The relation between pH and sperm quality

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ABSTRACT. The pH of semen varies with the animal species. For the bull, some authors give a variety of values such as Rollinson who reported 6 to 6.4; Bonadonna 6.6 and Juma 6.4 and 6.5. Anderson stated that hydrogen ion concentration is a test of sperm quality since when the pH is high the sperm have a reduced fertility and motility and are less concentrated. Fructose is the main energy source for the sperm and therefore the cell glycolytic activity is very important and this depends on pH.

We have studied 224 animals which were classified into three groups: I Animals in production. II Healthy animals out of production. III Azoospermic animals.

Significant differences were found to exist between pH values of groups I and II as well as of II and III and of I and III. When choosing a sample of sperm, it is very important to measure the pH correctly.

RESUMEN. El pH de la esperma varía según las diferentes especies animales. En el bovino algunos autores señalan valores discordantes, así Rollinson reportó de 6 a 6.4. Bonadonna de 6.6 y Juma entre 6.4 y 6.5. Anderson decía que la concentración de iones pH es una prueba para investigar la calidad de la esperma, ya que a pH alto existe una escasa fertilidad, así como una disminución de la motilidad y la concentración. La principal fuente de energía para los espermatozoides es la fructosa, por lo que es muy importante la actividad glicolítica de la célula, donde juega un importante papel el pH.

Hemos estudiado 224 animales los cuales clasificamos en tres grupos: I Animales de producción. II Animales sanos fuera de producción. III Animales azoospérmicos.

Habiéndose encontrado que existen diferencias significativas entre los valores de pH de los grupos I y II, así como entre el II y III y el I y III. Por la gran importancia que tiene la medición estricta de este parámetro, recomendamos se tome en cuenta como uno de los factores a valorar cuando se quiera elegir una muestra de esperma.

INTRODUCTION

The pH of semen varies with the animal species. Authors give varying values to bull semen. For instance, Rollinson, *(1950)* stated that the most fertile semen has a pH between 6.0 and 6.4. Romijin, *(1948)* gave average values of 6.65; Bertagni, *(1940)* stated that the pH of bull semen is always over 6.64. Bonadonna, *(1967)* gave the average pH as 6.68; Derivaux, *(1966)* gave values between 6.5 and 6.8 and Juma, *(1969)* between 6.4 and 6.5.

The pH of bull semen can reach neutral (7.0) and higher values approaching alkalinity. According to Rollinson, *(1950)*, alkalinity denotes normal fertility. In azoospermic semen, the pH is almost always near to or above 7.0. Higher values were also found in bulls subjected to a regime of excessive mounting *(Bidot, 1973)* as also was found when ejaculation was incomplete or in cases of orquiepididymitis or vesiculitis. Blom *(1950)* determined the pH values at different positions: tail of epididymis (6.2 to 6.4); vesicle gland (5.6 to 6.15); prostate gland and bulbourethral glands (7.5 to 8.24).

According to Anderson, *(1946)*, hydrogen ion concentration is a test to determine sperm quality since higher pH values are associated with reduced fertility as well as a decrease in the motility and concentration of the sperm, that is, the lower the initial pH, the higher will be the initial motility and the number of cells per cubic milimeter.

Other authors *(Derivaux, 1966)* consider that the rate of increase of acidity after ejaculation is much more important in the determination of sperm quality than the initial pH value, since this depends on the cell glycolytic activity and the main energy source for spermatozoa is fructose. When this is in high concentration, the pH decreases rapidly due to lactic acid accumulation. Thus a rapid increase in pH indicates a high vitality of the cells present in the sample. The fundamental aims of our study were directed to determining the changes that the pH of semen undergoes in animals under different conditions.

MATERIALS AND METHODS

A total of 224 animals of Holstein, Brown Swiss, Cebú, Short Horn, Criollos and Santa Gertrudis races from different centers of Havana

province, clinically tested, with tuberculosis and brucella negative, were used.

They were divided into three groups according to the following characteristics:

Group I. 164 animals being used for production of semen and subjected to a twice a week mount regime.

Group II. 26 healthy animals which had been removed from production a year before and subjected to two mounts only fifteen days before the experiment.

Group III. 34 azoospermic animals which had later previously shown conditions such as orquitis, orquiepididymitis and vesiculitis but were subjected to a mount regime of twice a week.

The enviromental conditions for all the groups of animals were similar as well as the daily ration of food.

The semen samples were obtained using the standard method of an artificial vagina. Several methods have been described for the determination of the pH of semen. These include the standard test paper, colorimetric method such as that using brominethymol described by Theret *(1950)* and potentiometric methods. A very stable Schlumberger SM-101A portable pH meter calibrated to 0.1 was used. Measurements were taken immediately after extraction.

Readings with test paper were also carried out to the nearest 0.1 and values between 7.0 and 7.5 were obtained. Motility, density and volume were also determined.

The volume was read directly from the graded collector. A drop of semen (0.01 approximately) was immediately observed under the microscope to determine motility and density. the former being measured by using a decimal system and the latter using the percentage of rectilineal movement *(Blom, 1946).*

RESULTS AND DISCUSSION

Volume. The volumes of the samples are shown in table I. The average volume for animals in group I was 5.6 $\pm$ 1.8; for animals in group II

3.9 ± 1.0, and for those in group III 4.6 ± 1.6 ml. With the above data we can affirm that the animals in group I ejaculated more than the animals no longer being regulargy used in group II, which indicates that a prolonged interruption of normal mounting decreases the volume of semen ready for ejaculation.

TABLE I

Volumes obtained

	Volume
Group I	5.6 ± 1.8
Group II	3.9 ± 1.0
Group III	4.6 ± 1.6

Motility. The average motility of the groups is shown in table II. For group I it was 68.4 ± 22.1 and for group II it was 57.2 ± 26.2.

Group III contains azoospermic animals.

TABLE II

Motility in the three groups

	Motility
Group I	68.4 ± 22.1
Group II	57.2 ± 26.2
Group III	0

It can be observed that the motility of the sperm of animals in production is greater than among those animals removed from production a year previously. This is because the latter have a greater accumulation of aged cells and so a decrease in motility *(Bidot, 1973).*

Density. The results obtained are shown in table III.

TABLE III

Density per groups

	Density
Group I	77.1 ± 17.8
Group II	67.8 ± 13.2
Group III	0

In group I the average density is 77.1 ± 17.8 and in group II, 67.8 ± 13.2. Density in group III is zero. This parameter decreases also in animals out of production. When this happens, a reabsorption of older cells takes place, mainly, in the most distal portion of the different vessels. *(Stigler, 1918; Polowzow, 1927).*

According to Bonfert *(1956)*, the prolongation of sexual rest can produce a progressive worsening of seminal material with functional degradation of the semen.

According to B. M. Kerruish *(1955)* a very long sexual rest in bulls decreases the libido and fertility and it makes them produce a seminal product of little use and of low quality.

pH. The pH values observed are shown in table IV. For animals in production the pH is 6.5 ± 0.2, which agrees with the majority of the authors including Holy (6.2 — 6.9), and Rollinson (6.0 — 6.4). Semen from animals in group II has a pH of 6.8 ± 0.3. The higher pH in this group denotes a tendency to alkalinity.

According to Rollinson *(1950)*, this tendency denotes an abnormal fertility due to the low quality of the sperm.

For animals in group III, the pH observed was 7.03 ± 1.3. Rollinson *(1950)* affirmed that an azoospermic semen is always near to or above 7.

TABLE IV

pH in the three groups

	pH
Group I	6.5 ± 0.2
Group II	6.8 ± 0.3
Group III	7.03 ± 1.3

This always occurs after conditions such as orquitis, orqui-epididymitis or vesiculitis. All vesiculitis the above data appear in table V.

TABLE V

Statistics by groups

Groups	Statistics	pH	Volume	Motility	Density
I. Animals for production	$\bar{x}$	6.5	5.6	68.4	77.1
	S^2	0.04	3.09	491.26	318.42
	S	0.21	1.76	22.16	17.84
	C.V.	3.18	31.51	32.39	23.13
II. Healthy animals out of production	$\bar{x}$	6.8	3.9	57.2	67.8
	S^2	0.07	1.01	690.22	175.29
	S	0.26	1.00	26.27	13.24
	C.V.	3.78	25.30	45.94	19.52
III. Azoospermic animals	$\bar{x}$	7.03	4.6	—	—
	S^2	1.62	2.53	—	—
	S	1.27	1.59	—	—
	C.V.	18.08	34.69	—	—

The following correlations were found between the parameters in the three groups.

In group I a significant inverse correlation ($r = -0.3251$) was found with $p < 0.01$ between the pH and the volume and a significant direct correlation ($r = 0.7739$) with $p < 0.001$ between motility and density. The rest of the comparisons were not significant (Table VI).

In group II, when density and pH were compared the significant differences were found with $r = -0.7020$ for $p < 0.01$.

TABLE VI

Correlations. Group I

	Motility	Density	pH
Volume	r = 0.004517	r = 0.138961	r = —0.325081**
Motility	——	r = 0.773927***	r = —0.171350
Density	——	——	r = —0.137845

** P<0.01

*** P<0.001

The other comparisons were not significant (Table VII).

TABLE VII

Correlations. Group II

	pH	Volume	Motility	Density
Volume	r = —0.2577	——	r = 0.2848	r = 0.1994
Motility	r = —0.3332	——	——	r = 0.4014
Density	r = —0.7020**	——	——	——

** P<0.01

In group III significant no differences were found (Table VIII).

TABLE VIII

Correlations. Group III

	pH
Volume	r = —0.1586

CONCLUSIONS

From the above results we draw the following conclusions:

1. All animals out of production for a prolonged period undergo a quantitative decrease in sperm quality.
2. Orquitis, orqui-epididymitis and vesiculitis in some cases produce considerable alterations in sperm quality (azoospermic semen) which in most cases are irreversible.
3. We recommend as a parameter of great importance the accurate measurement of pH when trying to determine the quality of a sample of semen.
4. Whenever possible, potentiometric methods should be used to measure pH since standard test papers have very low sensitivity.

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