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# Development and quality of sheep embryos cultured in commercial G1.3/G2.3 sequential media

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**Abstract**

Present study assesses the developmental ability and quality of ovine IVP embryos derived from culture in sequential media G1.3/G2.3. A total of 1474 cumulus–oocyte complexes were matured in M199 supplemented with EGF and FCS for 24 h in 5% $CO_2$ in humidified air at 39 °C. Oocytes were co-incubated in SOF medium with $1 \times 10^6$ spermatozoa/ml at the same temperature and gas conditions (Day 0 p.i.). Presumptive zygotes at 20 h p.i. were denuded, washed and placed in culture in SOF (control; $n = 742$) or G1.3 media supplemented with 3 mg/ml of BSA ($n = 732$) under mineral oil in a humidified and controlled atmosphere at 39 °C. Embryos in the treated group were changed to G2.3 medium on Day 3 of culture. A group of blastocysts in each group were frozen by conventional method (SOF, $n = 55$; G1.3/G2.3, $n = 48$). In vivo embryos ($n = 72$) were recovered at Day 7 from the uterus of progestagen + eCG treated females and they were cultured in defined medium ($n = 38$) or frozen ($n = 34$) directly after recovery. Cleavage rate of IVP embryos recorded at 48 h p.i. was similar for control and treated embryos (49.8 versus 47.5%). There were no significant differences in blastocyst development from the two groups on Day 6 (26.0 versus 25.6%), 7 (42.1 versus 38.6%) or 8 (50.8 versus 43.2%). Blastocyst development rates from total oocytes cultured were comparable (24.1 versus 21.5%). However, the proportion of hatched blastocysts was significantly higher for control embryos (86.6 versus 44.3%, $P < 0.0001$). In addition, embryos cultured in SOF had higher re-expansion rates post-thawing at 24 h (38.2 versus 6.2%), 48 h (36.4 versus 4.1%) and 72 h (34.5 versus 4.1%) and hatching rate (32.8 versus 2.0%) than embryos cultured in sequential media ($P < 0.0001$). In vivo embryos showed higher hatching rate (61.7%) than IVP groups (SOF, $P < 0.01$; G1.3/G2.3, $P < 0.0001$) but

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lower than their fresh cultured counterparts (86.8%; $P = 0.01$). In conclusion, G1.3/G2.3 media supported high developmental rates of embryos in vitro but the quality of the embryos was impaired.


*Keywords:* Cryosurvival; Embryo; Ewe; IVF; Sequential medium

## 1. Introduction

Suboptimal culture conditions in vitro can led to the development of embryos incapable of withstanding cryopreservation or establishing pregnancies (Bavister, 2000). Supplementation of ruminant embryo culture media with serum is used widely. It has been shown that prolonged exposure to serum can greatly alter embryo morphology and biochemistry (Gardner et al., 1994; Thompson et al., 1995) and it has been associated with the large offspring syndrome (Walker et al., 1996; Young et al., 1998). The use of serum-free culture systems that support high rates of development in culture of high-quality embryos is thus desirable.

Recent developments have included the supplementation of media with amino acids (Biggers et al., 1997; Steeves and Gardner, 1999) and the use of sequential media for the extended culture of pre-implantation embryos (Lane et al., 2003). Physiological sequential media are formulated to reflect the carbohydrate levels of the reproductive tract and reduce cellular stress on the embryo (Lane et al., 2003), on the basis of the temporal sensitivities of embryos (Menezo et al., 1998). The early cleavage stage embryo has a low metabolic activity with a limited ability to utilize glucose; it generates energy from low levels of oxidation of pyruvate/lactate and non-essential amino acids (Barnett and Bavister, 1996). Conversely, the post-compaction embryo has a high metabolic activity, uses glucose as the preferred nutrient and requires nonessential and essential amino acids for cell proliferation and differentiation as well as specific vitamins to maintain oxidation (for review, see Bavister, 1995). These sequential media would mimic the change in environment experienced by the developing embryo in vivo, enabling the biochemical and morphological changes such as the maternal zygotic transition, compaction and blastocoel formation and expansion (Swain et al., 2001).

It could be very useful to develop chemically defined medium in ovine embryos for the further analysis of embryos such as physiology, molecular biology and biochemistry. However, the effect of sequential culture media has not been tested previously in ovine embryos. The aim of this study was to assess the ability of the commercially available sequential media G1.3/G2.3 to support ovine embryo development to the blastocyst stage and to examine the quality of the blastocysts produced, measured in terms of survival after conventional freezing, compared with those produced in synthetic oviduct fluid (SOF) medium and those derived in vivo.

## 2. Materials and methods

### 2.1. In vivo embryo production

The work was conducted using ethical guidelines for care and use of agricultural and/or laboratory animals for research. Embryos were recovered from crossbred ewes ($n = 30$) maintained on pasture with free access to water at Lyons Research Farm, University College Dublin (53°18′N). They were superovulated as described by Papadopoulos et al. (2002). Briefly, the estrous cycles were synchronised using an intravaginal progestagen pessary (FGA; Chronogest,