# Effect of endocrinopathies on fertility in the mare
# Part 1: Equine metabolic syndrome

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## Abstract

Obesity and insulin resistance (IR) have been associated with subfertility in the mare. There has been a substantial increase in the prevalence of overweight and obese horses in recent years (45 - 50% from 4.5% in 1998). Some of these mares, with a history of good sport performance, enter breeding programs with high expectations of embryo production or pregnancy. Without losing perspective of the reproductive problems associated with ageing and obesity, it is very important to consider metabolic problems (e.g., equine metabolic syndrome [EMS] and pituitary pars intermedia dysfunction [PPID; i.e., equine Cushing's disease]) that may directly impact fertility. However, EMS and PPID differ widely in their pathophysiology; PPID is neurological in origin, whereas EMS is the result of overfeeding horses with high metabolic efficiency, especially during winter. Generally, EMS and PPID are associated (and often confused with each another) because they share several clinical manifestations, especially, when or if PPID has concurrent insulin dysregulation manifestations (abnormal and excessive distribution of adipose tissue, IR, laminitis, and subfertility). A large proportion of obese and IR mares continue to cycle during winter, have longer inter-ovulatory intervals, and a higher incidence of anovulatory follicles. Diagnosis of EMS requires a detailed history and physical evaluation combined with appropriate laboratory tests. Once identified, EMS should be primarily managed with a low-carbohydrate diet and a good exercise program. If necessary, medication (e.g., levothyroxine or metformin) can be used. However, there are no critical studies regarding safety or efficiency of these drugs.

**Keywords:** Obesity, mare, fertility, insulin resistance, anovulatory follicles

## Introduction

Equine metabolic syndrome (EMS) has been associated with infertility in the mare. The mechanisms by which this condition occurs has not been completely elucidated. In preparation for winter, during autumn months horses begin to accumulate energy in the form of body fat. This process is mainly controlled by the hypothalamic-pituitary axis and the secretion of peptides (adrenocorticotropic hormone [ACTH], alpha melanocyte stimulating hormone [αMSH], corticotropin-like intermediate peptide [CLIP], β-endorphins) from the pituitary pars intermedia that stimulate an increase in appetite, adipogenesis, and the development of a long, thick haircoat.[1] In EMS, excess body fat occurs because similar level of feeding is maintained during winter that is perceived by the body as an 'excess,' especially carbohydrates, because physiologically it should be a period of feed shortage.[2]

EMS is suspected in obese or normal mares that have ectopic fat deposits, abnormal estrous cycles, anovulatory follicles, estrous cycles during winter, and laminitis.

## Equine metabolic syndrome

Equine metabolic syndrome is defined as a 'collection of risk factors for endocrinopathic laminitis'.[3] These risk factors are insulin dysregulation (ID) and/or IR, obesity, adipose dysregulation, and less frequently, cardiovascular changes; regardless, the key factor is ID. Adipose dysregulation also has an important role by increasing blood concentrations of leptin and proinflammatory cytokines, and by decreasing adiponectin. Leptin is responsible for maintaining body condition by suppressing appetite and increasing energy outflow.[3] Persistent high leptin concentrations are associated with hyperinsulinemia in nonpregnant ponies.[4] Adiponectin increases insulin sensitivity and reduces inflammation. Lower than normal concentrations of adiponectin have been associated with an increase in serum amyloid-A, a marker for inflammation, and with ID.[3]

Horses with EMS have a history of or are predisposed to laminitis, and frequently exhibit abnormal estrous cycles.[5] Obesity is manifested by regional, localized, or ectopic deposition of subcutaneous adipose tissue in the nuchal ligament (i.e., 'crested' or bulging neck), tail base, shoulders, mammary

gland and prepuce, especially in geldings. These horses appear to be genetically predisposed to need fewer calories to maintain adequate body weight (i.e., 'easy keepers'). Some breeds (e.g., Ponies, Quarter Horses, Morgans, Arabians, and Saddlebreds) are predisposed to have this condition.[6]

Pathophysiology

Amount of insulin needed for glucose uptake into a cell is highly regulated and is called 'insulin sensitivity.' When a horse develops IR, there is a decrease in insulin-dependent glucose uptake in skeletal and adipose tissue, creating an increase in plasma concentrations of glucose.[2,6] This in turn generates hyperinsulinemia in an effort by the pancreas to compensate for cellular insensitivity that perpetuates this condition. Information on predisposing factors to ID is limited; however, obesity appears to be a recurring factor. Obesity is not observed as the sole cause of EMS, since this syndrome also diagnosed in lean horses; however, when present, it exacerbates ID.[3] In horses with EMS, the fat deposits are composed of enlarged adipocytes and infiltrated macrophages that behave like endocrine tissue. Excess fat deposits will release cortisol, adipokine peptides (e.g., leptin, adiponectin), and proinflammatory cytokines (e.g., tumor necrosis factor-α, (TNF-α), interleukin (IL)-1 and IL-8. These hormones, proteins and factors may adversely affect glucose absorption by cells, producing inflammation and possibly vascular endothelial damage.[2,7] Elevated TNF-α concentrations would inhibit normal function of cellular insulin receptor favoring IR. They also reduce secretion of adiponectin (anti-inflammatory peptide and insulin sensitivity promoter) by adipocytes creating a proinflammatory state and increasing IR. Conversely, secretion of leptin is increased with obesity, insulin, and TNF-α; however, as with insulin, these mares appear to develop 'leptin resistance,' failing to regulate appetite or energy balance.[8]

Obesity in horses, especially ponies, is a risk factor for development of laminitis. The simplest explanation is excessive weight on the hoof and inter-laminar surface.[2,4,6,9] Mechanisms by which obesity and ID causes laminitis have not been completely elucidated. Current theories include changes in intracellular insulin signaling resulting in endothelial dysfunction and mechanisms involving insulin-like growth factor 1 (IGF-1).[3,10,11] Obesity and IR appear to be responsible for mares continuing to cycle during winter, having longer inter-ovulatory intervals, prolonged luteal phases, and a higher incidence of anovulatory follicles.

Possible systemic and local mechanisms of action of insulin on reproduction in the mare

A few studies linked EMS, IR and/or hyperinsulinemia with subfertility. It was proposed that signals sent to the brain regarding metabolic status may somehow control seasonality, especially the occurrence and length of seasonal anestrus.[8] Aggressive feed restriction led to cessation of reproductive activity during winter whereas obese mares displayed continuous estrous cycles during winter.[12] Similarly, a large proportion of obese mares continue to cycle during winter compared to proper weight or slender mares.[13] After several investigations it was concluded that the cause for the loss of the seasonal anestrus was not plasma leptin concentrations but the excess energy balance.[12] In mares, physiological recognition of the availability of metabolic substrate for cyclicity would be more important than the perception of a change in the photoperiod.

Subsequent studies reported aberrations in estrous cycle length and progesterone concentrations in obese/IR mares compared to normal mares.[14-16] Transient induction of hyperinsulinemia in mares using exogenous lipid-heparin infusion lengthened the inter-ovulatory interval with a marginal increase of the luteal phase, and higher peaks of progesterone concentrations; however, with no effect on LH concentrations compared to control mares.[14] Insulin in horses, like in women, may act directly at the level of ovary by stimulating steroidogenesis since progesterone concentrations were higher in mares with IR without an increase in LH concentrations.[14] Obese mares had longer inter-ovulatory intervals, prolonged luteal phases or elevated progesterone concentrations, and a higher incidence of anovulatory follicles compared to obese mares with moderate feed restriction.[15] Progesterone concentrations remained elevated between 37 and 78 days in 83% of obese mares whereas in mares subjected to feed restriction, increased progesterone concentrations did not exceed 22 days.[15] It was speculated that the persistence of elevated progesterone concentrations in obese mares was due to persistent corpus luteum (CL) or luteinization of anovulatory follicles.[15,16] Transrectal ultrasonographic evaluation in obese mares demonstrated the presence of persistent anovulatory follicles during elevated progesterone concentrations, suggesting that 1 or more mechanisms for triggering ovulation is affected.[15,17] Some preliminary data indicated that transient IR did not lead to differences in LH or FSH concentrations compared to normal mares and suggested that elevated insulin concentrations may directly affect the ovary rather than the hypothalamic-pituitary axis.[14]

With respect to the link between insulin and maternal recognition of pregnancy, exogenous insulin treatment during days 7 - 17 of diestrus had no effect on luteal size, diestrus length, inter-ovulatory interval, or circulating LH concentrations.[18] Similarly, obesity produced by overfeeding in peripartum did not adversely affect subsequent estrus duration, time to first and second postfoaling ovulations, and rates of pregnancy or early embryonic death.[19]

Possible mechanisms that associate hyperinsulinemia/ID with ovarian dysfunction and anovulatory follicles in mares

Polycystic ovary syndrome (PCOS) in women has been used as a model to understand effects of hyperinsulinemia on fertility in mares. Equine metabolic syndrome and PCOS have similar clinical signs (e.g., ovulatory dysfunction, obesity, and hyperinsulinemia/IR). In women, chronic hyperinsulinemia/IR has been associated with an increase in the duration of follicular phase due to a high incidence of ovarian (anovulatory) follicles.[20] Supposedly, high insulin concentrations stimulated the selective release of GnRH/LH and increased ovarian steroid secretion.[20-22] Increased pulses of GnRH selectively stimulate the secretion of LH, but not FSH. LH stimulates the expression of cytochrome P450c17 in follicular cells that increases the production of 17α-hydroxyprogesterone and testosterone. Increases in testosterone concentrations adversely affected the secretion of FSH. Hormonal environment of increased LH

and decreased FSH concentrations reduced or inhibited follicular maturation, decreased the number of granulosa cells, decreased aromatization and estrogen production, and inhibited ovulation.[20]

Metformin treatment to women with metabolic syndrome and PCOS decreased plasma concentrations of insulin, LH, and androgens, thus supporting the leading role of hyperinsulinemia in this endocrine alteration.[20,23] However, there are 2 fundamental differences between the effect of hyperinsulinemia on the reproductive system of mares compared to women. Mares with IR have a longer estrous cycle due to a longer diestrus period, and not a longer follicular phase. More importantly, higher concentrations of insulin did not affect the hypothalamic pituitary axis in mares since LH and FSH concentrations are similar for mares with or without IR.[14] Therefore, in mares, the mechanism of action appears to be at the ovarian level. Higher concentrations of insulin may act as an LH-like molecule, bind to insulin or the IGF-1 receptors at the theca cells, and stimulate production of 17α-hydroxyprogesterone and testosterone. This synergic action with endogenous LH will have a negative impact on follicular development, maturation, and ovulation.

In ponies that developed hemorrhagic anovulatory follicles (HAF) repeatedly, higher estradiol concentrations occurred 3 days before the formation of HAF.[24] In these ponies, follicular diameter, LH, FSH, and progesterone concentrations were similar on day 0 (ovulation) and day -1 (previous) compared to follicles that ovulated, suggesting that the focus (formation of HAF) should not be strictly on hypothalamic-pituitary-gonadal axis. Interestingly, the body condition of mares used for this experiment (4 - 16 years old) was higher throughout the experiment (body score > 7) and 3 of 4 'HAF repeaters' mares did not enter the anovulatory season later in the year. Although the metabolic status of these mares is not known, obesity and/or hyperinsulinemia might have affected ovarian steroidogenesis. Furthermore, substantial correlation of insulin, leptin, adiponectin, and cytokines concentrations with serum and follicular fluid suggested the role of hyperinsulinemia.[25] Granulosa cells expressed receptors for insulin, leptin, adiponectin, TNF-α, IL-6 and IL-1B indicating that they have the potential to respond to these hormones and factors that have a local effect on steroidogenesis, granulosa cell proliferation, oocyte maturation and early stages of embryo development.[25] Additionally, gene expression of tissue inhibitor of metalloproteinase -2 (TIMP2) in granulosa cells was increased, essential for activation of matrix metalloproteinase-2 (MMP-2). Inadequate TIMP2 expression will inhibit activation of MMP-2 and disrupt tissue remodeling necessary for ovulation. This could contribute to obesity-related formation of persistent anovulatory follicles.[26] Disruption of normal follicular concentrations by these factors may affect follicular development and maturation, ovulation, pregnancy, and even the metabolic future of the offspring. Impacts of insulin resistance on follicular growth, size at ovulation and response to human chorionic gonadotrophin (hCG) were studied.[27] Dominant follicle (F1) had similar size at ovulation in IR mares compared to unaffected mares. Mares with IR had more subordinate follicles, and their second largest follicle (F2) was larger in diameter than in normal mares, almost establishing codominance with F1. The impact of these findings on fertility was not studied. Although it was not significant, because of the number of mares per group (n = 4), hCG treatment induced ovulation before 48 hours in 2 of 4 mares with IR inducing ovulation in all (n = 4) normal mares.[27] As practitioners, we consider the lack of response to ovulatory drugs as 1 of the first signs of an anovulatory follicle.

In summary, most studies point towards an alteration in steroidogenesis at the ovary in mares with EMS. Insulin has gonadotrophic and steroidogenic effects in other species. In mare, the excess insulin may have an LH-like effect on theca cells, producing an increase in androgen/estrogen concentrations affecting normal follicular development and ovulation.[20,24] It may also affect steroidogenesis once a CL is formed, or in anovulatory follicles by increasing progesterone production, as shown in early studies.[14,15]

### Equine metabolic syndrome and pregnancy

Normal pregnant mares have slower glucose clearance and greater insulin secretion than nonpregnant mares. It appears to be a kind of 'physiologic IR' created to have high glucose concentrations, since uptake by the feto-placental unit depends on the existing concentration gradient. Pregnant mares with EMS have an exacerbated insulin and glucose response to high carbohydrate diets during pregnancy.[28-30]

### Diagnosis

Common clinical features in horses with EMS are obesity, and the characteristic regional distribution of body fat (neck, shoulders, base of the tail). These horses also need less feed to maintain body weight (i.e., 'easy keepers'), or they may have difficulty losing weight. In addition, these horses have a predisposition, or history of previous bouts of laminitis and abnormal estrous cycles. There are static and dynamic tests to identify IR/ID.

### Static tests

Insulin and glucose concentrations: This test is ideal for the identification of horses with moderate to severe insulin dysregulation. Baseline insulin values > 20 µu/ml are indicative of ID and values > 30 µu/ml are considered diagnostic.[6, 8,11]

Adipokines: Blood concentrations of adiponectin is associated with obesity and ID, and low blood concentrations are considered a risk factor for laminitis.[3,31-33]

### Dynamic tests

Oral sugar test: This is easily performed and is very sensitive to identify mild to moderate cases of ID.[3,5]

Combined glucose-insulin test: Evaluates tissue insulin sensitivity and beta cell responsiveness.[3,5,34]

Treatment

Diet and exercise: Treatment is aimed at decreasing excess weight gain and, if necessary, gradual weight loss. The goal is also to keep insulin concentrations to < 30 µu/ml.

Affected horses should have a diet composed of grass hay; low (i.e., < 10%) nonstructural carbohydrates (NSC- sugar, starches and fructanes); and a ration balancer to meet mineral and vitamin requirements. Exercise, if possible, is recommended to burn calories and to stimulate glucose absorption. Mares should have continuous access to forage, especially if they have dietary restrictions due to EMS, since it is proven to enhance fertility.[8,35] Grain and concentrates should be given in small amounts or eliminated from the diet, and time on pasture should be limited.[36] Pregnant mares with EMS should be fed in the same way as nonpregnant EMS mares for the first 2 trimesters. The necessary increase in calories needed during the last trimester and lactation should be coming from fat and fiber.[28-30] Exercise has a proven beneficial effect on insulin sensitivity in nonpregnant mares. It is recommended to do no more than 30 minutes of trot exercise/day in the third trimester.[8]

Pharmaceuticals: They are supposed to stimulate weight loss and insulin sensitivity. However, there are no studies regarding safety or efficacy of these drugs. Supplementation with oral 24 - 96 mg/day of levothyroxine (Thyro L®, Lloyd Inc, Shenandoah, IA) is recommended if the mare loses weight very slowly, or cannot reach the desired weight, cannot exercise due to laminitis or, her hyperinsulinemia persists even after reaching the desired weight.[6,37] This approach is not recommended in pregnant mares since it may produce congenital goiter in the foal, as reported in humans.[38] In humans, metabolic syndrome is successfully treated with biguanide (Metformin) that is also safe to use in pregnant women. However, this drug is not well absorbed in horses, and that there is low bioavailability and minimal systemic effects on ID.[39] The exact mechanism of action in horse is not known, but it may decrease the absorption of glucose at the level of enterocytes. Recommended dose is oral 15 - 30 mg/kg (twice or thrice a day) 30 minutes before each meal.

## Conclusion

Pituitary pars intermedia dysfunction and EMS have been associated with subfertility in the mare, probably through their effects on the estrous cycle and ovarian follicular dynamics. However, confounding factors are age and insulin dysregulation. It is important to make an accurate diagnosis since treatment on 'a trial-and-error basis' is not without adverse consequences. Use of appropriate laboratory tests in conjunction with clinical signs is paramount in the diagnosis and management of this condition.

## Conflict of interest

None to declare.

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