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Review Article

Clinical Applications of Preimplantation Genetic Testing in Equine, Bovine, and Human Embryos

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ABSTRACT

The use of preimplantation genetic testing (PGT) in reproductive medicine and animal production has increased significantly in the last few years. Preimplantation genetic testing starts with the collection of a few cells from the embryo and continues with the genetic analysis of the DNA in the sample. The last step is the interpretation of the results and the decision whether to transfer the embryo or not. New genetic tools have broadened the application of PGT. In humans, preimplantation genetic diagnosis helps select against embryos carrying known genetic disorders, and preimplantation genetic screening is used to help prevent the transfer of embryos with aneuploidies. In cattle, PGT is increasingly used to select embryos carrying genetic traits of interest for meat or milk production. The availability of single nucleotide polymorphism (SNP) microarray chips at a low cost has helped the incorporation of this technology in genetic selection programs. In the horse, embryos have been mainly analyzed for sex selection and for the detection of known genetic disorders. Recently, chips with up to 600K SNPs have become commercially available for horses. This technology, in combination with the recent success of embryo cryopreservation, will probably lead to the inclusion of PGT as a tool for horse breeding in the future.

1. Introduction

Preimplantation genetic testing (PGT) is increasingly being used in reproductive human medicine and domestic species production programs and comprises Preimplantation genetic diagnosis (PGD) and preimplantation genetic screening (PGS). In humans and in domestic species, PGD is used to test an embryo for known genetic abnormalities and decrease the transmission of genetic disorders such as Tay-Sachs disease or Hyperkalemic Periodic Paralysis, or to detect a known genetic trait, such as sex or coat color. Preimplantation genetic screening is the overall screening of an embryo for duplications or deletions of chromosomes and chromosomes segments and is used mainly with human embryos to improve pregnancy rates by transferring only euploid embryos. This is used in older women, couples with previous pregnancy failures, and couples with severe male factor infertility [1]. Preimplantation genetic screening in not currently used in domestic species, but this technique could become a useful tool if, in the future, embryos from older mares are found to carry a higher incidence of aneuploidy. Both PGD and PGS involve obtaining one or more cells from and embryo and subsequent genetic analysis of the sample. In addition, the polar body of oocytes and the blastocoele fluid of embryos have also been used as a source of DNA for genetic analysis [2–5]. The result obtained from this analysis is used to decide whether the embryo should be transferred or not.

In humans, PGT is used for the detection of specific genetic disorders or aneuploidy [6]. In cattle, the result

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obtained is most commonly used for genomic selection (GS) and helps to select animals for breeding [7,8]. Although the first birth produced by transfer of an equine biopsied embryo was reported almost 20 years ago [9], it is only recently that interest in this technology has risen with more efficient methods of biopsy [10] and its application in a large-scale commercial program [11]. In general, PGD in the horse is of interest to determine the sex of the embryo and to diagnose certain diseases associated with specific mutations as well as the selection of certain genetic traits of interest such as coat color.

This article reviews the clinical application of PGT in equine, bovine, and human embryos, as well as the different type of oocyte and embryo sampling techniques and the genetic tools available for testing. We will also discuss the degree of progress obtained in the equine species as well as the future perspectives.

2. Biopsy Samples

The starting point of a successful PGT is performing the right biopsy. Highly trained personnel and specific equipment are required to obtain oocyte and embryo biopsy samples. During the biopsy procedure, enough DNA for analysis should be obtained in such a way to ensure the viability of the embryo is not affected. Biopsy samples may be obtained from the oocyte by removal of the first and/or second polar body, from the early cleavage stage embryo by removal of a single blastomere, from the morula by collecting blastomeres, or from the blastocyst by removal of trophoblast/trophectodermal cells or aspiration of blastocoele fluid (Fig. 1).

First and second polar body biopsies are less invasive than collecting cells at the cleavage or blastocyst stage, but the effect of the paternal genome is ignored. Polar body biopsy is useful in certain cases, to detect abnormal patterns of maternal chromosome segregation. In human oocytes and embryos, it has been demonstrated that the accuracy of the information obtained with this type of approach is lower when compared to biopsy samples obtained at the cleavage or blastocyst stage [12]. The level of mosaicism may be higher at the cleavage stage than the blastocyst stage [13,14]. In addition, clinical data show that biopsy of human embryos at the cleavage stage has a negative effect on embryo development, resulting in slower development and increasing the rate of embryo death [15–17]. Given all this evidence, there is a growing tendency in human medicine to obtain embryonic samples at the morula or blastocyst stage. In human embryos, the techniques for collection are more standardized than in embryos from domestic species, and micropipettes are used to collect cells from morula or blastocysts, aided by laser [18]. Recently, the presence of sufficient DNA for amplification by polymerase chain reaction (PCR) was found in blastocoele fluid and human in vitro–produced embryos were sexed successfully using blastocoele fluid as the only source for DNA [2]. In addition, the DNA in blastocoele fluid from human blastocysts could be amplified by whole genome amplification (WGA) [5] and used for genetic screening for aneuploidy [4,19].

In bovine and equine embryos, cells are obtained at the morula or blastocyst stage, from embryos produced in vivo or in vitro [8,10,11,20]. Polar body biopsy is not routinely used in livestock production systems.

In cattle, there are mainly three methods, which are commonly used to collect biopsy samples [21]. The first one uses a microblade to cut a portion of the morula or trophectoderm from the blastocyst. In the second method, a

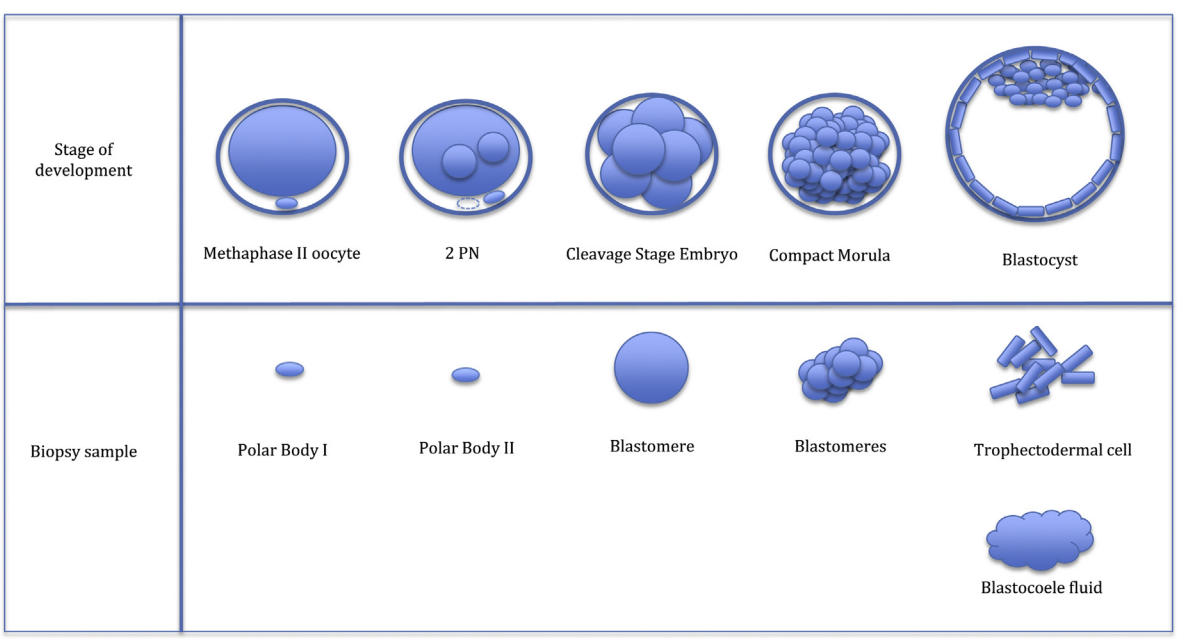

Fig. 1. Different types of biopsy samples obtained at various stages of early development. PN, pronuclei.