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INTERSEXUALITY IN THE PIG^{*})

By

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The interest in studies of anomalies in the sex organs of man and animals has a very old tradition. One of the most important reasons for this is the possibility to throw light upon the sex differentiation and the development of different sexes. Of course there is also the clinical importance of anomalies as causes of sterility. In pigs there is a practical consequence in addition. Most intersex pigs will be declassified at slaughter since they are often cryptorchids.

According to *Armstrong* (1964) differentiation and development of different sexes in mammals depend on the following main factors: 1. The sex chromosomes. 2. Development of the gonads. 3. The accessory sexual glands and the external genitalia. 4. The psychogenically determined sex character.

A number of definitions of the entity of intersexuality have been proposed. According to *Biggers & McFeely* (1966) intersexes present an inborn variation of the geno- and phenotype of the sex which makes it difficult or impossible to present an exact diagnosis of the sex. This definition holds scarce biological information but the variation of the defect is so great that it seems to us to be the only one to cover all variants. Usually one speaks about true intersexes or hermaphrodites when the gonads of both sexes are present whereas pseudohermaphrodites have gonads of one sex but the remaining characteristics of the sex organs of the opposite sex.

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Frequency

Among the farm animals intersexuality is most common in goats and pigs. In Sweden *Andersson* (1956) found a frequency of 0.5 % in pigs from 5 slaughter-houses. *Freudenberg* (1957) and *Koch* (1963) found the same incidence, 0.2 %, in the German pig population. *Albertsen* (1951) in Denmark reported on frequencies ranging from 0.1 to 0.6 %. He also estimated the parts shared by true hermaphrodites and pseudohermaphrodites to 81.5 % and 18.5 % respectively. *Breeuwsma* (1969) found in a large material in Holland 0.4 % intersexes.

Clinical picture

In almost all references the defect pigs are characterized clinically as females with external genitalia varying from a fairly normal vulva with slightly enlarged clitoris to individuals with a considerably enlarged penis-like clitoris. This sometimes can be dislocated cranially. Vulva is then underdeveloped or replaced by a prepuce. Sometimes a cone shaped skinfold in the umbilical region is reported as characteristic for the intersex pigs. Inguinal hernia is found occasionally. Testicles can be seen in a well developed scrotum or can be palpated in the inguinal hernia.

Libido of a male character and the typical smell of boar can often be found in older intersexes (*Arthur* 1959). On the other hand there are also reports on fertile intersexes, functioning as females (*Petersen* 1952; *Hulland* 1964; *Ryberg* 1965).

Morphology

The embryology and morphology of the gonads and other genital organs of intersexes have been the object of much interest (*Bruner-Lorand* 1964; *Armstrong* 1964; *Biggers & McFeely* 1966; *McFeely et al.* 1967). The intersex pigs have generally been characterized as male pseudohermaphrodites with testicular gonads, Müllerian and Wolffian ducts and male accessory sexual glands of varying development. The enlarged clitoris completes this picture (*Holz* 1941; *Freudenberg*; *Makino et al.* 1962; *Henricson & Bäckström* 1963; *Koch*; *Hard & Eisen* 1965; *McFeely et al.* 1967). True hermaphrodites with both male and female gonads have also been described (*Baker* 1926; *Holz*; *Albertsen*; *Johnston et al.* 1958).

Freudenberg, Henricson & Bäckström, Hard & Eisen and

others found at histological examination of testicular tissue no signs of spermatocytogenic activity. The case reported by *Makino et al.* (1962) showed incomplete spermatogenic activity. Ovarian tissue of intersex pigs have shown active oocytogenesis (*Brambell* 1929; *Pound et al.* 1961). This is of course a necessary condition for the above-mentioned reports of fertile intersex sows.

Genetical predisposition

Several authors present evidence that the predisposition for intersexuality in the pig should be inherited (reviewed by *Koch et al.* 1957; *Freudenberg*; *Johnston et al.*). The most extensive material has been presented by *Johnston et al.* These authors argue that a recessive gene should be the main cause even if additive genes could influence the manifestation of the defect.

In goats *Soller & Angel* (1964) showed evidence for a linkage between the genes causing hornlessness and intersexuality. With reference to the report of *Freudenberg* of multiple malformations connected to hermaphroditism *Brandsch* (1962) proposed the possibility of a similar situation also in pigs.

Karyotype and sex chromatin

Pig intersexes have mostly turned out to have a female karyotype, showing the sex chromosome constitution XX (*Makino et al.*; *Henricson & Bäckström*; *Hard & Eisen*; *McFeely et al.*). The results of previous investigations based on sex chromatin made by *Cantwell et al.* (1958) have thus been confirmed. Lately sex chromosome mosaicism has been found by *McFee et al.* (1966), *Lojda* (1968) and *Hansen-Melander & Melander* (1969). *Lojda* in a big offspring group of a sow also found pure XX- or XY-karyotypes. *Gluhovschi et al.* (1969) among 22 intersexes had 21 with 38 (XX) and 1 with 39 (XXY) chromosomes. The first mosaic reported by *McFee et al.* (1966) has been proposed by *McFeely* (1967) to be a case of freemartinism. There are early reports on vessel anastomosis between fetuses of different sexes of pigs (*Hughes* 1927, 1929; *Hoadley* 1928).

The aim of the present investigation was to cover different aspects of the intersexuality in pigs, mainly the frequency in Swedish pigs, the karyotype, the macro- and microscopic anatomy of the gonads and the possible genetic predisposition. Some organoleptic investigations have also been performed.

MATERIAL AND METHODS

Different projects have demanded different kinds of material and will therefore be presented separately.

The frequency investigation

The frequency of intersexes and some data referring to the litters holding intersexes (litter size, sex quotient, frequency of other malformations) have been studied in 4 separate materials.

1. One-hundred-and-sixty herds were selected at random from herds connected to the pig health control program in the southern part of the county of Älvsborg. One of us (*Bäckström*) inspected 2,383 piglets in 250 litters from 13/1 to 5/5 1964. In addition to the data being a result of the ocular investigation additional data on the number of stillborn piglets and of piglets having died from accidents or diseases have been reported by the farmers.

2. An identical procedure has been applied on a sample of 372 herds from the county of Skaraborg from 10/11 1965 to 31/3 1966. A total of 980 litters and 10,636 piglets were included.

3. The owners of 7 farms in the county of Älvsborg reported on the status and fate of 331 litters with 3,659 piglets from February 1964 to January 1966.

4. Data on 14 litters and including 145 piglets were collected in connection to the investigation on intersex individuals which will be presented below.

Intersex individuals

Individuals for detailed investigation were selected at random from the materials mentioned above and from cases subjected to the veterinary college in Stockholm. They form a total of 45 individuals.

Karyotype studies were performed with the leucocyte culture techniques of *Moorhead et al.* (1960) and *Arakaki & Sparkes* (1963) on 42 cases. On 30 cases also the skin culture technique of *Basrur et al.* (1963) was performed.

The intersex pigs have been slaughtered at different ages (cf. Table 4). The sexual organs were carefully inspected and material for histological investigation was collected. Fixation was performed in acetic acid-alcohol 1 + 3. Five micron sections were prepared and stained in Gomori's hematoxyline or eosin-hematoxyline.

The uterus content of 21 cases has been subjected to bacteriological examinations. Routine techniques were with incubation on agar and blue agar and incubation for control on anaerobal bacteria.

Organoleptic investigations have included 22 intersexes and 18 normal controls. Pork specimens were tested by a panel of 6 people at the food product laboratory of the cooperative association in Stockholm. The specimens were tested for smell when fried or boiled and for taste after frying. A specimen got a note for bad smell or taste when at least 4 out of 6 testers gave this remark.

Pedigrees have been prepared for intersex individuals as far as data on ancestors have been available.

RESULTS

Frequency studies

In Table 1 the frequency of intersexes in the 3 random samples are given. There is no significant heterogeneity between the 3 frequencies ($\chi^2 = 2.4$; d.f. = 2; $P > 0.05$) and so the average frequency of 0.2 % should be a good estimate of the condition in a fairly big producing area of Sweden.

Table 1. Frequency of intersex pigs in three random samples.

Material	Number of			% freq. of intersexes
	farms	litters	piglets born	
I*)	160	250	2,843	0.25
II	372	980	10,636	0.19
III*)	7	331	3,659	0.33
Total	538	1,559	17,121	0.21

*) one farm with 17 pigs in 2 litters is included in both materials I and III.

The proportion between the sexes in the normal material (Table 2) is about 52 % male pigs and 48 % females. The male sex is overrepresented to an extent generally seen in mammals. In litters holding intersexes there are 45 % males, 42 % females and 13 % intersexes. Since on account of their chromosome constitution (see below) the latter ones should be characterized as genetical females, there is an almost significant underrepresenta-

Table 2. Frequency of different sexes.

A = litters without intersexes.

B = litters with intersexes.

Material	No. of litters	Percentage of		
		males	females	intersexes
I A	244	51.72	48.28	—
II A	735	51.77	48.23	—
III A	293	52.15	47.85	—
Total A	1,272	51.86	48.14	—
I B*)	6	50.00	36.54	13.46
II B	14	45.16	42.74	12.10
III B*)	7	43.06	41.66	15.28
Total B	25	45.02	41.99	12.99

*) cf. Table 1.

tion of males in these litters ($\chi^2 = 4.3$; d.f. = 1; $0.01 < P < 0.05$). Comparing this result to the litter size of normal and intersex litters (Table 3), one finds a corresponding reduction of the average number of piglets in the last mentioned litters. An increased embryonic death rate of males in intersex litters thus seems probable.

Table 3. Litter size, pig losses including stillborn pigs, inborn malformations.

A = litters without intersexes.

B = litters with intersexes.

Material	No. of litters	No. of piglets born		pig losses	Percentage of		
		totally	per litter		intersexes	scrotal hernia	other malformations
I A	244	2,789	11.42	16.36	—	0.97	0.68
II A	961	10,438	10.86	19.29	—	1.04	1.03
III A	323	3,574	11.07	17.62	—	1.40	0.73
Total A	1,528	16,799	10.99	18.45	—	1.11	0.91
I B*)	6	56	9.33	7.14	12.50	1.79	1.79
II B	19	198	10.42	9.60	10.10	2.02	0.51
III B*)	8	85	10.63	5.88	14.12	4.71	0
IV B	14	145	10.36	15.86	9.66	1.38	4.14
Total B	45	465	10.33	10.54	10.75	2.37	1.72

*) cf. Table 1.

The pig losses (Table 3) within 5 weeks of age are highly significantly higher in normal than in intersex litters ($\chi^2 = 18.5$; d.f. = 1; $P < 0.001$). On the other hand there is a tendency to higher frequency of different malformations in the intersex litters. Concerning scrotal herniae this difference is almost significant ($\chi^2 = 5.3$; d.f. = 1; $0.01 < P < 0.05$). In intersexes the frequency of scrotal herniae was 16 % and the overall frequency of malformations 22 %.

Genetical predisposition

One landrace boar (N) having produced 25 litters in the same farm is included in our material. The frequency of intersexes in his offspring was $\frac{12}{286} = 4.2$ %. The difference between this frequency and a general frequency of 0.2 % is of course highly significant ($\chi^2 > 100$). In the same herd another boar had produced 21 litters without any intersex. Six sows had been mothers in both offspring groups. Hereditary predisposition for this defect is consequently most probable.

As to the nature of a hereditary predisposition our material does not give much positive information. We can probably exclude a monogenous recessive inheritance for the following reasons. The boar N had produced 12 intersexes with 8 sows in 14 litters with a total of 160 piglets. The frequency $\frac{12}{160} = 0.075$ is most obviously separated from the frequency of 0.25, which would have been expected according to a monogenous recessive theory.

Pedigree studies of intersex individuals might have added some information on the genetics of the defect. The pedigrees being possible to use were, however, very incomplete especially on female ancestors. No closer inbreeding lay behind the intersex litters.

The boar N had an overall frequency of malformations (excl. intersexes) of 2.45 %.

Investigation on individuals

In Table 4 the clinical, patho-anatomical and bacteriological investigations on the 45 intersexes have been summarized.

Table 4. Summary of the clinical, pathoanatomical and bacteriological investigations.

Fig no.	Age mths	Ext. genit.	Vagina	Cervix	Uterus		Ovaries	Acc. sex. glands	Testicles				Bact. inv.	
					l	r			left	right	size	Hern. inguin.		
					a	s	a	s	a	s	l	r	l	r
1	5	1	1	0	3+2+3	0	0	0	1	1	1	1	1	—
2	5	1	2+3	3+4	4,3+2+3	0	0	0	2	1	1	2	x	—
3	6	2	1	3	2	0	0	0	2	2	1	1	x	—
4	7	5	0	0	0	0	x	1	1	1	2	2	—	—
5	8	1	1	2	2	0	x	2	1	1	2	1	—	—
6	7	1	2+3	1	3+1+0	0	0	1	1	1	2	2	—	—
7	7	1	2	2+5	2	1 ²)	x	1	1	1	1	2	—	—
8	7	1	1+3	2+4	3+2+3	0	0	2	2	2	2	2	—	—
9	7	1	2	2	2	0	0	1	1	1	3	3	—	—
10	7	1	2+3	3+4+5	2	0	0	1	1	1	2	2	—	++3
11	8	2	1+3	3+4+5	3+1+3	0	x	1	1	1	2	2	—	+++1
12	7	1	1	1	2	0	0	1	1	1	1	2	0	0
13	7	3	0	1	2	0	0	1	1	1	2	2	0	0
14	7	1	2	3	3+2+3	2	2	0	1	1	2	2	—	++1
15	8	1	2	3+5	2	0	0	1	1	1	3	3	x	0
16	10	1	2	2+4	2	0	0	1	1	1	2	2	—	+1
17	8	2	0	1	1	0	x	1	1	1	1	1	0	0
18	7	2	2	1	3+2+3	1 ²)	0	2	2	2	2	2	0	0
19	9	1	2	2	3+1+3	2 ²)	0	1	1	1	1	1	—	+++1,3
20	8	1	2	1	3+1+3	2 ²)	0	1	1	1	1	1	—	0
21	7	1	2	2	2	2	0	1	1	1	1	1	—	0

*Explanation of symbols in Table 4**Extern. genitalia*

1. "Vulva-clitoris" type. Vulva fairly normal. Slightly to moderately enlarged clitoris.
2. "Clitoris-penis" type. Highly enlarged penislike clitoris, partly or totally envolved in a prepuce. Localized at the normal place of vulva which is missing.
3. "Nipple-penis" type. The "penis" as in 2, but dislocated cranio-ventrally.
- 3¹⁾ = 3, but the urethra ends blindly before entering the "penis". In cases nos. 29 and 30 an artificial fistula had been opened up.
Most cases had the above-mentioned skin fold in the umbilical region.

Vagina

- Not examined.
 0 Aplastic.
 1 Hypoplastic-canalized.
 2 Normal.
 3 Filled with fluid.

Cervix

- Not examined.
 0 Aplastic.
 1 Hypoplastic-canalized.
 2 Normal with normal folds.
 3 Without folds.
 4 Filled with fluid.
 5 Ends blindly cranially or caudally.

Uterus

- 0 Aplastic.
 1 Hypoplastic-canalized.
 2 Normal.
 3 Filled with fluid.
 4 Parts of the uterine horns in an inguinal hernia.
 l Left.
 r Right.

Ovaries

- 0 Aplastic.
 1 Ovarian tissue.
 1²⁾ Cystic ovarian tissue.
 2 Ovotestis.
 2²⁾ Cystic degeneration of 2.

Accessory sexual glands

- Not examined.
 0 Aplastic.
 x { Bulbo-urethral glands } present
 { Prostate }
 { Vesiculae seminales }

Testicles

- a Abdominal localization.
 s Scrotal "
 0 Aplastic.
 1 Testicle present. No spermatogonia detected.
 1³⁾ " " " A few dividing sp.g. detected.
 2 = 1 + seminocele.
 Size 1 Small 2 Normal 3 Enlarged.

Bacteriological investigation of the uteral mucosa

—	Not examined.	
0	No bacterial growth.	
+	Slight	} bacterial growth
++	Moderate	
+++	Heavy	
1	Coccae (usually α - and β -hemolyzing).	
2	Coliform bact. and Esch. coli.	
3	Cor. pyogenes.	

External genitalia. The majority of the 45 cases belonged to type 1 (29 cases), 10 belonged to type 2 and 6 to type 3.

Vagina was aplastic or hypoplastic in 13 and normal in 26 out of 39 cases.

Cervix was normal in 14, and showed different forms of abnormality in 23 out of 37 cases.

Uterus was totally aplastic only in 1 case, in another the right horn was aplastic. About half the number of uteri were filled with fluid.

Ovaries were missing on both sides in 32 out of 45 intersexes. Four cases had both ovaries and 1 had a single ovary. Eight cases had ovotestes, 6 of which on both sides. Ovarian tissue was present primarily in intersexes of type 1 (12 out of 13). On histological examination primary follicles, growing and Graafian follicles were detected. Follicular cysts were common. Twelve out of 23 ovaries were highly cystic degenerated. As only a few sections were taken from each ovary little could be said about the quantity of follicles. However, the impression was that of follicular hypoplasia.

Accessory sexual glands were present in 8 out of 32 pigs. The cases having accessory sexual glands were generally those having a male type: intersex type 3, testicles in the scrotum.

Testicles were present in all 45 intersexes, in 1 only single-sided. In the majority of cases the testicles were situated in the abdomen. On the surface of 12 of the 89 testicles a brown-yellowish tissue could be seen by ocular inspection. It varied in size from that of a pea to cases when it covered about a quarter of the total surface. Histologically these protrusions were identified as testicular tissue and should be characterized as seminocle.

The testicular sections showed a marked degree of tubular hypoplasia. The basement membrane was covered with cells, most of which evidently being Sertoli cells. In 2 out of 45 cases a few mitoses were seen in the cell layer close to the basement

membrane. This should be a proof of the existence of spermatogonia. The possibility of some active spermatogonia even in additional cases cannot be excluded, since only a limited number of sections were inspected. No primary spermatocytes were detected.

Interstitial cells seemed to be present in a normal amount.

Bacteriological investigation

Two thirds (14 out of 21) of the investigated cases were bacteriologically positive. *Corynebact. pyogenes* was the most common positive finding. The combination open vagina-cervix and uterus with liquid content was positive in all the 4 cases investigated. Even without external communication a liquidous vagina-uterus was positive in 6 out of 8 cases.

In 2 cases the uteral infection was combined with nephritis and cystitis. One of these pigs was totally prohibited at slaughter (coli sepsis). Three additional cases were complicated by local lymphnode reaction and local abscesses in the peritoneum.

Karyotype studies

Between 25 and 50 metaphases from cultured leucocytes and/or skin cells were scored in 42 intersex pigs. All metaphases showed an apparently normal female karyotype (38/XX).

Organoleptic investigation

In Table 5 the organoleptic tests are summarized. In the first 2 rows intersexual pigs have been compared to controls. Although there was a clear numerical difference, this was not significant at the 5 % level. The intersexes have been grouped according to different types in the following rows. A clear tendency for those types having most male characteristics to be diverging from a normal smell and taste can be seen. This is most evident and significant when male secondary sexual glands are present.

DISCUSSION

The most common external phenotype of the intersexual pig is not very much different from a normal female. The only symptom is an enlarged clitoris. Very often the enlargement is minimal and could sometimes probably escape clinical observation. The clinical frequency (0.2 % in our material) is then probably an

Table 5. Organoleptic investigations. Frequency of notes for bad smell or taste of pork from intersex pigs and controls.

A = smell at boiling.

B = smell at frying.

C = taste after frying.

Category	No. of animals	No. (frequency %) of "bad" notes		
		A	B	C
Controls	18	6 (33)	3 (17)	0
Intersexes	22	15 (68)	11 (50)	5 (23)
Intersexes age 5—7 mths	15	9	8	1
" " 8—10 "	7	5	2	2
Intersex type 1 (cf. Table 4)	16	10	7	1
" " 2 "	4	4	3	3
" " 3 "	2	2	1	1
Intersex with one or both testicles in the scrotum	8	7	5	3
Intersex with abdominal testicles	14	9	6	1
Size of testicles 1—3 (cf. Table 4)	11	8	4	2
" " " 4—6 "	11	8	7	2
Male second. sex glands present	5	5	2	4
" " " " absent	17	11	9	1
Ovarian tissue present	7	4	2	0
" " absent	15	12	9	4
Vagina or uterus with content	10	5	6	1
" " " without "	12	11	5	4
Bacteriologically positive	6	3	5	1
" negative	7	6	2	1

* χ^2 for difference = 6.0, $P < 0.05$.** " " " = 8.2, $P < 0.01$.

underestimation of the real frequency. *Breeuwisma* (1969) reported a frequency of 0.4 % in a large Dutch slaughter-house material.

Uterus and testicles are very constant findings in the intersexes. The size of the uterus varies as a consequence of presence or absence of fluid content. The internal phenotype also varies concerning ovaries and accessory sexual glands.

In the present material no variation concerning chromosome constitution could be demonstrated in blood leucocytes. All cases had 38/XX chromosomes. Sex chromosome aneuploidia can very probably be excluded as a cause of the most common forms of

intersexuality in the pig also in view of many other scientific reports. However, there have been some reports lately (*McFee et al.* 1966; *Lojda* 1968 and *Hansen-Melander & Melander* 1969) demonstrating the existence of sex chromosomal mosaicism (XX/XY) in some of these cases. As only a limited number of cells were inspected in our material, we cannot exclude the presence of such mosaicism at least in some cases.

If male germ cells cannot be produced by a chromosome set lacking the Y-chromosome, this chromosome had to be present at least during that part of embryonic life when spermatogonia were formed. We have found 2 cases having testicles with spermatogonia. This cell type was, however, very rare and consequently the presence of it in different cases is also a function of scoring enough sections.

In the case of vessel anastomoses between fetuses of different sexes only a few cells of the XY-type (a mosaic XX > > XY) might be sufficient to initiate male characteristics. The XY-cell line could later on be very difficult to detect. According to such a theory almost all pig intersexes might be a consequence of fetal vessel anastomoses between unlike sexed sibs. Extending this theory the hereditary predisposition to intersexuality could work via predisposition to freemartinism.

There are, however, additional results of the present investigation speaking in favour of a different type of genetical predisposition. Other defects except intersexuality were relatively common in litters producing intersexes. Besides there was an underrepresentation of male piglets speaking in favour of increasing embryonic mortality of male embryos. A mutant gene acting in early embryonic life with a varying expressivity or modified by other genes could be responsible for different types of malformations including intersexuality.

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SUMMARY

In 3 relatively large samples intersexuality was found in 0.2 % of the pigs. Litters with intersexes had a deficit of male piglets. The total number of piglets in such litters was correspondingly reduced. Pig losses until the age of 5 weeks were reduced in intersex litters. Other malformations, especially scrotal hernia, were more common in these litters than in normal ones.

Forty-five intersexes showed big variation in the development of sexual organs. All had testicles, however. Thirteen (29 %) were so-called true hermaphrodites whereas 32 were male pseudohermaphrodites.

All 42 cases being karyotyped were genetical females. There are arguments for genetical predisposition for the defect. The hereditary transmission is probably complicated. In any case it is not monogenic recessive.

Practical consequences of the defect are: almost complete sterility, slaughter losses due to bad smell and taste of the meat and increased risk of cassation (local or general inflammations), increased frequency of other malformations in litters holding intersexes.

SAMMANFATTNING

Intersexualitet hos svin.

I tre relativt stora stickprovsundersökningar från Västergötland har intersexualitet påvisats hos 0,2 % av svinen. Kullar med intersexer har ett underskott på galtgrisar, som motsvaras av ett mindre antal grisar i kullarna. Smågrisförlusterna intill 5 veckors ålder är i ungefär motsvarande grad mindre i intersekkullarna. I dessa senare är andra typer av missbildningar, särskilt pungbräck, rikligare företrädade än normalt.

De 45 undersökta intersex-individerna företedde en rikhaltig variation beträffande könsorganens utveckling. Samtliga hade dock testiklar. 13 individer (29 %) var s.k. äkta hermafroditer, medan resterande var s.k. hanliga pseudohermafroditer.

Samtliga 42 kromosomundersökta fall var genetiska honor. Det föreligger argument för genbunden disposition. Nedärvningsmönstret är sannolikt komplicerat. I varje fall föreligger ej monogen recessiv nedärvning.

Till de praktiska konsekvenserna av defekten hör: Praktiskt taget 100-procentig sterilitet, sämre slaktutbyte till följd av dålig lukt och smak hos fläsket samt ökad risk för kassationer vid slakten (av de 45 intersexerna måste 5 rensas till följd av lokala inflammationer och 1 totalkasseras) och ökad frekvens av andra missbildningar, i synnerhet bräck.

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