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Dairy cattle exploratory and social behaviors: Is there an effect of cloning?

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Abstract

While an increasing number of animals are produced by means of somatic cloning, behavioral studies on cloned animals are still rare. The aim of this study was to investigate whether the somatic cloning procedure has an influence on locomotion, exploratory, vocal and social behaviors of heifers. Ten heifers were used in the present study. Five of them were cloned heifers derived from somatic cells of three different *Prim'Holstein* cows and five others were same-age control heifers produced by artificial insemination. In addition to observations of social behaviors in the stable group, each animal was placed individually for a short time in an unfamiliar environment. Our results failed to show any statistical differences between clones and their controls both in frequencies of agonistic and non-agonistic behaviors. However, cloned heifers showed significantly more non-agonistic and less agonistic behaviors towards other cloned partners than towards control ones. This result also stood for control heifers. As far as their Hierarchical Index was concerned, three cloned heifers were highest ranking and two others lowest ranking. In this herd, social dominance appeared to be linked to body weight and age rather than to a cloning effect. In an unfamiliar environment, cloned and control subjects exhibited the same level of locomotion and vocalization. However, cloned heifers showed more exploratory behaviors than did control ones. This difference could be due to environmental factors during the postnatal period rather than to cloning.

Keywords: Cattle; Somatic cell nuclear transfer; Social and exploratory behaviors; Locomotion; Vocalizations

1. Introduction

Since Dolly the sheep [1], several mammalian species, cattle [2], goat [3], mouse [4], pig [5], rabbit [6], cat [7], horse [8], rat [9] and more recently dog [10], have been cloned from somatic cells. A strong interest has developed for cloning cattle [11–14], mainly for scientific and economical reasons. Cloning can be a successful process, though a high incidence of fetal loss is observed [15]. Somatic cloned calves have been characterized by high birth weight, frequent delivery by caesarean section and increased perinatal mortality [16–18]. Researchers thus are trying to identify causes of these problems in order to guarantee the health and well being of animals issued from cloning [19]. Cloning by somatic cell nuclear transfer is still a new method of reproduction and there are very few studies about its

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consequences on the behavior of offspring. Does the cloning process affect the adaptive behavior of domestic animals? To ensure the well being of cloned animals and for a better knowledge of possible effects induced by cloning, behavioral studies are needed.

There have been very few behavioral studies on animals cloned from somatic cells, due to the limited number of such cloned animals. In mice, Tamashiro et al. [4] failed to show any effect of cloning on locomotor activity in home cage and on spatial performances in a Morris water task. In cattle, a study by Savage et al. [20] reported that four cloned heifers exhibited a higher level of curiosity, more grooming activities and were more aggressive and dominant than controls. These authors also described that these clones issued from the same donor preferred each other as companions to unrelated conspecifics, which may suggest a process of kin recognition. In that study, all the cloned heifers were derived from a single 13-year-old Holstein cow and the design did not allow disentangling putative effects of cloning and those due to the donor's genetic background.

The aim of the present study was to investigate whether or not somatic cloning had an influence on locomotion, vocalizations, exploratory and social behaviors of heifers derived from several donors. This was investigated both in an undisturbed mixed herd (cloned heifers and their age-matched controls) and through an isolation test in an unfamiliar environment.

2. Materials and methods

2.1. Animals and housing

A total of 10 *Prim'Holstein* heifers belonging to two categories (cloned heifers and control heifers) were involved in the present study (Table 1). Five cloned heifers were produced from adult somatic cells of three different *Prim'Holstein* genotypes (A, B and C). The five control *Prim'Holstein* heifers were age matched and produced by artificial insemination from four different bulls in the same farm. The group was formed with 6 to 13.5 months old individuals. Before 6 months of age all clones and controls were housed in the same nursery in individual box stalls in similar conditions. At 6 months all subjects have been introduced in a social transition group (ST group). The composition and the size (16–24 animals) of this group varied depending on introduction and removal of individuals. The proportion of cloned and matched control individuals was stable and balanced. The duration of the subjects' social experience in ST group ranged from 0.5 to 6 months. Six subjects (three clones and three matched controls) stayed in the ST group for 6/7 months, the last four subjects (two clones and two matched controls) for 0.5–2 months. At the end of this social experience in the ST group the 10 animals, aged from 6.5 to 13.5 months, were grouped together to constitute the experimental groups in the same loose house system (11 m × 12 m). The mean surface available was of approximately 13 m^2 per animal.

The animals were housed at the INRA experimental farm in Bressonvilliers with artificial and/or natural light between 6 a.m. and 7 p.m. Each heifer was identified with an I.D. number printed on two ear tags. In addition, prior to the study, one of us (M. Coulon) was trained recognizing individual coat patterns.

Throughout the observational period, heifers were maintained as a stable group with free unrestricted access to a unique standard diet (grass silage, hay, corn straw and mineral).

2.2. Behaviors

2.2.1. Social behaviors

Observation sessions occurred from 5 p.m. to 7:30 p.m., four times a week, during 8 weeks after the introduction of the 10 heifers. Each heifer was observed during each session for three 5 min periods (focal animal sampling) [21]. The time interval duration between each period was 50 min, and the order of observed individuals was randomly assigned each day. All observations of coded behaviors were completed by means of hand written method: types and frequencies of social behaviors were noted as well as the identity of the donor and the receiver. The following social behaviors were noted: agonistic behaviors with offensive behaviors (offensive approaches with threats, butts and fights) and defensive behaviors (spontaneous withdrawals, escapes) and non-agonistic behaviors (non agonistic approaches with sniffing, licking, rubbing, supporting head on the back of another animal) [22,23]. Number of behavioral occurrences was noted for each subject and for each dyad.

A Hierarchical Index, HI, was calculated for each individual across the 8 weeks. It corresponds to the ratio between the number of offensive behaviors and the sum of agonistic behaviors (offensive plus defensive) given by one individual [24]. The Hierarchical Index varies between 0 and 1. The calculation formula is given below (where x is a given individual).

$$\text{Hierarchical Index}(x) = \frac{\text{Offensive behaviors}(x)}{\text{Offensive} + \text{defensive behaviors}(x)}$$