Equine metabolic syndrome and Cushing's disease: Possible role in infertility in the horse

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Abstract

Over the last several years, there has been a clear increase in the geriatric horse population. Some of those mares are expected to become donors for embryo transfer or to become pregnant, forgetting the consequences of a long life expectancy. For example; illnesses and problems that commonly affect the aged horse - such as Cushing's disease (CD) and equine metabolic syndrome (MS) - have become more prevalent. These metabolic conditions share traits, e.g., insulin resistance (IR), often in conjunction with obesity, which have been associated with the development of abnormal reproductive function and laminitis in the horse. A large number of obese and IR mares continue to cycle during winter, have longer inter-estrus intervals, prolonged luteal phases and a higher incidence of anovulatory follicles. Diagnosis of MS or CD requires a good clinical assessment in combination with the proper laboratory testing. Once identified, MS should be managed with a diet poor in carbohydrates and an exercise program that stresses a moderate body condition. Treatment for CD comprises appropriate health care and the administration of pergolide, cyproheptadine or a combination. Even though a link between infertility and these metabolic conditions has been established, there is a clear need for further investigation.

Keywords: Cushing's disease, metabolic syndrome, insulin resistance, infertility, mare

Introduction

Several endocrine and metabolic conditions had been empirically linked to infertility in the mare including hypothyroidism, metabolic syndrome (MS-insulin resistance) and Cushing's disease (CD) or pituitary pars intermedia disease (PPID). The mechanisms by which these conditions lead to infertility are not completely understood. These metabolic aberrations are gaining attention by veterinary scientists and equine nutritionists as the population of geriatric, obese horses in the USA is increasing and owners are requesting additional care for affected horses. This review discusses the pathophysiology of MS and CD, how they may adversely affect fertility, diagnostics and recommended therapies.

During the fall months and in preparation for winter, the horse begins to store energy in the form of body fat. The process is controlled by the hypothalamic-pituitary axis as it induces an increased secretion of pro-opiomelanocortin peptides[1] and results in an increase in appetite and adipogenesis in conjunction with the development of a thick hair coat. This "survival mechanism", disappears by the end of winter in healthy horses but not in horses with MS or CD. Metabolic synrrome and, to a certain degree CD, are associated with the acquisition of excessive fat and, more importantly, the persistence of body fat and its negative consequences on the mare's general heath and reproduction.[2] Clinical findings that should make one suspicious of MS or CD include obesity in older horses that do not shed their winter coats completely or do so much later in the spring than the remainder of the herd, abnormal estrous cycles, anovulatory follicles and repeated bouts of endometritis. A number of tests are available for diagnosing MS or CD. However, there is no single "gold standard".

Equine metabolic syndrome

Equine MS is closely associated with two disorders: obesity and insulin resistance (IR or glucose intolerance). Horses affected with MS also have a predisposition to developing laminitis and mares may exhibit abnormal estrous cycles.[3] The obesity is evident as subcutaneous regional adiposity ("cresty neck", tailhead, shoulders and prepuce in geldings) and affected horses tend to be genetically predisposed requiring fewer calories to keep normal body weight ("easy keepers"). Not all obese horses have IR even though obesity increases the horse's risk of developing the disease. Some breeds appear to be more commonly affected than others, including ponies, Quarter Horses, Morgans, Arabians and Saddlebreds. There is no gender bias.[4]

Pathophysiology: When foods rich in carbohydrates are ingested, they are broken down into glucose. This is followed by a physiological hyperglycemia which triggers the release of insulin from the pancreas. Insulin promotes glucose uptake by cells. Once in the cell, glucose is either used immediately or it is stored as glycogen or fat. The amount of insulin required for inducing glucose uptake into cells is tightly regulated and is referred to as insulin sensitivity. If a horse develops IR, there is a reduction in the insulin driven uptake of glucose by the skeletal and adipose tissue creating hyperglycemia.[2,4] This, in turn, generates hyperinsulinemia in an effort by the pancreas to compensate for the insensitivity.

Little is known about the factors predisposing to IR; however, obesity appears to be the recurrent trait in horses. It has been suggested that certain adipocites behave as endocrine tissue releasing cortisol, pro-inflammatory cytokines and leptin, which may interfere with glucose uptake by the cells.[2] Age and diet appear to be important contributing factors to the development of IR. Ageing is believed to decrease sensitivity to insulin.

Obesity in horses and especially in ponies appears to be a risk factor for mild laminitis. The simplest explanation is excessive weight at the hoof–lamellar interface.[4] The fact that ponies are at higher risk even though they bear less weight, suggests the genetic predisposition of this breed to MS and IR.[2,4,5] Glucose is essential for the health and strength of the hoof-lamellar interface. IR creates cell-glucose starvation which may increase the risk of laminitis more than obesity per se.

Diagnosis

Characteristics that are common in horses with MS or IR include increased age (8 to 20 years old), obesity, distinctive distribution of body fat (neck, shoulders, rump), "easy keepers" that have difficulty losing weight, a history, presence or predisposition for laminitis, abnormal estrous cycles and decreased pregnancy rates. Resting levels of insulin are good indicators of IR since compensatory hyperinsulinemia is a common finding. Plasma samples should be taken early in the morning after an overnight fast. Horses should be removed from pasture and only fed course roughage overnight. Roughage should be available as some horses will become increasingly agitated if no food is available. Resting insulin concentrations > 20 μU/mL are suggestive of insulin resistance and > 30 μU/mL are considered diagnostic.[4,6] Even though resting levels of insulin are good markers, they are not helpful in mild or early IR where the rise of insulin is too small to reach or exceed the reference value. Reference values differ among laboratories and should be taken into consideration when interpreting results (insulin >30 μU/mL at University of Tennessee vs. >43 μU/mL at Michigan State University).[6]

If one is presented a horse with physical findings consistent with MS or CD that has a normal resting plasma insulin level, a combined glucose-insulin test should be performed.[6] This test consists of collecting a baseline glucose blood sample before infusing 150 mg/kg of 50%

dextrose solution. Immediately after the dextrose infusion, insulin is administered intravenously at 0.10 U/kg. Serum samples are collected at 1, 5, 25, 35, 45, 60, 75, 90, 105, 120, 135 and 150 min. However for practical purposes, the test can be limited to 60 minutes. A horse is considered to have insulin resistance if blood glucose levels remain above baseline for more than 45 min.[6] Stress should be avoided during the test since it can cause transient IR. Horses should be kept quiet, allowed to graze and the jugular catheter should be placed the night before for blood collection.

Metabolic syndrome and CD share a number of clinical signs including abnormal distribution of fat, hyperinsulinemia and insulin resistance, predisposition to laminitis and infertility. Cushing's disease can be differentiated from MS by performing a dexamethasone suppression test (DST; see PPID), as this test will be positive only for PPID. Indeed, MS has been described as the presence of insulin resistance without detectable presence of PPID.[7]

Treatment

Treatment is directed at lowering body fat and consequently insulin levels to <30 μU/mL. Horses should be placed on a low carbohydrate diet to reduce caloric intake and partake in a consistent exercise program to increase energy outflow and glucose uptake. Grain should be removed from the diet (alone or mixed with molasses) and access to pasture should be limited. Medical treatment with levothyroxine sodium (Thyro L; Vet-A-Mix, Shenandoah, IA, USA) is warranted if weight loss occurs too slowly or hyperinsulinemia persists after reaching the desirable body weight.[4] Levothyroxine sodium is administered at a dose of 48 mg/day PO for 3 to 6 months to accelerate weight loss in horses, especially those which cannot exercise due to laminitis. Persistent hyperinsulinemia/hyperglycemia is treated with a combination of levothyroxine sodium (24 mg/day PO) and a biguanide (metformin; 15 mg/kg PO BID). The

mechanism of action is not known but it appears to inhibit gluconeogenesis and increases glucose uptake.[4] Its safety in the horse has not been established. In addition, administration of chromium at a dose of 5 to 25 mg/day may improve insulin efficacy in transporting glucose into the cells.[4,7]

Cushing's disease or pituitary pars intermedia dysfunction

Cushing's disease or PPID is a relatively prevalent condition in horses due to an increase in the geriatric population.[8,9] Onset of clinical signs usually occurs around 18 to 23 years of age but horses as young as 7 years have been affected. There is no sex predisposition and all breeds can be affected. Ponies and Morgan horses appear to be at a higher risk.[10] First described by Pallaske 75 years ago, this syndrome is characterized by adrenocortical hyperplasia and excessive glucocorticoid, mineralocorticoid and androgenic steroid secretion in response to a surplus release of adrenocorticotrophic hormone (ACTH).[11] In the horse, there is a hypothalamic or pituitary-dependant hyperadrenocorticism. The presence of a possible benign adenoma in the pituitary gland or the degeneration of dopaminergic neurons at the hypothalamic level had been held responsible.[6,9,12]

Pathophysiology. The hypothalamus controls secretion of ACTH by the anterior pituitary through the release of corticotrophin releasing hormone (CRH). Neurons secreting CRH are located in the anterior portion of the paraventricular nuclei and their axons terminate in the pituitary median eminence. Corticotrophin releasing hormone stimulates secretion of ACTH, beta-lipotropin (ß-LPH) and ß-endorphins. All three originate from the same precursor called pro-opiomelanocortin (POMC),[9,11] and all have the same secretory dynamics, that is release is increased in response to stress and hypoglycemia and decreased in response to glucocorticoids.

Negative feedback inhibition of ACTH by glucocorticoids is absent in PPID. Therefore, ACTH and POMC related hormones persist despite cortisol elevation.[12]

Chronic exposure to excessive glucocorticoids results in a variety of physical and clinical abnormalities. The most characteristic clinical sign is hirsutism (47 to 100% of affected horses) and the inability to shed the winter coat. It is suggested that hirsutism results from increased melanocyte-stimulating hormone (MSH) released from the pars intermedia or increased production of androgens by the adrenal cortex. In women, hirsutism has been associated with clinical hyper-androgenism.[13] In addition there are changes in weight, unusual fat accumulation at the neck, tail head and over the croup (29 to 67%). Obesity is believed to be the underlying factor for horses with MS to develop PPID at a younger age than average. Chronic obesity is thought to result from a pro-inflammatory and pro-oxidative condition that may accelerate degeneration of dopaminergic neurons.[7] Other characteristics are muscle atrophy (protein catabolism), immunosuppression (and consequent predisposition to retarded wound healing and infections such as endometritis and sole abscesses), polydipsia and polyuria (25%), lethargy, tachypnea, hyperhidrosis (65%) and occasionally, neurologic impairment.[6,14] Laminitis occurs in 50% of advanced cases. One explanation is that excess of corticoids promote laminar vasoconstriction. The presence of IR seems to exacerbate the condition. Broodmares may show persistent lactation (hyperprolactinemia), low grade endometritis (immunosuppression) and anovulatory follicles during the cyclic season.[7,9] In humans, 23% of Cushing's patients with persistent lactation and amenorrhea (due to GnRH inhibition by prolactin) have increased prolactin concentrations. The increase in prolactin is due to excessive production and secretion by corticotroph adenomas or an alteration of the regulation at the dopaminergic neurons.[6,9,12]

The most common biochemical abnormality in PPID is hyperglycemia and hyperinsulinemia/IR. Other findings include increased hepatic enzymes, cholesterol and triglycerides. Horses can also have mild anemia, neutrophilia and lymphopenia.

Diagnosis

Presence of hirsutism had greater diagnostic accuracy than any endocrinological test alone or in combination.[10] Affected horses are usually aged (8 to 20/30 years old), overweight, with the characteristic distribution of body fat (neck, shoulders,), laminitis, and abnormal estrous cycles.[7,8,9,10]

Dynamic endocrinologic diagnostic tests

The dynamic endocrinologic diagnostic tests consist of the overnight dexamethasone suppression test (DST), thyrotropin-releasing hormone (TRH) stimulation test and the cortisol rhythm assay.

DST. In normal horses, administration of dexamethasone (20 mg IM/500 kg) decreases the ACTH release from the pituitary, resulting in a cortisol serum concentration of < 1 μg or 10 ng/dl approximately 19 hours after injection. Conversely, in horses with PPID, serum cortisol concentration does not decrease after dexamethasone administration due to continuous ACTH release. Unfortunately, this gold-standard test loses accuracy (sensitivity and specificity) early in the condition when there is still some feedback from the hypothalamic-pituitary axis, as it appears that loss of suppression by dexamethasone is a late event in the pathogenesis.[10] The DST results are also affected by the season of the year since the pituitary pars intermedia produces more hormones during the fall ("survival mechanism"). One study showed that false-positive overnight DST results were common when horses or ponies were tested in the fall.[1]

TRH stimulation test. Horses with PPID show an increase (30 to 50% from baseline) in serum cortisol concentration 30 to 90 min after TRH administration (0.5 to 1 mg, IV) whereas normal horses do not.[15] Normally, TRH produced by the hypothalamus stimulates TSH release from the pituitary. However, in PPID, exogenous TRH stimulates ACTH and hence, cortisol release. It appears to be due to an up-regulation and expression of TRH receptors in hyperplastic/adenomatous corticotropes in the pituitary pars distalis and intermedia.[10,14] This test is safe to perform in laminitic horses. It has not been critically evaluated in horses early in the disease process.

(Diurnal) Cortisol rhythm assay. This assay is based on the observation that horses with PPID have a loss of the diurnal cortisol rhythm (normally high in morning, low at midnight).[10,16] When performing this test, plasma cortisol concentration is measured at 8 AM and again at 4 PM. A difference of less than 30% between the morning and afternoon cortisol concentration is considered to be suggestive of PPID. However, this test has not been validated and several factors such as fasting, changes in stabling or laminitis can increase plasma cortisol concentration in the horse.

Combined DST and TRH stimulation test. Administration of dexamethasone before TRH should suppress ACTH release from the pituitary par distalis therefore, any increase in cortisol after TRH administration is attributed to pars intermedia corticotropes. In performing this test, a baseline plasma cortisol concentration is obtained followed by administration of dexamethasone (20 mg, IM/500 kg). Three h after the dexamethasone is given, TRH (0.5 to 1 mg, IV) is administered. Cortisol should be suppressed by the TRH. Plasma cortisol concentrations are measured 30 min and 21 h after administration of TRH (24 h after dexamethasone administration). A plasma cortisol concentration > 1 μg/dl 24 h after administration of

dexamethasone or a $\geq$ 66% increase in cortisol 30 min after TRH administration is considered diagnostic for PPID.[8,10] The test is expensive and may be impractical for the ambulatory clinician.

Accuracy of each test when compared with histological findings is DST/TRH 81%; DST 71%; TRH 71%. Accuracy of hirsutism alone is 86%.

Single sample endocrinologic tests

Two single sample endocrinological tests are available and include measurement of endogenous release of plasma adrenocorticotrophic hormone (ACTH) concentration and insulin concentration.

ACTH concentration test. Measuring a single sample of endogenous plasma ACTH, which is supposedly higher in horses with PPID, is not more accurate than the DST test, does not detect early cases, and it undergoes daily and seasonal variations. A reference value of > 50 pg/ml is considered elevated.[10] Sample handling is intricate. Blood must be collected in cold plastic tubes containing EDTA, as the ACTH can be absorbed onto glass and degraded by proteolytic enzymes in blood or serum. It needs to be centrifuged as soon as possible (within 30 to 60 min) and the plasma transferred immediately to a plastic tube for freezing and shipping (should arrive to the laboratory below 60 ^{0}F). This test is recommended when one is trying to avoid administration of dexamethasone to laminitic animals.

Insulin concentration. Insulin resistance/hyperinsulinemia has been recognized in horses with PPID. Affected horses have increased insulin blood levels attributed to excessive circulating concentrations of cortisol since they have antagonistic metabolic effects. Excessive insulin concentrations vary between laboratories and reference values must always be consulted when interpreting the test. Cutoff point for normal insulin concentration varies from 25 μU/ml to 57

μU/ml[7,10] however, > 30 μU/ml is considered diagnostic of hyperinsulinemia. The accuracy of this test compared to histological findings is 92%. This test has the advantage of a single sample for the diagnosis, however, MS should be ruled out since hyperinsulinemia is also present in this condition.

Douglas has proposed that testing for suspected cases of PPID and MS should start by simply collecting two serum samples 8 to 10 h apart.[7] No grain should be given 4 h before both samples and stress should be avoided since both parameters can result in false positives. Serum samples are collected for measurement of cortisol, insulin and thyroxine (TT4). The absolute values of cortisol are 20 to 90 ng/ml, however, the test specifically looks at a difference of less than 30% between the two samples since that indicates a loss in the diurnal cortisol rhythm which is considered suggestive of PPID. A consistent insulin value > 25 to 30 μU/ml will be indicative of IR.

A typical endocrine profile in a horse with IR and PPID

Sample time	**TT4** **Normal=12.0 ng/ml**	**Insulin** **Normal=<25 μU/ml**	**Cortisol** **Normal 20-90 ng/ml**	**Cortisol** **Normal>30 % difference**
8 to 10 AM	11.9 ng/ml	35 μU/ml	58 ng/ml	11 %
4 to 6 PM	12.2 ng/ml	55 μU/ml	65 ng/ml	

From: Douglas RH. Endocrine assessment and management of insulin resistance and PPID.[7]

Horses suspected of having early signs of PPID ("Pre-Cushingoid") that have normal values from earlier, appropriate diagnostic tests, should be re-tested at 4 to 6 month intervals avoiding the fall months. The evaluation should include a physical examination, CBC, serum chemistry, ACTH, insulin concentrations and DST as a baseline profile.[10]

Treatment

Management of PPID is essential to assure appropriate health care of older animals: body clipping, regular hoof and dental care, enhanced nutrition, and intermittent or long term antibiotics may be required. The dopamine agonist, pergolide mesylate (1 to 5 mg/PO/q 24 h/500 kg horse) is most commonly administered since the disease appears to be associated with a loss of hypothalamic dopaminergic innervations. Pergolide should be discontinued in pregnant mares approximately 3 weeks before their anticipated foaling date to avoid agalactia (PRL suppression). The most common adverse effect of pergolide administration is a mild decrease in appetite. Serotonin antagonists (cyproheptadine 0.25 mg/kg/PO/q 12 or 24 h, or 200 to 400 mg/500 kg/PO/q 24 h) are also used alone or in conjunction with pergolide. This therapy is based on the secretagogue effect of serotonin on ACTH in rat pars intermedia.[10,17] The use of cyproheptadine is controversial since results appear to be similar to the clinical improvement obtained with good management alone.

It has been suggested, that a combination of 1 mg of pergolide and 200 mg of cyproheptadine/day accelerates the clinical response to medication, increasing the chances of successfully breeding a mare with history of development of anovulatory follicles or chronic endometritis.[6]

Recent studies have examined the use of trilostane (0.4 to 1.0 mg/kg/q 24 h in feed) in horses with PPID.[10,18] This drug is a competitive inhibitor of 3-ß hydroxysteroid dehydrogenase,

an adrenocortical enzyme needed for cortisol production. Even though some treated horses exhibited a decrease in clinical signs, there is reservation toward using this treatment because less than 20% of horses with PPID have adrenocortical hyperplasia.

Aging, obesity, insulin resistance and infertility

Recent studies associate obesity and insulin resistance in horses with development of abnormal reproductive function and laminitis.[19] Equine MS and CD or PPID share traits that provide plausible explanations for the clinically perceived increased incidence of fertility problems in horses.

a) The shared clinical manifestations of both diseases are: aged horse, obesity, regional subcutaneous adiposity (neck, tail head, prepuce), a history, presence or predisposition for laminitis and/or infertility.
b) The shared metabolic alterations include: increased insulin and or insulin resistance, +/_ increased glucose (and in some cases increased leptin and "leptin resistance", cortisol and fatty acid).
c) The shared presence of excessive fat, in the form of obesity or regional adiposity, creates at the cellular-molecular level an increase in "adipocytokines" such as tumor necrosis factor-α (TNF-α). Therefore, PPID and MS may also have an increased level of TNF-α, a pro-inflammatory cytokine which inhibits the proper function of the insulin receptor. Increased fatty acids impair glucose transport through the membrane. Increased 11ß-hydroxysteroid dehydrogenase-1 (11ß-HSD1) converts cortisone to cortisol generating more adipogenesis and IR.[2]

Similar to humans, old horses have been identified as developing a condition referred to as "inflamm-aging". This condition is a persistent low grade, systemic, chronic, inflammation caused by an increased production of serum levels of IL-6 and TNF-α.[20] Increased body fat, associated with obesity, may be a contributing factor since adipose tissue leads to increased production of inflammatory cytokines.[20,21] Adams et al, showed that fat, old, horses between 20 and 28 years old, had greater mRNA expression of TNF-α and INF-γ from lymphocytes and monocytes and had higher serum concentrations of TNF-α protein than thin old horses.[21] The impact of this increase in pro-inflammatory cytokines and infertility is unknown. In humans, elevated TNF-α plays a direct role in the development of obesity-associated insulin resistance and it appears that inflammation may be a key link between obesity and IR in horses as well.[19]

In women, chronic insulin resistance and hyperinsulinemia are associated with increased duration of the follicular phase of the menstrual cycle. Polycystic ovarian syndrome (POS) in women is often associated with obesity and characterized by several clinical signs, neuroendocrine and metabolic alterations. Clinical signs that are diagnostic in women are: ovulatory dysfunction, clinical hyperandrogenism (hirsutism, alopecia, acne) and IR/hyperinsulinemia.[13]

Neuroendocrine disturbances associated with POS include an increase in GnRH pulses that selectively stimulate LH release but not FSH. LH stimulates the expression of the P450c17 cytochrome in the theca cells, increasing the production of 17α-hydroxyprogesterone and testosterone. This hormonal environment (↑LH/↓FSH) impairs follicular maturation, decreases the number of granulosa cells and aromatization (↓E_2), and inhibits normal ovulation.[13] Insulin resistance/hyperinsulinemia may have some role since administration of metformin decreases the plasma levels of insulin, LH and androgens in women.[13,22] The increased level of insulin and

insulin like growth factor-1 (IGF-1) directly target the theca cells acting in synergism with LH, stimulating P450c17 cytochrome production and hyperandrogenism. They also decrease the hepatic secretion of the sexual hormone binding globulins (SHBG) and the IFG binding proteins (IGFBP-1) producing hyperandrogenism by increasing the free testosterone fraction and the IGF-1 bioactivity.[13]

There are limited studies in the mare on the endocrine effects of IR, MS, or PPID on reproductive function. One study showed that a great proportion of obese mares continued to cycle during the winter compared to lean mares.[23,24] It was speculated that metabolic alterations in the form of excessive energy imbalance may lead to continuous cycling during winter.[23,25] Later studies found that obese mares had aberrations in the duration of the estrous cycle: longer inter-estrus intervals (36.72$\pm$4.77 vs. 26.00$\pm$0.54 days), prolonged luteal phases (30.00$\pm$5.55 vs. 17.67$\pm$0.18 days) and a higher incidence of anovulatory follicles compared to lean mares.[23,26] Progesterone remained elevated for periods of 37 to 78 days in 83% of the obese mares while the longest time progesterone was elevated in lean mares was 22 days.[24] The persistence of elevated serum levels of progesterone in obese mares was thought to be associated with a persistent corpus luteum or luteinization of an anovulatory follicle.[27] Ultrasonic examination of the reproductive tract of these mares showed large, persistent anovulatory follicles during periods of elevated progesterone suggesting that one or more mechanisms involved in ovulation may be suppressed or inhibited.[28] Obese mares exhibited lower levels of serum concentrations of thyroxine and leptin and higher levels of insulin and insulin insensitivity compared to lean mares.

The mechanism of how hyperinsulinemia and IR may disrupt normal reproductive activity is unknown but the following data suggest a direct effect on the ovary rather than the

hypothalamic-pituitary-ovary axis. In domestic species, insulin in combination with IGF, promotes follicular development and modulates ovarian steroids secretion as seen in women with POS.[13] Sessions et al., showed that induction of transient insulin resistance in the mare lengthens the interovulatory interval (mean duration 19.57±2.66 vs. 15.57± 0.97 d), creates higher serum peak concentrations of progesterone (12.87±4.04 vs. 8.78±2.23 ng/mL) but does not affect serum LH concentrations (3.9±0.34 vs. 3.29±0.13 ng/mL) when compared to control mares.[29]]

In women, hyperinsulinemia affects the menstrual cycle by alterations of the GnRH-mediated LH release and has a direct effect on the ovary by increasing steroid secretions.[30,31] This phenomenon is well documented in women with POS where there is an arrest of follicular development. Apparently, small follicles that just attained LH receptors, in the presence of insulin and high LH, produce estradiol levels that are similar to levels produced by mature follicles, thereby inhibiting growth and arresting follicles in the immature stage. A similar phenomenon may be occurring in the mare with insulin resistance; however it appears to be due to a direct effect of insulin on the ovary since the hypothalamic-pituitary axis was not affected during an induced short term hyperinsulinemia. Peak concentrations of progesterone were higher in mares experiencing transient IR compared to control mares suggesting an increased steroid secretion by the ovary. It has been proposed that insulin has a stimulatory effect on progesterone production by luteal tissue and this effect is mediated by changes in the developing follicle. In studying the repeatability of hemorrhagic anovulatory follicles (HAF) in ponies, Ginther also deflected the focus from the hypothalamic-pituitary-ovary axis.[32] He showed that follicular diameter, levels of LH and progesterone were similar in ovulating and HAF mares before and at the day of ovulation. The impact of his findings on obese mares remains unknown.

The longer interovulatory interval in obese mares appears to be due to luteinization without ovulation of the dominant follicle (failure to ovulate) extending the luteal phase. One plausible explanation is what happens in women with POS; insulin directly targets the theca cell stimulating P450c17 cytochrome production and hyperandrogenism. Increased androgen inhibits FSH secretion and this hormonal environment (↑androgens/↓FSH) impairs follicular maturation, decreases the number of granulosa cells and aromatization (↓E_2), and inhibits normal ovulation. Further studies need to be performed to confirm this theory by measuring androgens and FSH in mares with persistent IR.

Another explanation, which does not exclude the prior, is that elevated insulin affects follicle remodeling mechanisms necessary for ovulation. Normal follicular growth, ovulation and atresia require remodeling events which are carried out by matrix metalloproteinases (MMP) and tissue inhibition of metalloproteinases (TIMP). Recent studies indicate that exposure to elevated insulin affects the *in vitro* expression of MMP and TIMP in preovulatory and atretic follicles. Moreover, it specifically disrupts the expression of TIMP-2 modifying the ratio of MMP/TIMP expression by equine granulosa cells of large follicles. This alteration may be responsible for the inability of the follicular wall to remodel for development, migration and ovulation.[26,33]

Conclusion

Although the pathophysiology of metabolic diseases such as IR, MS or CD is not completely known, there is increasing evidence that these conditions may adversely impact fertility of the mare. Diagnostic testing for these conditions should be considered in old, obese mares that repeatedly form anovulatory follicles, experience bouts of laminitis or endometritis. While accuracy of diagnostic tests for detecting disease may not be as high as one would like, a combination of tests along with clinical assessment will identify the vast majority of mares. A

strict exercise regimen plus removal of feed stuffs rich in carbohydrates from the diet and supplementation with thyroid hormone and/or pergolide should improve fertility in affected mares.

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