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From the Department of Obstetrics and Gynaecology, Swedish University of Agricultural Sciences, Uppsala, Sweden.

SEMEN QUALITY AND FERTILITY AFTER HEAT STRESS IN BOARS

By
Lena Malmgren and *Kjell Larsson*

MALMGREN, LENA and KJELL LARSSON: *Semen quality and fertility after heat stress in boars.* Acta vet. scand. 1984, 25, 425—435. — Sperm morphology and the fertilizing capacity of ejaculated spermatozoa were examined in 6 Swedish Landrace boars before and after heat stress. The boars were exposed to 35°C during 100 h in a climatic room. Fertility was measured by insemination of gilts before and at various times after heat stress. Each gilt (n=44) was inseminated with a total of 5×10^9 spermatozoa diluted to 100 ml with EDTA-glucose diluent and fertilization was assessed by examining recovered ova 2 days after insemination.

Changes in semen quality varied among the boars from a very weak response in 2 boars to pronounced semen alterations occurring 2—6 weeks after heat stress in the other boars. A close relationship was found between seminal changes and fertilization rates, all ejaculates which had high fertilization rates being of the same quality as the pre-exposure ejaculates. The ejaculates that had poor fertility were characterized by lowered sperm motility and increased numbers of spermatozoa with abnormal heads, proximal cytoplasmic droplets and nuclear pouch formations.

sperm morphology; fertilization; boar.

Lowered fertility and/or lowered total sperm counts and decreased ejaculate volumes have been found in boars during or shortly after the warm summer period in several countries (e.g. *Okauchi & Hirakata* 1963, *Thibault et al.* 1966, *Einarsson* 1968, *Stone* 1977). In experimental studies various forms of heat stress have been used to induce similar changes. Lowered ejaculate volumes and total sperm counts have been found in some studies (*Christenson et al.* 1972, *Stone* 1982), but in other experiments (*Wetteman et al.* 1976, *Cameron & Blackshaw* 1980, *Einarsson & Larsson* 1982, *Larsson & Einarsson* 1984) ejaculate volumes and total sperm counts remained unaffected. In most studies an in-

crease of abnormal spermatozoa has been found (e.g. *Wetteman et al.* 1976, *Einarsson & Larsson* 1982, *Larsson & Einarsson* 1984). However, the results vary among experimental boars and also due to the different regimes for heat stress that have been used.

Lowered courting activity of the boars was found during heat stress (*Winfield et al.* 1981, *Stone* 1982) and apparent endocrine changes in plasma levels of cortisol and testosterone were found during and after heat stress (*Einarsson & Larsson* 1980, *Larsson et al.* 1983). The fertility rate was decreased 2—6 weeks after heat stress(*Christenson et al.* 1972, *Wetteman et al.* 1976, *Stone* 1982). In most of the aforementioner studies considerable variations among boars in their response to heat stress have been found. Furthermore the relationship between seminal changes and fertility have not been fully clarified. The purpose of the present study was to investigate the seminal changes in boars after exposure to heat stress and to relate these changes to the fertilizing capacity of the spermatozoa.

MATERIALS AND METHODS

Six mature (7—8 months) Swedish Landrace boars were used for the experiments. The boars originated from the same herd and were brought to the clinic at approximately 7 months of age. At the clinic they were kept in individual pens at barn temperature (18°C) and were fed a standard ration of grain mixture and concentrates.

Semen collection

Semen was collected twice weekly during the whole experimental period, beginning 6—8 weeks before heat stress and continuing until the semen quality was again normalized according to the standards of our laboratory (6—13 weeks after the heat stress). Semen collections were not performed while the boars were in the climatic room. The boars were allowed to mount the dummy sow in their own pens and the gloved hand technique was used for collection of semen. At collection the gel portion of the eajculate was filtered away through double gauze and the remaining part of the ejaculate was collected into a plastic bag in a preheated vacuum-flask.

Semen evaluation

Ejaculate volumes were measured by means of 100 ml graded cylinders. Sperm motility was estimated under a phase-contrast microscope at 37°C and at a maximum of ×400 magnification. Semen morphology was assayed by phasecontrast microscopy of formol-saline fixed wet preparations and by light microscopy of William's stained dry smears. Sperm concentrations were measured by haemocytometric counting. At least 8 ejaculates with normal semen quality, according to the standards of our laboratory, were collected from each boar before exposure to heat stress. One of the boars showed increased numbers of spermatozoa with single-bent tails before exposure to heat stress. He was used in the experiments without fulfilling the demands on normalized sperm morphology before termination of semen collection.

Climatic room

The exposure to elevated temperature took place in a 3.5× 3.5 m temperature controlled room. The temperature in the room was kept at 35°C and the relative humidity at 40 %. The boars were kept in the climatic room for 100 h.

Fertility evaluation

A total of 44 gilts (crossbred Swedish Landrace × Swedish Yorkshire, 7—8 months of age) were used for the fertility tests. It was originally planned that gilts should be oestrus-synchronized with Oxolven® (*Mayer et al.* 1977) for insemination with semen collected immediately before and 1, 3 and 6 weeks after heat stress. However, in too many cases ovarian cysts occurred (cf. *Malmgren et al.* 1983) and therefore only 10 gilts showing normal heat after synchronization were used and the remaining 34 were inseminated in spontaneous oestrus. For this reason the timing of inseminations relative to heat stress was less accurate than the original plan (see Table 2).

All gilts were checked for oestrus twice daily with the aid of a vasectomized boar and insemination was done once at 10—30 h after onset of standing heat. The inseminations were performed with a rubber spiral tip catheter according to *Melrose & O'Hagan* (1961). The gilts were inseminated with 5×10^9 spermatozoa diluted to 100 ml in EDTA-glucose diluent. All ejaculates used

for insemination were examined as previously described and inseminations were performed within 6 h after semen collection. All gilts were slaughtered 2 days after insemination and the genital tract was removed immediately after stunning and bleeding. After a careful macroscopical examination the oviducts and upper parts of the uterine horns were flushed with saline for recovery of ova. The numbers of fresh corpora lutea (CL) in each ovary were recorded. The flushing solution was collected in Petri dishes which were then examined for presence of ova under a stereo-microscope at × 50 magnification. Recovered ova were morphologically examined under an inverted phase-contrast microscope at × 400 magnification. Ova with symmetrical cleavage (2- to 8-cell stages) were recorded as fertilized, whereas asymmetrically divided or undivided ova were classified as unfertilized. Ova with broken zona pellucida and empty zonae pellucidae were not included in the calculations of fertilization rate.

Postmortem examination of the boars

The boars were slaughtered when the semen qualitiy was again normal after heat stress. Immediately after stunning and bleeding the genital organs were removed and examined carefully. Specimens for histological examination were taken from two parts of each testicle. The specimens were fixed in Bouin's fixative and, after processing, slides were stained with haematoxylin-eosin and Van Gieson's stain.

Statistical methods

Standard statistical methods were used for evaluation of data. The relationship between seminal characteristics and fertilization rate was based only on the ejaculates that had been used for inseminations and, to illustrate this relationship, ejaculates that had a fertilization rate of more than 50 % were compared with those that yielded less than 50 % fertilization rate.

RESULTS

Seminal changes and sperm morphology are presented in Table 1. The values referred to as "before" represent 8 ejuculates from each boar. With the exception of the boar that had increased percentages of single-bent sperm tails, all boars had normal

semen before exposure to heat stress. The high percentage of coiled and bent tails is due only to the aforementioned boar, which had an average of 26 % single-bent sperm tails.

Table 1. Semen characteristics of the boars before, 2—3, 4—6 and 7—9 weeks after heat stress. Overall means ± s.

	Before	Week 2—3	Week 4—6	Week 7—9*
Motility (%)	68.0± 4.7	56.4±17.7	53.8±17.6	62.8±10.3
Ejaculate volume (ml)	141.6±46.4	181.0±57.7	155.8±62.5	167.9±55.9
Total sperm number × 10^9	37.1±14.4	39.9±13.4	36.8±20.8	44.6±22.0
% sperm abnormalities:				
Abnormal heads	2.0± 1.0	11.8±20.2	14.1±20.3	1.9± 2.0
Prox. cytopl. droplets	0.4± 0.6	3.5± 3.9	7.4± 8.5	0.8± 1.1
Coiled and bent tails	5.6±12.2	11.0±18.9	12.5±21.7	9.6±18.7
Abnormal acrosomes	0.6± 0.6	2.1± 3.1	7.7±11.0	0.7± 0.9
Nuclear pouch formations	0	5.5±12.0	3.6±14.3	0
Abnormal midpieces	2.2± 3.5	3.7± 3.0	5.0± 4.5	3.1± 3.8
No of boars	6	6	6	4
No of ejaculates	48	25	30	25

* The 4 boars with latest normalization of semen.

There were no consistent changes in ejaculate volumes and total sperm counts after heat stress, while there was an apparent decline in sperm motility during weeks 2—6 after heat stress. In 3 boars increased numbers of sperm abnormalities were found during weeks 2—6 after heat stress and in a fourth boar increased numbers of abnormal spermatozoa were found during weeks 4—7 after heat stress. The remaining 2 boars only had a minor increase in abnormal sperm heads (up to 11 %) during a short period (week 3) after heat stress. The data summarized in Table 1 are overall means for all boars and the reason for the relatively slight increases seen in the table is the different patterns among the boars. The high percentage of coiled and bent tails is entirely related to the boar that had this abnormality already before heat stress, and the data for this boar are summarized in Table 1a.

The lowest fertilization rate was obtained with semen collected 2—3 weeks after termination of heat stress (Table 2). An im-

Table 1 a. Semen characteristics of the boar with increased incidences of bent and coiled tails.

	Before	Week 2—3	Week 4—6	Week 7—9	Week 10—12
Motility (%)	65.0± 7.1	13.3± 7.6	8.3± 5.8	48.0±18.0	11.9±17.7
Ejaculate volume (ml)	131.9±59.3	180.0±30.4	231.7±43.1	210.0±51.3	207.1±43.0
Total sperm number × 10^9	42.5±21.3	56.0±11.0	82.3±21.0	55.6±17.4	62.3±27.7
% Sperm abnormalities:					
Abnormal heads	1.4± 0.6	6.0± 5.7	9.1± 4.9	1.6± 1.4	5.3± 4,3
Prox. cytopl. droplets	0.4.± 0.6	4.3± 2.4	7.7± 6.5	0.6± 0.6	1.0± 1.2
Coiled and bent tails	26.3±19.5	53.2±20.4	70.3±13.1	35.8±24.1	66.2±13.3
Abnormal acrosomes	0.5± 0.5	0.7± 0.8	2.5± 1.8	0.3± 0.4	2.6± 5.8
Nuclear pouch formations	0	5.7± 9.8	0	0	0
Abnormal midpieces	7.8± 5.2	4.3± 1.6	11.7± 6.6	6.4± 4.4	6.1± 6.3

provement was seen 5—6 weeks after heat stress and for the late recovering boars the fertilization rate was very high for semen collected 7—10 weeks after heat stress. The data presented in Table 2 include all gilts inseminated during these periods and semen from all boars was used. Four of the inseminated gilts are not presented in the table because 1 was inseminated in week 1 and the other 3 in weeks 11—13; data from these gilts are included only in Table 3.

Table 2. No. of inseminated gilts, no. of recovered and fertilized ova before and after heat stress of the boars.

	No. of gilts inseminated	gilts/boar	No. of c.l.	No. of ova recovered	% fertilized ova
Before heat stress	14	1—3	154	115	78 a,c
2—3 weeks after	12	1—3	144	92	63 b
5—6 weeks after	6	1—3	73	51	67 a,b
7—10 weeks after	8	1—3	85	53	89 c

Fertilization rates with different superscripts differ significantly ($P < 0.05$).

The greatest effect on fertility was seen when ejaculates with low sperm motility and high numbers of abnormal spermatozoa were used. Most ejaculates collected before heat stress had a more than 50 % fertilization rate and, as seen in Table 3, the ejaculates with a more than 50 % fertilization rate collected after heat stress

had a semen quality close to that found before heat stress. The ejaculates that had low fertilization rates were characterized by lowered sperm motility and increased numbers of sperm abnormalities.

Table 3. The relationship between fertilization rates and ejaculate characteristics (mean ± s).

%	≤ 50 % fertilized ova	> 50 % fertilized ova	r
Motility	44.2±28.1	63.4±10.5	0.53***
Abnormal heads	18.8±29.1	4.0± 6.5	—0.47**
Prox. cytopl. droplets	5.4± 6.5	1.6± 2.9	—0.43**
Coiled and bent tails	22.2±28.1	7.1±16.2	—0.41**
Abnormal acrosomes	4.5± 4.3	1.7± 2.2	—0.24 n s
Nuclear pouch formations	5.3±13.5	0.7± 3.2	—0.33*
Abnormal midpieces	5.2± 4.7	2.1± 2.7	—0.25 n s
No gilts	13	31	
No of cl	159	354	
No of recovered ova	119	237	
No of fertilized ova	17	220	
$\bar{x}$ fertilization %	14.0 (0—50)	93.0 (63—100)	

$P < 0.05^*$, $P < 0.01^{**}$, $P < 0.001^{***}$, $P > 0.05$ n s.

Significant correlations were found between fertilization rate and sperm cell motility, % abnormal sperm heads, % spermatozoa with proximal cytoplasmic droplets, % coiled and bent tails and % spermatozoa with nuclear pouch formations (Table 3). The correlations were calculated for each semen characteristic without correcting for any possible combined effects.

Most of the fertilized ova had 10 or more spermatozoa attached to the zona pellucida, whereas very few of the unfertilized ova had any spermatozoa in the zona pellucida (Table 4). Furthermore, when low quality semen was inseminated, few ova had any spermatozoa attached to the zona pellucida (Table 5).

Table 4. The relationship between fertilization and spermatozoal attachement to the zona pellucida.

Number of spermatozoa attached to the zona pellucida	Number of ova	
	fertilized	unfertilized
0	17	96
1—10	72	13
>10	150	8

Table 5. The relationship between the numbers of spermatozoa attached to zona pellucida and ejaculate characteristics (mean ± s).

%	Number of spermatozoa attached to zona pellucida 0	1—10	>10
Motility	37.2±30.2	61.0±11.9	66.0± 4.6
Abnormal heads	14.9±27.0	3.0± 2.9	2.6± 2.3
Prox. cytopl. droplets	5.7± 6.2	1.9± 3.6	1.3± 2.3
Coiled and bent tails	28.9±26.2	10.4±17.9	1.6± 3.3
Abnormal acrosomes	4.9± 6.4	2.3± 7.0	1.3± 1.3
Nuclear pouch formations	4.5±11.3	1.0± 3.3	0 ± 0.1
Abnormal midpieces	5.6± 3.9	3.4± 3.1	2.5± 2.7

None of the boars had any macroscopical alteration in the genital organs. Histological examination of the testicles revealed that most tubuli seminiferi were normal. In all of the boars a few tubuli with signs of degeneration were seen.

DISCUSSION

The seminal changes found after heat stress are in close agreement with those found in previous experiments in our laboratory, utilizing the same climatic room and similar animals (*Einarsson & Larsson* 1980, *Larsson & Einarsson* 1984). However, in the present study the variations in response among the experimental boars were more apparent. The variations are most likely explained by differences among the boars in heat resistance or differences in the ability to adapt to the new environment.

Apart from the previously described seminal alterations, i.e. lowered sperm motility and increased numbers of spermatozoa with proximal cytoplasmic droplets and abnormal sperm heads, also increased numbers of spermatozoa with nuclear pouch formations were found during the period after heat stress. The abnormality was not found in a single ejaculate before heat stress. The abnormality was not found in a single ejaculate before heat stress nor in the ejaculates collected after normalization of other sperm characteristics. This observation is in agreement with the results of *Bane & Nicander* (1965), who found the pouch formations to be related to a disturbance in spermiogenesis.

In the present study fertility was only estimated as fertilization rate shortly after insemination. Thus further negative effects on embryonic survival, were not accounted for. The present results clearly indicate a relationship between seminal changes

and the fertilizing ability of the spermatozoa. The present results are in agreement with the results presented by *Wetterman et al.* (1976, 1977).

The time lapse from heat stress to the occurrence of lowered sperm motility and increase of abnormal spermatozoa in the ejaculates indicates that spermiogenesis, but not earlier stages of the spermatogenesis, was negatively affected. As indicated by the increased of spermatozoa with proximal cytoplasmic droplets, also epididymal sperm maturation might have been influenced. Although the percentage of abnormal spermatozoa was very high in single ejaculates, the mean numbers in the ejaculates that had poor fertilization rates are surprisingly low. However, the fact that the unfertilized ova rarely had any spermatozoa attached to the zona pellucida indicates that the fertilizing ability and/or the uterine-oviductal transport was affected also in morphologically normal spermatozoa.

The results of this study indicate that it is possible to use semen evaluation as an indicator of fertility in a boar that has been exposed to heat stress or presumably to any other kind of environmental disturbance.

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SAMMANFATTNING

Inverkan av förhöjd omgivningstemperatur på spermiekvalite och fertilitet hos galt.

Morfologi och befruktningsförmåga hos ejakulerade spermier undersöktes hos 6 stycken Svenska Lantrasgaltar, före och efter värme-

stress. Galtarna vistades i en klimatkammare där de exponerades för 35°C i 100 tim. Fertiliteten bedömdes genom att gyltor (n=44) inseminerades med sperma som samlades före, och vid olika tidpunkter efter värmestressen.

Till varje insemination användes 5×10^9 spermier utspädda till 100 ml med EDTA-glukos lösning. Gyltorna slaktades 2 dygn efter insemination och befruktningsförmågan bedömdes genom att antal befruktade respektive obefruktade ägg räknades.

Förändringarna i spermiekvaliten varierade hos galtarna, från ringa förändringar hos 2 av galtarna till uttalade spermiemorfologiska förändringar som varade i 2—6 veckor efter värmestressen hos de övriga 4 galtarna. Ett nära samband påvisades mellan de spermiemorfologiska förändringarna och befruktningsförmågan, vilket innebar att ejakulat som gav en hög befruktningsfrekvens också uppvisade en spermiekvalite som ej avvek från de ejakulat som samlats före värmestressen. Ejakulat med låg befruktningsförmåga karakteriserades av sänkt spermiemotilitet och ökat antal spermier med patologiska huvudformer, proximala droppar och kärnsäckar.

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Reprints may be requested from: Lena Malmgren, the Department of Obstetrics and Gynaecology, Swedish University of Agricultural Sciences, S-750 07 Uppsala, Sweden.