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Genomic stability and physiological assessments of live offspring sired by a bull clone, Starbuck II

H. Ortegon [a,1], D.H. Betts [b], L. Lin [b], G. Coppola [b], S.D. Perrault [b], P. Blondin [c], W.A. King [b,*]

[a] *Department of Population Medicine, Ontario Veterinary College, University of Guelph, Ontario, Canada N1G 2W1*
[b] *Department of Biomedical Sciences, Ontario Veterinary College, University of Guelph, Ontario, Canada N1G 2W1*
[c] *L'Alliance Boviteq Inc., Saint-Hyacinthe, Quebec, Canada J2T 5H1*

Abstract

It appears that overt phenotypic abnormalities observed in some domestic animal clones are not transmitted to their progeny. The current study monitored Holstein heifers sired by a bull clone, Starbuck II, from weaning to puberty. Genomic stability was assessed by telomere length status and chromosomal analysis. Growth parameters, blood profiles, physical exams and reproductive parameters were assessed for 12 months (and compared to age-matched control heifers). Progeny sired by the clone bull did not differ ($P > 0.05$) in weight, length and height compared to controls. However, progeny had lower heart rates (HR) ($P = 0.009$), respiratory rates (RR) ($P = 0.007$) and body temperature ($P = 0.03$). Hematological profiles were within normal ranges and did not differ ($P > 0.05$) between both groups. External and internal genitalia were normal and both groups reached puberty at expected ages. Progeny had two or three ovarian follicular waves per estrous cycle and serum progesterone concentrations were similar ($P = 0.99$) to controls. Telomere lengths of sperm and blood cells from Starbuck II were not different ($P > 0.05$) than those of non-cloned cattle; telomere lengths of progeny were not different ($P > 0.05$) from age-matched controls. In addition, progeny had normal karyotypes in peripheral blood leukocytes compared to controls (89.1% versus 86.3% diploid, respectively). In summary, heifers sired by a bull clone had normal chromosomal stability, growth, physical, hematological and reproductive parameters, compared to normal heifers. Furthermore, they had moderate stress responses to routine handling and restraint.

Keywords: SCNT; Animal cloning; Progeny assessments; Telomere; Chromosome

1. Introduction

Animal cloning by somatic cell nuclear transfer (SCNT) has been reported for several mammalian species. Since Dolly, the sheep clone [1], several other species have been cloned, e.g. mice [2], cattle [3,4], goats [5], pigs [6,7], rabbits [8], cats [9], mules [10], horses [11–13] and most recently, the dog [14]. However, there are two main interrelated limitations for the widespread commercial implementation of this technology: the first one is low efficiency, which in cattle is only approximately 6% surviving to term [4]. The other one is the ''cloning syndrome'' characterized by a series of developmental abnormalities resulting in early embryonic death, high rates of abortion, prolonged gestation, large offspring syndrome, placental and fetal malformations and immune system complications [15]. A high

* Corresponding author. Tel.: +1 519 824 4120/54927; fax: +1 519 767 1450.
E-mail address: waking@uoguelph.ca (W.A. King).
[1] Present address: Alberta Agriculture Food and Rural Development Edmonton, AD, Canada T6H 4P2.

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incidence of postnatal mortality and increased phenotypic variation has also been reported [16]; these are of concern to the general public and government institutions, with consequences for the acceptability, use and regulation of animal clones and their products.

Many factors may be involved in SCNT failure. The in vitro handling of reconstructed embryos has been implicated in immediate- to long-term adverse consequences [17]. Epigenetic alterations and ''maturity'' of the recipient cytoplasm [17], as well as donor cell-derived chromatin alterations, all have an effect on the impaired reprogramming capabilities of the newly transferred nucleus and on embryonic chromosomal abnormalities [18–20]. Evidence of these alterations following SCNT is manifested by inappropriate expression of certain genes at various stages of development, such as imprinted and non-imprinted genes [21–23], and by aberrant chromatin modifications [24] such as X-chromosome inactivation status [25] and DNA methylation patterns [19,26,27]. Furthermore, an increased incidence of chromosomally abnormal cells in live–born cattle clones has also been reported [28].

One molecular marker to evaluate nuclear reprogramming efficiency and chromosomal stability in clones is telomere length changes. The telomere length alterations present in many animal clones were apparently correlated to the type of donor cell utilized, the age of donor individual and duration of donor cell in vitro culture [29–31]. With the exception of progeny sired by caprine clones [32], it appears that the offspring of clones have telomeres of normal length in both somatic and germline cells, as well as a very low incidence of cells with chromosomal anomalies [31,33]. Therefore, the abnormal telomere lengths in SCNT animals are reversed to normal sizes in their progeny. Other genetic and overt phenotypic alterations in clones appeared not to be transmitted to their offspring, suggesting that epigenetic and genetic alterations are reset in the germline of clones.

In terms of overall health, cattle clones may range from clinically normal to exhibiting severe respiratory distress syndrome and left-sided cardiopathy with ventricular dilation and cardiopulmonary hypertension [16]. Apparently, ''healthy'' clones may have a higher body temperature at birth, together with higher erythrocyte mean cell volume and plasma leptin concentrations [34]. Thymic aplasia has been reported in cattle clones, resulting in compromised immune function, which may be associated with an increased incidence of infectious disease [35]. A detailed physical examination and hematological characterization provided a good indication of the presence or absence of these conditions [15]. Conversely, other reports indicated that the incidence of frequent health complications in clinically healthy clones was similar to that in control animals [15]. Growth rates of clone heifers were similar to those attained for normal age-matched controls [15]. Similarly, fertility rates for natural mating and artificial insemination were similar to those achieved in normal heifers, and there were no apparent differences in pregnancy and parturition [15,36].

The commercialization and practical use of cloning technology is presently minimal. From a production perspective, the benefit of cloning high-quality individuals will be to increase the number of descendents of elite genomes in the breeding population via enhanced and prolonged production of a large number of progeny. Since it is likely that the offspring of clones will be used in commercial production settings, with their edible products entering the food chain more frequently than the clones that produced them [37], it is important to monitor the offspring of clones. Overall health, growth and reproductive parameters have not been extensively studied for the progeny of cattle clones due to limited numbers of offspring [15,38]. Based on these minimal observations, progeny of cattle clones displayed greatly improved long-term viability compared to the clones themselves, with hematological and biochemical profiles within normal ranges. The incidence of disease is very low and comparable to normal non-clone animals. The objective of the present study was to investigate, from weaning to puberty, the growth, hematological, reproductive, genetic, physical and overall health parameters in a group of heifers sired by a bull clone, Starbuck II, compared to aged-matched controls.

2. Materials and methods

2.1. Animals

A total of 30 calves (19 females and 11 males) produced at different times from artificial insemination using frozen–thawed semen collected from Starbuck II, a bull clone produced in Que., Canada (Centre d'Insémination Artificielle du Québec L'Alliance Boviteq Inc and the Faculté de Médecine Vétérinaire, Université de Montréal) to breed normally cycling Holstein recipients. Shortly after birth, seven of the heifers were re-located to the Ponsonby Research Station of the University of Guelph (Guelph, Ont. Canada), where they were monitored on a monthly basis until puberty. Concurrently, a group of breed- and age-matched controls were similarly monitored. The duration of the observation period in both groups was 12 months.