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Pig Reproductive and Respiratory Syndrome Virus (PRRSV) Does Not Attach to Boar Sperm; It Affects Only the Velocity Pattern and the Mobility Pattern

Néstor Méndez Palacios[1*], Netzi Naidí Mendez Palacios[1], Felicitas Vázquez Flores[1], José Alfredo Galicia Domínguez[1], Edgar Guadalupe Beltrán Rosas[2], Maximino Méndez Mendoza[1]

[1]Facultad de Medicina Veterinaria and Zootecnia-Benemérita, Universidad Autónoma de Puebla, Puebla, Mexico
[2]Independent Advisor in the Porcine Area, Puebla, Mexico
Email: nmp63mx@gmail.com

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Abstract

The purpose of the study was to evaluate the sperm viability of semen infected with PRRSV viral particles, observing the effect of the Virus on the motility of boar sperm. The work was carried out at the FMVZ-BUAP Genetics and Reproduction Laboratory. 5 stallions were used. Each sample contained 1×10^6 sperm, the PRRS virus strain was ATCC-VR-2332 (0, 10^2, 10^4 and 10^6 copies of RNA/mL in triplicate), it was observed daily at the CASA; Hamilton Thorne®. Cells with MT ($P < 0.05$) on days 1, 3, 5, 7 and 10 of evaluation with 201 ± 7.3, 167 ± 10.1, 165 ± 14.6, 134 ± 8.2 and 120 ± 8.8, respectively. The % MP between control and virus concentrations ($P \geq 0.05$). The LCV on day 1 and 7 PI at $10X^2$ and $10X^6$ ($P < 0.05$) vs control. In the Correlation Matrix, where it is observed that there is a correlation between VSL and VAP, VSL and VCL, VCL and ALH, VAP with ALH. There is a correlation of VSL and ALH, STR and ALH. In this study there were ($P \leq 0.01$) in the VCL, in the concentrations (10^2) 162.81 ± 10.65 and (10^6) 177.12 ± 5.77 vs 193.04 ± 4.62 of control. This indicates that altering these parameters would be related to fertility and the PRRS virus affects the LCV. Regarding the VSL, it was observed that the sperm infected with viruses 10^2, 10^4 and 10^6 of 48.00 ± 3.38, 49.88 ± 1.83 and 50.55 ± 2.24 Vs. 56.66 ± 1.68 of control respectively, the control would have greater possibilities of fertilizing the oocyte. In this study, it was found ($P \leq 0.01$) in the VAP with 102 of 77.26 ± 5.16, 10^4 with 83.35 ± 2.41 and 10^6 with 81.29 ± 3.14 vs the control with 90.56 ± 2.07. Regarding the ALH there is ($P < 0.05$) a 10^4 with 8.70 ± .26 and 10^6

with 9.64 ± 0.23 vs control 8.50 ± 0.27. The presence of different concentrations of PRRSV in boar semen induces changes in different types of sperm motility. Infection of ejaculates with the PRRS virus affects sperm motility on days 1, 3, 5, 7, and 10 post-infections.

Keywords

PRRSV, Boar Sperms, Velocity Pattern and Mobility Pattern

1. Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) has caused significant economic losses in the swine industry worldwide [1] [2]. Losses range from 75 thousand euros in a farm with a thousand sows with a "light" infection, to losses of 698 thousand euros [2] and 664 million dollars per year in the USA [2]) or 1.8 million dollars per day [3].

PRRSV causes significant economic losses to the farm, regardless of the pathogenicity of the strain, since a primary infection can cause losses from an outbreak and to this are added the losses due to the permanence of the disease in endemic form, with the possibility of re-emerging as an outbreak. acute after a long period of the last outbreak and are outbreaks that take 2 to 3 months to subside. Losses on the farm due to the endemic form are slight, but constant due to a decrease in fertility rates and weight gain, and increases costs by associating it with other respiratory diseases [4].

It is important to remember that PRRSV leaves serious economic losses on large or small farms, both in abortions and neonatal mortality, as well as in growing and fattening areas, and mostly causes many fertility problems that delay production. PRRSV is an RNA virus that exploits all known mechanisms of genetic variation to ensure its survival. Distinctive features of RNA virus replication include high mutation rates, high yields, and short replication times. As a consequence, RNA viruses replicate as complex and dynamic mutant swarms, called viral quasispecies, such that they are a major problem for swine production.

According to Meulenberg *et al.* [5] the causal agent is an RNA virus called PRRSV. This virus belongs to the order Nidovirus, family Arteriviridae and genus Arterivirus, [6] [7] [8]. The PRRS virus particle has a diameter between 50 and 65 nm, with a central isometric nucleocapsid approximately 30 to 35 nm in diameter [7]. The genome of the PRRS virus is 15 kb positive-strand RNA, containing seven open reading frames (ORFs).

PRRSV, being a small enveloped virus, has a positive-sense stranded RNA genome [4] [9], and is ~15 kb in length [3] [4]. It contains 11 open *reading frames* (ORFs), including ORF1a, ORF1b, ORF2a, ORF2b, ORF3, ORF4, ORF5a, ORF5, ORF6, ORF7, and a short trans frame (TF) ORF. *transframe*) in the *non-structural proteins* 2 (nsp2) region. These ORFs encode 16 *non-structural*

proteins (nsp1α, nsp1β, nsp2-nsp6, nsp7α, nsp7β, nsp8-nsp12, nsp2TF and nsp2N) and eight *structural proteins* (SP), which are: GP2, E, GP3-GP5, GP5a, M, and N [3].

The ORF 1a and 1b genes, located at the 5 end, occupy 80% of the genome and encode the replicase and proteins involved in the replication and transcription of the viral RNA. ORFs 2-5 encode GP2, GP3, GP4 and GP5, and ORF6 and ORF7 encode the M protein and the nucleocapsid (N), respectively [10].

In stallions, clinical manifestations include anorexia, lethargy and low libido [11] [12], consequently, there are alterations in semen quality, including a decrease in sperm motility, an increase in morphoanomalies. and increase in sperm with distal cytoplasmic droplets between days 35 and 42 post-infection [13], alterations in semen quality are directly correlated with fertility parameters [14].

The virus has been found in the seminiferous tubules, reaching the germ cells by migration of macrophages to the interstitial cells of the testis and by hematogenous dissemination [15]. Another route of PRRSV transmission is artificial insemination when semen from infected insemination centers is handled. Due to the above, the study is focused on evaluating the effect of PRRSV on the motility of Boar sperm, when infected with viral particles, because motility is an essential property of the cell and its alterations have been associated with problems of fertility [16] and [17]. Individuals with low sperm motility are considered infertile [17].

2. Material and Methods

The work was carried out in the Genetics and Reproduction laboratory of Facultad de Medicina Veterinaria and Zootecnia from the Benemérita Universidad Autónoma de Puebla.

2.1. Location

The semen was obtained from the "Porcina Santa Rosa" Insemination Laboratory located in the Town of Santa Rosa de Lima, Tecamachalco, Puebla, Mexico at 1998 meters above sea level at 18˚52'48 North Latitude and 097˚47'21 West Longitude.

2.2. Animals

Five stallions of 18 months of age on average (York-Shire, Duroc Jersey, Landrace, Berk-Shire and Berk-Shire-Duroc) were used. The semen was at a concentration of 4×10^9 sperm in 100 ml for each seminal dose. used a long-lasting diluent (MRA®). A semen and blood sample was taken from each stallion to confirm that they were negative for the PRRS Virus using a Real-Time PCR technique.

The concentrations used for each dose of semen were: 0, 1×10^2, 1×10^4, and 1×10^6 viral particles and each dose was tested in triplicate. Each of the doses

containing 1×10^6 spermatozoa, the PRRS Virus of the ATCC-VR-2332 strain was applied (0, 10^2, 10^4 and 10^6 copies of RNA/ml), they were observed daily and the parameters were recorded using the Analysis system. Computer-Assisted Sperm Analysis (CASA), Semen Analyzer (Hamilton Thorne®).

A 15 µL aliquot of control semen (no virus) and another 15 µL aliquot of PRRS virus-infected semen were placed on a slide, then the slide was placed on the preheated 37˚C stage of the CASA system. At least 200 sperm cells were quantified at 10x in triplicate in each preparation. Likewise, two aliquots of the same ejaculate of normal semen and semen infected with the PRRS virus (from the same sample) stored at 17˚ were evaluated. The concentration was 1 million sperm per sample. For analysis, 1×10^6 sperm were incubated in a 25-well microculture plate in the presence of different concentrations of PRRS virus of the ATCC-VR-2332 strain (0, 10^2, 10^4 and 10^6 RNA copies/ml). In practice, semen doses should be kept at 15˚C - 20˚C [18].

For its study, dilutions were made that would allow homogeneity in all samples and the response would be more authentic.

Table 1 shows the dilution form of the semen, to which the PRRSV particles were added in one million sperm.

Dilutions:

The concentration was 1 million sperm per sample.

PRRS Virus concentrations: Table 2 shows the way in which the PRRSV samples are distributed. In a 20-well microculture plate, 1×10^6 sperm were incubated in the presence of different concentrations of PRRS virus ATCC-VR-2332 strain (0, 10^2, 10^4 and 10^6 RNA copies/ml).

Table 1. Semen dilution.

Male	µL OF SEMEN	µL OF DILUENT
1	65	935
2	55	945
3	90	910
4	55	945
5	40	960

Table 2. Distribution of concentrations of Viral particles per sample.

A	B	C	D	E	N
0	0	0	0	0	3
10^2	10^2	10^2	10^2	10^2	3
10^4	10^4	10^4	10^4	10^4	3
10^6	10^6	10^6	10^6	10^6	3

2.3. Variables Evaluated

Velocity Pattern: VCL: Curvilinear Velocity, VSL: Rectilinear Velocity, VAP: Linear Velocity; Trajectory Pattern: LIN: Linearity (LIN = VSL/VCL × 100), STR: Straightness Index (STR = VSL/VAP × 100); Mobility Pattern: ALH: Lateral Displacement Amplitude, BCF: Beat Frequency, MOV: Cells with Movement, MP: Cells with Progressive Movement.

2.4. Statistic Analysis

Análisis Estadístico

Compliance with the assumptions of the analysis of variance (homogeneity of variances, independence and normality of the data graphically) was reviewed, finding that these assumptions are met... The changes in the different sperm motility parameters over the days and between the different virus concentrations were analyzed with a two-way ANOVA, in which the days were considered as blocks. Post hoc differences were analyzed with Tuckey. $P < 0.05$ was considered significant. The analysis was performed using R 9.8.2 software on a MacBook running Mac OS x 10.6.

3. Results

The semen was evaluated every 24 hours for ten days, taking as reference the Total Cells Observed, Virus Concentration and Days Post Infection. ($P \leq 0.05$) were observed regarding the number of cells with Total Mobility on the different evaluation days. 201 ± 7.3, 167 ± 10.1, 165 ± 14.6, 134 ± 8.2 and 120 ± 8.8 for days 1, 3, 5, 7 and 10, respectively.

At the same time, there was no ($P \geq 0.05$) between the control and virus concentrations with respect to the Progressive Mobility percentages.

Table 3 shows that the concentration of sperm cells decreases as the days post-infection increase.

In Table 4, it is observed that the Curvilinear Velocity on days 1 and 7 Post-Infection at the $10X^2$ and $10X^6$ concentration exists a significant difference ($P < 0.05$) compared to the control group.

In Table 5. It is observed that the Rectilinear Speed on day 1 Post-Infection at the concentrations $10X^2$, $10X^4$ and $10X^6$ is a significant difference ($P < 0.05$) compared to the control group.

Table 6. It is observed that the Linear Velocity on day 1 post-infection at the concentration $10X^2$, $10X^4$ and $10X^6$ there is a significant difference $P < 0.05$ compared to the control group

Table 7. The amplitude of Lateral Displacement shows that on days 5 and 7 Post-Infection at the concentration $10X^4$ and $10X^6$ there is a significant difference $P < 0.05$ compared to the control group.

Table 8. Progressive Motility shows that on day 1 and 3 Post-Infection at the concentration $10X^2$ and $10X^4$ there is a significant difference $P < 0.05$ compared to the control group.

Table 3. Total cells observed on different days Post-Infection.

DPI*	1	3	5	7	10
**TCÉL.OBSRV.	201 ± 7.3	167 ± 10.1	165 ± 14.6	134 ± 8.2	120 ± 8.8

*DPI = Days Post-Infection **TCEL. OBSERV. ¨ = Total Cells Observed.

Table 4. Curvilinear Velocity (VCL).

DPI	CONTROL	$10X^2$	$10X^4$	$10X^6$
1	193.04 ± 4.62	**162.81 ± 10.65****	187.88 ± 4.99	**177.12 ± 5.77****
3	173.91 ± 5.75	174.21 ± 5.47	172.92 ± 7.85	186.94 ± 5.76
5	162.04 ± 5.95	156.96 ± 7.10	165.59 ± 6.81	158.98 ± 10.75
7	144.22 ± 6.65	146.64 ± 8.07	153.03 ± 7.03	**161.07 ± 4.34***
10	132.15 ± 9.79	127.72 ± 12.69	130.96 ± 7.72	112.71 ± 8.90

*DPI = Days Post Infection.

Table 5. Straight Line Speed (VSL).

DPI	CONTROL	$10X^2$	$10X^4$	$10X^6$
1	56.66 ± 1.68	**48.00 ± 3.38*****	**49.88 ± 1.83****	**50.55 ± 2.24****
3	46.19 ± 2.27	47.56 ± 1.93	51.04 ± 2.67	50.54 ± 2.16
5	43.43 ± 2.20	46.48 ± 2.73	44.82 ± 1.64	41.02 ± 3.16
7	41.85 ± 1.73	40.45 ± 1.92	38.15 ± 1.83	44.25 ± 2.01
10	40.20 ± 3.23	38.86 ± 3.18	37.62 ± 1.97	36.53 ± 1.63

*DPI = Days Post Infection.

Table 6. Linear Velocity (VAP).

DPI	CONTROL	$10X^2$	$10X^4$	$10X^6$
1	90.56 ± 2.07	**77.26 ± 5.16*****	**83.35 ± 2.41****	**81.29 ± 3.14****
3	77.05 ± 3.28	78.84 ± 2.91	81.94 ± 3.26	84.47 ± 3.26
5	71.80 ± 3.03	73.01 ± 3.76	73.68 ± 3.07	69.96 ± 4.84
7	65.92 ± 2.72	65.90 ± 3.41	64.96 ± 2.61	72.88 ± 2.50
10	61.96 ± 5.21	60.17 ± 5.74	59.26 ± 3.42	53.72 ± 3.22

*DPI = Days Post Infection.

Table 7. Amplitude of Lateral Displacement (ALH).

DPI	CONTROL	$10X^2$	$10X^4$	$10X^6$
1	10.34 ± 0.16	9.69 ± 0.74	9.98 ± 0.16	9.91 ± 0.24
3	9.57 ± 0.25	9.60 ± 0.25	14.97 ± 5.16	9.71 ± 0.17
5	9.33 ± 0.24	8.66 ± 0.36	9.13 ± 0.31	**7.51 ± 0.85***
7	8.50 ± 0.27	9.15 ± 0.39	**8.70 ± 0.26***	**9.64 ± 0.23***
10	8.32 ± 0.37	8.48 ± 0.37	7.93 ± 0.33	7.48 ± 0.40

*DPI = Days Post Infection.

Table 8. Progressive Motility (PM).

DPI	CONTROL	$10X^2$	$10X^4$	$10X^6$
1	34.73 ± 1.49	30.93 ± 3.25	**29.06 ± 2.04****	31.80 ± 2.00
3	31.85 ± 2.41	**24.46 ± 2.57****	33.86 ± 1.92	32.60 ± 1.26
5	28.40 ± 3.10	27.53 ± 2.12	25.07 ± 2.32	24.60 ± 3.23
7	28.14 ± 2.47	29.00 ± 2.09	26.42 ± 2.88	23.92 ± 2.92
10	15.33 ± 2.73	20.77 ± 3.75	22.91 ± 2.87	23.00 ± 3.39

*DPI = Days Post Infection.

Figure 1 shows the relationship between STR and ALH, which the heads carry out in their curvilinear path from one side to the other of their average linear path. We observe that there is a correlation between VCL and STR (%) is the percentage relationship between VSL and VAP × 100. In the same way, there is a correlation between VAP and STR (%) It is the percentage relationship between the VSL VAP × 100 and there is a correlation between STR with LIN VSL and VCL × 100).

In Figure 2, photographs of PRRSV are shown, indicating that this virus is only found in the fluid (semen) without adhering to the sperm as seen in the photographs presented below.

The arrows point to the PRRSV viruses, the boar sperm are free of the virus and are observed in the evaluated semen.

4. Discussion

The presence of PRRSV has been detected in different organs of naturally infected pigs; However, so far they have not been detected in sperm [15]. Something similar happens when the disease is induced and viruses have only been found in semen.

Likewise, the movement speeds of these cells (VSL, VAP and VCL) are analyzed, which are motility parameters that serve as indicators of the male's fertility; spermatozoa with a high VSL are more likely to contact the oocyte, while those with more VCL than VSL are associated with low levels of fertilization [19] [20]. However, in this study it was found that there is a significant difference ($P \leq 0.01$) in the Curvilinear Velociy in the concentrations (10^2) 162.81 ± 10.65 and (10^6) 177.12 ± 5.77 where this parameter is lower than the control 193.04 ± 4.62; These results corroborate what mentions; [19] [20]; which indicates that when these parameters are affected it would be related to fertility and the PRRS virus affects curvilinear speed. Regarding Straight Line Velocity (VSL), it was observed that sperm infected with viruses at a concentration of 10^2, 10^4 and 10^6 had significant differences when compared to the control group with parameters of 48.00 ± 3.38, 49.88 ± 1.83 and 50.55 ± 2.24 Vs. 56.66 ± 1.68 respectively. Which is confirmed with what was mentioned (Liu *et al.*, 1991), this means that the control or non-infected group would have greater possibilities of fertilizing the oocyte. In this study, it was found that there is a significant difference ($P \leq 0.01$)

in the Linear Velocity (VAP) in the concentrations (10^2) 77.26 ± 5.16, (10^4) 83.35 ± 2.41 and (10^6) 81.29 ± 3.14 this parameter is less than the control 90.56 ± 2.07; These results confirm what is mentioned [19].

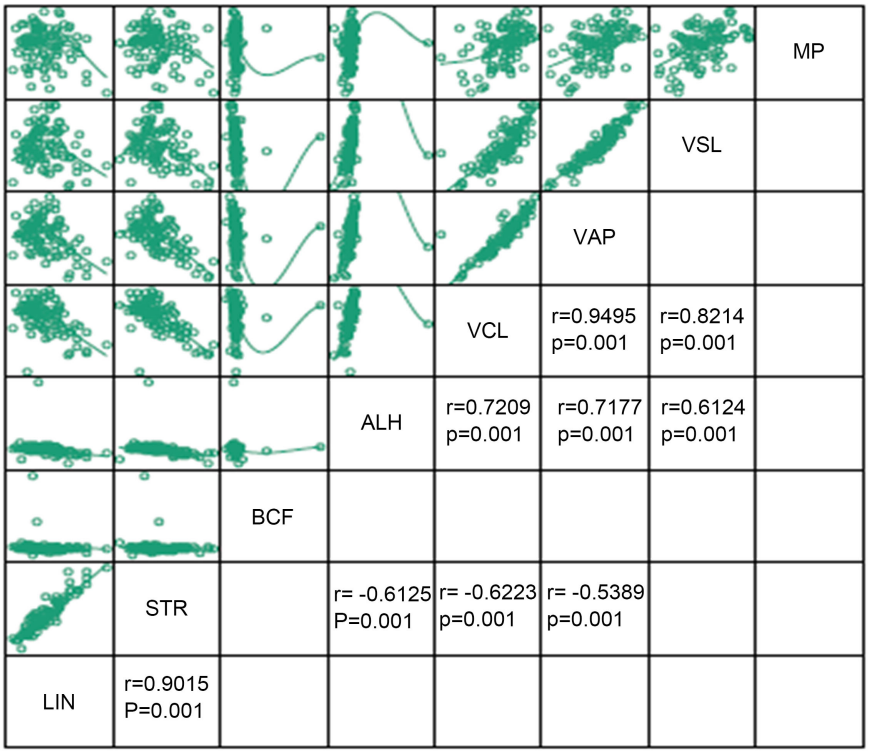

Figure 1. Matriz de Correlaciones de los Parámetros de Movilidad Espermática.

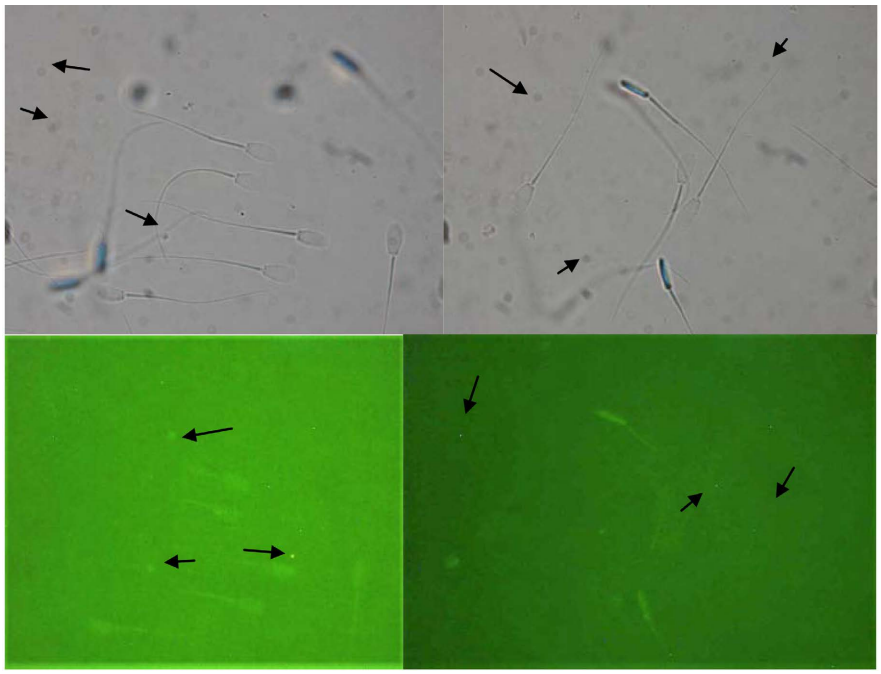

Figure 2. Microscopic photographs and/or fluorescence microscopy of the Pig Reproductive and Respiratory Syndrome Virus (PRRSV) in boar semen.

Shanis *et al.* [21] suggest that both BCF and ALH are of great importance in penetration into the oocyte. These authors observe that in cervical mucus, ALH decreases while BCF increases to help the penetration of the sperm cell into said mucus. With respect to the Amplitude of Lateral Displacement (ALH), a significant difference is observed at the concentration (10^4) 8.70 ± 0.26 and (10^6) 9.64 ± 0.23 with respect to the control group 8.50 ± 0.27 with a $P < 0.05$. This is corroborated by what Shanis *et al.* mention. [21]. Regarding Beat Frequency (BCF), no significant difference was found.

Different types of sperm movement have been related to fertility [22]. Sperm that move progressively are those that have the ability to move faster and have a greater amplitude of the flagellum; They are also sperm that can potentially fertilize the oocyte [17].

Traditionally, the assessment of sperm movement has been carried out subjectively with the help of a microscope. Some authors such as Grunert *et al.* [23] have reported that the use of the CASA system does not present any advantage over manual techniques. However, obtaining precise data based on sperm movement allows us to describe the physiological aspects of the sperm found in an ejaculate [24] [25].

The results show that the presence of the PRRS virus in semen modifies total mobility. These observations coincide with those of Swenson *et al.*, [26], who evaluated total mobility subjectively, before and after infecting the stallion. The results show that the PRRS virus affects the different types of sperm motility analyzed with the CASA system.

Based on the correlation matrix obtained, it is observed that Total Mobility is correlated with Progressive Mobility, (VAP) and VCL, M P, VSL and VCL, in ejaculates from Hampshire-Duroc, Landrace, Duroc, Yorkshire and Landrace pigs. terminal line evaluated in the CASA Minitube Sperm Vision System® system. However, the results obtained do not show any correlation of rectilinear speed with respect to MT, MP, VCL, VSL, ALH, BCF, STR, LIN; these differences can be explained by the management of the concentration used (5×10^7 sperm/mL) and even the pig breeds used by these authors, which suggests that when using a CASA System, there is a consistency of the results obtained, using different breeds. The confusion phenomenon appears when the association observed between the study factor (sperm) and the PRRSV effect (disease) can be totally or partially explained by the known variables (Motion Pattern and Speed Pattern) that would be the (confusion factor), in this way by including the variables under study that were carried out during the test, it allows explaining the relationship that exists between the sperm, the PRRSV and the Speed and Movement Patterns, eliminating to a certain extent the phenomenon of confusión

The results obtained from the VSL, ALH and BCF, in the work, are similar to those reported by Abaigar *et al.*, [27], in ejaculates from Landrace and Large White pigs evaluated in a Hobson Sperm Traker® analyzer; However, the Curvilinear Velocity is higher and differs from our results. This difference could be

explained by the concentration used (20×10^6 sperm/mL) and the number of sperm evaluated (100 cells).

In the study carried out by Hirai *et al.*, [22], the reported values of MT, VAP and LIN in ejaculates from the Pietrain pig breed evaluated in a Mika Motion Analyzer® are higher and differ from our results, these differences. They may be due to the type of analyzer used and the management of the concentration (50×10^6/mL) used. In our study, a concentration of 1×10^6 sperm/mL was used. Likewise, the study by O'Conell *et al.* [28], carried out on human ejaculates and evaluated in a Hamilton Thorne Research CASA system and which is similar to the one used in this study, shows that the values reported for MT, MP, VAP, VSL, VCL, are slightly higher than those reported in this study.

The differences between the values derived from a CASA system are due to the configurations of the CASA system and the model used [29]. A concentration of 100 and 50×10^6 sperm/mL leads to errors in the area average, concentrations of 30 and 20×10^6 sperm/mL differ significantly in high VAP and VCL and low BCF, SRT and LIN with respect to concentrations of 10 and 5×10^6 sperm/mL. mL and finally the concentration of 5×10^6 sperm/mL differs in low ALH and BCF from the concentration of 10×10^6.

The speeds of movement of these VSL, VAP and VCL cells are motility parameters that serve as indicators of the male's fertility; Sperm with a high VSL are more likely to contact the oocyte, while those with more VCL than VSL are associated with low levels of fertilization [16] [17].

BCF and ALH evaluations are also indicative of the existence of disorders in sperm movement parameters. In fact, a low BCF indicates the "lazy" nature of the sperm cell and reaffirms its inability to survive for a long period of time. These spermatozoa present a very stable movement, with very few variations, due to intracellular disorders in the internal processes of energy production and transduction.

5. Conclusions

1) Computer-Assisted Sperm Analysis indicated that there is a relationship between the different types of sperm motility, resulting in the addition of PRRS Virus at different concentrations in uninfected porcine semen, which induces changes in the Velocity and Pattern Pattern. Movement of sperm

2) Infection of ejaculates with the PRRS virus does affect sperm motility on days 1, 3, 5, 7, and 10 post-infections.

3) The Correlation Matrix showed that there were positive correlations with respect to the different parameters of the CASA System, indicating that when these sperm mobility indices are altered (VCL, VSL, VAP) they suggest problems in the Velocity and Mobility patterns, as observed during sperm exposure to PPRSV in the post-infection days, in a research laboratory.

4) In the Fluorescence microscope photographs, the Pig Reproductive and Respiratory Syndrome Virus is observed in the seminal fluid, which indicates that the Virus does not adhere to the boar sperm during infection.

Suggestions

1) Continue the study with PRRSV-infected semen in females in heat through Artificial Insemination (complex and expensive).

2) Measure symptoms of the disease in females and fertility, as well as; abortions, mummies, live births, stillborns, and neonatal death, among other parameters.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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