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THE EFFECT OF EXOGENOUS ADMINISTRATION OF OESTROGENS ON THE FUNCTION OF THE EPIDIDYMIS AND THE ACCESSORY SEX GLANDS IN THE BOAR

By

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LINDE, CATHARINA, STIG EINARSSON and BÖRJE GUSTAFSSON: *The effect of exogenous administration of oestrogens on the function of the epididymis and the accessory sex glands in the boar.* Acta vet. scand. 1975, *16*, 456—464. — Three normal, sexually mature young boars of Swedish Yorkshire breed were treated with daily intramuscular injections of diethylstilboestrol and/or oestradiolbenzoate for a total period of 13—15 weeks. Whole ejaculates were collected on a dummy sow with an artificial vagina twice a week. The ejaculate was examined as regards semen volume, sperm concentration, sperm motility and sperm morphology. The seminal plasma was analysed for Na, K, Cl, Mg, inorganic phosphate, total protein and fructose. Testicles, epididymides and the accessory sex glands were obtained at slaughter. Material from epididymal segments A, B, D_b, F_a and F_b was collected and examined for sperm morphology. Spermatocrit, osmotic pressure and plasma concentrations of Na, K, Cl, total protein and GPC were assessed in material from epididymal segment F_a. The sex glands and the accessory sex glands were examined macroscopically and microscopically.

The semen volume, the sperm concentration and consequently the total sperm count per ejaculate showed a gradual increase during the course of the investigation. This is to be expected as the boars used were comparatively young at the beginning of the experiment. These values were all within normal limits for adolescent boars.

Taking all the characteristics examined into consideration no conclusive evidence was found that an exogenic administration of oestrogens to intact boars has any influence on the function of the epididymis and the accessory sex glands.

boar; diethylstilboestrol; oestradiolbenzoate; epididymal function; accessory sex glands.

A case of presumed epididymal dysfunction in a boar was reported by *Einarsson & Gustafsson* in 1973. The sperm morphology was characterized by a low sperm motility and a marked

increase in the frequency of sperm tail abnormalities. The pathological changes arose during the epididymal transit. In addition the composition of the caudal epididymal content differed from what is considered normal. In the bull *Gustafsson* (1965, 1966) and *Gustafsson et al.* (1974) observed a connection between low sperm motility, a high incidence of sperm tail defects and an abnormal composition of the epididymal plasma. Similar morphological sperm changes can be experimentally induced in the bull by exogenous administration of oestrogens (*Cupps et al.* 1960, *Gustafsson* 1966).

The main object of the present investigation was to study the effect of exogenously administered oestrogens on the sperm morphology, on the epididymal function and on the function of the accessory sex glands in boars.

MATERIAL AND METHODS

Three boars of Swedish Yorkshire breed were used. The boars were stabled at the Department of Obstetrics and Gynaecology for about 3 weeks before the experiment started. During that time the boars were trained to mount and ejaculate on a dummy

Table 1. The duration of the experimental periods I—III. Hormonal compound and dosage used.

	Experimental period					
	I		II[a]		III	
Boar no.	number of weeks	hormone compound and dosage used	number of weeks	hormone compound and dosage used	number of weeks	hormone compound and dosage used
1	3	—	11	oestradiol-[b] benzoate 5 mg/day	4	oestradiol-benzoate 20 mg/day
2	3	—	7	oestradiol-benzoate 20 mg/day	6	diethylstil-[c] boestrol 10 mg/day
3	3	—	8½	oestradiol-benzoate 20 mg/day	6½	diethylstil-boestrol 10 mg/day

[a] Between periods II and III boars nos. 2 and 3 were left untreated for 3 weeks.

[b] Ovex vet®, LEO, Hälsingborg.

[c] Stilbol®, ACO, Solna.

sow. At the start of the experiment the boars were between 7½ and 8 months old. After a pre-experimental period of 3 weeks during which time regular semen collections were made (experimental period I, Table 1) the boars were given daily intramuscular injections of oestradiolbenzoate or diethylstilboestrol for a total period of 13--15 weeks each. The dosage used and the duration of the 3 experimental periods are given in Table 1.

Whole ejaculates were collected as a rule twice a week (Monday and Thursday) throughout the experiment. Immediately after collection the bulbo-urethral gland secretion was filtered off through double gauze. The seminal volume was measured after filtration. The sperm motility was assessed with fresh semen under a cover slip at 37°C. The sperm concentration was determined in a Bürker haemocytometer. Examination and assessment of sperm morphology were done as recommended by *Holst* (1949) and *Bane* (1961). The spermatozoa were separated from the seminal plasma by centrifugation at 3,200 × g for 15 min. The seminal plasma was analysed for concentrations of sodium, potassium, chloride, inorganic phosphate, magnesium, total protein and fructose with the methods described by *Einarsson* (1971).

The boars were slaughtered on the first post-treatment day after period III. The sexual organs were secured and examined. Epididymal contents were sampled from segments A, B, D_b, F_a and F_b and after fixation in buffered formol-saline examined for sperm morphology. (Epididymal segments as defined by *Crabo* 1965). Spermatocrit, osmotic pressure and plasma concentrations of sodium, potassium, chloride, total protein and glycerylphosphorylcholine (GPC) were assessed in material collected from epididymal segment F_a by methods described by *Einarsson* (1971) and *Crabo*.

The sexual organs were examined macroscopically, and pieces of tissue were taken from proximal and distal regions of the testis, from caput and cauda epididymidis and from seminal vesicles and bulbo-urethral glands. These were fixed in Bouin's solution for histological examination.

RESULTS

Ejaculated semen

The mean values for volume, sperm concentration and total sperm count per ejaculate during the 3 experimental periods are

Table 2. Semen volume, sperm concentration and total sperm count per ejaculate during the 3 experimental periods. Mean ± s.

Boar no.		Experimental period		
		I	II	III
1	Semen volume (ml)	94 ± 33	111 ± 27	118 ± 27
	Sperm concentration ($\times 10^3$/mm^3)	246.8 ± 130.7	424.7 ± 117.9	516.9 ± 128
	Total sperm count per ejaculate ($\times 10^9$)	23.1	47.1	61
2	Semen volume (ml)	170 ± 18	133 ± 35	197 ± 26
	Sperm concentration ($\times 10^3$/mm^3)	335.4 ± 23.9	244.8 ± 191.9	419.4 ± 238.4
	Total sperm count per ejaculate ($\times 10^9$)	57	34.3	82.6
3	Semen volume (ml)	122 ± 13	174 ± 41	213 ± 31
	Sperm concentration ($\times 10^3$/mm^3)	293.3 ± 163.6	396 ± 99.9	442.6 ± 205.2
	Total sperm count per ejaculate ($\times 10^9$)	35.8	68.9	94.3

given for each individual boar in Table 2. The semen volume and the sperm concentration and consequently the total sperm count per ejacutale increased gradually during the experimental periods. In boar 2, however, these characteristics showed a slight decrease during experimental period II, but rose again during period III and reached higher levels than during period I. A contributory cause for the declining values during period II was a pronounced difficulty for the boar to complete the ejaculatory act because of leg weakness.

The sperm motility was, with 1 occasional exception, 70—90 % in all the ejaculates collected from the 3 boars.

The incidence of sperm abnormalities did not change throughout the experimental periods with the following few exceptions. An enhanced frequency of single bent sperm tails was observed in 1 ejaculate from boar 2 and in 3 ejaculates from boar 3. In the latter case these deviations in sperm morphology occurred sporadically during the experimental periods and not in consecutive ejaculates.

The concentrations of electrolytes, total protein and fructose in the seminal plasma varied between collections but did not differ significantly between experimental periods. This was the

Table 3. Sperm morphology in different epididymal segments from the 3 boars treated with oestrogens and from normal boars. Normal boars mean ± s. (*Gustafsson et al.* 1970).

Epididymal segment	Proximal cytoplasmic droplets (%)				Distal cytoplasmic droplets (%)				Detached heads (%)				Total abnormal tails (%)			
	boar no.				boar no.				boar no.				boar no.			
	1	2	3	normal boars	1	2	3	normal boars	1	2	3	normal boars	1	2	3	normal boars
A	—	97.0	93.0	—	—	0	1.0	—	—	1.0	1.0	—	—	0	0	—
B	—	100	98.0	89.2±19.1	—	0	0	1.1±4.0	—	0	1.0	3.2±4.2	—	0	0	0.3±0.4
D_b	—	2.0	12.0	1.0± 1.1	—	95.3	76.5	90.6±7.8	—	0.3	1.5	1.6±1.4	—	0.5	0	0.7±0.4
F_a	5.5	1.5	15.3	1.3± 0.8	93.0	95.5	80.0	93.1±7.1	1.3	0.5	0.8	1.1±1.2	0.4	1.0	0.8	1.2±0.5
F_b	—	1.3	7.5	—	—	96.3	85.8	—	—	0.8	0.3	—	—	0	6.5	—

case irrespective of the oestrogen compound used (oestradiolbenzoate or diethylstilboestrol) and the dosage given (5 or 20 mg oestradiolbenzoate/day).

Epididymal content

The results of the examinations of the sperm morphology in epididymal segment F_a in boar 1 and segments A, B, D_b, F_a and F_b in boars 2 and 3 are shown in Table 3. The observed percentages did not differ from the normal values as reported by *Gustafsson et al.* (1970). The percentage of proximal cytoplasmic droplets was slightly increased in segment F_a in boar 3. However no increase of the frequency of proximal droplets had been observed in ejaculated semen from this boar during the experimental periods.

Table 4. The composition of the contents of cauda epididymidis (F_a) in boars nos. 2 and 3 and in normal boars. Normal boars mean±s.

	Boar 2	Boar 3	Normal boars
Spermatocrit (%)	46	41	34.6 ± 5.4[1]
Osmotic pressure (mOsm/l)	357	366	330.7 ±13.4[2]
Sodium (meq./l)	17.0	20.5	30.2 ± 8.9[2]
Potassium (meq./l)	27.5	30.0	31.3 ± 4.1[2]
Chloride (meq./l)	21.5	19.0	19.3 ± 2.6[2]
Total protein (g/100 ml)	1.6	2.2	2.77 ± 0.61[2]
GPC (mM/l)	47.4	33.2	38.4 ± 8.5[3]

[1] *Crabo* 1965.
[2] *Einarsson* 1971.
[3] Unpublished results 1975.

The spermatocrit, the osmotic pressure and the results of the chemical analyses of the epididymal plasma (segment F_a) from boars 2 and 3 are given in Table 4. All the values obtained were within normal limits of variation (*Crabo, Einarsson* 1971, *Einarsson & Gustafsson*).

No gross or histological deviations from normal could be demonstrated of the testes, the epididymides and the accessory sex glands.

DISCUSSION

The number of boars used in this study is fairly small, but the morphological and physico-chemical examinations of the semen were extensive, and the investigation was going on for a rather long period of time. The results obtained are unanimous and seem to allow certain general conclusions.

The volume, the sperm concentration and the total sperm count per ejaculate rose gradually during the experimental periods. As the boars were comparatively young at the beginning of the experiment, these parameters are normally expected to increase. By comparison with the physiological increase of these characters in growing young boars (*Bane et al.*, personal communication 1965) the values obtained in this study must be considered within normal limits.

The motility and the sperm morphology in ejaculated semen differed only in exceptional cases from the mean values given by *Bane et al.* for boars of the same age. The composition of the seminal plasma remained fairly constant throughout the experiment in all the boars and were in accordance with the normal values presented by *Einarsson* (1971). The sperm morphology and the chemical composition of the epididymal content were also in agreement with normal values (Table 4). No conclusive effect of the exogenously administered oestrogens on the function of the epididymides and the accessory sex glands could thus be demonstrated in this study.

In the bull exogenous administration of oestrogens brings about a pronounced change in the sperm morphology and a changed composition of the epididymal plasma (*Gustafsson* 1966). The sensitivity for exogenously administered oestrogens thus appears to differ between bulls and boars. This difference is at present difficult to explain especially as it has been shown that daily administration of oestradiolbenzoate to a normal boar resulted in a marked rise in the peripheral plasma level of oestradiol-17-β and a corresponding decrease in the level of testosterone (unpublished). Apparently the oestrogen is absorbed from the site of injection and does exert an influence on the peripheral plasma levels of oestrogen and testosterone. Whether these hormonal changes are reflected also locally in testes, epididymides and accessory sex glands has, however, not been determined. Hormonal assays of blood from spermatic vein and

artery and of fluids from different levels of the reproductive tract will probably highly contribute in answering the question about the apparent insensitivity of the boar genital organs to exogenously administered oestrogens.

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SAMMANFATTNING

Effekten av parenteralt administrerade östrogener på bitestikelfunktionen och på de accessoriska könskörtlarnas funktion hos galt.

Tre normala, könsmogna unga galtar av Svensk Yorkshireras behandlades med dagliga intramuskulära injektioner av diethylstilboestrol och/eller oestradiolbenzoat under 13—15 veckor. Helejakulat uppsamlades på en suggfantom med en artificiell vagina två gånger i veckan. Ejakulaten undersöktes med avseende på volym, spermiekoncentration, motilitet och spermiemorfologi. Spermaplasman analyserades på sitt innehåll av Na, K, Cl, Mg, oorganiskt fosfat, total-

protein och fruktos. Testiklarna, bitestiklarna och de accessoriska könskörtlarna tillvaratogs vid slakten. Material från bitestikelregionerna A, B, D_b, F_a och F_b uppsamlades och undersöktes med avseende på spermiemorfologi. Spermatokrit, osmotiskt tryck och plasmahalter av Na, K, Cl, totalprotein och GPC undersöktes i material från bitestikelregion F_a. Könskörtlarna och de accessoriska könskörtlarna undersöktes såväl makroskopiskt som mikroskopiskt.

Ejakulatvolymen, spermiekoncentrationen och följaktligen totalantalet spermier per ejakulat ökade successivt under försökets gång. Detta är vad man kan förvänta då galtarna var förhållandevis unga vid försökets början. Dessa värden ligger alla inom de normala variationgränserna för växande galtar.

Vid beaktande av alla de undersökta parametrarna fanns inga bevis för att en exogen tillförsel av östrogener till normala galtar skulle ha någon effekt på bitestikelfunktionen eller på de accessoriska könskörtlarnas funktion.

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