Institute of Experimental Morphology and Anthropology with Museum
Bulgarian Anatomical Society

Acta morphologica et anthropologica, 13
Sofia • 2008

In Vitro Manipulation Influence on Embryogenesis

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This review summarizes the consequences of biotechnological used methods for farm animal reproduction such as multiple ovulation and embryo transfer (MOET), in vitro embryo production (IVEP) after oocyte and zygote collection and culture, cloning by nuclear transfer (NT) and transgenesis (TG) such as freezing of sperms (SF), oocytes (OF) and embryos (EF).

There is evidence that MOET and IVEP can result in a recipient of deleterious side-effect commonly known as the large offspring syndrome (LOS). On the other hand, NT may lead to incompletely reprogramming of the transferred genome. Also TG may constitute an additional set of factors that may negatively affect the expression of the transgene and the concomitant synthesis and release of a protein. The freezing programmes for SF, OF and EF harmful cell membranes and viability after thawing has specific tolerance depending on animal species.

It is suggested that the introduction of biotechnology methods into farm animal husbandry should be carefully used and monitored animals used in experiments or routinely treated must be accompanied with a comprehensive analyzing protocol.

Key words: biotechnology, in vitro manipulation, embryogenesis.

Introduction

The aim of this study is to provide further information on the consequences of biotechniques employed in farm animal breeding. Biotechnological experiments on animals have been considerably expanded. That has produced a positive effect on the development of scientific findings, but it has also recorded some negative consequences on the animal's ontogenesis (2, 24, 29, 30). In Bulgaria biotechnological approaches and in vitro manipulations involved are applied in limited mainly for experimental needs [22].

Materials and Methods

In the animal reproduction the following biotechnologies have been routinely applied — multiple ovulation (MO), oestrus cycle synchronisation (OCS), oocyte collection (OC), in vitro embryo production (IVEP), and the subsequent embryo transfer (ET) to suitable recipients. Simultaneously like an extra niche cloning approaches using nuclear transfer (NT) and transgenesis (TG) are being developed but they are

still in process of development and are being applied largely for experiments. Furthermore cryogenic techniques for a long-term preservation of gametae, zygotae and preimplantation embryos are used for sperm freezing (SF), oocyte freezing (OF) and embryo freezing (EF) in liquid nitrogen at a temperature of — 196°C.

Results and Discussion

Biotechnologies IVEP and ET have been broadly adapted in the breeding programmes of advanced countries. However, the existing data suggest that biotechnologies in use lead to asynchronisation of folliculogenesis [10], chromosome aberration [11], oogenesis and hormonal disfunction [12], asynchronisation of the uterus cycle and embryogenesis [13], prolonged pregnancy with signs of uterus distonya [24]. According to researchers after induced MO a total of 27 - 45 % of the embryos have abnormal karyotype and are expected to be eliminated before, during or after implantation [11].

Recently a convincingly evidence has been collected and showed that a newborn weight of IVEP obtained calves is on 30% over 50 kg in comparison with in vivo controls at birth [29, 30]. Besides offspring cows and sheep by IVEP are less active and avital at the same time. Also increasing the percentage of anomalies of hydroalantois and congenital malformations including abnormal limbs and vertebral column are detected [12]. The observed anomalies were summarized as a large offspring syndrome (LOS) of these animals [30]. Furthermore in calves obtained by IVEP signs of embryogenetic disorders in various organs — heart, liver, kidneys, and adrenal glands have been reported, as well as usual postnatal deviations [7, 25]. Anomalies related to overweight body at birth are explained with the demetilation of the recombinant gene IGF2 [15[. For the LOS escape the evaluation criteria and effective reliable morphological control of foliculogenesis, gametes and embryos must be strictly observed. It also seems that the added at in vitro culture serum, hormones and inhibitors are impact factors on the genome or cellular mitochondria that visibly change the intracellular metabolism which needs constant parameters [4].

Manifestation of LOS symptoms after NT can be connected with common manipulations by objects but also with factors specifically dependent on NT [3]. First the nucleus of the donor somatic cell must past through the process of genetic reprogramming which is connected to transforming the origins of the gene expression characteristic of the donor cell to one specific of early embryo development. This process may be incomplete and leads to unsuitable origins of gene expression. Secondly NT involves exposure to reconstructed oocytes of different oblique stimulations aiming to facilitate the fusion between the nucleus and the recipient cytoplasm as electric shock or treating with various protein inhibitors [5]. These stimulants can lacerate the epigenetic modifications or transcripted genes. Theoretically in any procedure or in any stage of the subsequent processes when carrying out manipulations embryo development, fetus features and the newborn can be affected [27].

A transgenic animal contains genome in which DNA from exogenous source has been introduced by experimental manipulation. In the beginning the application of this technique was focused on studying the genetic factors related to human disease development [8]. Lately TG focused on animals because it aimed at improving farm animal productivity [6, 28]. Congenital malformations and a high rate of prenatal mortality have been reported in transgenic experiments carried out on cows using microinjection [26]. Readings of transgenic lambs and calves obtained through NT also show high rates of prenatal mortality and distinct prenatal and neonatal pathology and immunity disorders [19].

The cryobiological approaches of gametes, zygotes and preimplantation embryo freezing have a selective effect on the viability of the thawed objects [18]. Considerable and more fundamental research has been conducted for freezing male gametes of different kinds of animals. It permits to introduce the technologies on a massive scale mainly in cattle and sheep breeding [1, 9]. It was established that female gametae in different stages of the gametogenesis are more frost-tender and suffer relatively a lower percentage (15%) undergoing freezing and thawing procedures [20, 23]. The claims that meiotic maturing oocytes are more susceptible to the damaging effect of the cryogenic factors forced researchers to focus their efforts on objects with reconstructed diploid genome and the cells are divided by mitosis [7]. Our results of embryo freezing show that the crystal structure of the frozen water damage not only the membrane components in the cell but also biopolymers with protein, lipid and carbonhydrate content (Fig. 1). Under deep frozen condition to −196°C in liquid nitrogen only some physician-chemical reactions are going on and it is considered that they have no practical impact on the cell genome [21]. Whether this is also true of the long-term storage is an issue still to be proved by the future when the objects stored in the cryobanks will be used [22]. Over the last few years contrary to the conventional freezing methods vitrification of the biological object is being employed more convincingly [9, 14, 16]. But in this method they are still looking for the optimal ratio between the concentration of the cryoprotectant used and the time present in the cell. The high cryoprotectant concentration inhibits metabolism and creates conditions for membrane lesion [17].

Conclution

The current research presents convincing evidence according to which after particular interference in ontogenesis of farm animals different anomalies occur in embryogenesis being reflected in the postnatal development as well. Therefore it must be

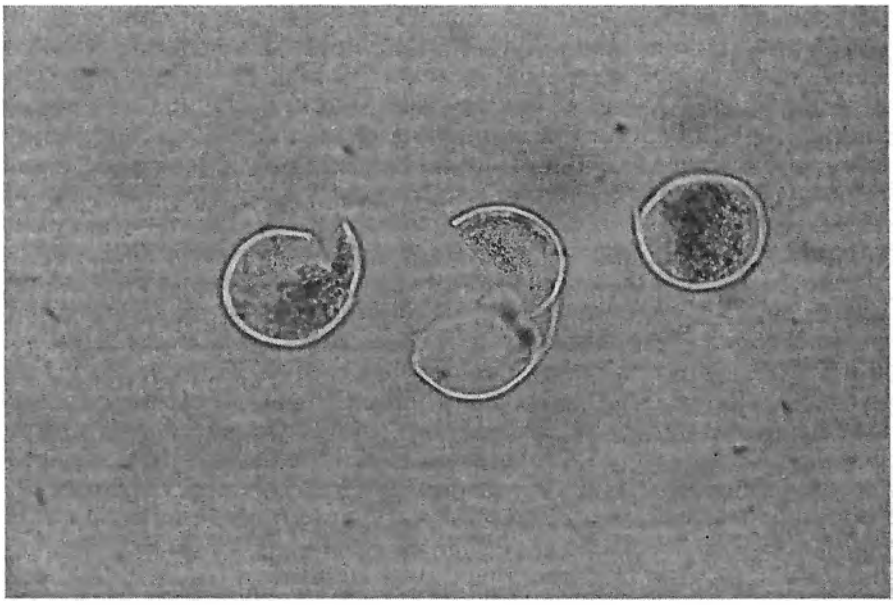

Fig. 1. Damaged frozen-thawed sheep embryos after long-term storage. Native, × 400

concluded that employed reproductive biotechnologies on farm animals should be carefully introduced by monitoring of their condition. The data including the total number of animals and treated groups, their observed normal and pathological status as well as some paraclinic and clinic prenatal and neonatal parameters of the development should be summarised in comprehensive protocol. The obtained results should be systematically analyzed so that it could be scientifically used for experiments and routine biotechnical approaches employed in animal reproduction.

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