Long term culture and characterization of chicken primordial germ cells.

Introduction:
As precursors of germ cells, avian primordial germ cells (PGCs) are foreseen as promising tools to develop avian transgenesis and preservation of genetic resources of endangered species. PGCs can be isolated from blood during their circulating phase, expanded in vitro and genetically manipulated while maintaining their germ cell properties and can colonize the genital ridges of recipient embryos. We developed original culture methods that allow long term propagation of undifferentiated PGCs and their efficient cryopreservation.

Material and methods:
PGCs culture and cryopreservation:
Blood samples were collected from embryos originating from three commercial layer breeds (White Leghorn, Isa Brown and Dekalb White) and two Belgian endangered breeds (Herve and Ardennes breeds). Samples were pooled and cultivated on cell culture inserts in selective medium in the presence of mitotically inactivated Buffalo Rat Liver cells.
PGCs were cryopreserved using either slow freezing (KnockOut-DMEM containing 50% FBS and 5% DMSO) or a newly developed vitrification method. Vitrification was performed as follow: cells were successively submitted to three vitrification solutions (proprietary formulation - Dr F. Ectors), gradually increasing the cryoprotectants concentration. Cells were resuspended in the third solution (vitrificant solution semen struice), loaded in a straw and rapidly immersed in liquid nitrogen.
PGCs characterization:
PGCs were labelled for the ES cells marker, SSEA-1, whose expression was evaluated by flow cytometry. Expression of germ line markers (CVH, CDH, DAZL), pluripotency markers (PouV, Sox2, Nanog), telomerase and CKX8 receptor was evaluated by RT-PCR.
The proportion of female cells in stabilized cell lines was estimated by means of a quantitative PCR method, based on the amplification of a chromosome W specific sequence.
Migratory ability of PGCs was assessed by injecting cell-track labelled cells in recipient embryos.

Results:
Upon primary culture of pooled blood samples, PGCs emerge in 3 to 4 weeks. Undifferentiated cells display a round morphology and grow as unattached single cells or small clusters (Fig. 1a). Adherent cells are systematically eliminated. Overall, 35% of pooled samples give rise to cells lines. Cells lines expressed SSEA-1 marker (Fig. 1b). Flow cytometry analysis showed expression rates varying from 90 to 99% (Fig. 2).

Conclusion:
In conclusion, we provide here an original method allowing to efficiently promote PGCs expansion in an extended period of time. Long-term cultured PGCs still expressed all tested specific markers and showed high ability to colonize embryonic gonads even after one cryopreservation step. In addition, we demonstrated that all stabilized cell lines present a male phenotype, confirming published observations (van de Lavoir et al., Nature, 2006).