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

M. Coulon a,b,*, C. Baudoin a, M. Depaulis-Carre a, Y. Heyman b, J.P. Renard b, C. Richard c, B.L. Deputte a,d

a Université Paris 13, CNRS UMR 7153, Laboratoire d’Ethologie Expérimentale et Comparrée, 99 avenue J-B. Clément, F-93430 Villetaneuse, France
b INRA, Biologie du Développement et Reproduction, UMR INRA ENVA 1198, F-78352 Jouy-en-Josas, France
c UCEA-INRA, Bressonvilliers, F-91630 Leudeville, France
d ENVA, Ethologie, F-94704 Maisons-Alfort Cedex, France

Received 16 March 2007; received in revised form 22 June 2007; accepted 2 August 2007

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.

© 2007 Elsevier Inc. All rights reserved.

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
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 m² 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).

Hierarchical Index(x) = \frac{\text{Offensive behaviors}(x)}{\text{Offensive + defensive behaviors}(x)}
We also calculated an Agonistic Index among clones and among controls (intracategories of heifers) and between clones and controls (intercategories of heifers) across the 8 weeks. This index corresponds to the ratio between the number of agonistic behaviors (offensive and defensive) during a dyadic encounter between a subject \((x)\) and a conspecific \((y)\) and the sum of sociable (= non-agonistic) behaviors \([25]\).

\[
\text{Agonistic Index}(x, y) = \frac{\text{Agonistic behaviors}(x) + \text{Agonistic behaviors}(y)}{\text{Sociable behaviors}(x) + \text{Sociable behaviors}(y)}
\]

The Agonistic Index is positive and greater than 1 when the number of agonistic behaviors recorded for a dyad is greater than the number of sociable (= non-agonistic) behaviors during the encounter. This Agonistic Index allows comparison of levels of agonistic and non-agonistic behaviors for dyads during encounters, rather than comparing individual behaviors, thus allowing a better understanding of social organization.

2.2.2. Individual test: exploratory behaviors, locomotor activity and vocalizations

An individual test was performed after the observational period of the group, 8 weeks after the experimental group formation. Heifers were removed individually from their social group and introduced into an unknown enclosure \((3 \text{ m} \times 6 \text{ m})\) for 5 min (individual test). This enclosure was divided into six areas of \(3 \text{ m}^2\). To assess their level of locomotor activity, the total number of areas crossed was noted. An area was considered as crossed when one animal had, at least, its two forelegs inside this area. The number of exploratory behaviors (sniffing, lickings at some part of the environment) and of vocalizations were also noted. After the 5-min test, the heifer was reintroduced in their group without inducing any immediate agonistic interaction.

2.3. Statistical analysis

Due to small sample size and not normally distributed data only non-parametric statistics were used. We checked firstly that the number of behaviors did not vary during the successive weeks of observation using a Kruskal–Wallis test \((H\text{ statistics})\) \([26]\). Then frequencies of agonistic and non-agonistic interactions were compared using a Wilcoxon test \((T\text{ statistics})\) \([26]\). We used the Kendall partial correlation coefficient \([26]\) to sort out the effect of age and body weight on HI. Social behaviors, Agonistic Index of cloned and control heifers for individual behavior and for dyads were compared using the Mann–Whitney test \((U\text{ statistics})\) \([26]\) as well as all behaviors observed in a new environment. A two-tailed probability level of 0.05 was used throughout. All means were presented with the standard error, S.E. All analyses were performed with the statistical package Statistica\(^R\).

2.4. Ethical note

Animal care and all procedures were performed in accordance with EU directives and authorization B91 332 101 and 93-031 of the French Ministry of Agriculture. The protocol, registered as “number 06-002”, was approved by the French Regional Ethical Committee for Animal Experimentation of Ile de France-Sud.

3. Results

For all social behaviors, no difference appeared between the 8 weeks of observation \((H = 11.8; p = 0.1)\). Consequently, data across the whole period were pooled together.
3.1. Social behaviors in a loose housed mixed herd

Overall heifers presented more non-agonistic behaviors than agonistic ones ($T = 1; p < 0.01$) with control heifers exhibiting significantly more non-agonistic than agonistic behaviors ($T = 0; p = 0.04$), while this difference was not significant for clones ($T = 1; p = 0.08$).

There were no significant differences between clones and controls for frequency of social behaviors ($U = 4; p = 0.07$), whatever their kind, agonistic or non-agonistic (respectively, $U = 11.5; p = 0.83$ and $U = 10; p = 0.6$; Fig. 1).

Heifers were ranked according to their Hierarchical Index (Fig. 2). Three cloned heifers (from the same donor animal) appeared in the first three ranks but the other two (from clones A and B) appeared in the last two ranks. Body weight (as measured at the beginning of study) was significantly positively correlated to Hierarchical Index (HI and body weight $T_{HI-BW} = 0.69; p < 0.01$; Fig. 3A). However, as age was also positively correlated to both variables ($T_{HI-Age} = 0.75; p < 0.01$; Fig. 3B), we used the Kendall partial correlation coefficient to sort out the effect of age and body weight on HI. HI was actually more correlated with age than with body weight as the correlation between HI and body weight dramatically decreased when variation of age was kept constant (Kendall partial correlation coefficient, $T_{HI-BW, Age} = -0.10$).

During observational period, significantly less agonistic behaviors ($U = 669; p < 0.01$) and more non-agonistic behaviors ($U = 698; p = 0.01$) occurred in intracategories of dyads of heifers (within clones or within controls) than between dyads of clones and controls (intercategories of heifers, Fig. 4). The Agonistic Index is significantly lower between dyads of heifers of a same category (cloned or control heifers) than between dyads of clones and controls ($U = 57; p < 0.001$; Fig. 5). However, no difference appeared between clones and controls ($U = 45; p = 0.7$; Fig. 5).

3.2. Behaviors in a new environment (individual test)

Cloned heifers explored significantly more the new environment than did controls ($U = 2.5; p = 0.03$; Fig. 6). No differences were found between clones
and controls regarding locomotor activity ($U = 11.5; p = 0.83$) and vocalizations ($U = 11; p = 0.75$).

### 4. Discussion

In the context of the group, social behaviors of cloned heifers, derived from several donors, were similar to those of matched controls. Non-agonistic interactions among clones or among controls were more frequent than between clones and controls. When tested individually in a new environment, clones and controls showed similar locomotor activity and vocalizations, however, cloned heifers explored more than their controls.

In our study, cloned heifers behaved in the same way as control AI animals and their social behaviors appeared very similar to behaviors already described in various breeds of cows [27–31]. Cloned heifers, like their controls, presented relationships based on agonistic and non-agonistic behaviors. In general, non-agonistic interactions were more frequent than agonistic interactions, which is a characteristic of a stable herd and can reinforce social bonds [31]. These bonds are strongly influenced by the duration since the group was formed and animals have been together [30].

Our results slightly differed from those of Savage et al. [20] who observed that cloned heifers behave more aggressively than controls in their group, leading to a clone’s dominance. In this study [20], the four clones were derived from the same donor who also exhibited behavioral dominance and these observations could indicate a possible correlation between the common genotype and the observed dominance. Clones in our study originated from three different donors, and did not perform more agonistic behaviors than did their controls. The only social situation where more agonistic behaviors were recorded was when cloned heifers were interacting with controls and in that case the more aggressive heifer was the oldest one. In this herd, social dominance among heifers appeared classically linked to body weight and age rather than to clones’ characteristics contrary to the study by Savage et al. [20]. In their study, these authors controlled the characteristics (age, weight, experience) of cloned heifers and their controls, they did not differ and consequently they did not allow to study their effect on the behavioral dominance. In our study another factor influencing the Hierarchical Index could be the onset of puberty, which occurs generally at 9–12 months, but one pre pubescent heifer (#427) was observed within the first four ranks, perhaps indicating that this factor is not the only one involved.

Moreover, clones and controls showed similar locomotor activity and vocalizations when observed individually in a new environment. These results are consistent with earlier observations by Savage et al. [20]. The locomotor activity exhibited during this kind of test is thought to measure anxiety, since greater activity reflects higher emotional reactivity [28]. Our results, i.e., similar locomotor activity in both groups, suggest that all heifers react with the same emotional

---

**Fig. 4.** Frequencies of agonistic and non-agonistic behaviors observed among clones or among controls (“Within” dyads (clone–clone plus control–control) = stipple bars and “Between” dyads (clone–control) = hatched bars) [**$p < 0.01$**].

**Fig. 5.** Dyadic Agonistic Index (mean ± S.E.M.) observed among clones (filled bars), among controls (open bars), among clones or among controls (“Within” dyads = stipple bars) and between clones and controls (“Between” dyads = hatching bars) [***$p < 0.001$**].

**Fig. 6.** Frequencies of exploration, locomotor activity and vocalizations of clones (filled bars) and controls (open bars) during the individual test in a new environment [*$p < 0.05$*].
level. However, we observed that clones explored more than controls did. This last result fits with the observations of Savage et al. [20]. Indeed, although the conditions of management of animals were controlled, the clones could benefit from more intensive care during the postnatal period and had more interactions with humans than controls, the former being more fragile during the first weeks of life [17]. This environmental difference could have favored their exploration performances.

Cloned and control heifers showed more non-agonistic behaviors, and less agonistic behaviors, when interacting with heifers of their own category than with the other category. The Agonistic Index was lower for dyads of heifers of the same category (either control–control or clone–clone) than for control–clone dyads. In this study the heifers of the two categories (clones and controls) were managed under similar conditions. These results confirm and extend those of Savage et al. [20] concerning regroupings and stronger interactions between the clones of a group. In Savage et al.’s study [20], the four clones were issued from the same donor and they interpreted their results as possibly indicating genetic kin recognition processes. In our study, the five cloned heifers originated from three different genotypes and they were more frequently associated between them than expected if kinship was the most important factor influencing these associations. In our group with only three clones from the C donor, we never observed closer interactions between them than with the other two cloned heifers. Association among clone heifers, as well as grouping among controls, could be due also to similar behaviors within each category of heifers. Both cloned and control heifers are likely to show non-agonistic behaviors with others, the behavioral profiles of which are similar [32,33]. Our group of clones was constituted with heifers derived from three genotypes, which introduces more genetic diversity than in the study of Savage et al. [20]. In further studies, it would be interesting to observe if closer interactions occur between heifers issued from a same clone than between heifers from different clones.

In conclusion, in the mixed herd, cloned and control heifers present the same behavioral characteristics. Complementary studies of cloned heifer behavior under various environmental conditions would be of interest for the detection of potential behavioral differences between heifers born from somatic nuclear transfer when compared to control heifers born from conventional reproduction. Moreover, longitudinal studies on the development of social behavior in controlled environments are now needed for explaining individual differences due to environmental influences. Those studies would also consider the possible variations of behavior occurring in animals of different ages.

Acknowledgements

We thank the staff of the experimental farm INRA-Bressonnvilliers for special care to the animals. We are indebted to Coralie Taquet to previous observations and to Prof. F. S. Dobson and Dr. Christine Moinard for helpful comments.

References


[27] Plusquellec P, Bouissou MF. Behavioral characteristics of two dairy breeds of cows selected (Herens) or not (Brune des Alpes) for fighting and dominance ability. Appl Anim Behav Sci 2001;72:1–21.


