Short-term effect of ovariectomy on measures of insulin sensitivity and response to dexamethasone administration in horses

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Objective—To evaluate the effect of ovariectomy on insulin sensitivity in horses and determine whether the effects of suppression of the hypothalamo-pituitary-adrenal axis differ before and after ovariectomy.

Animals—6 healthy mares.

Procedures—The horses underwent an IV glucose tolerance test (IVGTT), an insulin sensitivity test, and a dexamethasone suppression test before and 5 weeks after ovariectomy. Body weight, serum cortisol and plasma ACTH concentrations, serum insulin-to-blood glucose concentration ratios, and changes in blood glucose concentration with time after injection of glucose or insulin were compared before and after ovariectomy.

Results—The dexamethasone injection resulted in a decrease in serum cortisol concentration before and after ovariectomy. In all horses, baseline plasma ACTH concentrations were within the reference range before and after ovariectomy. For each mare, results of an IVGTT before and after ovariectomy were considered normal. No significant differences in basal blood glucose concentration or time to reach baseline glucose concentration after an IVGTT were observed. Basal serum insulin concentration and serum insulin-to-blood glucose concentration ratios were not significantly different before or after ovariectomy, nor was the mean time to attain a 50% decrease in blood glucose concentration after insulin injection.

Conclusions and Clinical Relevance—Results indicated that ovariectomy does not appear to modify dexamethasone response in horses and that it does not modify short-term measures of insulin sensitivity. Findings suggested that horses undergoing ovariectomy are not at higher risk of developing equine metabolic syndrome or hypothalamo-pituitary-adrenal axis dysfunction and associated morbidity. (Am J Vet Res 2013;74:1506–1513)

Bilateral ovariectomy is not a common elective surgery in horses, but as standing, noninvasive techniques improve, it is being more commonly performed in equine practice. Ovariectomy is performed in the unusual instance of bilateral ovarian disease and more frequently to improve temperament or prevent reproductive cycling.1,2 Ovariectomized mares can act as embryo recipients because there is no need to synchronize estrus between donor and recipient mares.3 Although pregnancy is established in ovariectomized horses and in sexually intact horses at the same rate, the need of ovariectomized horses to receive daily progesterone supplementation after embryo implantation adds a level of inconvenience that does not occur when sexually intact animals are used. Perhaps for this reason, ovariectomy is currently not a common practice in embryo recipients.

Despite the relative infrequency of clinical application, various surgical techniques for ovariectomy in horses have been reported. Standing minimally invasive procedures such as laparoscopy or flexible endoscopy have been advocated because they are associated with minimal postoperative pain and allow an early return to function.4–6

In other species, ovariectomy results in a wide variety of endocrine alterations. These effects are not limited to the hormones needed for reproductive function but also extend to the hormones of the hypothalamo-

ABBREVIATIONS

DST  Dexamethasone suppression test
IVGTT  IV glucose tolerance test
QUICKI  Quantitative insulin sensitivity check index
pituitary-adrenal axis and to factors that influence insulin sensitivity and glucose handling. In humans, rats, and sheep, it has been shown that ovarian hormones exert considerable control over insulin resistance and cellular lipid homeostasis and that this control is suppressed within 7 weeks after ovariectomy.\textsuperscript{12-15} It has also been shown in humans, dogs, and rats that ovariectomy causes an increase in fat mass and a decrease in insulin-stimulated glucose transport as early as 3 weeks after gonadectomy.\textsuperscript{12-15} Gonadal steroids influence the glucocorticoid response to stress in rats and sheep.\textsuperscript{16} Ovariectomized ewes have an altered hypothalamo-pituitary-adrenal axis, compared with that of sexually intact animals, in both stressed and control states.\textsuperscript{16} Therefore, it could be hypothesized that a similar response could be observed in mares following ovariectomy.

Equine metabolic syndrome is a recently described condition in which horses develop a variety of clinical signs including a tendency to become obese, abnormal adipose deposition, and laminitis.\textsuperscript{17} The key defect is insensitivity to insulin and insulin resistance.\textsuperscript{15} If ovariectomy in horses leads to the development of insulin resistance, it would be important to feed and exercise ovariectomized horses appropriately to prevent the development of clinical equine metabolic syndrome with its associated morbidity.

The effect of ovariectomy on insulin sensitivity in horses has not been reported, to our knowledge. Horses have been shown to respond normally to an ACTH stimulation test after ovary removal,\textsuperscript{18} but the response of ovariectomized horses to suppression of the hypothalamo-pituitary-adrenal axis has not been previously described. The purpose of the study reported here was to determine whether horses have short-term alterations in insulin sensitivity (as measured by commonly described clinical tests) or changes in the response to dexamethasone administration after ovariectomy.

**Materials and Methods**

**Horses**—The Purdue University Animal Care and Use Committee approved the study protocol. Six mares, with a median age of 16 years (range, 8 to 18 years), enrolled in a comparative study\textsuperscript{8} investigating 2 surgical ovariectomy techniques were used in this study. Breeds included Paint Horse (n = 4), Quarter Horse (1), and Appaloosa (1). Weight range at the time of admission was 368 to 523 kg (median weight, 426 kg). The mares were fed grass hay and water ad libitum. Three days before surgery, the horses were fed pelleted feed and water ad libitum. Pelleted feed was removed 24 hours prior to surgery, but mares had free access to water. After surgery, the horses were returned to their normal diet. Postoperative monitoring and analgesic protocols applied in the present study have been described in detail.\textsuperscript{8} Included in the postoperative monitoring was evaluation of a standardized pain score, which was performed every 6 hours for the first 24 hours, then at least daily for 11 days. Postmortem examinations were performed at the end of the comparative study.

**Experimental design**—One week before surgery, a DST was performed for each horse by administering dexamethasone\textsuperscript{4} (40 µg/kg, IM) at 5:00 PM (designated as 0 hours). A blood sample (9 mL) was collected for determination of plasma ACTH and serum cortisol concentration just before the dexamethasone injection was administered. Blood samples for assessment of plasma ACTH concentration were collected into tubes containing EDTA, and samples for assessment of serum cortisol concentration were collected into sterile evacuated tubes without anticoagulant. Blood samples were collected at 15 (8:00 AM the following day) and 24 (5:00 PM the following day) hours after dexamethasone administration. Plasma and serum samples were obtained and frozen at –18°C. Samples were analyzed at the Animal Health Diagnostic Center at Cornell University with a benchtop immunoassay analyzer.\textsuperscript{9} The assays have been validated for use in horses.

After a 3-day washout period, an IVGTT was performed as previously described.\textsuperscript{19,20} Briefly, a catheter was placed in the right jugular vein of each horse. Dextrose was rapidly administered (time point designated as 0 minutes) in the left jugular vein at a dose of 150 mg/kg. Blood samples (9 mL) were collected through the IV catheter placed in the right jugular vein 5 minutes before (baseline) and 3, 15, 30, 45, 60, 90, 120, 150, and 180 minutes after dextrose infusion. The first 6 mL of blood withdrawn from the catheter at each time point was discarded, and the following 3 mL were used for analysis. Blood glucose concentration was instantaneously measured with a point-of-care glucometer.\textsuperscript{5} When blood glucose concentration returned to predextrose administration values, the experiment was discontinued and the time was recorded.

Blood collected at the baseline time point (5 minutes prior to dextrose administration) was placed in an evacuated tube, and the serum was harvested for insulin determination. Serum insulin concentration was determined at the Animal Health Diagnostic Center at Cornell University with a radioimmunoassay. Insulin responsiveness was evaluated by calculating the baseline plasma insulin-to-plasma glucose concentration ratio.\textsuperscript{30} Insulin sensitivity (tissue responsiveness to insulin) was evaluated by calculating the reciprocal (the baseline plasma glucose-to-plasma insulin concentration ratio) from the baseline whole blood glucose and serum insulin concentrations obtained 5 minutes before dextrose administration. Insulin sensitivity was also evaluated by calculating the QUICKI. The QUICKI provides an indirect evaluation of insulin sensitivity.\textsuperscript{20,21} The index is calculated by use of an equation as follows:

\[ \text{QUICKI} = 1/(\log_{10} \text{basal insulin concentration} + \log_{10} \text{basal glucose concentration}) \]

After a 24-hour washout period, an insulin tolerance test was performed as previously described.\textsuperscript{19,22} Briefly, human regular insulin\textsuperscript{8} (0.1 U/kg) was rapidly administered to each horse through the IV catheter placed in the right jugular vein. Blood samples (9 mL each) were collected immediately prior to insulin injection (0 minutes or baseline) and at 3, 15, 30, 45, 60, and 90 minutes to determine blood glucose concentration by use of a handheld glucometer. When blood glucose concentration was < 20 mg/dL at 2 consecutive time points or when the horse developed clinical signs...
of hypoglycemia, dextrose (150 mg/kg, IV) was administered and the horse was immediately fed high-starch concentrates. Signs indicative of hypoglycemia included sweating, muscle fasciculations, and ataxia. For each horse, euglycemia was documented before the end of the test. Horses were considered insulin sensitive when a 50% reduction from baseline (0 minutes) blood glucose concentration was attained. Jugular catheters were removed immediately following the final blood sample collection. For each horse, the time to attain a 50% reduction of baseline blood glucose concentration was calculated by a simple connection between the 2 time points surrounding the 50% threshold (linear regression) as previously described.\textsuperscript{21}

After the initial series of tests, each horse was ovariectomized with either a transvaginal natural orifice transluminal endoscopic surgery or a laparoscopic approach.\textsuperscript{3,9} Briefly, the left and right ovaries were removed by use of a vessel-sealing device\textsuperscript{c} via a transvaginal approach with a flexible endoscope for visualization in the natural orifice transluminal endoscopic surgery group (3 mares) or via a conventional bilateral laparoscopic approach (3 mares). Comparison of the 2 surgical techniques by sequential measurements of plasma fibrinogen and serum amyloid A concentrations, evaluation of peritoneal fluid, and clinical examinations revealed that the 2 procedures were equivalent in terms of pain and surgical inflammation.\textsuperscript{7} Horses were observed closely for 2 weeks following the procedure and then were turned out to pasture for 10 weeks. Analysis of the ovaries after surgery indicated that 2 mares were in estrus (designated as horses 1 and 3) and 4 mares were in diestrus (designated as horses 2, 4, 5, and 6).

Five weeks after ovarioectomy, the same endocrine tests were performed again for each horse after a 3-day adaptation period, during which the horses were housed in individual box stalls and fed hay. In addition, a complete physical examination was performed and body weight determined. The 3-week time frame was elected considering that, as occurs in humans, dogs, and rodents, significant effects of ovarioectomy on insulin sensitivity were observed after 3 weeks. Initial testing was performed in July, and follow-up testing was performed in September.

**Statistical analysis**—Normal distribution was determined by the Shapiro-Wilk test. Mean ± SD were calculated for data that followed normal distribution and median (range) for data with nonnormal distribution. Normally distributed data obtained before and after ovarioectomy were compared by means of a paired t test. Other data were compared by means of a Wilcoxon signed rank test. In particular, body weight; baseline concentrations of whole blood glucose, serum insulin, serum cortisol, and plasma ACTH; time to attain baseline blood glucose concentration after dextrose administration; and time to reach 50% of baseline blood glucose concentration after insulin administration were compared. Changes in blood glucose concentrations after dextroside or insulin injections and changes in plasma ACTH and serum cortisol concentrations after dexamethasone injections were compared before and after ovarioectomy by means of repeated-measures ANOVA. Commercial software\textsuperscript{1} was used for analysis, and significance was defined as $P < 0.05$.

**Results**

The horses were weighed before and 5 weeks after ovarioectomy. The mean ± SD weight before surgery was 450 ± 62 kg, and the mean weight after surgery was 437 ± 55 kg. Although the mean weight 5 weeks after ovarioectomy was not significantly ($P = 0.15$) lower, 1 mare in estrus (horse 1) did lose 48 kg after surgery. As reported, heart rates and pain scores were mildly elevated within the first 18 hours after surgery but did not necessitate additional analgesic administration.\textsuperscript{8}

Before ovarioectomy, the IM injection of dexamethasone resulted in a significant and prolonged decrease in serum cortisol concentration at 15 ($P = 0.003$) and 24 ($P = 0.003$) hours, compared with baseline. The serum cortisol concentration was < 1 µg/dL at 15 and 24 hours after dexamethasone injection in all horses, indicating a normal response to the DST (Figure 1). After ovarioectomy, the IM injection of dexamethasone resulted in a significant and prolonged decrease in serum cortisol concentration at 15 ($P = 0.002$) and 24 ($P = 0.003$) hours, compared with values at baseline. However, in 1 mare in diestrus (horse 6), the IM injection of dexamethasone did not result in a serum cortisol concentration < 1 µg/dL at any time after ovarioectomy, indicating an abnormal response to the DST. There were no significant differences between the serum cortisol concentration at baseline before and at baseline after ovarioectomy ($P = 0.29$) nor between the responses to dexamethasone injection (at 15 hours, $P = 0.24$; at 24 hours, $P = 0.50$; and for the comparison of the complete responses, $P = 0.47$).

Before ovarioectomy, the IM injection of dexamethasone resulted in a significant decrease in plasma ACTH concentration at 15 ($P = 0.002$) but not at 24 ($P = 0.40$) hours in all horses (Figure 2). After ovarioectomy, the IM injection of dexamethasone resulted in a significant and prolonged decrease in plasma ACTH concentration at 15 ($P = 0.001$) and 24 ($P = 0.001$) hours for all of the horses. In all instances, before and after ovarioectomy, baseline ACTH concentrations were within reference range (9 to 35 pg/mL). There was a significant ($P = 0.01$) increase in the plasma ACTH concentration.

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**Figure 1**—Mean ± SD serum cortisol concentration after injection of dexamethasone (40 µg/kg, IM) in 6 healthy horses before (gray line; diamonds) and after (black line; squares) ovarioectomy. Value is significantly ($P < 0.05$) different from baseline (data obtained prior to treatment at 0 hours).
at baseline following ovariec- tomy, but the responses to dexamethasone injections were not significantly different (at 15 hours, P = 0.02; at 24 hours, P = 0.50; and for the comparison of the complete responses, P = 0.10).

The baseline blood glucose concentrations before (84 ± 12 mg/dL) and after (85 ± 3 mg/dL) ovariec- tomy were not significantly (P = 0.84) different. All horses tolerated the IVGTT well. Blood glucose concentrations for all horses returned to baseline within 3 hours after dextrose infusion before and after ovariec- tomy, indicating a normal response to the IVGTT. The mean times for the concentration to return to baseline before (108 ± 66 minutes) and after (99 ± 51 minutes) ovariec- tomy were not significantly (P = 0.85) different. Before and after ovariec- tomy, the blood glucose concentration versus time curves after injection of dextrose did not differ significantly (P = 0.81) at any time point (Figure 3).

Before ovariec- tomy, blood glucose concentration after insulin administra- tion reached the 50% reduction threshold in 5 horses, indicating that 5 horses were insulin sensitive and 1 horse in estrus (horse 1) was insulin resistant. In 1 mare in diestrus (horse 2), dextrose was administered IV; this horse received dextrose at 45 minutes after insulin injection because its blood glucose concentration was 16 mg/dL and it had mild signs of hypoglycemia. Horse 2 was closely monitored, and euglycemia was confirmed 30 minutes after glucose injection. No other adverse effect was evident. After ovariec- tomy, all horses had a > 50% reduction in blood glucose concentration within 45 minutes after insulin administration, indicating that all of them were insulin sensitive. One horse in estrus (horse 3) received dextrose at 45 minutes after insulin injection because its blood glucose concentration was 14 mg/dL, although it did not have clinical signs of hypoglycemia. The mean time to reach the 50% threshold was not significantly (P = 0.69) different before (20 ± 4 minutes) and after (20 ± 5 minutes) ovariec- tomy. The horse in estrus that was insulin resistant before ovariec- tomy (horse 1) became insulin sensitive after ovariec- tomy, and it attained the 50% reduction in blood glucose concentration below baseline at 22 minutes after insulin injection. Before and after ovariec- tomy, the blood glucose concentration versus time curves after injection of dextrose did not differ significantly (P = 0.89) at any time point (Figure 4).

The mean serum insulin concentration (food not withheld) was not significantly (P = 0.24) different before (21.98 ± 7.88 µU/mL) and after (17.49 ± 8.91 µU/mL) ovariec- tomy. Horses had access to grass hay but not concentrate when blood samples were collected for insulin concentration determination. Food was not withheld from the study horses before blood samples were collected for insulin assay, and the hay was not analyzed. However, the same grass hay was available before and after ovariec- tomy, and no grain or other concentrate was fed at any time. None of the horses were hyperinsulinemic before or after ovariec- tomy.

There was no significant difference in the surrogate tests (ie, basal plasma glucose-to-plasma insulin concentration ratio; P = 0.12), basal plasma insulin-to-plasma glucose concentration ratio (P = 0.25), or QUICKI (P = 0.12) before and after ovariec- tomy. The horse in estrus that was determined to be insulin resistant on the basis of the insulin-response test (horse 1) was not found to be insulin resistant on the basis of the data for the QUICKI, basal plasma glucose-to-plasma insulin concentration ratio, or basal plasma insulin-to-plasma glucose concentration ratio.

**Discussion**

The morbidity associated with performing ovariec- tomy on standing horses has decreased dramatically in the recent past as laparoscopic techniques have been developed and improved. Most horses tolerate ovariec- tomy with few complications, and the procedure is actively promoted by several institutions and refer-
eral surgical practices. In addition to removal of a diseased ovary, owners request the procedure when mares have undesirable estrus-associated behaviors that are extreme or of long duration. These behaviors can be dangerous to other horses and handlers and may include kicking and striking. At our hospital over the past 5 years, 50% of the ovariectomies in horses were performed because of owner complaints about behavior. In addition, removal of reproductive potential is a means of ensuring that mares that are carriers of genetic diseases such as hyperkalemic periodic paralysis, lethal white syndrome, and hereditary equine regional dermal asthenia do not procreate. Indeed, ensured sterility may be requested for registration purposes. Postoperative monitoring and analgesic protocols applied in the present study have been described in detail. Included in the postoperative monitoring was evaluation of a standardized pain score, which was performed every 6 hours for the first 24 hours, then at least daily for 11 days. As reported previously, heart rates and pain scores were mildly elevated within the first 18 hours after surgery but did not necessitate additional analgesic administration.

The ethics of performing gonadectomy on otherwise healthy horses have not been discussed in the veterinary medical literature, to our knowledge, despite the large number of publications describing various surgical techniques for performing ovariectomy. A great deal of ethical discussion surrounds the spaying of dogs. Spaying is a procedure promoted without qualification by the AVMA and other groups, presumably because the transient pain, risk of surgical complications, or death, and increase in risk for a small number of disease conditions attendant with ovariectomy are outweighed by the increased likelihood that a neutered pet will not have adverse behaviors, which may lead to it being surrendered for euthanasia, and by the global importance of minimizing the numbers of dogs and cats that are euthanized in shelters. In a similar vein, ovariectomy in horses increases the likelihood that they will not be euthanized as a result of dangerous behaviors or that they will not contribute to horse overpopulation. As such, it behooves researchers to investigate the physiologic effects of ovariectomy and to identify any metabolic abnormalities that may result from the procedure.

The results of the present study have suggested that ovariectomy does not alter the response to dexamethasone administration and does not change insulin sensitivity in mares, at least within the time frame of 5 weeks. The administration of dexamethasone to the study horses induced a significant and prolonged decrease in serum cortisol concentration, as has been previously reported. This inhibition of ACTH secretion by glucocorticoids, causing the prolonged decrease in serum cortisol concentration, acts on the pars distalis. In cases of pituitary pars intermedia dysfunction, the ACTH-secreting tumor originates in the pars intermedia and the inhibition caused by dexamethasone is less effective. Overall, unlike what has been observed in ovariectomized ewes, there was no significant alteration in the hypothalano-pituitary-adrenal axis, as suggested by the lack of difference between the responses to dexamethasone before and after ovariectomy in the horses of the present study.

In all horses of the present study, IM injection of dexamethasone resulted in a decrease in plasma ACTH concentration. Although plasma ACTH concentration did decrease, the ACTH and serum cortisol concentrations did not mirror one another as might be expected. Serum cortisol concentrations remained decreased for a much longer period. This is consistent with findings of another study, which indicated that there was a poor correlation between plasma ACTH and serum cortisol concentrations in horses. Horses with pituitary pars intermedia dysfunction often have markedly high plasma ACTH concentration and serum cortisol concentration within reference ranges.

In the horses of the present study, baseline plasma ACTH concentration was significantly higher after ovariectomy than before the procedure. Pituitary pars intermedia dysfunction is a cause of high endogenous ACTH concentration. However, because of the short time frame involved in this study and the fact that only 1 of the 6 horses had an abnormal DST result, this was not considered probable. It has been shown that plasma ACTH concentration varies throughout the year, with a physiologic increase occurring in fall. In the present study, the horses were first tested in July and then tested again in September; thus, the increase in baseline plasma ACTH concentration was attributed to its normal seasonal variation rather than an effect of ovariectomy. Ideally, the pre- and postovariectomy testing should have occurred at a time that avoided the months between August and November. However, limitations of the study did not allow for this.

For all the horses of the present study, results of the IVGTT both before and after ovariectomy were considered normal. The marked postinjection hyperglycemia and the subsequent exponential decrease of the blood glucose concentration toward baseline are explained by the rapid glucose uptake after endogenous insulin secretion and by the availability in glucose-kinase in peripheral tissues. In the present study, the short-term response to dextrose administration did not appear to be changed by ovariectomy. During both tests, the mean time to reach the baseline was approximately 100 minutes. This is consistent with previous reports of times to return to preinjection blood glucose concentration ranging from 90 to 180 minutes.

In horses, the injection of human insulin induces a marked decrease in blood glucose concentration and can be used to assess equine insulin sensitivity. The glucose response to insulin in horses is composed of 2 phases. The first phase is a decrease from time to injection to a blood glucose concentration nadir. This was reached between 40 and 60 minutes in the present study. This rapid decrease is attributed to the insulin-stimulated uptake of glucose by peripheral tissues, primarily in the muscle. The second phase is a return to preinjection blood glucose concentrations, which occurred within 120 minutes after injection in the present study. At the end of the second phase, posthypoglyc-
cemic hyperglycemia can sometimes be observed (Somogyi effect). This slow recovery is caused by the suppression of liver output of glucose from glycogenolysis, gluconeogenesis, or both.25 The data obtained from the horses of the present study suggested that ovariectomy did not induce any short-term changes in insulin sensitivity. The gold standard to measure insulin resistance in horses and other species is the euglycemic-hyperinsulinemic clamp technique.26,27 The IVGTT and the insulin sensitivity test results reveal separate aspects of a horse’s ability to handle glucose and together provide more information than either would alone. The IVGTT primarily indicates whether a horse’s pancreas can respond appropriately to a high blood glucose concentration. If blood glucose concentration decreases quickly after IV administration of a glucose load, that can happen only because the pancreas has secreted an appropriate amount of insulin. On the other hand, the insulin tolerance test measures the ability of the peripheral tissues to respond to the insulin and increase glucose uptake at the cellular level. Although the measures of insulin sensitivity that were used in the present study did not demonstrate differences before and after the horses underwent ovariectomy, it is possible that a more precise technique may have revealed some subtle differences. However, considering the absence of significant differences in baseline blood glucose and serum insulin concentrations and results of the IVGTT, insulin tolerance test, and surrogate tests, it was concluded that ovariectomy did not induce changes in the horses’ glucose and insulin dynamics within the time frame of this study. It is possible that differences would have been observed if the horses had been tested for a longer period after ovariectomy. The horses were tested during 2 seasons, yet no differences in dexamethasone response, IVGTT results, or insulin sensitivity were detected. These findings were consistent with those reported previously in humans,28 which indicated that glucose and insulin dynamics are minimally affected by seasonal factors. Horses of the present study were given free access to grass hay during the testing procedures. Diet, particularly the soluble carbohydrate concentration, has been shown to influence response to measures of insulin sensitivity.29 Hay analysis was not performed; thus, the amount of soluble carbohydrate the mares ingested was not known. Because this was a comparative study, in which all horses ate hay from the same source at both test time points, it was believed that any dietary influence on insulin dynamics would be similar.

Overall, only 2 of the 6 horses had abnormal test results either before or after ovariectomy. One horse that was in estrus (horse 1) was initially insulin resistant as measured by the insulin response test, and another horse (horse 6) had an abnormal DST result after ovariectomy. Before ovariectomy, the horse in estrus was shown to be insulin resistant because it failed to reach a 50% decrease in blood glucose concentration after insulin injection. Although not hyperinsulinemic at the time of testing, this horse’s insulin concentration was toward the upper limit of the reference range (34.20 μU/mL; reference range, 10 to 40 μU/mL) before ovariectomy. Furthermore, compared with the other 5 study horses, this horse had the highest body condition score at the beginning of the study. Interestingly, none of the surrogate tests identified horse 1 as insulin resistant before ovariectomy. Such disparate test findings could be explained by the fact that, although used in human and equine medicine, none of the surrogate tests have been validated for use in horses.21 After ovariectomy, horse 1 was insulin sensitive. The horse’s transition from insulin resistance to insulin sensitivity could be explained by the fact that it lost 48 kg between the first and second set of tests. The reason for the weight loss was explained by the fact the horse was turned out on pasture and received no feed supplement. It has been previously shown that body weight and sensitivity to insulin are associated.30,31,32 Another possible explanation for the transition from insulin resistance to insulin sensitivity in that horse could be its estrous cycle stage at the time of first testing. It has been described that progesterone inhibits glucose uptake and could lead to insulin resistance.33 Although progesterone concentration was not measured in this horse, analysis of its ovaries after ovariectomy revealed that it was in estrus at the time of first testing, suggesting a low circulating progesterone concentration. Therefore, the change in the insulin response in this horse was attributed to weight loss rather than ovariectomy.

For the other horse (horse 6), the result of the DST after ovariectomy was not considered normal. A possible explanation for such a result could be that the horse was developing hypothalmo-pituitary-adrenal axis dysfunction. However, the fact that the horse was tested in September may have confounded the results, given that ACTH secretion and dexamethasone response in horses follow a seasonal pattern.28–30 After ovariectomy, horse 6 had the highest body condition score among all study horses, and although it was not hyperinsulinemic, its blood insulin concentration was toward the upper limit of the reference range (33.65 μU/mL). Given that there was no general indication that ovariectomy influenced test results or that the 2 horses with abnormal results had high or changing body condition scores, it was concluded that the abnormal results for those 2 horses were more likely caused by individual variation than by an effect of ovariectomy.

A possible reason why the mares in the present study did not have more differences in glucose and insulin dynamics before and after ovariectomy, compared with findings in other species, may be that 5 weeks is not an adequate amount of time for all the effects of ovary removal to develop in horses. In sheep and rats, significant alterations in endocrine function have been detected 6 weeks after ovary removal.29,33,34 The lack of differences may be attributable to the large concentrations of sex steroid hormones secreted by the adrenal glands in horses. Horses are the only domestic animal that may achieve estrus following ovariectomy, which is evidence of the amount of estrogens produced by the adrenal gland.35,43 There was no evidence of abdominal inflammation on postmortem examinations of any of the horses.8 Thus, the presence of chronic inflammation or stress was unlikely to affect the results of the present study. It is also possible that the number of horses used in the present study was too small to detect
differences between pre- and postovariectomy results; however, considering that post hoc sample size calculations (\(\alpha = 0.05; \text{power} = 0.80\)) indicated that, depending on the comparison, between 63 and 1,005 mares would have been needed to detect a significant difference, it is very likely that similar results would have been observed with a larger sample and that therefore the sample size was adequate.

The results of the present study have provided insight into the forms of medical care required by mares undergoing bilateral ovarioctomy. On the basis of the data obtained from the study horses, there is no evidence that horses become markedly insulin resistant or develop alterations in their hypothalamic-pituitary-adrenal axis after ovary removal in the short term. It would appear that there is no need to modify the diet or institute other management changes designed to prevent the morbidity of insulin resistance in ovarioctomized horses. However, it is important to maintain the horses’ ideal body condition score and weight because these factors do influence insulin sensitivity.

References