Severe alterations in sperm chromatin identified by transmission electron microscopy interfere in in vitro development of bovine embryos

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INTRODUÇÃO

- When observed under transmission electron microscopy, bull sperm chromatin is usually extremely electron-dense and homogeneous. However, changes from small spots to larger lighter regions are also often observed.
- The aim of this study was to verify the importance of these sperm chromatin changes identified by transmission electron microscopy on the in vitro fertilization and early embryonic development.

MATERIAIS E MÉTODOS

- For this study, five ejaculates from five different Giroland bulls with different levels of fertility were used. Part of each semen sample was used for in vitro embryo production (IVEP) routines and part was used for evaluation of sperm chromatin by transmission electron microscopy. One hundred and twenty IVEP routines were performed according to the protocol [1] of the Biology of Reproduction Laboratory of the Universidade Federal de Uberlândia, with approximately 40 oocytes per routine, totaling 4916 oocytes, all from slaughterhouse-collected ovaries. Cleavage rates of experimental bulls were determined 48 hours after in vitro fertilization, represented by the percentage of mature oocytes that started cleavage. Blastocyst rates were determined seven days after fertilization and were represented by the percentage of oocytes that started cleavage and reached the blastocyst stage. The rest of the semen samples were used for the evaluation of sperm chromatin by transmission electron microscopy [2]. On average 150 sperm head images of each sample were captured and classified into five grades of defects, as shown in figure 1. Pearson's correlation test was applied between each chromatin defect type and the cleavage and blastocyst rates

RESULTADOS

- **Table 1**. Pearson's correlation coefficients and p-value between the grades of chromatin defects and cleavage and blastocysts rates obtained in PIVEs

Defect category	Cleavage		Blastocyst	
	Coefficient	p-value	Coefficient	p-value
Grade 0	0,91	0,03	0,99	0,0002
Grade 1	0,55	0,33	0,82	0,09
Grade 2	-0,09	0,89	-0,48	0,41
Grade 3	-0,93	0,02	-0,98	0,003
Grade 4	-0,88	0,05	-0,98	0,003

DISCUSSÃO

- The high positive and significant correlations between cleavage and blastocyst rates and the percentage of sperm without chromatin defects show that, in general, the absence of changes in sperm chromatin favors the process of fertilization and the early embryonic development. Regarding blastocyst rates, as they were calculated based on the oocytes that started cleavage and not on the total oocytes that were placed for maturation, they can also be considered as a good parameter to evaluate the early embryonic development [3]. Therefore, the lack of significant correlation between cleavage rate and milder defects (grades 1 and 2) indicates that these defects have little or no effect on the oocyte fertilization process. Likewise, the lack of significant correlation between these defects and the blastocyst rate indicates that when sperm carriers with this type of chromatin defect fertilized the oocyte, the initial embryonic development is not impaired. Several study methods evaluate sperm chromatin. Most of them evaluate DNA fragmentation. However, other methodologies simply evaluate chromatin compaction [4]. What at first would be a methodological deficiency is actually a broader scope.
- In general, fragmentation is accompanied by chromatin decompression; however, the reverse is not true. An area of decompression is undoubtedly more sensitive to DNA damage, but it can also be a morphological expression of DNA defects or simply epigenetic alterations [5,6]. The most severe defects (grades 3 and 4) showed significant negative correlations with cleavage and blastocyst rates, indicating that these types of defects can interfere with the fertilization process and when a sperm with these defect types fertilizes the oocyte, the embryonic development initial is impaired. A possible explanation for this would be that among the most severe defects in chromatin (grades 3 and 4), some could lead to changes in sperm head shape, altering sperm hydrodynamics, leading to unsatisfactory motility for the fertilization process [5]. However, some of the defects classified as 3 and 4 may not be enough to interfere with the fertilization process, allowing oocyte fertilization. In general, this type of sperm is accompanied by epigenetic alterations and / or DNA fragmentation [6], would make embryonic development unfeasible [7].

CONCLUSÃO

- Severe alterations in sperm chromatin identified by transmission electron microscopy interfere in the oocyte fertilization process and mainly in the in vitro development of bovine embryos

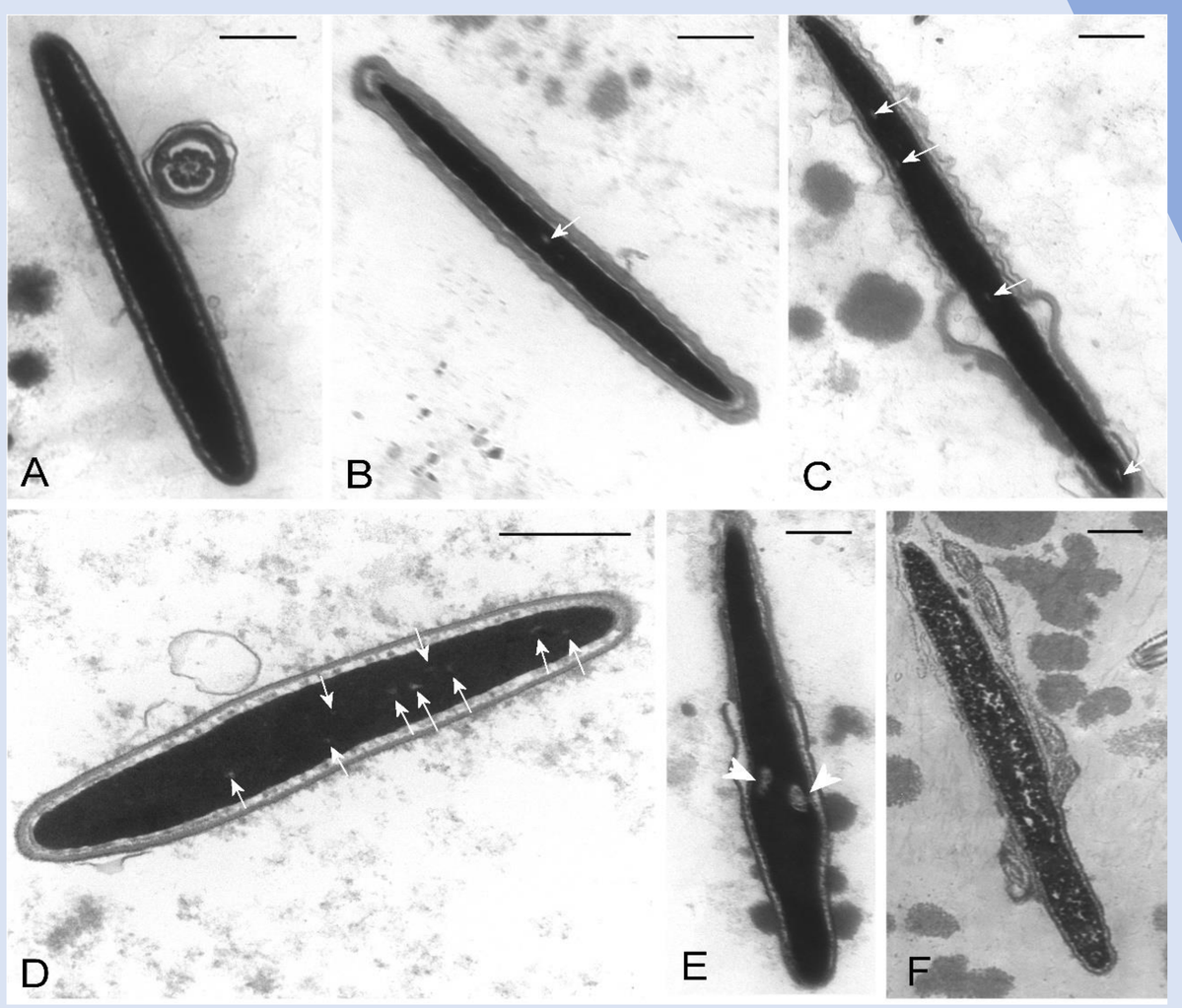

Fig. 1. Examples of sperm heads with each grade of chromatin defect. Grade 0 - absence of chromatin defects (A). Grade 1- presence of up to 3 small bright spots in chromatin (B). Grade 2 - presence of up to 6 small bright spots (C). Grade 3 - presence of several light points (above 6) (D) or lighter region(s) occupying up to a quarter of the sperm head (E). Grade 4 - lighter region(s) occupying above a quarter of the sperm head or large regions with granular chromatin (F). (arrow= bright spot; head arrow= lighter region; bar= 0.5µm)

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References
[1] A.C. Lucio et al., Theriogenology, 86 (2016) 924.
[2] J.M. Soares; M.E. Beletti, Braz. J. Vet. Res. An. Sci., 43 (2006) 554.
[3]M.S. Ortega MS et al., Biol. Reprod. 99 (2018) 1244.
[4] D. P. Evenson, Anim. Reprod. Sci., 169 (2016) 56.
[5] R. Oliva, Hum Reprod Update,12 (2006) 417.
[6] M. Muratori; C. De Geyter, Best. Pract. Res. Clin. Endocrinol. Metab., 33 (2019) 117.
[7] S. Colaco; D. Sakkas, J. Assist. Reprod. Genet., 35 (2018) 1953.