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Sperm chromatin stability and their relationship with fertilization rate in Sheep of the Junín race

Estabilidad de la cromatina espermática y su relación con la tasa de fecundación en ovejas de la raza Junín

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ABSTRACT

The objective of this research was to evaluate the effect of sperm on chromatin stability and its relationship with the membrane integrity structural – physiological and the rate of fertilization of female sheep. Ejaculates of sperm (2×10^9 sperm·mL^{-1}) with 70% of motility were collected using an artificial vagina (n=5, 2 years old. For this, each ram was served with fifteen female sheep (n=75), generating thus five different Groups (A, B, C, D, and E). A control Group also was considered. Sperm nuclear chromatin stability (NCS) was evaluated using the Borate Buffer (BB), Sodium Dodecyl Sulfate (SDS), and the mixture of Ethylenediaminetetraacetic acid (EDTA) + SDS. The fertilization rate was evaluated after 16-18 hours post sperm injection. Sperm concentration showed a significant difference (P<0.05) between Groups. In Contrast, seminal volume, and sperm motility do not show a significant difference (P>0.05). A high correlation (r^2=0.52) was observed between morphology and motility, and the fertilization rate was 74.6% (n=56). It was concluded in general that techniques to evaluate nuclear condensation values do have a high likelihood to give a diagnosis about the future potential of sperm populations in Junín ram.

Key words: Sperm chromatin; membrane integrity; Peruvian rams

RESUMEN

El objetivo de esta investigación fue evaluar el efecto de los espermatozoides sobre la estabilidad de la cromatina y su relación con la integridad de la membrana estructural – fisiológica y la tasa de fertilización de las ovejas hembras. Las eyaculaciones de espermatozoides (2×10^9 espermatozoides·ml^{-1}) con un 70 % de motilidad se recogieron mediante una vagina artificial (n=5, 2 años). Para ello, cada carnero se servía con quince ovejas hembra (n=75), generando así cinco grupos diferentes (A, B, C, D y E). También se consideró un grupo de control. La estabilidad de la cromatina nuclear (NCS) de los espermatozoides se evaluó utilizando el tampón de borato (BB), el dodecil sulfato de sodio (SDS) y la mezcla de ácido etilendiaminotetraacético (EDTA) + SDS. La tasa de fertilización se evaluó después de 16-18 horas después de la inyección de espermatozoides. La concentración de espermatozoides mostró diferencias significativas (P<0,05) entre los grupos. Por el contrario, el volumen seminal y la motilidad de los espermatozoides no mostraron diferencias significativas (P>0,05). Se observó una alta correlación (r^2=0,52) entre morfología y motilidad, y la tasa de fecundación fue del 74,6% (n=56). Se concluyó en general que las técnicas para evaluar los valores de condensación nuclear tienen una alta probabilidad de dar un diagnóstico sobre el potencial futuro de las poblaciones de espermatozoides en carneros de Junín.

Palabras clave: Cromatina espermática; integridad de la membrana; carneros peruanos

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INTRODUCTION

Infertility is a significant concern not only in humans but also in several species of animals [18]. In farm animals, infertility can cause negative effects on animal welfare and considerable economic loss [8]. Infertility is related to various causes such as physiological disturbances, infectious causes, and bad nutrition, which may work in combination or separately [2, 28]. Likewise, infertility may arise due to damage to Deoxyribonucleic acid (DNA) and high levels of loosely packaged chromatin [9]. As sperm cells have the important mission of delivering vehicles for chromatin cargo within, the oocyte, which is composed of paternal DNA information and its associated proteins, its integrity is considered a keystone of reproductive success [31]. Therefore, a correct chromatin package level is necessary for successful insemination [32]. In the last decades were reported that chromatin sperm stability contributes to both adequate embryonic development and successful fertilization [9].

The variability between species is attributed to the proportion of protamine; in contrast, sperm from the bull (*Bos taurus*), Rat (*Rattus novergicus*), ram (*Ovis aries*), pig (*Sus scrofa domesticus*), and guinea pig (*Cavia porcellus*), have only one type of protamine (P1), while humans have a second (P2), which is deficient in cysteine residues [1]. In addition, membrane integrity is very important for metabolism, sperm training, the acrosomal reaction, and the binding of sperm to the surface of the oocyte. Plasma membrane damage can cause loss of normal sperm function, which involves the decrease of motility, viability, and fertilizer capacity.

The quality of sperm in animals and human are typically assessed through the measuring of sperm density, motility, total count, and morphology [7]. Therefore, this work aimed to assess the stability of the chromatin in ram sperm and relate it to the membrane integrity structural – physiological and the rate of pregnancy.

MATERIALS AND METHODS

Study site

The handling and sampling of the animals were carried out at the Experimental Station IVITA-El Mantaro, located 3300 meters above sea level, in the Huancayo Province, Junin-Peru with a latitude 11°49'13"S and longitude 75°23'17"W. This station is developing research to generate knowledge and new technologies based on the livestock production systems. Its climate is characterized by an annual average temperature of 12.4°C, annual average precipitation of 707.9 millimeters (mm), and relative humidity of 77%.

Animals and semen collection

The present study was carried out using 5 male sheep (ram, about 120 kilograms -kg-) of 2 years old (YO) and 75 female sheep (ewes) (about 85 kg) of 3-5 YO of the Junín sheep breed. During the day (d), animals were kept daily grazed for 12 hours (h) on grass pastures of Reed canarygrass (*Phalaris arundinacea* L.), and afternoons were fed with alfalfa (*Medicago sativa*) hay to maintain a body condition. Each ram was served with fifteen ewes, generating five (A, B, C, D, and E) different Groups of analysis. For better control, rams and female sheep belonging to the same Group were identified with their earrings and colored wired laces. All rams were sexually active and under strict control. For each ram, semen ejaculates were collected every month (mos) for five mos (a total of 25 semen samples) via an artificial vagina (robust tubular rubber (11 centimeter length) casing with rounded opening, with a valve that allowed air to be added for adjustment of inner pressure) and kept in a water bath (Thermo Scientific, Model 265, USA) at 30°C for 8-10 minutes (min) until examination of the initial semen quality (after 16-18 h post sperm injection) for concentration, sperm motility, volume, viability, and sperm plasma integrity through eosin Y test and hypoosmotic (HOS) swelling test [26].

For this purpose, the split-plot design was applied. The samples collected were transported to the Reproductive Biotechnology laboratory of the National University of Center of Peru.

Seminal quality

Seminal volume, sperm morphology, sperm motility, and sperm morphology were evaluated according to guidelines recommended by World Health Organization (WHO) [30].

Sperm motility

Sperm motility was measured using a computer-assisted sperm motion analyzer (Stromberg Mika, Germany). Semen was diluted to a concentration of 30×10^6 sperm·millilitre^{-1} (sperm·mL^{-1}) with a Buffer (Hepes balanced saline (HPS) at 7.6 pH) and then placed approximately 10 microlitre (µL) on a 10 micrometer (µm) of depth disposable chamber slide (Leja Products, Netherlands) [21]. The image was digitized via a camera (10-phase contrast objective x and 3.3-ocular lens x) mounted on a phase-contrast microscope (x400) (IM3-MET, OPTIKA, Italy). In total, five small squares; one central and four angulars were evaluated.

Sperm concentration

Sperm (spz) concentration was measured using a pre-calibrated photometer (NucleoCounter SP100, ChemoMetec, Denmark) after dilution 25 µL of ram semen with 500 µL of 0.9% NaCl solution containing 0.32 millimolar (mM). Sperm concentration was computed by counting cells in five large squares of a Neubauer hemocytometer (Countess 3 Automated Cell counter, Thermo Fisher Scientific, USA) chamber under a phase contrast microscopy [21].

Eosin Y test and hypoosmotic swelling test

The structural integrity of the membrane of the sperm was evaluated with the Eosin Y-water (EY) test method, and the functional integrity was assessed through the hypoosmotic (HOS) swelling test [21]. Results were expressed in percentage according to the degree of reaction. It was considered a minimum count of 100 sperm per test.

- **Eosin Y – water method:** here 0.5 grams (g) of eosin Y and 0.9 g of sodium chloride (NaCl) were dissolved in 100 mL of distilled water. At the prepared eosin Y solution was added 10 g of nigrosine, and after, boiled, and allowed to cool at room temperature. Then, the staining mixture was filtered using filter paper and poured into a dark bottle. The tests were performed using a drop of semen placed on a heated microscope slide and added two drops of eosin-nigrosine staining solution, and then mixture carefully using a pipette tip for making a smear, and finally dried to room temperature for 5 min. The smear was evaluated under a phase-contrast microscope (Labomed Lx400, USA). At least 100 sperm were assessed per slide, and the percentage of swollen tail sperm was computed [20].

- **Hypoosmotic swelling test (HOS):** efficacy of the HOS test was evaluated using fructose (1351 milligrams -mg-), Na-citrate (735 mg), and 100 mL distilled water solution with three different osmolarities: 50, 100, and 150 osmolarities (mOsm·kg^{-1}). For this, 0.1 mL ram semen was added to 0.9 mL of hypoosmotic solutions (50 and 100 mOsm·kg^{-1} fructose) and incubated (Model 4130, Thermo Fisher Scientific, Brazil) at 37°C for 45 min at room temperature [19]. After incubation, one drop of incubated semen was placed on a microscope slide and assessed under phase contrasts x40 microscope. A minimum of 100 sperm were assessed per slide, and percentages of sperm with swollen were computed [19].

Sperm chromatin stability assessment

Sperm nuclear chromatin stability (NCS) was assessed following the Gonzales and Sánchez [11] methodology. For this, 20 µL of fresh semen were deposited in three test tubes coded from 1 to 3. In tube 1 (control) was added 180 µL of Borate Buffer (BB), in tube 2 was added 180 µL of Sodium Dodecyl Sulfate (SDS) at 1%, and in tube 3 was added 180 µL of a mixture of Ethylenediaminetetraacetic acid (EDTA) + SDS. These solutions were incubated (GTOP model 260, Olabo, China) at 40°C for 60 min. Finally, 200 µL of glutaraldehyde at 2.5% was added, mixed, and left at rest for 10 min. Each solution obtained was smeared onto a glass slide, dried, fixed with methanol (5 to 10 min), stained Giemsa solution (at 50%) for 40 min, and observed at the microscope with x400 magnification. The minimum count was 100 sperm for each of the three laminae. Using this method, it is possible to find three grades: Grade 0: no condensation of head sperm head; Grade 1: moderate condensation of head sperm; and Grade 2: high condensation of the sperm head, all related to chromatin response [9]. If the percentage of no-decondensed sperm head in the presence of SDS + EDTA is greater-than 30%, the samples are classified as having high sperm head stability [21].

Pregnancy rate related to the sperm chromatin stability

The pregnancy rate was evaluated using the birth rate, which was calculated on the number of offspring born, to determine female fertility.

Statistical analysis

The Wilcoxon rank-sum test was applied to compare the average percentages of sperm concentration, seminal volume, normal morphology, and sperm motility. Pearson correlation was computed to assess the relationships among the eosin Y test, hypoosmotic test, morphology, and motility. All data were treated with R-free software, version 3.3.6 [24]. A comparison was considered significant when *P* was less-than 0.05.

RESULTS AND DISCUSSION

Seminal parameters quality

Average and standard deviation (SD) of sperm concentration, seminal volume, normal sperm morphology, and sperm motility, from sheep semen of five Groups of rams are presented in TABLE I. In that TABLE I has been observed differences ($P<0.05$) among Groups for sperm concentration, but no differences were found for seminal volume, normal morphology, and sperm motility. Higher sperm concentration was presented by the ram belonging to Group A, while the ram belonging to Group E showed the highest values for seminal volume, normal morphology, and sperm motility. Sperm motility ranged from 4.03 to 4.81 suggesting that the semen is of good quality.

TABLE II has presented the percentages and SD of eosin swollen tail sperm based on the Eosin Y test (structural integrity) and Hypoosmotic test (physiological integrity). The percentage of sperm in Eosin Y ranged from 58 to 77%, while the Hypoosmotic test ranged from 60 to 81%. The percentage average between methods no-showed a significant difference ($P>0.05$). Likewise, no differences ($P>0.05$) were found between each Group. A higher percentage of sperm was observed in Group E in both methods.

Regarding the structure and membrane physiology of sperm, these variables are quite consistent in the contingency tables for membrane structure, where eosin evidences a difference between cells that do respond to the membrane test; while for the hypoosmotic test it was more significant because the sperm populations of the animals show a greater difference between the swollen and non-swollen cells. Thus, results found in ejaculated evidence to be optimal conditions for the sperm populations to fulfill their role of fertilizing the female gamete. The reliability of the test results is supported by the correlation of these in the measurement of an association with the same trend.

TABLE I
Seminal parameters from ejaculated ram semen

Group	Sperm concentration sperm (10^6·mL^{-1}) ($\bar{x}$ ± SD)	Seminal volume (mL) ($\bar{x}$ ± SD)	Normal morphology (%) ($\bar{x}$ ± SD)	Sperm motility ($\bar{x}$ ± SD)
A	2304 ± 386 a	0.96 ± 0.41 a	84 ± 7 a	4.61 ± 0.54 a
B	2198 ± 220 b	1.12 ± 0.22 a	70 ± 11 b	4.63 ± 0.55 a
C	2110 ± 196 b	0.90 ± 0.33 a	89 ± 4 a	4.22 ± 0.83 a
D	2228 ± 140 c	0.88 ± 0.34 a	91 ± 4 a	4.03 ± 1.02 a
E	2090 ± 263 b	1.56 ± 1.31 a	92 ± 5 a	4.81 ± 0.45 a
Total	2186 ± 246	1.08 ± 0.68	85 ± 11 a	4.44 ± 0.71

n= 5: number of ejaculations, ($\bar{x}$ ± SD): average and standard deviation, values on each vertical column followed by the same letter do not differ significantly at $P<0.05$

TABLE II
Percentages and standard deviation of eosin swollen tail spermatozoa based on Eosin Y test (structural integrity) and Hypoosmotic test (physiological integrity)

Group	Eosin Y test 100 sperm (%)	Hypoosmotic test 100 sperm (%)
A	68 ± 15 a	60 ± 12 a
B	58 ± 8 a	61 ± 7 a
C	72 ± 4.5 a	75 ± 12 a
D	72 ± 26 a	68 ± 32 a
E	77 ± 11 a	81 ± 8 a
Average	69.4 ± 13 A	69.0 ± 14 A

n= 5: number of ejaculations, ($\bar{x}$ ± SD): average and standard deviation

Pearson correlation between observed parameters found in ram semen was presented in TABLE III. There were no significant correlations between motility and the Eosin Y test and the Hypoosmotic test (P<0.001), except for morphology. A significant correlation (r^2=0.81) was observed between the Eosin Y and Hypoosmotic test (P<0.001). Besides, morphology and Hypoosmotic tests showed statistical correlation.

Morphological and kinetic aspects of sperm are important factors for fertilization [10]. For this, microscopic analysis is necessary to contrast the mass and individual motility of sperm the most before possible. Thus, to avoid thermal shock, a platen adaptable to the base of the microscope was used.

Osmotic pressure is of great importance, not in the ejaculate itself whose variations do not exceed normal limits, but in terms of the composition of the diluents [6]. In general terms, it can be admitted that sperm requires for its normal maintenance isotonic means [15]. Variations in the osmotic pressure of solvents or culture media are not compatible with sperm vitality. However, different slightly hypotonic means have been tested, with the idea that this means greater possibilities of natural detoxification of the cell spermatic, a phenomenon that would favor the survival and the fecundating capacity of the sperm. It seems that ruminant sperm are the most resistant to the osmotic pressure, while the gametes of animals of internal fertilization require for their normal biology very few variations of the same. In this case, the hypoosmotic test was used to test the functional efficiency of the sperm membrane, which gives an acceptable percentage in the sperm population subjected to hypoosmotic conditions, indicating an adequate fertile potential, with an optimal functionality of their sperm membrane. High percentages of sperm cells that were subjected to the hypoosmotic test suggest that sperm populations subjected to membrane integrity tests (eosin and hypoosmotic testing) are crucial for viability and physiological changes that occur on the membrane surface [18], so it can be argued that both tests would have a value in testing sperm fertility.

Membrane integrity is important for sperm metabolism, training, acrosomal reaction, and sperm binding to the surface of the oocyte [4]. In contrast, damage to membrane integrity can cause loss of normal sperm function, such as motility, viability, and fertilizing capacity. It has been shown that exposure of mammalian sperm to hypoosmotic conditions can cause water flow through the membrane, resulting in increased sperm volume and swelling of the plasma membrane. The ability of the sperm tail to swell in the presence of a hypoosmotic solution is a sign that the transport of water through the membrane occurs normally. Swelling of the spermatic nucleus and decondensation of chromatin in mammalian sperm occurs shortly after penetration of the sperm to the cytoplasm of the oocyte. The decondensation of chromatin was doubled when the pre-ejaculates of ram and cattle were incubated *in vitro*. There was an increase in the time of decondensation when stored *in vitro* at 25 °C, which reflected an increase in the junctions within the sperm by histones for the formation of disulfide bridges. This can increase embryonic mortality when it is observed that sperm was stored *in vitro* before insemination [16].

Chromatin compaction degrees

Interactions between animals (Groups A to E), methods (BB, SDS, and SBS + EDTA), months, and compaction levels are presented in TABLE IV. From TABLE IV, it was observed no significant (P>0.05) differences among methods. No differences may be related to the that the medium used would depend on the degree that has each ejaculate. However, compaction degrees were observed with significant differences (P<0.05) when the methods interacted with the periods or mos in which the semen was collected. In the case of mos that interact with the degrees of compaction was found significant differences (P<0.001). Interaction mos*compaction degrees being significant ensures that each mos evaluated maintains different conditions relating to the compaction degree, and suggests that any animal may be evaluated. Likewise, it was found that the compaction degree was affected (P<0.0001), by the interaction (mos*compaction degree, (P<0.001)) with the mos in which the ejaculation was obtained. Besides, was observed interactions between the compaction degrees with the methods (P<0.001) and mos (P<0.01) evaluated. Unlike the degrees of compaction, no simple effect was found in the animals or the mos that the semen was collected. However, differences between the methods were significant when interacting with the mos and the degrees of compaction.

TABLE III
Pearson correlation between observed parameters of ram semen

Observed semen parameters	Eosin Y test (%)	Hypoosmotic test (%)	Morphology (%)	Motility (0-5)
Eosin Y test (%)	1			
Hypoosmotic test (%)	0.81 (P<0.001)	1		
Morphology (%)	0.56 (P<0.001)	0.46 (P=0.001*)	1	
Motility (0-5)	0.22 (P<0.05)	0.22 (P=0.002*)	0.52 (P<0.001)	1

*: significative value

Differences in compaction degrees found among the males studied showed dependence on the effect of the period in which the ejaculates were obtained (compaction interaction*mos: significant). This result suggests the presence of the seasonal effect in the process of spermatogenesis on the compaction of nuclear chromatin [25]. However, rams are not mostly susceptible to the effect of seasonality [29]. This was corroborated with results found in seminal parameters quality (concentration, normal morphology, and sperm motility) which were not altered. However, it was observed an effect on the compaction degrees of the chromatin.

In the interaction with methods, the highest percentage occurred between grades 0 and 1, which expresses appropriate stability of chromatin, good quality of the sperm, and excellent fecundation potential [14]. In the present case, the BB method reported a higher percentage of sperm compared to other methods, with a grade of 0 in all rams. This could be because SDS is a detergent that acts on the disulfide bridges of chromatin, which makes sperm chromatin less stable when subjected to SDS detergent. As the EDTA is a tinging agent that acts on zinc by removing it; this mineral gives stability to the disulfide bridges of sperm chromatin [3]. This finding suggests a line of good quality that guarantees their fertilizer capacity.

TABLE IV
Split plot analysis showing interaction levels between animals, methods, months, and compaction degrees

Source	DF	Type SS	Square	F - value	*P*
Animals	4	7.11	1.78	0.02	0.999
Month	4	4.89	1.22	0.01	0.999
Error (a)	16	12.89	0.806	0.01	1.000
Month	2	8820.2	4410.1	46.65	0.0001
Compaction*compaction	8	2519.8	314.9	3.33	0.0018
Error (b)	40	17177	429.42	4.54	0.0001
Methods	2	1.56	0.78	0.01	0.9918
Method*month	8	8.44	1.06	0.01	1.0000
Method*compaction	4	27587.8	6896.9	72.96	0.0001
Method*month*compaction	16	2808.9	175.6	1.86	0.0312
Error (c)	120	11343.3	94.53		
Total	224	70291.6			

TABLE V showed the physiological state and compaction degree of sperm chromatin. From TABLE V, there were no differences between pregnancy and non-pregnancy rates. There is also no statistical relationship (*P*>0.05) between compaction degrees (0, 1, and 2) and their relationship with animals that were not as prey. However, a higher rate of prey (74.6%) was found.

In the pregnancy rate and its relationship with the stability of chromatin, it was found a non-significant correlation, which would suggest that there is no adequate degree for the good stability of chromatin, which did not coincide with being the most frequent in pregnancy. For this, standard semen analysis is required, including concentration, morphology, and motility analysis, which are widely used as an indicator of fertility [13]. Thus, the parameters above mentioned may influence the physiological, structural, and stability aspects of the sperm chromatin. Based on all that, here the method used allowed obtain sperm of the best quality (in terms of morphology and motility), with minimum contamination by other cellular or non-cellular structures such as granulations and crystals [27].

TABLE V
Physiological state and compaction degree of sperm chromatin

Physiological state	Compaction degrees						Total	
	0 (< 0.63)		1 (0.63 to 1.17)		2 (> 1.18)			
	n	%	n	%	n	%	n	%
No prey	6	24.0	5	20.0	8	32.0	19	25.3
Prey	19	76.0	20	80.0	17	68.0	56	74.7
Total	25		25		25		75	100

Ejaculates of Ovid's and caprids, as well as that of ruminants in general, are composed of four fractions or liquid emissions, the product of the litter glands, bulbourethral, prostate, Henle blisters, and vesicular glands [5]. The largest volume corresponds to the prostate glands and blisters of Henle; hence, the function of this biological phenomenon. Here, it is possible to admit that the ejaculate of sheep (*Ovies aries*) and goats (*Capra aegagrus hircus)* is of low volume and high sperm concentration since in all species the volume of the ejaculate depends on the secretory capacity of the gland's paragenitals.

Pregnancy rate related to the sperm chromatin stability

TABLE VI presents the number of sheep by group sent for reproduction, served, calved, and served percentage. From TABLE VI, Group B (93.30%) showed a higher percentage of sheep served,

TABLE VI
Number of sheep by group sent for reproduction, served, calved, and served percentage

Group	sheep for reproduction	Sheep served	Calved sheep	Served percentage (%)
A	15	12	10	80.00
B	15	14	13	93.30
C	15	13	12	86.67
D	15	13	11	86.67
E	15	12	10	80.00
Total	75	64	60	

followed by Group C and D (both with 86.67%), and Group A and E (both with 80.0%). About calved sheep, percentage of prolificity, and sex of the offspring, Group B obtained the highest number of calved sheep (13), followed by Group C and D (both 12), and Group A and E (both 10). The average percentage of prolificity was 114%, Group B (123%) presented the highest percentage, followed by Group D (118%), Group C (116%), Group A (110%), and Group E (100%). Regarding Pearson's correlation between pregnancy rate and sperm chromatin stability, it was found a negative correlation (-0.11) indicating that there is no statistical significance (P>0.05). It is possible to suggest that the higher the degree of de-condensation of chromatin the rate of calving remains. The dispersion of the degrees of compaction tends to increase the percentage of calving, thus, the point "66.67" (66.67, 0.99), the point "80" (80, 0.93), and the point "86.67" (86.67, 0.89), from which it follows that by increasing the percentage of calves tends to decrease the degree of compaction, from 0.99 to 0.82.

The results obtained regarding the timely decondensation of chromatin when incubated in BB as a primary and common mean at three degrees of condensation at a temperature of 40 °C for approximately 45 min, in no way resemble being stored for a few h or d, indicating the importance of temperature variation is for the case of artificial insemination (AI) programs and the considerations that must be given for obtaining good results, that is, high percentages of pregnancy and viability of embryos. During the late stage of spermiogenesis, the nucleus of the spermatid becomes very condensed and adopts an elongated shape characteristic of the species. Although there is no full understanding of the forces that determine nucleus shape, it is known that these visible modifications are accompanied by biochemical changes involving the removal of Ribonucleic acid (RNA) from the nucleus at the beginning of nuclear elongation and the replacement of lysine-rich histone with a more basic arginine-rich protein.

Sperm obtained from sheep healthy were induced to decondensation of chromatin with dithiothreitol (DTT) and SDS *in vitro*. A high range of stable no-condensed nuclei (79%) was observed in ram semen with normal fertility. In contrast, semen of rams with low fertility presented 31% of chromatin stability. Seminal plasma and other constituents (Zn) inhibited the decondensation chromatin of sperms, while when added EDTA shows the opposite. Results suggest that the addition of chromatin decondensation using SDS under controlled conditions may be a reliable method for predicting fertile capacity [23]. The results also showed the EDTA action to eliminate Zn, and borate buffer, allowing their growing inside the sperm medium [17]. Likewise, it was observed that there was no inhibition of the decondensation chromatin as happened in the tube containing SDS and BB. It is important to mention that the methodologies evaluated in this work are different, due here applying the protamine P2, which is held by the bull, ram, rat, pig, guinea pig, and humans [12].

This makes chromatin less stable in humans than in other species [7]. These results coincide with the obtained results. In this sense, the sperm subjected to the EDTA does not have a very decondensed form, which does not mean that there has not been decondensation. This is probably by the minimum presence of protamine P1, which makes the chromatin of sheep more stable.

These results offer the idea that the condensation of chromatin in mammals tends to vary because of the pregnancy rate. In this study, the pregnancy rate in Junín sheep was 86.67%.

Although it is true not there is a degree of condensation that differs in function from the fertilization rate, which is explained by the number of sheep pregnant and not pregnant. The degrees of condensation of chromatins 1 and 2 are the least stable, compared to the zero degrees which are the most stable [22].

TABLE VII shows the rate of fertilization of each Group based on the pregnancy and not the pregnancy of the sheep. No significant differences (P<0.05) were found between the animal Group. These results indicate that none of the rams was more effective in reproduction than the others.

CONCLUSIONS

The concentration of sperm showed differences among the five groups analyzed. Based on the parameters assessed, the seminal volume and sperm motility do not show significant differences among groups. Besides, a high correlation (r^2=0.52) was found between motility and morphology. A high rate of fertilization rate (74.6%.) was

TABLE VII
Rate of fertilization from the ram that pregnant and did not pregnant the sheep

Fertilization	Statistics	Group of animals					Total
		A	B	C	D	E	
Prey	Recount (% of total)	10 (13.3%)	13 (17.3%)	12 (16.0%)	11 (14.7%)	10 (13.3%)	56 (74.7%)
No prey	Recount (% of total)	5 (6.7%)	13 (17.3%)	12 (16.0%)	11 (14.7%)	10 (13.3%)	56 (74.7%)
Total	Recount (% of total)	15 (20.0%)	15 (20.0%)	15 (20.0%)	15 (20.0%)	15 (20.0%)	56 (74.7%)

found. It was concluded in general that techniques to evaluate nuclear condensation values do have a high likelihood to give a diagnosis about the future potential of sperm populations in Junín ram.

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BIBLIOGRAPHIC REFERENCES

[1] BALHORN, R.; STEGER, K.; BERGMANN, M.; SCHUPPE, H.C.; NEUHAUSER, S.; BALHORN, M.C. New Monoclonal Antibodies Specific for Mammalian Protamines P1 and P2. **Syst. Biol. Reprod. Med**. 4(6): 424–447. 2018. https://doi.org/jctc.

[2] BINDARI, Y.R.; SHRESTHA, S.; SHRESTHA, N.; GAIRE, T.N. Effects of Nutrition on Reproduction- A Review. **Pelagia Res. Libr.** 4(1): 421–429. 2013.

[3] BJÖRNDAHL, L.; KVIST, U. Human Sperm Chromatin Stabilization: A Proposed Model Including Zinc Bridges. **Mol. Hum. Reprod**. 16(1): 23–29. 2009. https://doi.org/djrrvx.

[4] BUCCI, D.; SPINACI, M.; GALEATI, G.; TAMANINI, C. Different Approaches for Assessing Sperm Function. **Anim. Reprod**. 16(1): 72–80. 2018. https://doi.org/jctd.

[5] CARVALHO, L.E.; SILVA-FILHO, J.M.; PALHARES, M.S.; SALES, A.L.R.; GONCZAROWSKA, A.T.; OLIVEIRA, H.N.; RESENDE, M.; ROSSI, R. Physical and Morphological Characteristics of the First Three Jets of Pêga Jackasses Sperm-Rich Fraction. **Arq. Bras. Med. Vet. Zoot**. 68(4): 845–852. 2016. https://doi.org/jctf.

[6] COOPER, T.G. The Epididymis, Cytoplasmic Droplets and Male Fertility. **Asian J. Androl**. 13(1): 130–138. 2011. https://doi.org/dfv5ww.

[7] EVENSON, D.; JOST, L. Sperm Chromatin Structure Assay Is Useful for Fertility Assessment. **Springer.** 22(2): 169–189. 2000.

[8] GALIOTO, F.; PAFFARINI, C.; CHIORRI, M.; TORQUATI, B.; CECCHINI, L. Economic, Environmental, and Animal Welfare Performance on Livestock Farms: Conceptual Model and Application to Some Case Studies in Italy. **Sustain**. 9(9): 1–22. 2017. https://doi.org/gb4rb6.

[9] GARCÍA, J.; NORIEGA-HOCES, L.; GONZALES, G.F. Sperm Chromatin Stability and Its Relationship with Fertilization Rate after Intracytoplasmic Sperm Injection (ICSI) in an Assisted Reproduction Program. **J. Assist. Reprod. Genet**. 24(12): 587–593. 2007. https://doi.org/d3v9pf.

[10] GARCÍA-VÁZQUEZ, F.; GADEA, J.; MATÁS, C.; HOLT, W. Importance of Sperm Morphology during Sperm Transport and Fertilization in Mammals. **Asian J. Androl**. 18(6): 844–850. 2016. https://doi.org/jctg.

[11] GONZALES, G.F.; SÁNCHEZ, A. High Sperm Chromatin Stability in Semen with High Viscosity. **Syst. Biol. Reprod. Med**. 32(1): 31–35. 1994. https://doi.org/bkbvpb.

[12] GU, N.H.; ZHAO, W.L.; WANG, G.S.; SUN, F. Comparative Analysis of Mammalian Sperm Ultrastructure Reveals Relationships between Sperm Morphology, Mitochondrial Functions and Motility. **Reprod. Biol. Endocrinol**. 17(1): 1–12. 2019. https://doi.org/jcth.

[13] HAMILTON, J.A.M.; CISSEN, M.; BRANDES, M.; SMEENK, J.M.J.; DE BRUIN, J.P.; KREMER, J.A.M.; NELEN, W.L.D.M.; HAMILTON, C.J.C.M. Total Motile Sperm Count: A Better Indicator for the Severity of Male Factor Infertility than the WHO Sperm Classification System. **Hum. Reprod.** 30(5): 1110–1121. 2015. https://doi.org/f7c9q8.

[14] HEKMATDOOST, A.; LAKPOUR, N.; SADEGHI, M.R. Sperm Chromatin Integrity: Etiologies and Mechanisms of Abnormality, Assays, Clinical Importance, Preventing and Repairing Damage. **Avicenna J. Med. Biotechnol**. 1(3): 147–160. 2009.

[15] HOLMES, E.; BJÖRNDAHL, L.; KVIST, U. Hypotonic Challenge Reduces Human Sperm Motility through Coiling and Folding of the Tail. **Androl.** 52(11): 1–7. 2020. https://doi.org/gjhxjr.

[16] KURYKIN, J.; HALLAP, T.; JALAKAS, M.; PADRIK, P.; KAART, T.; JOHANNISSON, A.; JAAKMA, Ü. Effect of Insemination-Related Factors on Pregnancy Rate Using Sexed Semen in Holstein Heifers. **Czech J. Anim. Sci**. 61(12): 568–577. 2016. https://doi.org/f9r8jm.

[17] MAARES, M.; HAASE, H. A Guide to Human Zinc Absorption: General Overview and Recent Advances of in Vitro Intestinal Models. **Nutrients**. 12(3): 1–45. 2020. https://doi.org/gqfrs7.

[18] MULLER, C.J.C.; CLOETE, S.W.P.; BOTHA, J.A. Fertility in Dairy Cows and Ways to Improve It. **S. Afr. J. Anim. Sci**. 48(5): 858–868. 2018. https://doi.org/jctj.

[19] MUNUCE, M.J.; CAILLE, A.M.; BERTA, C.L.; PERFUMO, P.; MORISOLI, L. Does the Hypoosmotic Swelling Test Predict Human Sperm Viability? **Arch. Androl.** 44(3): 207–212. 2000. https://doi.org/d8bx6g.

[20] NUR, Z.; SEVEN-CAKMAK, S.; USTUNER, B.; CAKMAK, I.; ERTURK, M.; ABRAMSON, C.I.; SAĞIRKAYA, H.; SOYLU, M.K. The Use of the Hypo-Osmotic Swelling Test, Water Test, and Supravital Staining in the Evaluation of Drone Sperm. **Apidologie**. 43(1): 31–38. 2012. https://doi.org/bkt786.

[21] RAMÓN, M.; SALCES-ORTIZ, J.; GONZÁLEZ, C.; PÉREZ-GUZMÁN, M.D.; GARDE, J.J.; GARCÍA-ÁLVAREZ, O.; MAROTO-MORALES, A.; CALVO, J.H.; SERRANO, M.M. Influence of the Temperature and the Genotype of the HSP90AA1 Gene over Sperm Chromatin Stability in Manchega Rams. **PLoS One**. 9(1): 1–9. 2014. https://doi.org/jctk.

[22] RIBAS-MAYNOU, J.; GARCIA-BONILLA, E.; HIDALGO, C.O.; CATALÁN, J.; MIRÓ, J.; YESTE, M. Species-Specific Differences in Sperm Chromatin Decondensation Between Eutherian Mammals Underlie Distinct Lysis Requirements. **Front. Cell Dev. Biol**. 9(4): 1–11. 2021. https://doi.org/jctm.

[23] RODRIGUEZ, H.; OHANIAN, C.; BUSTOS-OBREGON, E. Nuclear Chromatin Decondensation of Spermatozoa *in vitro*: A Method for Evaluating the Fertilizing Ability of Ovine Semen. **Int. J. Androl.** 8(2): 147–158. 1985. https://doi.org/cnt9dq.

[24] R TEAM CORE. A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna Austria. R Foundation for Statistical Computing: Vienna, Austria. Version 3.6.2. Pp 3879. 2019.

[25] SILVA, S.F.M.; OLIVEIRA, L.C.A.; DIAS, F.C.R.; CORDERO-SCHMIDT, E.; VARGAS-MENA, J.C.; SILVA, I.G.M.; BÁO, S.N.; LUNA, J.L.S.; LIMA, R.R.M.; JÚNIOR, R.F.A.; FARIAS, N.B.S.; MOURA, C.E.B.; MATTA, S.L.P.; MORAIS, D.B. Seasonal Evaluation of Spermatogenesis of the Hematophagous Bat Desmodus Rotundus in the Caatinga Biome. **PLoS One**. 15(12): 1–19. 2020. https://doi.org/jctn.

[26] TAYLOR, M.A.; GUZMÁN-NOVOA, E.; MORFIN, N.; BUHR, M.M. Improving Viability of Cryopreserved Honey Bee (*Apis Mellifera* L.) Sperm with Selected Diluents, Cryoprotectants, and Semen Dilution Ratios. **Theriogenol**. 72(2): 149–159. 2009.

[27] UGUR, M.R.; SABER ABDELRAHMAN, A.; EVANS, H.C.; GILMORE, A.A.; HITIT, M.; ARIFIANTINI, R.I.; PURWANTARA, B.; KAYA, A.; MEMILI, E. Advances in Cryopreservation of Bull Sperm. Front. **Vet. Sci**., 6(6): 1–15. 2019. https://doi.org/gg8vj5.

[28] WATHES, D.C.; OGUEJIOFOR, C.F.; THOMAS, C.; CHENG, Z. Importance of Viral Disease in Dairy Cow Fertility. **Engineering**. 6(1): 26–33. 2020. https://doi.org/jctp.

[29] WETTERE, W.H.E.V.; KIND, K.L.; GATFORD, K.L.; SWINBOURNE, A.M.; LEU, S.T.; HAYMAN, P.T.; KELLY, J.M.; WEAVER, A.C.; KLEEMANN, D.O.; WALKER, S.K. Review of the Impact of Heat Stress on Reproductive Performance of Sheep. **J. Anim. Sci. Biotechnol.** 12(1): 1–18. 2021. https://doi.org/jctq.

[30] WOLD HEALTH ORGANIZATION (WHO). WHO Laboratory Manual for the Examination and Processing of Human Semen, 5th. Ed.; World Health Organization. Pp 271. 2010.

[31] WU, T.F.; CHU, D.S. Sperm Chromatin: Fertile Grounds for Proteomic Discovery of Clinical Tools. **Mol. Cell. Proteomics**. 7(10): 1876–1886. 2008. https://doi.org/dt79p3.

[32] ZHENG, W.W.; SONG, G.; WANG, Q.L.; LIU, S.W.; ZHU, X.L.; DENG, S.M.; ZHONG, A.; TAN, Y.M.; TAN, Y. Sperm DNA Damage Has a Negative Effect on Early Embryonic Development Following in Vitro Fertilization. **Asian J. Androl.** 20(7): 75–79. 2018. https://doi.org/gbkh4x.