In Vitro Embryo Production: Growth Performance, Feed Efficiency, and Hematological, Metabolic, and Endocrine Status in Calves

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ABSTRACT

The potential management benefits of in vitro embryo production have been offset by an increased incidence of health-related problems in resulting calves [increased birth weight, congenital abnormalities, and peri- and postnatal mortality (large-offspring syndrome)] and of recipient cows (prolonged gestation, dystocia, increased hydroallantois, abortion). The aim of the present research was to determine whether relevant metabolic, endocrine, or hematological traits could be related to the causes of enhanced growth performance of in vitro fertilized calves. Growth performance and feed efficiency as well as hematological, metabolic, and endocrine traits studied in calves derived from in vitro-produced embryos (IVP; n = 11) and in calves derived from artificial insemination (AI; n = 8). Donor cows from which oocytes for in vitro fertilization were obtained had a heterogeneous background, thus excluding genetic maternal influences. On the other hand, semen for in vitro fertilization and for artificial insemination was from the same bull, and recipient cows were held under the same husbandry and feeding conditions as AI cows, thus reducing the variability. Blood samples were collected preprandially on d 1, 2, 3, 4, 7, 14, 28, 56, and 112 of life and every 20 min between 0830 and 1630 h on d 7 and 112 for the evaluation of growth hormone secretory patterns. Gestation of IVP cows was longer than that of AI cows, but birth weights were similar in both groups. Feed intake, average daily gain, and body length during the experimental period, body weight from wk 8 to 16, and gain/feed ratio during the first month of life were higher in IVP than in AI calves. At birth, potassium, 3,5,3′-triiodothyronine, and thyroxine concentrations were lower in IVP than in AI calves. Concentrations of sodium and potassium on d 7, of triglycerides on d 28, and of albumin on d 56 were higher in IVP than in AI calves. In conclusion, IVP calves had higher feed intake and growth rate during the entire growth period and improved feed efficiency in the first month of life than AI calves, but this was not mirrored by consistent changes of hematological, metabolic, or endocrine traits, whose concentrations were in the normal range. Additional work is needed to study IVP calves under field conditions.
(**Key words:** calves, growth performance, metabolism, endocrinology)

Abbreviation key: BE = base excess, **GH** = growth hormone, **IVP** = calves derived from in vitro-produced embryos, **LOS** = large offspring syndrome, **MR** = milk replacer, **NfE** = nitrogen-free extract, **pCO$_2$** = partial carbon dioxide pressure, **pO$_2$** = partial oxygen pressure, **T$_3$** = 3,5,3′-triiodothyronine, **T$_4$** = thyroxine.

INTRODUCTION

In vitro production (**IVP**) of calves has advantages for breeding programs because embryos can be gained from most donor cows, donors can be combined with many more sires, and more embryos can be obtained per time unit than with AI (van Wagtendonk-de Leeuw et al., 1998). However, several problems during gestation, calving, and postnatal life of IVP calves have been reported, such as increased birth weight (Walker et al., 1996), prolonged gestation period (Kruip and den Daas, 1997), dystocia (Hasler, 2000), a greater incidence of hydroallantois (van Wagtendonk-de Leeuw et al., 1998), increased incidence of abortions (Hasler et al., 1995), congenital abnormalities (Schmidt et al., 1996), and enhanced peri- and postnatal mortality (Behboodi et al., 1995). The "large offspring syndrome" (**LOS**) is of particular interest. Causes of LOS are thought to be factors present in culture systems used for preimplanted stages of embryos, such as high amounts of ammonia (McEvoy et al., 1997), enhanced

activity of growth factors present in fetal serum used in culture media, and paracrine growth factor influences associated with embryonic cell interactions in the co-culture (van Wagtendonk-de Leeuw et al., 2000). Possibly contributing to the development of LOS, embryonic manipulations (such as nuclear transfer) and the surrounding environment before implantation may result in inappropriate epigenetic modifications of imprinted genes and affect gene expression during later fetal development (Young and Fairburn, 2000; Young et al., 2001). To date, most studies on IVP calves have focused on the perinatal period, characterizing problems such as the viability of IVP bovine embryos (Taverne et al., 2002), abnormally high birth weight (Yang et al., 2001), calving, and metabolic and endocrine status (Jacobsen et al., 1999, 2000a,b, 2002; Sangild et al., 2000). Based on the few long-term studies during the postnatal period (McEvoy et al., 1998; Chavatte-Palmer et al., 2002), it is still not clear how IVP calves compare physiologically with AI calves. In the present study, the hypothesis was tested that apparently normal IVP and AI calves differ with respect to growth performance, feed intake, feed efficiency, and hematological, metabolic, and endocrine traits. Of prime interest were factors that are centrally involved in postnatal growth regulation, growth performance, body composition, and feed intake [growth hormone (**GH**), glucagon, IGF-I, insulin, leptin, 3,5,3′-triiodothyronine (**T_3**), and thyroxine (**T_4**)].

MATERIALS AND METHODS

Animals

In vitro embryo production. Bovine ovaries were collected at random at a local slaughterhouse and transported to the laboratory in PBS to which 1% fetal bovine serum (FBS 2910149, ICN Pharmaceuticals, Orangeburg, NY) was added. Cumulus-oocyte complexes were collected through a filter, followed by washing of the filter with HEPES-buffered Medium 199 (Gibco BRL, Paisley, UK) that was supplemented with 10% fetal bovine serum at 25°C. After transfer to 4-well dishes (Gibco, Basle, Switzerland), in vitro maturation was performed in 30-μL drops of maturation medium (Medium 199; Sigma, Schnelldorf, Germany) supplemented with 15% fetal bovine serum, penicillin (60 UI/mL; Gibco), streptomycin (60 μg/mL; Gibco), and human chorionic gonadotropin (10 IU/mL, 5 IU/mL hCG, PG 600; Intervet, Boxmeer, the Netherlands) under paraffin oil (K 27537460; Merck, Darmstadt, Germany) for 24 h in an incubator with an atmosphere of 5% CO_2 in air.

Ova from the different cows were pooled before fertilization. The in vitro fertilization was performed in 30-μL drops of fertilization medium (Le Gal and Massip, 1999). The semen was from a Limousin bull (name: Lemming; ID number: 910.0870.5426.2; origin: Switzerland). This bull was previously tested by the Swiss Simmental-Red Holstein breeder organization. The nonreturn rate of sired daughters was −1.5%, there were normal calvings in 94%, and the bull enhanced net weight and carcass gain of his sons by 107%. Frozen-thawed semen of this bull was layered on a Percoll solution (45 and 90%) and centrifuged at $700 \times g$ for 30 min. The sperm pellet was resuspended in 5 mL of sperm washing solution (fertilization medium without heparin) and centrifuged at $100 \times g$ for 10 min. Sperm were added to the oocytes and coincubated under paraffin oil for 18 h in an incubator with an atmosphere of 5% CO_2 in air.

At the end of the fertilization process, presumptive zygotes were centrifuged to remove cumulus cells, washed 3 times in HEPES-buffered Medium 199 and then transferred in 30-μL drops of modified synthetic oviductal fluid (SOFaa; Holm et al., 1999) that was supplemented with 5% fetal bovine serum. The in vitro culture was under paraffin oil for 6 to 7 d in an incubator with an atmosphere of 5% CO_2 and 5% O_2 in air. Only blastocysts of excellent quality were frozen and used for embryo transfer in 4 Simmental × Red Holstein, 1 Brown Swiss, 2 Holstein × Angus, 3 Brown Swiss × Blonde d'Aquitaine, and 1 Holstein × Blonde d'Aquitaine.

AI calves. Calves born by heifers and cows (9 Simmental × Red Holstein, 2 Holstein) that were inseminated by standard methods with semen from the same bull as above, served as controls.

Calvings. The calves, all mixed-breed, were born at the research station (Posieux, Switzerland) between August and December 2002. Eleven calves (6 males, 5 females) were single-born from the 11 IVP recipients. Calvings were unassisted except for one calf that required heavy extraction and one calf that was delivered by cesarean section. Eleven calves (3 males, 7 females, 1 stillborn) were single-born following AI. Calvings were unassisted except for one calf that required slight extraction and one calf that was stillborn after subcutaneous injection of prostaglandin $F_{2\alpha}$ (500 mg of Cloprostenol, Estrumate; Essex, Friesoythe, Germany). One male AI calf was slaughtered on d 41 of life because of weakness and another calf on d 27 of life, and one AI female calf died at birth with neurological symptoms. The 3 calves were excluded from the study. Because of the loss of one calf at birth and 2 losses during growth, only 8 AI calves finished the trial.