

INCIDENCE OF TWINNING AND FREEMARTINISM IN FRIESIAN CATTLE WITH HISTOPATHOLOGICAL STUDIES OF THE FREEMARTIN GENITALIA

(With One Tables & 6 Fig.)

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(Received at 19/10/1994)

نسبة حدوث التوائم والآنثى العقيمة التوائم لذكر في الأبقار الفريزيان ودراسة التغيرات المستوباثولوجية المصاحبة لها

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اشتمل هذا البحث على دراسة عدد ٢٩٧١ حالة ولادة من ضمنها ٤١ حالة توائم ، وتبين أن النسبة الكلية لحدوث التوائم هي ٣٨ ٪ كما ان نسبة التوائم متشابهة الجنس هي ٧ ٪ و٥٣ ٪ وان نسبة التوائم مختلفة الجنس ٣ ٪ و٤٦ ٪ . أيضاً ان النسبة الجنسية لذكور الاناث عند الولادة هي ١ : ٣ كما أن وزن العجول المولودة فردياً أعلى من وزن العجول المولودة توائم .
تبين ان نسبة ٧ ٪ من الاناث المولودة توائم لذكر تكون عقيمة وان هذه الحالات يمكن تشخيصها بالفحص الكلينيكي عند بلوغ سن التلقيح . وبالفحص تبين وجود صفر شديد بحجم المبيض والرحم وعدم وجود عنق الرحم ووجود زوجين من الغدد تشبه الغده المنويه للذكر .
أيضاً وجود ضيق شديد بالمهبل تبين انسداده ، وان الشكل العام للأنثى يشبه الذكر .
بالفحص المجهرى تبين ان المبيض يتكون من نسيج ضام وأيضاً خلايا كبيرة متجمعه تشبه خلايا الجسم الاسفر . وأيضاً عدم وجود قناة فالوب ووجود حزمة النسيج الضام الكولاجيني بدلا منها - وجود غده متشابهه للغده المنويه الذكريه .

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SUMMARY

The data of 2971 calvings including 41 twin calving were studied for the incidence of twinning and freemartinism in Friesian dairy herds. The overall incidence of twinning was 1.38% with 0.76% homosexual and 0.64% heterosexual. An incidence of 94.7% of females that borne co-twin with males were freemartins "Sterile". In such cases, the gonads were small and undifferentiated. The gonadal histopathological structures were mainly stromal connective tissue and aggregated large cells. The uterus was small without cervix and the vagina was blind. Two pairs of vesicular glands were always present, they consists of small alveoli lined by cuboidal epithelium. The body weights of single borne calves were significantly higher ($P < 0.001$) than twins at parturation.

Keywords: Incidence, twinning, freemartinism, freisian cattle, histo pathological.

INTRODUCTION

The incidence of twinning in cattle is considered to be undesirable and its spread is often a disastrous in animal breeding. The causes of this condition may be attributed to environmental and/or hereditary factors (ROBERT, 1971). The incidence of twinning were studied by several authors (JOHANSSON and HANSSON, 1943; GILMORE, 1952; ARTHUR, 1959; WILLIAMS *et al.*, 1963 and DAVID *et al.*, 1976). They reported that incidence of twinning may be related to a breed difference. Many studies of bovine heterosexual twins cited that about 90-95% of female twins were sterile (GILMORE, 1952; WILLIAMS *et al.*, 1963 and MARCUM, 1974). Different theories explained the causes of freemartinism in cattle have been suggested by KELLER and TANDLER (1916), LILLIE (1917), MASON *et al.* (1959). JAINUDEEN and HAFEZ (1965), HERSCHLER and FECHHEIMER (1967) and OHNO *et al.* (1976). These theories indicated that freemartin development may be due to hormonal, cellular and/or immunological factors. The interplacental blood vessel anastomosis that allowing interchange of blood between heterosexual twins is responsible for the development of freemartinism (LAING, 1978). This vascular anastomosis allows male hematopoietic cells to collonize the body of the female,

create chimera with partial conversions of the ovaries to testis and modify the internal genitalia (HELEN[<] 1988). However WACHTEL et al. (1980) reported that H-y antigen is disseminated in the male, transmitted to the female serum and mounted by gonad specific receptors that causing gonadal alteration.

Histopathological alteration in freemartin reproductive organs varies according to the degree of masculinization. The gonadal histopathology was described by RAJAKOSKI and HAFEZ (1963), SHORT (1969) and VIGIER et al. (1977). MOREOVER, ROTHE et al. (1961) and LASTER et al. (1971) recorded the presence of vesicular glands in the majority of freemartins which is used in differentiating freemartinism from severe cases of aplasia of the paramesonephric duct derivatives.

The aim of our study is to record the incidence of twinning and freemartinism in some Friesian herds. Also the histopathological picture of reproductive organs in freemartin heifers was described.

MATERIALS AND METHODS

This work had been done in three Friesian herds that belong to Assiut Governorate. The data of 2971 parturations were collected, then calssified into males and females for both single and twins calvings. The overall incidence of twinning, and sex ratio were calculated. The birth body weights of single and twinn-born calves were compared statistically by using T-test (STEEL and TORRIE, 1980). In the farm, the twin calves were raised as the single born calves. At age of 18 months or 350 Kg body weight, all heifers were examined gynecologically before breeding to cull those with genital abnormalities.

At this age freemartin heifers were diagnosed rectally by absence of the cervix and the presence of cord - like structure instead of the normal uterus (ARTHUR, 1959). The potency of the vagina was measured by introducing a lubricated test tube or insemination pipette into the vagina.

For the purpose of histopathological studies the reproductive organs of two slaughtered freemartin heifers were separated, dissected and photographed. Ten blocks were taken from the gonads, Fallobian tubes, endometrium and the seminal glands like structures. These samples were fixed in 5% buffered glutraldehyde for 48 hr., then washed in cacodylate buffer. Samples were post fixed in 1% osmium tetraoxide in the same buffer for 2 hr. Semithin sections were prepared according to GUPTA (1983) and stained with toluidine blue, then examined microscopically and photographed. Some other samples from the seminal gland-like structure were fixed in 10% neutral buffer

formalin. The fixed samples were processed and embedded in paraffin. Paraffin sections (for wide orientation) were prepared and stained with H&E stain (BANCROFT and STEVENS, 1977) then examined microscopically and photographed.

RESULTS

A. Incidence of twinning:

The obtained results concerned with incidence of twinning and freemartin syndrome are presented in Table (1). From the table, it is clear that out of 19 heterosexual twins, 18 heifers (94.7%) were diagnosed as freemartins by rectal examination, while one heifer (5.3%) was diagnosed clinically as normal fertile heifer and it became pregnant. Concerning the body weights at the time of parturition, non significant differences were recorded between males and females of both homo- and heterosexual twins. However, the body weights of single born calves recorded a significant increase ($P > 0.001$) rather than any type of twin calves.

B. Anatomy and histopathology of the genitalia of the freemartin heifers:

The facial look of freemartin heifers is masculine type with long horns and looks like a bull (Fig. 1). The animal has small vulva with a coarse tuft of hair. Moreover, there is a dark line on the skin extending between the perineal region to near the umbilicus. The udder and teats appeared small in size and firm in consistency. The external characteristics of the animal resembles a steer.

After slaughtering of the animal and dissecting the genital system, the gonads appears small in size, flat and undifferentiated. There is a band of connective tissue connecting the gonads with the tip of uterine horns. The uterus appears small in size (less than one finger in diameter and shorter than normal) and lacking a cervix. The vagina is blind and narrow with two pairs of seminal glands-like structure connected laterally on the middle of the vagina (Fig. 3).

Histopathological studies of the different parts of the genital system showed that gonadal tissue (Fig. 4) was formed mainly of stromal connective tissue and aggregated large cells. These cells showed vesicular nucleus with a large amount of cytoplasm which contains numerous fat globules and empty vacuoles. These cells resemble lutein cell. Some of the primary and secondary follicles could be observed. The tissue of the fallopian tube is composed mainly of a collagenous connective tissue. Its lumen and cell structure could not be

differentiated. The endometrial tissue (Fig. 5) was composed of a covering columnar epithelium and a thick layer of connective tissue. The endometrial glands are few and found deeply in the endometrial stroma. The epithelium of the glands are of columnar type as shown in figure 5. The two pairs of seminal gland-like structure consist of relatively small alveoli lined by cuboidal epithelium in a prominent fibrous tissue stroma (Fig. 6). The lumen of the alveoli contains a small amount of haemogenous eosinophilic secretory material.

DISCUSSION

Our results recorded 1.38% for twinning, this result is similar to that of *GILMORE (1952)* and *ARTHUR (1959)*. They recorded an incidence 1.04 to 2.0% in dairy cattle and 1.0% in beef cattle. Due to breed difference, the incidence could be varied between 1.0-8.85% (*JOHANSSON and HANSSON, 1943; MEADOWS and LUSH, 1957* and *DAVID et al., 1976*). Moreover, *LAING (1979)* reported that twins occur in about 2.0% in dairy cattle and 0.5% in beef cattle. Following twin births, delayed uterine involution, retained placenta, septic metritis and temporary or permanent sterility are common (*Robert, 1971*).

Sex ratio is usually expressed as the percentage of male births, in this respect our results recorded 48.7%. *GILMORE (1952)* stated that sex ratio of calves averaged 51.12% males with variations from 42.6 to 53.7%. Moreover, *BRANDS et al. (1965)* recorded an incidence of 50.6% male calves at birth. The variation of approximately 50.0% may be due to higher percentage of males at time of conception than at birth. Also, a higher percentage of males that being absorbed, aborted or expalled dead at term (*WILLIAMS, 1943* and *HNEVKOSKY et al., 1964*).

Concerning the body weights of twinning at parturation, our results showed a significant increase in both normal single birth males or females rather than in case of twinning. *GILMORE (1952)* ststed that single bovine males weigh 25-45% more than twin males, and single females weigh from 40 to 50% than twin females. This reduced weight of individual twin is possibly due to reduced placental area or reduced amount of available nutrients of each fetus, and to the shortened gestation period.

One of undesirable reasons of twinning, is the incidence of freemartinism. The present study recorded an incidence of 94.7% of females that borne co-twins to a male calves were sterile (freemartin). Moreover, one (5.3%) of them was clinically fertile, this was confirmed by pregnancy detection. These results are similar to that recorded by *GILMOR (1952)*.

TWINNING, FREEMARTINISM & FRIESIAN CATTLE

WILLIAMS et al. (1963), ROBERT (1971), MARCUM (1974) and DAVID et al. (1976). The incidence of freemartin in heterosexual twins depends upon the interplacental blood vessel anastomosis causing chromosomal chimeras and prevents the normal development of the genital organs. WILLIAMS et al. (1963) found that approximately 90-92% of all bovine twins develop placental anastomosis. Moreover, LAING (1979) stated that in cases of monochochual heterosexual twin pregnancies, vascular placental anastomosis develops the production of freemartin syndrome in almost 100% of cases. However, in bichornual pregnancies freemartin occurs in about 80%. Through this placental anastomosis a transference of steroidal factor that stimulate the growth of Wolffian ducts and nonandrogenic glycoprotein factor that inhibiting the growth of Mullerian duct (LAING, 1979). In cases of fertile females that borne co-twin to a male, the anastomosis may have not occurred or occurred later after complete differentiation of gonadal ridge to an ovary (ROBERT, 1971) and SMITH et al., 1977).

In the reproductive organs of freemartins (Fig. 3), the ovaries appear small in size and undifferentiated. SHORT (1969) revealed that freemartin gonads vary from that of small testis to approximately normal appearing ovary. RAJAKOSKI and HAFEZ (1963) found that in some freemartin cases is a large gonad containing follicles and masses of tissues resembling corpora-lutea. The luteal or interstitial cell masses varies in size from approximately 1 mm to those as large as normal corpora-lutea. Uterine tubes had been described by ROTHER et al. (1961) and LASTER et al. (1971), they found a well developed but somewhat small uterus that communicate with the vagina, the cervix usually does not develop. The vagina is of normal size caudally, but rapidly tapered cranially. Unfortunately, we lack any literature concerning the histopathological picture of freemartin uterus to compare our results. The two pairs of tubular structures (Fig. 3) were confirmed by histopathological examination to be a seminal gland (Fig. 6). Similar findings were recorded by LASTER et al. (1971) and HELEN (1988). They were in agreement that seminal glands are usually present in freemartin syndrome and they are useful in differentiating freemartin from severe cases of aplasia of paramesonephric duct derivatives.

Conclusively, the condition of twinning is undesirable due to economic losses and genital anomalies rather than of health. Moreover, according to the findings of DUNN et al. (1979) they advise not to use the males that were borne co-twin with freemartins as sires in artificial insemination centers. This

is due to low sperm count or high incidence of abnormal spermatozoa.

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LEGEND

- Fig. 1: Facial look of freemartin heifer.
 Fig. 2: Small vulva with coarse tuft of hair in freemartin heifer.
 Fig. 3: Genitaltract of freemartin showing underdeveloped uterus with two pairs of seminal gland-like structure.
 Fig. 4: Histopathology of freemartin gonads showing stromal connective tissue and some of primary and secondary follicles.
 Fig. 5: Histopathology of endometrium in freemartin heifer showing a thick layer of connective tissue and deep endometrial glands its columnar epithelium.
 Fig. 6: Histopathology of seminal gland-like structure consist of small alveoli lined by cuboidal epethelium.

Table (1). Different criteria concerning the incidence of twinning and freemartin syndrome in Friesian cattle.

Criteria	Index
1- Total number of calvings	2971
a) Number of single borne females	1483
b) Number of single borne males	1447
c) Number of twins calvings	41
d) Overall incidence of twinning	1.38 %
e) Sex ratio at birth (male : female)	48.70 %
2- Incidence of different types of twinning	
I. Homosexual twinning	22 (53.6 %)
a) Male with male	12 (54.5 %)
b) Female with female	10 (45.5 %)
II. Heterosexual twins (freemartin)	19 (46.4 %)
3- Fertility of heterosexual twins	
a) Sterile females (freemartin)	18 (94.7 %)
b) fertile female	1 (5.3 %)
4- Body weights of twinning at birth (Kg)	
I. Heterosexual	
a) Males	23.16 ± 0.59
b) Females	24.25 ± 0.76
II. Heterosexual	
a) Males	23.00 ± 0.91
b) Females	22.68 ± 0.87
5- Body weights of single borne calves at birth (Kg)	***
a) Males	30.45 ± 0.62
b) Females	29.15 ± 0.81

TWINNING, FREEMARTINISM & FRIESIAN CATTLE



