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## JOURNAL ARTICLE

# Separation of ram spermatozoa bearing X and Y chromosome by centrifugal countercurrent distribution in an aqueous two-phase system

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The availability of reliable quantification techniques of X and Y chromosome-bearing spermatozoa in a given insemination dose would allow further approaches to their separation, which is a topic of unquestionable interest in animal production. The aim of the current work was the development of a combined approach of polymerase chain reaction and countercurrent distribution to address both objectives. First, using Sac/polymorphisms for ZFX/ZFY loci in sheep deoxyribonucleic acid, a linear correlation has been established between the densitometric quantification of the restricted fragment length polymorphisms corresponding to the amplified loci ZFX/ZFY by polymerase chain reaction and the theoretical proportions of X and Y chromosomes in standard solutions. The method, subsequently applied to semen samples, estimated an equal proportion of spermatozoa bearing each chromosome. Second, by using centrifugal countercurrent distribution in a sensitive-charge aqueous two-phase system, we achieved the separation of a sperm population enriched in Y chromosome-bearing ram spermatozoa (75%) with a high viability rate (57%).

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