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Perinatal death associated with ET, IVP and cloning in cattle

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During the last decades, assisted reproduction has found wide application in cattle, but with increasing manipulation of the embryos the mortality of the fetus and offspring have been shown to increase. Manipulation of early embryos induces subtle modifications of the developmental capacity resulting in changes of the maternal-embryonic interaction and the fetal adaptation and in abnormalities, which become apparent during pregnancy and delivery.

By definition, assisted reproductive techniques in the bovine include ET, MOET, IVP and cloning [1-3]:

Embryo transfer (ET) is the process of transferring embryos from donors to recipients which are synchronised to the appropriate day after heat. Embryos for transfer can either be developed in vivo (MOET) or in vitro (IVF).

MOET (multiple ovulations and embryo transfer) is the technology which is used worldwide to produce 80% of embryos for commercial purposes. The embryos are produced by superovulation of the donor cow with eCG or FSH, which are administered in the mid-luteal phase of the estrus cycle of the donor over a 3 to 4 days period and are combined with induced luteolysis and estrus. The donor is inseminated during estrus, and 7 days later the uterus is flushed to recover on average four to six transferable good quality embryos. Half of the commercially produced embryos are transferred fresh and the other half is frozen for later transfer.

IVP is the in vitro production of embryos where immature oocytes are recovered from the ovaries in the live cow by transvaginal aspiration, so called OPU (ovum pick-up) using an ultrasound scanner equipped with a transducer and a guided needle. Alternatively, when the female parental origin is not required to be known, the oocytes are collected from slaughterhouse ovaries. Several culture protocols can be used to mature, fertilize and culture the oocytes for about one week to blastocyst stage which is suitable for transfer or freezing. IVP embryos contribute with 20% to the commercially transferred embryos, which are mainly transferred freshly. Pregnancy rates are generally about 40–45% after fresh transfers, but become more variable amongst different laboratories with frozen embryos.

Cloning is the process of fusing a donor cell (often a differentiated cell from an adult) with an oocyte, from which the nucleus, containing the DNA, has been removed. Under this process, the genome of the donor cells is transferred to the recipient oocyte and reprogrammed to the status of an undifferentiated cell, which can undergo embryonic development. Thus, genetically identical offspring of already existing individuals can be created. Cells harvested from preimplantation embryos, fetuses or adult animals – typically fibroblasts, cumulus and granulosa cells or mammary epithelial cells (Dolly) – can be used to produce these clones. The efficiency is still low and only about 5% of the cloned bovine blastocysts transferred to recipients survive to term. So far, 12 different animal species have been cloned, but cloning has not yet become commercially important. This technology may, however

find its main application in the production of transgenic animal models for biomedical industry.

Mortality during pregnancy

In vitro produced and cloned embryos are acutely sensitive to environmental conditions, but adverse repercussions of such sensitivities may not be manifest, until much later in development and the long-term consequences may have different times of sensitivity within the developing embryo. Conceptuses, fetuses and offspring resulting from those embryos can differ in their viability, morphology and physiology compared with in vivo controls [4-8]. A better understanding of these mechanisms will not only facilitate the refinement of IVP and cloning systems, but may also improve our management of pregnant animals.

After transfer of cloned embryos, 60% or more of the pregnancies are lost in the first trimester of pregnancy. This is followed by a small but constant loss of the remaining pregnancies up to the 8 months, which is an unusual pattern in the extent and timing of pregnancy loss. These pregnancy losses can be attributed to a defective placental function, as demonstrated by an initial reduction of cotyledon development. Later in the pregnancy the abortions are associated with placenta hypertrophy, enlarged cotyledons and thickening of the umbilical cord [9-11].

Under certain culture conditions (coculture and use of serum-supplemented medium) IVP embryos and cloned embryos may develop large offspring syndrome (LOS), in which late fetal losses are associated with excess fetal size, abnormal placental development and vasculature (i.e. hydroallantois, enlarged edematous placentomes in reduced numbers), extremely large umbilical veins and arteries and abnormal, asynchronous growth of organs with musculoskeletal deformities. Overall, there is a higher incidence of LOS in clones compared to in vitro derived embryos, and especially in clones produced from somatic cells compared with clones produced from embryonic cells [7,10,12-14].

Chromosomal abnormalities are a well-known cause of pregnancy failure and chromosomally abnormal cells are commonly found in IVP or cloned embryos. However, the frequency reported varies with the method of embryo production. The most frequently observed deviation from the diploid karyotype is mixoploidy resulting from aberrant cell division causing polyploidy in a variable proportion of an embryo's cells [15,16].

Mortality at delivery

Most of the born calves derived from in vitro produced embryos appear normal, but a number of deviations have been reported that together have been termed as LOS. These include weak labor in the recipients, extended gestation, dystocia, increased incidence of hydrallantois, congenital malformations, increased birth weight and higher incidence of perinatal mortality. Live offspring occasionally exhibit heart insufficiency and respiratory distress with pulmonary hypertension. A wide range of other illnesses have been reported in clones, including infections, somatic overgrowth and omphalocele. Apparently normal clones show physiologic particularities such as alterations in temperature regulation and increased abdominal fat and leptin concentrations. Some of these calves require considerable intensive care to support their initial survival [10,11,17-23].

One possible approach to reduce the perinatal morbidity/mortality of offspring derived from assisted reproductive technologies is to provide the appropriate assistance during delivery. A number of procedures such as inducing parturition have been developed for this purpose; however, these protocols did not significantly reduce parturient and neonatal disorders following transfer of IVP or NT embryos [24]. Even when the adverse effects of dystocia are prevented by cesarean section, prenatal problems persist and evidence suggests that these are consequences of energy metabolism defects resulting from placental insufficiency. The majority of parturient complications may be explained by an inadequate signaling between mother and fetus associated with deficiencies in the establishment of placental vasculature.

Until an age of 5 months, the mortality of cloned calves is up to 40% [25] and between weaning and 4 years of age, the annual mortality rate in cattle cloned from somatic cells is approximately 10%. The LOS is the consequences of abnormal programming occurring during early embryonic development, which becomes manifest throughout gestation, the neonatal period and during adulthood in the cloned animal, but it does not appear to be transmitted to subsequent offspring following sexual reproduction [26,27].

The increased perinatal mortality in calves born after assisted reproduction involves ethical and economic concerns which have to be attended solved if the methods are to be used for commercial purposes such as breeding and production of transgenic animals for pharmaceutical production.

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