

Le déficit en cellules germinales est loin de suffire à expliquer, à lui seul, l'arrêt de croissance des gonades. Le déficit en tissu somatique est plus important. La différence de volume entre les gonades des freemartins et des fœtus témoins est due pour 95 p. cent à une déficience en tissu somatique chez les fœtus de 49 à 59 j et pour 70 p. cent environ chez les fœtus de 60 à 110 j. Cependant, chez les freemartins de 49 à 59 j, le nombre des cellules germinales par unité de volume gonadique est identique à celui des témoins. Il y a donc proportionnalité entre la réduction du nombre des cellules germinales et la réduction du volume de tissu somatique; ceci ne s'observe plus après 59 j.

La méiose apparaît chez tous les freemartins de plus de 77 j, mais à chaque stade, elle ne concerne qu'un petit nombre des cellules germinales présentes. De plus, elle ne semble pas évoluer au delà du stade pachytène. Après le stade où sont apparues les premières figures de méiose, le nombre des cellules germinales contenues dans les gonades diminue beaucoup, il en subsiste encore quelques-unes après 150 j, mais aucune n'est en méiose.

### **Cytogenetic and Reproductive studies of Bulls Born Co-Twin to Freemartins**

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Thirteen A.I. Center bulls, born twin to freemartins, were karyotyped. Records of reproductive performance were available on 13 and necropsy specimens on 8. Fifty or more metaphases from cultured lymphocytes were scored to establish the XX/XV ratios. The percentage of XX cells, which ranged from 6 p. cent to 93 p. cent, were used to identify the bulls.

Five bulls (13XX, 36XX, 43XX, 60XX, and 71XX) either failed to produce semen or the semen produced was below standards for sperm motility, concentration and morphology, and as young bulls they were culled. The highest percentage of abnormal sperm were observed in 15 successive, unsatisfactory ejaculates of 60 XX.

The remaining eight bulls were used for artificial breeding. Four (6XX, 30XX, 63XX, 84XX) are now in-waiting for A.I. proofs. With the exception of 63XX, which produced a high percentage of sperm with acrosomal defects, their sperm production and fertility as young bulls were satisfactory. Thirty-three p. cent of the frozen semen of the young bull 29XX was discarded, but freezability of his sperm appeared satisfactory as a 6-year-old when he was culled for A.I. production. Fertility of 33XX was below breed average. Two Holsteins, 85XX and 93XX, were used as A.I. proven sires. Their sperm production as young bulls was below the average of their randomly chosen stablemates. There was a highly significant decline ( $P < 0.01$ ) in 85XX's sperm production as a 6-year-old, and he was culled as an 8-year-old despite his good fertility record. Fertility of 93XX was slightly below breed average but semen samples were satisfactory until severe spondylosis at age 10 complicated his semen collection.

The sex ratios of offspring of 29XX, 33XX and 85XX were obtained. A skewed male:female sex ratio of 29:71 in offspring of 33XX was significant ( $P < 0.05$ ). There was no evidence of germ cell chimerism based on blood typing of three offspring of 33XX and 23 offspring of 85XX.

Necropsy specimens from 13XX, 29XX, 36XX, 43XX, 60XX, 71XX, 85XX and 93XX revealed various levels of testicular degeneration.

These results indicate that XX/XV chimeric bulls are high risk sires for breeding purposes, probably as a result of hormonal deficiencies or invasion of the gonads with XX primordial germ cells.

### **Le chimérisme XX/XV ne joue pas de rôle dans la phase initiale du freemartinisme chez les bovins**

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Le développement des anomalies sexuelles chez les fœtus freemartins passe par deux phases successives distinctes: — entre 50 et 70 j, une phase initiale exclusivement d'inhibition (arrêt du développement de la gonade et régression des canaux de Müller comme chez les mâles) — après

70 j, une phase de masculinisation (développement de certaines caractéristiques masculines du tractus génital interne et différenciation de structures testiculaires dans la gonade).

L'analyse du chimérisme XX/XY, effectuée chez les fœtus freemartins durant la phase initiale d'inhibition (jusqu'à 70 j), ne montre aucun parallélisme entre le pourcentage de cellules XY dans le foie (très probablement des cellules hématopoïétiques) et le degré d'inhibition des gonades et des canaux de Müller, et il ne semble pas y avoir de chimérisme dans les tissus somatiques autres que les tissus hématopoïétiques.

De plus, lorsque dans les gestations multiples, on laisse s'établir les échanges cellulaires qui sont précoces (à partir de 30 j), et que l'on empêche expérimentalement les passages hormonaux en séparant chirurgicalement *in utero* les fœtus jumeaux avant l'apparition des premières anomalies sexuelles (50 j), les jumeaux femelles peuvent montrer (à 60 j) un important chimérisme XX/XY dans le foie (jusqu'à 56 p. cent de cellules XY) sans être affectés par l'inhibition caractéristique des gonades et des canaux de Müller.

Ces résultats indiquent que la phase initiale du freemartinisme chez les Bovins ne dépend pas du chimérisme XX/XY mais plutôt d'une hormone; peut-être le facteur testiculaire d'inhibition des canaux de Müller.

### Single-born XX/XY chimaeric bulls with normal phenotype

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In repeated blood cultures of a *German Simmenthal* × *Hungarian Simmenthal* A.I.-bull out of 1363 cells evaluated 98,61 p. cent female and 1,39 p. cent male were found. His sperm-production and fertility is out-standing (65,7 p. cent from 4 525 first inseminations; the average of the other 113 bulls was 51,3 p. cent in the same period). The sex-ratio of calves born from this bull as well from his father was found as normal. His dam, three paternal half-sisters, seven half-brothers and eight sons were found as karyotypically normal. In one single-born paternal half-brother out of 125 lymphocytes investigated 98,7 p. cent were XX and 2,4 p. cent XY. This second chimaera was producing also sperm and was found as normal according to the necropsy and histological investigation of the testis. In his bone marrow two XX, in the kidney six male and one female, in the testis one male mitoses were found. The two bulls were born in two well-known cooperative-farms, so their birth data are acceptable. The two dams were not relatives. No singleborn intersexes were observed in the mentioned groups. No blood-chimaerism was detected using the direct methods; the blood-type of the XY-cell-line of the A.I.-bull was reconstructed on the basis of his normal sons accepted according to the blood-type of their paternal grand-parents. All of the blood-factors (blood-group, Tf, Hb) of the XX- and XY-cell-lines agree at least one allele.

It is supposed, that these consequently identical alleles in the blood-type of both XX- and XY-cell-lines are of maternal origin and this suggests to the fusion of two early embryos originated from the fertilisation of the ovum and the second polar body (both originating from the same meiosis II and so having the same gene complements). The fact, that these two bulls are paternal half-brothers suggests to the possible role of a factor being in the sperm in the polocyte fertilisation and early embryo-fusion.

### A freemartin calf with XX/XXY mosaicism

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The *friesian* heifer was born twin to a dead bull calf. The vulva was aplastic and the long anogenital distance was unusual for the classic freemartin condition. Neither clitoris nor penis were palpable, but the urethral orifice lay just above the level of the mammary gland. At laparotomy neither gonads nor Wolffian nor Mullerian structures could be found in the broad ligament. The level of circulating testosterone was extremely low (200 pg/ml). Cytogenetic and blood typing work showed erythrocyte chimaerism and XX/XY/XXY lymphocytes. Skin cells showed XX/XXY mosaicism and a small number of cells with a translocation anomaly. There