<https://helda.helsinki.fi>

Expression of uterine oxytocin receptors and blood
progesterone, 13,14-dihydro-15-keto-prostaglandin
ionized calcium levels in dystocic bitches

Tamminen, Tuire

2019-09-01

Tamminen , T , Sahlin , L , Masironi-Malm , B , Dahlbom , M , Katila , T , Taponen , J &
Vapaavuori , O 2019 , ' Expression of uterine oxytocin receptors and blood progesterone,
13,14-dihydro-15-keto-prostaglandin F₂±, and ionized calcium levels
Theriogenology , vol. 135 , pp. 38-45 . <https://doi.org/10.1016/j.theriogenology.2019.06.007>

<http://hdl.handle.net/10138/316029>

<https://doi.org/10.1016/j.theriogenology.2019.06.007>

cc_by_nc_nd

acceptedVersion

Downloaded from Helda, University of Helsinki institutional repository.

This is an electronic reprint of the original article.

This reprint may differ from the original in pagination and typographic detail.

Please cite the original version.

18 **Abstract**

19

20 This study aimed to examine the etiology of canine dystocia by measuring the relative expression of
21 oxytocin receptor (OXTR) mRNA and the concentration of serum progesterone, plasma
22 PGF_{2α} metabolite (PGFM), and blood ionized calcium (iCa) near term and in dystocia. Altogether 58
23 bitches were included in this study, 41 of which underwent cesarean section (CS). The four CS groups
24 were based on history: complete uterine inertia (CUI; n = 7), partial uterine inertia (PUI; n = 13),
25 obstructive dystocia (OD; n = 10), and elective cesarean section (ECS; n = 11). An additional group
26 of medically treated dystocia without CS (MD; n = 8) and a control group (C; n = 9) with normal
27 parturition (without CS and medical treatment) were also formed. Blood samples were taken prior to
28 CS or medical treatment. Progesterone concentrations were highest in the ECS and a significant
29 difference ($p < 0.05$) was observed between the ECS and the OD and between the ECS and the
30 combined dystocia (CUI, PUI, OD, MD) groups (COMB). Highest concentrations of PGFM was
31 observed in the C, the difference being significant ($p < 0.05$) between the C and the ECS and between
32 the C and the COMB group. The progesterone:PGFM ratio was significantly ($p < 0.05$) higher in the
33 ECS than in the C and the COMB group. No significant difference ($p > 0.05$) was observed in iCa
34 concentrations between the groups. Relative OXTR mRNA expression was evaluated with real-time
35 PCR from full-thickness uterine samples taken from the incision site during CS. The expression was
36 highest in the ECS and the difference in expression was significant ($p < 0.05$) between the ECS and
37 the OD and between ECS and the combined dystocia (CUI, PUI, OD) groups (COMB2). The study
38 supports previous reports of decreasing progesterone and increasing PGFM during parturition
39 luteolysis. Upregulation of OXTR occurs near term. In obstructive dystocia, a prolonged influence of
40 oxytocin and uterine exhaustion may lead to downregulation of OXTR. Complete primary uterine
41 inertia may have a different etiology as no clear decrease in OXTR was observed in CUI as in OD. It

remains unclear if parturition ceases because of uterine inertia or if uterine inertia occurs because of ceased parturition and desensitization of receptors.

Keywords:

Canine, uterus, birth, progesterone, prostaglandin F_{2α}

1. Introduction

Parturition is a complex event and includes hormonal and behavioral changes, neural activity, and interaction between the dam and the offspring. Near term, canine plasma PGF_{2α} levels increase leading to luteolysis, followed by a decrease in peripheral plasma progesterone levels [1,2,3] that allow for contractions of the uterus and for parturition to proceed [1]. The secretion of PGF_{2α} in the bitch is suggested to originate from placental trophoblast cells [4]. During parturition, increase in peripheral plasma cortisol [2,5,6], vasopressin [6], and oxytocin (OT) [6,7] occur. However, changes in cortisol levels vary greatly between individuals during parturition [2,5,6]. While estrogen concentrations are somewhat higher in the last trimester of pregnancy in the bitch, there is no marked prepartum increase as detected in many other species [8,9,10]. Two days prior to parturition, estrogen levels of the bitch decrease suddenly during prepartum luteolysis indicating its luteal source [11].

Occurrence of dystocia in bitches varies greatly depending on the population studied; the average is estimated to be below 5% [12]. In a group of 200 000 insured bitches (excluding Boston Terrier, English Bulldog, and French Bulldog) in Sweden, dystocia occurred in 16% of parturitions [13]. In the UK, the occurrence varied from 0% to 92% among 151 breeds (22, 005 litters) [14]. While

66 dystocia seems to be more common in miniature and small breeds [13,15,16], several medium- and
67 large-size breeds also have a higher than average proportion of litters born by cesarean section (CS)
68 [14]. Approximately 60% of dystocia cases undergo CS [13,15,16,17]. In brachycephalic breeds, the
69 proportion of CS is very high [14,18]. There may be a risk of bias in statistics of dystocia in these
70 breeds due to the popularity of elective CS (ECS).

71
72 Dystocia is sometimes difficult to diagnose. Therefore, a complete history and physical examination
73 is required. The suggested causes vary slightly according to different authors [12,19,20]. There are
74 several, sometimes simultaneous, causes of dystocia. Maternal factors are more common than fetal
75 factors. The most common maternal cause is primary uterine inertia, which can be complete or partial
76 [12]. In complete primary uterine inertia, the uterus fails to initiate parturition due to absence of
77 uterine contractions and thus no puppies are born [12,17]. In partial primary uterine inertia, the bitch
78 may have weak uterine contractions or contractions that cease without any obvious reason (such as
79 obstruction) before all puppies are born [12,17]. Secondary uterine inertia is caused by prolonged
80 parturition due to obstruction in the birth canal [12,17].

81
82 Oxytocin is a nonapeptide hormone produced mainly in the hypothalamus and stored in the posterior
83 pituitary gland. Oxytocin is released after suitable stimulus, such as intracervical pressure. As one of
84 the most potent uterotonic hormones, OT enhances the contractility of the uterus. During parturition,
85 plasma OT concentration increases [6,7]; this may not occur in dystocia [21,22]. The effect of OT in
86 the uterus is mediated through specific, class I G-protein-coupled transmembrane receptors known as
87 oxytocin receptors (OXTR) [23]. Near term, during prepartum luteolysis, OXTR are upregulated
88 [24,25,26]. In humans, continuous exposure to OT leads to desensitization of OXTR by reduction of
89 OT binding sites in the myometrial cell membrane and by downregulation of OXTR mRNA in
90 myometrial cells [27]. While desensitization may also have a role in canine dystocia due to prolonged

influence of OT, there is no published evidence of OXTR desensitization in bitches. The aim of this study was to examine the relative expression of OXTR mRNA in the canine uterus near parturition and in dystocia. Levels of serum progesterone, plasma prostaglandin F_{2α} metabolite (PGFM), progesterone:PGFM ratio, and blood ionized calcium (iCa) were also analyzed to clarify possible causative factors for dystocia.

2. Materials and methods

The study was approved by the Ethics Committee of the Viikki Campus, University of Helsinki, Finland. Blood sampling from bitches with normal parturition was authorized by the National Animal Experiment Board (ESAVI, Hämeenlinna, Finland), license number ESAVI/3802/04.10.03/2011.

2.1. Groups

Client-owned pet bitches that had CS performed either at the Small Animal Clinic of Mäntsälä or the Veterinary Teaching Hospital of the University of Helsinki were enrolled in the study (Table 1). The inclusion criteria were a diagnosis of dystocia resulting in CS or ECS due to small litter size or previous dystocia. In addition, one group was established from bitches with medically treated mild dystocia that gave birth without CS. Bitches with normal parturitions served as controls for blood parameters. The owners of the bitches were requested to sign a written consent and complete a questionnaire to obtain the history of the bitch including previous and present parturitions. Any systemic disease was an exclusion criterion.

115 The following study groups were formed: 1) complete primary uterine inertia (CUI; n = 7, no puppies
116 born, parturition does not proceed, discharge of fetal fluids >3 hours or green discharge, no response
117 to vaginal stimulus), 2) partial primary uterine inertia (PUI; n = 13, at least one puppy born, parturition
118 ceases without obstruction), 3) obstructive dystocia (OD; n = 10, fetal oversize/narrow birth canal,
119 malpresentation, malformation), 4) elective caesarean section (ECS; n = 11, 58-66 days from mating,
120 previous dystocia, one or two puppies, before the onset of the stage 1 of parturition), 5) medically
121 treated dystocia (MD; n = 8, no CS, medical treatment), 6) control (C; n = 9, no CS, no medical
122 treatment, normal parturition). Dystocia groups were also combined (COMB: CUI, PUI, OD, MD
123 and COMB2: CUI, PUI, OD) to compare with ECS and C. The diagnosis and treatment decisions
124 were performed by the veterinarian on call. After blood sampling, the bitches were treated, if
125 necessary, with calcium glubionate (Calcium-Sandoz[®], Sandoz A/S, Copenhagen, Denmark) and
126 oxytocin (Vetox[®], Vetcare, Salo, Finland) (Table 1).

127

128 The individual and average data of the bitches are presented in Table 1. Altogether 35 different breeds
129 were included in the study. Four bitches in the ECS group had had previous history of dystocia and
130 CS. In the PUI and MD groups, each had one bitch with mild, medically treated dystocia without CS
131 in the previous pregnancy. The previous parturitions of the other multiparous bitches were normal.
132 The gestation length was calculated from ovulation day (at progesterone level 16-32 nmol/l) and from
133 the first and the last mating according the available information (Table 1).

134

135 2.2. Blood sampling

136

137 Blood samples were taken prior to CS or medical treatment from the vena cephalica into a syringe
138 (Radiometer Safe Pico, ref: 956-610, Radiometer Medical, Copenhagen, Denmark), to an EDTA tube
139 (Vacuette[®], Mekalasi Oy, Nurmijärvi, Finland) with 5000 KIU aprotinin/ml EDTA blood (Aprotinin,

140 Roche Diagnostics GmbH, Mannheim, Germany) and a serum tube with clotting activator
141 (Vacuette[®], Mekalasi Oy, Nurmijärvi, Finland). Blood samples were taken prepartum in the ECS
142 group and peripartum (second stage of parturition) in the other groups. EDTA tubes and syringes
143 were stored in an ice-water bath and serum tubes at room temperature. Blood samples were
144 centrifuged (Eppendorf Centrifuge 5810R, Eppendorf Nordic A/S, Hørsholm, Denmark) as follows:
145 EDTA tubes at 4 °C, 1200 x g, 10 min, and serum tubes at 22 °C, 1700 x g, 10 min. Plasma and serum
146 were divided into aliquots, frozen at -20 °C, and stored at -70 °C until analyzed.

147

148 2.3. Progesterone assay

149

150 Serum progesterone concentrations were measured in one run using a commercial RIA kit
151 (Progesterone Coat-A-Count[®] RIA, Siemens Healthcare Diagnostics Oy, Espoo, Finland) according
152 to the manufacturer's instructions. The concentrations were measured in duplicate with a gamma
153 counter (1272 GliniGamma, LKB Wallac Oy, Turku, Finland). The intra-assay coefficient of
154 variation was 3.4% at a serum concentration of 4.4 nmol/L and 2.0% at a concentration of 32.5
155 nmol/L.

156

157 2.4. PGF_{2α} metabolite assay

158

159 Concentrations of the major metabolite of PGF_{2α}, 13,14-dihydro-15-keto-prostaglandin F_{2α} (PGFM),
160 were measured from plasma using a commercial immunoassay kit (DetectX[®] 13,14-dihydro-15-keto-
161 PGF_{2α} (PGFM) Enzyme Immunoassay Kit, Arbor Assays, Michigan, USA) according to the
162 manufacturer's instructions. Prior to performing the assay, plasma samples were diluted 1:15 with
163 the assay buffer provided in the kit. The optical density of each well was measured with a Multiscan
164 GO Spectrophotometer with SkanIt software 4.1 (Thermo Fisher Scientific Oy, Vantaa, Finland).

165 The intra-assay coefficient of variation of duplicates was 12.0%. The inter-assay coefficient of
166 variation was 13.0% at a plasma concentration of 15.8 nmol/L and 3.5% at a concentration of 58.5
167 nmol/L. The linearity of the assay was evaluated by diluting the canine plasma sample (1/10, 1/20
168 and 1/40) with the assay buffer provided in the kit. Observed to expected ratios were calculated for
169 the dilutions. The mean recovery of the expected PGFM concentrations in different dilutions was
170 102% and dilutions of the canine plasma sample showed linearity over the studied range
171 ($R^2=0.996$). The detection limit was 0.13 nmol/L.

172

173 2.5. *iCa*

174

175 Blood *iCa* was analyzed instantly with Roche Electrolyte Analyzer (9180, Fisher Scientific Oy,
176 Vantaa, Finland) from Safe Pico syringes stored in an ice-water bath. The syringes contained 60 IU
177 of dry electrolyte-balanced heparin. Contact with air was minimized with a specific cap to remove
178 possible air bubbles.

179

180 2.6. *Uterine samples*

181

182 Uterine samples were obtained only from bitches undergoing a CS. Immediately after the removal of
183 the puppies from the uterus, a full-thickness sample of uterine wall (approximately 5 x 30 mm) was
184 taken from the incision site (interplacental area, uterine body or proximal horn). The sample was
185 immediately frozen in liquid nitrogen and stored at -70 °C for PCR analysis to measure the relative
186 expression of OXTR mRNA.

187

188 2.7. *PCR*

189

190 2.7.1. RNA preparation and reverse transcription

191 The full procedure has been described previously [28]. In brief, a 2-μg aliquot of total RNA from
192 each canine uterine sample was reverse transcribed at 37°C for 60 min in a final volume of 20 μL
193 with a reaction mixture (Qiagen) containing 1× RT buffer, dNTP mix (0.5 mM each dNTP), 600 ng
194 random primers (Invitrogen, Paisley, UK), 2 units of RNase inhibitor (Qiagen), and 4 units of
195 Omniscript™ reverse transcriptase (Qiagen).

196

197 2.7.2. Real-time PCR analysis

198 The real-time PCR analysis and the primers used have been described previously [28]. The
199 oligonucleotide primer pair for the OXTR was designed with NCBI/Primer-BLAST. To standardize
200 the quantification method, RPL27 and HPRT1 were selected as non-regulated reference genes with
201 primer pairs obtained from Silva et al. [29] and Bhatti et al. [30], respectively. The primers were
202 based on the sequences of the canine genes, and were the following: OTR forward primer: 5'-
203 TGCTGGCCTTCATCGTGTGCT-3'; OTR reverse primer: 5'-
204 GATGAAAGCCGAGGCTTCCTTGGG-3' from NM_001198659.1 with predicted size 95 bp;
205 RPL27 forward primer: 5'-ACAATCACCTCATGCCCACA-3'; RPL27 reverse primer: 5'-
206 CTTGACCTTGGCCTCTCGTC-3' from NM_001003102.2 with the predicted size 122 bp; HPRT1
207 forward primer: 5'-AGCTTGCTGGTGAAAAGGAC-3'; HPRT1 reverse primer: 5'-
208 TTATAGTCAAGGGCATATCC-3' from NM_001003357.1 with predicted size 104 bp. All samples
209 were run in duplicate and the purity of PCR products was confirmed by a melting-curve analysis in
210 all experiments. Each PCR assay included a negative control containing an RNA sample without
211 reverse transcription. The PCR amplification rate and the cycle threshold (C_t) values were analyzed
212 using iCycler™ iQ 3.1 software (Bio-Rad). The OXTR product was normalized against the mean of
213 RPL27 and HPRT1 products to yield the relative expression of OXTR mRNA.

214
215
216
217
218
219
220
221
222
223
224
225
226
227
228
229
230
231
232
233
234
235
236
237

2.8. Statistical analysis

Data were analyzed using IBM SPSS Statistics 24 software for Windows. The non-parametric Kruskal-Wallis one-way ANOVA test with Bonferroni correction was used to detect possible differences in serum progesterone levels, plasma PGFM levels, blood iCa, and relative expression of OXTR mRNA between the groups. Differences were considered statistically significant at $p<0.05$.

3. Results

3.1. Progesterone

Serum progesterone concentrations in the different groups are presented in Fig. 1a. The concentrations were highest in the ECS group; the largest variation in levels was also observed in this group. There was a significant difference ($p<0.05$) between the ECS and the OD and between the ECS and the COMB groups.

3.2. PGFM

Plasma PGFM concentrations were highest in the C and lowest in the ECS group (Fig. 1b). A significant difference ($p<0.05$) was detected between the C and the ECS and between the C and the COMB groups.

238 3.3. Progesterone:PGFM

239

240 The progesterone:PGFM ratio was highest in the ECS group (Fig. 1c). A significant difference
241 ($p<0.05$) was observed between the ECS and the C and between the ECS and the COMB groups.

242

243 3.4. iCa

244

245 Blood iCa concentrations were lowest in the PUI group but no significant difference ($p>0.05$) was
246 observed between the groups (Fig. 1d). No hypocalcemia was detected (reference interval 1.16-1.40
247 nmol/l).

248

249 3.5. qPCR

250

251 The mean relative expression of OXTR mRNA was highest in the ECS group (Fig. 1e). The difference
252 was significant ($p<0.05$) between the ECS and the OD and between the ECS and the COMB2 groups.
253 There was no significant difference between bitches treated or not treated with calcium glubionate
254 and OT.

255

256

257 4. Discussion

258

259 Our study indicates that in complete primary uterine inertia the etiology may not be the absence or
260 downregulation of OXTR, as there was no difference in OXTR expression in comparison of CUI to
261 bitches near term but before the first stage of parturition (ECS group). Upregulation of OXTR occurs

262 near term, and the prolonged influence of OT and uterine exhaustion in obstructive dystocia may lead
263 to downregulation of OXTR.

264

265 Our results support previous reports [1,2,3] on decreasing progesterone and increasing PGFM levels
266 during prepartum luteolysis in pregnant bitches. As expected, progesterone levels were higher in the
267 ECS group than in the other groups, as CS was performed in this group before the onset of parturition
268 (before stage 1). A sudden decrease of progesterone is observed in near term pregnant bitches at the
269 end of the luteal phase [11]. Termination of corpora lutea function in non-pregnant bitches is
270 suggested to be more likely regressive than the active luteolytic process found in pregnant bitches,
271 which indicates a different regulation mechanism [31]. Failure of luteolysis can lead to prolonged
272 gestation [32]. Except in the ECS group, all bitches in this study had undergone luteolysis.

273

274 One possibility for the etiology of complete primary uterine inertia could be a problem in parturition
275 initiation. Excessive progesterone and insufficient PGF2 α levels could prevent sufficient uterine
276 contractions and thus interfere with parturition. However, our results suggest that this might not be
277 the case, as the progesterone and PGFM levels in CUI group were similar to other dystocia groups.
278 This may indicate that the etiology is more likely at the level of uterine function, such as myometrial
279 distention beyond its capacity to contract or the lack of cervical pressure to stimulate OT release. The
280 progesterone:PGFM ratio was highest in the ECS group, where the highest progesterone and lowest
281 PGFM concentrations were also found. This indicates that luteolysis had not yet occurred in this
282 group. A high progesterone:PGFM ratio has been reported in dystocic bitches with complete primary
283 uterine inertia in comparison to a control group [22]. In our study no such difference was observed.

284

285 Calcium and OT injections are used as a treatment for uterine inertia to enhance contractions of the
286 uterus [19,20]. Batra [33] reported that OT-induced myometrial contractions in the rat depend on the

287 influx of extracellular calcium, and this influx is directly increased by OT. The action of OT has also
288 been postulated to occur by inhibiting the Ca^{2+} -extrusion pump in humans [34]. Hypocalcemia was
289 not diagnosed in any of the bitches in this study. However, there are reports of hypocalcemia in risk
290 groups of uterine inertia [35] and in dystocic bitches [36]. In our study, a single treatment with
291 calcium glubionate and OT did not seem to affect the expression of OXTR mRNA or distribution of
292 OXTR. In dystocia, the uterus has been under the influence of OT, and exhaustion and desensitization
293 may prevent medical treatment to induce uterine contractions. However, in this study the number of
294 bitches treated or not treated was low and further investigation is necessary.

295
296 Veiga et al. [26] reported higher expression of OXTR mRNA in both endometrium and myometrium
297 of late pregnant and parturient bitches than in earlier stages of pregnancy. In our study, full-thickness
298 samples were used for real-time PCR; endometrium and myometrium thus cannot be compared
299 separately. The samples of this study were run together with samples from our earlier report on non-
300 pregnant bitches [28], and the relative expression of OXTR mRNA was higher in pregnant bitches
301 than in non-pregnant ones. Expression of OXTR in the canine uterus is probably not regulated only
302 by a decrease of progesterone. In anestrus bitches with basal levels of progesterone, OXTR
303 expression does not differ from diestrus bitches with uteri under the influence of progesterone [28];
304 the expression is thus likely a part of more complex regulatory pathways. In the OD group, OXTR
305 mRNA expression was significantly decreased. In the PUI group the decrease also approached
306 significance. A large variation of OXTR mRNA expression in the CUI group may be due to the
307 heterogeneity of this group. It is also possible that in the CUI group the mechanism of dystocia is
308 different than that of the PUI and OD groups. The uterus does not contract in complete primary uterine
309 inertia, which may be due to the lack of cervical stimulus and insufficient release of OT to systemic
310 circulation. Thus, desensitization might not occur and expression of OXTR mRNA could remain
311 high. Bergström et al. [21] reported lower plasma OT concentrations in primary uterine inertia cases

312 than in bitches with normal parturition. In obstructive dystocia, and possibly in partial uterine inertia,
313 uterine exhaustion possibly with paracrine or autocrine signaling may result in OXTR
314 downregulation.

315

316 Although strict criteria were defined to include the bitches in the groups in this study, some
317 heterogeneity probably exists. Breed diversity also increases the heterogeneity of the groups. In this
318 study, the bitches with normal parturition were used as controls only for the blood parameters. For
319 OXTR gene expression only ECS samples from prepartum bitches were used. Further studies are
320 necessary to compare OXTR gene expression also with samples from bitches with normal parturition.
321 The number of bitches was quite low (particularly in the CUI group), which may affect the results. A
322 greater number of individuals is necessary to more properly evaluate the effect of calcium and OT
323 treatment on OXTR. Furthermore, an uterokinetic study *in vitro* with myometrial muscle strips, as
324 described by Gogny et al. [37], may provide information on myometrial contractions and
325 desensitization under prolonged influence of OT. Further studies of genetic background with breeds
326 and lines susceptible to complete primary uterine inertia are needed.

327

328

329 **5. Conclusions**

330

331 This study provides evidence of prepartum upregulation of OXTR in the canine uterus. Expression
332 of OXTR was increased near term. A decrease in expression was observed in obstructive dystocia
333 and may also occur in partial primary uterine inertia. However, no clear decrease in expression was
334 observed in the CUI group, which may indicate a different etiology for inertia than in OD. The
335 etiology in complete primary uterine inertia is more likely at the level of uterine function, such as
336 myometrial distention beyond its capacity to contract or the lack of cervical pressure to stimulate OT

337 release. A decrease of OXTR may also occur during normal parturition; the role of desensitization
338 of OXTR in dystocia should to be clarified. It remains unclear if parturition ceases because of uterine
339 inertia or if uterine inertia occurs because of ceased parturition and desensitization of receptors.

340

341

342 **Acknowledgements**

343

344 The authors would like to thank the following from the University of Helsinki: Kati Holmsten, Merja
345 Pöytäkangas, and Satu Sankari for assistance in laboratory analyses. The staff of the Veterinary
346 Teaching Hospital of the University of Helsinki and the Veterinary Clinic of Mäntsälä is
347 acknowledged for assistance in sample collection. This study was financially supported by the Finnish
348 Foundation of Veterinary Research, the Finnish Veterinary Foundation, and the European Veterinary
349 Society for Small Animal Reproduction (EVSSAR).

350

351

352 **References**

353

- 354 [1] Concannon PW, Isaman L, Frank DA, Michel FJ, Currie WB. Elevated concentrations of 13,14-
355 dihydro-15-keto-prostaglandin F-2 alpha in maternal plasma during prepartum luteolysis and
356 parturition in dogs (*Canis familiaris*). *J Reprod Fertil* 1988;84:71-7.
- 357 [2] Veronesi MC, Battocchio M, Marinelli L, Faustini M, Kindahl H, Cairoli F. Correlations among
358 body temperature, plasma progesterone, cortisol and prostaglandin F2 α of the periparturient bitch. *J*
359 *Vet Med A* 2002;49:264-8.
- 360 [3] Nohr B, Hoffmann B, Steinetz BE. Investigation of the endocrine control of parturition in the dog
361 by application of an antigestagen. *J Reprod Fertil Suppl* 1993;47:542-3.

362 [4] Kowalewski MP, Beceriklisoy HB, Pfarrer C, Aslan S, Kindahl H, Küçükaslan I et al. Canine
 363 placenta: a source of prepartal prostaglandins during normal and antiprogesterin-induced parturition.
 364 Reproduction 2010;139:655-64.

365 [5] Concannon PW, Butler WR, Hansel W, Knight PJ, Hamilton JM. Parturition and lactation in the
 366 bitch: serum progesterone, cortisol and prolactin Biol Reprod. 1978;19:1113-8.

367 [6] Olsson K, Bergström A, Kindahl H, Lagerstedt A-S. Increased plasma concentrations of
 368 vasopressin, oxytocin, cortisol and the prostaglandin F2 α metabolite during labour in the dog. Acta
 369 Physiol Scand 2003;179:281-7.

370 [7] Klarenbeek M, Okkens AC, Kooistra HS, Mol JA, Bevers MM, Taverne MAM. Plasma oxytocin
 371 concentrations during late pregnancy and parturition in the dog. Theriogenology 2007;68:1169-76.

372 [8] Yoshinaga K, Hawkins RA, Stocker JF. Estrogen secretion by the rat ovary in vivo during the
 373 estrous cycle and pregnancy. Endocrinology 1969;85:103-12.

374 [9] Robertson HA, Smeaton TC. The concentration of unconjugated oestrone, oestradiol-17 alpha and
 375 oestradiol-17 beta in the maternal plasma of the pregnant ewe in relation to the initiation of parturition
 376 and lactation. J Reprod Fertil 1973;35:461-8.

377 [10] Robertson HA. Changes in the concentration of unconjugated oestrone, oestradiol-17alpha and
 378 oestradiol-17beta in the maternal plasma of the pregnant cow in relation to the initiation of parturition
 379 and lactation. J Reprod Fertil 1974;36:1-7.

380 [11] Concannon PW, Hansel W, Visek WJ. The ovarian cycle of the bitch: Plasma estrogen, LH and
 381 progesterone. Biol Reprod 1975;13:112-21.

382 [12] Linde-Forsberg C. Abnormalities in pregnancy, parturition, and the periparturient period. In:
 383 Ettinger SJ, Feldman EC, editors. Textbook of veterinary internal medicine, 7th ed. St. Louis: Elsevier
 384 Saunders; 2010, p. 1890–1901).

385 [13] Bergström A, Nødtvedt A, Lagerstedt AS, Egenvall A. Incidence and breed predilection for
386 dystocia and risk factors for cesarean section in a Swedish population of insured dogs. *Vet Surg*
387 2006;35:786-91.

388 [14] Evans KM, Adams VJ. Proportion of litters of purebred dogs born by caesarean section. *J Small*
389 *Anim Pract* 2010;51:113-8.

390 [15] Gaudet DA. Retrospective study of 128 cases of canine dystocia. *J Am Anim Hosp Assoc*
391 1985;21:813-8.

392 [16] Münnich A, Küchenmeister U. Dystocia in numbers – Evidence-based parameters for
393 intervention in the dog: Causes for dystocia and treatment recommendations. *Reprod Dom Anim*
394 2009;44:Suppl 2:141–7.

395 [17] Darvelid AW, Linde-Forsberg C. Dystocia in the bitch: A retrospective study of 182 cases. *J*
396 *Small Anim Pract* 1994;35:402-7.

397 [18] Linde Forsberg C, Persson G. A survey of dystocia in the Boxer breed. *Acta Vet Scand*
398 2007;49:8.

399 [19] Johnston SD, Root Kustritz MV, Olson PNS. *Canine and feline theriogenology*. 1st ed.
400 Philadelphia: WB Saunders; 2001.

401 [20] Davidson A. Problems during and after parturition. In: England G, von Heimendahl A, editors.
402 *BSAVA Manual of canine and feline reproduction and neonatology*, Gloucester: British Small
403 *Animal Veterinary Association*; 2010, p. 121–34.

404 [21] Bergström A, Fransson B, Lagerstedt A-S, Olsson K. Primary uterine inertia in 27 bitches:
405 aetiology and treatment. *J Small Anim Pract* 2006;47:456-60.

406 [22] Bergström A, Fransson B, Lagerstedt AS, Kindahl H, Olsson U, Olsson K. Hormonal
407 concentrations in bitches with primary uterine inertia. *Theriogenology* 2010;73:1068-75.

408 [23] Gimpl G, Fahrenholz F. The oxytocin receptor system: structure, function, and regulation.
409 *Physiol Rev* 2001;81:629-83.

- 410 [24] Derussi AA, de Souza RW, Volpato R, Guaitolini CR, Ackermann CL, Taffarel MO et al.
411 Progesterone (PR), oestrogen (ER-alpha and ER-beta) and oxytocin (OTR) gene expression in the
412 oviduct and uterus of pregnant and non-pregnant bitches. *Reprod Domest Anim* 2012;47:Suppl
413 6:197-9.
- 414 [25] Gram A, Boos A, Kowalewski MP. Uterine and placental expression of canine oxytocin receptor
415 during pregnancy and normal and induced parturition. *Reprod Domest Anim* 2014;49:Suppl 2:41-9.
- 416 [26] Veiga GA, Milazzotto MP, Nichi M, Lúcio CF, Silva LC, Angrimani DS, et al. Gene expression
417 of estrogen and oxytocin receptors in the uterus of pregnant and parturient bitches. *Braz J Med Biol*
418 *Res* 2015;48:339-43.
- 419 [27] Phaneuf S, Asbóth G, Carrasco MP, Europe-Finner GN, Saji F, Kimura T, et al. The
420 desensitization of oxytocin receptors in human myometrial cells is accompanied by down-regulation
421 of oxytocin receptor messenger RNA. *J Endocrinol* 1997;154:7-18.
- 422 [28] Tamminen TM, Sahlin L, Masironi B, Taponen J, Laitinen-Vapaavuori O, Katila T. Oxytocin
423 receptors in dioestrous and anoestrous canine uteri. *Reprod Domest Anim*. 2017;52:153-9.
- 424 [29] Silva E, Leitão S, Ferreira-Dias G, Lopes da Costa L, Mateus L. Prostaglandin synthesis genes
425 are differentially transcribed in normal and pyometra endometria of bitches.
426 *Reprod Domest Anim* 2009;44:Suppl 2:200-3.
- 427 [30] Bhatti SF, Rao NA, Okkens AC, Mol JA, Duchateau L, Ducatelle R et al. Role of progestin-
428 induced mammary-derived growth hormone in the pathogenesis of cystic endometrial hyperplasia in
429 the bitch. *Domest Anim Endocrinol* 2007;33:294-312.
- 430 [31] Kowalewski MP. Luteal regression vs. prepartum luteolysis: regulatory mechanisms governing
431 canine corpus luteum function. *Reprod Biol*. 2014;14:89-102.
- 432 [32] Irons PC, Nöthling JO, Volkmann DH. Failure of luteolysis leads to prolonged gestation in a
433 bitch: a case report. *Theriogenology*. 1997;48:353-9.

- 434 [33] Batra S. Effect of oxytocin on calcium influx and efflux in the rat myometrium. Eur J Pharmacol.
435 1986 Jan 14;120:57-61.
- 436 [34] Popescu LM, Nutu O, Panoiu C. Oxytocin contracts the human uterus at term by inhibiting the
437 myometrial Ca^{2+} -extrusion pump. Biosci Rep. 1985;5:21-8.
- 438 [35] Hollinshead FK, Hanlon DW, Gilbert RO, Verstegen JP, Krekeler N, Volkmann DH. Calcium,
439 parathyroid hormone, oxytocin and pH profiles in the whelping bitch. Theriogenology.
440 2010;73:1276-83.
- 441 [36] Frehner BL, Reichler IM, Keller S, Goericke-Pesch S, Balogh O. Blood calcium, glucose and
442 haematology profiles of parturient bitches diagnosed with uterine inertia or obstructive dystocia.
443 Reprod Domest Anim 2018;53:680-7.
- 444 [37] Gogny A, Mallem Y, Destrumelle S, Thorin C, Desfontis JC, Gogny M et al. In vitro comparison
445 of myometrial contractility induced by aglepristone-oxytocin and aglepristone-PGF 2α
446 combinations at different stages of the estrus cycle in the bitch. Theriogenology 2010;74:1531-8.

447

448

449 **Author contributions**

450 TMT: design of the study, RNA isolation, real-time PCR, data and statistical evaluation, manuscript
451 writing. OV, MD, JT, TK: design of the study, data and statistical evaluation, manuscript editing. LS,
452 BM: RNA isolation, real-time PCR, manuscript editing.

453

454

455 **Conflicts of interest**

456 The authors have no conflicts of interest to declare.

457

a)

b)

c)

d)

e)

Table 1. The individual and average data of 58 bitches divided in six groups.

Parity includes the current parturition. Medication: 0, no medication; 1, calcium and oxytocin after blood sample. n/a: not available.

Group	Mean age, min-max (years)	Mean weight, min-max (kg)	Mean litter size, min-max	Breed	Parity	Gestation length (days from the last and first mating)	Gestation length (days from ovulation)	Duration of the first stage of parturition (hours)	Duration of the second stage of parturition before intervention (hours), discharge	Litter size	Number of puppies born before dystocia/by cesarean section	Medication
1. Complete primary uterine inertia (CUI) n = 7	4.1, 2.3-6.2	23.7, 4.7-35.0	6.4, 1.0-14.0	Border Collie	1	56-61	n/a	26	3, fetal fluids	4	0/4	1
				Airedale Terrier	1	60-62	63	45	6, fetal fluids	7	0/7	0
				Karelian Bear Dog	3	68	n/a	n/a	n/a, green fluids	1	0/1	0
				Dogo Argentino	1	65	n/a	29	6, green fluids	2	0/2	0
				Chinese Crested Dog	1	62	n/a	16	4, fetal fluids	6	0/6	1
				English Springer Sp.	2	60	n/a	48	6, fetal fluids	14	0/14	1
				Labrador Retriever	1	59	60	65	6, fetal fluids	11	0/11	0
2. Partial primary uterine inertia (PUI) n = 13	5.1, 2.6-8.0	29.2, 6.5-65.0	7.0, 5.0-15.0	Mixed	1	64-65	n/a	n/a	4	5	1/4	1
				Labrador Retriever	2	60-62	62	5	4	8	5/3	1
				Dalmatian	3	63	63	6	4	6	1/5	0
				Poodle, Standard	1	61-62	62-63	7	9.5	5	3/2	0
				Labrador Retriever	2	63	n/a	10	5	8	6/2	1
				Miniature Schnauzer	1	60-61	n/a	24	3.5	7	1/6	1
				Tibetan mastiff	2	59-61	62	24	4	5	4/1	1
				Mixed	1	62	n/a	27	n/a	7	1/6	1
				Border Collie	1	57-61	n/a	22	5.5	6	4/2	1
				Bullmastiff	2	56-57	57	65	n/a, fetal fluids	15	1/14	0
				Great Dane	1	62	n/a	26	6	6	2/4	1
				Bearded Collie	1	54-56	58	14	5.5	8	4/4	1
				Giant Schnauzer	2	58	60	12	10	5	2/3	1
				Cairn Terrier	3	64	66	n/a	4	1	0/1	0
				Border Collie	3	59-63	63	n/a	n/a, green fluids	7	0/7	1
3. Obstructive dystocia (OD) n = 10	5.1, 2.3-8.3	23.7, 7.0-65.0	5.0, 1.0-9.0	Saluki	2	61	64	18	4	3	0/3	0
				Cavalier King Ch. Sp.	1	59-60	62	24	4	7	0/7	0
				French Bulldog	1	61-63	n/a	9	6	3	0/3	0
				Dog de Bordeaux	2	65-67	n/a	11	4	9	0/9	1
				Smooth Collie	3	60-61	n/a	24	8	6	3/3	1
				Cavalier King Ch. Sp.	1	56-58	59	n/a	3	4	1/3	1
				Spanish Mastiff	1	62	62	11	n/a, fetal fluids	3	0/3	1
				Border Collie	3	60-61	61	24	3	7	0/7	1
				Cavalier King Ch. Sp.	3	n/a	60	0	0	4	0/4	0
				Border Collie	1	64-65	64	0	0	2	0/2	0
4. Elective cesarean section (ECS) n = 11	4.6, 3.1-6.6	23.9, 6.9-65.0	3.8, 1.0-10.0	St. Bernard	2	n/a	61	0	0	2	0/2	0
				Cavalier King Ch. Sp.	3	58-60	60	0	0	5	0/5	0
				Great Dane	2	62-63	62-63	0	0	1	0/1	0
				Shetland Sheepdog	4	60	n/a	0	0	1	0/1	0
				Boston Terrier	2	63-63	62	0	0	2	0/2	0
				Newfoundlander	1	58-64	n/a	0	0	10	0/10	0
				Cavalier King Ch. Sp.	3	59-60	59	0	0	8	0/8	0
				Boston Terrier	1	63-65	n/a	0	0	3	0/3	0
				Golden Retriever	1	64-66	65	0	0	4	0/4	0
				Dachshund	1	58-60	n/a	1	2	4	0/-	1
5. Medically treated dystocia, no cesarean section (MD) n = 8	4.7, 2.6-6.5	13.3, 4.5-20.0	5.1, 2.0-8.0	Dachshund, miniature	1	63	n/a	15	5	6	0/-	1
				Finnish Lapphund	1	n/a	n/a	n/a	7	5	4/-	1
				Jack Russell Terrier	2	58-61	n/a	n/a	4	2	0/-	1
				Australian Kelpie	1	60	n/a	15	7	8	6/-	1
				English Springer Sp.	3	n/a	n/a	12	10	4	2/-	1
				Finnish Lapphund	2	n/a	n/a	n/a	5	6	3/-	1
				Lapponian Herder	2	n/a	63	7	5	6	2/-	1
				Cavalier King Ch. Sp.	1	n/a	60	n/a	n/a	5	-	0
6. Control, normal parturition (C) n = 9	4.20, 1.9-6.5	10.9, 8.0-30.0	5.4, 4.0-10.0	Labrador Retriever	3	n/a	60	n/a	n/a	10	-	0
				Cavalier King Ch. Sp.	4	n/a	59	n/a	n/a	4	-	0
				Pug	2	n/a	61-62	n/a	n/a	5	-	0
				Pug	1	n/a	61	n/a	n/a	5	-	0
				Cavalier King Ch. Sp.	4	61	n/a	n/a	n/a	5	-	0
				Cavalier King Ch. Sp.	1	62	n/a	n/a	n/a	4	-	0
				Cavalier King Ch. Sp.	2	n/a	58-59	n/a	n/a	5	-	0
				Cavalier King Ch. Sp.	2	n/a	60	n/a	n/a	6	-	0