Review Article
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Effect of seminal plasma on hipoosmotic swelling test in fresh alpaca spermatozoa

Summary

A study was designed with the objective of evaluating the effect of seminal plasma on the response to the hypoosmotic swelling test (HOST) in alpaca spermatozoa, for which three experimental groups were organized as follows: Group 1(n=15) plasma free sperm seminal (obtained from the vas deferens, aspirated in PBS), Group 2(n=15) free seminal plasma sperm reconstituted with seminal plasma (obtained from the vas deferens, aspirated in PBS, mixed in 50/50% with seminal plasma) and Group 3(n=15) whole semen (obtained by artificial vagina), The samples were incubated in a hypoosmotic solution adjusted to 100mOsmol (sodium citrate+fructose+2H_2Ocsp 100mL). 0.1mL of semen+0.9mL of hypoosmotic solution was mixed, incubated for 30minutes in a water bath at 37°C and the reaction was stopped with 0.1mL of 4% formaldehyde. A count of at least 200 spermatozoa was performed per sample, using an optical microscope with immersion objective (100X), the vitality was evaluated by supravital eosin staining (0.7%)-nigrosin(1%), the results indicate that it does not exist a detrimental effect of the seminal plasma on the endosmotic response, being, on the contrary, superior in the whole semen; the vitality of the spermatozoa with and without seminal plasma is similar, however it decreases when it is reconstituted with seminal plasma, possibly due to the seminal plasma of another animal; there is no positive correlation between endosmosis and vitality, indicating that the latter would not necessarily reflect the integrity of the membrane, which is why it is recommended to perform this test routinely in alpaca semen exams.

Keywords: endosmosis, seminal plasma, spermatozoon, alpaca

Volume 2 Issue 4 - 2017

Pacheco JI,[1] Mamani Cato RH,[2] Vélez VM[1]
[1]Department of Veterinary Medicine, National University of San Marcos, Perú
[2]National Institute of Agrarian Innovation (INIA), Perú

Correspondence: Pacheco JI, Department of Veterinary Medicine- National University of San Marcos, Perú, Email mvz_joelpc@hotmail.com

Received: December 04, 2017 | **Published:** December 22, 2017

Introduction

The process of fertilization can't be attributed only to the number of live, motile and normal sperm deposited within the female but especially to its functionality.[1] The hypoosmotic test or hypoosmotic test (HOST) is a functional test, by which the live sperm are subjected to an incubation in a hypoosmotic solution, the sperm with the functional membrane will allow the entry of water by osmosis that will be evidenced by swelling and curling of the tail.[2] In ejaculates of llamas obtained by artificial vagina, and subjected to said test, endosmosis percentages of 40% were observed using a solution of 100mOsmol.[3] Other reports in flames indicate 33.48% in semen collected by electro ejaculation and 30.15% in semen collected by artificial vagina using hypoosmotic solution adjusted to 50mOsmol;[4] and a last report indicates 36% in semen sperm collected by burning electro ejaculation using also hypoosmotic solution adjusted to 50mOsmol.[5] In alpaca ejaculates obtained by electro ejaculation, percentages of endosmosis were obtained with a range of 20 to 62% using 100mosmol hypoosmotic solution,[6] while in alpacas whole semen 23.5% was reported using hypoosmotic solution of 150mOsmol,[7] Alpaca epididymal spermatozoa were subjected to the hypoosmotic test at an osmolarity of 100mOsmol, obtaining a response of 89.08%, evidencing a greater response in free sperm from seminal plasma.[8] The artificial vagina method allows obtaining complete ejaculates of alpacas,[9] free seminal plasma sperm can be obtained by means of the deferential vas deferens technique permanently,[10] Under these considerations, the objective of the present investigation was to evaluate the endosmotic response of spermatozoa of alpaca in the presence and absence of seminal plasma and reconstituting the seminal plasma to sperm obtained from the vas deferens.

Methodology

Six male alpacas were used, three males donated whole semen using the artificial vagina technique, said semen was also used to obtain seminal plasma by centrifugation, at 2000g/20 minutes and in three males free seminal plasma sperm were obtained, which were obtained by the deferential vas deferens technique.[10] The sperm samples were divided into three groups per treatment, making a total of 45 samples: Group 1 (n=15) free seminal plasma sperm (obtained from the vas deferens, aspirated in PBS), Group 2 (n=15) free seminal plasma spermatozoa reconstituted with seminal plasma (obtained from the vas deferens, aspirated in PBS, mixed in 50/50% with seminal plasma), and group 3 (n=15) whole semen (obtained by artificial vagina). The samples were incubated in a hypoosmotic solution adjusted to 100mosmol (sodium citrate+fructose+2H_2O c.s.p. 100mL). 0.1mL of semen+0.9mL of hypoosmotic solution was mixed, incubated for 30 minutes in a water bath at 37°C and the reaction was stopped with 0.1mL of 4% formaldehyde. The count of sperm positive to endosmosis was performed, counting not less than 200 spermatozoa per sample using an optical microscope with immersion objective (100 X), the vitality was evaluated by supravital eosin staining (0.7%)-nigrosin (1%). The statistical design used corresponded to a DCA, with previous transformation to the sine of the arc of the percentages of endosmosis and vitality influenced by each of the experimental groups, and Duncan's test to separate the average; also, the Pearson correlation test was used to relate the values of endosmosis and vitality; in both cases a probability value of 95% was used, the data were analyzed using the SAS V9.0 program.[11]

Results and discussion

The data on the effect of endosmosis and vitality influenced by the presence or absence of seminal plasma, are detailed in (Table 1). The endosmosis found in free seminal plasma sperm obtained from the vas deferens (38.66%), is lower than the report of Rodríguez,[8] who indicates 89.08% in epididymal sperm, possibly the sperm suffer changes in the permeability of its membrane during transit through the vas deferens. The spermatozoa that were reconstituted with seminal plasma presented a similar endosmotic response than those free of seminal plasma, which could be due to the amount of seminal plasma used, which corresponded to only 50% of the total volume prior to incubation, the decrease in vitality could be attributed to the use of seminal plasma from another animal; this finding is similar to the response reported by Carretero et al.[5] who found 36% of endosmosis in flame semen obtained by electro ejaculation, where said semen is less viscous than the semen obtained by artificial vagina. The endosmosis of the whole semen with presence of seminal plasma was higher than groups 1 and 2, would indicate that the seminal plasma does not negatively influence this response as expected, being similar to the responses observed in llama spermatozoa in ejaculates obtained by vagina artificial,[3] superior to those obtained by electroejaculation[4] and similar to that described in semen of alpacas obtained by electroejaculation,[6] and superior to what was obtained by Pacheco et al.,[7] who used a 150mOsmol hypoosmotic solution, while in the present study a 100mosmol hypoosmotic solution was used. On the other hand, the correlations found between endosmosis and vitality were negative (less 0.2 group 1, les 0.53 group 2 and 0.02 group 3), which indicates that there is an inversely proportional relationship, mainly in group 2, indicating that at lower vitality there is greater endosmosis, similar to that described by Pacheco et al.,[7] who indicate that there is no direct relationship between the two tests, which is why they recommend performing this test routinely since vitality does not necessarily indicate the functionality of the membrane of said sperm. There is no negative effect of seminal plasma on the response to the hypoosmotic swelling test in alpaca spermatozoa, with endosmosis in whole semen being higher; vitality is similar between sperm without seminal plasma and whole semen, however, it decreases when seminal plasma is added to free seminal plasma sperm; there is no positive correlation between endosmosis and vitality indicating that vitality does not necessarily reflect the integrity of the plasma membrane, so it is recommended to routinely perform this test on alpaca sperm.

Table 1 Alpaca sperm endosmosis in the presence and absence of seminal plasma

Espermatozoa	n	Host (%)	D.S.	CV	Mín	Máx	Vitality (%)
Without Seminal Plasma	15	38.66[a]	7.82	20.07	26.19	59.49	69.12[a]
Recombinant with Seminal Plasma	15	38.95[a]	7.67	19.83	24	51	57.46[b]
With Seminal Plasma	15	54.70[b]	8.91	15.96	43	73	68.50[a]

Acknowledgements

None.

Conflict of interest

The author declares no conflict of interest.

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