a pregnancy with a reasonable expectation of leading to a healthy born individual. However, carrying out embryo transfer (ET) and getting pregnancies to term is not always possible in the expensive experiments using domestic animal species. In this scenario, estimations of embryonic viability, designed as non-expensive and direct laboratory techniques able to replace embryo transfer and pregnancy follow-up with precise and reliable information, are goals of major interest. In this chapter we will analyze maternal factors that determine viability of embryos, describe validated models and techniques necessary to extract knowledge from the sometimes difficult to access and analyze reproductive system, with a special focus on the ability of embryos to survive cryopreservation.

1. Embryonic Development and Viability in Livestock Species

The fundamental events of fertilization and early embryonic development have been investigated in depth and are basically similar in all mammalian species (Figure 1).

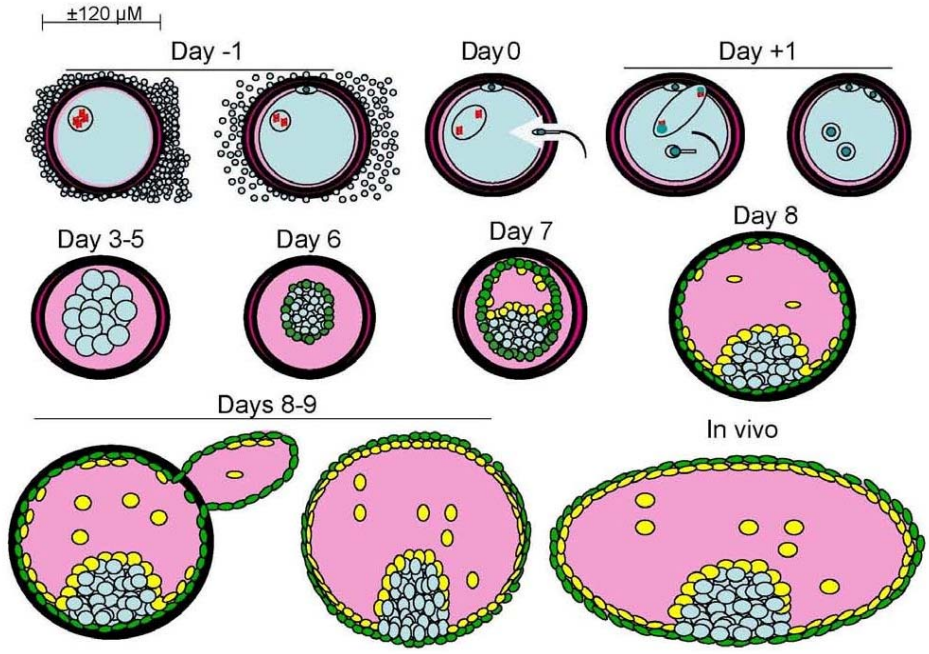

Figure 1. Schematic view of oocyte maturation and embryo development in the bovine species.

Development throughout embryonic stages is essentially the same for both, *in vivo* and *in vitro* embryos, although timing variations can be observed *in vitro* depending on culture conditions. Contrary to primates and mice, embryos from pigs and ruminants elongate before implantation.

In ruminants and pigs, a significant part of the embryonic development takes place during approximately two-thirds to three-quarters of a normal estrous cycle. The embryo itself must impede the resumption of the cyclic activity by using specific signaling molecules. Most if not all embryo technologies in livestock species have a common end-point that is embryo transfer, which allows full term pregnancies in the uterus of a host female that is called embryo-recipient (Figure 2). However, not all embryonic stages are appropriate for embryo transfer and/or cryopreservation, and normally morulae and blastocysts (Figures 3 and 4) are preferred.

Figure 2. Recipient experimental herd from Centro de Biotecnología Animal (SERIDA) in Deva, Gijón, Spain. Cattle are of local (Asturiana de los Valles and Asturiana de la Montaña) and mixed breeds.

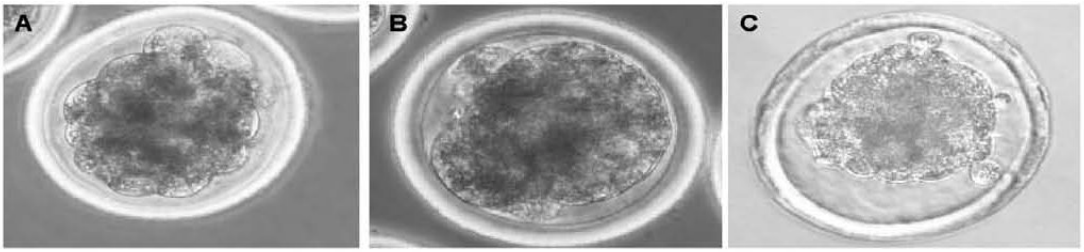

Figure 3. *In vitro* produced, Day-5 bovine morulae at a pre-compaction stage (A; note the scallop trim shape), in vitro produced, Day-6 compact morulae (B), and in vivo recovered, Day-7 morulae from an artificially inseminated, non-superovulated donor

(C). Note that the perivitelline space is larger in the *in vivo* recovered morula (C), suggesting that compaction has been more pronounced.

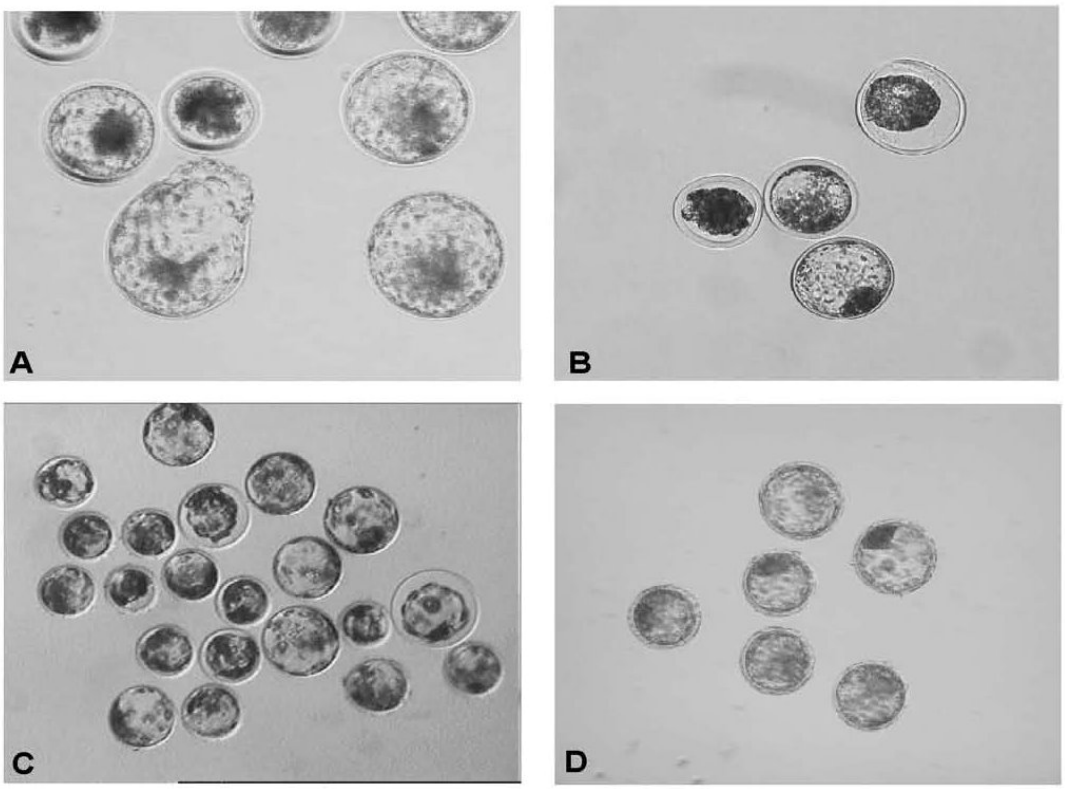

Figure 4. *In vitro* produced bovine (A) and porcine (C) blastocysts; *in vitro* produced bovine embryos that developed in the cow uterus from Day-5 to Day-8 (B); *in vivo* recovered pig blastocysts (pig blastocysts pictures are a courtesy from Dr E.Martínez).

These embryonic stages, when entirely produced in the live animal, present higher pregnancy rates upon transfer, and survival after cryopreservation superior to earlier embryonic stages, especially in pigs.

However, when dealing with IVP embryos (approached elsewhere in this chapter), survival after cryopreservation is compromised, and normally only blastocysts show acceptable cryopreservation survival rates.

Therefore, all concerns related to embryonic development and viability in this chapter will be referred to as blastocyst formation and viability, although subsequent developmental periods will also be discussed as necessary. Embryos produced by nuclear transfer (i.e. clones), and genetically modified and/or micromanipulated embryos, will be excluded from our discourse.

In the live animal, the zygote results from an ovulated oocyte received in the infundibulum that is fertilized in the oviductal ampulla within a specific window of time. The oviduct exhibits an appropriate environment for interactions between oocytes and spermatozoa and the initial embryonic stages.

Transportation of early embryos to the uterus occurs in the oviduct by the orchestrated actions of cilia and smooth muscle. It seems that local signals act in synchrony with gametes and embryonic requirements at specific times, so that there is not a continuous embryo transportation motion, but a regulation over short distances. The oviductal secretion is viscous and maximal around ovulation.

The oviductal isthmus is an extremely thin tubal organ, and is completely filled with oviductal secretion; in the isthmus, myosalpingeal contractions are reduced. Oviductal mucosa bears a complex anfractuosity, although endocrine signals regulate shape and function, so that the appearance will depend on the phase of the estrous cycle. During the estrogenic phase, secretory cells are prominent and ciliated cells are abundant; in the luteal phase, however, the ciliated cells are scarce.

The above traits, together with the very small size of gametes, unfortunately make the oviduct a difficult organ to access and to analyze *in vivo*. Furthermore, in the genital tract it is difficult to identify the respective female factors (i.e. ovary and oocyte; embryo; oviduct and uterus, etc) that contribute to making a viable embryo, so that modeling specific strategies is necessary if we wish to identify factors influencing embryonic viability.

This is especially interesting as our aim is to produce IVP embryos with improved quality. The earliest signaling between the embryo and the mother, known as embryo-maternal interactions, occurs in the oviduct.

However, signaling during the early development (prior to the implantation window) occurs exclusively through local circuits, leading to a lack of specific molecules detectable in blood so that the mother is unaware of the pregnancy.

The embryo enters the uterus around the 16-cell morula stage. In the uterus, the early embryo is surrounded by a thin fluid layer stabilized by glycoproteins that protect the embryo against osmotic changes and fluctuations.

Similar to the oviduct, this microenvironment would avoid dispersal of ions and essential compounds, particularly during cilia beating or muscular contraction.

Progesterone and estradiol trigger peripheral changes leading to temporal modifications within the endometrium (Figure 5) and the oviduct, which allows an appropriate substrate for the ovum and the embryo throughout their development.

The composition of the uterine fluid (UF) would therefore be a reflection of general modifications dependent on estrus cycle phases and interactions between the embryos and the genital tract.

In monovulatory species, analysis of local interactions between early embryos and the genital tract *in vivo* still remains a challenge because of intrinsic technical difficulties in the exploration of the uterine environment surrounding the embryo.

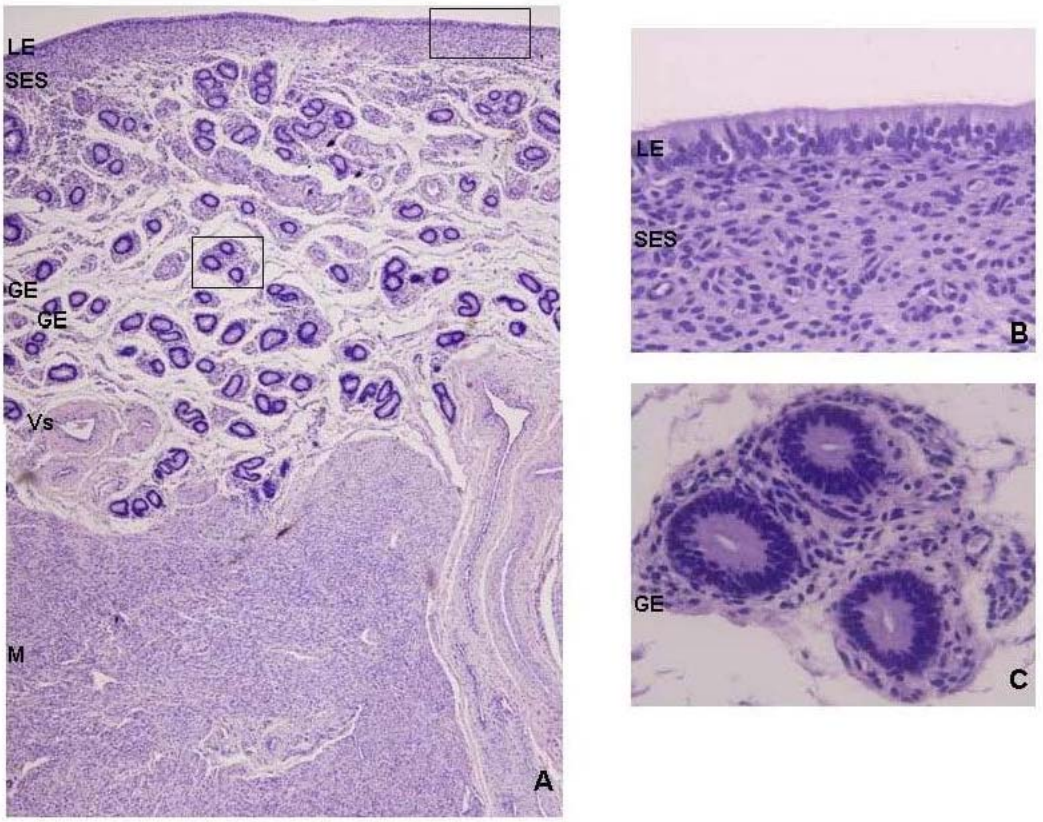

Figure 5. Main cellular compartments of the bovine endometrium at D8 of the estrous cycle, stained with hematoxylin-eosine. A) Full thickness section on the bovine endometrium: GE, glandular epithelium; LE, luminal epithelium; M, myometrium; SES, subepithelial stroma; Vs, blood vessel. B) High magnification photograph of the luminal epithelia (LE), which consists of a single layer of columnar epithelium, resting on the subepithelial stroma (SES). C) High magnification photograph of three uterine glands. Note that the nuclei of the epithelial cells are shifted to the basal pole, which is characteristic of the secretory phase.

Throughout the genital tract, the maternal uterus corrects for a number of differential effects that can be observed in embryos produced *in vitro* (approached in another chapter). Generally, *in vitro* systems do not provide the nutritive and environmental conditions with an exact molecular timing. These specific conditions arise from an efficient early embryo maternal communication.

A definition of embryonic viability should be based on successful pregnancy expectations and offspring health. If either of these two requirements is not fulfilled, embryonic viability should be considered compromised. A number of events can occur that alter pregnancy progression, which include early embryonic losses and miscarriage. Similarly, a number of alterations that are first observed usually at birth include abnormalities that can lead to enhanced mortality and derive from an inappropriate peri-conceptional and/or early embryonic environment. Ultimately, these alterations can also affect adult life. Embryonic viability, therefore, would consist of the probability of an embryo to establish pregnancy with a reasonable expectation to deliver to term a healthy offspring.

Evaluation of embryonic viability is of enormous interest in livestock reproductive technology. However, due to restrictions that include economical, practical and scientific reasons, analysis of embryonic viability by embryo transfer and pregnancy follow-up is often difficult to accomplish in an experimental framework. As a consequence, there is intense research on analytical techniques focusing on producing both embryos with improved viability starting from new or improved embryo production systems, media and/or treatments. Evaluation of the latter would not require single embryo culture or individual embryonic follow-up (experiments in livestock are normally based on group culture systems). A second research line deals with identification of markers of embryonic viability, which requires individual embryo follow up and/or single embryo culture.

Techniques analyzing viability require validation; that is to say, that ET is or should have been necessary to a certain extent in order to correlate laboratory results with the actual pregnancy rates attributable to markers identified in single embryos. Unfortunately, as we will see below, evaluation after ET is not a rule.

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