Frank Rattray Lillie's Study of Freemartins (1914-1920) [1]

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Frank Rattray Lillie's research on freemartins from 1914 to 1920 in the US led to the theory that hormones [3] partly caused sex differentiation [4] in mammals. Although sometimes applied to sheep [5], goats, and pigs, the term freemartin [6] most often refers to a sterile cow [7] that has external female genitalia and internal male gonads and was born with a normal male twin. Lillie theorized that a freemartin [6] is a genetic female whose process of sexual development from an undifferentiated zygote [8] was suppressed or antagonized by her twin's release of male hormones [3] via their shared blood circulation *in utero*. Despite publications of similar findings by physician Julius Tandler in Vienna, Austria, in 1910 and physician Karl Keller in Wiesensteig, Germany in 1916 prior to Lillie's research, Lillie often receives credit for the hormonal theory of sex differentiation [4] in the freemartin [6]. Lillie's study of freemartins, and the subsequent research by graduate students in Lillie's laboratory at the University of Chicago [9] in Chicago, Illinois, prompted many embryologists to research sex differentiation [4] and hermaphroditism in mammals.

Aristotle [10], who in the fourth century BCE lived in Athens, Greece, described hermaphroditism in animals; but scholar Marcus Terentius Varro, who lived in Rieti, Italy, for most of his life and died in 27 BCE, first wrote about a sterile cow [7] born with a male twin. In 1779 John Hunter [11], surgeon to King George III in London, England, detailed the anatomy of three freemartins and he observed that each freemartin [6] was born only with a male twin. Before DNA analysis of chromosomes in the late 1950s, scientists determined the organism's sex by the internal sex organs, which, in the freemartin [6], are male structures. In 1909 David Berry Hart in Edinburgh, Scotland, and in 1910 Leon Jacob Cole at the University of Wisconsin in Madison, Wisconsin, independently concluded that the freemartin [6] resulted from a single egg [12] division to form twins, and that the freemartin [6] was actually a male whose sex glands were underdeveloped. Between 1910 and 1916, prior to Lillie's study of the freemartin [6], Tandler and Keller published articles about the freemartin [6] and determined in their analyses, like Lillie, that the freemartin [6] was a female cow [7]. Furthermore, Tandler and Keller identified the shared blood circulation *in utero* of the twins as a cause of the sex differentiation [4] changes to the freemartin [6].

Lillie conducted his research on the freemartin [6] at the University of Chicago [9] in Chicago, Illinois, where he chaired the Zoology Department from 1910 to 1931. He first expressed interest in determining the cause of sex differentiation [4] in "The Biological Significance of Sexual Differentiation: A Zoological Point of View" published in 1907. Lillie's research on the freemartin [6] began in 1914 when he received a pair of calf fetuses in their fetal membranes from the manager of Lillie's farm, where he had a herd of pure-bred Holstein-Friesian cattle. Because of the proximity of the Union Stockyards to the University of Chicago [9] and the cooperation of Swift and Company's foreman, Lillie received ninety-seven uteri and their respective ovaries when available, containing twins from 1914 to 1920.

The first issue Lillie addressed in his examination of twin fetuses was whether they arose from a single fertilized egg [13] (monozygotic twins) from two fertilized eggs (diplozygotic twins). If the twins were monozygotic, they would be genetically identical, and the freemartin [6] would therefore be male as it was always born with a male twin. Lillie initially hypothesized that he would find the fetuses in separate fetal membranes (chorions) if they were diplozygotic. After examining twenty-two sets of twins, Lillie determined that twins in cattle share a single chorion [14].

Lillie noted that the fetus [15] begins to grow in the horns of the uterus [16]. Wrapped in the uterine membrane, the fetus [15] first grows in a downward direction, where it descends through a constricted aperture from the horn to the body of the uterus [16], and then also grows laterally into the other uterine horn. The twin fetus [15] also grows in that manner; such growth creates the opportunity for the chorions to fuse as they lay next to each other. Lillie then turned his observation to the ovaries, looking for the ductless gland which appears in the ovaries after ovulation [17] (corpus luteum [18]). In thirty-six uteri with both ovaries attached, thirty-five cases had either a corpus luteum [18] in each ovary [19], or two in one ovary [19]. Lillie concluded that twins were diplozygotic and thus that the freemartin [6] must be female as according to Lillie, mere association of two males *in utero* was an unlikely cause for the transformation of one male into a freemartin [6].

To bolster his argument that a freemartin [6] was female, Lillie performed a statistical analysis of his data. According to his analysis, it was statistically more likely that the freemartin [6] was a genetic female than a male. He also observed that a

freemartin [6] and her twin did not resemble each other and therefore were not identical twins.

Lillie concluded that the twins were diplozygotic after observing twenty-seven cases, and then he examined the anatomy of the chorion [14] to explain the indeterminate sex characteristics of the freemartin [6]. Through injections of the entire arterial and venous sytems, and careful dissections of the chorions, Lillie determined that the fetal freemartin [6] and its twin shared blood circulation, or anastomosis, in the chorion [14]. Normal females born with a male twin did not exhibit any anastomosis in the chorion [14].

As the freemartin [6] and her male twin share blood circulation, Lillie postulated that hormones [3] released by the male twin affect the sex differentiation [4] of the female. As the male twin is always fertile and shows no alteration of typical sex characteristics, while the female exhibits varying degrees of male sex characteristics, Lillie theorized that the timing of the anastomosis and flow of the male sex hormone [20] to the female fetus [15] accounted for the variation seen in the freemartin [6]. He examined the gross anatomy of the male and female twins and, with histological examination of the cells by graduate student Catherine Lines Chapin, Lillie established that the testis develops earlier than the ovary [19]. To Lillie, the earlier formation of the testis explained how the transfer of male hormone [20] early in development of the female affects her sexual differentiation [4] while the later release of female hormone [20] does not alter the male.

Lillie published the results of his study of the freemartin [6] in a number of articles, four of which are described here. The first article, "The Theory of the Free-Martin" was published in 1916. It was a preliminary report of the study on forty-one cases of bovine twins. Lillie wrote the article in response to Leon J. Cole's 1916 abstract "Twinning in Cattle, with Special Reference to the Free Martin." Lillie challenged Cole's hypothesis that freemartins are genetically male using the statistical analysis of Cole's cases as well as of his own. Lillie also described his theory of sex hormones [21]' responsibility for the indeterminate sex of the female.

Lillie's second article, "The Free-martin: A Study of the Action of Sex Hormones in the Foetal Life of Cattle," was published in 1917. The article described the study on fifty-seven pairs of twins and Lillie's theory of hormonal action. The article also included illustrations of a few of these cases.

After the 1917 article, Lillie learned about Tandler and Keller's works. He wrote "Tandler and Keller on the Free-Martin," in 1919 to acknowledge their work and to identify its similarities to his own. He said that he hadn't heard of their work because their initial publication was in a journal unknown to US biologists and that World War I [22] had hindered references to their work by other scientists.

Lillie's 1923 article discussed his study on freemartins, "Supplementary Notes on Twins in Cattle." Lillie described the sets of twins numbered fifty-eight to ninety-seven. Lillie asserted that the additional information derived from those cases supported his conclusions from 1917. Lillie also addressed challenges to his theory from other scientists. Lillie included some of the information previously published by Tandler and Keller in another statistical analysis to challenge David Berry Hart in Edinburgh, Scotland, who had said that the freemartin [6] was a male. Lillie countered arguments from Carl Richard Moore, also at the University of Chicago [9], and from Knud Sand at the University of Copenhagen [23] in Copenhagen, Denmark. Both had concluded that sex gland antagonism did not account for hermaphroditism. Lillie countered this conclusion by noting that timing of the release of the male hormone [20] inhibits the growth of the ovary [19] in the freemartin [6]. Carl Gottfried Hartman at University of Texas in Austin, Texas, had hypothesized that some hermaphrodites in mammals resulted from female sex hormone [20] altering the sex of the male. Lillie stated that Hartman's argument was flawed, as the female hormone [20] is not produced early in the fetal life when male sex differentiation [4] could be altered. Julian Sorell Huxley at King's College London in London, England, had contended in the 1920s through the 1930s, based upon the freemartin [6], that sex hormones [21] could completely invert the sex of one type into another. In addressing Huxley's argument, Lillie referred to his own study and emphasized that the changes to the freemartin [6]'s sexual organs were variable, and never a complete conversion.

DNA analysis later confirmed Lillie's position that the freemartin [6] is genetically female. By the 1960s, however, scientists no longer held that male sex hormones [21] partly caused the freemartin [6]. In 1984 the Anti-Müllerian Hormone (AMH) was purified and ultimately shown to inhibit ovaries from developing.

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Subject
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