

Field study reveals morphological and neuroendocrine correlates of seasonal breeding in female water voles, Arvicola terrestris

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1 Title

- 2 Field study reveals morphological and neuroendocrine correlates of seasonal breeding in
- 3 female water voles, *Arvicola terrestris*.

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18 Abstract (150 words)

Seasonally breeding mammals display timely physiological switches between reproductive 19 activity and sexual rest, which ensure synchronisation of births at the most favourable time of 20 the year. These switches correlate with seasonal changes along the hypothalamo-pituitary-21 22 gonadal axis, but they are primarily orchestrated at the hypothalamic level through environmental control of KISS1-dependent GnRH release. Our field study shows that births 23 24 of fossorial water voles, Arvicola terrestris, are concentrated between March and October, which indicates the existence of an annual reproductive cycle in this species. Monthly field 25 26 monitoring for over a year further reveals dramatic seasonal changes in the morphology of the ovary, uterus and lateral scent glands, which correlate with the reproductive status. Finally, 27 we demonstrate seasonal variation in kisspeptin expression within the hypothalamic arcuate 28 nucleus. Altogether, this study demonstrates a marked rhythm of seasonal breeding in the 29 water vole and we speculate that this is governed by seasonal changes in photoperiod. 30

31 Keywords

Seasonal breeding; Field study; hypothalamo-pituitary-gonadal axis; Kisspeptin; Water voles;
 Arvicola terrestris

34 1. Introduction

35 In seasonally breeding mammals, annual sexual activity is temporally restricted to allow synchronised births when environmental conditions, such as temperature and food 36 37 availability, are most favourable to the survival of the juveniles and mothers (Vasantha, 2015). The annual cycle of sexual activity and sexual rest of seasonal mammals depends upon 38 major central regulations of the neuroendocrine axis which controls the reproductive function, 39 namely the hypothalamo-pituitary-gonadal axis (for review see Simonneaux et al., 2013). 40 Two populations of kisspeptin neurons, respectively located in the anteroventral 41 periventricular nucleus (AVPV) and arcuate nucleus of the hypothalamus, play distinct but 42 pivotal roles in the central control of reproduction. Kisspeptin is the most potent secretagogue 43 of GnRH (Messager et al., 2005), which stimulates the release of FSH and LH in the 44 bloodstream by the gonadotropic cells of the pituitary gland (Belchetz et al., 1978). In adult 45 46 females, FSH and LH act on ovaries to support follicular growth, allow ovulation and formation of *corpora lutea* (Greep et al., 1942) and trigger the production of the sex-steroids 47 estrogen and progesterone. In turn, sex steroids influence the growth of the uterus (Brody and 48 Wiqvist, 1961; Galand et al., 1971) and secondary sexual characteristics, as well as sebaceous 49 glands (Frost et al., 1973; Thiessen et al., 1971). At least in rodents, estrogen exerts positive 50 feedback onto kisspeptin neurons located in the AVPV, which triggers the pre-ovulatory LH 51 52 surge, but negative feedback onto kisspeptin neurons of the arcuate nucleus, which is key for the pulsatile secretion mode of LH (Gottsch et al., 2009; Smith et al., 2005). Furthermore, in 53 seasonal breeders, there is strong evidence that these Kiss1-expressing neurons of the arcuate 54 nucleus relay photoperiodic information and are crucial for the timely orchestration of 55 56 seasonal switches in reproductive states (Simonneaux, 2020)

Our understanding of the mechanisms regulating the HPG axis in temperate zone non-57 hibernating fossorial species is currently limited. To address this gap, we have studied 58 Arvicola terrestris, which is the fossorial ecotype of the water vole, Arvicola amphibius, a 59 small semi-aquatic rodent species that displays widespread distribution across Eurasia 60 (Mahmoudi et al., 2020; Shenbrot and Krasnov, 2005). Fossorial water vole mainly colonizes 61 mid-mountain permanent meadows and orchards where it displays a mostly underground 62 63 lifestyle. It digs extensive networks of galleries, which are used for foraging and storing food, especially during autumn and winter, and it builds nests (Airoldi, 1976). 64

3

Births of water voles are mainly observed from March to October (Airoldi, 1978; Stoddart, 65 1971; Ventura and Gosálbez, 1990; Villette et al., 2020) with an average of three litters per 66 year. It has been hypothesized that the sexual activity of water voles displays an annual 67 rhythm, which has been empirically supported by the annual fluctuation in the activity of 68 androgen-dependent lateral scent glands of adult males. These sebaceous glands were 69 characterised by a hypertrophied epidermis and the absence of hair follicles and appear to be 70 71 implicated in the attraction of sex-partner and territoriality (Saucy, 1988; Stoddart, 1972; Stoddart et al., 1975). However, the sexual activity of fossorial water voles may be 72 73 maintained during mild winters in some populations located in Switzerland (Meylan and Airoldi, 1975). Likewise, when exposed to favourable weather conditions, as occurs in the 74 75 northwest of Spain, fossorial voles can display year-round breeding (Somoano et al., 2017). In 76 this context, the regulation of the annual rhythm of sexual activity of water voles needs 77 further investigation. Here, we explored the hypothesis that seasonal regulations, mediated by kisspeptin, along the hypothalamo-pituitary-gonadal axis were responsible for the alternation 78 79 between periods of breeding and sexual rest.

To this end, we have performed monthly monitoring of trapped fossorial female water voles from February 2019 through to June 2020 in order to characterise seasonal regulations of breeding at different levels, i.e. along the hypothalamo-pituitary-gonadal axis and at the level of the steroid-dependent lateral scent glands. We expected to observe, during the breeding period, growth of genital organs (ovaries and uterus) and lateral scent glands compared to winter. Furthermore, we anticipated that these changes would correlate with changes in kisspeptin immunoreactivity in the hypothalamic arcuate nucleus.

87 2. Material and methods

88 2.1. <u>Ethical statement</u>

The trapping of water voles was authorised in the French department of Puy-de-Dôme by prefectural authorization 19-00100. This study was carried out in accordance with European directive 2010/60/UE and was approved by an ethical committee for animal experimentation (C2EA-02, project 21994-201907510411944).

93 2.2. <u>Study species</u>

The European water vole belongs to the Cricetidae family, which also includes voles of the genera *Microtus* and *Myodes* and hamsters (Wilson and Reeder, 2005). The fossorial ecotype of water voles in the study area (Auvergne, France) has an adult weight of 75-160g and approximately measures fifteen centimetres, twenty with the tail. The reproductive capacities of the water vole are high, the gestation period lasts 21 to 22 days, with four to seven embryos per litter, 3 litters per year, and sexual maturity is reached at about five weeks.

100 2.3. <u>Monthly physiological monitoring</u>

From February 2019 through to June 2020, 13 successive rounds of captures were carried out 101 to perform monthly physiological monitoring (Table 1). Lack of data for some months is due 102 to winter climatic conditions, which made animal trapping unfeasible. Captures were 103 performed during the day on permanent meadows around Angle-bas (45° 42' 47" N, 2° 46' 104 105 12" E, alt. 887 m) in the French department of Puy-de-Dôme. Female fossorial water voles (Arvicola terrestris) were caught with traps (Topcat®, Andermatt, France) placed into their 106 galleries. Traps were checked regularly (~every 15min) and sampling was performed right 107 away. For each animal, we recorded body weight and size (head and body, without tail) and 108 performed visual inspection of vaginal opening to assess sexual maturity. The number of 109 embryos was counted for each pregnant female, and non-pregnant lactating females were 110 identified by their developed nipples. Uterus and ovaries were collected and fixed in Bouin's 111 112 solution (Sigma, France) for 48h before being weighed and stored in 70% ethanol. The lateral scent glands were frozen in dry ice and stored at -80°C. 113

For the analyses of monthly variations, only data from non-pregnant females were used. The weights of the uterus, ovaries, and lateral scent glands were related to the body weight for each animal, while the surface of lateral scent glands was related to the body size. Uncorrected values are available in supplementary data (Sup.Table 1).

118 Monthly weather data (sunshine, rainfall, and average temperature) corresponding to the119 monitoring period were obtained from the website www.infoclimat.fr.

120

2.4. Tissue collection for immunohistochemistry

Non-pregnant females caught in summer (July and August), and winter (November) (Figure 1A) were anesthetised with isoflurane vapours, then euthanised by injecting an overdose of pentobarbital and finally intracardially perfused with a sodium nitrite solution followed by 4% paraformaldehyde (PFA) in phosphate buffer. Brains, ovaries, uterus, and lateral scent glands

were collected and postfixed in 4% PFA for 48h then weighed before being cryoprotected in
30% sucrose. Pituitaries were collected in NaCl buffer before being weighed.

127 2.5. <u>Immunohistochemistry</u>

Before immunohistochemical labelling, brains were frozen at -18°C, sliced in coronal free-128 floating sections of 30µm, and stored in cryoprotective solution until analysed. Then, brain 129 sections were washed three times for 5 min in Tris buffer saline (TBS). Sections were 130 incubated for 30 min with 0.3% H₂O₂ in TBS to inhibit endogenous peroxidase activity and 131 for 2h in TBS containing 0.1% triton X100 with 2% normal donkey serum (NDS) to block 132 non-specific binding. Sections were then incubated for 72h at 4°C in TBST-NDS with a rabbit 133 anti-kisspeptin antibody (1:50000, AC564, (Franceschini et al., 2006), Table 2). After three 5 134 min washes, sections were incubated for 2h at room temperature (RT) in TSBT-NDS with a 135 136 donkey anti-rabbit antibody (1:1000, Jackson ImmunoResearch). Sections were washed three times for 5 min and incubated for 1h at RT with an avidin/biotin system for signal 137 amplification (VECTASTAIN® Elite® ABC HRP Kit, PK-6100). Sections were then rinsed 138 three times for 5min in Tris-HCL and revealed with DAB 0.15 mg.mL-1 dissolved in Tris-139 HCL containing 0.01% H₂O₂ for 15 min. Finally, sections were mounted in TBS, dehydrated, 140 and coverslipped in DPX. The specificity of immunolabeling was checked on consecutive 141 sections of the arcuate nucleus by pre-adsorption for 24h of the primary antibody with the 142 kisspeptin 10 peptide (GeneCust, France) (Sup. figure 1). 143

144 2.6. <u>Histological staining</u>

One horn of each uterus was sectioned in its middle portion for sampling. Uterine horn 145 samples and ovaries were rinsed in phosphate buffer saline (PBS) for 24h. Then, they were 146 dehydrated in successive baths of 50%, 70%, and 90% ethanol, followed by three baths of 147 absolute ethanol for 30 min each. After one night at 4°C and 2h at RT in butanol, samples 148 were impregnated in paraffin two times at 60°C before final embedding. Once dried, samples 149 were sliced into 5µm sections and arranged on slides. Before staining, sections were 150 deparaffined in xylene two times for 5 min. Then, sections were rehydrated with successive 151 baths of absolute, 90%, 70% ethanol, and a final bath of tap water, two times for 5 min each. 152 Sections were then stained in hematoxylin for 30 sec and cleared under tap water. After a 153 quick bath in 70% ethanol, sections were stained again in eosin for 5 sec. Finally, sections 154 were dehydrated and coverslipped in DPX. 155

Lateral scent glands were embedded in OCT (Tissue-Tek), frozen in dry ice, and sliced into 10 μ m sections using a cryostat. Then, sections were dried and stained with the same protocol as described above.

159

2.7. RNA extraction, Reverse Transcription-PCR and Cloning

The complete open reading frame for Kiss1 was obtained using hypothalamic mRNA from a 160 single male water vole. All procedures have been described in detail elsewhere (Lomet et al., 161 2018). RNA was extracted using TriReagent (Sigma). Concentration and purity of the sample 162 were determined with Nanodrop 2000 (ThermoScientific), and integrity was checked by 163 standard agarose gel electrophoresis. cDNA was synthesised using Omniscript RT kit 164 (Qiagen) and Oligo-dT primers (Eurofins, Germany). cDNA was cloned using a standard 165 homology procedure whereby multiple rodent Kiss1 sequences were aligned to guide PCR 166 primer design (see Table 2). PCR was performed using AccuPrime GC-rich DNA polymerase 167 (from Thermofisher). PCR reactions were loaded on a 1.5% agarose gel and migrated at 80-168 110V in Tris-EDTA-Acetic acid (TEA) buffer for ~30min. PCR fragments were extracted on 169 gel and cloned in pBS (Promega). Six independent clones were sequenced (Eurofins). 170

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172 2.8. <u>Image acquisitions and quantifications</u>

173 Images of sections of brains, uterine horns, ovaries, and lateral scent glands were acquired174 with AxioScan.Z1 slide scanner (Carl Zeiss France).

The measure of the uterine horn surface was performed on two sections separated by 100 µm 175 with the combination of the surface of myometrium and endometrium without the lumen. To 176 evaluate the existence of a period of anoestrus, which would be characterized by anovulation 177 and therefore by the absence of corpora lutea, the number of corpora lutea was counted on 178 three sections distributed over the entire surface of one ovary for each animal. Haemorrhagic 179 and mature corpora lutea with well-defined luteinised and non-necrotic theca and granulosa 180 cells were defined as being "recent". The two ovaries were inspected to determine the 181 182 percentage of females with recent corpora lutea in summer (July + August, n=11) and in winter (November + January, n=9). 183

Quantifications of kisspeptin-ir fibre density were performed on three sections at the anterior, median, and caudal levels of the arcuate nucleus (corresponding to the plates 43, 48, and 51 of the Paxinos and Franklin mouse brain atlas). The counting surface was 0.029 mm² on each side of the third ventricle. Images were analysed with ImageJ software (NIH, ver.1.52p). 188 Images were binarised, background was subtracted, and the area covered by the fibres was 189 measured after thresholding. No difference was detected between the three levels of arcuate 190 nucleus. So, statistical analysis was performed on the average of the quantifications of these 191 three levels to have a global overview of the arcuate nucleus.

Only few kisspeptin-ir fibres and cell bodies were detected in the AVPV (anteroventralperiventricular nucleus) which prevented us from performing any relevant quantification.

194 2.9. <u>Statistical analysis</u>

Results are shown as Tukey's boxplot with each individual (including outliers) represented by 195 196 a circle; means are indicated by a cross. Normality of data distribution was verified with the Shapiro-Wilk test. According to the normality, data were analysed either by one-way 197 ANOVA followed by Tukey's multiple comparisons test or by Kruskal-Wallis followed by 198 Dunn's test. To simplify interpretation of the data related to the annual monitoring, only 199 posthoc comparisons with the month of January are presented. All other pairwise comparisons 200 201 are available in supplementary data (Sup. Table 2). The effect of season on the weight of pituitary and the density of kisspeptin-ir fibres were analysed by unpaired Student's t-test. 202 GraphPad Prism 8.4.3 (GraphPad Software Inc, San Diego, California USA) was used to 203 204 perform statistical analyses and to draw the graphs.

205 **3. Results**

The average body weight of non-pregnant females significantly differs throughout the year (Table 1, one-way ANOVA, p=0.0013). The maximum average body weight was observed during spring, from April through June, while the minimum was observed during winter, in January (98g vs 75g, p=0.0028). The average body size also differed throughout the year (Table 1, Kruskal-Wallis, p=0.0053) even though no pairwise comparison reached statistical significance.

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Pregnant females were observed from the end of February through October 2019, then again from April 2020 onwards (Figure 1A). The maximum percentage of pregnant females (58% of sexually mature females) was observed in June 2019. During this month, among the nonpregnant females, 28% were lactating. Despite the presence of sexually mature females, a lack of pregnant females was observed between November 2019 and the end of February 2020. The average number of embryos per female (Figure 1B) was close to 4, but varied from 1 to

219 7, and did not display statistically significant variation.

220

221 The weight of the ovaries displayed a significant annual rhythm (Figure 2A, one-way 222 ANOVA, p<0.001). The heaviest ovaries were observed at the end of April 2019, while the lightest ovaries were found in January 2020 (0.0219%.BW vs 0.0018%.BW, p<0.001). In the 223 ovaries, the number of corpora lutea was quantified, and a significant difference was detected 224 (Figure 2B&C, Kruskal-Wallis, P<0.001) with a maximum number of corpora lutea at the 225 end of April 2019 and a minimum in January 2020 (3.16 vs 0.25, p=0.0350). Recent corpora 226 lutea were found only in females trapped in summer (July/August) compared to winter 227 (November/January) (8/11 vs 0/9, Fisher's exact test, p=0.0014, Figure 2C). 228

229

230 Consistent with data for body and ovarian weight, the uterine weight showed a significant monthly variation (Figure 2E, Kruskal-Wallis, p<0.001). These variations followed a 231 232 sinusoidal-like profile with a progressive increase, up until the maximum was reached in June 2019, then followed by a large 10-fold decrease until minimum weight was attained in 233 234 January 2020 (0.1988% of BW, 0.0186% of BW, p<0.001). Then, from January through to June 2020, the uterine weight increased again. As for the uterine weight, a significant monthly 235 variation of the surface of uterine horn sections was detected (Figure 2F and G, one-way 236 ANOVA, p<0.001). The surface of the myometrium and endometrium was significantly 237 238 larger in June compared to January (2.06 vs 0.27 mm², p=0.0027).

239

Histological observations of sections after staining of the lateral scent glands showed that 240 numerous sebaceous glands are located under the epidermis. The lipidic secretions are 241 produced by the vacuolated cells contained inside alveoli of different lobules (Figure 3A&B). 242 243 These lateral scent glands showed a significant monthly variation of both their size and weight (Figure 3C&D, Kruskal-Wallis, p<0.001 for both). Similar to the uterus and ovaries, 244 245 variations of lateral scent glands size and weight followed a sinusoidal-like profile with 246 maximal development in September 2019, followed by regression until a nadir was reached in January 2020 (p<0.001 for both). 247

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The weight of the pituitary was significantly higher in voles trapped in summer (July and August) compared to the ones trapped in winter (November) (4.08mg vs 2.83mg, Student ttest, p=0.0118; Figure 4C). In the arcuate nucleus of the hypothalamus, a significantly lower density of immunoreactive kisspeptin fibres was detected in voles trapped in summer
compared to the ones trapped in winter (Student-t-test, p<0.001; Figure 4A&B).

254

255 The complete open reading frame for *Kiss1* was obtained after cloning and sequencing by using hypothalamic mRNA from a single male European water vole (Arvicola terrestris). The 256 cDNA for Kiss1 has been deposited in Genbank under accession number MT922570. The 257 Kiss1 cDNA is predicted to yield a precursor protein of 136 amino acids, which shows very 258 high similarity with KISS1 sequences of multiple other rodents (Figure 4D). In particular, the 259 260 sequence of the water vole KP10 peptide (last 10 amino acids at the C-term of the mature peptide) is identical to that of all other rodents. Akin to what has been found for other 261 mammals, the European water vole Kiss1 open reading frame exhibits a high 66% GC content 262 (mouse 67%, human 67%, sheep 74%, horse 75%). 263

264 **4.** Discussion

Our extensive field study, relying on monthly physiological monitoring performed over a period of ~1.5 years uncovers dramatic seasonal rhythmicity in multiple morphological and neuroendocrine markers along the hypothalamo-pituitary-gonadal axis of female fossorial water voles. These features indicate that *Arvicola terrestris* displays marked seasonal breeding, akin to what is found in other long-day breeders.

270 In our female fossorial water voles, a change in the body weight was observed throughout this monitoring with heavier and lighter animals observed during spring/summer and winter, 271 272 respectively, which was previously described in other rodents known as seasonal breeders 273 (Jones et al., 2020; McKeever, 1963; Wade and Bartness, 1984). This change in body weight can also be linked to the renewal of the population. In the wild, 274 275 even though a study reported that some water voles may live up to 2 years, the average lifespan is 4-5 months (Stoddart, 1971). In another study, the lifespan was estimated to be 276 277 between 2 and 2.5 years, with a marked decrease in the number of older animals in autumn 278 (Ventura et al., 1991). No difference in body size of animals was detected between winter and 279 any other season. The vaginal opening inspection associated with the measure of body size 280 confirmed the sexually mature state of females.

281

Pregnant and lactating females were observed between March and October. While sexuallymature females were occasionally found also for the remainder of the year (from November to

February), none of these were identified as being pregnant. This is fully consistent with earlier findings, which defined the breeding period of water voles as being discontinuous throughout the year (Airoldi, 1978). Pregnant females were mainly observed from March through to October, even though some could also be observed during mild winters (Meylan and Airoldi, 1975). Our current observations strengthen the existence of a consolidated period of sexual rest during winter in female water voles.

290 We then assessed whether seasonal breeding rhythmicity could be correlated with rhythmic anatomical and neuroendocrine variations at all levels of the hypothalamo-pituitary-gonadal 291 292 axis. An increase in the weight of the ovaries was observed between late winter and early 293 summer, followed by a progressive regression until winter as previously described in Belding 294 ground squirrel and Pine voles (McKeever, 1963; Valentine and Kirkpatrick, 1970). This 295 increase in ovarian weight was associated with a higher number of corpora lutea in spring and 296 summer, thus indicating a higher rate of ovulation than in winter, as reported in bank and field 297 voles (Brambell and Rowlands, 1936; Thibault et al., 1966). In addition, no recent corpora 298 *lutea* were found in winter, which may suggest a possible winter anoestrus in some female water voles. 299

A seasonal change in uterine weight was also observed. Female water voles trapped in spring and early summer presented the heaviest uterus with myometrium and endometrium considerably more developed than in winter. Uterine tissues are known to be highly sensitive to oestradiol (Brody and Wiqvist, 1961; Galand et al., 1971), and the increase of uterine weight observed in spring and early summer suggests a higher level of estradiol production by ovaries during this period.

In parallel to the reproductive organs, seasonal changes of lateral scent glands were identified 306 307 with maximal development in summer and a dramatic regression in winter, as described earlier in male water vole (Stoddart, 1980). These sebaceous glands were shown to be 308 androgen-dependent in the male water vole (Stoddart, 1972), as in male and female hamsters 309 and gerbil (Frost et al., 1973; Lucky et al., 1986; Thiessen et al., 1971; Vandenbergh, 1973). 310 311 Indeed, the development of these glands was used as an external marker of gonadal activity in gerbil (Thiessen et al., 1968). In water vole, it was further proposed that the scent glands are 312 313 involved in the attraction of the sexual partner (Stoddart et al., 1975). In this context, we recently identified season-specific signatures of the volatile olfactory compounds produced by 314 the lateral scent glands (Nagnan-Le Meillour et al., 2019). 315

Moving backwards along the hypothalamo-pituitary-gonadal axis, we found that the pituitary 316 is heavier in animals trapped in summer, compared to those trapped in winter. Similar 317 observations were made in females field voles and Belding ground squirrel (Al-Khateeb and 318 319 Johnson, 1971; McKeever, 1963). Furthermore, male field voles also show increased pituitary weight during the breeding season, and this is associated with increased levels of LH and 320 FSH, both in the pituitary and in the plasma (Craven and Clarke, 1986). The increased level of 321 gonadotropins observed in female field and prairie voles during the breeding period is 322 consistently correlated with higher ovulation rates (Martinet and Meunier, 1975; Moffatt et 323 324 al., 1995). The heavier pituitary weight in summer may also be due to an increased number of lactotroph cells, which produce prolactin to stimulate milk production in nursing females 325 326 (Carretero et al., 2003).

Finally, we investigated potential seasonal regulation at the central hypothalamic level, by 327 328 assessing levels of kisspeptin peptide, the most potent secretagogue of GnRH. We first cloned 329 the Kiss1 cDNA in this species. The deduced sequence of the water vole KISS1 precursor 330 protein is highly similar to the sequence of other rodents. Moreover, the water vole KP10 sequence is identical to the mouse KP10 sequence against which the AC564 antibody is 331 directed. Using this KP10-specific antibody, we showed that the density of kisspeptin-ir fibres 332 in the hypothalamic arcuate nucleus is much lower in female voles trapped in summer than in 333 those trapped during winter. In seasonal species, kisspeptin levels in the arcuate nucleus are 334 often correlated to the reproductive state, with more neurons and fibers observed during the 335 breeding season (summer for rodents, and winter for sheep of temperate regions of the world), 336 compared to the non-breeding season. This is consistent with the role of kisspeptin as a major 337 GnRH secretagogue (Messager et al., 2005). Our findings in female water voles may therefore 338 339 appear counterintuitive, in that levels of kisspeptin-ir are opposite to this general rule. However, similar results have been observed in Siberian (Phodopus sungorus) and European 340 341 hamsters (Cricetus cricetus), in which a low density of kisspeptin-ir is seen in animals maintained under long photoperiod (summer condition) compared to those maintained under 342 343 short photoperiod (winter) (Greives et al., 2007; Rasri-Klosen et al., 2017; Sáenz De Miera et 344 al., 2014). This result could be due to the potent negative feedback exerted by sex hormones, 345 particularly estradiol, onto arcuate kisspeptin neurons during the reproductive period (Gottsch et al., 2009; Smith et al., 2005), which vanishes during the period of sexual rest. The highest 346 347 density of kisspeptin fibres in winter might also result from diminished secretion, hence the accumulation of the peptide, which is actively secreted during the breeding period (Rasri-Klosen et al., 2017).

Altogether, our results considerably substantiate the hypothesis that female water voles 350 display a marked annual cycle of reproduction, with breeding during spring/summer and a 351 352 sexual rest period during autumn/winter. Our data provide evidence for these seasonal regulations at all levels along the hypothalamo-pituitary-gonadal axis (Figure 5). In most 353 354 seasonally breeding mammals living at temperate latitudes, daylength is the main proximate driver of seasonal breeding, as demonstrated in the field vole (Baker and Ranson, 1933, 355 356 1932a, 1932b), through mechanisms that involve the neurohormone melatonin produced and 357 secreted by the pineal gland (for review see Dardente et al., 2019). However, it is noteworthy 358 that additional cues, among which social interactions, stress and food availability, are able to modulate the impact of photoperiod to some extent (Dardente et al., 2016; Delgadillo et al., 359 360 2020). Voles are no exception to this general statement. Indeed, the density of vole population and quality of the feed resource can impact fertility of female voles in the field (Martinet et 361 al., 1969; Pinot et al., 2014). Moreover, mild temperature associated with better resource 362 availability seems to explain the continuous reproduction along the year of the fossorial water 363 voles located in Northwest Spain (Somoano et al., 2016). 364

In summary, our data demonstrate that female water voles are seasonal breeders. Regulations of the reproductive axis throughout the year are responsible for the alternation between periods of breeding and sexual rest. While photoperiod is the main environmental factor governing seasonal changes in mammals, its direct involvement in seasonal breeding of the water vole remains to be demonstrated rigorously. Finally, the relative impact of photoperiod and additional cues such as food availability and temperature remains to be determined in both female and male water voles.

13

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384 **References**

- Airoldi, J.-P., 1978. Etude par capture et recapture d'une population de campagnols terrestre,
 Arvicola terrestris scherman shaw (Mammalia, Rodentia). La Terre la Vie 32.
- Airoldi, J.-P., 1976. Le terrier de la forme fouisseuse du campagnol terrestre, Arvicola
 terrestris scherman Shaw (Mammalia, Rodentia). Rev. suisse Zool. 41, 23–42.
- Al-Khateeb, A., Johnson, E., 1971. Seasonal changes of pelage in the vole (Microtus agrestis).
 I. Correlation with changes in the endocrine glands. Gen. Comp. Endocrinol. 16, 217–
 228. https://doi.org/10.1016/0016-6480(71)90033-5
- Baker, J.R., Ranson, R.M., 1933. Factors affecting the breeding of the field mouse (Microtus agrestis). Part III.—Locality. Proc. R. Soc. B 113, 486–495.
- Baker, J.R., Ranson, R.M., 1932a. Factors affecting the breeding of the field mouse (microtus agrestis). Part II.—Temperature and food. Proc. R. Soc. London. Ser. B 112, 39–46.
 https://doi.org/10.1098/rspb.1932.0076
- Baker, J.R., Ranson, R.M., 1932b. Factors affecting the breeding of the field mouse (Microtus agrestis). Part I.—Light. Proc. R. Soc. B 110, 313–322.
 https://doi.org/10.1098/rspb.1932.0027
- Belchetz, P.E., Plant, T.M., Nakai, Y., Keogh, E.J., Knobil, E., 1978. Hypophysial responses
 to continuous and intermittent delivery of hypothalamic gonadotropin-releasing
 hormone. Science (80-.). 202, 631–633. https://doi.org/10.1126/science.100883
- Brambell, F.W.R., Rowlands, I.W., 1936. Reproduction of the Bank Vole (Evotomys glareolus, schreber). I The oestrous cycle of the female. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 226, 71–97. https://doi.org/10.1098/rstb.1936.0003
- Brody, S., Wiqvist, N., 1961. Ovarian Hormones and Uterine Growth: Effects of Estradiol,
 Progesterone and Relaxin on cell growth and cell division in the Rat Uterus.
 Endocrinology 68, 971–977. https://doi.org/https://doi.org/10.1210/endo-68-6-971
- Carretero, J., Rubio, M., Blanco, E., Burks, D.J., Torres L., J.L., Hernández, E., Bodego, P.,
 Riesco, J.M., Juanes, J.A., Vázquez, R., 2003. Variations in the cellular proliferation of
 prolactin cells from late pregnancy to lactation in rats. Ann. Anat. 185, 97–101.
 https://doi.org/10.1016/S0940-9602(03)80068-7
- Craven, R.P., Clarke, J.R., 1986. Gonadal development and gonadotrophin secretion in the
 male vole (Microtus agrestis) after an abrupt change in photoperiod. J. Reprod. Fertil. 76,
 513–518. https://doi.org/10.1530/jrf.0.0760513
- 416 Dardente, H., Lomet, D., Robert, V., Decourt, C., Beltramo, M., Pellicer-Rubio, M.T., 2016.
 417 Seasonal breeding in mammals: From basic science to applications and back.
 418 Theriogenology 86, 324–332. https://doi.org/10.1016/j.theriogenology.2016.04.045
- 419 Dardente, H., Wood, S., Ebling, F., Sáenz de Miera, C., 2019. An integrative view of
 420 mammalian seasonal neuroendocrinology. J. Neuroendocrinol. 31, 1–17.
 421 https://doi.org/10.1111/jne.12729
- Delgadillo, J.A., Lemière, A., Flores, J.A., Bedos, M., Hernández, H., Vielma, J., GuerreroCervantes, M., Zarazaga, L.A., Keller, M., Chemineau, P., 2020. Undernutrition reduces
 the body weight and testicular size of bucks exposed to long days but not their ability to
 stimulate reproduction of seasonally anestrous goats. Animal 2562–2569.

- 426 https://doi.org/10.1017/S1751731120001329
- Franceschini, I., Lomet, D., Cateau, M., Delsol, G., Tillet, Y., Caraty, A., 2006. Kisspeptin
 immunoreactive cells of the ovine preoptic area and arcuate nucleus co-express estrogen
 receptor alpha. Neurosci. Lett. 401, 225–230.
 https://doi.org/10.1016/j.neulet.2006.03.030
- 430 https://doi.org/10.1016/j.neulet.2006.03.039
- Frost, P., Giegel, J.L., Weinstein, G.D., Gomez, E.C., 1973. Biodynamic studies of hamster
 flank organ growth: hormonal influences. J. Invest. Dermatol. 61.
 https://doi.org/10.1111/1523-1747.ep12676217
- Galand, P., Leroy, F., Chrétien, J., 1971. Effect of oestradiol on cell proliferation and
 histological changes in the uterus and vagina of mice. J. Endocrinol. 49, 243–252.
 https://doi.org/10.1677/joe.0.0490243
- Gottsch, M.L., Navarro, V.M., Zhao, Z., Glidewell-Kenney, C., Weiss, J., Jameson, J.L.,
 Clifton, D.K., Levine, J.E., Steiner, R.A., 2009. Regulation of Kiss1 and Dynorphin gene
 expression in the murine brain by classical and nonclassical estrogen receptor pathways.
 J. Neurosci. 29, 9390–9395. https://doi.org/10.1523/JNEUROSCI.0763-09.2009
- Greep, R.O., Dyke, H.B. van, Chow, B.F., 1942. Gonadotropins of the swine pituitary.
 Endocrinology 30, 635–649.
- Greives, T.J., Mason, A.O., Scotti, M.A.L., Levine, J., Ketterson, E.D., Kriegsfeld, L.J.,
 Demas, G.E., 2007. Environmental control of kisspeptin: Implications for seasonal
 reproduction. Endocrinology 148, 1158–1166. https://doi.org/10.1210/en.2006-1249
- Jones, E.J., Poole, K.C., Sollini, J., Town, S.M., Id, K.B., 2020. Seasonal weight changes in
 laboratory ferrets. PLoS One 15, 1–14. https://doi.org/10.1371/journal.pone.0232733
- Lomet, D., Piégu, B., Wood, S.H., Dardente, H., 2018. Anti-angiogenic VEGFAxxxb
 transcripts are not expressed in the medio-basal hypothalamus of the seasonal sheep.
 PLoS One 13, 1–23. https://doi.org/10.1371/journal.pone.0197123
- Lucky, A.W., McGuire, J., Nydorf, E., Halpert, G., Nuck, B.A., 1986. Hair follicle response
 of the golden syrian hamster flank organ to continuous testosterone stimulation using
 silastic capsules. J. Invest. Dermatol. 86, 83–86. https://doi.org/10.1111/15231747.ep12283883
- Mahmoudi, A., Maul, L.C., Khoshyar, M., Darvish, J., Aliabadian, M., Kryštufek, B., 2020.
 Evolutionary history of water voles revisited: Confronting a new phylogenetic model
 from molecular data with the fossil record. Mammalia 84, 171–184.
 https://doi.org/10.1515/mammalia-2018-0178
- Martinet, L., Meunier, M., 1975. Plasma and pituitary levels levels of LH in fields voles,
 Microtus arvalis, reared under two different photoperiods. J. Physiol. (Paris). 70, 539–
 547.
- Martinet, L., Meunier, M., Lhoste, J.-M., 1969. Influence Des Variations Saisonnières De La
 Luzerne Sur La Croissance, La Mortalité Et L'Établissement De La Maturité Sexuelle
 Chez Le Campagnol Des Champs (Microtus Arvalis). Ann. Biol. Anim. Biochim.
 Biophys. 9, 451–462. https://doi.org/10.1051/rnd:19690401
- McKeever, S., 1963. Seasonal changes in body weight, reproductive organs, pituitary, adrenal
 glands, thyroid gland, and spleen of the belding ground squirrel (Citellus beldingi). Am.
 J. Anat. 113, 153–173. https://doi.org/10.1002/aja.1001130111

- 469 Messager, S., Chatzidaki, E.E., Ma, D., Hendrick, A.G., Zahn, D., Dixon, J., Thresher, R.R.,
- 470 Malinge, I., Lomet, D., Carlton, M.B.L., Colledge, W.H., Caraty, A., Aparicio, S.A.J.R.,
 471 2005. Kisspeptin directly stimulates gonadotropin-releasing hormone release via G
- 472 protein-coupled receptor 54. Proc. Natl. Acad. Sci. 102, 1761–1766.
- 473 https://doi.org/10.1073/pnas.0409330102
- 474 Messager, Sophie, Chatzidaki, E.E., Ma, D., Hendrick, A.G., Zahn, D., Dixon, J., Thresher,
 475 R.R., Malinge, I., Lomet, D., Carlton, M.B.L., Colledge, W.H., Caraty, A., Aparicio,
- 476 S.A.J.R., 2005. Kisspeptin directly stimulates gonadotropin-releasing hormone release
- via G protein-coupled receptor 54. Proc. Natl. Acad. Sci. U. S. A. 102, 1761–1766.
 https://doi.org/10.1073/pnas.0409330102
- 479 Meylan, A., Airoldi, J.-P., 1975. Reproduction hivernale chez Arvicola terrestris sherman
 480 shaw (Mammalia, Rodentia). Rev. suisse Zool. 82, 689–694.
- 481 Moffatt, C.A., Gerber, J.M., Blom, J.M.C., Kriegsfeld, L.J., Nelson, R.J., 1995. Photoperiodic
 482 Effects on Steroid Negative Feedback in Female Prairie Voles (Microtus ochrogaster).
 483 Gen. Comp. Endocrinol. 100, 92–95.
- 484 https://doi.org/https://doi.org/10.1006/gcen.1995.1137.
- Nagnan-Le Meillour, P., Descamps, A., Le Danvic, C., Grandmougin, M., Saliou, J.M.,
 Klopp, C., Milhes, M., Bompard, C., Chesneau, D., Poissenot, K., Keller, M., 2019.
 Identification of potential chemosignals in the European water vole Arvicola terrestris.
 Sci. Rep. 9, 1–12. https://doi.org/10.1038/s41598-019-54935-z
- Pinot, A., Gauffre, B., Bretagnolle, V., 2014. The interplay between seasonality and density:
 Consequences for female breeding decisions in a small cyclic herbivore. BMC Ecol. 14.
 https://doi.org/10.1186/1472-6785-14-17
- 492 Rasri-Klosen, K., Simonneaux, V., Klosen, P., 2017. Differential response patterns of
 493 kisspeptin and RFamide-related peptide to photoperiod and sex steroid feedback in the
 494 Djungarian hamster (Phodopus sungorus). J. Neuroendocrinol. 29, 1–13.
 495 https://doi.org/10.1111/jne.12529
- 496 Sáenz De Miera, C., Monecke, S., Bartzen-Sprauer, J., Laran-Chich, M.P., Pévet, P.,
 497 Hazlerigg, D.G., Simonneaux, V., 2014. A circannual clock drives expression of genes
 498 central for seasonal reproduction. Curr. Biol. 24, 1500–1506.
 499 https://doi.org/10.1016/j.cub.2014.05.024
- Saucy, F., 1988. Dynamique de population, dispersion er organisation sociale de la forme
 fouisseuse de campagnol terrestre, (Arvicola terrestris scherman (shaw), Mammalia,
 Rodentia). Université de Neuchatel.
- Shenbrot, G.I., Krasnov, B.R., 2005. An atlas of the geographic distribution of the arvicoline
 rodents of the world (Rodentia, Muridae: Arvicolinae). Pensoft Publishers.
- Simonneaux, V., 2020. A Kiss to drive rhythms in reproduction. Eur. J. Neurosci. 51, 509–
 530. https://doi.org/10.1111/ejn.14287
- Simonneaux, V., Ancel, C., Poirel, V.J., Gauer, F., 2013. Kisspeptins and RFRP-3 act in
 concert to synchronize rodent reproduction with seasons. Front. Neurosci. 7, 1–11.
 https://doi.org/10.3389/fnins.2013.00022
- Smith, J.T., Cunningham, M.J., Rissman, E.F., Clifton, D.K., Steiner, R.A., 2005. Regulation
 of Kiss1 gene expression in the brain of the female mouse. Endocrinology 146, 3686–

- 512 3692. https://doi.org/10.1210/en.2005-0488
- Somoano, A., Miñarro, M., Ventura, J., 2016. Reproductive potential of a vole pest (Arvicola scherman) in Spanish apple orchards. Spanish J. Agric. Res. 14.
 https://doi.org/10.5424/sjar/2016144-9870
- Somoano, A., Ventura, J., Miñarro, M., 2017. Continuous breeding of fossorial water voles in northwestern Spain: Potential impact on apple orchards. Folia Zool. 66, 37–49. https://doi.org/10.25225/fozo.v66.i1.a6.2017
- Stoddart, D.M., 1980. the Ecology of Vertebrate Olfaction. https://doi.org/10.1007/0-387 23622-8
- Stoddart, D.M., 1972. The lateral scent organs of Arvicola terrestris (Rodentia : Microtinae). j.
 zool 166, 49–54.
- Stoddart, D.M., 1971. Breeding and Survival in a Population of Water Voles. J. Anim. Ecol.
 40, 487–494. https://doi.org/10.2307/3257
- Stoddart, D.M., Aplin, R.T., Wood, M.J., 1975. Evidence for social difference in the flank
 organ secretion of Arvicola terrestris (Rodentia : Microtinae). j. zool 1799, 529–540.
- Thibault, C., Courot, M., Martinet, L., Mauleon, P., du Mesnil du Buisson, F., Ortavant, R.,
 Pelletier, J., Signoret, J.P., 1966. Regulation of breeding season and estrous cycles by
 light and external stimuli in some mammals. J. Anim. Sci. 25 Suppl, 119–142.
 https://doi.org/10.2527/jas1966.25supplement119x
- Thiessen, D.D., Friend, H.C., G. Lindzey, 1968. Androgen Control of Territorial Marking in
 the Mongolian Gerbil. Science (80-.). 160, 432–434.
- Thiessen, D.D., Owen, K., Lindzey, G., 1971. Mechanisms of territorial marking in the male
 and female mongolian gerbil (meriones unguiculatus). J. Comp. Physiol. Psychol. 77,
 38–47. https://doi.org/10.1037/h0031570
- Valentine, G.L., Kirkpatrick, R.L., 1970. Seasonal Changes in Reproductive and Related
 Organs in the Pine Vole, Microtus pinetorum, in Southwestern Virginia. J. Mammal. 51,
 553–560.
- Vandenbergh, J.G., 1973. Effect of Gonadal Hormone on the Flank Gland of the Golden
 Hamster. Horm. Res. Paediatr. 4, 28–33.
- 541 https://doi.org/https://doi.org/10.1159/000178287
- Vasantha, I., 2015. Physiology of Seasonal Breeding: A Review. J. Vet. Sci. Technol. 07.
 https://doi.org/10.4172/2157-7579.1000331
- Ventura, J., Gosálbez, J., 1990. Reproductive cycle of Arvicola terrestris (Rodentia,
 Arvicoidae) in the Aran Valler, Spain. Rev. suisse Zool. 55, 383–391.
- Ventura, J., Gosálbez, J., Lopez-Fuster, M.J., 1991. Structure de population d'Arvicola
 terrestris (Linnaeus, 1758) (Rodentia, Arvicolidae) du Nord-Est Ibérique. Mammalia 55,
 85–90. https://doi.org/10.1515/mamm.1991.55.1.85
- Villette, P., Afonso, E., Couval, G., Levret, A., Galan, M., Goydadin, A.C., Cosson, J.F.,
 Giraudoux, P., 2020. Spatio-temporal trends in richness and persistence of bacterial
 communities in decline-phase water vole populations. Sci. Rep. 10, 1–19.
 https://doi.org/10.1038/s41598-020-66107-5
 - 18

- Wade, G.N., Bartness, T.J., 1984. Effects of photoperiod and gonadectomy on food intake,
 body weight, and body composition in Siberian hamsters. Am. J. Physiol. 246.
 https://doi.org/https://doi.org/10.1152/ajpregu.1984.246.1.R26
- Wilson, D.E., Reeder, D.M., 2005. Mammal Species of the World : A Taxonomic and
 Geographic Reference. The Johns hopkins University Press.
- 558

559 **Figure captions:**

Table 1. Summary for each capture of the number of adult females not pregnant, the number of samples used for staining and immunohistochemistry, and the average body weight and height. Only significant posthoc comparisons to January are indicated on graphs (* p < 0.05. ** p < 0.01).

Table 2. Characteristics of antibodies and primers used for Immunohistochemistry and PCR.

Figure 1. Female voles caught in the field display a marked annual rhythmicity in pregnancy. (A) Percentage of pregnant, non-pregnant lactating and non-pregnant females observed throughout the monitoring. (B) The average number of embryos per female. (Results are shown as Tukey's boxplot with each female represented by circles and means indicated by a cross).

Figure 2. Female voles caught in the field display a marked annual rhythmicity in their 570 **reproductive organs.** Graphs show monthly variations in (A) ovarian weight. (B) corpora 571 lutea number. (E) uterine weight and (F) uterine horn section. Only significant posthoc 572 573 comparisons to January are indicated on graphs (Results are shown as Tukey's boxplot with outliers represented by circles and means indicated by a cross. * p<0.05. ** p<0.01. 574 575 ***p<0.001). (C) Percentage of females with recent corpora lutea in summer (July+August, 576 n=11) or in winter (November+January, n=9). (D&G) Histological staining of ovaries and uterine horn sections to illustrate the difference between July and January. (e: endometrium; 577 m: myometrium; CL: corpora lutea). 578

Figure 3. Female voles caught in the field display a marked annual rhythmicity in their lateral scent glands. (A) Photography indicates the position and details the appearance of the lateral scent gland. (B) Images of hematoxylin and eosin stains of sections of lateral scent glands. The double arrow indicates the thickness of the sebaceous gland layer. (SG: sebaceous gland; e: epidermis). (C&D) Monthly changes in the size and weight of lateral scent glands of female water voles. Only significant posthoc comparisons to January are indicated on graphs 585 (Results are shown as Tukey's boxplot with outliers represented by circles and means 586 indicated by a cross. * p<0.05. ** p<0.01. ***p<0.001).

Figure 4. Female voles caught in the field display a seasonal change in the level of 587 kisspeptin and pituitary weight. (A&B) Representative images and graph of 588 589 immunoreactive kisspeptin fibres density in the arcuate nucleus of summer-trapped and winter-trapped females (n=7 for both). Red squares indicate the counting surface. (ARC: 590 591 arcuate nucleus, 3V: Third ventricle, ME: median eminence). (C) Pituitary weight of female water voles trapped in summer (n=8) or in winter (n=7) (Results are shown as Tukey's 592 boxplot and means indicated by a cross. * p<0.05, ***p<0.001). (D) Alignment of kisspeptin 593 sequence in Arvicola terrestris and other selected rodents (Microtus ochrogaster, 594 595 Mesocricetus auratus, Cricetus griseus, Peromyscus leucopus, Meriones unguiculatus, Arvicanthis niloticus, Rattus norvegicus and Mus musculus). Yellow: positions unique to 596 597 Arvicola terrestris ; Bold Green: Kp10; Bold Red: dibasic cleavage site + amidation (based on 598 mouse Kp52 sequence). Asterisks indicate positions which have a single fully conserved residue. Colons indicate conservation between amino acid groups of strongly similar 599 properties. Periods indicate conservation between amino acid groups of weakly similar 600 properties. 601

Figure 5. The alternation of the breeding and sexual rest periods correlates with the 602 annual rhythmicity in regulations of the hypothalamo-pituitary-gonadal axis; and in 603 parallel with the indication of the day length, the monthly average temperature, sunshine, and 604 605 rainfall throughout the monitoring (infoclimat.fr). Hypothesis: the lowest kisspeptin fibres 606 immunoreactivity in arcuate nucleus during the breeding season compared to the nonbreeding season might be due to enhanced secretion of kisspeptin (i.e. less storage) along with 607 608 strong negative feedback of estradiol onto the arcuate kisspeptin neurons, thereby mitigating *Kiss1* at the transcriptional level. In this scenario, accumulation of kisspeptin in winter would 609 610 reflect reduced kisspeptin secretion (i.e. storage) along with reduced estradiol feedback. (1) 611 (Sophie Messager et al., 2005); (2) (Belchetz et al., 1978); (3) (Brody and Wiqvist, 1961; 612 Galand et al., 1971; Greep et al., 1942; Stoddart, 1972). (GnRH, Gonadotropin release hormone: LH, Luteinizing hormone: FSH, Follicle-stimulating hormone; E2, Estradiol; P4, 613 614 Progesterone).



Pregnants Lactating Non-pregnants

Β

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Jun

Jan Feb Apr







LSG surface









Micr_ochr Arvi_terr Meso_aura Cric_gris Pero_leuc Meri_ungu Arvi_nilo Ratt_norv Mus_mus MTALASWQLLLLCITTFGEP-----LANPGPTGQQSG-SQELINAWKKGPRCAEGKSGVTGLRAR-RTSPCPPVDS MTALASWQLLLLCITTFGEPLAKVAPLANPGPTGQQSG-SQELINVWEKGPRCAEGKSGVAGLRARTRTIPCPPVES MTTLASWQLLLLCVATFGEPLAKVAPVANPGPTGQRPG-PQELINAWEKGPRCAQKKPGVAGLRAR-RTSPCPPVES MTALASWQLLLLCVATFGEPLAKVAPVANPGPTGQRPG-PQELINAWEKGPRCAERKPGVAGLRAR-RTSPCPPVES MPLASWQLLLLCVATFGEPLAKVAPVNPGPTGQRPG-PQELINAWEKGPRCAERKPGVAGLRAR-RTSPCPPVES MPLASWQLLLLLFVTTFGEPLAKVAPVVNPGPTGQRPG-SQELVNAWEKGPRCAERKPGVAGLRAR-RTSPCPPVES MISLASWQLLLLLCVATFGEPLAKVAPVVNPGHTGQWSG-SQELVNAWEKGPRCAERKPGAAGPRAR-RTSPCPPVES MISLASWQLLLLLCVATFGEPLAKVAPVVNPGHTGQWSG-SQELVNAWEKSPRVAESKPGAAGLRAR-RTSPCPPVES MISLASWQLLLLCVATFGEPLAKVAPVVNPGHTGQWSG-SQELVNAWEKSPRVAESKPGAAGLRAR-RTSPCPPVEN MISMASWQLLLLCVATFGEPLAKVAPVVNPGPTGQQSG-PQELVNAWEKSPRVAESKPGAAGLRAR-RTSPCPPVEN MISMASWQLLLLCVATFGEPLAKVAPVVNPGTGQQSG-PQELVNAWEKSPRVAESKPGAAGLRAR-RTSPCPPVES * ********

Micr_	ochr
Arvi	terr
Meso	aura
Cric	gris
Pero	leuc
Meri	ungu
Arvi	nilo
Ratt	norv
Mus_n	nus



Dat	te of capture		Nb of voles	Staining Ovaries - Uterus	IHC & Pituitary	Body weight (g)	± SEM	Body size (cm)	± SEM
2019	Febuary	26	19	6-6		85.8	± 2.0	14,5	± 0,1
	April	03	10	6-6		92.9 *	± 4.2	14,5	± 0,3
	April	30	11	5-6		95.9 **	± 3.3	14,5	± 0,3
	June	05	7	5-6		98.0 **	± 5.3	16,0	± 0,3
	July	11	8	5-5	3-4	95.3 *	± 4.1	15,0	± 0,4
	August	21	23	6-6	4	86.1	± 2.1	14,5	± 0,2
	September	18	10	6-6		87.3	± 2.7	14,5	± 0,3
	October	19	12	5-5		90.3	± 2.9	15,5	± 0,2
	November	26	15	5-5	7	89.3	± 2.2	15,0	± 0,2
2020	January	30	9	4-6		75.0	± 3.1	15,0	± 0,3
	Febuary	24	8	5-6		82.6	± 3.5	15,0	± 0,4
	April	01	7	5-6		96.7 *	± 6.4	15,0	± 0,3
	June	22	5	5-5		89.8	± 6.5	15,5	± 0,3

Antibodies	Catalog # and RRID	Dilution
Rabbit anti- kisspeptin	AC564 ; AB_2296529 Home	1:50000
Biotin SP Donkey anti-rabbit	711-065-152 Jackson ImmunoResearch	1:1000

PCR Primers	5' → 3' sequence
Forward	GAACTGCCTCTTCCTGCCAG
Reverse	ACTGGGCTCGCAATCCACC