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The prevalence of embryonic remnants following the recovery of post-hatching bovine embryos produced in vitro or by somatic cell nuclear transfer

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ABSTRACT

The reliable collection of peri-implantation embryos in the bovine has important ramifications to post-transfer consequences, particularly in the elucidation of mechanisms associated with post-hatching embryo development and to perturbations in developmental growth following transfer. This study analyzed both in vitro produced (IVP) and somatic cell nuclear transfer (SCNT) embryo-like structures (ELS) recovered at Day (D) 14 and D21. The recovered ELS were subsequently processed for histological examination. At D14 and D21, many of the embryos recovered in the IVP group conformed to the appropriate stage of development. However, a significant number of anomalies were present in the SCNT groups when examined in more detail. Histological examination revealed that irrespective of whether these embryos had undergone trophoblast expansion to an ovoid, tubular or filamentous morphology, many had a degenerated hypoblast layer and a large proportion did not possess an epiblast and therefore could not differentiate into any of the three germ layers as would be expected at the neural groove or somite stage. The prevalence of this developmental pattern was random and did not correlate with treatment (IVP or SCNT) or with types of structures recovered. The rapid embryo elongation period also coincides with the time of greatest embryonic loss and these observations could have important implications for

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assessing the recovery of embryos post-transfer where incorrect morphological assessment could lead to false implantation and pregnancy determination rates. The implementation of additional methodology is required to adequately characterize the quality of IVP and SCNT-derived embryos collected post-transfer.

1. Introduction

In ruminants, over 40 years of research has been applied to the development of pre-elongation embryos ex vivo where it is now a reliable research tool for observing developmental biology, advancing-assisted reproductive technologies (ART) and for improving breeding through genetic selection (Hansen, 2006; Thompson et al., 2007). However while various ART such as in vitro production (IVP) and somatic cell nuclear transfer (SCNT) have improved significantly and resulted in similar rates of morulae and blastocyst formation (Niemann and Wrenzycki, 2000; Korfiatis et al., 2002), the overall quality of blastocysts produced is impaired when compared to their in vivo counterparts (Niemann and Wrenzycki, 2000; Farin et al., 2004).

The first stage in the assessment of an embryo's developmental competence is its ability to produce viable offspring after transfer to a recipient. Historically, morphology and the proportion developing to the blastocyst stage are used as criteria to assess developmental competence. These criteria remain the most practical method of choice for selection of viable embryos prior to transfer (Van Soom et al., 2003). In the bovine, an embryo grading system (Lindner and Wright, 1983; Stringfellow, 1998) with only minor modifications (Stringfellow, 1998; Hasler, 2001) is used extensively in both research and commercial practice. The advent of new ART (IVP and SCNT) has shown that embryos adapting to a variety of culture and induced environmental conditions can undergo significant alterations to their fetal developmental pathway without obvious changes in pre-implantation morphology (Behboodi et al., 1995; Agca et al., 1998; Enright et al., 2000; Crosier et al., 2001; Edwards et al., 2003; Chavatte-Palmer et al., 2004; Wells, 2005; Farin et al., 2006).

The overall efficiency of IVP indicates that between 80 and 90% of immature bovine oocytes undergo nuclear maturation in vitro and 80% then fertilize. Only 30–40% of fertilized embryos develop to the blastocyst stage and around 50% of the transferred embryos establish and maintain pregnancy to produce viable offspring (Betteridge and Loskutoff, 1993; Farin et al., 2001). While SCNT has similar blastocyst development rates when compared to IVP, the proportion that initiate pregnancy resulting in offspring is considerably lower (10–15%) (Paterson et al., 2003). In addition, a proportion of the SCNT-derived embryos after transfer are affected by the "large offspring syndrome (LOS)" (Niemann and Wrenzycki, 2000; Lazzari et al., 2002; Ushijima, 2005; Farin et al., 2006). The morphology of the pre-transfer embryo is therefore not an accurate predictor of subsequent embryo developmental potential in vivo.

In the bovine species, blastocysts are formed approximately 7 days after fertilization. However their apposition to the uterus takes place about 2 weeks later (21 days post-fertilization) (Noden and de Lahunta, 1985; Betteridge and Flechon, 1988a; Guillomot, 1995). During this interval, the blastocyst initiates the process of gastrulation and the trophoblast undergoes dramatic elongation from 150 μm to 300 mm (Chang, 1952; Greenstein et al., 1958; Grimes et al., 1958; Betteridge and Flechon, 1988b; Maddox-Hyttel et al., 2003). This dramatic growth involves a significant transition in bovine trophoblast development, the formation of the embryonic disc (ED) and the subsequent process of gastrulation, which results in differentiation of the germ layers (Grealy et al., 1996; Dunne et al., 2000; Maddox-Hyttel et al., 2003; Degrelle et al., 2005).

The onset of implantation (D8–16 after fertilization) is also the time of greatest embryonic loss (Diskin and Sreenan, 1980; Roche et al., 1981), which has a significant economic impact on both animal production and research programs. Studies with artificial insemination (Diskin and Sreenan, 1980; Roche et al., 1981; Dunne et al., 2000) and IVP (Farin and Farin, 1995; Peterson and Lee, 2003; Farin et al., 2004) with a variety of medium formulations (Thompson et al., 1998; van Wagtendonk-de Leeuw et al., 1998) report losses in this period between 40 and 60% (Ayalon, 1978). For those embryos generated by SCNT procedures, embryonic losses have been reported between 50 and 100% (Edwards et al., 2003).