

## Twinning in muskox and the cytogenetic investigation of a freemartin

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**Abstract:** The occurrence of twinning has been documented for muskox in both wild and captive populations. Of two known captive twin births only one set survived beyond 120 days. In both cases the twins were male-female pairs and both females showed abnormal sexual development. Two sets of stillborn twins have also been recorded. All four stillborn fetuses were female and none showed anomalies of the reproductive tract upon post-mortem examination. Blood cultures from the surviving male and female twins revealed that both were chimeric, indicating the admixture of fetal blood. Fibroblast cultures were normal for the respective sex of each individual. The freemartin heifer had anatomical abnormalities of the clitoris as well as the secondary sex characteristics of a male.

**Key words:** twins, reproductive dysfunction

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### Introduction

Twinning has been previously reported in the muskox, *Ovibos moschatus*, in both free-ranging and captive populations (Alendal, 1979; Pattie, 1986; Reindl, 1987; Wilkinson, 1971). Investigation of two wild populations in Alaska revealed that 4 of 102 pregnant cows were carrying twins during the latter half of pregnancy (Dineford and Anderson, 1984). The numbers, sexes, and reproductive ability of surviving twins in these free-ranging populations is unknown.

Four sets of twins have been born in captive herds (Table 1.), (Reindl, 1987; Watson, pers. comm.). No anomalies were observed in any of the four females born to female/female twin pairs. By contrast, both females born to the male/female twin pairs had abnormal sexual

characteristics (Watson, pers. comm. and personal observation). Both of these females were probably freemartins, although only one was available for investigation. Marcum, (1974) presents a classic description of the freemartin syndrome.

The abnormalities observed for the freemartin, studbook number 248, included an increased physical size, an enlarged horn boss (Fig. 1), an enlarged clitoris (Fig. 2), and masculine behavioral changes. During a physical examination, it was noted that the genitalia appeared near normal for a female though the clitoris was enlarged. The vagina did not appear blocked. Rectal palpation did not confirm developed ovaries.

Table 1. Summary of captive born twins.

Location	Sex	Date of birth	Comments
Musk Ox Dev. Corp.	F/F	10 May - 70	stillborn
Minnesota Zoo	M/F	29 May - 84	male died 26 Oct. -90
Musk Ox Dev. Corp.	M/F	6 June - 85	female died ≈ 35 days male died ≈ 90 days
Minnesota Zoo	F/F	24 March - 90	aborted fetuses

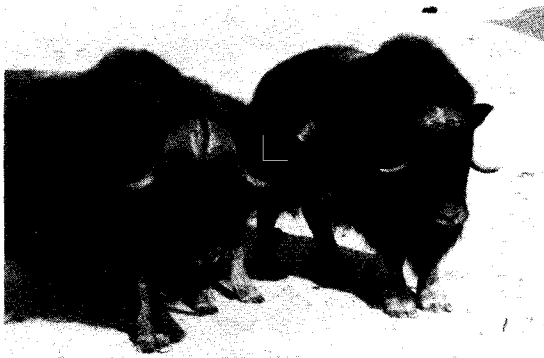


Fig. 1. The differences in physical size and horn development between the freemartin, #248, on the left and the normal cow, #234, on the right, is apparent (photographed at ages five and eleven respectively). The horn boss width of 32.5 cm is nearly identical to that of its male co-twin (32.0 cm) and double the width of its dam (16.0 cm).

Behavioral changes were recorded during the development of the freemartin as anecdotal comments in the keeper daily reports. In July of 1989, as a five-year old, #248 was reported as behaving like a bull in rut by chasing cows and a young bull, banging tires and vocalizing. Other reports stated that #248 was aggressively chasing all other muskox away from feed and that she broke a butting post off at ground level.

Blood and tissue samples were collected from both twins for cytogenetic analysis.

### Cytogenetic methods and materials

Chromosome preparations of lymphocytes were made from 10 drops of whole blood, which was cultured for 72 hours in RPMI-1640 me-

dium containing 20% fetal calf serum and 3% Pokeweed mitogen. The cultured cells were harvested following 2 hours treatment with Colcemid (0.01  $\mu\text{g}/\text{ml}$ ), followed by 15 minutes of 0.075 M KCL hypotonic treatment, and fixed using 3:1 methanolacetic acid. Chromosome spreads were made, stained and observed microscopically.

Chromosome preparations of fibroblasts were made from cultured skin biopsies. The skin biopsies (approximately 8 millimeters in diameter) were washed free of blood in balanced salt solution, and aseptically minced into 1-2 mm pieces with a scalpel. The tissue fragments were distributed on 25  $\text{cm}^2$  culture flask bottoms, the excess fluid removed, and allowed to attach for 30 minutes. Then 2 ml of Complete Eagle's medium containing 10 % fetal calf serum were added to the flask carefully so as not to disturb the tissue fragments. Every 48-72 hours of incubation the medium was replaced with 10 ml



Fig. 2. The external genitalia of the freemartin, #248, with the vulval lips parted to reveal the enlarged clitoris.

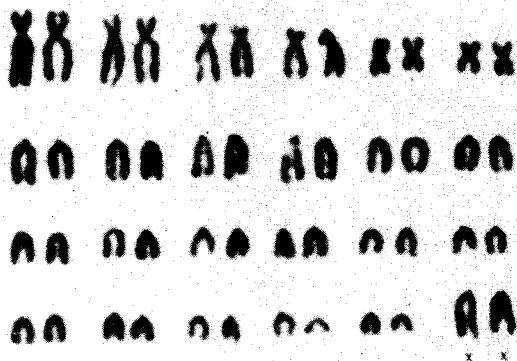


Fig. 3. Normal female chromosome spread and karyotype of a cultured blood lymphocyte from the freemartin muskox, #248.

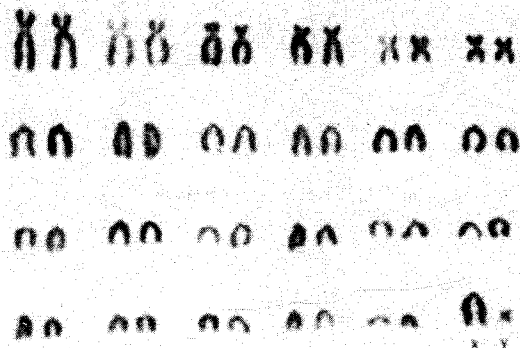


Fig. 4. Normal male chromosome spread and karyotype of a cultured blood lymphocyte from the freemartin muskox, #248. The Y chromosome is identified by the arrow.

of fresh media. Approximately 10–12 days later the near monolayer outgrowth of fibroblasts was trypsinized and divided into 2 flasks. During the last two hours of a 30 hr incubation period Colcemid (0.01  $\mu\text{g}/\text{ml}$ ) was added to one flask. Following this routine air-dried chromosome spreads were prepared, and Giemsa stained spreads were examined microscopically.

### Results and discussion

Fifty blood lymphocyte spreads were examined for each of the male and female co-twins. In the freemartin female 6 spreads were normal female (xx) and 44 were male (xy). The male co-twin was similar, 5 spreads were female (xx) and 45 were male (xy). The 50 fibroblast spreads from the skin of the freemartin were all female (xx)

and 30 spreads from the male skin biopsy were all male (xy). Both of the twin muskox are chimeric, the result of the exchange of fetal blood.

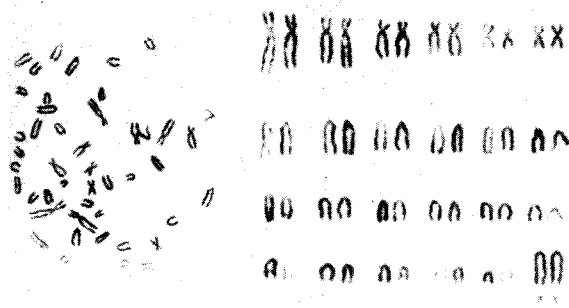


Fig. 5. Normal female chromosome spread and karyotype of a cultured skin fibroblast from the freemartin muskox, #248.

## References

- Alendal, E.** 1979. Muskox cow most likely with twins in the Dovre Mountains, Norway. *Norsk Polarinst. Årbok*. 1978: 88-92.
- Dinneford, W. B. and Anderson, D. A.** 1984. Fetal twinning rates, pregnancy rates, and fetal sex ratio in two Alaskan muskox populations. - *In: Proceedings of the First International Muskox Symposium, Biological Papers of the University of Alaska, Special Report. no. 4.*
- Marcum, J. B.** 1974. The Freemartin Syndrome. - *Animal Breeder Abstract* 42: 227-242.
- Pattie, D. L.** 1986. Muskox density and calf numbers on Devon Island's north coast. - *Journal of Mammalogy* 67 (1): 190-191.
- Reindl, N. J.** 1987. Twin birth of captive muskox. - *Der Zoologische Garten* 57: 370-371.
- Wilkinson, P. F.** 1971. The first verified occurrence of twinning in the muskox. - *Journal of Mammalogy* 52: 238.

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