Relationship of residual feed intake with semen parameters and testicular ultrasound of Nellore bulls

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Abstract

Residual feed intake (RFI) is one of the main tools used to identify feed efficiency. However, there is no consensus on the true impact of RFI on male reproductive traits in cattle. The study aimed to evaluate characteristics of the testicular parenchyma and vascular parameters of the pampiniform plexus obtained by ultrasound, semen quality parameters, and sperm freezability in Nellore bulls classified based on RFI. Twenty-seven bulls (21.82±0.88 months of age) evaluated for feed efficiency were sampled for the study, including 15 with low RFI (-0.592±0.09 kg dry matter/day) and 12 with high RFI (0.792±0.10 kg dry matter/day). In ultrasound and Doppler assessment, the most efficient animals (low RFI) showed higher pulsatility and resistive indexes, as well as a tendency (*P*=0.061) towards greater heterogeneity of the testicular parenchyma (0.625±0.032 vs. 0.508±0.032; 1.012±0.072 vs. 0.802±0.072 and 12.9±0.96 vs. 10.2±0.96, respectively, for low vs. high RFI). However, these animals tended (*P*=0.0652) to have lower peak diastolic velocity (5.19±0.50 for low RFI vs. 6.54±0.50 for high RFI). Analysis of fresh semen showed a lower percentage of minor defects in low RFI animals (2.67±1.19%) compared to high RFI animals (8.10±1.19%), without differences in the other parameters in fresh or thawed semen and after thermoresistance testing. Evaluation of flow cytometry parameters showed a higher quality of mitochondrial respiration in semen samples of low RFI animals (22.04±2.50%) compared to high RFI animals (12.29±2.71%). In conclusion, although RFI exerts an effect on the Doppler parameters of the pampiniform plexus, it was not sufficient to affect the quality of fresh or thawed semen.

Introduction

Investments in feed account for more than half of the total cost of cattle production; consequently, improvements in feed efficiency are important for the growth of the livestock sector, directly affecting the profitability of livestock farming (Forbes, 2007). Growing attention has been given to the development of strategies that can increase herd efficiency, reducing both feed intake and the environmental impact of methane production by cattle (Herd et al., 2002; Sakamoto et al., 2021). Studies have been conducted over the past decades to better understand residual feed intake (RFI), which has proved to be an important tool that can be used in the genetic improvement of beef cattle (Grion et al., 2014; Ceacero et al., 2016).

Studies have reported an unfavorable relationship between fertility indices and feed efficiency in young cattle (Arthur et al., 2005; Basarab et al., 2007). Within this context, reports suggest that young bulls with low RFI (most efficient) produce ejaculates characterized by lower sperm motility (Awda et al., 2013) and lower semen quality based on sperm morphology (Hafla et al., 2013; Awda et al., 2013). However, Rossi (2017) and Kowalski et al. (2017) found no effect of RFI on the reproductive parameters of young bulls. Similarly, Ferreira Júnior et al. (2018) observed a low genetic and phenotypic correlation between RFI and scrotal circumference (SC), indicating a low or zero effect of feed efficiency on fertility traits of bulls.

Sperm quality is very important for the reproductive success of bulls, with direct effects on the results of field and *in vitro* fertility, which can even cause changes in the quality of the produced embryos (Saacke,

2009). Therefore, studies have investigated sperm function in detail in order to estimate the fertilization potential of bulls and to predict long-term male fertility from the collected semen samples (Freitas-Dell'Aqua et al., 2009).

Ultrasonography is a complementary technique to andrological examination and can be an excellent tool to examine the integrity of the testicular parenchyma, in addition to being a rapid and non-invasive method. Spectral Doppler ultrasound can be used to assess blood flow indices in the testicular arteries and pampiniform plexus and thus to estimate blood perfusion in this region (Ortiz-Rodrigues et al., 2017; Claus et al. 2019; Rodrigues et al., 2020). Studies have evaluated the relationship of these parameters with the semen quality of various species based on the testicular health of individuals (Pinggera et al., 2008; Ahmadi et al., 2013; Tomlinson et al., 2017; Camela et al., 2019; Gloria et al., 2018; 2020). Within this context, some studies observed lower echogenicity in low RFI animals when compared to high RFI animals, a finding that may indicate lower cellularity (Fontoura et al., 2016; Bourgon et al., 2018). On the other hand, Kowalski et al. (2017) found no difference between animals with distinct RFI.

The aim of the present study was to evaluate the characteristics of the testicular parenchyma obtained by B-mode ultrasound, vascular parameters of the pampiniform plexus measured by spectral Doppler, semen parameters, and sperm freezability in Nellore bulls classified for feed efficiency. We tested the hypothesis that selection for RFI affects the reproductive parameters of Nellore bulls.

Material And Methods

Study location and animals

The experiment was conducted at the Institute of Animal Science, Sertãozinho, SP, Brazil. Twenty-seven young Nellore males (17 born in 2016 and 10 born in 2017) were evaluated. The animals had participated in postweaning (7 months) feed efficiency tests, including 145 animals in 2017 and 128 animals in 2018. In the performance test, the animals were fed in collective pens equipped with electronic feed bunks for the recording of feed intake (GrowSafe® Systems Ltd., Airdrie, Alberta, Canada), with 28 days of adaptation and 77 ± 10 days of test.

The RFI was calculated as the residual of the regression equation according to the model proposed by Koch et al. (1963) within the contemporary group: $DMI = \beta0 + \beta W*BW^{0.75} + \beta G*ADG + \varepsilon$, where DMI is the mean dry matter intake during the test; β0 is the intercept of the equation; $BW^{0.75}$ is the mean metabolic body weight; ADG is the average daily weight gain; βW and βG are the regression coefficients of DMI on $BW^{0.75}$ and ADG, respectively, and ε is the residual of the equation, corresponding to RFI.

For this study, animals with extreme low (most efficient animals) and high RFI (least efficient animals) were chosen to compose the total samples. After the performance test, the animals were kept on *Brachiaria brizantha* pasture with free access to water and proteinated salt until the start of the

experimental period, when the animals were 21.5 ± 0.886 months old and had reached a body weight of 495 ± 62.2 kg.

The experiment lasted 21 days to obtain the ultrasonographic parameters and to perform the sperm evaluation of fresh and thawed semen from low and high RFI animals, as illustrated in Fig. 1.

Testicular ultrasound

Two testicular ultrasound assessments were performed, one at the beginning of the experimental period and one after 21 days. A Z5 Vet ultrasound apparatus (Mindray, Shenzhen, China) coupled to a linear 7.5-MHz transducer was used. Scans were performed in the longitudinal and transverse planes of the right and left testes. The images were analyzed using the Image Pro Plus 7.01 software (Media Cybernetics Inc., San Diego, CA, USA), with numerical grayscale pixel values ranging from 0 (absolute black) to 255 (absolute white) (Griffin et al., 2009).

Doppler ultrasound was applied to the region of the spermatic cord to determine the mean diameter of the testicular artery (Figure 2). Spectral Doppler was used for the measurement of vascular parameters using three waves for calculation: peak systolic velocity (PSV), end-diastolic velocity (EDV), vascular resistive index [RI = (PSV – EDV)/PSV], and pulsatility index [PI = (PSV – EDV)/M, where *M* is the mean PSV and EDV] (Wood et al., 2010; Feliciano et al., 2012).

Andrological evaluation

The SC was measured with a millimeter tape measure as recommended by the CBRA (2013). The animals were submitted to andrological evaluation every 7 days, totaling four assessments, with the first two for standard sperm evaluation (Figure 1). In the last two assessments, the semen was also cryopreserved. The semen samples were collected with an Autojac® electroejaculator (Neovet, Brazil).

Sperm concentration was measured with a photometer (SDM1, Minitube, Germany), calibrated for bovine semen, as the number of total spermatozoa per mL ejaculate. Sperm motility kinetics were analyzed by computer-assisted semen analysis (CASA; Hamilton Thorne Research, IVOS-14, USA). For this purpose, 10 µL of diluted semen sample was placed in a previously heated (38ºC) Makler chamber (SEFI Medical Instruments Ltd.®, Haifa, Israel) and five random fields were observed. The following CASA parameters were obtained: total motility (TM, %); progressive motility (PM, %); rapid motility (RAP, %); average path velocity (VAP, µm/s); straight line velocity (VSL, µm/s); curvilinear velocity (VCL, µm/s); amplitude of lateral head displacement (ALH, µm); beat cross frequency (BCF, Hz); straightness (STR, %), and linearity (LIN, %).

For the analysis of sperm morphology, the semen samples were stored in 500 µL of 4% saline-buffered formalin for examination by differential interference contrast (DIC) microscopy (Eclipse Ni-U, Nikon®, Tokyo, Japan). A total of 200 cells were counted and defects in the head, midpiece, tail, and acrosome were recorded. The anomalies were classified as major, minor, and total sperm defects (CBRA 2013).

Cryopreservation of semen

The semen collected in the last two samples was cryopreserved, totaling two batches of cryopreserved semen samples per bull. The semen was packaged at room temperature into 0.5-mL straws (IMV® Technologies, France), at a final concentration of 25×10^6 spermatozoa/straw (50×10^6 spermatozoa/mL). The diluent used was BotuBov (BotuPharma®, Botucatu, Brazil) containing 6.4% glycerol as cryoprotectant. A programmable portable semen cryopreservation system was used for refrigeration and cryopreservation (TK 4000®, Tetakon, Uberaba, Brazil), following a curve of 0.25ºC/min (±25ºC to 5ºC). After stabilization for 4 hours at 5ºC, cryopreservation was performed (-20ºC/min; 5ºC to -120ºC). Next, the straws were immersed directly in liquid nitrogen (-196ºC) and stored until the time of post-thaw analysis.

Post-thaw semen evaluation

Two cryopreserved straws of each sample per bull were thawed in a water bath at 37ºC for 30 seconds. The samples were submitted to thermoresistance testing (TRT) in a water bath at 46ºC for 30 min (Barnabé et al., 1980; Crespilho et al., 2008). After thawing and after TRT, the semen samples were analyzed by CASA as described above.

Analysis of membrane integrity

A BD LSR II flow cytometer (Becton Dickinson, Mountain View, CA, USA) equipped with blue (488 nm, 100 mW), red (640 nm,40 mW), and violet (405 nm, 100 mW) lasers was used for flow cytometry. The filter configurations for the photomultiplier tubes measuring fluorescence emission of the applied fluorochromes were 450/50 nm (H342), 530/30 nm (FITC), 660/20 nm (APC), and 694/50 nm (PI). Data were analyzed using the BD FACSDiva v6.1 software.

The semen samples were diluted in TALP-PVA (100 mM NaCl, 3.1 mM KCl, 25.0 mM $NaHCO_3$, 0.3 mM NaH_2PO_4, 21.6 mM DL-sodium lactate 60%, 2.0 mM $CaCl_2$, 0.4 mM $MgCl_2$, 10.0 mM acid-free Hepes, 1.0 mM sodium pyruvate, 1.0 mg/mL polyvinyl alcohol-PVA, and 25 µg/mL gentamicin) at a concentration of 5×10^6 spermatozoa/mL, supplemented with Hoechst 3342 (7 µM diluted in distilled water; H342; 14533, Sigma Aldrich, Darmstadt, Germany) for the elimination of debris, according to the method of Freitas-Dell'Aqua et al. (2012).

Propidium iodide (P4170; Sigma Chemical Company, St. Louis, MO, USA) and fluorescein isothiocyanate-conjugated *Pisum sativum* agglutinin (FITC-PSA; L0770, Sigma) were used for the evaluation of plasma and acrosome membrane integrity. A 200-µL semen sample was diluted in TALP-PVA medium to a concentration of 5×10^6 spermatozoa/mL and mixed with 1.5 µM propidium iodide and 0.5 mL FITC-PSA (2 mg/mL) [49]. The subpopulation identified in this analysis were spermatozoa containing intact plasma and acrosomal membranes (MPAI).

Mitochondrial membrane potential and plasma membrane stability were assessed using the combination of MitoStatusRed (MST; mitochondrial potential) and Yo-Pro® (YP; Y3603 Life Technologies, Darmstadt, Germany). For this purpose, YP (25 nM) and MST (20 µM) were added to the 500-µL semen aliquots extended in TALP-PVA and the samples were incubated for 20 min at 37ºC.

Lipid peroxidation was assessed using C11-BODIPY as fluorescent probe (D-3861; Molecular Probes, Carlsbad, CA, USA). Each semen aliquot (2 million sperm/mL TALP-PVA extended in 489.5 µL) was added to C11-BODIPY 581/591 (0.5 µL, solution 1 mg/mL). After incubation, the samples were washed two times at 300 × *g* for 5 min and the pellet was resuspended in 500 µL TALP-PVA and analyzed by flow cytometry (Guasti et al., 2012).

Statistical analysis

The results were submitted to analysis of variance considering repeated measures using the PROC MIXED procedure of the Statistical Analysis System (SAS) program. The following statistical model was adjusted: y = µ + RFI + evaluation + RFI × evaluation + age + e, where *y* = response variable; *µ* = overall mean; RFI = effect of RFI class (low, high); evaluation = effect of evaluation class (i = 1, 2 or i = 1,..., 4, depending on the variable); age = linear effect of the covariate age of animal at evaluation, and *e* = error. The repeated measures of the same animal were modeled considering compound symmetry (CS) as residual (co)variance structure.

Means were adjusted by the least squares method (LSMEANS) and compared by the probability of difference (PDIFF), when necessary. Statistical significance was set at $P<0.05$ and a tendency was considered when $0.05>P<0.1$.

Results

Animals classified as low RFI exhibited higher RI and PI ($P=0.019$ and $P=0.049$, respectively) than high RFI animals. However, EDV tended to be lower in low RFI animals compared to high RFI ($P=0.065$). In addition, a tendency towards greater testicular heterogeneity was observed in low RFI animals ($P=0.061$). There were no differences in the other ultrasound variables evaluated between low and high RFI animals (Table 1).

Table 1
Least square means of ultrasound-measured testicular traits of Nellore bulls according to residual feed intake class

Trait	RFI		SEM	*P*-value
	Low	High		
RFI (kg DM/day)	-0.592	0.792	0.0654	<0.0001
Diameter (mm)	2.62	2.56	0.0863	0.592
PSV (cm/s)	13.9	13.9	0.773	0.995
EDV (cm/s)	5.19	6.54	0.498	0.0652
RI	0.625	0.5083	0.0334	0.0196
PI	1.012	0.8022	0.0726	0.0492
Mean pixel intensity	65.6	62.3	2.44	0.339
Minimum pixel intensity	32.9	31.6	1.29	0.463
Maximum pixel intensity	127	122	2.39	0.164
Heterogeneity	12.9	10.24	0.964	0.061

RFI: residual feed intake; SEM: standard error of the mean; Diameter: diameter of the testicular artery; PSV: peak systolic velocity; EDV: end-diastolic velocity; PI: pulsatility index; RI: vascular resistive index.

No differences were observed in the characteristics of fresh semen between RFI groups, except for minor defects whose percentage was lower in semen of low RFI animals compared to high RFI animals (Table 2). Similarly, there were no differences in the sperm kinetic parameters evaluated between most and least efficient animals after thawing and after rapid TRT (Table 3).

Table 2
Least square means of sperm kinetic parameters of fresh semen from Nellore bulls according to residual feed intake class

Parameter	RFI		SEM	*P*-value
	Low	High		
RFI (kg DM/day)	-0.592	0.792	0.046	<0.0001
SC (cm)	33.08	33.05	0.673	0.963
TM (%)	83.9	84.6	1.87	0.793
PM (%)	60.67	62.61	2.12	0.519
RAP (%)	80.94	81.31	2.11	0.899
VAP (μm/s)	106.7	105.7	2.31	0.825
VSL (μm/s)	83.6	84.3	2.018	0.819
VCL (μm/s)	182.4	182.6	6.49	0.985
ALH (μm)	7.05	7.15	0.234	0.8002
BCF (Hz)	33.8	29.9	3.56	0.460
STR (%)	79.5	80.7	1.06	0.439
LIN (%)	49.6	49.7	0.991	0.935
Major defects (%)	12.2	9.78	3.22	0.583
Minor defects (%)	2.61	8.27	1.19	0.0024
Total defects (%)	14.9	17.9	3.21	0.511

RFI: residual feed intake; SEM: standard error of the mean; SC: scrotal circumference; TM: total motility; PM: progressive motility; RAP: rapid motility; VAP: average path velocity; VSL: straight line velocity; VCL: curvilinear velocity; ALH: amplitude of lateral head displacement; BCF: beat cross frequency; STR: straightness; LIN: linearity.

Table 3
Least square means of sperm kinetic parameters after thawing and rapid thermoresistance testing of Nellore bulls according to residual feed intake class

Parameter	RFI		RFI		SEM	*P*-value		
	Low	High	Low	High		RFI	Time	RFI*TRT
Semen	Thawed		After TRT					
TM (%)	48.8	47.2	36.3	33.2	6.59	0.788	<0.001	0.792
PM (%)	38.3	37.6	30.67	28.4	5.43	0.836	0.0021	0.762
RAP (%)	44.7	43.0	33.3	30.8	6.32	0.8102	<0.001	0.874
VAP (μm/s)	32.8	31.5	23.3	24.6	2.09	0.9901	<0.001	0.528
VSL (μm/s)	29.8	27.9	22.6	23.2	1.71	0.839	<0.001	0.575
VCL (μm/s)	54.6	53.6	38.3	37.3	3.24	0.777	<0.001	0.991
ALH (μm)	2.65	2.54	2.10	2.01	1.17	0.648	<0.001	0.944
BCF (Hz)	14.9	15.7	14.8	15.3	0.56	0.236	0.665	0.788
STR (%)	13.2	11.8	10.06	9.94	1.44	0.5306	<0.001	0.333
LIN (%)	17.8	16.9	15.7	15.0	0.75	0.349	0.0107	0.918
LP (AU)	98.4	84.0	207.3	206.9	9.98	0.084	<0.001	0.380

RFI: residual feed intake; SEM: standard error of the mean; TRT: thermoresistance testing; TM: total sperm motility; PM: progressive motility; RAP: rapid motility; VAP: average path velocity; VSL: straight line velocity; VCL: curvilinear velocity; ALH: amplitude of lateral head displacement; BCF: beat cross frequency; STR: straightness; LIN: linearity; LP: lipid peroxidation.

P-value. RFI: between RFI classes in the analysis of thawed semen; Time: between time 0 (thawed semen) and TRT (30 min at 46^{o}C); RFI*TRT: interaction between RFI and TRT.

Figure 1 shows the results of flow cytometry analysis. The percentages of cells with a stable plasma membrane (PMStable, Fig. 2A) and MPAI (Fig. 2B) were similar between low and high RFI animals. There was also no difference in the percentage of cells with high mitochondrial potential between low and high RFI animals (HMP, Fig. 2C). However, the quality of high mitochondrial potential of stable cells (HMPStable, Fig. 2D) was greater in low RFI animals compared to high RFI animals (P = 0.013).

Fig. 2. Flow cytometry analysis of cryopreserved semen from bulls with low and high residual feed intake (RFI). A: Percentage of spermatozoa with a stable plasma membrane (PMStable). B: Percentage of cells with intact plasma and acrosome membranes (MPAI). C: Percentage of cells with high mitochondrial

potential (HMP). D: Quality of the mitochondrial potential of stable cells (HMPStable). The asterisk indicates a significant difference (P<0.05).

Discussion

Spectral Doppler ultrasound analysis of the testicular artery showed a tendency towards a lower EDV in low RFI animals when compared to high RFI animals. The PSV and EDV represent the velocity at which blood flows through the analyzed blood vessel and reaches the tissue. According to Ortiz-Rodriguez et al. (2017), the higher the velocity at which blood passes through the testicular artery, the better the blood perfusion in the testis. Studies have reported a relationship between blood flow velocity and male reproductive capacity in different animal species (Kutzler et al., 2011; Ortiz-Rodriguez et al., 2017; Gacem et al., 2020; Lemos et al., 2020).

In the present study, higher RI and PI were observed in low RFI animals compared to high RFI animals. According to Pozor and McDonnel (2004), PI and RI are more sensitive markers of blood flow than EDV and PSV since these indexes provide not only information about velocity but also about vascular impedance. The PI and RI represent the difficulty of blood to flow through the vessel; the higher these indexes in the testicular artery, the lower the testicular tissue perfusion and consequently the supply of oxygen and nutrients to the testes (Strina et al., 2016; Fávaro et al., 2020). Parenchymal organs require continuous blood flow and the arteries that supply these structures typically have low resistance (Carvalho et al., 2008).

Analysis of pixel intensity of the testicular parenchyma revealed no differences between high and low RFI animals. This result corroborates the findings of Kowalski et al. (2017) who did not observe a difference between RFI classes in young developing Purunã bulls. On the other hand, Fontoura et al. (2016) and Bourgon et al. (2018) found higher maximum pixel intensities in animals with lower feed efficiency (high RFI). This difference might be related to the age of the animals in the cited studies since pixel intensity of the testicular parenchyma is higher before and during puberty (Brito et al., 2004; Rodrigues et al., 2020). The hypothesis to explain this difference is that spermatogenesis starts at a certain stage of development of the testicular parenchyma during puberty (Kastelic and Brito, 2012). Furthermore, the breed may also be a determinant factor, as reported by Rodrigues et al. (2020) who observed differences in testicular pixel intensity between zebu (Nellore) and taurine (Caracu) animals.

Observing the ultrasound results, the tendency towards a difference (P=0.061) between RFI classes might be related to differences in the number or diameter of the seminiferous tubules, which could affect the heterogeneity of the testicular parenchyma (Brito et al., 2012). The testicular tissue is homogenous and moderately echogenic. This state can change during puberty or in the presence of testicular pathology that can alter homogeneity and increase the pixel intensity as a result of fibrotic processes (Kastelic and Brito, 2012). Despite these differences in the ultrasound parameters of the testicular artery and vascular parameters of the pampiniform plexus between low and high RFI animals, they were not sufficient to cause differences in SC or in the quality of sperm motility in these animals.

The mean SC did not differ between low and high RFI animals. Similar results have been reported in previous studies comparing bulls with distinct RFI values (Hafla et al., 2013; Wang et al., 2012; Fontoura et al., 2016; Kowalski et al., 2017). On the other hand, Awda et al. (2013) and Bourgon et al. (2018) observed a greater SC in high RFI animals but the difference decreased when the animals received better-quality diet, suggesting that this difference in reproductive parameters between low and high RFI animals is due to the energy distribution for maintenance and production and reproductive traits. The authors suggested that, in animals with low RFI, reproductive parameters may have a lower priority, a fact delaying sexual maturity.

Although several studies have associated vascular parameters with semen quality (Gloria et al., 2018; Hedia et al., 2019; Gacem et al., 2020), the higher EDV in least efficient animals and the higher RI and PI in most efficient animals observed in the present study were not sufficient to cause alterations in the seminal parameters studied. Evaluation of the parameters of fresh and thawed semen and after TRT showed that feed efficiency did not affect sperm kinetics since no differences in CASA parameters were detected between low and high RFI animals. The results of the present study corroborate other studies that evaluated sperm motility or progressive motility in low and high RFI animals (Hafla et al., 2013; Awda et al., 2013; Fontoura et al., 2016; Bourgon et al., 2018). However, some authors changed the division of low and high RFI classes and reported different results. Including body composition traits in the equation for calculating RFI, Fontoura et al. (2016) found higher total and progressive sperm motility in least efficient (high RFI) animals when compared to most efficient (low RFI) animals, which was not observed in the present study. On the other hand, Wang et al. (2012) observed lower sperm motility in low RFI animals. However, this difference was not sufficient to reduce the fertility of breeding animals, with the most efficient animals having a larger number of offspring. It should also be noted that the mean values reported by the cited authors are considered excellent for andrological examination (Kennedy et al., 2002; Penny, 2010; CBRA, 2013).

When the sperm morphology of fresh semen was analyzed, we found only differences in the percentage of minor defects, with most efficient (low RFI) animals exhibiting a smaller number of defects than least efficient (high RFI) animals. This result contradicts most of the studies that compared sperm morphology between low and high RFI bulls and did not observe any difference (Wang et al., 2012; Bruinjé et al., 2019) or observed higher percentages of sperm pathologies in low RFI animals (Hafla et al., 2013; Fontoura et al., 2016; Bourgon et al., 2018). Despite the significant difference in sperm morphology observed here between animals with distinct RFI, the mean values are within the range recommended by different andrology handbooks (Kennedy et al., 2002; Penny, 2010; CBRA, 2013; Chenoweth; McPherson, 2016).

The percentages of spermatozoa with a stable plasma membrane, with intact plasma and acrosome membranes, and with high mitochondrial potential were similar between low and high RFI animals. These results corroborate some of the findings reported by Bruinjé et al. (2019); however, these authors reported a higher percentage of mitochondrial respiration activity in spermatozoa from low RFI animals, while the proportion of cells with low mitochondrial potential was higher in these animals. Other studies observed

higher rates of mitochondrial activity in liver tissue (Lancaster et al., 2014), in *longissimus dorsi* muscle (Kolath et al., 2006), and in lymphocytes (Ramos; Kerley, 2013) of most efficient animals (low RFI).

In the present study, although there was no difference in the proportion of cellular respiration of sperm between the two RFI classes, most efficient animals (low RFI) exhibited a better quality of cellular respiration of stable cells in cryopreserved semen. These results may explain why the lower blood flow observed in the testicular artery of low RFI animals was not sufficient to change the sperm kinetics of fresh or thawed semen. One hypothesis would be that sperm cells of low RFI bulls are more efficient in energy production, requiring less blood supply, or that they are adapted to a lower nutritional demand since mitochondria can be partially influenced by the surrounding environment, particularly by other organelles (Keil et al., 2011).

The results suggest that RFI does not influence sperm kinetics nor the sensitivity of sperm to cryopreservation; however, feed efficiency influences blood flow in the vascular cone, increasing the difficulty of blood to pass through the testicular arteries and to reach the testes in most efficient animals. However, cellular metabolism may have compensated for the lower availability of nutrients for sperm cells.

In conclusion, low RFI bulls have lower blood flow in the pampiniform plexus, resulting in greater heterogeneity of the testicular parenchyma evaluated by B-mode and Doppler ultrasound. On the other hand, the reduced blood flow in the pampiniform plexus of low RFI bulls was not sufficient to change sperm kinetics, indicating that the RFI class does not affect the quality of fresh semen, thawed semen, or semen after rapid TRT.

Declarations

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Competing Interests. The authors have declared that no competing interests exist.

Ethics Approval. The Animal Use Ethics Committee of the Faculty of Agricultural and Veterinarian Sciences, UNESP, Jaboticabal SP, Brazil, approved the project. The study was conducted in accordance with the Ethical Guidelines on Animal Experimentation adopted by the National Council for the Control of Animal Experimentation (CONCEA) (Protocol 08791/19).

Consent to participate. Not applicable

Consent to publish. Not applicable

Data Availability. All relevant data are within the manuscript. All original data are available on request from the authors.

Code Availability. Not applicable

Author Contributions. Marcelo Sant'Ana Borges: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing **Marina de Oliveira Silva:** Investigation **Luana Gomes Fernandes:** Investigation **Naiara Nantes Rodrigues:** Investigation **Guilherme Fazan Rossi:** Conceptualization, Methodology, Investigation **Camila de Paula Freitas Dell'Aqua:** Investigation, Writing – original draft **Sarah Figueiredo Martins Bonilha:** Resources **Maria Eugênia Zerlotti Mercadante:** Conceptualization, Formal analysis, Resources, Writing – original draft, Writing – review & editing **Fábio Morato Monteiro:** Conceptualization, Methodology, Formal analysis, Investigation, Resources, Data curation, Writing – original draft, Writing – review & editing

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Figures

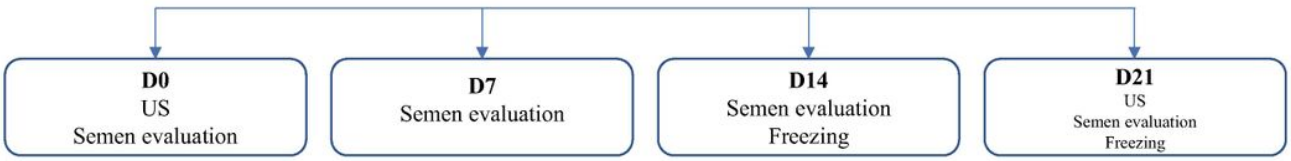

Figure 1

Flow diagram of the experimental period. US: ultrasound.

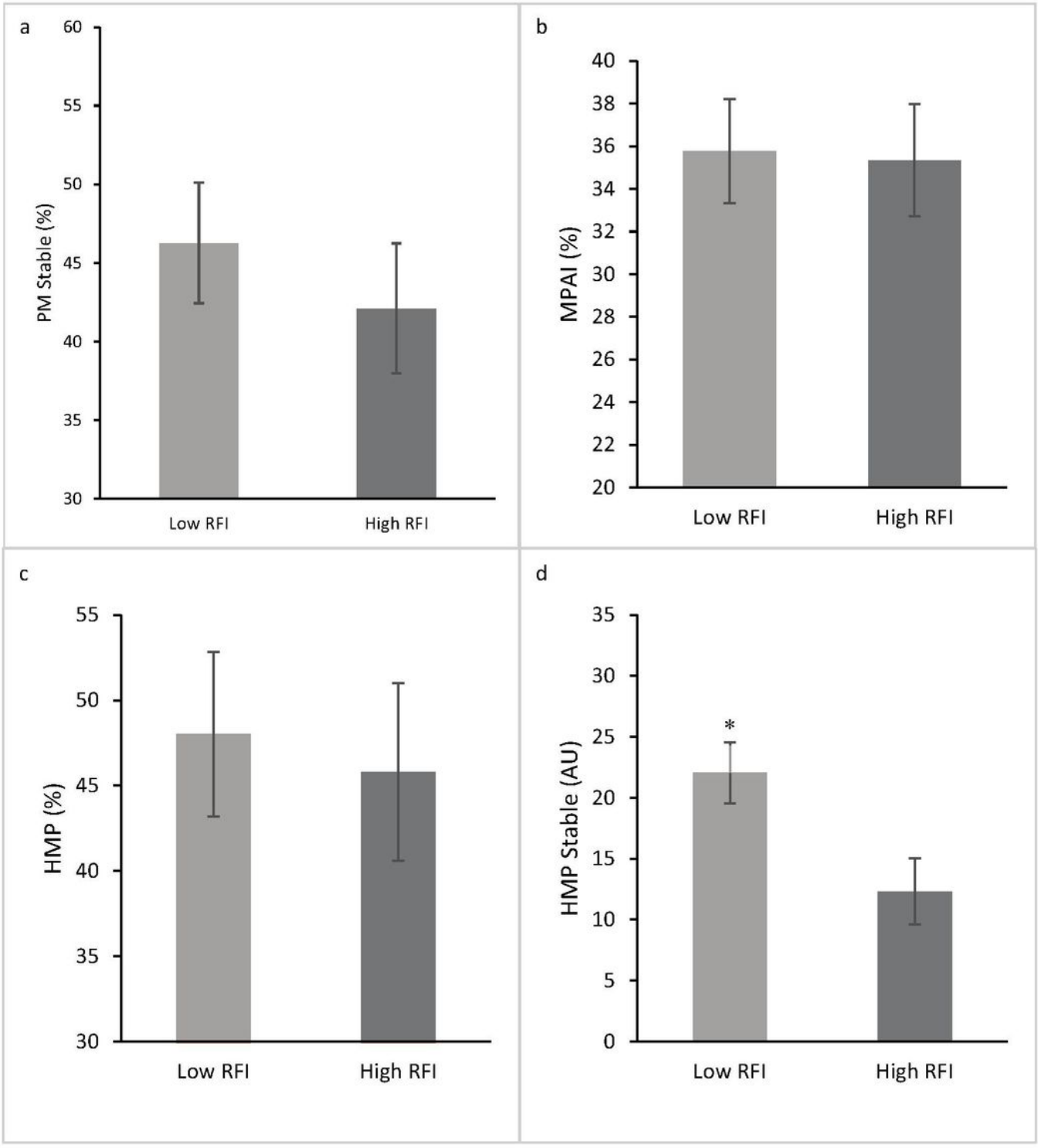

Figure 2

Flow cytometry analysis of cryopreserved semen from bulls with low and high residual feed intake (RFI). A: Percentage of spermatozoa with a stable plasma membrane (PMStable). B: Percentage of cells with intact plasma and acrosome membranes (MPAI). C: Percentage of cells with high mitochondrial potential (HMP). D: Quality of the mitochondrial potential of stable cells (HMPStable). The asterisk indicates a significant difference ($P<0.05$).