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Cytogenetic, histological, hormonal and semen studies in male goats with developed udders

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Cytogenetic, hormonal, histological and semen quality examinations were performed in two unrelated male goats with developed udders (*gynaecomastia*). The bucks were neither stimulated for lactation nor milked. The progressive enlargement of the udder was observed between first and second breeding season (10-22 months). Karyotyping showed normal males. Plasma testosterone levels were within the range noted for normal bucks. Plasma concentrations of prolactin, growth hormone, FSH and LH were high, but comparable to normal bucks.
Structure of the mammary glands was similar to that found in females. The quality parameters of semen of goats with *gynaecomastia* was comparable to that considered common for the species.

KEY WORDS: gynaecomastia / goats / karyotype / hormone levels / semen quality / udder

Gynaecomastia or well developed male mammary glands, sometimes with secretory activity was reported in adult male goats by Rieck *et al*. [1975], Basrur

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and Yadav [1990], Dafalla *et al.* [1990] and Panchadevi and Pandit [1997]. Causes of this condition, recognized as glandular tissue proliferation leading to unilateral or bilateral enlargement of mammary glands in males may be hormonal or mechanical [Oskam *et al*. 2005]. Hereditary predisposition, chromosomal aberrations, or presence of testicular tumors can play a role in the onset of this abnormality [Rieck *et al*. 1975, Braunstein 1993, Smith and Sherman 1994, Lofstedt *et al*. 1994, Wooldridge *et al*. 1999]. The udder of lactating bucks possesses functional lactiferous glands with extensive parenchyma consisting of groups of tubulo-alveolar secretory units separated by interlobular connective tissue septa [Basrur and Basrur 2004]. The age of bucks at the onset of *gynaecomastia* varied from 7 months to several years and milk yield from 20 to 1500 ml per day [Marx *et al*. 1975, Lofstedt *et al*. 1994, Basrur and Yusoff 1997].

In this paper, cytogenetic, histological, hormonal and semen quality investigations are described of two bucks with developed udders

Material and methods

Animals

Two unrelated Polish White Improved male goats (buck 1 and 2) born and maintained in a breeding flock at the experimental farm of the Institute of Genetics and Animal Breeding, Jastrzębiec, were examined as having spontaneously developed udders. Until the first breeding season both bucks displayed normal male phenotype, were neither stimulated for lactation nor milked. Between the first and the second breeding season (*i.e.* at the age of 19-22 months) bilateral mammary glands enlargement began. The bucks were kept under normal photoperiodic conditions, fed commercial concentrate and hay and were not treated with any hormone.

At the age of 22 months, cytogenetic, histological, hormonal and semen quality examinations were performed. As bucks were born in twin litters, their co-twin females were also assessed in order to exclude leukocyte chimaerism. Chromosome preparations were obtained from a leukocyte culture using standard methods.

Hormonal investigations

Blood plasma was isolated and freezed until analysed for testosterone (T), growth hormone (GH), prolactin (PRL), follicle stimulating hormone (FSH) and luteinizing hormone (LH). Blood samples for hormonal investigations were collected four times every two hours between 7.30 to 13.30 h.

Testosterone was determined with a radioimmunoassay (RIA) method, described by Stupnicki [1985]. The sensitivity of the assay was 0.6 ng/ml, and the intra- and inter-assay variation coefficients were 12.4 and 14.6%, respectively.

Plasma GH concentration was assayed by a RIA double-antibody method, using antibovine-GH and antirabbit-gammaglobulin antisera and bovine GH standard

(NIDDK-GH-B-1003A) according to Dvorak *et al*. [1978]. The assay sensitivity was 0.68 ng/ml, and the intra- and inter-assay variation coefficients were 5.9 and 10.2%, respectively.

Plasma concentration of PRL was assayed by a RIA double-antibody method, using antiovine-PRL and antirabbit-gammaglobulin antisera according to Wolinska *et al*. [1977]. The assay sensitivity was 2 ng/ml and the intra- and inter-assay variation coefficients were 9 and 12%, respectively.

Plasma FSH concentration was determined by a RIA double-antibody method using antiovine-FSH NIDDK-NIH antibody. The standard and antibody were both supplied by dr. L.E. Reichert Jr., from the Tucker Endocrine Research Institute LLC, Atlanta, Georgia, USA. The sensitivity of the assay was 1.56 ng/ml and the intra- and inter-assay variation coefficients were 3.3 and 11.3%, respectively.

The concentration of LH was determined by a routine RIA double-antibody method, using antiovine-LH and antirabbit-gammaglobulin antisera and ovine LH standard (NIH-LH-B6), according to Stupnicki and Madej [1976]. The assay detection limit was 0.312 ng/ml per sample. The variation coefficient calculated for control samples at the concentration of 1 and 5 ng/ml of LH was 10 and 4%, respectively.

Semen quality assessment

Investigations of semen quality and clinical examinations of reproductive organs were performed during the second breeding season. The semen was collected to the artificial vagina in the present of a doe in *oestrus*. The following qualitative and quantitative parameters were estimated: volume, colour, consistency, granulation, mass motility, concentration of spermatozoa, and per cent of spermatozoa with morphological primary and secondary defects.

Histological investigations

The samples 4-5 mm thick for histological examination of mammary glands were collected immediately *post mortem* and fixed in a buffered 4% formaldehyde. After 48 h the specimens were rinsed in running water. Next, after dehydration in a number of vessels containing ethyl alcohol, tissue specimens were embedded in paraffin at 60^{o}C. Paraffin sections 5 µm thick were stained with hematoxylin and eosin.

Results and discussion

Both bucks showed normal male phenotype from birth to puberty. The progressive enlargement of the udders began between the first and the second breeding season (month 10-22 of life).

During the second breeding season bucks had large, well developed and pendulous udders with symmetric teats in front of testicles (Photo 1 and 2) and the following dimensions (cm):

	buck 1	buck 2
udder circumference	57	48
udder length	29	23
left teat length	7	1.5
right teat length	5	2

Photo 1. Male goat with *gynecomastia*.

Photo 2. Udder of gynaecomastic male goat.

Chromosome analysis performed on 300 lymphocyte metaphases of each buck revealed a normal caprine karyotype (60,XY). Also their female litter-mates showed a normal 60,XX karyotype.

The genetic component and environmental factors of caprine *gynaecomastia* are not fully recognized so far. Examination of a Fawn German buck with functional active *gynaecomastia* and yielding 20 ml milk a day showed mosaicism (X/XY) with variable deletions of the chromosome Y [Rieck *et al*. 1975]. Another gynaecomastic buck yielding 250-300 ml milk a day showed 45% neutrophils with sex chromatin (Barr body) indicating the presence of additional X chromosome [Panchadevi and Pandit 1979].

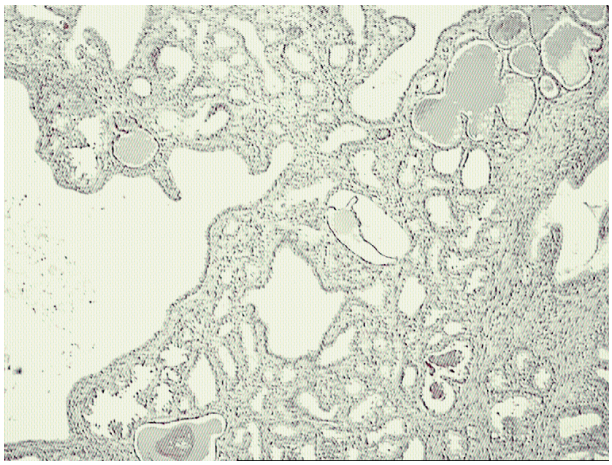

Photo 3. Excretory structures differing in size of the mammary gland of gynaecomastic male goat.

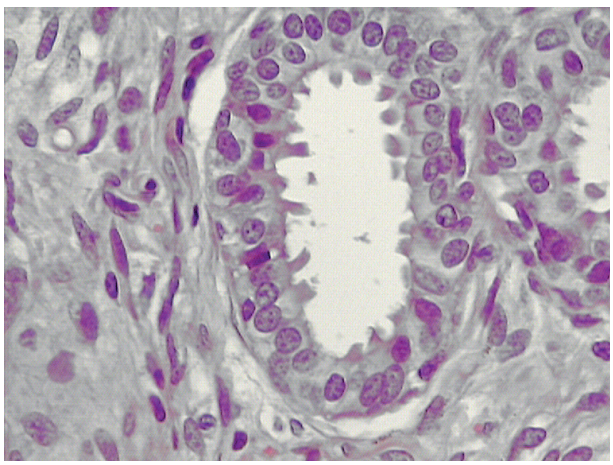

Photo 4. Tearing of apical parts of glandular epithelial cells of the mammary gland of a gynaecomastic male goat

Mammary gland parenchyma contained lobular glandular structures separated with a slight amount of fibrous connective tissue with locally observed aggregates of adipocytes. Irregular in shape and with differentiated diameter, the secretory segments were lined with follicular cells (Photo 3). In most areas it was simple columnar epithelium or simple cuboid epithelium with abundant cytoplasm. Cells showed the traits of apocrine secretion (Photo 4). In some locations, the secretory epithelium had the shape of the pseudostratified epithelium with the majority of tall, columnar cells. In their apical parts, the cells formed tearing-off vesicles (Photo 5). On the other hand, some secretory segments were formed from cuboidal epithelium, but very low one, passing into the squamous epithelium (Photo 6).

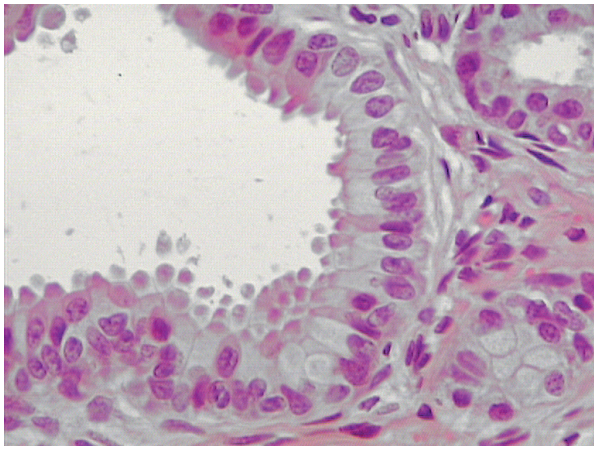

Photo 5. Fragment of a vesicle of a pseudostratified epithelium of a mammary gland in gynaecomastic male goat with signs of apocrine secretion.

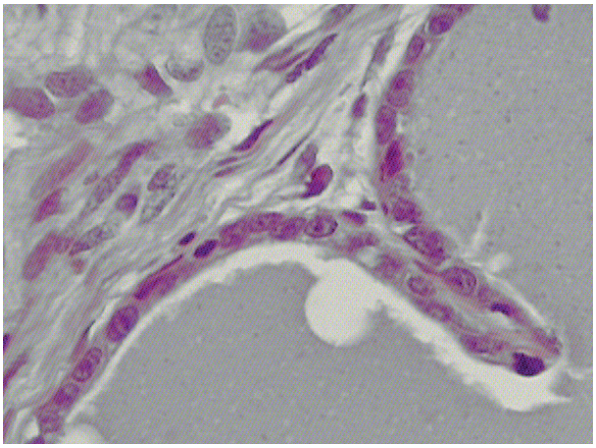

Photo 6. Fragment of a vesicle with squamous epithelium in a mammary gland of gynaecomastic male goat.

Histological image of the mammary gland pointed to a local differentiation of the activity of its secretory vesicles. In many areas they took a typical shape of the cisterns with different diameter and in some regions an extended net of tubular structures in which the epithelial cell nuclei were surrounded with a slight amount of cytoplasm. The lumen of their secretory segments corresponded with the two- or three-fold height of the epithelial cells, which pointed to their low secretory activity or its total lack in those regions. Most of the secretory segments presented typical traits of apocrine secreting cells, owing to the numerous apical cellular fragments of tearing-off vesicles visible at the lumen side. As it is commonly known, such type of secretion is represented by the epithelial cells of the mammary gland fat-secreting vesicles. The general structure of the mammary glands in question was found similar to the udder structure typical of female goats.

Mean plasma values for T, GH, PRL, FSH and LH in bucks as measured four times a day are presented in Table 1. Concentration of T and PRL occurred higher in buck 1 than in buck 2. A significant inter-individual difference in bucks' T and PRL plasma levels was reported by Bosu and Barker [1982] and Lofstedt *et al*. [1994]. This indicates the considerable individual variation in the T plasma level. In the present report the overall mean plasma T concentrations in bucks with *gynaecomastia* were within the range recorded for clinically normal bucks and intersex goats by Zlotnik [1973] and Georgie *et al*. [1985]. Higher levels of plasma PRL and GH (Tab. 1) were generally similar to the values reported for lactating male goats by Lofstadt *et al*. [1994]. In the present investigation the bucks were not stimulated for lactation, and not milked.

Table 1. Plasma hormone concentration (ng/ml) in bucks with developed udders

Buck	Sampling time (h)	T	GH	PRL	FSH	LH
1	07.30	5.65	3.73	7.42	2.27	6.73
	09.30	9.36	2.43	5.42	2.63	6.56
	11.30	7.72	4.27	61.39	4.31	5.87
	13.30	8.7	5.34	84.53	4.92	6.10
mean		7.86	3.94	39.69	3.53	6.32
SE		±0.81	±0.61	±19.78	±0.64	±0.20
2	07.30	4.08	3.89	3.72	4.45	6.29
	09.30	3.79	2.48	2.97	2.87	6.38
	11.30	3.12	5.46	20.93	4.22	4.4
	13.30	3.88	5.42	23.26	3.46	5.57
mean		3.72	4.31	12.72	3.75	5.66
SE		±0.21	±0.71	±5.44	±0.36	±0.46

The gynaecomastic males described in this report showed also high circulating levels of FSH and LH as compared to normal bucks [Oscam *et al*. 2005].

External and internal parts of reproductive system available for clinical examination (testes, epididymes, penis) showed a normal size and consistency.

Parameters of semen quality are presented in Table 2.

Table 2. Results of semen quality assessment in bucks with developed udders

Parameter	Buck 1	Buck 2
Semen volume (ml)	0.8	1.0
Semen colour	yellow-citron	ivory-cream
Semen consistency	cream	cream
Semen granulation	z(z)	Zzz
Mass motility	++(+)	+++
Spermatozoa with progressive motility (%)	70	80
Spermatozoa concentration	1.650.000/µl	2.650.000/µl
Spermatozoa with defects (%)		
Primary	6.5	4.8
Secondary	17.3	10.3

The quality parameters of semen of both bucks occurred comparable to those typical of the species. No pathologies were found in the testes and epididymes such as aplasia or atrophy. Both animals displayed high libido, were fertile and during the three breeding seasons buck 1 sired 39 and buck 2 - 103 kids. However, male goats with *gynaecomastia* may show a depressed libido and decreased sperm count as well as reduced motility of spermatozoa and low serum testosterone level [Bloch *et al*. 1988, Daffala *et al*. 1990, Smith and Sherman 1994].

Summing up, it may be concluded that gynaecomastia found in both bucks and described here cannot be interpreted on the basis of hormonal, semen, or cytogenetic aberrations. No cancer structure of glands was detected. The results are especially interesting when compared to the other cases of gynaecomastia described in the literature.

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Charakterystyka cytogenetyczna, histologiczna i endokrynologiczna oraz jakość nasienia kozłów z wykształconymi gruczołami mlekowymi

S t r e s z c z e n i e

Wykorzystano dwa niespokrewnione ze sobą kozły z wykształconymi gruczołami mlekowymi (wykazujące ginekomastię), których nie stymulowano hormonalnie ani nie dojono Stopniowy rozrost wymion obu zwierząt obserwowano między pierwszym a drugim sezonem kopulacyjnym (w wieku 10-22 miesięcy), a przeprowadzone badania wykazały kariotyp typowy dla samców (60, XY). Poziom testosteronu, prolaktyny, hormonu wzrostu, FSH i LH w osoczu krwi zawarty był w granicach notowanych dla normalnych kozłów. Struktura tkanki wymienia była zbliżona do występującej w wymionach samic. Parametry jakości nasienia okazały się typowe dla gatunku. Oba kozły były płodne. Kozioł 1 był ojcem 39, a kozioł 2 - 103 koźląt.