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Short communication

Behavioural study of a progeny of a bull clone

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ABSTRACT

Behaviour of progeny of a bull clone was compared to that of offspring from the donor bull. A total of fifteen Friesian female calves were used. Seven of them were progeny of a bull clone (CLp) and eight were offspring from its donor bull (DONp). During the collection of blood samples for hemocromocytometric analysis, behavioural response of calves to restraint was examined. Calves were individually restrained for blood sampling at 24–48 h, and at 1, 2, 3, 4, 8, 12, 16, 20, 24 weeks of age. Number of struggling movements (STR) and vocalisations (VO) occurring within the first minute of blood sampling were recorded by direct observation. The calves were also monitored at 2nd, 3rd, 4th, and between 5th and 8th week of age for lying, standing, nutritive and non-nutritive oral, sniffing, self-grooming behaviours and vocalisation. Each calf was observed in a morning and an afternoon time band, each lasting 3 h.

No difference was found between CLp and DONp on the overall mean for STR and VO at restraint. The study of general behaviour did not reveal difference between CLp and DONp for the average proportion of any of the considered traits.

Our results suggest that the adaptive ability of progeny of cloned bull to challenges and conditions imposed by a farm environment are comparable to what is normally observed in dairy calves reared under similar conditions.

1. Introduction

Cloning livestock by somatic cell nuclear transfer implies reprogramming a somatic cell into a totipotent embryonic cell. An incomplete nuclear reprogramming due to epigenetic dysregulation is considered to be the main reason of abnormalities and impaired health observed in livestock clones in peri-natal period (EFSA, 2012). These aspects have raised ethical and scientific concerns about the welfare of both clones and their progeny, due the possibility that epigenetic abnormalities observed in cloned subjects could be transmitted to the offspring.

Several studies were carried out on the progeny of cattle clones. Results of these studies showed that the offspring of clones displayed haematological and biochemical profile (Abeni et al., 2012), as well as growth and reproductive parameters (Abeni et al., 2012; Heyman et al., 2004; Kasai et al., 2007; Panarace et al., 2007; Watanabe and Nagai, 2008) comparable with healthy, non-clone animals, suggesting that epigenetic possible alterations present in clones were reset in the germ line of clone. This was further supported by Couldrey et al. (2011) who analysed the sperm DNA methylation patterns of Friesian bull clones.

Few studies were carried to investigate the effects of cloning on behaviour of cattle; these studies show that

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bovine clones exhibit less playing behaviour, higher levels of curiosity, more grooming activities (Savage et al., 2003) and more exploratory behaviour than conventional controls (Coulon et al., 2007). No difference was found between clones and conventional heifers for cognitive capacities and kin discrimination (Coulon et al., 2010).

To our knowledge no specific study on behaviour of progeny of bull clones has been done.

In farm animals, behaviour is an important and early indicator of proper maturation of physiological systems underlying the vital functions and ability to cope with the environment.

Restraint, even in absence of painful stimuli, has been shown to be a powerful stressor in several species; in cattle, behavioural responses to restraint can be used as indicators of coping ability and fearfulness, which are traits mediated by underlying biological functions.

Also the behavioural repertoire, the rhythms of rest and activity, the development of behaviour with age are potential indicators of the level at which the farm animals are adapted to their environment. Calves need to spend a lot of time resting and sleeping; Hänninen et al. (2005) found a positive correlation between the total duration of rest and the growth rate in calves; sleep is essential for regulation of several hormones and brain development in growing animals.

The aim of the present study was to assess whether there was a difference between progeny of a bull clone and offspring of the corresponding donor bull in (a) the level of excitability to routine handling, measured by the behavioural response, and (b) the general behaviour expression, as indicators of adaptive ability to farm environment.

2. Materials and methods

2.1. Animals and management

The study was carried out at the experimental dairy farm of CRA-FLC, located in Cremona (Italy). A total of fifteen Holstein female calves were used. The calves were born at different times from Holstein recipient heifers implanted with embryos obtained by IVF with frozen semen of a bull clone ($n=7$; CLp) or its nuclear transfer donor bull ($n=8$; DONp, as control); oocytes for in vitro production of embryos were collected from six Holstein donor cows. Deliveries were concentrated between November and January; median of birth dates were December 13th and December 23rd for CLp and DONp, respectively; standard deviation of birth dates was higher in CLp (42 days) than in DONp (21 days) due to one CLp born on September. The mean of birth weight were 38.4 kg (SD=8.2 kg; range=26.0–52.0) and 42.7 kg (SD=8.9 kg; range=27.0–54.0) for CLp and DONp, respectively. Calves were separated from their respective surrogate mothers immediately after birth and moved to a calf barn, where they were housed in individual straw-littered cages. Cages (182×90 cm^2) had solid sides, with an opening in the front to allow the calf access to bucket of milk and feeder. A heat lamp was directed onto the cage for the first 48 h of life. Calves were fed colostrum by bottle for the first two day postpartum. Starting from 3rd day of age, calves were fed reconstituted milk, twice daily (06:00–15:00), by nippled milk bucket (2 L/meal, on average). At approximately one week of age, the milk diet was supplemented with calf-starter and ad libitum roughage in the form of hay, distributed daily, immediately after the morning milk meal. At 8 weeks of age, calves were transferred outdoors into group pens (6.15×3.33 m^2; to a maximum of 4 calves/pen). The calves were weaned in the successive 2 weeks, by a progressive decrease in milk supply and after the assessment of an adequate solid feed intake (approximately above 700–800 g/day of DM).

All calves were healthy at birth, but during the first moth of life three DONp died for neonatal diarrhoea.

2.2. Data collection

2.2.1. Behaviour at restraint

Behavioural data were collected during sample session for the study of haematological profile and clinical chemistry. According to that protocol, each calf was subjected to manual restraint for sampling blood at jugular vein at 24–48 h and 1, 2, 3, 4, 8, 12, 16, 20, 24 weeks of age. Blood sampling sessions were performed between 06:00 and 07:00, before milk distribution. On each occasion, an operator entered the cage and manually restrained the calf firmly against a wall of the cage until the end of blood sample collection, while another operator outside the cage kept the calf's head. Blood sampling started immediately after immobilisation of the calf. Blood was drawn from external jugular vein using a 20 G needle connected to vacuum tube. From the insertion to removal of the needle passed at least 1 min per calf because of the need to collect from 3 to 4 samples. This was obtained by changing vacuum tubes, without removal of the needle from jugular vein. At the end of blood sampling the calf was released. In calves raised into group pens, restraint consisted of head immobilisation by the headlocks; a rope was also placed around the muzzle. Blood sampling procedure is described above. In cattle, it was already shown that acute restraint per se elicits a variety of physiological and behavioural responses, where struggling is the most common behavioural expression. Struggling movement was defined as vigorous trunk movements in attempting to free oneself from the squeeze by operator or head immobilisation by headlocks. Number of struggling movements (STR) and vocalisations (VO) occurring within the first minute of blood sampling were recorded by direct observation. Struggling movements were considered as a distinct event when a single or a series of trunk movements were interrupted by a period of body immobility of at least 10 s.

2.2.2. Behaviour over the pre-weaning period

Each calf was observed during four daily sessions at 2nd, 3rd, 4th, and between the 5th and 8th week of age; on the basis of preliminary observations of conventional calves reared on the same experimental farm, a morning (MTB) and an afternoon time band (ATB) were chosen for observations. Morning time band started 2 h after milk meal distribution and lasted 3 consecutive hours; observation during this time band aimed to compare the two groups mainly for resting behaviour; in ATB, calves were observed 1 h before,