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Obtaining farm animal embryos *in vitro*[*]

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ABSTRACT

This paper reviews the basic knowledge about obtaining farm animal embryos *in vitro* with special focus on differences among species and application of this procedure in the future. *In vitro* production of farm animal embryos consists of *in vitro* maturation (IVM) of oocytes, *in vitro* fertilization (IVF) of matured oocytes, and *in vitro* culture (IVC) of embryos. Oocytes can be collected from live animals (by laparotomy, laparoscopy, Ovum Pick Up) or from slaughtered ones (by puncture, sectioning). Usually immature oocytes are isolated, and during IVM they reach maturity. Matured oocytes are cultured with sperm (IVF), leading to the formation of zygotes. In the case of fertilization problems (horse, pig), intracytoplasmic sperm injection is used. The zygotes are usually cultured (IVC) to the morula and blastocyst stages. These embryos can be transferred to recipients or frozen/vitrified. Offspring have been obtained after transfer of cattle, sheep, goat, pig and horse embryos. This procedure can be used in animal breeding, biotechnology, medicine, and basic research.

KEY WORDS: oocytes, sperm, embryos, *in vitro*, farm animals

INTRODUCTION

Obtaining farm animal embryos *in vitro* is a biotechnique used both commercially (*in vitro* production, IVP), in basic research, as well as in infertility

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treatment (Gordon, 2004; Thibier, 2005; Betteridge, 2006; Galli and Lazzari, 2008; Duszewska et al., 2010; Figueirêdo Freitas et al., 2010; Dang-Nguyen et al., 2011; Zhao et al., 2011). Commercially, it is used in animal breeding and biotechnology, hence it is called *in vitro* production of embryos (IETS-www.iets.org). The goal of this procedure is entirely different in basic research and infertility treatment, where it plays the same role as the assisted reproduction techniques (ART) used in humans (Gardner et al., 2011). For this reason it is hard to call this biotechnique *in vitro* production of embryos, and instead it is called obtaining embryos *in vitro* (Duszewska et al., 2010).

Obtaining farm animal embryos *in vitro* is a multi-step procedure comprising: *in vitro* maturation (IVM), *in vitro* fertilization (IVF), and *in vitro* culture (IVC) (Scheme 1). Offspring of some farm animal species have been obtained after transfer of embryos obtained *in vitro* to recipients, including sheep (Gandolfi et al., 1987), cattle (Lu et al., 1988), pigs (Mattioli et al., 1989), and goats (Crozet et al., 1993). For many years, obtaining horse embryos *in vitro* presented the biggest challenge. In 1991 two foals were born, albeit from naturally matured oocytes that were fertilized and cultured *in vitro* (Palmer et al., 1991).

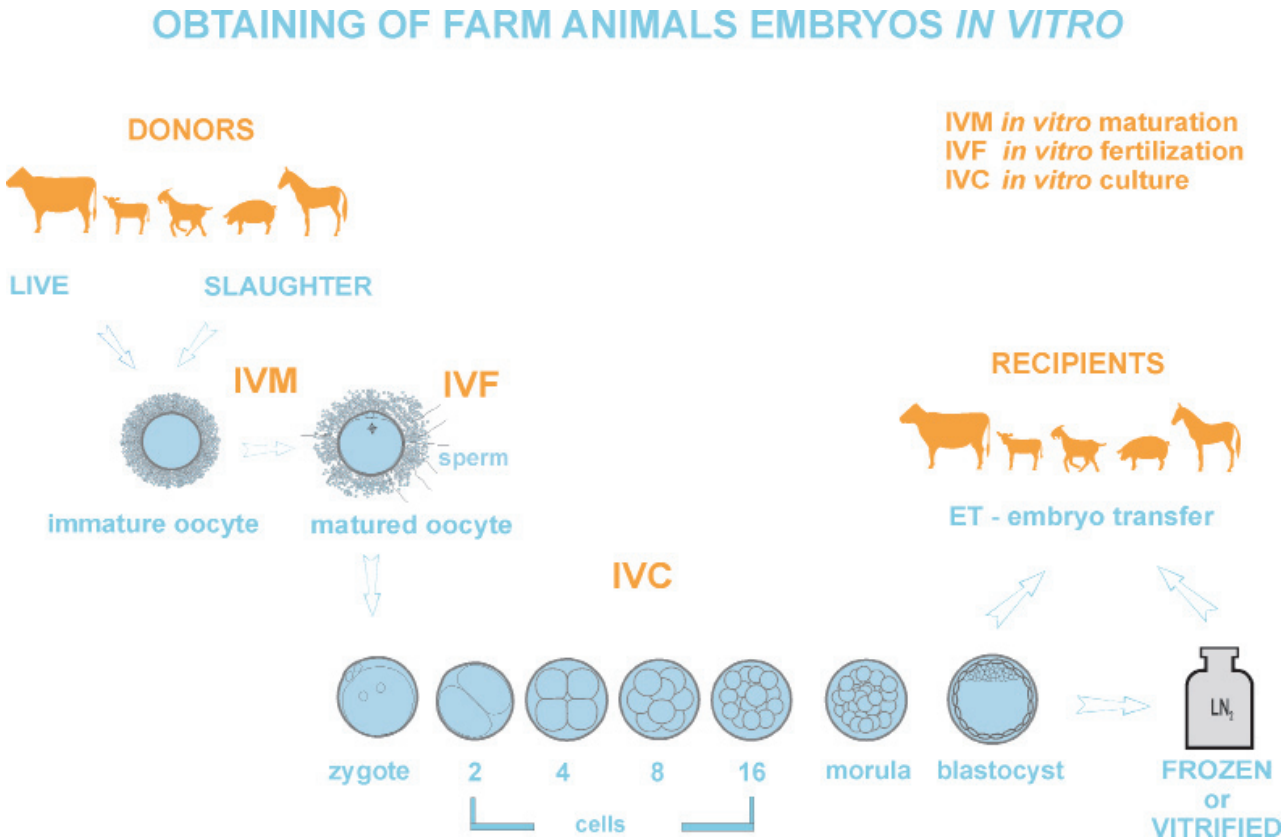
The most important factors responsible for success in obtaining embryos *in vitro* are: the source of oocytes and sperm (including the age and physiological state of female and male), technical factors, and highly skilled laboratory and veterinary staff. The key technical factors are: media for maturation, fertilization, and embryo culture (containing water, amino acids, vitamins, hormones, growth factors, and additives obtained from albumin, serum, etc.), conditions of maturation, fertilization, and culture (temperature, CO_2 level, humidity), and the timing of oocyte maturation, fertilization, and embryo development (Duran, 2000; Camargo et al., 2006).

All of these requirements are characteristic of the respective farm animal species and have been described in detail in the literature: cattle (Hoshi, 2003; Lonergan, 2007; Lonergan and Fair, 2008), pigs (Abeydeera, 2002; Nagai et al., 2006; Lonergan and Fair, 2008; Gil et al., 2010; Dang-Nguyen et al., 2011), sheep and goats (Cognie et al., 2003; Paramio, 2010), and horses (Squires at el., 2003; Betteridge, 2006, 2007; Hinrichs, 2010).

PROCEDURE OF OBTAINING EMBRYOS *IN VITRO* FROM FARM ANIMALS

In vitro maturation of oocytes (IVM)

Oocytes are collected from the follicles located in the cortex of the ovary. There are differences in the localization of the cortex among farm animal species. In cattle, sheep, goats, and pigs the cortex is the outermost layer, however, in



Scheme 1. Obtaining of farm animals embryos *in vitro*. In the first stage (IVM), oocytes are collected from the donors (live or slaughtered) and subsequently are subject to *in vitro* maturation. In the next stage *in vitro* fertilization matured oocytes are incubated with sperm which leads to fertilization. In the last stage *in vitro* culture, the embryos obtained from IVF are cultured *in vitro*, usually up to blastocyst stage, when they can be transferred to recipients (ET) or can be frozen or vitrified and used at a later date

mares the cortical layer is located inside the ovary, which poses some problems in oocyte collection.

Oocytes can be collected from live or slaughtered animals. The methods used for collection from live animals are: laparotomy (in all species), laparoscopy (sheep, goats and swine, as well as horses and cattle) and ovum pick up (OPU) (cattle, horses). OPU entails puncture of ovarian follicles. In sheep and goats, laparoscopy combined with OPU is a popular method (Pieterse et al., 1991; Carter et al., 2000; Galli et al., 2001; Abeydeera, 2002; Baldassarre et al., 2002; Cogine et al., 2003, 2004; Squires et al., 2003; Colleoni et al., 2007; Paramio, 2010).

Oocytes can be collected from females during natural hormonal cycles or after hormonal stimulation. Pregnant or young females can be used as oocyte donors (Romaguera, 2011). Mature oocytes (Metaphase II) can be isolated, although in the majority of cases, immature ones are harvested (Miyano, 2003). The frequency of oocyte isolation is often a function of ovarian follicle development and maturation, which is species specific (Hendriksen, 2000; Hirao, 2011; Sirard, 2011).

The most frequently used methods of obtaining material from slaughterhouse samples are: puncture of an ovarian follicle with a needle connected to a suction pump (in all species), sectioning of ovaries (mostly in mares), and isolation of ovarian follicles and subsequent collection of oocytes (in all species) (Abeydeera, 2002; Cogine et al., 2003; Squires et al., 2003).

Immature oocytes are surrounded by several tightly packed layers of granulosa cells called the corona radiata, with which they form the cumulus-oocyte complex (COC), and a cluster of cells forming the cumulus oophorus (Abeydeera, 2002; Cognie et al., 2003; Hoshi, 2003; Nagai et al., 2006; Lonergan, 2007; Lonergan and Fair, 2008; Gil et al., 2010; Paramio, 2010; Dang-Nguyen et al., 2011). In mares, oocytes can be surrounded by both tightly packed and dispersed layers of the corona radiata, but both types of oocytes are competed for maturation (Squires et al., 2003; Betteridge, 2006; Hinrichs, 2010, 2010a).

Immature oocytes are at the prophase stage of the first meiotic division, namely in GV (germinal vesicle) stage. Oocyte maturation encompasses simultaneous nuclear and cytoplasmic changes. The key factors regulating meiosis are MPF (maturation promoting factor) and MAP (mitogen activated protein kinase), whose activity is necessary for the oocytes to reach the metaphase II stage (Gilchrist and Thompson, 2007).

In the majority of mammals, resumption of meiosis *in vitro* is spontaneous and is commenced by germinal vesicle break down (GVBD) and completed by reaching the metaphase II stage of meiotic division by the oocyte, the stage when it can be properly fertilized (Miyano and Manabe, 2007). Cytoplasmic maturation includes a number of modifications - both morphological (organelle migration, increase in the number of certain organelles, such as mitochondria, and formation

of the cytoskeleton and spindle apparatus) and biochemical (cell cycle regulation, metabolic processes, accumulation of informational and storage material) (Watson, 2007).

The granulosa cells of the corona radiata play an important role in the maturation of the oocyte, being responsible for contact of the oocyte with the environment and, therefore, mediating the hormonal and metabolic regulation of this process (Abeydeera, 2002; Cognie et al., 2003; Hoshi, 2003; Nagai et al., 2006; Lonergan, 2007; Lonergan and Fair, 2008; Gil et al., 2010; Paramio, 2010; Dang-Nguyen et al., 2011).

The matured oocyte is characterized by an evenly dispersed corona radiata and cytoplasm without any signs of degeneration, fragmentation, or vacuolization. In some farm animal species, the first polar body can be observed, however, in the majority, it is usually obscured by the opaque oocyte cytoplasm (due to its high lipid content), therefore, the stage of maturation is determined indirectly on the basis of dispersion of the granulosa cells of the corona radiata (Abeydeera, 2002; Cognie et al., 2003; Hoshi, 2003; Nagai et al., 2006; Lonergan, 2007; Lonergan and Fair, 2008; Gil et al., 2010; Paramio, 2010; Dang-Nguyen et al., 2011).

There are also differences in the timing of oocyte maturation *in vitro* among species (Banwell and Thompson, 2008). In cows, sheep, and goats, oocytes reach maturity (metaphase II), after 24 h culture, and in horse, between 24 and 30 h. Maturation takes the longest in pig oocytes, where M II is reached within 40-48 h of culture (Abeydeera, 2002; Cognie et al., 2003; Hoshi, 2003; Nagai et al., 2006; Lonergan, 2007; Lonergan and Fair, 2008; Gil et al., 2010; Paramio, 2010; Dang-Nguyen et al., 2011).

In vitro fertilization of matured oocytes

The next step in obtaining embryos *in vitro* is the fertilization of matured oocytes. Usually, thawed sperm is used for fertilization, however, since in sheep and horses problems with freezing sperm occur, fresh sperm is used in these species (Morrell and Rodriguez-Martinez, 2010) Also, XY sperm separation can be performed and the obtained sperm used for fertilization (Seidel, 2003; Cran, 2007).

Spermatozoa used in *in vitro* fertilization have to undergo capacitation, which starts with removal of the glycoprotein coat. This subsequently leads to structural modifications and changes in the intramembrane protein topography of the head and tail of the spermatozoon. The changes in the sperm head enable the acrosomal reaction to occur, whereas changes in the tail lead to hyperactivation (augmented movement of the sperm). *In vitro* capacitation is a reversible process, in contrast to the acrosomal reaction, which is an irreversible process allowing the sperm to

penetrate the zona pellucida (Tulsiani et al., 2007; Bailey, 2010; Gadella, 2011).

The acrosomal reaction consists of the release of enzymes that allow the sperm to penetrate the zona pellucida. The entry of sperm leads to oocyte activation, completion of the II meiotic division resulting in the extrusion of the II polar body. Subsequently, two pronuclei are formed, DNA replication taking place in each of them, and the first mitotic division is initiated when the chromosomes of the oocyte and sperm form a common metaphase plate. Differences among species can pertain to asynchrony in pronuclei formation and the moment of replication initiation (Gadella, 2010; Ikawa et al., 2010; Visconti and Florman, 2010; Tulsiani et al., 2011).

Fertilization in pigs and horses poses many problems, because polyspermy (the entry of more than one sperm into the oocyte) can often be observed both *in vivo* and *in vitro*. Interestingly, in the case of *in vitro* matured oocytes, the frequency of polyspermy is much lower than in *in vivo* matured oocytes. Polyspermy leads to creation of polyploid embryos. In some cases triploid embryos can develop up to the blastocyst stage, however, at later stages they degenerate and undergo resorption. Because of the high lipid content in the cytoplasm it is not possible to evaluate the quality of these embryos in the way it is done with human embryos (Abeydeera, 2002; Cognie et al., 2003; Hoshi, 2003; Nagai et al., 2006; Lonergan, 2007; Lonergan and Fair, 2008; Gil et al., 2010; Paramio, 2010; Dang-Nguyen et al., 2011).

In pigs, polyspermy has been successfully overcome by modification of the fertilization medium, and in horses it is circumvented by the use of intracytoplasmic sperm injection (ICSI) (Squires, 1996). In pigs, chromatin decondensation defects are sometimes observed in the formation of the male pronucleus (Gil et al., 2010).

In horses, *in vitro* fertilization problems are related to sperm entry into the oocyte, and, specifically, to the penetration of the zona pellucida (Alm et al., 2001; Roasa et al., 2007). To date there are only two foals that were obtained with the use of conventional fertilization (Palmer, 1991). To circumvent this problem, the ICSI technique, that is, the introduction of the spermatozoon into the cytoplasm of *in vitro* matured oocyte, is routinely used (Palermo et al., 1992; Squires et al., 2003), and has been further modified by the use of piezoICSI (Choi et al., 2002; Galli et al., 2002).

The ICSI technique can be used in all farm animal species (Garcia-Rosello et al., 2009), although in cattle, sheep, goats, and pig *in vitro* fertilization poses no problems (Goto and Yanagita, 1995; Catt et al., 1996; Kolbe and Holtz, 2000).

In all farm animal species, the timing of fertilization is similar and takes place 18-20 h from insemination. Slight differences are the result of introduction of the sperm to the oocyte in the ICSI procedure, which can lead to earlier formation of

the zygote (Goto and Yanagita, 1995; Catt et al., 1996; Kolbe and Holtz, 2000; Garcia-Rosello et al., 2009; Nakai et al., 2011).

In vitro culture of embryos

The last stage of obtaining embryos *in vitro* is their culture, that is the development of the embryos from zygote to the morula and blastocyst stages. This encompasses nuclear and cytoplasmic modifications and their mutual interactions, changes in embryo metabolism, embryonic genome activation, modifications of gene expression, formation of the morula and compaction of blastomeres, formation of the blastocyst with a characteristic structure named the blastocyst cavity that arises as a result of cavitation, and formation of additional structures such as the capsule in the horse embryos (Abeydeera, 2002; Cognie et al., 2003; Hoshi, 2003; Nagai et al., 2006; Lonergan, 2007; Lonergan and Fair, 2008; Gil et al., 2010; Paramio, 2010; Dang-Nguyen et al., 2011). The capsule is a structure located between trophoblast and zona pellucida, which is generated by the embryo. Its role is to protect the embryo and it is found in embryos obtained both *in vitro* and *in vivo* (Tremoleda et al., 2003).

One of the most important processes that take place during embryonic development both *in vitro* and *in vivo* is the acquisition of control by the embryonic genome - the maternal-zygotic transition (MET) (Vigneault et al., 2004). Activation of the zygotic genome takes place at the 4-blastomere stage in pigs, and the 8-16 blastomere stage in cattle, sheep, goats, and horses. Up until the moment of embryonic genome activation, embryonic development is based on the material stored during oocyte maturation (Sirard, 2010).

Differences occur among species in *in vitro* obtained blastocyst morphology that concern the size of the embryos and the number of blastomeres forming the inner cell mass (ICM) and the trophectoderm. The significant differences in the number of blastomeres in horse embryos between blastocysts obtained *in vivo* (1761 blastomeres) and *in vitro* (255 blastomeres) deserve special attention. In other species the observed differences are not that great (Pomar et al., 2005).

The duration of embryonic development up to the blastocyst stage is characteristic of a given species and is connected with the length and number of consecutive cell cycles. Pig embryos have the shortest development time, reaching blastocyst stage in only 144 h, while cattle, ovine, and goat blastocysts are formed within 168 h. Equine embryos develop the longest (168-192 h of culture) (Abeydeera, 2002; Cognie et al., 2003; Hoshi, 2003; Nagai et al., 2006; Lonergan, 2007; Lonergan and Fair, 2008; Gil et al., 2010; Paramio, 2010; Dang-Nguyen et al., 2011).

Embryo transfer

After the transfer of farm species embryos obtained *in vitro* to recipients, the percentage of offspring is lower than in case of embryos obtained *in vivo* (Abeydeera, 2002; Cognie et al., 2003; Hoshi, 2003; Nagai et al., 2006; Lonergan, 2007; Lonergan and Fair, 2008; Gil et al., 2010; Paramio, 2010; Dang-Nguyen et al., 2011).

The percentage of calving, kidding, or foaling after transfer of *in vivo* and *in vitro* embryos is, respectively, cattle, 70 vs 45; sheep, 75 vs 30; horses, 75 vs 20; goats, 75 vs 30; pigs, 60 vs 5. In the case of pigs the percentage is extremely low (Pomar et al., 2005).

Embryos obtained *in vitro* can be frozen or vitrified and later transferred to recipients. Their developmental abilities after transfer are much lower, however, than non-frozen embryos obtained *in vitro*. In the majority of farm animal species, progeny is obtained after transfer of embryos frozen at the early or mature blastocyst stage. On the other hand, much less offspring can be obtained after transfer of frozen, morula-stage *in vitro* embryos. Great hopes lie in vitrification, which allows for higher survival rates of embryos and, moreover, enables obtaining more progeny after their transfer (Dobrinsky, 2002; Vajta and Kuwayama, 2006).

CRYOPRESERVATION OF EMBRYOS AND OOCYTES

Cryopreservation of oocytes and embryos is performed in two ways: slow freezing and vitrification. Slow freezing is a conventional method based on a low concentration of cryoprotectant and slow temperature reduction. Vitrification is an alternative method characterized by a high cryoprotectant concentration and ultra-rapid cooling rates, which makes it possible to avoid water precipitation, thus preventing intracellular ice crystal formation. In this case, the short time of vitrification and elimination of costly programmable freezing equipment is of great importance. There are many modifications of freezing and vitrification procedures (Vajta and Kuwayama, 2006; Ledda et al., 2007; Pereira and Marques, 2008; Prentiz and Anzar, 2011; Saragusty and Arav, 2011).

Interspecific differences exist in cryopreservation resistance of both oocytes and embryos. Oocytes can be cryopreserved at the GV stage and in metaphase II, but compared with embryos, oocytes are much more sensitive. As a result of cryoconservation they develop morphological and functional defects (Martins et al., 2005; Contreras et al., 2008; Pereira and Marques, 2008), therefore, the cryopreservation efficiency is low. The specificity of oocyte cryopreservation is due to the granulosa cells surrounding them; because of their role in oocyte maturation they can not be removed. Further problems with oocyte cryopreservation are

related to the morphology of the metaphase II oocyte cell membrane and cortical granules and spindle apparatus (Pereira and Marques, 2008).

With the use of the conventional, slow method of freezing oocytes, offspring have been obtained from many species, such as cattle (Otoi et al., 1996), sheep (Woods et al., 2004), horse (Maclellan et al., 2002). After vitrification, offspring have been obtained in cattle (Sripunya et al., 2010), pigs (Liu, et al., 2008), sheep (Succu et al., 2007), and horse (Bogliolo et al., 2006).

Cryopreservation of farm animal embryos obtained *in vitro* does not pose as many problems as that of oocytes, however, even in that case the efficiency of freezing depends on the species, developmental stage of the embryos, and their origin (*in vitro* or *in vivo*), as well as the method of cryoconservation and choice of cryoprotectant. Cattle and sheep embryos are the most resistant, the least - pig and horse embryos (Pereira and Marques, 2008). In the 1980s and 1990s, progeny was obtained after freezing and thawing of farm animal embryos. Also after vitrification of *in vitro* embryos, progeny of many farm animals has been obtained (Dobrinsky, 2002; Vajta and Kuwayama, 2006; Saragusty and Arav, 2011).

DEVELOPMENT POTENTIAL OF EMBRYOS OBTAINED *IN VITRO*

The aim of the procedure of *in vitro* embryo production is to obtain a large number and high quality of blastocysts. The number of embryos obtained depends on the number of collected oocytes and their quality. On the other hand, the quality of the blastocysts also depends on the conditions during oocyte maturation and embryo culture and on the bull's influence. Both quality and quantity of blastocysts determines the number of offspring and its healthiness after transfer of those embryos into recipients (van Wagtendonk-de Leeuw et al., 2000; Rizos et al., 2002; Lonergan et al., 2003; McEvoy et al., 2006; Hansen et al., 2010; Lazzari et al., 2010; Romaguera et al., 2010).

The development potential of embryos obtained *in vitro* is lower in comparison with embryos obtained *in vivo* due to suboptimal conditions during oocyte maturation, their subsequent fertilization, and embryonic development (Wright and Ellington, 1995; Young et al., 1998; Lonergan et al., 2006; Metwally and Ledger, 2011).

Evaluation of embryos is based mainly on the timing of mitotic cleavage and on embryo morphology. In the morphological aspect, during the oocyte and early embryonic development stages, one can observe inhibition of development, fragmentation, vacuolization, and lysis. Analysis of embryonic development indicates that in all species of farm animals, high mortality occurs at the early stages of *in vitro* development. Among the types of death of single blastomeres, and sometimes of all the blastomeres of the embryo, the most prominent are

necrosis and senescence. Far less frequent is programmed cell death (apoptosis), which can be observed mainly in horse embryos. More recently other types of cell death have been attracting greater attention, specifically, autophagy and mitotic catastrophe (Okada et al., 2004). One can not exclude other, so far unknown, types of cell death (Betts et al., 2001; Okada et al., 2004).

These events do not always lead to embryo death, sometimes they will only cause perturbations of development, which can become manifest much later, after implantation or during postnatal development. Among the reasons of high mortality and occurrence of disorders are: changes of metabolism during oocyte maturation and embryo development, anomalous activation of the embryonic genome and defective gene expression, chromosomal aberrations (polyploidy, aneuploidy, mixploidy) (van Wagtendonk-de Leeuw et al., 2000). Epigenetic modifications, i.e. the posttranslational changes in gene expression (including genomic imprinting) that can occur *in vitro* present an interesting problem. Epigenetic modifications during maturation and embryonic development, and probably also during fertilization, can alter preimplantation, postimplantation, and also postnatal development of individuals. This can lead to various disorders, including: hydrocephalus, brain hypoplasia or damage, abnormal limb development, heart and liver enlargement, respiratory problems, as well as large offspring syndrome (LOS). Epigenetic modifications can lead to the death of individuals in the pre- and postnatal period (resorption, abortion, or death shortly after birth) (Young et al., 2000; Weaver et al., 2009; Bartolomei and Ferguson-Smith, 2011; Li and Sasaki, 2011).

It should be stressed, however, that in most cases the progeny obtained after transfer of the farm animal embryos obtained *in vitro* is indistinguishable both anatomically and physiologically from that obtained under natural conditions.

APPLICATION OF PROCEDURES FOR OBTAINING EMBRYOS *IN VITRO* FROM FARM ANIMALS

Obtaining livestock embryos *in vitro* has applications in animal breeding, biotechnology, medicine, and basic research (Betteridge, 2006; Hansen, 2006; Galli and Lazzari, 2008; Duszewska et al., 2010).

In animal breeding the procedure of obtaining embryos *in vitro* can be used as an alternative technique to insemination or a multiple ovulation and embryo transfer (MOET) programme, which consists of inducing multiple ovulations and transferring embryos to recipients. It is possible to use females only as oocyte donors without the burden of the further stages of this procedure. Immature or pregnant females can also be used as oocyte donors. Oocytes and embryos can be frozen or vitrified, which enables banking them (Gardner et al., 2011).

Obtaining embryos *in vitro* is of the greatest importance in biotechnology because this technique facilitates embryonic and somatic cloning, creating transgenic individuals and their clones, and also creating chimeras. The great hopes of human medicine lie in use of pigs as organ donors in xenotransplantation (Duszewska et al., 2010).

Basic research in both oocyte maturation, oocyte fertilization and early embryonic development is an important field that makes use of embryos obtained *in vitro*. Early development of bovine embryos is similar to human embryos and, therefore, cattle is considered to be a model species (Niemann et al., 2000).

In recent years more attention has been paid to the application of this biotechnique in infertility treatment in farm animals, where it plays the same role as assisted reproduction techniques (ART) used in humans. There are many indications connected with farm animal infertility for the application of the *in vitro* embryo obtaining procedure, including ICSI. Moreover, in special cases, the microsurgical epididymal sperm aspiration (MESA) or testicular sperm aspiration (TESA) (testicle biopsy) procedures can be applied, with subsequent use of ICSI (Greve and Callesen, 2005; Duszewska et al., 2010).

Recommendations for use of procedures for obtaining embryos *in vitro* can include:

1. Oviductal obstruction; in this case females are used only as oocyte donors.
2. The use of females only at the most desired moment, which is facilitated by cryoconservation of gametes and embryos, that is, freezing and vitrification of immature and mature oocytes and embryos, which enables banking them similarly to freezing sperm, and for subsequent transfer of embryos to the mother or surrogate mother.
3. Sex-linked genetic aberrations; in this case it is possible to regulate the sex in a herd by use of sexed semen for *in vitro* fertilization.
4. Carrier-state of lethal genes can be detected at the early stages of embryonic development and makes it possible to eliminate those embryos from the population.
5. Polyspermy, that is, the penetration of more than one sparmatozoa into the oocyte, generates polyploidy, e.g., triploid embryos, which develop only until the blastocyst stage and undergo resorption at the later stages of development.
6. Immunological infertility.
7. Insufficient number of motile spermatozoa (oligospermia) or excessive thickness of the zona pellucida, which prevents sperm penetration, are indications for ICSI.

8. Preimplantation genetic diagnosis (PGD) can supplement prenatal diagnostics, which is a separate field in human medicine in infertility treatment. Among the many directions of PGD, the most important include diagnostics of chromosomal aberrations, including hereditary diseases.
9. Others.

Thanks to progress in many fields of science it has become possible to treat infertility not only in humans, but also in farm animals by use of assisted reproduction techniques and techniques used in biotechnology.

CONCLUSIONS

The procedure of obtaining farm animal embryos *in vitro* is commonly used and is considered to be one of the most important biotechniques in reproduction since it combines animal breeding with biotechnology as well as human and veterinary medicine. Obtaining embryos *in vitro* is of the greatest importance in biotechnology because this technique facilitates embryonic and somatic cloning, creating transgenic individuals, their cloning, and also creating chimeras. The great hopes of human medicine lie in use of pigs as organ donors in xenotransplantation. Obtaining farm animal embryos *in vitro* may be used in infertility treatment.

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