Reproductive developmental dynamics during the early and intermediate prepuberal periods in Nelore breed (*Bos indicus*) calves

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Short title: Reproductive and biometric changes in prepubertal calves

Abstract

The demand for calves as oocyte donors for in vitro embryo production is growing. However, Bos indicus have a late puberty, and some aspects of the reproductive physiology during the prepubertal period remain unclear. We characterized endocrine and morpho-functional reproductive features in Nelore calves (n=8) at 2- 5 (early prepubertal period, EPP) and from 8-11 months old (mo., intermediate prepubertal period, IPP). The calves' ovaries and uterus were B-mode transrectal ultrasonography examined, and blood samples were collected every second week. The antral follicles number and size, and ovarian and uterine horn diameters, were recorded, and plasma FSH and LH concentrations were measured (RIA). Non-pregnant, non-lactating cyclic Nelore cows (n=8) were used as controls for endocrine endpoints. Somatic development was monitored by monthly weighing, and 3D scanning of the rump area. The somatic and endocrine endpoints were compared within and between EPP and IPP, and between each period and control cows. Associations were determined by the Spearman correlation method, and the developmental rates were determined by non-linear regression. All morphological endpoints, except antral follicle count, increased ($P < 0.001$) from the EPP to the IPP. However, within each period differences occurred only at EPP. During the EPP LH and FSH plasma concentrations were similar ($P > 0.05$), whereas during the IPP LH was lower ($P < 0.05$) and FSH was higher ($P < 0.001$) than control cows. The EPP calves showed moderate to high positive correlations among ovarian, uterine, and somatic endpoints. Conversely, the IPP such correlations were mostly weak. In summary, distinct

ovarian activity and development patterns of primary and secondary sexual characteristics occurred in Nelore calves at 2- 5 mo compared to 8-11 mo.

Keywords: *Bos indicus*, puberty, reproductive physiology, rump development.

Introduction

The period from birth until puberty is marked by a number of changes that will lead to the maturity of the hypothalamus-hypophysis-ovary axis and prepare the reproductive system for the successful generation of viable offspring. In cattle, as in many other species, the ovary is active during this period, and follicle recruitment and growth up to the antral phase will occur [1-4]. Sexual steroids produced by growing ovarian follicles promote the development of the tubular part of the reproductive system, but they also play a crucial role in somatic development, acting in fat deposition [5] and in muscle [reviewed by 6] and bone development [5, 7, 8]. However, final follicle maturation and subsequent ovulation will not occur before puberty, mainly due to a higher sensitivity of the hypothalamus to the negative feedback of ovarian estradiol [9], which prevents further increases in FSH and LH.

In the past, studies on cattle puberty focused on the physiology of the events during the peripubertal period, particularly from 8 mo on, aiming to reduce the age at puberty [10]. However, the possibility of generating offspring from prepubertal cattle by the use of *in vitro* embryo production (IVEP) technologies renewed the interest in understanding the reproductive physiology during earlier prepubertal phases. The production of offspring

62 from prepubertal cattle by IVEP has been reported since the 1990s [11, 12]. However,
63 low embryo rates and the technical difficulties for oocyte recovery overcame the economic
64 advantages of using donors with genetic merit still undetermined. This scenario changed
65 with the recent advances in genomics and the development of commercial microchips to
66 evaluate the genomic-predicted transmitting ability (GPTA) for type and production traits
67 in different cattle breeds [13]. Selection of genetically superior cattle can now be
68 determined from birth [13], and this has boosted the demand for the use of prepubertal
69 calves as oocyte donors.

70 Two main techniques are currently used to recover oocytes from prepubertal
71 female cattle, namely laparoscopic and ultrasound-guided follicle aspiration (LOPU and
72 OPU, respectively). The LOPU is used mainly in calves up to 5 mo, when rump
73 development limits the rectal access to the ovaries. On the other hand, OPU has been
74 the technique of choice for older calves and prepubertal heifers [14]. In this regard, the
75 sequence of events that occur in these early time windows are key to understand how to
76 improve the developmental potential of oocytes recovered from calves. Despite the
77 occurrence of antral follicle growth before puberty, oocytes recovered from prepubertal
78 calves have lower developmental potential compared to those recovered from pubertal
79 heifers or cows [15, 16]. The causes underlying this difference are not fully understood,
80 but changes in gonadotropin stimulation and in subsequent steroid production play a
81 central hole in oocyte developmental potential. Transplantation of grafts from calf ovary

82 to adult cows restores oocyte potential [9], whereas treatment with exogenous FSH or
83 eCG increases the blastocyst rates obtained with calf oocytes [10].

84 The prepubertal period can be subdivided in phases characterized by distinct
85 morphological and endocrine features. In male calves, the infancy period lasts up to 3 mo
86 and is associated with a low LH secretion. After that, LH pulse frequency increases
87 throughout the prepubertal period. In females, ovary size, antral follicle count (AFC), and
88 dominant follicle (DF) maximum size increase up to 4 mo, plateau between 4 and 8 mo,
89 and resume increase until puberty [1]. It should be noted that, however, most studies
90 addressing the reproductive physiology during the prepubertal period were done in *Bos*
91 *taurus* breeds [1, 16, 17]; *B. taurus indicus* cattle is less precocious than European breeds
92 (*B. Taurus* [18, 19]), with significant differences in ovarian physiology, such as a higher
93 antral follicle count (AFC) and a lower diameter of the dominant follicles [20]. Thus, it is
94 likely that the timing or the magnitude of the events that will take place in the early
95 prepubertal period may also differ from those reported for *B. taurus*.

96 The aim of the present study was to investigate the endocrine and morpho-
97 functional reproductive characteristics of Nelore calves in two time windows, namely at
98 the early and intermediate prepubertal periods. We hypothesized that these periods are
99 characterized by distinct patterns of ovarian activity, which in turn drive reproductive and
100 somatic development patterns in each phase.

101

102

Materials and Methods

Animals and location

This study was conducted at the Sucupira Experimental Farm, Embrapa Genetic Resources and Biotechnology, Brasilia, Brazil. The contemporaneous Nelore calves (n = 8) used in the present study were generated by timed artificial insemination (TAI) with X-sorted semen. For endocrine endpoints, non-pregnant and non-lactating pluriparous cows (n = 8) from the same herd were used as controls. Calves were raised under pasture (*Brachiaria decumbens*), with *ad libitum* access to water and minerals. Weaning occurred at 7 mo. The average daily weight gain during the experiment was 0.57 ± 0.2 kg/day (from an average of 90.9 ± 8.3 kg BW at the beginning to 245.0 ± 12.3 kg BW at the end of the experiment).

Experimental design

Two evaluation periods were defined: 2 to 5 mo, referred to as early prepuberal period (EPP), and 8 to 11 mo, referred to as intermediate prepubertal period (IPP). These time windows were chosen to represent the initial and intermediate prepubertal periods, taking in account the expected age at puberty in the Nelore breed (22 to 36 mo, [21]) as well as the ages at which LOPU and OPU are usually performed in calves. In each period, calves were examined weekly by transrectal ultrasonography to measure ovarian and uterine horn diameters (mm), total antral follicle count (AFC), the number of follicles ≤ or > 4 mm, and

123 the diameter of the largest follicle (mm). Blood samples were collected once a day for FSH
124 and twice a day for LH plasma evaluations, at the same day of the ultrasonographic
125 exams. Once a month, the calves were weighted and underwent rump biometric
126 measurements. When calves reached 15 mo, monthly ultrasound scanning was used to
127 determine the age at puberty, based on the first detection of a corpus luteum.
128

129 ## Ultrasonographic evaluations

130 Ovarian and uterine evaluations were performed using a B-mode ultrasound
131 (MyLab 30 VetGold, Esaote. Genova, Italy) equipped with a 5- to 7.5-MHz linear rectal
132 probe. For all exams, settings related with frequency (7.5 MHz), focus depth, and gain
133 adjustments were standardized. The AFC and the number of follicles ≤ or > 4 mm were
134 determined visually during ovarian scanning. Linear measurements of the diameter of the
135 ovary, of the largest follicle present, and of the uterine horns were done using the internal
136 caliper of the ultrasound. During the first evaluation period (2 to 5 mo, EPP), a probe
137 guide was used to enable transrectal examination without rectal manipulation of the
138 genital tract, whereas for the second period (8 to 11 mo, IPP), as well as for cows,
139 conventional transrectal scanning procedures were used.
140

141 ## Blood samples and hormonal measurements

142 Blood samples were collected by jugular vein puncture using 21G, double-lumen
143 needles and vacuum tubes with EDTA. The samples were kept on ice until being
144 centrifuged (3,000 x g for 15 min), and 1-mL aliquots of plasma were stored at -20°C. The
145 LH and FSH analyses were performed by radioimmunoassay (RIA) as described by [22]
146 at the Laboratory of Endocrinology of the Sao Paulo State University (UNESP, Araçatuba,
147 SP, Brazil). Assay sensitivity was 0.126 ng/mL for LH and 0.390 ng/mL for FSH. The intra-
148 assay coefficient of variation was < 11%.

149

150 ## Rump biometric data

151 Rump width, length, and geometry were calculated using as reference points the
152 prominences of the *tuber ischiae* and the *tuber coxae* of the pelvic bones. Three-
153 dimensional images of the rump area were acquired by structured infrared light scanning,
154 using a portable sensor (iSense, 3D Systems, Rock Hill, SC, USA) connected to a tablet
155 computer (iPad Air 2, Apple Inc., Cupertino, CA, USA). This computer was equipped with
156 a real-time scanning app (https://itunes.apple.com/us/app/isense/id807510940). The
157 nominal resolution of this equipment at 0.5 m distance was 0.9 mm for the x/y axes and
158 1.0 mm for the z axis (depth). The points cloud data were transformed in a geometric
159 surface and stored as OBJ files. The 3D images were then edited using the open-source
160 software MeshLab (SourceForge, USA) to delete unspecific scans from the nearby

161 objects and to perform the measurements. As a validation procedure, manual
162 measurements were taken using a metric tape. There were no significant differences in
163 rump width or length, as measured directly with scales or obtained in the 3D images, and
164 only the latter were used in this study. The rump area was defined as the area of the
165 trapezium formed by the width at the ilium, the width at the ischium, and the rump length.
166 Changes in rump geometry were demonstrated in snapshots taken from the back view of 3D
167 images from calves at 2, 5, 8, and 11 mo. The trapeziums were drawn using the same
168 reference points as for biometric measurements.
169

170 ## Statistical analysis

171 Data of continuous outcome variables were first examined for normality and
172 homogeneity of variances using the Shapiro-Wilk and Bartlett tests. Variables with normal
173 distribution were evaluated by ANOVA, differences between parity groups (calves vs.
174 cows) or between prepubertal moments (EPP vs. IPP) were compared using the Student
175 t test, otherwise analyzed using the Mann-Whitney test. The association between
176 reproductive and biometric data was evaluated using Spearman's correlation. The
177 reproductive developmental rate was evaluated by non-linear regression analysis
178 (Gompertz curve), and the validation of this curve was tested by the deviance of the fitted
179 curve compared to a straight line. The Gompertz model equation was Z *exp (-b*exp (a*x),
180 where "Z" is the maximum data value, "b" is the integration constant, and "a" corresponds
181 to the acceleration rate. This non-linear regression analysis (Gompertz curve) was also

182 used to compare rump geometry changes at different ages. All statistical procedures were
183 performed using the GraphPad Prism (v. 6) or the R (v. 3.6.1) software, and a P value ≤
184 0.05 was considered as statistically significant. Results are shown as mean ± SEM.
185

186 # Results

187 The results of the ultrasonographic evaluation of the calves' ovaries and uterus
188 during the EPP and IPP are shown in Table 1. The ovarian and the uterine diameter, the
189 number of follicles larger than 4 mm, and the diameter of the largest follicle were greater
190 in IPP calves compared to EPP calves ($P < 0.001$). However, there was no difference (P
191 > 0.05) in AFC between EPP and IPP. The EPP calves had LH and FSH plasma
192 concentrations similar ($P > 0.05$) to cows, whereas IPP calves had lower LH ($P < 0.05$)
193 and higher FSH ($P < 0.001$) concentrations than cows (Fig 1, A-D).
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201 **Table 1. Average values (mean ± SD) of reproductive and somatic endpoints in**
202 **Nelore calves during two time windows in the prepubertal period: from 2 to 5 and**
203 **from 8 to 11 months old.**

	Age group		
Endpoint	2–5 months	8–11 months	P value
Ovarian diameter (mm)	16.5 ± 2.4 [A]	18.1 ± 2.1 [B]	< 0.0001
Antral follicle count (n)	20.7 ± 6.7	22.0 ± 8.9	0.5412
Number of follicles > 4mm	3.4 ± 1.6 [A]	4.9 ± 2.2 [B]	< 0.0001
Diameter of the largest follicle (mm)	7.2 ± 1.5 [A]	7.9 ± 1.3 [B]	0.0001
Uterine diameter (mm)	8.4 ± 1.0 [A]	10.1 ± 1.0 [B]	< 0.0001
Average body weight	118.8 ± 22.9 [A]	227.0 ± 18.7 [B]	< 0.0001
Mean rump area (cm^2)	514.2 ± 101.4	856.7 ± 79.1	-------

204 Different letters in the same line are different. T-test and Mann-Whitney analysis, P<0.05.
205
206 **Fig 1 (A-D). Comparison of plasma LH (A, B) and FSH (C, D) concentrations (ng/mL)**
207 **in Nelore calves during two prepubertal periods (black bars) and non-pregnant, non-**
208 **lactating pluriparous Nelore cows (gray bars).** A: LH, 2- to 5-mo calves vs. cows; B:
209 LH, 8- to 11-mo calves vs. cows; C: FSH, 2- to 5-mo calves vs. cows; D: FSH, 8- to 11-
210 mo calves vs. cows. P values were determined using T-test (A, B) or Mann-Whitney test
211 (C, D). Results are shown as mean ± SEM.
212

213 The correlation matrix for reproductive and biometric endpoints is shown in Fig 2
214 (A, B). During the EPP, moderate to high positive correlations were observed among
215 ovarian diameter, number of follicles > 4.0 mm, diameter of the largest follicle, uterine
216 horn diameter, and rump biometric endpoints (R values ranging from 0.30 to 0.77, P <
217 0.05). On the other hand, during IPP, most of these associations were weak and non-
218 significant (P > 0.05). In both periods, AFC and the number of follicles ≤ 4 mm only
219 presented a significant positive correlation with ovarian diameter, whereas in the IPP
220 AFC, the number of follicles ≤ 4 mm was negatively correlated with rump width at the
221 ischium, rump area, and body weight (R = -0.10, -0.16, and -0.10, respectively, P < 0.05).
222
223 **Fig 2 (A-B). Spearman's correlation between reproductive and biometric**
224 **parameters in Nelore calves at A: 2 to 5 mo and B: 8 to 11 mo.** Endpoints evaluated:
225 1: rump width at the ilium (cm), 2: rump width at the ischium (cm), 3: rump length (cm),
226 4: rump area (cm^2), 5: ratio between ilium's and ischium's length, 6: body weight (Kg), 7:
227 ovarian diameter (mm), 8: antral follicle count, 9: number of follicles ≤ 4.0 mm, 10: number
228 of follicles > 4.0 mm, 11: diameter of the largest follicle (mm), 12: uterine horn diameter
229 (mm). * is P < 0.05 and ** is P < 0.01.
230
231 The Gompertz model was used to analyze whether the differences observed
232 between correlations in the EPP and IPP were caused by differences in the rate of
233 reproductive development. Despite of the relatively weak values for r^2 (ranging from 0.2 to

234 0.4), we could determine the reproductive developmental rate ("a" value in Gompertz
235 equation) for calves at this early age (Fig 3A). During this period, the ovarian diameter
236 and the number of follicles > 4.0 mm increased by 33%, the diameter of the largest
237 follicle increased by 24%, and the uterine horn diameter increased by 19%. To ensure
238 that the slope of the curves was positive, they were compared to a straight line. The
239 curves for all endpoints, except follicles ≤ 4.0 mm, differed ($P < 0.05$) from the straight
240 line, i.e., represented growing trends (Table 2). Conversely, during the IPP, the equations
241 for the same endpoints showed r^2 values below 0.01 (Fig 3B), and the curves did not differ
242 from straight lines ($P > 0.05$, Table 2). The following equations were calculated for the
243 EPP:
244 Ovarian diameter: 23.57*exp(-1.00983*exp(-0.33040*x)); $r^2 = 0.44$
245 Follicles > 4.0 mm: 8*exp(- 2.52892 *exp(-0.33549*x)); $r^2 = 0.23$
246 Diameter of the largest follicle: 11.30*exp(- 0.99893*exp(-0.24732*x)); $r^2 = 0.21$
247 Uterine horn diameter: 11.20*exp(-0.53403*exp(- 0.19523*x)); $r^2 = 0.16$
248 Rump area: 711.40 * exp (-2.05001 * exp (-0.64280 * x)); $r^2 = 0.748$
249 The following equations were calculated for the IPP:
250 Ovarian diameter: 23.65*exp(- 0.43306*exp(-0.05201*x)); $r^2 = 0.009$
251 Follicles > 4.0 mm: 14*exp(- 0.84750*exp(-0.02280*x)); $r^2 = 0.002$
252 Diameter of the largest follicle: 10.65*exp(-0.62063*exp(-0.08197*x)); $r^2 = 0.014$
253 Uterine horn diameter: 13.15*exp(-0.55009*exp(-0.08051*x)); $r^2 = 0.024$
254 Rump area: 1026.22 * exp (- 3.7862 * exp (-0.3416 * x)); $r^2 = 0.303$

255 **Fig 3 (A-B). Gompertz curves for reproductive parameters in Nelore calves from 2**
256 **to 5 mo (A) and from 8 to 11 mo (B).** The parameters evaluated and respective r^2 values
257 at the periods A and B, respectively, are as follows: 1: antral follicle count (r^2 = 0.056 and
258 0.001), 2: follicles ≤ 4.0 mm (r^2 = 0.014 and 0.002), 3: ovarian diameter (r^2 = 0.442 and
259 0.009), 4: diameter of the largest follicle (r^2 = 0.207 and 0.014), 5: uterine horn diameter
260 (r^2 = 0.157 and 0.024), and 6: ovarian follicles > 4.0 mm (r^2 = 0.232 and 0.001). The R
261 software output presents mean values in the Y axis in an arbitrary scale so that all
262 endpoints fit in the same picture.

263 **Table 2. Comparison between the Gompertz curves for reproductive parameters (referred**
264 **to in Fig 3) and a straight line in 2- to 5- and 8- to 11-month-old Nelore calves. Results are**
265 **shown as sum of squares (SQ), F, and P-values. When P < 0.05, the curve slope was**
266 **considered positive.**

267

	Age group					
	2 to 5 months			8 to 11 months		
Endpoint	SQ	F	P-value	SQ	F	P-value
Ovary diameter (mm)	-238.9	74.6	<0.001	-4.3	0.9	0.3
AFC (n)	-244.0	5.6	0.02	-16.3	0.1	0.6
Follicles ≤ 4.0 mm (n)	-61.1	1.3	0.26	-19.8	0.2	0.6
Follicles > 4.0 mm (n)	-60.1	28.4	<0.001	-0.9	0.2	0.6
Largest follicle (mm)	-42.3	24.5	<0.001	-2.4	1.5	0.2
Uterine diameter (mm)	-15.2	17.5	<0.001	-2.9	2.6	0.1

268 Changes in rump geometry are shown in Fig 4 (A-D). The non-linear regression
269 analysis demonstrated a greater developmental rate in rump area during EPP, which
270 increased by 64% (r^2 = 0.75) compared to 34% (r^2 = 0.31) during the IPP (Fig 5 A, B).
271 During the EPP, there was also a greater change in rump geometry, which changed from
272 a more squared to a trapezoidal shape (Fig 4 A, B). The average rump area was 1,684.2
273 ± 173,8 cm^2 for cyclic cows (control) and 514.2 ± 101.4 cm^2 and 856.72 ± 79.1 cm^2 for
274 EPP and IPP calves, respectively. The ratio rump width at ilium: at ischium differed
275 between EPP and IPP ($P < 0.05$). The calves reached puberty at an average of 20.5 mo
276 (ranging from 17 to 24 mo).
277
278 **Figure 4 (A-D). Changes in rump geometry during prepubertal development in**
279 **Nelore (*Bos indicus*) calves. Back-view 3D images of Nelore females' rump at 2 (A)**
280 **and 5 (B) and at 8 (C) and 11 (D) mo.** Trapezoids show changes in rump geometry. To
281 build the trapeze, the iliac crest and tuber ischiadicum were used as reference points.
282
283 **Figure 5 (A-B). Non-linear regression curves with r^2 values of rump geometry**
284 **measures in calves at 2 to 5 mo (A) and 8 to 11 mo (B).**
285

286 # Discussion

287 The goal of this study was to characterize the reproductive, biometric, and hormonal
288 patterns in the initial and intermediate prepubertal periods in Nelore (*B. indicus*) calves.

289 The present results support the hypothesis that the dynamics of reproductive and biometric
290 development during the prepubertal period differs over time.

291 There was a progressive increase in ovarian activity in calves, indicated by the difference
292 in the number of growing follicles and by the maximum size of the largest (presumptive dominant)
293 follicle and indirectly by uterine horn diameter between EPP (2 to 5 mo) and IPP (8 to 11 mo)
294 calves. However, the dynamics of ovarian and uterine development differed within periods, with
295 a higher growth rate during the EPP, as demonstrated by the differences in the non-linear
296 Gompertz curves. In EPP calves, there was a significant increase in all endpoints other than AFC,
297 whereas in IPP calves, the curves were not significantly different from a straight line, i.e., there
298 was a relative stabilization in reproductive development. The faster development
299 observed in younger calves is in line with the so-called “mini puberty” previously described
300 in *B. taurus* [1] as well as in sheep [4] and humans [reviewed by 3]. Coherently, ovarian
301 activity showed positive and significant correlations with ovarian and uterine horn
302 diameters as well as with biometric endpoints (length at ilium, length in ischium, rump
303 length, rump area, ratio between rump width at ilium: at ischium, and body weight) during
304 the EPP. On the other hand, most of these correlations became nonexistent or even
305 negative in IPP calves.

306 The marked changes observed in EPP calves are likely driven by the increasing
307 production of estradiol (not evaluated in this study) by growing follicles. The circulating
308 estradiol promotes both uterine growth [23] and somatic development [5], and ovarian
309 activity at this early age seems to be important for the acquisition of primary and

310 secondary sexual characteristics. In IPP calves, on the other hand, increases in body
311 weight or size were associated with a stabilization rather than proportional changes in
312 ovarian activity. Previous studies have described that, in the first months of life, there is a
313 faster development of the reproductive tract, followed by a plateau and by a resumption of
314 progress near puberty [1, 24]. However, in the current study, this plateau in reproductive
315 development occurred in the period referred to as the intermediate prepubertal period
316 (IPP, 8 to 11 mo). To make a parallel, it would correspond, in precocious *B. taurus* breeds,
317 to the peripubertal period, in which remarkable neuroendocrine and physiological
318 changes are in progress, with concurrent changes in the reproductive tract. The age at
319 puberty in Holstein calves is, on average, 250 days (8.3 mo), with approximately 30% of
320 the calves reaching puberty as early as at 7 mo [25]. The Angus, a beef breed, reaches
321 puberty at about 13 mo [26]. Conversely, our calves attained puberty at an average of
322 20.5 mo, consistently with the age previously reported by others for the Nelore breed [18].
323 In this regard, the present study demonstrates the differences in the timing of the events
324 in prepubertal *B. indicus* calves, with the plateau phase extending to the period here
325 defined as the IPP.

326 It is noteworthy that changes in rump geometry (from squared to trapezoid) were
327 observed only in EPP calves, and further development was characterized mainly by
328 increases in rump size rather than in shape. Rump characteristics are associated with
329 pregnancy failure, anestrus, and dystocia [27-29] in heifers, highlighting the importance
330 of the changes in rump development that take place during the early prepubertal phase.

331 A novelty of the current study was the use of 3D image technologies to demonstrate these
332 changes in rump geometry. Structured light scanning allows the fast and accurate
333 generation of 3D models from cattle, which can be freely rotated in a 3D virtual space and
334 used to define reference marks and acquire biometric measurements [30]. In the current
335 study, rump size and geometry were calculated using as references the prominences of
336 the *tuber ischiae* and the *tuber coxae* of the pelvic bones, which can be easily spotted in
337 the 3D models.
338 Differently from the other ovarian endpoints evaluated, the number of sonographic-
339 detectable antral follicles (AFC) did not differ between age groups, and analysis by the
340 Gompertz curve showed little or no trend of increase in AFC values within the periods
341 analyzed. Coherently, in both periods, AFC was positively correlated only with ovarian
342 diameter and, in older calves, negatively correlated with rump development and body
343 weight. The association of AFC and ovary size has previously been described in pubertal
344 cattle [31], and in the current study, we now show that this relationship also occurs in
345 early prepubertal calves. As AFC presents a high individual repeatability from prepubertal
346 to post-pubertal heifers [32], our findings suggest that oocyte donor selection can be
347 performed in young calves, with no expected effect on other characteristics associated
348 with prepubertal sexual development. However, the association between AFC and
349 somatic development remains to be elucidated.
350 The progressive increase in the number of growing follicles and in the maximum size of
351 the largest follicle observed in this study in the EPP calves was consistent with the

352 hypothesis that a higher hypophyseal release of FSH and LH occurs during early
353 calfhood. In fact, plasma concentrations of FSH and LH in EPP calves were similar to
354 those in control cows. The hypophyseal gonadotropins support follicle growth and
355 steroidogenesis (reviewed by [33]), which in turn may have promoted uterine
356 development and the changes in rump geometry, as observed in the current study.
357 Mauras et al. [34] observed that, in children, the increase in GnRH pulse amplitude leads
358 to a greater FSH and LH release and subsequent estradiol production, which in turn
359 increases the production of growth hormone (GH) and insulin-like growth hormone (IGF-
360 1), as well as calcium absorption and skeleton mineralization.

361 On the other hand, the events observed during IPP support the hypothesis of an
362 increased sensitivity of the hypothalamic-hypophyseal axis as a response to the negative
363 feedback from estradiol. Previous studies have shown that after the transient
364 hypothalamic-pituitary-gonadal axis activation soon after birth, there is a decrease in
365 plasma concentration of gonadotropins [1, 4]. In fact, in our study, LH concentrations were
366 lower in IPP calves than in control cows. The lower amplitude and frequency of LH pulses
367 may have reduced estradiol production and, thus, the rate of development of primary and
368 secondary sexual characteristics. Moreover, the lower negative feedback on FSH
369 production [35] would explain the plasma concentrations of FSH in IPP calves, which were
370 higher than those in the control cows.

371

372

Conclusions

The EPP in Nelore calves is characterized by increased ovarian activity, uterine development, and rump geometry change, contrasting with the relative stabilization observed in the IPP. Additionally, the plateau phase in Nelore calves extends to a moment when *B. taurus* breed calves are already in the peripubertal period. These findings highlight the importance of the early prepubertal period for the development of primary and secondary characteristics and shed light on the implications of the differences in physiology between these periods for the use of calves as oocyte donors.

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References

[1] Rawlings NC, Evans ACO, Honaramooz A, Bartlewski PM. Antral follicle growth and endocrine changes in prepubertal cattle, sheep and goats. Anim Reprod Sci. 2003; 78: 259-270. DOI: 10.1016/s0378-4320(03)00094-0

[2] Hernandez-Medrano JH, Campbell BK, Webb R. Nutritional influences on folliculogenesis. Reprod Domest Anim. 2012; 47: 274-282. DOI: 10.1111/j.1439-0531.2012.02086.x

[3] Kuiri-Hänninen T, Sankilampi U, Dunkel L. Activation of the hypothalamic- pituitary-gonadal axis in infancy: minipuberty. Horm Res Paediatr. 2014; 82: 73-80. DOI: 10.1159/000362414

411 [4] Torres-Rovira L, Succu S, Pasciu V, Manca ME, Gonzalez-Bulnes A, Leoni GG, et al.
412 Postnatal pituitary and follicular activation: a revisited hypothesis in a sheep model.
413 Reproduction. 2016; 151: 215-225. DOI: 10.1530/REP-15-0316
414 [5] Connelly KJ, Larson EA, Marks DL, Klein RF. Neonatal estrogen exposure results in
415 biphasic age-dependent effects on the skeletal development of male mice. Endocrinology.
416 2015; 156: 193-202. DOI: 10.1210/en.2014-1324
417 [6] Smith MF, Geisert RD, Parrish JJ. Reproduction in domestic ruminants during the past
418 50 yr: discovery to application. J Anim Sci. 2018; 96: 2952-2970. DOI: 10.1093/jas/sky139
419 [7] Oursler MJ, Cortese C, Keeting P, Anderson MA, Bonde SK, Riggs BL, Spelsberg TC.
420 Modulation of transforming growth factor-β production in normal human osteoblast-like
421 cells by 17 β-estradiol and parathyroid hormone. Endocrinology. 1991; 129: 3313-3320.
422 DOI: 10.1210/endo-129-6-3313
423 [8] Michael H, Härkönen PL, Väänänen HK, Hentunen TA. Estrogen and testosterone use
424 different cellular pathways to inhibit osteoclastogenesis and bone resorption. J Bone
425 Miner Res. 2005; 20: 2224-2232. DOI: 10.1359/JBMR.050803
426 [9] Day ML, Anderson LH. Current concepts on the control of puberty in cattle. J Anim Sci.
427 1998; 76:1-15. DOI: 10.2527/1998.76suppl_31x
428 [10] Presicce GA, Jiang S, Simkin M, Zhang L, Looney CR, Godke RA, et al. Age and
429 hormonal dependence of acquisition of oocyte competence for embryogenesis in
430 prepubertal calves. Biol Reprod. 1997; 56: 386-392. DOI: 10.1095/biolreprod56.2.386

431 [11] Brogliatti GM, Adams GP. Ultrasound-guided transvaginal oocyte collection in
432 prepubertal calves. Theriogenology. 1996; 45: 1163-1176. DOI: 10.1016/0093-
433 691x(96)00072-6
434 [12] Majerus V, De Roover R, Etienne D, Kaidi S, Massip A, Dessy F, et al. Embryo
435 production by ovum pick up in unstimulated calves before and after puberty.
436 Theriogenology. 1999; 52: 1169-1179. DOI: 10.1016/S0093-691X(99)00209-5
437 [13] Jiang J, Ma L, Prakapenka D, VanRaden PM, Cole JB, Da Y. A large-scale genome-
438 wide association study in US Holstein cattle. Front genet. 2019; 10: 412. DOI:
439 10.3389/fgene.2019.00412
440 [14] Baldassarre H. Laparoscopic ovum pick-up followed by *In vitro* embryo production
441 and transfer in assisted breeding programs for ruminants. Animals. 2021; 11: 216. DOI:
442 10.3390/ani11010216
443 [15] Batista EOS, Guerreiro BM, Freitas BG, Silva JCB, Vieira LM, Ferreira RM, et al.
444 Plasma anti-Müllerian hormone as a predictive endocrine marker to select *Bos taurus*
445 (Holstein) and *Bos indicus* (Nelore) calves for *in vitro* embryo production. Domest Anim
446 Endocrinol. 2016; 54: 1-9. DOI: 10.1016/j.domaniend.2015.08.001
447 [16] Baldassarre H, Currin L, Michalovic L, Bellefleur AM, Gutierrez K, Mondadori RG, et
448 al. Interval of gonadotropin administration for in vitro embryo production from oocytes
449 collected from Holstein calves between 2 and 6 months of age by repeated laparoscopy.
450 Theriogenology. 2018; 116: 64-70. DOI: 10.1016/j.theriogenology.2018.05.005

451 [17] Bernal-Ulloa SM, Heinzmann J, Herrmann D, Hadeler KG, Aldag P, Winkler S, et al.
452 Cyclic AMP affects oocyte maturation and embryo development in prepubertal and adult
453 cattle. Plos One. 2016; 11(2): e0150264. DOI: 10.1371/journal.pone.0150264
454 [18] Monteiro FM, Mercadante MEZ, Barros CM, Satrapa RA, Silva JAV, Oliveira LZ, et
455 al. Reproductive tract development and puberty in two lines of Nellore heifers selected for
456 postweaning weight. Theriogenology. 2013; 80: 10-17. DOI:
457 10.1016/j.theriogenology.2013.02.013
458 [19] Abeni F, Petrera F, Le Cozler Y. Effects of feeding treatment on growth rates,
459 metabolic profiles and age at puberty, and their relationships in dairy heifers. Animal.
460 2019; 13: 1020-1029. DOI: 10.1017/S1751731118002422
461 [20] Sartori R, Gimenes LU, Monteiro Jr PL, Melo LF, Baruselli OS, Bastos MR. Metabolic
462 and endocrine differences between *Bos taurus* and *Bos indicus* females that impact the
463 interaction of nutrition with reproduction. Theriogenology. 2016; 86: 32-40. DOI:
464 10.1016/j.theriogenology.2016.04.016
465 [21] Nogueira GP. Puberty in South American Bos indicus (Zebu) cattle. Anim Reprod Sci.
466 2004; 82-83: 361-372. DOI: 10.1016/j.anireprosci.2004.04.007
467 [22] Bolt DJ, Scott V, Kiracofe GH. Plasma LH and FSH after estradiol, norgestomet and
468 GnRH treatment in ovariectomized beef heifers. Anim Reprod Sci. 1990; 23: 263-271.
469 DOI: 10.1016/0378-4320(90)90040-M
470 [23] Jdidi H, Kouba FG, Aoiadni N, Abdennabi R, Turki M, Makni-Ayadi F, et al. Effects of
471 estrogen deficiency on liver function and uterine development: assessments of *Medicago*

472 *sativa*'s activities as estrogenic, anti-lipidemic, and antioxidant agents using an
473 ovariectomized mouse model. Arch Physiol Biochem. 2019; 18: 1-12. DOI:
474 10.1080/13813455.2019.1625927
475 [24] Honaramooz A, Aravindakshan J, Chandolia RK, Beard AP, Bartlewski PM, Pierson
476 RA, et al. Ultrasonographic evaluation of the pre-pubertal development of the reproductive
477 tract in beef heifers. Anim Reprod Scie. 2004; 80: 15-29. DOI: 10.1016/S0378-
478 4320(03)00136-2
479 [25] Bruinjé TC, Rosadiuk JP, Moslemipur F, Sauerwein H, Steele MA, Ambrose DJ.
480 Differing planes of pre- and postweaning phase nutrition in Holstein heifers: II. Effects on
481 circulating leptin, luteinizing hormone, and age at puberty. J Dairy Sci. 2021; 104: 1153-
482 1163. DOI: 10.3168/jds.2020-18810
483 [26] Allen CC, Tedeschi LO, Keisler DH, Cardoso RC, Alves BRC, Amstalden M, et al.
484 Interaction of dietary energy source and body weight gain during the juvenile period on
485 metabolic endocrine status and age at puberty in beef heifers. J Anim Sci. 2017; 95: 2080-
486 2088. DOI: 10.2527/jas.2016.1002
487 [27] Wall E, White IMS, Coffey MP, Brotherstone S. The relationship between fertility,
488 rump angle, and selected type information in Holstein-Friesian cows. J Dairy Sci. 2005;
489 88: 1521-1528. DOI: 10.3168/jds.S0022-0302(05)72821-6
490 [28] Holm DE, Webb EC, Thompson PN. A new application of pelvis area data as culling
491 tool to aid in the management of dystocia in heifers. J Anim Sci. 2014; 92: 2296-2303.
492 DOI: 10.2527/jas.2013-6967

493 [29] Holm DE, Nielen M, Jorritsma R, Irons PC, Thompson PN. Ultrasonographic
494 reproductive tract measures and pelvis measures as predictors of pregnancy failure and
495 anestrus in restricted bred beef heifers. Theriogenology. 2016; 85: 495-501. DOI:
496 10.1016/j.theriogenology.2015.09.031
497 [30] Viana JHM, Hinduja S, Bártolo PJS. Estimation of biometric parameters from cattle
498 rump using free-hand scanning and a 3D data processing algorithm. Virtual Phys
499 Prototyp. 2016; 11: 167-172. DOI: 10.1080/17452759.2016.1211292
500 [31] Ireland JJ, Smith GW, Scheetz D, Jimenez-Krassel F, Folger JK, Ireland JL, et al.
501 Does size matter in females? An overview of the impact of the high variation in the ovarian
502 reserve on ovarian function and fertility, utility of anti-Müllerian hormone as a diagnostic
503 marker for fertility and causes of variation in the ovarian reserve in cattle. Reprod Fert
504 Develop. 2011; 23: 1-14. DOI: 10.1071/RD10226
505 [32] Morotti F, Santos GMG, Júnior CK, Silva-Santos KC, Roso VM, Seneda MM.
506 Correlation between phenotype, genotype and antral follicle population in beef heifers.
507 Theriogenology. 2017; 91: 21-26. DOI: 10.1016/j.theriogenology.2016.12.025
508 [33] Kinder JE, Bergfeld EG, Wehrman ME, Peters KE, Kojima FN. Endocrine basis for
509 puberty in heifers and ewes. J Reprod Fert. 1995; 49: 393-407. PMID: 7623330
510 [34] Mauras N, Rogol AD, Haymond MW, Veldhuis JD. Sex steroids, growth hormone,
511 insulin-like growth factor-1: neuroendocrine and metabolic regulation in puberty. Horm
512 Res Paediatr. 1996; 45: 74-80. DOI: 10.1159/000184763

513 [35] Ginther OJ, Bergfelt DR, Kulick LJ, Kot K. Selection of the dominant follicle in cattle:
514 establishment of follicle deviation in less than 8 hours through depression of FSH
515 concentrations. Theriogenology. 1999; 52: 1079-1093. DOI: 10.1016/S0093-
516 691X(99)00196-X
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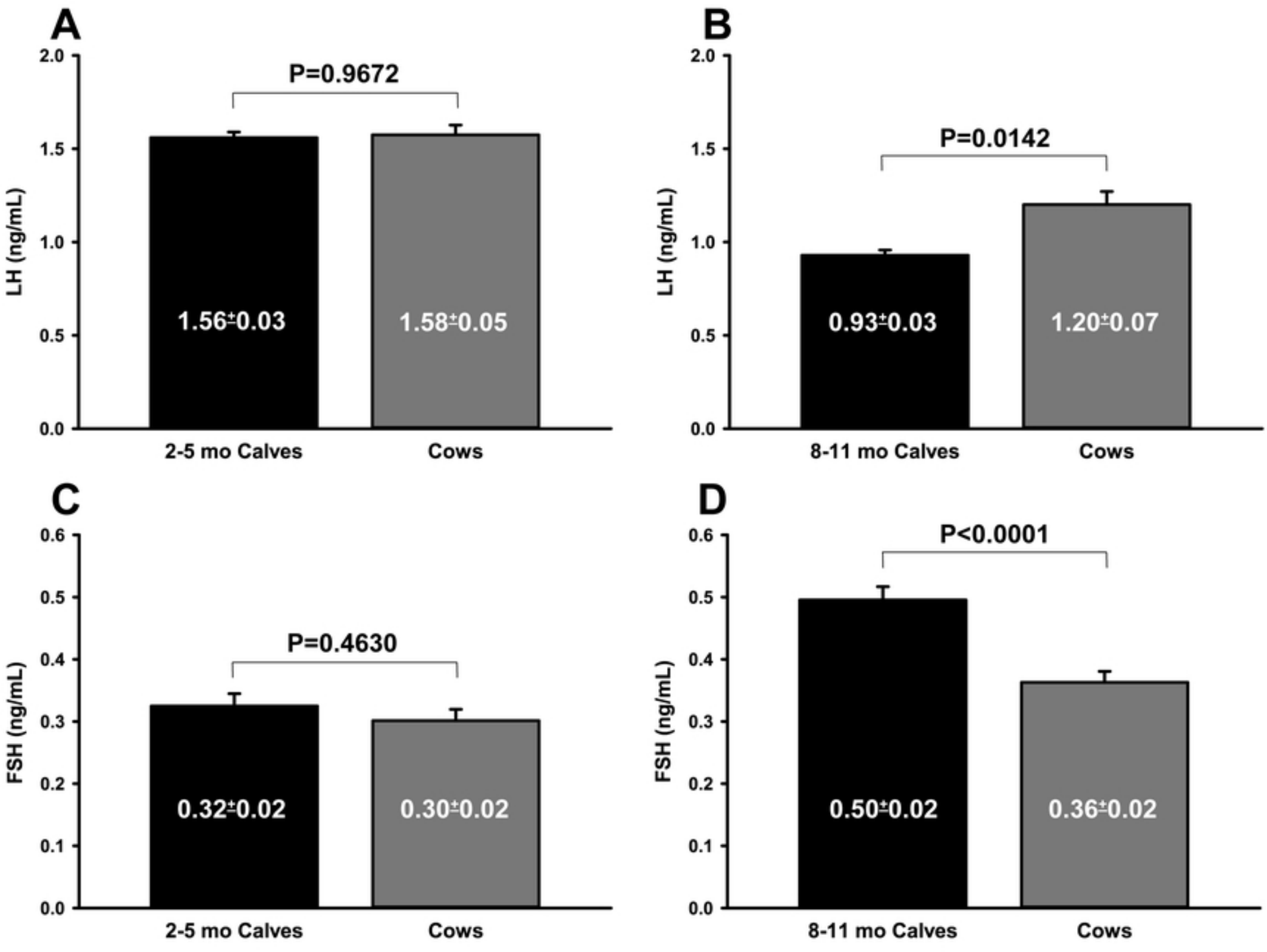
A
P=0.9672
LH (ng/mL)
2.0
1.5
1.0
0.5
0.0
1.56±0.03
1.58±0.05
2-5 mo Calves
Cows
B
P=0.0142
LH (ng/mL)
2.0
1.5
1.0
0.5
0.0
0.93±0.03
1.20±0.07
8-11 mo Calves
Cows
C
P=0.4630
FSH (ng/mL)
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0.32±0.02
0.30±0.02
2-5 mo Calves
Cows
D
P<0.0001
FSH (ng/mL)
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0.50±0.02
0.36±0.02
8-11 mo Calves
Cows

Figure 1

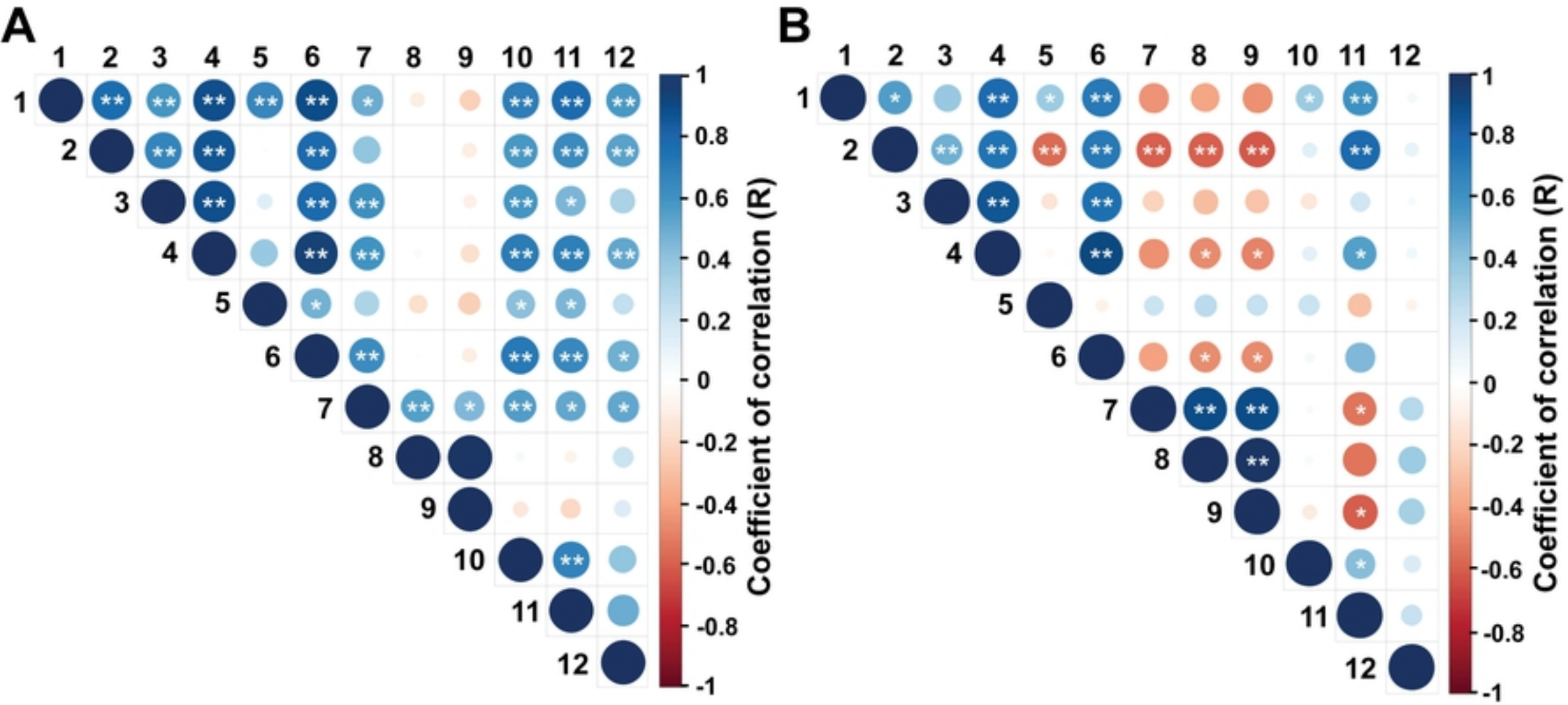
A
B
1 2 3 4 5 6 7 8 9 10 11 12
Coefficient of correlation (R)
1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
-1

Figure 2

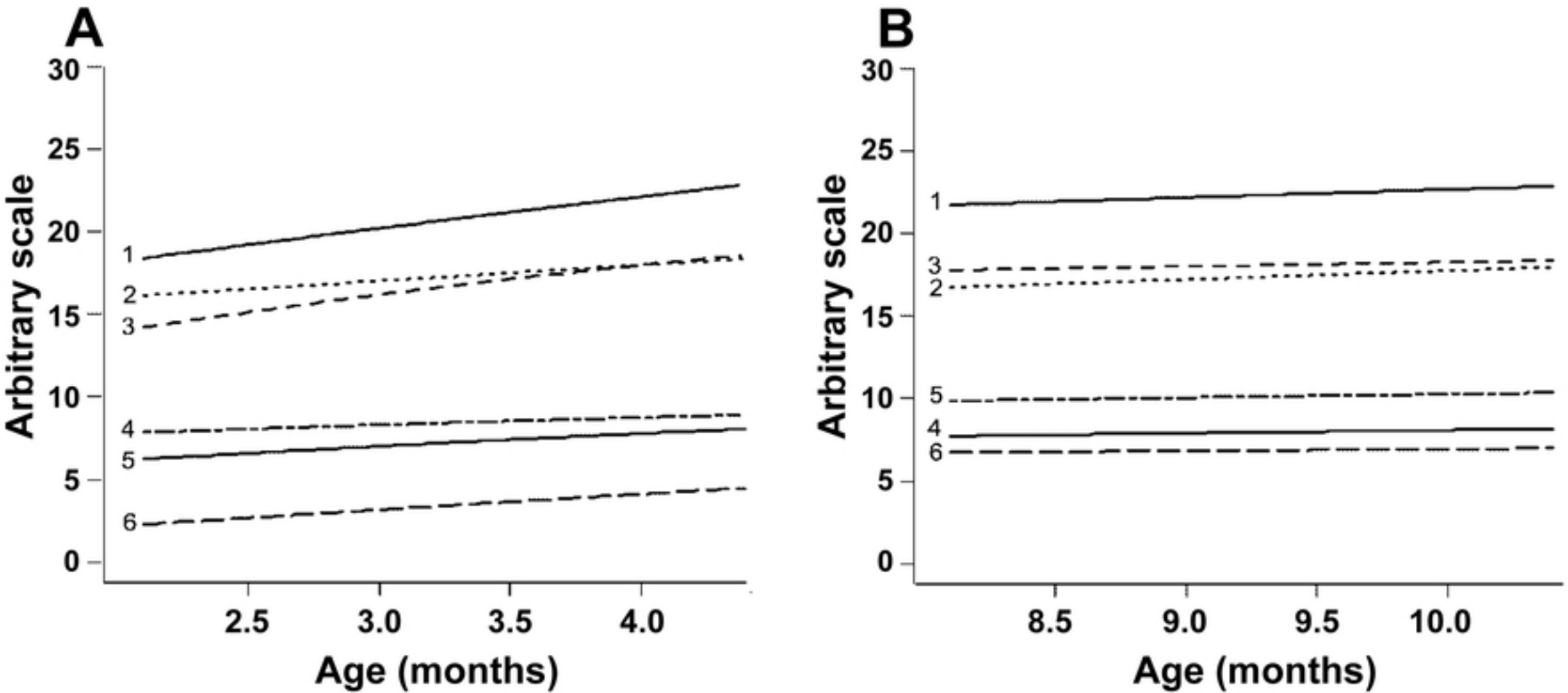
A
B
Arbitrary scale
Age (months)
30
25
20
15
10
5
0
2.5
3.0
3.5
4.0
8.5
9.0
9.5
10.0
1
2
3
4
5
6

Figure 3

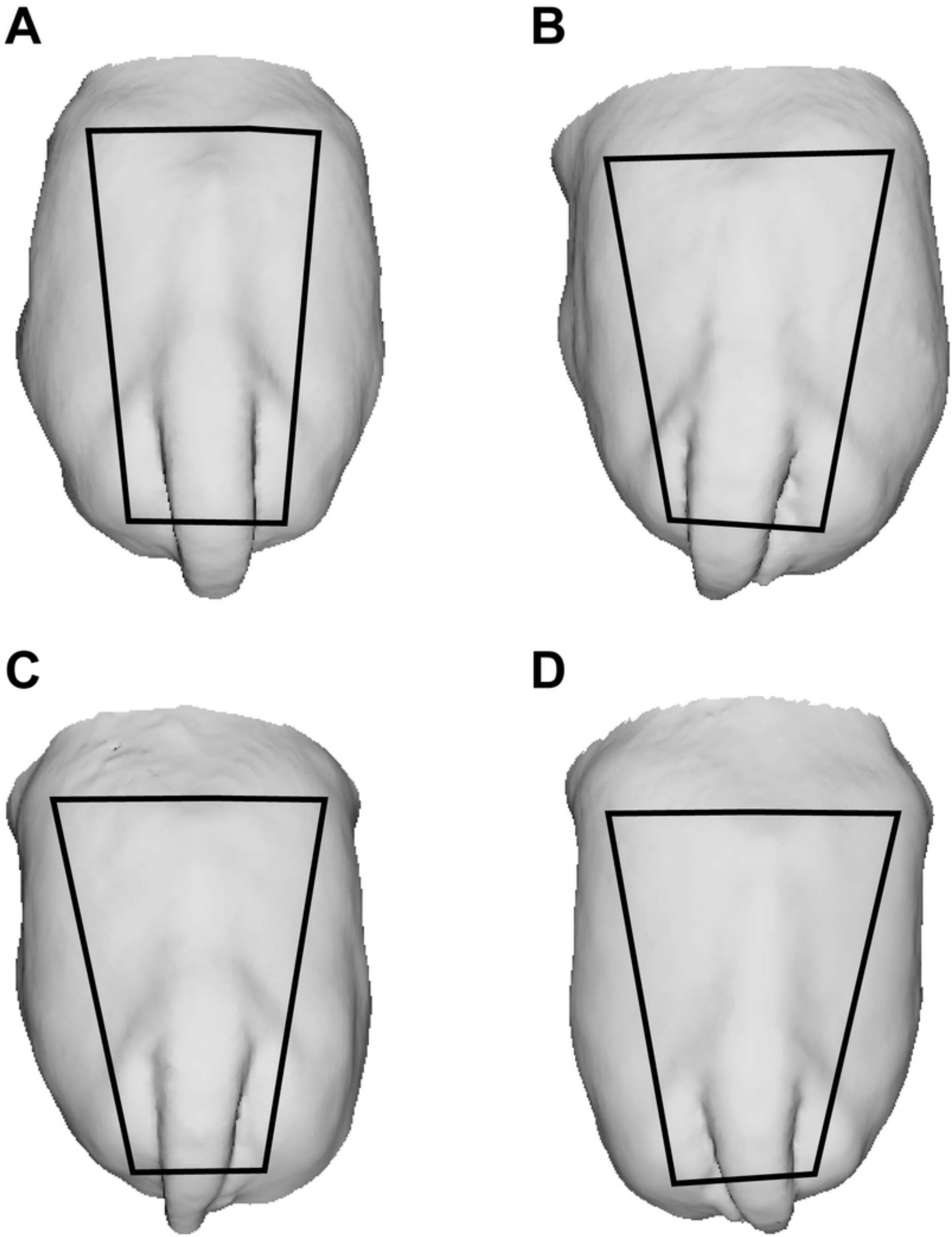

Figure 4

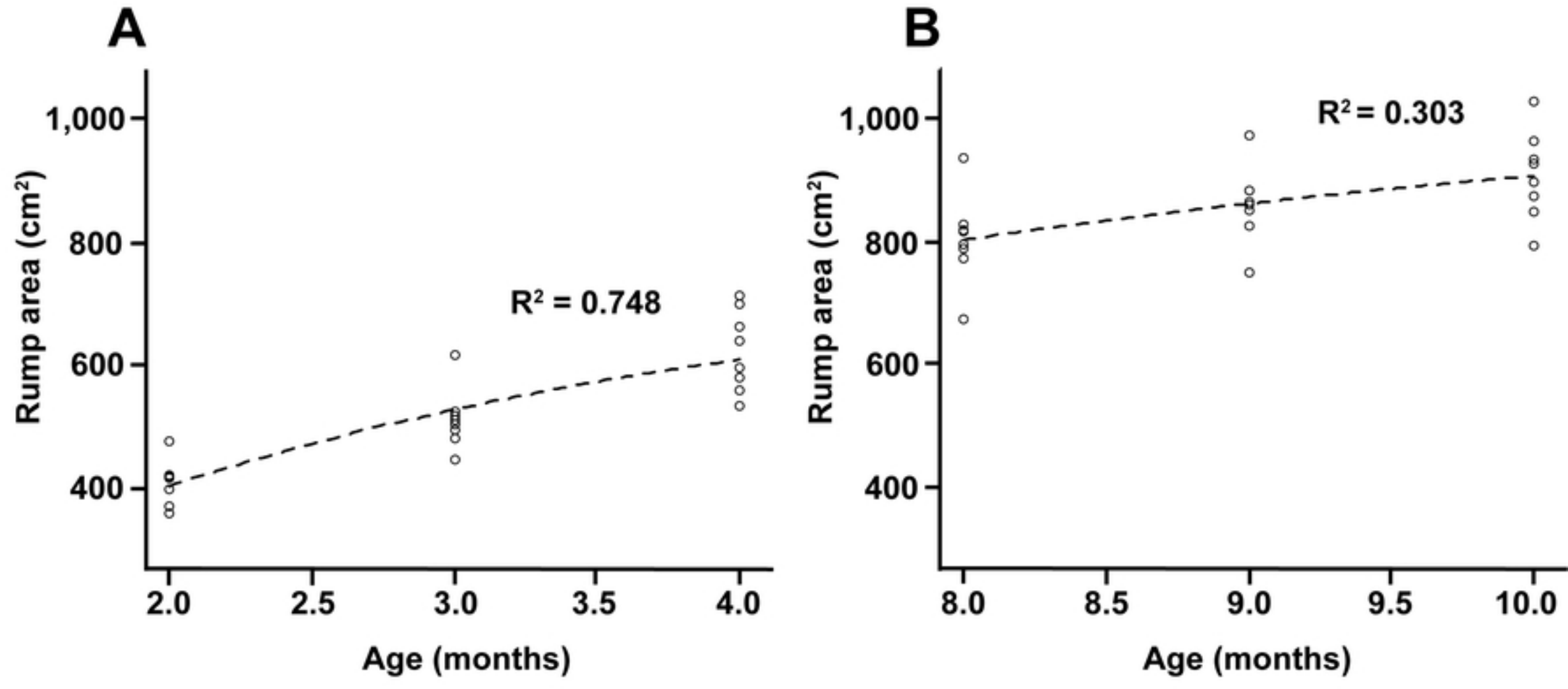
A
1,000
800
600
400
Rump area (cm²)
R² = 0.748
2.0
2.5
3.0
3.5
4.0
Age (months)
B
1,000
800
600
400
Rump area (cm²)
R² = 0.303
8.0
8.5
9.0
9.5
10.0
Age (months)

Figure 5