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## The application of a multicolor ZOO-FISH on secondary bovine oocytes showed its potential use for aneuploidy detection

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The female gametes are more susceptible to chromosome segregation errors during meiosis I division and therefore they are the major contributors to the embryo aneuploidies. The evaluation of aneuploidies in bovine oocytes is useful for monitoring the reproductive health of this species and FISH is the main method employed for this purpose. To date only 2-3 chromosomes were simultaneously investigated for aneuploidy detection in cattle oocytes. In this work we propose a multi-color ZOO-FISH by the simultaneous detection of 6 specific chromosome painting probes on secondary oocytes matured in vitro. Standard procedures were employed for 24h in vitro oocytes maturation, whereas specific autosomal probes were prepared by microdissection and DOP-PCR using river buffalo mitosis (2n=50). Probes were labelled with spectrum-green and -orange in a second DOP-PCR. Three sequential rounds of FISH were achieved for the same slides. Each round was realized using two probes simultaneously hybridized on MII oocytes with the corresponding first polar bodies (I pb). Slides were counterstained with DAPI in antifade. Digital images were captured in gray-scale and pseudo-colored by the software. Six specific probes, painting 3 out of 5 sub-metacentric river buffalo chromosomes (BBU 1p, 1q, 3p, 3q, 4p and 4q) were sequentially hybridized on BTA secondary oocytes with the corresponding (I pb). The different colors of the probes allowed the identification of 6 cattle chromosomes (BTA 1, 5, 8, 19, 27 and 28) both on MII and polar bodies evidencing no abnormalities for the investigated cells, but confirming their potential use for aneuploidy detection in bovine oocytes. This result opens further opportunity of investigation for clinical cytogenetic applications also in the other species with difficult CGH karyotype.

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