

# UC Davis

## UC Davis Previously Published Works

### Title

Abnormal mare behaviour is rarely associated with changes in hormonal markers of granulosa cell tumours: A retrospective study

### Permalink

<https://escholarship.org/uc/item/3xw3t0q3>

### Authors

Huggins, Lauren  
Norris, Jamie  
Conley, Alan  
et al.

### Publication Date

2023-06-21

### DOI

10.1111/evj.13967

Peer reviewed

## ORIGINAL ARTICLE

# Abnormal mare behaviour is rarely associated with changes in hormonal markers of granulosa cell tumours: A retrospective study

Lauren Huggins | Jamie Norris | Alan Conley  | Pouya Dini 

Department of Population Health and Reproduction, School of Veterinary Medicine, University of California, Davis, California, USA

**Correspondence**

Pouya Dini, Department of Population Health and Reproduction, School of Veterinary Medicine, University of California, Davis, CA 95616, USA.  
Email: [pdini@ucdavis.edu](mailto:pdini@ucdavis.edu)

**Abstract**

**Background:** Abnormal or undesired mare behaviours are often assumed to be associated with ovarian abnormalities.

**Objectives:** We aimed to determine the incidence of abnormal behaviours and their association with concentrations of one or more ovarian hormones associated with a granulosa cell tumour (GCT).

**Study design:** Retrospective descriptive.

**Methods:** A total of 2914 hormonal profile samples submitted with the words behave, behaviour, or behaving in the submission history were analysed. The association between reported abnormal behaviours and concentrations of testosterone, anti-Müllerian hormone (AMH), inhibins and inhibin-B were assessed. Statistical analysis was performed using a Chi-squared test of association.

**Results:** Of the 2914 cases that were submitted due to behaviour issues, 2506 (86%) did not have any of the measured hormones reach GCT-like concentrations. The remaining 408 cases had either one (63%), two (25.5%), or three (11.5%) hormones with concentrations consistent with those from confirmed GCT cases. Testosterone had the lowest percent of GCT-like values among the cases (7.7%), compared with AMH (9.4%), inhibins (9.6%) and inhibin B (8.7%). Stallion-like behaviour was significantly associated with increased concentrations of all four hormones. In contrast, aggression, oestrous and other abnormal behaviours were significantly less likely to be associated with increased concentrations of the hormones.

**Main limitations:** Retrospective study, using sample submission history.

**Conclusion:** Overall, the abnormal behaviours among mares, except the stallion-like behaviour, were not associated with increased ovarian hormones. These results highlight the common misassumption about the involvement of the ovaries in 'abnormal behaviours' or 'undesirable behaviours' of mares.

**KEYWORDS**

abnormal behaviours, GCT, granulosa cell tumour, horse, stallion-like behaviour

This is an open access article under the terms of the [Creative Commons Attribution-NonCommercial-NoDerivs](https://creativecommons.org/licenses/by-nc-nd/4.0/) License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2023 The Authors. *Equine Veterinary Journal* published by John Wiley & Sons Ltd on behalf of EVJ Ltd.

## 1 | INTRODUCTION

Assumptions are often made that abnormal or undesired behaviours of mares are associated with the secretion of ovarian hormones. It is a commonly held belief that oestrous cycle-associated behaviours can be deleterious to a mare's athletic performance and make them difficult or dangerous to handle.<sup>1-3</sup> A membership survey of the 1996 American Association of Equine Practitioners revealed that 90% of the 751 respondents agreed that a mare's competitive ability could be affected by the oestrous cycle.<sup>4</sup> As a result of these conceptions, veterinarians are often consulted to diagnose the cause of the abnormal or unwanted behaviour, a process often involving a multimodal approach, as clinical assessments of these complaints can range from establishing normal sexual behaviours to exploring medical conditions causing disease or pain from unknown sources.<sup>1,3,5,6</sup> It has been noted that in the majority of the diagnostic work ups of such cases at a referral hospital, the underlying cause is most often unrelated to ovarian function or pathology.<sup>5</sup>

The undesirable or abnormal behaviours are described predominantly as stallion-like behaviours, aggression and oestrous cycle-associated behaviours. Stallion-like behaviours include mounting other mares, being 'socially animated,' and physical changes such as cresty necks and weight gain.<sup>5,7</sup> Aggression often presents as biting and/or kicking towards other horses and/or people.<sup>5,8</sup> Oestrous cycle-associated behavioural problems can manifest in the form of hyperexcitability, pain or colic around the time of ovulation, suboptimal performance during oestrus and persistent or intermittent oestrus.<sup>1,7,9</sup> Many of these unwanted behaviours may be accompanied by nonreproductive afflictions such as musculoskeletal pain, abdominal discomfort, submissive guarding, or by either endogenous or exogenous androgen exposure.<sup>1,5</sup>

One of the few reproductive pathologies which could be associated with abnormal and aggressive behaviour is the ovarian granulosa cell tumour (GCT). The GCT, originating from sex cord-stromal tissue, is the most common reproductive tract tumour.<sup>10</sup> When endocrinologically active, GCTs can secrete anti-Müllerian hormone (AMH), inhibins (inhibin-B predominantly) and testosterone. The increased concentrations of these hormones are believed to be responsible for the commonly seen abnormal behaviours of anoestrus during the breeding season, stallion-like behaviour and aggression.<sup>7,10</sup>

Hormonal analysis plays an important role in the diagnosis of a GCT. AMH, a glycoprotein produced by the granulosa cells in antral follicles and GCTs, serves as a clinically valuable biomarker for GCTs that is more easily interpreted because the concentrations do not change significantly throughout the year, oestrous cycle, or during pregnancy.<sup>11-14</sup> AMH is the most sensitive hormone (98%) to diagnose GCTs when the concentration exceeds the normal reference range,<sup>15</sup> which was recently revised (upper limit of 6.9 ng/mL, UC Davis Endocrinology Laboratory) based on data from an extensive study of 1101 mares.<sup>16</sup>

After AMH, inhibin is the next most sensitive analyte (80%), though it is less specific than AMH as the concentrations fluctuate throughout the oestrous cycle, peaking at ovulation and can fluctuate

during pregnancy.<sup>13,15,17,18</sup> The original 'inhibins' radioimmunoassay was nonspecific, detecting both inhibin A and inhibin B isoforms and even free  $\alpha$ -subunit. In 2018, the UC Davis Endocrinology Laboratory switched to a more selective enzyme immunoassay that specifically measures the inhibin B isoform that is secreted by GCTs.<sup>19</sup>

Lastly, testosterone is the least sensitive (48%) hormone for GCT diagnosis.<sup>15</sup> This steroid hormone is produced by theca cells<sup>20</sup> and when a substantial thecal component exists and testosterone is clearly increased, tumours may qualify as true granulosa thecal cell tumours (GTCT){Neto, 2010 #20}. Testosterone is also the more variable of the three hormones, as it fluctuates significantly over short periods of time within individual mares, throughout the year and immunoreactivity to testosterone can increase significantly during pregnancy.<sup>13,15</sup>

Once a GCT has been diagnosed, the treatment of choice is removal via either unilateral or bilateral ovariectomy. Surgical removal of a GCT, or even of normally functioning ovaries, has been postulated to decrease unwanted behaviours when owners are surveyed months to years after the procedure.<sup>6,21-26</sup> Several studies have documented this overwhelmingly positive owner feedback over the years using various surgical techniques to remove normal ovaries and GCTs alike.<sup>6</sup> When normal ovaries were removed, the rates of owner-reported improved behaviour ranged from 4 of 10,<sup>6</sup> 14 of 17,<sup>23</sup> 14 of 20,<sup>9</sup> 19 of 23<sup>24</sup> and 22 of 27<sup>26</sup> and even more overwhelmingly, 9 of 9,<sup>22</sup> 8 of 10,<sup>6</sup> 10 of 12<sup>23</sup> and 17 of 18<sup>26</sup> owners reported improved rideability and performance. Interestingly, similar results were seen when bilateral ovariectomies are performed in mule mares with 10 of 10 owners satisfied with the improvement in behaviour and work performance.<sup>25</sup> A prospective study compared owner's perception of improvement after a preliminary work up to rule out extra-ovarian sources for behaviours prior to bilateral ovariectomy.<sup>6</sup> This study showed that there was no difference in perceived improved behaviour (4/10 vs. 6/14) and rideability (8/10 vs. 8/14) between mares with normal or pathological ovaries respectively.<sup>6</sup>

Recently, a nonsurgical approach was used to treat four mares with presumed GCTs using multiple injections of the GnRH vaccine.<sup>27</sup> While the trial was not systematically designed and the hormonal results were not conclusive, the owners of all four mares reported the cessation of the abnormal behaviours (predominantly aggression or stallion-like), while ultrasonographic examinations revealed no changes in ovarian size.<sup>27</sup> GnRH vaccines, which can be used with adjuvants, may suppress reproductive cyclicity with a very variable time to return to cycling.<sup>2,3,28</sup> Despite the lack of ovarian activity, however, it is reported that despite use of these vaccines some mares can continue to exhibit oestrus behaviours, often in irregular or continuous patterns.<sup>2,3,28</sup>

This display of persistent oestrous behaviour, concurrent with a documented lack of ovarian function, aligns with studies reporting that 30%–35% of mares continue to show apparent oestrous behaviour after bilateral ovariectomy.<sup>9,23</sup> The cause of persistent oestrous behaviour in the absence of ovaries or ovarian activity has been hypothesised to be due to extragonadal sex steroids, most likely from the adrenal cortex.<sup>19,29</sup> One study that investigated the link between the adrenal cortex and abnormal oestrous behaviour by measuring

steroid hormones after ACTH administration found an unexplained difference in the adrenal response, with the abnormal mares showing decreased adreno-cortical reactivity compared with controls.<sup>30</sup> Another hypothesis is that the presence of ovarian progesterone is more important in the suppression of sexual receptivity rather than the influence of oestrogen promoting it.<sup>19,29</sup>

Despite the widely held belief among owners or trainers that abnormal behaviour or decreased performance is often associated with abnormal increases in ovarian hormones, there is little evidence to support it. This retrospective study aimed to determine the prevalence of reported undesirable behaviours among mares from which samples were submitted for hormone assays and their association with concentrations of one or more ovarian hormones. In this study, reference ranges published for the diagnosis of a GCT as the cut off value (GCT-like hormonal values) were used,<sup>17,19</sup> since this ovarian abnormality is one of the few conditions which has been associated with abnormal behaviours in mares.

## 2 | MATERIALS AND METHODS

Data from mare samples ( $n = 27\,841$ ) submitted to the UC Davis Endocrinology Laboratory during the period of October 2011 to November 2020 were evaluated retrospectively. From these submissions those with the words behave, behaviour and/or behaving included in the history at the time of submission were selected for analysis.

The descriptive histories were read individually to categorise the abnormal behaviour or behaviours of interest by a researcher blinded to the associated hormonal data. Each case was assigned to combinations of the three major behavioural categories (stallion-like, aggression and oestrous related complaints such as persistent oestrus, strong heats, irregular cycle length) if so described in the history. If no indication of any of those three behavioural groups was documented, the case was classified under the generalised 'abnormal behaviour' category. A single researcher analysed all of the histories to mitigate misinterpretation of the historical descriptions and assigning the appropriate behaviour/s. The frequency of minor behaviours and conditions of interest such as colic, back pain and lameness, among others, were also recorded.

The samples were submitted for one of the two GCT panels or individual hormone analysis. The GCT panel I included testosterone, inhibins (prior to 21 August 2017) or inhibin B (thereafter) and

progesterone and the GCT panel II included testosterone, inhibins or inhibin B and AMH. Serum samples were evaluated using enzyme immunoassay for AMH, inhibin B and progesterone, while radioimmunoassay was used to evaluate testosterone and inhibins.<sup>13</sup> The reference ranges used in this study mirror the current ranges provided by the Clinical Endocrinology Laboratory at the University of California, Davis (Table 1). Statistical analysis was performed using the comparisons of proportions ('N-1' Chi-squared test) using MedCalc Software Ltd. and JMP (SAS Institute, version 14 Pro). The relationships between the hormonal values were assessed using Pearson correlations. To account for errors arising from multiple comparisons, significance was set to  $p < 0.01$ .

## 3 | RESULTS

From the original 27 841 mare submissions, 2914 (8.6%) included the words behaviour, behave and/or behaving in the history description. A total of 54 (1.8%) of these mares previously received either a unilateral (33) or bilateral (21) ovariectomy. The average age of the patients was 10.8 years old (standard deviation [SD] 5.3), with a median of 10 and a range of 1–34 years old.

Of the 2914 cases that were submitted for behaviour issues, 2506 (86%) did not have any of the measured hormones reach GCT-like concentrations (GCT-like value; Table 1). The remaining 408 cases represented those with either one (63%), two (25.5%), or three (11.5%) hormones with concentrations consistent with those from confirmed GCT cases (GCT-like, Table 1). Increased AMH (>8 ng/mL) alone was the most likely finding (583/2914, 20%; Figure 1).

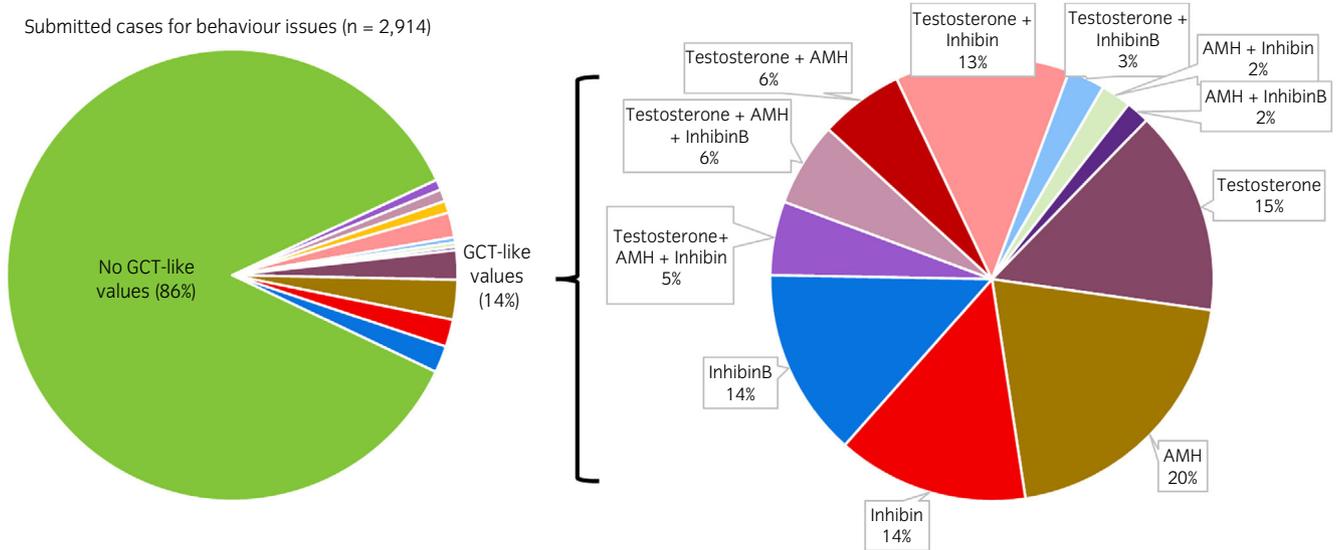
Across the four hormones analysed as a possible behavioural correlate, the incidence of cases with GCT-like values was very low with testosterone (197/2555), AMH (171/1814), inhibins (142/1477) and inhibin B (103/1189) (Figure 2). Testosterone was the most commonly used test (2555 submissions) as it appears in both GCT panels, unlike AMH (1814) and was not split between isomer-specific tests like inhibins (1477) and inhibin B (1189). Testosterone also had the lowest percent of GCT-like values among the cases (7.7%), compared with AMH (9.4%), inhibins (9.6%) and inhibin B (8.7%). There was a small portion (4.8%) of increased testosterone levels (70–100 pg/mL) that were below GCT-like levels and a relatively large portion (27.6%) of marginally increased levels (45–70 pm/mL) of testosterone.

Across all four hormones, the incidence of reported stallion-like behaviour was significantly increased when the respective hormone

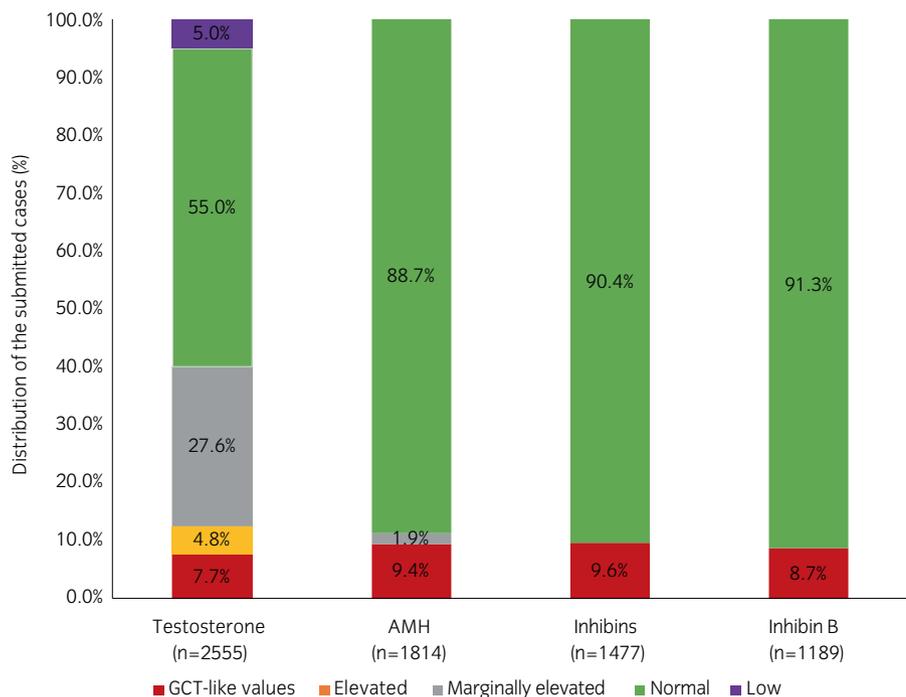
**TABLE 1** The reference ranges used in this study for each hormone.

HORMONE	Normal	Marginally increased	Increased	GCT-like
AMH (ng/mL)	0.1–6.9	-	7–7.9	>8
Inhibins (ng/mL)	0.1–0.7	-	-	>0.7
Inhibin B (pg/mL)	2–100	-	-	>100
Testosterone (pg/mL)	20–45	45–70	70–100	>100

Note: Conversion factors to SI units: AMH 1 ng/mL = 7.1429 pmol/L, Inhibins 1 ng/mL = 0.001 ng/L, Inhibin B 1 pg/mL = 1 ng/L, Testosterone 1 pg/mL = 0.00347 nmol/L.  
Abbreviation: AMH, anti-Müllerian hormone.



**FIGURE 1** Distribution of cases with granulosa cell tumour-like hormonal values among the submitted cases for behavioural issues. From the total 2914 cases that were submitted for behavioural issues, 2506 (86%) did not have any hormones reach GCT-like concentrations.

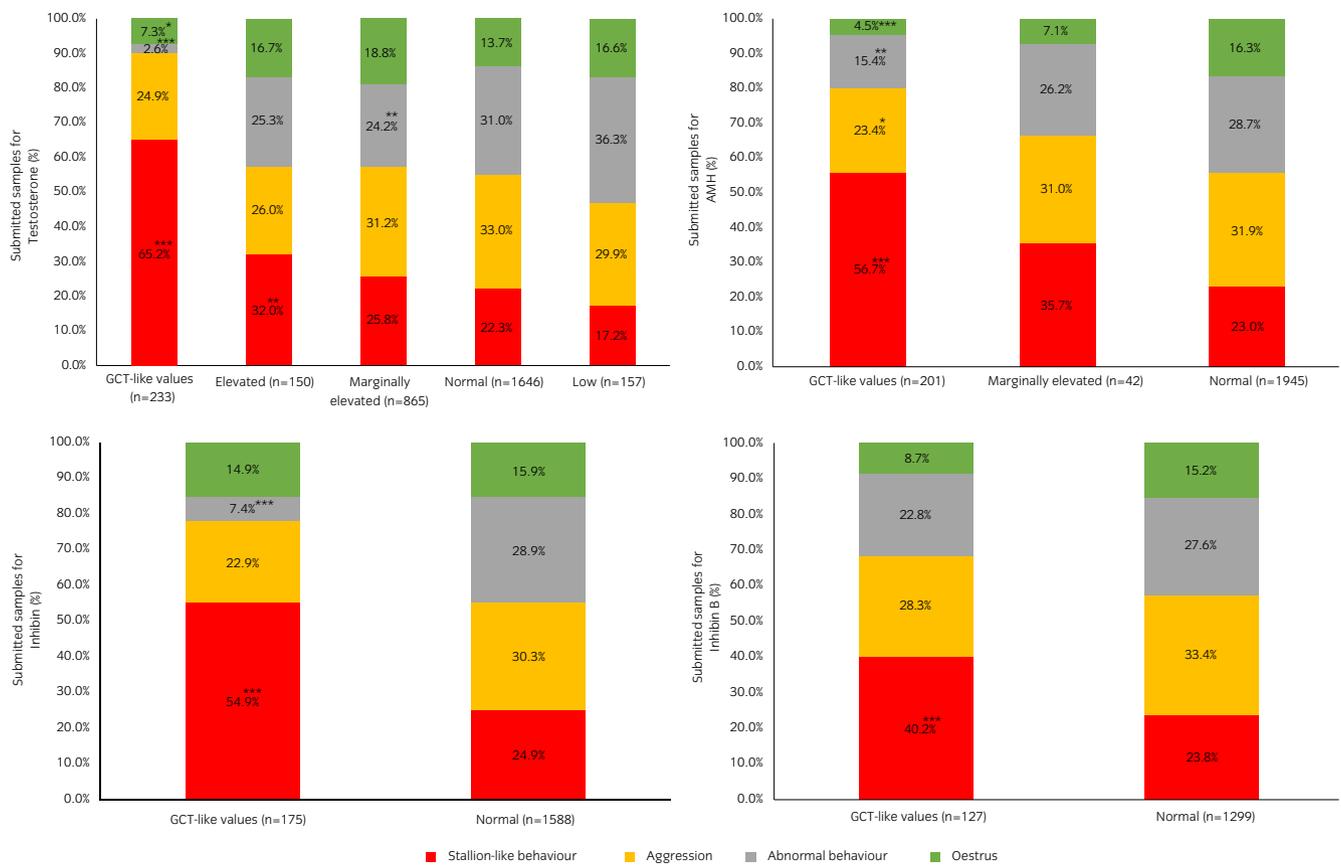


**FIGURE 2** Distribution of hormonal levels among the submitted cases for behavioural issues. The incidence of cases with granulosa cell tumour-like hormonal values was less than 10% of submitted cases.

was in the GCT-like range relative to the normal range ( $p < 0.0001$ ; Figure 3). Stallion-like behaviour was reported with greatest frequency when there were GCT-like concentrations in testosterone (65.2%), AMH (56.7%), inhibins (54.9%) and inhibin B (40.2%). There also were positive correlations between the concentrations of testosterone, AMH, inhibin and inhibin B among the samples with reported stallion-like behaviour (Table 2). The remaining three behavioural issues revealed an opposite trend. Those with GCT-like concentrations of testosterone and AMH had less frequently reported oestrus ( $p < 0.0006$ ) and generalised abnormal behaviours ( $p < 0.0001$ ) than those with normal hormone levels.

Among the 408 cases with at least one GCT-like hormonal value, stallion-like behaviour was the most reported behaviour problem (Figure 4). Stallion-like behaviour was reported in as many as 82.6% of cases with increased testosterone, AMH and inhibins ( $n = 22$ ), in contrast to only 22.9% of the cases that did not have any hormones reach concentrations like those observed in the GCT cases ( $n = 2506$ ). Unlike stallion-like behaviour, the other behaviours were all more likely to be reported among those with no hormonal abnormalities (Figure 4).

Among the submissions which have information from an ovarian examination by transrectal palpation, frequency of abnormal findings on ovaries were significantly higher in animals with GCT-like hormonal values



**FIGURE 3** Distribution of reported behaviours among four tested hormones. Comparison was made for each behavioural issue between the proportion of mares showing the issue with abnormal hormonal concentrations and the proportion of mares with normal values. Comparison was made between each condition (stallion-like behaviour, aggression, abnormal behaviour and oestrus) for each group (granulosa cell tumour-like values, increased, marginally increased and low) and the submitted samples with normal values (normal). \* $p < 0.01$ ; \*\* $p < 0.001$ ; \*\*\* $p < 0.0001$ .

**TABLE 2** Correlations of the four tested hormones values when stallion-like behaviour was reported.

	AMH (ng/mL)	Inhibins (ng/mL)	Inhibin B (pg/mL)	Testosterone (pg/mL)
AMH (ng/mL)	1	0.45 ( $p < 0.001$ )	0.48 ( $p < 0.001$ )	0.55 ( $p < 0.001$ )
Inhibins (ng/mL)		1	-	0.30 ( $p < 0.001$ )
Inhibin B (pg/mL)			1	0.24 ( $p < 0.001$ )
Testosterone (pg/mL)				1

Abbreviation: AMH, anti-Müllerian hormone.

( $p < 0.001$ ), except for increased AMH + inhibin B ( $p = 0.07$ ) and inhibin B ( $p = 0.36$ ), relative to those with normal hormone concentrations. The report of abnormal ultrasound findings tended to mimic abnormal palpation, but with decreased overall frequency (Figure 5).

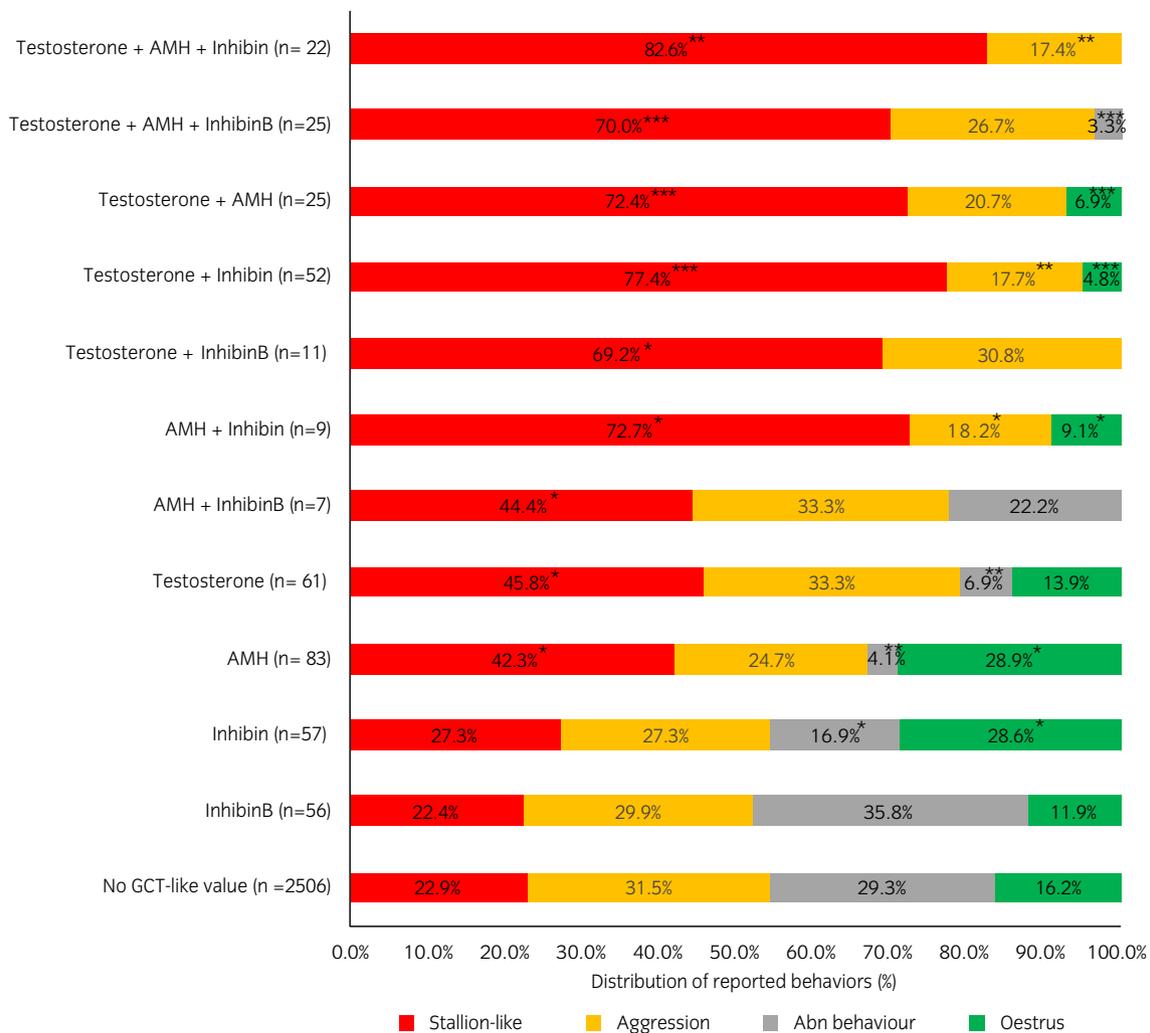
We further investigated the association of increased hormones with the other reported conditions. The conditions that are most likely to be reported in a case of significantly increased hormone were cresty necks (62.5%), weight loss (50%) and weight gain (40%), though the case number of each is very low (Figure 6). All of the other conditions and behaviours listed in Figure 6 are reported more frequently but fall below 17% incidence among the cases.

Of the 33 mares with a history of unilateral ovariectomy that were presenting with abnormal behaviour, 3 revealed significant

elevations of AMH/Inhibins, Inhibins and Inhibin B respectively. Among the 21 mares with a history of bilateral ovariectomy that were again showing abnormal behaviour, a single case revealed markedly increased testosterone (170.3 pg/mL) as well as increased progesterone (2.6 ng/mL). The history described possible ovarian tissue seen on transrectal ultrasound despite the spay and stallion-like behaviour in addition to persistent oestrus behaviour.

## 4 | DISCUSSION

The goal of the current study was to evaluate the association between abnormal behaviours reported by clients and ovarian hormone



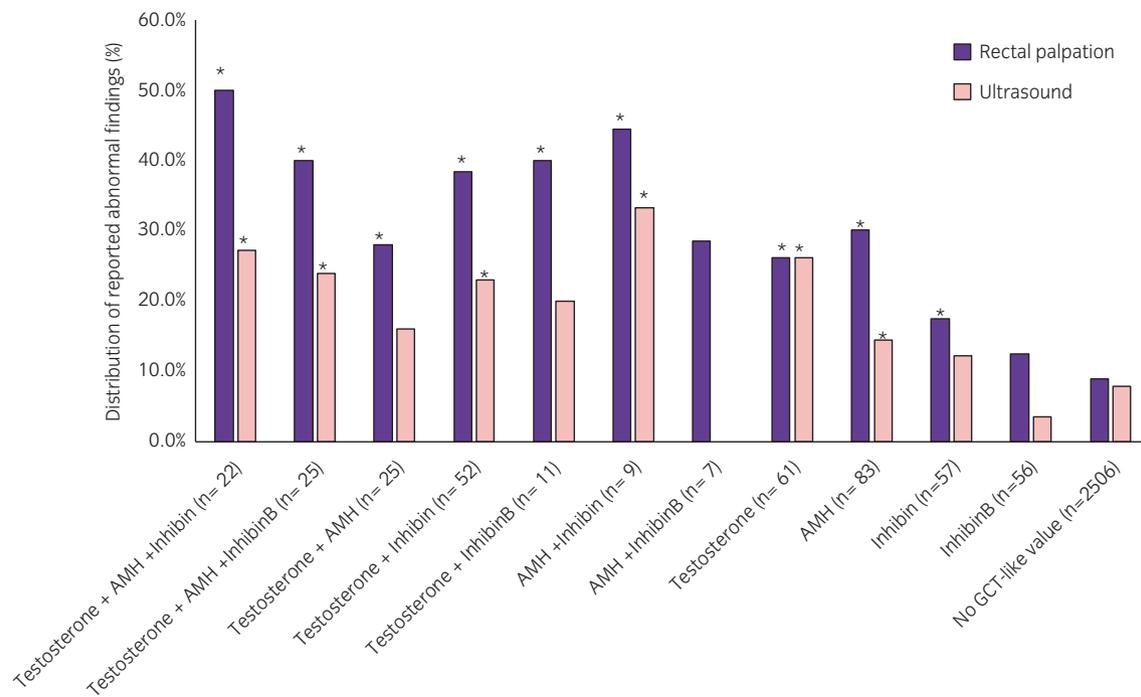
**FIGURE 4** Distribution of owner-reported behaviours based on the possible combinations of granulosa cell tumour (GCT)-like groups within a case. Comparison was made for each hormone (row) for each condition (stallion-like behaviour, aggression, abnormal behaviour and oestrus) to the submitted samples with no GCT-like values group. \* $p < 0.01$ ; \*\* $p < 0.001$ ; \*\*\* $p < 0.0001$ .

concentrations, using 2914 cases submitted for behavioural issues. The analysis demonstrated that the majority of behavioural problems were not associated with increased ovarian hormones. Among the behavioural problems reported, stallion-like behaviour was the only complaint associated significantly with increased ovarian hormones. This finding is aligned with previous reports, demonstrating stallion-like behaviour as the most common sign of confirmed cases of GCT, the ovarian condition commonly associated with this complaint.

In the study by Sherlock et al. with 52 confirmed cases of GCTs by histopathology, the incidence of reported behaviour was 50% stallion-like, 31% aggression, 19% prolonged/persistent oestrus and 8% anoestrus.<sup>31</sup> Other studies have found similar behavioural expression results, reporting 46% stallion-like, 22% prolonged/persistent oestrus and 32% anoestrus cases.<sup>32</sup> The increased frequency of anoestrus behaviour was consistent with a study (of only seven cases) that reportedly expressed 57% anoestrus, 28% stallion-like and 14% oestrus behaviours.<sup>33</sup> Anoestrus behaviour is typically vague and is likely only to be observed among mares being bred. In the study by

Sherlock et al., anoestrus was the least common behavioural complaint, quite possibly since only 18% of the 52 mares were broodmares.<sup>31</sup> Our results closely mirror the distribution reported by Sherlock et al. when looking at the frequency of each behaviour across all the mares with GCT-like hormonal values, revealing a relatively high incidence of stallion-like behaviour.<sup>31</sup>

Interestingly, in the same study, Sherlock et al. found no statistically significant difference in testosterone concentrations when stallion-like behaviour was reported.<sup>31</sup> This is in direct contrast to one of the more interesting results gleaned in the current study: that owner reported stallion-like behaviour was the most common behaviour among mares with significantly increased hormones. Most strikingly, this result was seen in all four hormones analysed, not just testosterone. In direct contrast to the findings of Sherlock et al., the incidence of stallion-like behaviour significantly decreased as the concentration of testosterone decreased, with even marginally increased testosterone levels (45–70 pg/mL) having significantly higher frequency of stallion-like behaviour in comparison to those mares with



**FIGURE 5** Distribution of reported incidence of abnormal palpation findings and abnormal transrectal ultrasound findings between the possible combinations of granulosa cell tumour (GCT)-like groups. Significance indicated by \* when  $p < 0.01$  comparing to no GCT-like value group.

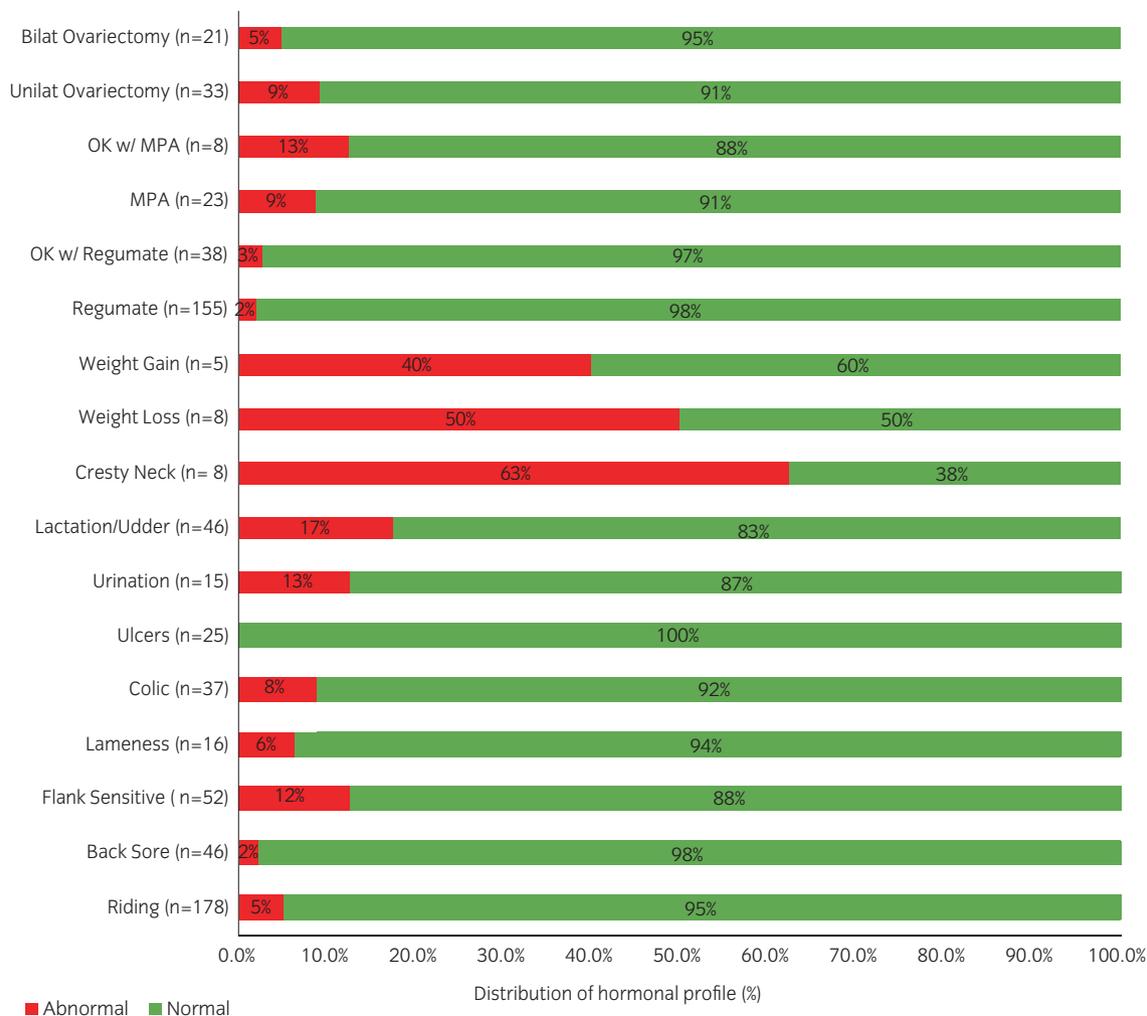
normal or low level of testosterone (Figure 3). Among the GCT-like groups, stallion-like behaviour was more likely (44%–82%) to be reported among those with two or three increased hormones than those with one (22%–45%). Surprisingly, even those with only increased AMH (42%) still had a significantly higher incidence of stallion-like behaviour relative to the cases without any increased hormones (23%). Unlike stallion-like behaviour, this study found no significant trends between aggressive behaviour and increased ovarian hormones (Figure 3), despite aggression being a relatively common complaint among mares with histologically proven GCTs.<sup>31</sup>

Unlike AMH, considering inhibin or inhibin B as a single analyte in the diagnosis of ovarian abnormalities such as GCT is less advisable due to the lower sensitivity and variability throughout the oestrous cycle and pregnancy.<sup>13,15,17,18</sup> Similar to the recommendations with AMH, it has been noted that increases of inhibins above 1 ng/mL (relative to the 0.7 ng/mL UC Davis Endocrinology Lab cut off) are most likely indicative of a GCT in a nonpregnant mare. A similar trend is seen with testosterone, the least specific and most variable of the three hormones. If concentrations are greater than 100 pg/mL in a nonpregnant mare, a GCT is the most likely cause as there is currently no other verified explanation in a mare, except exogenous administration of testosterone itself. Given that the endocrine nature of a GCT can change over time and the variability in the values of inhibin and testosterone, analysing hormone concentrations every 2–3 months or during different seasons can aid in diagnosis.<sup>13</sup>

While this study relies heavily on the behaviour and hormonal assays, the importance of pairing transrectal palpation and/or ultrasound findings with this information cannot be overstated. A recent

retrospective study out of the UC Davis Endocrinology Lab describing 14 cases of suspected GCTs highlighted the significance of pairing increased hormone levels with an abnormally large ovary on palpation to increase the confidence in the diagnosis. Renaudin et al. concluded that abnormal behaviour in the absence of abnormal palpation or hormonal levels is unlikely related to ovarian abnormalities.<sup>19</sup> This study supports these conclusions, as less than 10% of all of the cases with reported abnormal behaviours had increased ovarian hormones. Unfortunately, while they are indisputably helpful in reaching a diagnosis, transrectal palpation and ultrasound are not always included in the initial diagnostics. Often, a hormonal assay is performed initially due to abnormal behaviour and additional diagnostics are considered based on the results. The preference for conducting hormone analysis before transrectal palpation is likely influenced by many factors including the feasibility and safety given the environment or behaviour of the patient, additional diagnostic costs, lack of experience performing the procedure and/or access to appropriate equipment.

We further evaluated the possibility of association between increased ovarian hormones and less commonly reported problems such as colic, lameness and weight loss. Though weight loss was reported with very low frequency ( $n = 8$ ), 50% of the cases had at least one significantly increased hormone. Conversely, reports of colic (3/31, 8.1%) and lameness (1/16, 6.3%) had much lower proportions of abnormal hormonal profiles. The only other minor behaviours or conditions that showed similar trends (very low frequency but high proportion of abnormal hormonal values) were the physical masculinisation traits of a cresty neck (5/8, 62.5%) and weight gain (2/5, 40%). All other investigated behaviours and conditions were represented by



**FIGURE 6** Distribution of owner reported abnormal behaviours, diseases, conditions, or improvement or lack of improvement when using common oestrus-suppressing drugs with at least one granulosa cell tumour-like value required within one of the four hormones to be considered abnormal.

at most 18% and as little as 0% of cases having significant hormonal changes. These results agree with others that have stated that these reported abnormal behaviours and conditions are most likely caused by numerous other musculoskeletal, urogenital, abdominal, or other pathology unrelated to the ovary.<sup>5</sup>

In conclusion, reported abnormal behaviours in mares were associated with an increased concentration of either testosterone, AMH, inhibin, or inhibin B in less than <10% of submitted samples. Of the abnormal behaviours, stallion-like behaviour was the only one that was found to have significant association with increased concentrations of the tested hormones. Other abnormal, or undesirable behaviours or other perceived behaviours such as changes ‘under saddle’ or abdominal discomfort were very unlikely to be associated with ovarian hormones. Based on the analysis of many thousands of clinical submissions over several years, owner-reported, abnormal behaviours in mares is unlikely to be associated with an abnormal elevation of ovarian hormones in the majority of cases, except in mares with stallion-like behaviour.

#### AUTHOR CONTRIBUTIONS

Lauren Huggins contributed to study execution, data analysis and interpretation and preparation of the manuscript. Jamie Norris contributed to study design and data analysis and interpretation. Alan Conley contributed to study design, data analysis and interpretation and final approval of the manuscript. Pouya Dini contributed to study design, study execution, data analysis and interpretation and final approval of the manuscript. Pouya Dini had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

#### ACKNOWLEDGEMENTS

We thank the UC Davis Endocrinology Laboratory personnel for their assistance in extracting the data and acknowledge support from the John P. Hughes Endowment of the University of California, Davis.

#### FUNDING INFORMATION

No financial support.

## CONFLICT OF INTEREST STATEMENT

No competing interests have been declared.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request: Open sharing exemption granted by editor for this descriptive retrospective study.

## ETHICS STATEMENT

Research ethics committee oversight not required by this journal: retrospective study of clinical records.

## INFORMED CONSENT

Explicit owner consent for animals' inclusion in the study was not stated.

## ORCID

Alan Conley  <https://orcid.org/0000-0003-3798-7390>

Pouya Dini  <https://orcid.org/0000-0001-5553-5967>

## REFERENCES

- Pryor P, Tibary A. Management of estrus in the performance mare. *Clin Tech Equine Pract.* 2005;4:197–209.
- Dalin AM, Andresen O, Malmgren L. Immunization against GnRH in mature mares: antibody titres, ovarian function, hormonal levels and oestrous behaviour. *J Vet Med Ser A Physiol Pathol Clin Med.* 2002;49:125–31.
- Stout TAE, Colenbrander B. Suppressing reproductive activity in horses using GnRH vaccines, antagonists or agonists. *Anim Reprod Sci.* 2004;82–83:633–43.
- Jorgensen JS, Vivrette S, Correa M, Mansmann RA. Significance of the estrous cycle on athletic performance in mares. *Proc Am Assoc Equine Pract.* 1997;42:98–100.
- McDonnell SM. Behavior problem: ovaries or not? *Proc Am Assoc Equine Pract.* 2017;63:131–4.
- Melgaard DT, Korsgaard TS, Thoenfer MS, Petersen MR, Pedersen HG. Moody mares—is ovariectomy a solution? *Animals.* 2020;10:1–11.
- McCue PM, Roser JF, Munro CJ, Liu IKM, Lasley BL. Granulosa cell tumors of the equine ovary. *Vet Clin North Am Equine Pract.* 2006;22:799–817.
- Beaver BV. Aggressive behavior problems. *Vet Clin North Am Equine Pract.* 1986;2(3):635–644.
- Roessner HA, Kurtz KA, Caron JP. Laparoscopic ovariectomy diminishes estrus-associated behavioral problems in mares. *J Equine Vet Sci.* 2015;35:250–3.
- McCue PM. Neoplasia of the female reproductive tract. *Neoplasia.* 1998;98:739–49.
- Almeida J, Ball BA, Conley AJ, Place NJ, Liu IKM, Scholtz EL, et al. Biological and clinical significance of anti-Müllerian hormone determination in blood serum of the mare. *Theriogenology.* 2011;76:1393–403.
- Ball BA, Conley AJ, MacLaughlin DT, Grundy SA, Sabour K, Liu IKM. Expression of anti-Müllerian hormone (AMH) in equine granulosa-cell tumors and in normal equine ovaries. *Theriogenology.* 2008;70:968–77.
- Conley A, Ball B. Endocrine testing for reproductive conditions in horses. *Interp Equine Lab Diagn.* 2017 Dec 17:409–18.
- Claes ANJ, Ball BA. Biological functions and clinical applications of anti-Müllerian hormone in stallions and mares. *Vet Clin North Am Equine Pract.* 2016;32:451–64.
- Ball BA, Almeida J, Conley AJ. Determination of serum anti-Müllerian hormone concentrations for the diagnosis of granulosa-cell tumours in mares. *Equine Vet J.* 2013;45:199–203.
- Uliani RC, Conley AJ, Jo Corbin C, Friso AM, Maciel LFS, Alvarenga MA. Anti-Müllerian hormone and ovarian aging in mares. *J Endocrinol.* 2019;240:147–56.
- Conley AJ, Scholtz EL, Dujovne G, Cotterman RF, Legacki EL, Uliani RC, et al. Inhibin-A and inhibin-B in cyclic and pregnant mares, and mares with granulosa-theca cell tumors: physiological and diagnostic implications. *Theriogenology.* 2018;108:192–200.
- Bailey MT, Troedsson MHT, Wheaton JE. Inhibin concentrations in mares with granulosa cell tumors. *Theriogenology.* 2002;57:1885–95.
- Renaudin CD, Kelleman AA, Keel K, McCracken JL, Ball BA, Ferris RA, et al. Equine granulosa cell tumours among other ovarian conditions: diagnostic challenges. *Equine Vet J.* 2021;53:60–70.
- Assis Neto ACA, Ball BA, Browne P, Conley AJ. Cellular localization of androgen synthesis in equine granulosa-theca cell tumors: immunohistochemical expression of 17 $\alpha$ -hydroxylase/17,20-lyase cytochrome P450. *Theriogenology.* 2010;74:393–401.
- Smith LJ, Mair TS. Unilateral and bilateral laparoscopic ovariectomy of mares by electrocautery. *Vet Rec.* 2008;163:297–300.
- Allredge JG, Hendrickson DA. Use of high-power ultrasonic shears for laparoscopic ovariectomy in mares. *J Am Vet Med Assoc.* 2004;225:1578–80.
- Hooper RN, Taylor TS, Varner DD, Blanchard TL. Effects of bilateral ovariectomy via colpotomy in mares: 23 cases (1984–1990). *J Am Vet Med Assoc.* 1993;203:1043–6.
- Kamm JL, Hendrickson DA. Clients' perspectives on the effects of laparoscopic ovariectomy on equine behavior and medical problems. *J Equine Vet Sci.* 2007;27:435–8.
- Petrizzi L, Guerri G, Straticò P, Cuomo A, Vullo C, De Amicis I, et al. Laparoscopic ovariectomy in standing mule mares. *J Equine Vet Sci.* 2020;84:102857.
- Devick IF, Leise BS, McCue PM, Rao S, Hendrickson DA. Ovarian histopathology, pre- and post-operative endocrinological analysis and behavioral alterations in 27 mares undergoing bilateral standing laparoscopic ovariectomy. *Can Vet J.* 2020;61:181–6.
- Behrendt D, Burger D, Gremmes S, Szunyog K, Röhmeier S, Sieme H. Active immunisation against GnRH as treatment for unilateral granulosa theca cell tumour in mares. *Equine Vet J.* 2021;53(4):740–5.
- Imboden I, Janett F, Burger D, Crowe MA, Hässig M, Thun R. Influence of immunization against GnRH on reproductive cyclicity and estrous behavior in the mare. *Theriogenology.* 2006;66:1866–75.
- Asa CS, Goldfoot DA, Garcia MC, Ginther OJ. Sexual behavior in ovariectomized and seasonally anovulatory pony mares (*Equus caballus*). *Horm Behav.* 1980;14:46–54.
- Hedberg Y, Dalin AM, Forsberg M, Lundeheim N, Sandh G, Hoffmann B, et al. Effect of ACTH (tetracosactide) on steroid hormone levels in the mare. Part B: effect in ovariectomized mares (including estrous behavior). *Anim Reprod Sci.* 2007;100:92–106.
- Sherlock CE, Lott-Ellis K, Bergren A, Withers JM, Fews D, Mair TS. Granulosa cell tumours in the mare: a review of 52 cases. *Equine Vet Educ.* 2016;28:75–82.
- Meagher DM, Wheat JD, Hughes JP, Stabenfeldt GH, et al. Granulosa cell tumors in mares—a review of 78 cases. *Proc Am Assoc Equine Pract.* 1977;23:133–43.
- Crabtree J. Review of seven cases of granulosa cell tumour of the equine ovary. *Vet Rec.* 2011;169(10):251.

**How to cite this article:** Huggins L, Norris J, Conley A, Dini P. Abnormal mare behaviour is rarely associated with changes in hormonal markers of granulosa cell tumours: A retrospective study. *Equine Vet J.* 2023. <https://doi.org/10.1111/evj.13967>