EMBRYO PRODUCTION

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Summary

Researchers and technicians have developed several protocols for the production of embryos both *in vitro* and *in vivo* to be used both in animal breeding to increase the number of superior genotypes, and as a research tool. *In vivo* embryo production by Multiple Ovulation and Embryo Transfer (MOET) still accounts for the majority of embryos produced worldwide, but it has high costs and the number of produced embryos is often highly variable and unpredictable because of the donor effect. The application of MOET technologies requires the selection of valuable donors in perfect gynecological conditions and the administration of a superovulatory treatment to increase the number of growing follicles. Embryos are generally recovered 6-7 days after the induction of ovulation and either transferred to synchronized recipients or frozen. *In vitro* embryo production (IVP) is a newer and more flexible approach, although it requires specific laboratory expertise and equipment. Oocytes can be obtained both from ovaries collected at the slaughterhouse or from live donors by follicle aspiration. Cumulus-oocyte complexes are then selected and processed *in vitro*

for embryo production. This technology involves three major steps, oocyte maturation, fertilization and embryo culture up to the blastocyst stage, which require different culture media and incubation conditions. The goal of increasing efficiency of *in vitro* embryo production has been the driving force for much of the applied research in embryo biology and culture. Yet, *in vivo* produced embryos are still of higher quality, as evidenced by higher implantation rates after transfer and cryosurvival.

1. Introduction

Embryo production, both *in vivo* and *in vitro*, is a reproductive technology generally used to improve the number of offspring from selected females. *In vivo* embryo production through multiple ovulation and embryo transfer (MOET) is a well-established procedure, especially in cattle, and it is mostly used for commercial purposes.

The application of MOET technology requires, in mono-ovulatory species, the administration of hormones to stimulate follicular development and the subsequent stimulation of luteolysis. At estrus the donor is either artificially inseminated or naturally mated and approximately 7 days later the uterus is flushed to recover the embryos.

Thereafter the good quality embryos are selected and either transferred to synchronized recipients or cryopreserved. In general terms, MOET allows the production of good quality embryos, (i.e., embryos with high developmental competence to term after transfer into synchronized recipients), and high cryotolerance (i.e. ability to survive to the freezing and thawing procedures).

In vitro embryo production (IVP) is a more flexible procedure, which permits the production of embryos from oocytes collected both from abattoir materials and from live donors by follicle aspiration. It requires the presence of an incubator with controlled temperature and gas atmosphere and of laboratory equipment. After collection, good quality oocytes are selected and processed for *in vitro* embryo production. Embryos at the blastocyst stage, which is the stage of development suitable both for transfer and cryopreservation, are produced within a week.

Despite the great improvement in IVP efficiency over the last years, there is ample evidence showing that differences between *in vivo* and *in vitro* produced embryos still exist, which involve both morphological and molecular aspects. These differences are probably induced by several factors such as breed, oocyte quality, follicular environment, fertilization, and embryo culture environment. *In vitro* derived embryos usually have darker coloration, a lack of compactness of the cellular mass, alteration in the ratio of the inner cell mass to trophoblast cells, lower total cell number, greater mixoploidy, and alterations in gene expression and cell metabolism. These alterations may be involved in the low rate of embryo cryosurvival and phenotypic disorders observed in fetuses and offspring derived from *in vitro* produced embryos.

2. *In vitro* embryo production

In vitro embryo production (IVP) is a reproductive biotechnology that has great potential for speeding up genetic improvement in livestock, but it is also an important research tool for mammal embryology. Reliable procedures allow *in vitro* maturation and fertilization of bovine, sheep and goat oocytes and several culture protocols can be used to grow them for about a week up to the stage suitable for transfer or freezing. IVP was developed initially as a research tool and was applied to rescue follicular oocytes of slaughtered donors. In bovine, the use of IVP by commercial embryo companies has increased, and currently IVP embryos represent a considerable percentage of the total number of cattle embryos produced in the whole world.

In porcine, IVP technologies are less developed. The first successful production of piglets from *in vitro* matured and *in vitro* fertilized oocytes was described in 1989, but only in the present decade it has been demonstrated that porcine *in vitro* produced blastocysts could develop to full term. Although a great deal of progress has been made, current IVP systems still suffer from a low rate and poor quality of *in vitro* produced embryos compared with other species. The low efficiency of this technique may be due to several factors including a reduced incidence of male pronuclear formation, a high incidence of polyspermy and suboptimal conditions for embryo culture.

In vitro embryo production involves different phases: the collection of oocytes, which can be obtained either from abattoir derived materials or from live animals through the Ovum-Pick Up (OPU) technique; the selection of the good quality oocytes and their incorporation into the *in vitro* maturation system; the preparation of sperm and matured oocytes for *in vitro* fertilization; and the culture of the obtained embryos up to the expanded blastocyst stage of development.

2.1. Collection of Oocytes

2.1.1. Abattoir Material

Oocytes can be easily recovered from ovaries collected at the slaughterhouse. Ovaries should be transported to the laboratory within 1 h in Dulbecco's phosphate buffered saline (PBS) at temperature between 25 and 35°C in order to preserve oocyte developmental competence. It is known that the metabolic activities of cells are slowed down or completely arrested at low temperatures, whereas cellular autolysis could occur in ovaries during a long period of transportation at high temperatures. Many authors have reported, in several species, that ovary storage at 38°C for several hours decreased the rate of blastocyst formation. Ovary storage at low temperatures in bovine does not seem to affect oocyte maturation and the potential of oocytes to develop into blasctocyst. However, oocytes from porcine are more sensitive at low temperatures.

After washing the ovaries in fresh medium, oocytes can be collected from antral follicles either by aspiration or by slicing. In the first case, antral follicle > 2 mm in diameter are aspirated using a needle (usually 19-20 gauge) connected to a 5 mL syringe containing Tissue Culture Medium-199 (TCM-199) with Earle's salts and

bicarbonate supplemented with Hepes, heparin, antibiotics (most common used: penicillin/streptomycin solution or gentamycin) and polyvinyl alcohol (PVA). In the other case, ovaries are sliced using a micro-blade and the follicle content released in the same medium described above. Thereafter, cumulus–oocyte complexes (COCs; within the follicle oocytes are surrounded by several layers of somatic cells, the cumulus cells) are evaluated under a stereomicroscope and selected for *in vitro* maturation.

2.1.2. Ovum-Pick-Up (OPU) Technique

The Ovum-Pick Up (OPU) is a procedure which allows the aspiration of the follicles, and thus the collection of the oocytes, from live animals after the endoscopic or ultrasound visualization of the ovaries. The current technology of OPU/IVP aims at harvesting oocytes from preselected genetically superior living donors, followed by *in vitro* maturation, fertilization and culture until embryos have reached the morula or blastocyst stage. This procedure allows the repeated production of embryos from live animals of particular value and it is a serious alternative to superovulation. After the initial development of the OPU technique, many studies were undertaken to optimize the quality of the harvested oocytes and thus the subsequent embryo yield. The first studies aimed at optimizing the surgical procedure, and were focused on technical improvements (needle geometry, vacuum pressure, etc..). At the same time, biological factors such as the donor animal herself, hormonal prestimulation, timing and frequency of OPU and the experience of the OPU operators were also investigated. The most significant improvement with respect to improved oocyte yield and quality and thus subsequent embryo production was hormonal pre-stimulation prior to OPU using gonadotropins. Both in bovine and in small ruminants, it has been demonstrated that multiple treatment with exogenous gonadotrophins before follicular aspiration improves the number of oocytes recovered and increase embryo production of the processed oocytes compared to nonstimulated donors. However, systematic experiences showed that the quality of recovered oocytes can be affected by follicular dimension and health, hormonal profile, interval between the last exogenous gonadotrophin stimulation and follicle aspiration and treatment of donors.

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Biographical Sketch

Fiammetta Berlinguer was born in Sassari, Italy, the 5th of July 1975. She graduated as Doctor in Veterinary Medicine from Sassari University (Italy) in 1999 and was awarded PhD from Sassari University in 2003 for studies in assisted reproductive techniques in small ruminants. Major fields of study include application of assisted reproductive technologies in avian and mammal species.
She joined the Sassari University in 2004 with a research contract on reproductive physiology and preimplantation embryos development, followed by a teaching and research contract within the PhD course in "Animal Biology, Pathology and Reproduction". In the same years she was professor in Domestic Animal Endocrinology, course of study in Animal Reproduction, and professor in Etology and Adaptation, course of study in Animal Breeding. After a year as a postdoctoral fellow, in 2005 she earned a position as staff researcher at the Sassari University (Italy), which is her current job. She has published 36 peer reviewed publications and conference abstracts covering research into evaluation of intrinsic and extrinsic factors affecting oocyte quality and pre-implantation embryo development *in vitro*, oocyte and embryos cryopreservation, semen evaluation and cryopreservation in avian and mammal species, application of assisted reproductive technologies in endangered species management.