BIOTECHNOLOGY

Cloned Cattle Can Be Healthy and Normal

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The possibility of cloning humans has raised questions as to whether nuclear transfer can be used to reproducibly generate healthy adult animals. Reports in the popular and scientific press on genetic, immunological, and other developmental problems raise the question of whether there are "any normal clones in existence" (*1–4*). We evaluated a series of 24 sexually mature cattle, cloned from nonquiescent somatic cells as described (*5*, *6*).

A total of 496 blastocysts were transferred into progestin-synchronized recipients, and 110 of the 247 recipients (45%) were pregnant as determined by ultrasound 35 to 40 days after transfer. Eighty of these spontaneously aborted (73% compared with 7 to 24% for in vitro fertilization–derived pregnancies) (*7*), 30 developed to term (Web table 1) (*8*), and six died shortly after birth (Web table 2) (*8*). Necropsy results indicated that five of six deaths were due to cardiopulmonary problems secondary to placental insufficiencies; one calf died from enteric complications 149 days after birth. The remaining 24 Holstein cattle (80%) were vigorous (45 ± 2 kg at birth compared with 43 kg normal weight) (*9*) and remain alive and healthy 1 to 4 years later [survival from birth to reproductive maturity normally ranges from 84 to 87% (*10*, *11*)].

Results of physical examination were normal for all animals, including objective (temperature, pulse, and respiratory rate) and subjective (general appearance, eyes, lymph nodes, and cardiac and pulmonary auscultation) findings. Results of abdominal palpation per rectum (reproductive and gastrointestinal organs and kidneys) were normal. Body condition scores (1 = thin, 5 = fat) averaged 3.25 (range 3.0 to 3.5), which is appropriate for the animals' nutrition program. Social interaction and behavior of the cloned animals are normal. They have normal conditioned responses such as reacting to farm equipment used for feeding. They have developed a social dominance hierarchy and the full spectrum of behavioral traits. Reproductively, the cloned animals exhibited puberty at the expected age (10 to 12 months) and body weight (318 to 365 kg). Conception rates after artificial insemination have been excellent, with 87.5% conceiving on the first insemination and the remainder conceiving on the second insemination attempt. Two of the cloned animals have given birth to calves that appeared normal in all respects (Fig. 1).

Fig. 1. Normal clone (Victoria, right) at 3½ years of age and offspring (Vicky, left) at 3 months of age. The calf, 30 kg at birth, was born without assistance and is suckling and growing normally.

Routine clinical laboratory analysis on blood and urine showed these animals to be consistently within normal ranges expected for adult bovines, with an occasional minor deviation (Web table 2) (*8*). Values for hematocrit, hemoglobin, red blood cells, mean corpuscular volume, and mean corpuscular hemoglobin concentration were normal, and white blood cell differentials were within normal ranges and ratios. Routine blood chemistry showed normal concentrations of electrolytes, urea, creatinine, glucose, bilirubin, aspartate aminotransferase, sorbitol dehydrogenase, albumin, globulin, and total protein, although several of the latter (globulin and total protein) were slightly below normal. Urine was negative for glucose, ketones, bilirubin, blood, and protein and within normal ranges for specific gravity and pH. Random arterial blood samples were within normal limits for pO_2 (90.4 to 94.7 mm Hg) and pCO_2 (34.3 to 38.8 mm Hg). Quantitative analysis showed expected ratios of immunoglobulin G (IgG), IgA, and IgM.

An adaptive T cell response requires expression of major histocompatibility complex class I and class II genes for peptide presentation to $CD8^+$ and $CD4^+$ T cells, respectively. Peripheral blood lymphocytes (PBLs) from naturally conceived Holsteins and clones were compared for cell surface phenotypes and the ability to be stimulated by phytohemagglutinin (PHA) (Fig. 2). There were no significant differences between PBLs collected from natural and cloned animals for percentages of cells positive for (i) bovine leucocyte antigens class I and class II, (ii) CD4 and CD8, (iii) CD45 expressed on all bovine lymphocytes, and (iv) a B cell marker detected by the VPM30 antibody (*12*). Furthermore, T cells from natural and cloned cattle proliferated in response to the PHA mitogen with comparable levels of ^{3}H-thymidine uptake at a fixed time point. These results support the conclusion that these cloned Holsteins have functional immune systems. The animals have responded to periodic infections in the same manner as naturally conceived, control animals.

Consistent with published reports (*6*, *13*, *14*), several of the cloned calves experienced pulmonary hypertension and respiratory distress at birth and fever after vaccinations at 4 months. However, we did not observe genetic defects, immune deficiencies, gross obesity, or other drastic abnormalities cited by other researchers. It remains to be determined whether these abnormalities occur in other species and/or are due to differences in nuclear transfer techniques.

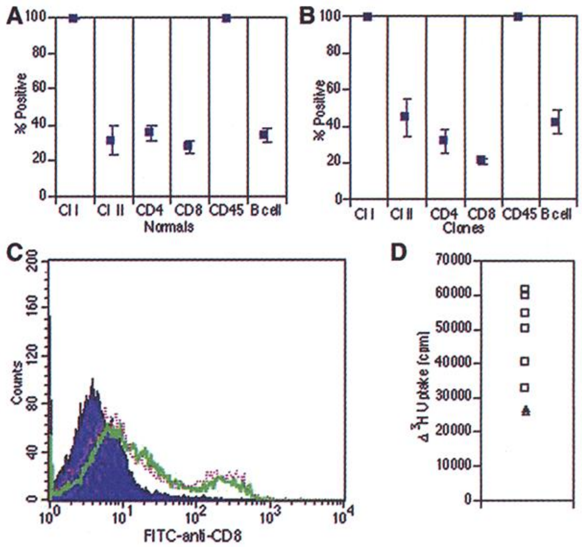

Fig. 2. Comparison of cloned and normal Holstein PBLs for cell surface phenotype and mitogen stimulation. (**A** and **B**) Mean percentages (±SE) of PBLs from normal (A) and cloned (B) Holsteins stained with specific monoclonal antibodies (Serotec, Oxford, England) and analyzed by flow cytometry with the use of isotype-matched control antibodies. (**C**) A single comparison of normal (red) and cloned (green) PBLs stained with fluorescein isothiocyanate (FITC) antibody to CD8 or FITC-isotype–matched control antibody (blue). (**D**) ^{3}H-thymidine uptake (cpm) by 5×10^5 clone (□) and normal PBLs (△) stimulated with PHA (2 μg/ml).

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15. We thank C. Blackwell, A. Spell, and J. Koster for their help in the collection of the data.

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ECOLOGY

Endangered Right Whales on the Southeastern Bering Sea Shelf

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The eastern North Pacific right whale (*Eubalaena japonica* Gray) was nearly extirpated by whaling by the 1960s. Today it is the most endangered population of large whale (*1*). Their historical summer distribution included the Gulf of Alaska and the southeastern Bering Sea, and that of the endangered western North Pacific population included waters east of Kamchatka, the Okhotsk Sea, and the Sea of Japan (*2*). During the intensive whaling era of the 1940s to 1960s, right whales in the southeastern Bering Sea concentrated in deep (>200 m) waters north of Unalaska Island, where they fed on the oceanic copepod *Neocalanus cristatus* (*2*). Here we report the summer distribution, prey, and primary habitat of a remnant population that now occupies a different habitat, the middle shelf of the southeastern Bering Sea, and forages on a different prey, *Calanus marshallae*. Given this region's large and possibly enhanced interannual and decadal-scale variability in climate and ecosystem response, as well as a long-term trend of warming summer sea-surface temperatures (SSTs) (*3*), the survival of these right whales is uncertain.

On the basis of our line-transect surveys in the southeastern Bering Sea, the eastern population of North Pacific right whales probably totals only tens of animals. Our study of a group of at least five, and possibly seven, right whales on 20 July 1997 on the middle shelf (57.13°N, 162.84°W; Fig. 1) involved the largest group of this species observed since the mid 20th century. The whales occurred in relatively warm (i.e., 10.4°C SST), well-stratified water in an extensive coccolithophore bloom of *Emiliania huxleyi*. Another sighting of one or two right whales on 21 July (57.16°N, 162.78°W; Fig. 1) reaffirmed the discovery. Near this region on 14 June 1999, soon after the seasonal sea ice retreat, we found a single right whale (56.56°N, 163.41°W; Fig. 1) in cold water (i.e., 4.4°C SST).

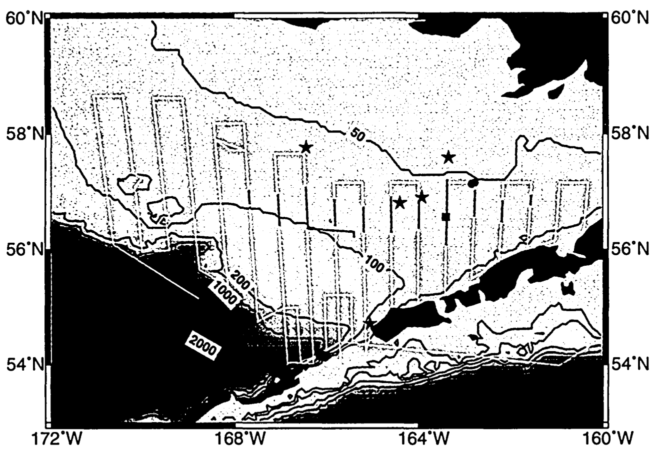

Fig. 1. Cruise track for cetacean surveys in the southeast Bering Sea, 17 July to 5 August 1997 and 10 June to 3 July 1999 (white lines), extent of a coccolithophore bloom (blue lines) in July 1997, and right whale sightings since 1985 (*7*) (★), 20 to 21 July 1997 (●), and 14 June 1999 (■).

These sightings are clustered in relatively shallow water (50- to 80-m depth) on the middle shelf between 162° and 166°W (Fig. 1). Right whales predictably occur near or south of the Inner Front (50-m isobath) in stratified waters. These patterns suggest that frontal features and stratification play an important role in the availability and concentration of right whale prey, calanoid copepods.

Right whales migrate to productive high-latitude waters where they target dense concentrations of prey for energetically successful feeding (*4*). *C. marshallae* (*5*) was the dominant copepod species in zooplankton samples collected near right whales on the middle shelf of the southeastern Bering Sea in 1997 and 1999. This Northeast Pacific shelf species is typical of the eastern Bering Sea middle-shelf assemblage of copepods (*6*). Right whales on the middle shelf appear to rely on patches of the larger, later copepodite stages (C III to C V) of *C. marshallae*. The copepodites of *C. marshallae* on the southeastern Bering Sea shelf were twice as heavy as those from a temperate region, the Oregon upwelling zone, and were also extremely rich in lipid. Right whales therefore should benefit from foraging on older stages of *C. marshallae* at higher latitudes. The middle-shelf concentrations of older copepodite stages of *C. marshallae* were an order of magnitude greater in the late 1990s than during the early 1980s. This increased production may explain why a remnant population of right whales now predictably occupies the middle shelf during summer.

Right whales, like other rare species, are not necessarily to be found in the center of their historical distribution, but rather at the edge of their once broad range. Ultimately, the distribution of foraging right whales is determined by effects of climatic forcing on the extent and duration of ice cover, the timing and magnitude of the spring bloom, and the consequent copepod production. Endangered right whales may be among the best bellwethers of large ecosystem shifts.

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