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Evaluation of the Spermate Quality of Cattle Players Through the use of Conventional Seminal Evaluation Systems and Casa System

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***Abstract*— The objective of this study was to compare the results of some parameters of seminal quality of bulls obtained by the Conventional method and also by the computerized CASA system, other objective was to determinate if there is any difference in seminal quality between Jersey, Holstein and Brown Swiss bulls. Semen samples were collected from 5 bulls and were analyzed by the conventional method which parameters are: Individual motility (%), Mass motility (1-5), Neubauer chamber concentration (106 spermatozoa/ml) and individual post-freezing motility (%). The same samples were analyzed by CASA method, which variables are: Total motility (%), Progressive motility (%), concentration (106 spermatozoa/ml) and total post-freezing motility (%). Results obtained by each breed of bulls in these parameters were compared using a Duncan multiple range test to assess statistical differences. The result was that Jersey bull's semen had the higher counts for most o the variables compared with the other two. The entire population of results by the Conventional method was also compared against the entire population of results by CASA, in those parameters that are equivalent between the two methods: motility (individual vs total), concentration (Neubabuer vs CASA) and post-freezing motility. This comparison was performed with a Student test that proved there were differences between the two methods in all the studied parameters. In the case of motility, the conventional method showed an average 79% against the 75.56% of the CASA system. In concentration, the conventional method had a result of 762.83 million spermatozoa/ml which was inferior that the 819.3 millions spermatozoa/ml of the CASA method. Finally, about the post-freezing motility the conventional method had the highest result with 65.36% against the 59.64% of CASA. The conclusion was that the numeric results of spermatic quality are different depending on which method is used.**

Keywords*— *Semen, CASA Evaluation, Conventional Evaluation, Motility, ConcentratioN.

I. INTRODUCTION

The analysis of seminal quality represents a valuable tool to evaluate the fertilizing potential of males of different species and complements the physical assessment of the animal (Montes, Torres, Rugeles, Almanza, & Guimarães, 2012). The seminal analysis or spermiogram includes a series of tests that evaluate various factors or functions of the sperm cell. (Quintero, 2003)

According to (Muiño, et al., 2005), among the disadvantages of the classical techniques used to evaluate the seminal quality are the subjectivity of the same, since the results depend in large part on the expertise of the evaluator. When semen is evaluated, seminal quality is studied, which is determined by the comparison of the parameters obtained with the values that are considered normal for an adult reproductive bull. (Vera Muñoz, 2008)

For several decades, many researchers have devoted much work and resources to try to eliminate the subjectivity inherent in the microscopic evaluation of semen quality. Fruit of these investigations was the development of computerized systems for the analysis of sperm motility. (Cuberlo & Rodríguez, 2013). The development of new technologies for the objective evaluation of sperm, generically called CASA (Computer - Assisted Sperm Analysis), has enabled the rapid and reliable assessment of both the kinetic and morphometric variables of the sperm. (Mailbox, 2013)

The analysis of computer-aided semen (CASA) has allowed an objective measurement of many parameters of semen mobility and morphology, offering more reliable, impartial and repeatable measurements with respect to visual examination (Magistrini et al., 1996; Colenbrander et al. 2003, Graham & Mocé, 2005)

Many researchers, specialists in animal reproduction are trying to design the "ideal seminal analysis", which adequately assess and predict the fertility of a seminal sample in the most accurate way possible. Thus, the analysis of ideal semen would be one that in a simple and effective way would allow to know in a predictive way the fecundating capacity of an ejaculate. (Mellisho, 2010)

In the following work the following objectives are proposed:

1. Compare data on seminal quality values by breed using the conventional evaluation system.

2. Compare data on seminal quality values by race using the Computer Aided Seminal Analysis (CASA) system.

3. Determine if there are differences between the values of the seminal quality determined by means of the conventional method and with the CASA system

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II. MATERIALS and METHODS

Materials – Study Area.

The research was carried out in the National Center of Production and Genetic Improvement "El Rosario", located in Tambillo, canton Mejía, province of Pichincha, belonging to the Ministry of Agriculture, Livestock, Aquaculture and Fisheries. Located at 3000 masl, with a cold temperate climate and an average temperature of 10º C. Samples will be collected, evaluated and processed in the Genetic Material Processing Laboratory of the aforementioned plant.

We selected 5 breeding bulls housed in the Central, distributed in the following breeds: 2 Holstein Friesian bulls, 1 Jersey bull and 2 Brown Swiss bulls, aged between 18 and 36 months.

For the collection of semen, the method of the artificial vagina was used, from each player 11 different ejaculates will be evaluated, the same ones that will be collected once a week.

Variables analyzed for

• Total motility

• Progressive motility

• Motionless

• Sperm concentration (CASA and Neubauer)

Experimental design and statistical tests

The data obtained were analyzed using the statistical program SPSS 22, according to a Completely Random Design. A Duncan media separation test was performed to determine significant differences in the seminal parameters between the three races included in this study.

Additionally, the measurements obtained through the conventional system and the CASA system of the variables under study were compared using the Student's means comparison test. A total of three comparisons were made: Individual (conventional) Motility vs. Total Motility (CASA), Neubauer (Conventional) vs. Concentration (CASA), and Post-Freeze Motility equally between both methods.

II. RESULTS AND DISCUSSION

Seed quality values by breed using the conventional evaluation system.

Individual Motility

When evaluating the individual motility percentage (MI), the Jersey race presented higher MI with an average 86.81% while for Holstein and Brown Swiss their percentages are lower 80.68% and 73.41% respectively; values that are lower than those obtained by (Moncayo, 2016) in which it varies between 85% and 96% for the Holstein and Brown Swiss races; in this sense Rubio et al. (2007) independently of the bovine race obtain MI of 62.91%; however, (Vera Muñoz, 2008) qualifies ejaculates with MI above 80% as very good corresponding to the values obtained for Holstein and Jersey and a Good rating for ejaculates with MI of 70 - 80% corresponding to Brown Swiss. The data obtained are within the minimum requirement for the classification of a bull as potentially satisfactory in the reproductive evaluation proposed by the International Society of Teriogenology, this is mentioned (Paez & Corredor, 2014)

Concentration - Neubauer

The sperm concentration obtained by the method of counting with the neubauer chamber presented a higher concentration for the Jersey race with an average of 1036.77 ± 3.22 x 106 spz / ml, followed by Holstein with an average of 816.20 ± 3.05 x 106 spz / ml and a lower concentration for Brown Swiss with an average of 572.41 ± 2.35 x 106 spz / ml, which agrees with the study carried out by (Moncayo, 2016), where it mentions that the Holstein breed showed a higher sperm concentration with an average of 699, 3 x 106 spz / ml and the Brown Swiss breed showed lower concentration among the breeds under study with an average of 391.7 x 106 spz / ml, this study related it to the results of (Medina, Sanchez, Velasco, & Cruz, 2007), in which they reported an average sperm concentration of 434.5 ± 41.6 x 106 spz / ml. On the other hand (Kumar U., et al., 2015) in the evaluation of semen quality in pure bulls and mestizos of jersey race, as a result obtains that, the average concentration of spermatozoa in the pure and crossed Jersey bulls was 1171.01 ± 56.09 and 1093.488 ± 48.25 million / ml.

According to (Palma, 2009), an ejaculate is considered good if it contains a number greater than 800 x 106 sperm per milliliter, on the contrary an ejaculate is bad when the number of sperm per milliliter is below 500 x 106. In the same way affirms Porras (2009) mentioning in his manual that, an ejaculate is considered of very good concentration to contain between 750 and 1000 x 106 sperm per milliliter and with a regular rating to contain between 250 and 400 x 106 sperm per milliliter .

(Palma, 2010), mentions that there is a considerable error in the determination of sperm concentration by means of the Neubauer chamber, since this varies in direct relation to the reduction of the surface considered in the count, when using uncalibrated coverslips, In addition, the error increases with the concentration of the sample (ejaculated 7 to 10%). To this they agree (Rodríguez, Franco, & Jiménez), where they mention that the variability of the results can be from 7.1 to 12%, however, it is a technique that requires experience to perform it properly and takes around 15 minutes per sample. (Palma, 2010), in his study also mentions that the variation could be significantly lower (Coefficient of variation, CV = 12.3), indicating that the accuracy of the determination varies with laboratories and personnel.

Table 1. Comparison of seminal parameters of the conventional method among three breeder breeds.

Raza	Motilidad individual (%)	Neubauer (10^6/ml)	Motilidad Postcongelación (%)
Jersey	86.81 ± 1.81Ω [a]	1036.77± 3.22 [a]	73.18 ± 2.36 [a]
Holstein	80.68 ± 1.11 [b]	816.20 ± 3.05 [b]	67.72 ± 1.53 [a]
Brown Swiss	73.41 ± 1.06 [c]	572.41 ± 2.35 [c]	59.09 ± 1.82 [b]

[abc] Medias con letras diferentes son estadísticamente diferentes
Ω Valores corresponden a media ± Erros estándar (n=22, para Jersey n=11)

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Motility Post freezing

In the case of Post-Freeze Motility Jersey it obtained the highest results 73.18%, which are statistically equal to those of Holstein 67.72%, both being better than Brown Swiss 59.09%.

In a study carried out by (Kumar U., et al., 2015) post-freezing motility is 46.11%, being lower than those obtained in this study, likewise, Cabrera and Pantoja (2012) in their investigation of sperm viability and acrosome integrity in frozen semen from national bulls they show values lower than 63%; while Madrid-Bury (2005) obtained greater responses to this study 76.7% - 85.5%. The lost percentage of post-freezing motility is minimal and they are within the parameters established by the Central to qualify as viable a lot.

Masal Motility

Table 2. Contingency table with the distribution of results of Masal Motility, parameter of the conventional method.

Raza	Motilidad masal (1 – 5)			
	2	3	4	5
Brown Swiss	0	13	9	0
Holstein	3	4	3	12
Jersey	0	0	6	5

The results of the measurements of mass motility are observed, which is measured on a scale of 1 to 5. None of the observations obtained a result of 1, and the only three observations that were assigned a value of 2, belong to Holstein. An exact Fisher test was carried out which resulted in a value of 32.3, which indicates with a degree of significance of> 0.001 that there was a relationship between the Race variable and the variable Mass motility, in short if there are statistical differences between the races; These resulting values are similar to those revealed by Muiño (2008).

These characteristics are within the parameters established for bulls in reproductive activity according to (Palma, 2011); In this regard Mellisho (2010) mention that for the evaluation of fresh semen the best indicator is the MM.

Seed quality values by breed using the CASA computerized evaluation system

Table 3. Comparison of seminal parameters of the CASA method among three breeder breeds.

Raza	Concentración (10^6/ml)	Motilidad total (%)	Motilidad progresiva (%)	Inmóviles (%)	Motilidad Postcongelación (%)
Jersey	1237.23±23.04$^{\Omega a}$	77.03± 2.2 ^{a}	70.23 ± 2.66 ^{a}	20.22 ± 0.95 ^{b}	60.43 ± 2.23 ^{ab}
Holstein	820.67±13.19 ^{b}	79.50 ± 1.99 ^{a}	72.53 ± 1.80 ^{a}	20.39 ± 0.99 ^{b}	63.24 ± 1.54 ^{a}
Brown Swiss	608.98±15.12 ^{c}	70.88 ± 1.62 ^{b}	63.20 ± 1.62 ^{b}	28.21 ± 1.20 ^{a}	55.65 ± 1.54 ^{b}

When a CASA system is used, it must be taken into account that the results it provides are only applicable and repeatable in another seminal analysis laboratory, as long as the same equipment is used.

There is a comparison between races of the parameters of the CASA method; in this respect for the parameters Concentration, Total Motility, Progressive Motility and Post-freezing Motility the Jersey race was the one that obtained the highest values, in some cases: total motility, progressive motility and post-freezing, there were no differences between Jersey and Holstein.

The only parameter in which Brown Swiss had statistically higher values was in the Immobiles.

Comparison of means between the conventional method and the CASA method.

Table 4. Comparison of means between analogous parameters of the conventional method and the CASA method.

Parámetro	Resultado CASA	Resultado Convencional
Motilidad (%)	75.56 ± 1.25$^{\Omega*}$	79.00 ± 0.98
Concentración (10^6/ml)	819.31 ± 14.06*	762.83 ± 19.8
Motilidad Post congelación (%)	59.64 ± 1.07*	65.36 ± 1.28

* Indicates significant difference, determined by Student's Test

Ω Values correspond to mean ± Standard error (n = 22, for Jersey n = 11)

In the case of motility, the analogous parameters were compared Individual Motility (Conventional) and Total Motility (CASA); for concentration were compared: Neubauer (conventional) with CASA concentration, and for post-freezing motility the comparison between the two homonymous variables of the two methods was made. The T values obtained in one of these comparisons were: 2.16, 2.08 and 3.42 respectively, all of which exceed the tabular T value, determining that there were differences in all cases with a significance of 0.033, 0.033 and 0.001 respectively.

Guzmán (2013) in a similar investigation, shows the statistical correlation between both methods, obtaining numerical differences, for the concentration of 0.866 and for the motility of 0.420, statistically does not obtain significant differences between the seminal characteristics.

(Agüero, 2012), in his study shows that he does not obtain differences between the individual motility measured through routine tests (57.1 + 5.6%) and the individual motility measured through the CASA system (58.2 + 7 , 0%), obtaining a high correlation between both methods (r = 0.60, P≤0.008), indicating that the latter method is an instrument that can be used in the routine evaluation of fresh semen in the laboratory.

Traditional techniques of evaluation of sperm motility, although they are common, fast and practical, present a subjective character that implies a great variation in laboratories that ranges from 30 to 60%. This may be due to the fact that they are subject to different interpretations that depend to a great extent on the operator's experience, which causes a human

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error, which plays a definitive role in the selection of reproducers Rodríguez and Martínez (2003). The different CASA instruments have demonstrated high levels of accuracy and reliability using different sperm classification methodologies (Verstegen, Iguer-Ouada, & Onclin, 2002)

Jouannet, P and collaborators (1976), mention that, motility is mainly determined by microscopic observation. Subjectivity, lack of precision and variability of results are important disadvantages. The main problem is related to the standardization and optimization of equipment and procedures.

III. CONCLUSION

On the results obtained in this research and when assessing the seminal quality in three races, it is concluded that both the values of the seminal quality evaluated by the conventional and computerized system show differences between breeds, obtaining high values for the Jersey breed, however the data thrown for each parameter evaluated are within the viable seminal ranges that are cited by several authors.

The conclusions derived in the determination of differences between the values of the seminal quality by means of the conventional method and with the CASA system, there are differences in all the cases, in this effect it can be mentioned that the systems seminal analysis assisted by computer provide measurements more reliable, impartial and repeatable, compared to the conventional exam.

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