Study Conception Rate and its Relationship with Motility Morphology Sperm and Holstein Dairy Cows to Pasture Systems

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ABSTRACT---- ***The objective was to analyze the results of fertility and relate morphology and number of sperm of bulls used in artificial insemination in grazing systems. 12780 backdate services were evaluated with 43 bulls to heifers and cows made from three dairy farms located in the province of Entre Rios - Argentina during the period 2011-2014. The variables evaluated were: sperm morphology, motile spermatozoa per dose, conception rate and number of services per pregnancy. The differences with statistical significance (P ≤ 0.05) were observed in the number of services that were needed to achieve pregnancy and conception rate. The variables taking into account the time of year when services are performed emerged that at the time from the beginning of autumn until spring, the amount of services required by cows and heifers was significantly lower (p ≤ 0.05) and conception rate also increased significantly (p≤0,5) if these variables with the results obtained during the summer are compared. It is concluded that conception rate is not related to the morphology and sperm motility in Holstein dairy cows in systems grazing, however if the time of year in which the services were performed, being less beneficial to service in the months where the ambient temperature he begins to rise.***

Keywords---- dairy cow, genotipos, conception rate, sperm morphology, sperm motility, grazing system.'

1. INTRODUCTION

Sperm morphology is an important indicator of fertility since knowing the relevance of each type of morphological abnormality and the frequency in which it is present in semen, will allow the estimation of its pregnancy capacity (18, 22, 23, 26). Frozen sperm is not a uniform product, this is due to the fact that – on one side – there is a wide range between the minimum values required to set out a consignment of frozen sperm into the market and high quality semen (5). The ISO 9002 standard for Artificial Insemination Centers set up that the inseminating doses must have at least 8 million spermatozoids with progressive motility. That number can be reduced to 6 million provided that the semen has more than 30% of spermatozoids with progressive motility and if the abnormality rate is equivalent to that obtained with 8 million. In 1998, Saacke and col., concluded that when the sperm morphology is within the acceptable values for the specie, the morphology of the sample does not provide enough evidence to judge the expected level of the semen fertility after the artificial insemination. This is independent if the valuation is subjective or a computerized system is used since a high number of spermatozoids per dose is able to compensate –at least partially – a high percentage of morphological abnormalities (24). There are antecedents which indicate that the loss of reproductive efficiency is associated with several variation sources. Besides the association with the increase in milk production (6, 16, 19), the reproductive indicators show an important seasonal variation, being the coldest seasons in general the most favourable (7, 12, 15). The lactation number is also a known factor which affects the reproductive indicators (27, 29). Another source of variation in the reproductive indicators is the type of service used (7, 13, 14, 17, 28). Fertility problems in milking rodeos

have several origins: environmental, from the animal itself (male and / or female), transport and conservation of semen, technique and inseminator.

The objective of this retrospective work was to analyze the results of fertility and relate them with the morphology and number of spermatozoids of bulls used in artificial insemination in pasture systems.

2. MATERIALS AND METHODS

Retrospective reproductive data was analyzed from 12780 services with 43 bulls in heifers and cows belonging to 3 milking premises located in the province of Entre Ríos (32º 00´ South and 59° 34´ West) during the period 2011-2014. The services were carried out in different times of the year, being the average summer temperature 23°C (maximum 37°C – minimum 10°C) and the average winter temperature 12ºC (maximum 18°C – minimum 5°C).

The analyzed dairy farms had an average of 120 Holstein milking cows. Their typical diet during the period under consideration was based on different seasonal provisions: direct alfalfa-based pasture, hay from alfalfa-based meadows, commercial concentration during the milking period (of 13 or 16 % of the PB according to nutritional requirements) and a portion in a mixture delivered by a rationing cart outside the farm which was made with whole maize, ground maize, wheat bran, soya expeller, mineral salts, powdered diatoms and liquid milk serum (a sub-product of the cheese factory from the same premises). In a particular year there were other items in the diet such as: citrus pulp, barley grains and summer or winter grazing which were not considered relevant in order to be taken into account for this work. The retrospective data come from periodic controls carried out by the technical veterinarian consultant responsible for the farms. The cows were inseminated with tested adult American – Canadian Holstein bulls from different Insemination Centres.

In the three systems under study, the variables assessed were:

1) Sperm morphology: quantity of spermatozoids with normal morphology out of a total of 200 cells, the result was expressed in percentage (%MN). It was assessed through an optical microscope (100X) with 30-minute previous Giemsa stain.

2) Motile spermatozoids per dose: quantity of sperm with motility per sample expressed in million (mill mov/ doses). This parameter was calculated on a Neubauer chamber, using 400X and keeping the sample at a temperature of 37°C.

3) Conception rate: expressed in percentage (% oestrus detection x % of pregnancy)

4) Number of services per pregnancy (NS / pregnancy)

In order to describe the variables mentioned above, the average and error standard was used. Several tests were used in order to see whether there were differences between medians: *t*-student test, the Tukey-Kramer HSD multiple test and the Wilcoxon test according to each group of datum under analysis. For the variables number of service per pregnancy and conception rate, the distribution of frequency was analyzed, the median was calculated and the comparison between groups was assessed through a median comparison test. Differences became statistically significant with $P<0,05$.

3. RESULTS

Table 1 shows the average and error standard estimated for each variable under analysis and according to the reproductive category of the animals (heifers and cows). The statistically significant differences are observed in the number of services that were necessary in order to get pregnancy and in the conception rate. Figure 1 shows the distribution of frequency for these two variables.

As regards the analysis of the variables considering the time of the year when the services are done, it came up that in the period between the beginning of Autumn and Spring, the quantity of services required per cows and heifers was significantly lower and the conception rate increased also significantly when compared with the results obtained during the Summer (Table 2 and 3)

4. DISCUSSION

The current high performance milking cow has been biologically set up for the market demands through the application of a selective, continuous, non-balanced and careless pressure, which has placed the animal close to its vital potential. It is becoming more and more difficult to provide the animals with a non-limiting environment, being that almost impossible to achieve during their initial milking stage. The considerable increase in the productive performance

and the size of modern high production cows has been possible because of a repeated and asymmetric use of a selection exclusively based on milk production. Even though this process has been accompanied by modifications in the nutritional environment, these have not been enough to avoid the decline of vital functions such as reproduction and survival (2). Scientific literature related to fertility of milking cows reveal a tendency to decrease the conception rate in US rodeos and in other parts of the world. The conception rate in milking cows has decreased from 66% in 1951, to 50% in 1975, to 40 % in 1997 (10). The infertility of milking cows during first lactation keeps being a critical problem which limits the profitability and sustainability of farms (20). In a work by Capitaine (2005) the annual average conception rate was 36 % in 250 dairy farms analyzed, in our work the conception rate was lower (33%) and higher for heifers (53.6%), being the latter lower than the 70 % mentioned by (10). This proves that the conception rate is higher in heifers than in cows, since fertility is directly related to the level of production. As production increases, the conception rate decreases and this is shown in every lactation and in every time of the year. As regards fertility during lactation, this is higher as high-production cows are closer to labour, and such production increases importantly as the cow recovers itself, certainly recovering from the requirements that high production demands. In Low-production cows there is not an important change as lactation goes on and just 40 days after labour they have high fertility. These relations were reported in a smaller group of cows and during a shorter time (4). The semen doses used in this work did not generally fulfilled with the required quality standards, considering quality in a dose: a concentration of at least thirty million total spermatozoids with a 30 % of progressive motility after de-frozen, or in different terms, a total of 9 to 10 million progressive motile spermatozoids. However, the results found coincide with those obtained by other authors, who –when inseminating with doses containing 6, 10 and 12 million of spermatozoids with progressive motility – did not find significant differences in the percentage of pregnancy at IATF (8) *Fixed Time Artificial Insemination*. There are other investigations, which showed that the number of motile cells - and therefore lineality, are parameters which can be used in order to predict the fertility of a bull, since it was found a significant correlation between the percentage of progressive motile cells and the percentage of fertilization (1).

On the other side, it is known that the stress produced by the heat impacts negatively on all the aspects related to milk production; this problem has been discussed for decades and even today the scientific community is still trying to find a way to diminish the impact that the high temperatures have on the conception rate, an undeniable parameter when analyzing milk production (25). The decrease in milk production and the reproductive losses during the Summer impact substantially on the economic potential of the dairy farms (11, 21). The Holstein cows were developed in warm climates, therefore, they are more productive with temperatures between 10 and 21°C. It is observed that the cows keep their percentage of pregnancy (35%) during Autumn, Winter, Spring; moreover, they increase it if compared with the average obtained regardless the season (33%), and drastically decrease it in Summer (21%). The number of mobile spermatozoids per dose is kept between 6 and 8 million regardless the season. The results found for heifers show the same trend: a good pregnancy rate during Autumn, Winter and Spring (66%), closer to the one cited by Fricke (2003), and a decrease in Summer (33%). It is noteworthy that the results obtained during Autumn, Winter and Spring, were achieved with a number of 4 million motile spermatozoids per dose, without affecting the pregnancy rate. These results of pregnancy rate coincide with that presented by Capitaine (2005) where conception through the year varies, high fertility starting by April, keeping it stable throughout October – November and then decreasing as temperatures increase. The decrease in the conception rate during the months with the highest average temperatures was also described when studying the reproductive behaviour of 23 dairy farms on pasture systems in the province of Buenos Aires, Argentina. In this case, the rate was about 5 points in Spring (October – November) and 2 % was added to that when analyzing the Summer (January – February) (9). This fact needs to be taken into consideration at the moment of defining a reproductive programme, that is to say a milk production system, since labours in Spring are due to services carried out during the Summer, when fertility is reduced and pregnancy is difficult to achieve.

5. CONCLUSION

It's concluded that conception rate is not related to the morphology and sperm motility in Holstein dairy cows in systems grazing, however if the time of year in which the services were performed, being less beneficial to service in the months where the ambient temperature he begins to rise.

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Table 1. Average and standard error of the variables analyzed by category of females

Animal Catgory	Number of bulls	Sperm morphology	Motile sperm per dose	Number of services per pregnancy	Conception rate
Cows	37	88,7 ± 7,6 a	7,1 ± 2,8 a	3 (2-8) a	33,0 ± 7, 3 a
Heifers	6	91,5 ± 4,3 a	6,2 ± 2,5 a	2 (1-3) b	55,0 ± 18,0 b

Different letters indicate significant differences column; P <0.05

Table 2. Average and standard error of the morphology and sperm motility, number of doses per pregnancy and conception rate per season in which the service was performed. Reproductive Category: Cows

Season	Sperm morphology	Motile sperm per dose	Number of services per pregnancy	Conception rate
A-W-S	90,55 ± 1,22 a	6,92 ± 0,49 a	2,87 ± 0,07 a	35,46 ± 0,96 a
S	84,14 ± 2,82 b	6,98 ± 1,27 a	4,61 ± 0,67 b	24,28 ± 3,05 b

Different letters indicate significant differences column; P <0.05

A-W-S: Autumn-Winter-Spring; **S**: Summer

Table 3. Average and standard error of the morphology and sperm motility, number of doses per pregnancy and conception rate per season in which the service was performed.

Reproductive Category: Heifers

Season	Sperm morphology	Motile sperm per dose	Number of services per pregnancy	Conception rate
A-W-S	90,28 ± 1,52 a	2,68 ± 0,75 a	2,34 ± 0,33 a	48,43 ± 6,74 a
S	87,50 ± 0,50 a	8,95 ± 1,35 b	2,90 ± 0,01 b	34,50 ± 0,50 b

Different letters indicate significant differences column; P <0.05

A-W-S: Autumn, Winter, Spring. **S**: Summer

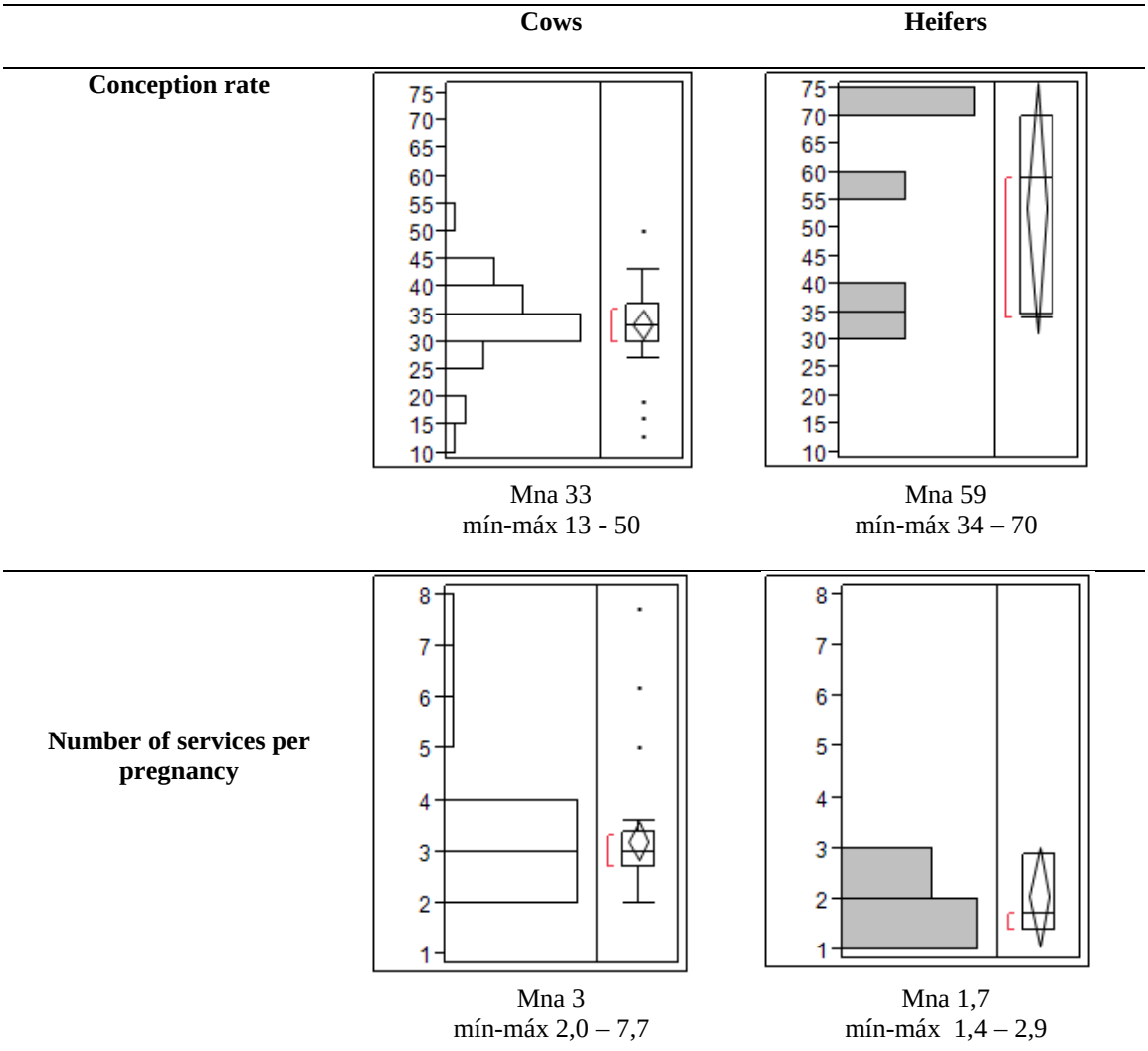

Figure 1. Frequency distribution, median, minimum and maximum (Mna, min - max) of conception rate and number of services per pregnancy, by category of animal