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# The Equine Model to Study the Influence of Obesity and Insulin Resistance in Human Ovarian Function

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**ABSTRACT**

***Background*:** Understanding ovarian folliculogenesis is critical to the study of fertility and in the development of fertility techniques as well as contraception. Mares and women share striking similarities in ovarian folliculogenesis, and in insulin resistance and obesity syndromes. The effects of insulin resistance and obesity on follicular development and the surrounding endocrinology and genes in mares may shed light on the causes and effects of metabolic and reproductive disorders such as polycystic ovarian syndrome (PCOS) in women using an appropriate research model.
***Review*:** Studies in laboratory animals (e.g. mice and rats) have demonstrated that, in general, these animals are not good research models to study ovarian function in women because of the remarkable physiological differences in ovarian folliculogenesis and luteogenesis/luteolysis. Therefore, there is an urgent need for the development of *in vivo* (whole animal) research models using species (e.g. mare and cow) that have a similar physiological ovarian function to the woman. The use of such models will allow for an understanding of the causes and effects of different pathological reproductive processes involved in diseases. Several studies from our group and others have shown that nowadays the mare seems to be one, if not the best, animal model to study ovarian function in women. In addition, the recent elucidation of the equine genome has provided evidence of the high gene conservation and similar chromosomal order of this species to humans, reinforcing the importance of this species for comparative studies with humans.
***Conclusions*:** The use of farm animal models is also relevant for agricultural and biomedical research because this might help to improve reproductive efficiency and health in animals and humans, as well as the quality of products (e.g. oocytes, embryos, etc.). This review will focus on the potential use of the mare as a model to study the effects of obesity and insulin resistance syndromes on ovarian function in women.

**Keywords:** insulin resistance, obesity, ovary, mares, women.

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## I. INTRODUCTION

The growing interest in research in insulin resistance and obesity is centered on the recognition that both conditions play a role in the pathogenesis of several adverse metabolic disturbances in humans and domestic animals, including horses. Reproductive disorders such as anovulation and Polycystic Ovarian Syndrome have been associated with the action of insulin on its target tissues. The striking similarities between mares and women in follicular dynamics and hormonal changes during the interovulatory interval and the ovulatory follicular wave [7,37,49,50,52,77], in ultrasonographic changes of the preovulatory follicle before ovulation [36,40-42,75,82], in reproductive aging processes [15,54,55], and in reproductive dysfunction, including development of HAFs (hemorrhagic anovulatory follicles) or LUFs (luteinized unruptured follicles [17,22,23,65,74,101]), demonstrate the importance of the mare as a relevant experimental model for the study of ovarian function in women.

Among the agriculturally important domestic species studied so far, the equine is the one with the highest incidence of metabolic syndrome (i.e. Equine Metabolic Syndrome). In addition, similar to what has been found in women, insulin resistance and obesity syndromes in mares have become serious and frequent problems for today's farm operations, associated or not with metabolic syndromes. Reproductive disorders at the ovarian level in women with these syndromes are very common and are usually a serious infertility problem (*i.e.* Polycystic Ovarian Syndrome). Few reproductive studies in mares with these syndromes have been reported. However, the few results available demonstrate that mares' ovarian function and estrous cycle also seem to be altered. The importance of the mare over other domestic species in the study of obesity and insulin resistance is warranted because of its monovular condition, significant similarity in ovarian folliculogenesis and metabolic disturbs (*e.g.* Equine Metabolic Syndrome) with humans, and the relative ease with which follicles are imaged, tracked, and targeted (*e.g.* follicle sampling, aspiration, injection) using transrectal and transvaginal ultrasonography (reviewed in [36,37]). Moreover, the similarities existing between mares and women in insulin resistance and obesity syndromes associated with reproductive disturbances during the estrous/menstrual cycle encourage the use of the equine model to test hypotheses using invasive technologies and may provide additional information that can also be considered for other farm animal species and in human clinical medicine. Additionally, studies in this area shall have beneficial impacts in agriculture allowing: (1) a better understanding of Equine Metabolic Syndrome; (2) improvement in the control and treatment of obese horses (males and females), cows, and other farm animals that might suffer from these syndromes; and (3) improvement on ovarian cyclicity, oocyte and embryo production, and fertility rates in farm animals with metabolic syndrome, insulin resistance and obesity. This review will focus on the similarities existing between mares and women in insulin resistance and obesity syndromes associated with reproductive disturbances during the estrous/menstrual cycle.

## II. GENERAL ASPECTS OF FOLLICLE DEVELOPMENT IN THE MARE

Ovarian follicle development in the mare is characterized by waves of several follicles that emerge and initially grow in synchrony (reviewed in [35,36]). Various numbers and types of follicular waves develop during an equine interovulatory interval (IOI) [47]. In a major wave, the largest follicle attains the diameter of a dominant follicle (=28-30 mm), whereas in minor waves, the largest follicle does not become dominant. The ovulatory wave emerges midway during an IOI of 21-24 days. After emergence at 6 mm [39], the follicles of a wave develop in a common-growth phase for several days [38]. At the end of the common-growth phase, a distinctive change in growth rates begins. This process is called deviation, and in mares it begins when the diameter of the two largest follicles are, on average, 22.5 mm and 19.0 mm [39,43,50]. The deviation in growth rates between the future dominant and subordinate follicles is a key event during the selection of the ovulatory follicle. After deviation ("follicle selection"), the developing dominant follicle maintains a constant growth rate until 1 or 2 days before ovulation [41] and the remaining follicles (subordinate follicles) grow at a reduced rate or establish a plateau phase and then regress.

The ovulatory waves, as well as major anovulatory waves and minor waves, originate from the stimulation of a follicle-stimulating hormone (FSH) surge, which reaches a peak when the largest follicle is about 13 mm [39]. The initial decline in the FSH surge appears to be a function of inhibin. Circulating estradiol does not begin to increase until about 2 days after the FSH peak or about 1 day before the beginning of deviation. Concentrations of luteinizing hormone (LH) during the ovulatory LH surge reach a transient plateau encompassing deviation of the ovulatory wave. The intrafollicular concentrations of estradiol, insulin-like growth factor 1 (IGF1), inhibin-A, and activin-A increase differentially in the future dominant follicle versus the future subordinate follicles about 1 day before the beginning of diameter deviation. These factors may be enablers for differentially enhancing the FSH and LH responsiveness of the future dominant follicle.

The characteristics and the intrafollicular and systemic hormonal events associated with the beginning of deviation have been reviewed for mares [50], mares and heifers [12,48], and have been compared between mares and women [49,52]. Briefly, dramatic changes in the IGF system lead to increased free IGF1 in the most developed follicle before the beginning of diameter deviation and play a crucial role in the events that lead to deviation in both horses and cattle. Estradiol and LH receptors also play a role, at least in cattle. The intrafollicular events prepare the selected follicle for the decreasing availability of FSH from the wave stimulating FSH surge and increasing availability of LH. Other follicles of the wave have a capability for future dominance similar to that of the largest follicle but do not have adequate time to attain the required preparatory stage. In this regard, the essence of deviation is a close two-way functional coupling between FSH and products of the follicles (inhibin, estradiol).

### III. SIMILARITIES IN FOLLICLE DEVELOPMENT AND SYSTEMIC HORMONES BETWEEN MARES AND WOMEN

As mentioned above, mares and women share striking similarities regarding ovarian folliculo genesis. Some of the similarities between the two species are: (1) emergence of the ovulatory follicle before the first subordinate follicle; (2) length of intervals between sequential emergence of follicles within a wave; (3) interval from emergence to deviation of the ovulatory follicle; (4) percentage growth of follicles during the common growth phase; (5) deviation in growth rates between the two largest follicles of a major wave (Figure 1); (6) percentage of a predeviation follicle that regressed an average of 1 day before deviation; (7) consistency of a mare:woman diameter ratio (approximately 2:1) of the ovulatory follicle; (8) prevalence of major anovulatory follicular waves during an interovulatory interval; (9) presence of major and minor follicular waves (Figure 2); (10) peak of the wave-stimulating FSH surge on the day of emergence of the follicular wave; (11) occurrence of follicle deviation during a decline in FSH concentrations; (12) estradiol and progesterone profiles during the periovulatory period; (13) long duration of the follicular phase; (14) increase in the length of the follicular phase and IOI with aging; (15) changes in concentrations of gonadotropins with aging; (16) cessation of follicular activity with aging; (17) preovulatory follicle characteristics; (18) anovulatory disturbs such as HAFs and LUFs [7,15,23,35-37,40,41,49,52,55]. Similar follicle dynamics between mares and women indicate the mare may be a useful experimental model for the study of folliculogenesis in women, with the advantage of larger follicle size.

It is still not clear whether or not obesity, insulin resistance, abnormal ovarian morphology and function, and/or altered endocrinology are causes or merely symptoms of ovarian disturbs in women with PCOS and in mares. The scientific literature pertaining to PCOS is vast and commonly based on clinical research, although some studies have focused on *in*

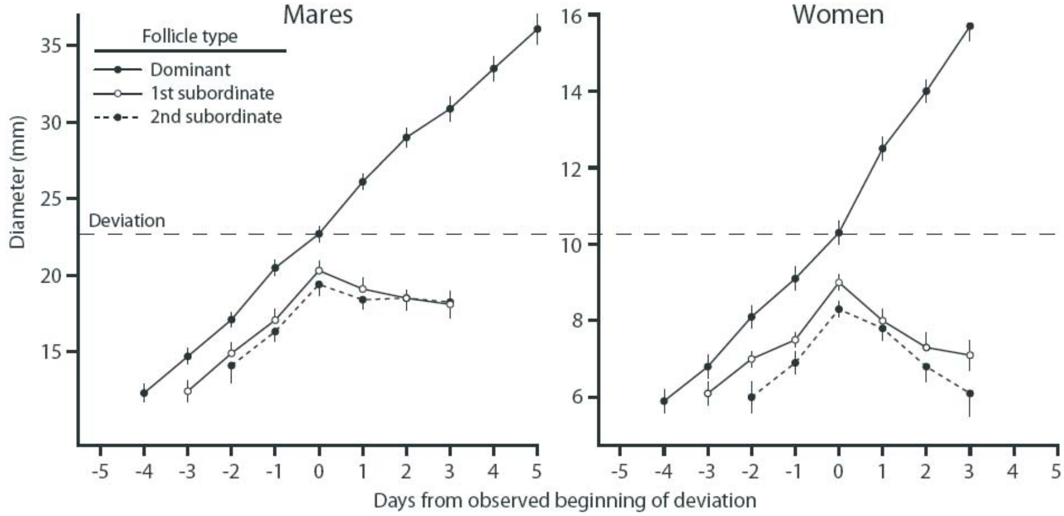


**Figure 1.** Means (±SEM) for diameters of four follicle types during ovulatory waves in 27 mares and 25 women. Numbers of waves for mares and women, respectively, with various follicle types were: dominant and first subordinate follicles (27 and 25), second subordinate follicles (14 and 9), and predeviation follicles (10 and 12). Dominant and subordinate follicles are centralized among individuals to the beginning of deviation (Day 0), and the predeviation follicle is centralized to the mean day at the beginning of its regression (Day 21). Adapted from [52].

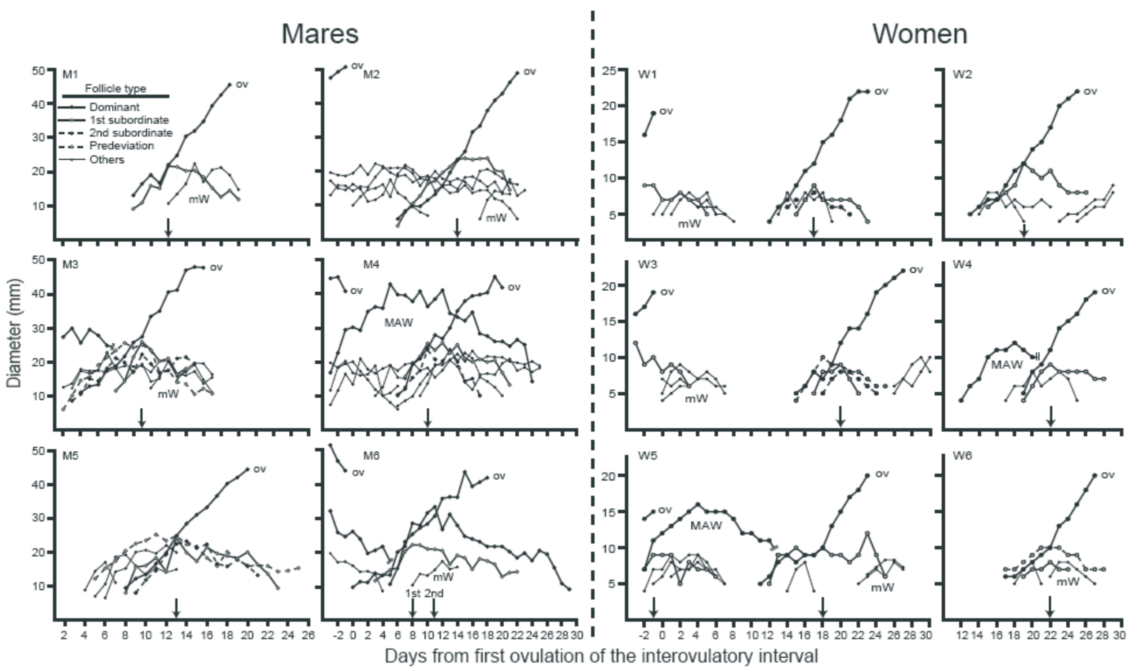


**Figure 2.** Comparative ovarian follicular wave patterns between mares and women. Presented on the left panel are individual follicle profiles in mares for three ovulatory waves (M1, M3, M5) and three interovulatory intervals (M2, M4, M6). Emerging follicles were not detected earlier in the interovulatory interval in M1, M3, and M5. Follicles of the ovulatory wave intermingled with regressing follicles from a previous ovulatory wave (M3, M6), a major anovulatory wave (M4), and static follicles from a previous wave (M2). A predeviation follicle (M3, M5) and a late regressing follicle (M4) are shown. Presented on the right panel are individual follicle profiles in women for three interovulatory intervals (W1, W3, W5) and three ovulatory waves (W2, W4, W6). Emerging follicles were not detected before 12 days postovulation in W2, W4, and W6. Note the predeviation follicle (W3, W6), a second-largest subordinate follicle (W1, W3), and the transiently arrested growth of the follicles during 13–18 days postovulation (W5). MAW = major anovulatory wave. mW = minor wave. OV = ovulation. An arrow indicates the beginning of deviation. Adapted from [52].

*vitro* culture and experimentation with follicular cells from PCOS-affected women. Using an animal model for controlled experiments to investigate the interrelationships between ovarian follicular development, reproductive endocrinology, and metabolic status at the whole-animal level and down to the cellular level may shed new light on some of the causes and effects of PCOS in women and ovarian disturbs in farm animals. In this regard, there is strong evidence that an appropriate animal model for this purpose can be the mare.

## IV. OBESITY

In domestic farm animals, research with an emphasis on nutrition was mainly related to the effects of poor nutrition on productivity and reproductive performances for a very long time. However, recent investigations have demonstrated an increasing awareness of obesity as a main detrimental factor that affects the health of companion [60,108] and some domestic animals, including horses [33,86,112]. Obesity is defined as an accumulation of excessive amounts of adipose tissue in the body. Human obesity or overweight diagnosis is relatively simple, requiring only the determination of the body mass index (BMI) by measurement of height and weight; however the measuring of the circumference of the individual's waist has been suggested as an excellent indicator of visceral adiposity [72]. In the equine field, various methods have been recommended for the purpose of assessing the relative adiposity of equine patients, including the body condition score (BCS; [58]) the equine BMI [66], and the use of ultrasonography to assess subcutaneous fat thickness near the tail head [28].

Every year, the scientific community and media dedicate increasing amounts of time and resources to the health complications caused by obesity in humans, which is currently considered one of the fastest-growing epidemics throughout the world. Obesity in humans is associated with an increased risk of mortality by a number of different diseases, and usually precedes many of the acknowledged metabolic disturbances including prediabetes, diabetes and cardiovascular disease. Obesity-related deaths are expected to exceed the number of all other causes of death for preventable conditions in the near future [14]. The occurrence of obesity-related diseases has been mainly linked to the visceral (abdominal) adiposity [3,72]. In horses and ponies, the association between regional adiposity and disease risk seems to be similar to the human [16]. Obese animals usually have elevated body condition scores and enlarged fat deposits on the neck ("cresty neck"), thoracic and/or tailhead regions [16].

In parallel with human obesity, there is a growing concern about the occurrence of obesity and insulin resistance in animals. Studies have shown a prevalence of obesity of 20 to 25% in dogs [31,92,108] and 26% in cats [4]. Few studies have examined the prevalence of obesity in horse populations. An estimate of only 4.5% overweight or obese horses was reported in 1998 in the U.S. population (NAHMS). However, the accuracy of this prevalence may be questioned because it was based on owner reporting, and not from results of physical measurements. More recent studies have shown that the prevalence of overweight and obese horses has increased to 45% and 51%, in a subpopulation of pleasure riding horses in North-West Glasgow in 2005 and Virginia in 2006, respectively [100,112]. Hence, the number of obese horses has shown a strong incline over time, and the reasons by which domesticated animals develop obesity are broadly similar to those reasons that have been attributed to obesity in humans [62]. In domesticated horses, for instance, obesity develops in light of the fact that they tend to be physically inactive and are provided with rations that are excessive in terms of energy [63].

Although health problems such as heart diseases and diabetes do not occur in horses as frequently as in humans, the clinical implications of obesity are just as serious. In human obesity, the underlying mechanism contributing to metabolic dysfunction appears to be the presence of low grade systemic inflammation (initiated in adipose tissue). Obese horses and ponies are known to have a higher risk of developing laminitis, an inflammation of the sensitive laminae of the hooves [70], and a lower fertility rate with a greater risk of dystocia [105]. Furthermore, higher fat mass leads to hyperthermia and an increased incidence of recurrent exertional rhabdomyolysis (RER), or tying-up [103]. Laminitis, with a prevalence of 7% [57], and RER, with a prevalence of 5 to 10% in Thoroughbreds [73], are considered two of the most common reasons for presenting a horse for veterinary consultation [57] and for exclusion of horses from competitions [73]. All these numbers stress the impact of obesity and associated disorders on equine health and welfare.

Obesity in women is often inextricably linked with ovarian function leading to clinical reproductive manifestations such as menarche onset, subfertility and Polycystic Ovarian Syndrome (PCOS; reviewed in [89]). PCOS is one of the most common endocrine disorders in women of reproductive age [29,34]. It is characterized by hyperandrogenic chronic anovulation, and has a mean prevalence of 7-8% (range, 4-12%) in the general population [5,6,25]. Insulin resistance has been recognized as the major factor related to PCOS and also a significant contributor to its reproductive and metabolic complications [26]. The potential mechanism related to reproductive disturbs seems to involve compensatory hyperinsulinemia, which stimulates ovarian androgen production, and also increases peripheral aromatization of androgens to estrogens; these factors may alter gonadotropin secretion, subsequently altering follicular development [24]. However, the molecular mechanism involving insulin resistance in the pathophysiology of PCOS remains obscure. PCOS is characterized by a group of features with no specific diagnostic and a large diversity in its clinical presentation [61]. As the name implies, PCOS is in part identifiable by abnormal ovarian morphology [98]. In the last 30 years, the development of ultrasound as a noninvasive diagnostic tool has resulted in numerous studies that have characterized abnormal ovarian morphology associated with PCOS. The most pertinent and clinically-relevant ultrasound diagnostic criteria include the presence of =12 follicles, 2-9 mm in diameter (small- to medium-sized follicles), and an ovarian volume >10 $cm^3$ that is approximately 2-fold greater than normal ovaries [10]. In a large study of 1,741 women diagnosed with PCOS by the presence of abnormal ovarian morphology, 38% were obese and 29% and 40% had elevated serum concentrations of LH and testosterone, respectively [9]. Currently, endocrinological abnormalities such as elevated systemic LH concentrations and hyperandrogenism are used for the diagnosis of PCOS [8].

The abnormal endocrinology associated with PCOS occurs locally at the level of the ovary and systemically at the hypothalamic-pituitary-adrenal axis. The gonadotropins are differentially altered in PCOS women. Elevated serum concentrations of LH [83,99] and normal [81,99] to low [83] concentrations of FSH were reported in women suffering from PCOS. The increase in systemic LH concentrations likely augment androgen production in the thecal cells of the follicle by activation of the LH receptor, and subsequent cAMP/Protein Kinase-A signaling increases the activation of enzymes responsible for the conversion of cholesterol to androstenedione [27]. In addition, increased insulin concentrations, which are associated with insulin resistance, may stimulate the enzyme 3âHSD in follicle and adrenal cells. This enzyme, mediated by IGF and paracrine factors, converts dehydroepiandrosterone (DHEA) to androstenedione [27]. Keeping in mind that androstenedione is a precursor of testosterone in the steroidogenic pathway, the increase in steroidogenic enzyme activity is a plausible reason for the reported increased serum concentrations of both androstenedione and testosterone in women with PCOS compared to normal women [81,83,99]. Despite increases in concentrations of androstenedione [83,99] and testosterone [80,83,99], serum estradiol concentrations in PCOS women were not different from normal women. A regulator of testosterone and estradiol activity and transport, the sex hormone binding globulin (SHBG), was reduced in blood samples of PCOS women compared to normal women [81].

In humans and rodents, some of the metabolic changes associated with obesity are the increase in free fatty acids (FFAs), altered adipokine production by adipose tissues, and elevated inflammatory cytokine concentration within the blood [91]. One explanation of the link between obesity and insulin resistance is the higher FFA concentrations in insulin-sensitive tissues that can result in a process referred to as *lipotoxicity* that can cause insulin resistance [97]. Adipokines are hormones produced by adipocytes that have local (paracrine) and remote (endocrine) effects on tissues. Leptin and adiponectin are the best known adipokines, and obesity has been associated with higher plasma leptin concentrations and lower plasma adiponectin concentrations in horses [67]. Recent studies have focused on leptin, which acts on the hypothalamus as a mediator of nutritional effects on reproductive function (reviewed in [20,110]. Low systemic leptin concentrations are associated with poor body condition in humans, ruminants, and horses [18,32,46,87]. In mares, systemic concentrations of leptin were lower in younger (<5 yr of age) than in older (>10 yr of age) animals [13,45]. However, lower circulating concentrations were also observed during the winter, independent of age or nutritional status and body condition [32,45]. Long-term dietary restriction resulted in both lower body condition scores and lower systemic leptin concentrations [45]. A recent study [44] demonstrated that mares submitted to a dietary short-term feed restriction presented decreased systemic and intrafollicular concentrations of leptin and tended to have greater intrafollicular concentrations of inhibin-A and VEGF. In follicular fluid, the concentration of leptin was positively correlated with free IGF1, and both intrafollicular leptin and free IGF1 were positively correlated with body condition score. Blood flow of the preovulatory follicular wall was greater in the restricted group and combined for both groups (control and treated) blood flow was negatively correlated with intrafollicular concentrations of leptin. Therefore, studies are necessary on the relationship of obesity and insulin resistance with the systemic and intrafollicular concentrations of insulin and IGF and its binding proteins as well as angiogenic factors (e.g. VEGF, NO).

## V. INSULIN RESISTANCE

Besides being involved in the process of carbohydrate metabolism, insulin takes part in an extensive range of physiologic processes, including stimulation of fatty acid and triglyceride synthesis in the liver and adipose tissue, inhibition of lipolysis, enhancement of protein anabolism, cell growth and survival, regulation of vascular endothelial function, and anti-inflammatory effects [56,109]. In ovarian function, insulin has been shown to have a major role, including the regulation of ovarian steroidogenesis, follicular development, and granulosa cell proliferation [1,84,111]. The reduction in sensitivity to the biological actions of insulin affects not only glucose metabolism, but also all aspects of insulin action. In this regard, the insulin-like growth factor system (e.g. IGF1 and IGF binding proteins) plays a crucial role in the ovary in processes of follicle selection and dominance in mares [12,51,53] as discussed before.

Insulin resistance or decreased insulin sensitivity is defined as the decreased biological response of cells to the action of insulin in transporting glucose from the bloodstream into the target tissue (e.g. liver, muscle and adipose tissue). It is important to emphasize that insulin resistance is not a disease itself, but a physiological status. The reduced insulin sensitivity and resulting hyperglycemia are usually compensated by increased insulin production by the pancreatic β-cells or by an increase in the glucose-mediated glucose disposal [69]. Insulin resistance has been well documented in humans [26,30] and horses [59,102]. In humans, obesity has been related to the development of insulin resistance. The link between obesity and insulin resistance has been suggested to be a consequence of inflammation in adipose tissue and the liver and the accumulation of by-products of nutritional overload (e.g. diacyglycerol) in insulin-sensitive tissues [78]. In horses, Vick *et al.* [104] reported associations between obesity and blood mRNA expression of tumor necrosis factor alpha (TNFα) and interleukin (IL-1β), suggesting that systemic inflammation may be involved in the development of insulin resistance.

Insulin resistance has been shown to play a central role in some health problems in humans [94] and also in horses [21,106]. In horses, insulin resistance has been associated with pathogenesis of diseases such as laminitis, pituitary adenoma, hyperlipidemia, osteochondritis, and also seems to influence negatively reproductive efficiency [105] and probably exercise [64].

Original research pertaining to the effects of obesity and insulin resistance on reproductive cyclicity and fertility in the mare is sparse, and the data from this limited amount of research have yielded no solid conclusions. Kubiak *et al.* [71] reported that there was no difference in the intervals from parturition to first ovulation and first to second ovulation and first cycle conception rate between postpartum mares fed to obesity and control mares. However, the results of the previous data are questionable considering the low numbers of mares used per experimental group. A few studies have reported on the effects of obesity and/or insulin resistance on the estrous cycle. Induction of transient insulin resistance via infusion of a heparinized lipid emulsion lengthened the interovulatory interval and reduced peak plasma progesterone concentrations during the luteal phase, but did not affect mean LH concentrations compared to control mares [96]. Vick *et al.* [105] reported that obese mares (BCS =7) with reduced insulin sensitivity had longer interovulatory intervals and luteal phases compared to feed-restricted mares; however, Metformin (anti-diabetic drug) treatment of obese mares did not alter the interovulatory interval or luteal phase. Daily intravenous infusion of insulin during the mid to late luteal phase (7-17 days after ovulation) did not alter the length of the estrous cycle, corpus luteum area, or plasma LH concentrations, but tended to lower plasma progesterone concentrations [90]. Surprisingly, to our knowledge, the effects of insulin resistance and obesity on the preovulatory follicular wave have not been studied in detail in mares or women.

### 5.1 Molecular mechanisms and defects of insulin activity in target tissues

Insulin signaling involves a wide number of substances and affects both transport and subsequent utilization of glucose, especially in relation to hexokinase, glycogen synthase, and other key enzymes in glucose and lipid metabolism [95]. A cascade of intracellular events is initiated when insulin binds and activates its receptor at the cell-surface, resulting in the tyrosine phosphorylation of several substrates, including the insulin receptor substrate. Insulin resistance may result from a reduction in the density of receptors, malfunction of insulin receptors, defective internal signaling pathways, and interference with the translocation or function of glucose transporter type 4 (GLUT-4) proteins [68]. Decreased levels of insulin receptors -1 and -2 tyrosine phosphorylation [19,88,107] and reduced numbers of insulin receptors [19] in adipose tissue from insulin resistant PCOS patients have been shown. Also, the expression of GLUT-4 was significantly decreased in PCOS adipocytes [93].

The presence of insulin receptors in both stromal and follicular compartments of the human ovary [85] and the ability of insulin to stimulate steroidogenesis in ovarian cells in vitro [11] has established the ovary as a target organ for insulin activity. Hyperinsulinemia can potentiate gonadotropin-stimulated steroidogenesis in granulosa and theca cells by increasing the low-density lipoprotein (LDL) receptor, 3β-hydroxysteroid dehydrogenase, 17α-hydroxylase, and 17,20 lyase expression [76,79,113]. Recently, the insulin signaling pathways on ovaries from obese and insulin resistant rats were studied [2]. The ovaries from obese and insulin resistant rats showed a reduction in the insulin receptor substract/phosphatidylinositol 3-kinase/AKT intracellular pathway, associated with an increase in FOXO3a, IL1B, and TNFα protein expression [2]. Similarly TNFα and IL-1â were elevated in obese insulin resistant mares [104].

### 5.2 Insulin resistance detection

A variety of tests, each with their own pros and cons, are available to test insulin resistance in human and veterinary medicine. There seems to be a consensus that the *hyperinsulinemic – euglycemic clamp procedure* is considered the “gold standard” for assessment of insulin sensitivity. The clamp procedure involves maintaining a constant supraphysiological insulin concentration while differentially controlling glucose infusions to maintain a steady state of glycemia. The greater the rate of glucose infusion, the more sensitive the subject is to insulin. A lower infusion rate indicates decreased insulin sensitivity (or higher insulin resistance). Single-point measurements of insulin and glucose during fasting states are convenient and practical; however, they do not offer the amount of information provided by the clamp or minimal model analysis [102]. The minimal model employs a frequently-sampled i.v. glucose tolerance test (FSIGT) with an i.v. insulin dose 20 min after the glucose bolus. Based on the differential effects of glucose on insulin and vice versa, the following endpoints are calculated [102]: 1) insulin sensitivity of tissue (SI); 2) difference between insulin-stimulated and insulin-independent glucose clearance (Sg); 3) pancreatic â-cell response to glucose (AIRg); and 4) comparison of insulin sensitivity to insulin response (DI).

## VI. CONCLUSIONS

Mares and women share striking similarities in ovarian folliculogenesis, and in insulin resistance and obesity syndromes. The effects of insulin resistance and obesity on follicular development and the surrounding endocrinology and genes in mares may shed light on the causes and effects of metabolic and reproductive disorders such as polycystic ovarian syndrome (PCOS) and anovulatory processes in women. Understanding the mechanisms related to this effect might help to improve reproductive efficiency and health in animals and humans, as well as the quality of products (e.g. oocytes, embryos, etc.). Moreover, the similarities existing between mares and women in insulin resistance and obesity syndromes associated to reproductive disturbances during the estrous/menstrual cycle encourage the use of the equine model to test hypotheses using invasive technologies and may provide additional information that can also be considered for other farm animal species and in human clinical medicine.

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