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# **The effect of mare obesity and endocrine function on foal birthweight in Thoroughbreds**

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No competing interests have been declared.

## **Ethical Animal Research**

Owner consent was obtained for all mares enrolled in the study

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## **Authorship**

The study design was established by N. Menzies-Gow, C. Marr, S. Smith and C. Dunnett, study execution and data analysis were done by S. Smith, data interpretation was done by S. Smith, N.Menzies-Gow, C. Marr and C. Dunnett. The preparation of the manuscript was done by S. Smith. All authors have approved the final version of the manuscript.

**Background:** Birthweight of Thoroughbred foals has increased in recent years. It is unknown whether this is associated with increased broodmare obesity or endocrine dysfunction.

**Objectives:** To determine insulin, leptin and triglyceride concentrations in Thoroughbred mares throughout gestation and investigate their association with obesity and foal birthweight.

**Study design:** Cohort study.

**Methods:** 66 mares were included from 40d post-breeding. Body condition score (BCS), weight and blood samples were obtained every 60d throughout gestation. Serum/plasma insulin, leptin and triglyceride concentrations and foal birthweight were recorded. Associations between hormone/triglyceride concentration with BCS, stage of gestation and birthweight were analysed using a linear mixed effects model.

**Results:** Serum insulin concentrations were greater at 1-60d (4.31uIU/ml) compared to 241-300d (3.13uIU/ml) and 61-120d (5.33uIU/ml) compared to 181-240, 241-300, 301-360d (3.78, 3.13, 3.37uIU/ml) gestation ( $p < 0.05$ ). There was no significant hyperinsulinaemia and no association of insulin concentration with BCS. Leptin concentration was greater at 181-240d (2.28ug/l,  $p < 0.0001$ ) compared to all other time points and correlated with BCS ( $p < 0.0003$ ). Triglyceride concentration was greater at 241-300d (0.245mmol,  $p < 0.02$ ) compared to earlier time points but was not associated with BCS. Foal birthweight was weakly positively correlated with BCS ( $r = 0.13$ ,  $p < 0.001$ ) and inversely correlated with leptin concentrations at 61-120d, 241-300d gestation ( $r = -0.64$ ,  $p < 0.05$ ).

**Main Limitations:** Reduction in sample size over the study and tight clustering of BCS.

**Conclusions:** Mare BCS correlated with foal birthweight; obese mares had heavier foals. Significant hyperinsulinaemia was not identified in this population. Increased leptin concentration in early and late gestation was associated with decreased foal birthweight. Further work is required to establish whether leptin concentration in late gestation could predict foal birthweight.

## Introduction

The birthweight of Thoroughbred foals has increased in recent decades [1-3] and there is a clinical impression that maternal obesity is leading to increased foal size. In other mammalian species maternal obesity leads to increased foetal size [4-7]. However, the effects of maternal obesity in horses are not fully understood and studies suggest instead that maternal overnutrition either does not alter foal size or has no detrimental effects [8-10]. In humans and experimental animal studies, maternal obesity and resultant increased offspring birthweight are associated with life-long adverse health effects [11,12]. There is limited information about the impact of increased foal size on equine health but it has been associated with an increased incidence of orthopaedic disease [3,13].

A physiological decrease in insulin sensitivity develops through the course of gestation in humans and overweight/ obese mothers are at increased risk of metabolic dysregulation during pregnancy [14]. Hyperinsulinaemia and decreased insulin sensitivity have also been identified in pregnant mares [15-17] and metabolic dysregulation including hyperinsulinaemia, hyperleptinaemia and hypertriglyceridaemia has been associated with obesity in horses [18-20]. However the combined effect of gestation and obesity on equine maternal endocrine function and foetal size is unknown.

The aims of this study were (1) to characterise insulin, leptin and triglyceride concentrations in Thoroughbred mares over the course of gestation and (2) to investigate the association of maternal obesity with foal birthweight.

## **Materials and methods**

### *Animals*

The study was performed with approval of the “*details to be provided*” Ethics and Welfare Committee. Sample size calculation showed that  $\geq 57$  mares would be required to generate a population with insulin dysfunction in 10% of the mares with 80% power and  $\alpha - 0.05$ . The prevalence of hyperinsulinaemia in the equine population has been reported to range from 10-38% [21,22]; a

value at the bottom end of this range was chosen as there is breed-related variation in insulin dysregulation [23]. A single stud farm was chosen for sampling convenience. All Thoroughbred mares resident at the farm in March 2013 were considered eligible for inclusion. Mares were enrolled in the study when they were confirmed pregnant by ultrasonographic evaluation at 40 days after mating. Diet, exercise and veterinary care of the mares were managed according to the standard protocol of the stud farm. In the first 7-8 months mares were at pasture 24h/day in groups assigned by the stud farm according to parity and stage of gestation. At this stage mares were fed approximately 1kg/24h of a high protein, low starch concentrate feed. From the beginning of the final third of gestation mares were stabled at night with unrestricted access to pasture during the day and were fed 3-5kg of a higher starch concentrate feed.

#### *Sample collection*

Mares were examined every eight weeks throughout gestation starting between 40-60 days after mating. Mares were examined over the same two-day period every 60 days for sampling convenience. Mares were stabled for 12 hours prior to examination. They were weighed on a weigh-bridge. Body condition score (BCS) was recorded by a single observer; six anatomic sites (neck, withers, loin, tailhead, ribs, shoulder) were graded 1-9 and the mean of these values taken (Kohnke, 1992).  $BCS \geq 7/9$  was defined as obese. Season was defined by dividing mares into those foaling January-March or April-June. Feed was withheld for approximately 6 hours prior to blood sampling and blood samples were obtained by jugular venipuncture between 0700 and 1000h. Blood for serum insulin concentration measurement was collected into plain blood tubes and allowed to clot at 37°C for at least 20 minutes. The remaining samples were collected into blood tubes containing fluoride oxalate for glucose concentration measurement, heparin for triglyceride concentration measurement and EDTA for leptin concentration measurement. Samples were immediately placed on ice and serum/plasma was separated within four hours of collection. All samples were centrifuged for ten minutes (3000 x g, 4°C). The supernatant was collected and stored at -80°C until analysis.

Parturition was attended by stud personnel in all mares. Foals were weighed within 24 hours of birth, at one week and one month after birth. Foal weight gain was calculated as a percentage of birthweight. Foals were weighed using weigh scales which were serviced and re-calibrated annually, or sooner if a fault was identified. Foals were weighed according to normal stud practice; as young foals normally suckle when woken/ disturbed, in the majority of foals weights were obtained soon after suckling.

### *Endocrine assays*

Glucose concentration was measured using a colorimetric assay<sup>A</sup> and insulin and leptin concentrations were measured using radioimmunoassay kits<sup>B,C</sup> validated for equine samples (Borer-Wier et al., 2012, Fitzgerald and McManus 2000). Triglyceride concentrations were measured by a commercial laboratory<sup>D</sup>.

Hyperglycaemia was defined as fasting glucose concentration  $>11\text{mmol/l}$  [24], hyperinsulinaemia as fasting serum insulin concentration  $>20\mu\text{IU/ml}$  [25], hyperleptinaemia as  $>7.3\text{ng/ml}$  [26] and hypertriglyceridemia as  $>1.13\text{mmol/l}$  [27].

### *Statistical analysis*

Data was tested for normality using a Shapiro-Wilk normality test and are expressed as mean ( $\pm$  S.D.) or median ( $\pm$  IQR) as appropriate.. Outcomes were assessed in separate models (model 1 - insulin concentration, model 2 - leptin concentration, model 3 - triglyceride concentration, model 4 - body condition score). A linear mixed effects analysis was performed to look for associations between each of the outcome and explanatory variables. Explanatory variables (fixed effects) assessed were mare body condition score,, weight, time in gestation and age which were continuous variables and obesity, parity and season which were categorical variables. Variables were assessed for inclusion in the model using univariable analysis. Random effect was the mares throughout. Backward step-down selection was performed to create the final models. Separate models were then created to assess

foal birthweight (model 5) and foal weight gain (model 6) as outcomes using the same method but also including insulin, leptin and triglyceride concentration as explanatory variables. Post-hoc comparison was performed with a Tukey's post-hoc comparison.

A Pearson correlation coefficient for parametric data or Spearman's rank correlation coefficient for non-parametric data was calculated between insulin, leptin or triglyceride concentration and BCS at each different time point and birthweight. Tests were performed using GraphPad Prism<sup>E</sup> and R statistical software<sup>F</sup>. Results were considered statistically significant if  $P \leq 0.05$ .

## **Results**

### *Study population*

66 mares were enrolled in the study, with an average of 44 mares seen at each time point; as not all mares were available for examination at every visit. The average age was 10 years  $\pm$  3.5 years, the median parity was 3 (2-4) with 8 maiden mares. Median BCS of mares over gestation was 7 (6-7) and there was no association of parity with BCS. The mares were in foal to 15 different stallions with no stallion covering more than 5 mares in this population.

### *Endocrine assays*

Serum glucose concentration did not change significantly over the course of gestation and no mares were hyperglycemic at any time point (data not shown).

Model 1 identified that serum insulin concentration was significantly ( $p < 0.05$ ) greater at 1-60 days gestation ( $5.27 \mu\text{IU/ml} \pm 0.33$ ) compared to 241-300 days ( $3.39 \mu\text{IU/ml} \pm 0.24$ ) and at 61-120 days ( $6.671 \mu\text{IU/ml} \pm 0.76$ ) compared to 181-240, 241-300 and 301-360 days (4.47, 3.39,  $3.89 \mu\text{IU/ml}$ ; figure 1). Hyperinsulinemia was identified in 3/66 mares and only at a single time point in each animal (61-120 days,  $n=2$  and 301-360 days,  $n=1$ ).

Model 2 identified that plasma leptin concentration was significantly ( $p=0.0001$ ) greater at 181-240 days ( $2.90\mu\text{g/l} \pm 0.27$ ) compared to 1-60, 61-120 days, 241-300 and 301-360 days (2.01, 2.13, 2.56,  $2.03\mu\text{g/l}$ ; figure 2). No horses were hyperleptinaemic at any time point.

Model 3 identified that plasma triglyceride concentration was significantly ( $p = 0.02$ ) greater at 241-300 days ( $0.38\text{mmol} \pm 0.09$ ) compared to 1-60, 61-120, 121-180 and 181-240 days (0.14, 0.16, 0.18,  $0.22\text{mmol/l}$ ; figure 3). One mare was hypertriglyceridaemic on a single occasion between 301-360 days.

### *Obesity*

Mean weight at enrollment into the study was  $579 \pm 48.0\text{kg}$  and 48/66 mares were obese. Model 4 identified that weight was significantly greater at 181-300 compared to 1-60 days ( $p < 0.05$ ) (figure 4). Throughout the study, BCS was determined on 262 occasions and was  $\geq 7/9$  on 143 occasions. A significantly ( $p=0.001$ ) smaller proportion of mares (39%) were obese at 301-360 days compared to all other time points (63% at 1-60 days, 47% at 61-120 days, 64% at 121-180 days, 44% at 181-240 days, 58% at 241-300 days).

Model 4 also showed that there was no association between BCS and insulin or triglyceride concentration. However there was a significant positive correlation between BCS and leptin concentration ( $p = 0.0003$ ,  $r=0.29$ ). The linear mixed effects models showed no significant association of BCS, insulin, leptin or triglyceride concentration with season, age or parity.

### *Foals*

Average foal weight ( $n=35$ ) was  $57\text{kg}$  (range 45-69kg) with 4 foals in at or below the 10<sup>th</sup> centile ( $52.4\text{kg}$ ) and 4 foals at or above the 90<sup>th</sup> centile ( $65.2\text{kg}$ ). Model 5 showed that average mare BCS was associated with foal birthweight throughout gestation ( $p = 0.001$ ) (figure 5) and a Spearman's



rank correlation showed a positive correlation of average mare BCS with foal birthweight ( $p = 0.001$ ,  $r = 0.13$ ). Model 5 also showed that there was an association of foal birthweight with leptin concentration ( $p=0.01$ ). There was a negative correlation of foal birthweight with leptin concentration at 61-120 days ( $p = 0.02$ ,  $r = -0.36$ ) and 241-300 days gestation ( $p = 0.005$ ,  $r = -0.64$ ).

Model 6 showed that there was no significant association of foal weight gain with average mare BCS, hormone or triglyceride concentration.

## **Discussion**

A high prevalence of obesity, 55%, was identified in this population of Thoroughbred mares from one farm, which is similar to that seen in the the UK pleasure horse population [28,29]. No previous data are available to assess whether obesity amongst broodmares is increasing as it is in many other equine and non-equine populations [28,30]. The changes in the percentage of obese mares over the course of gestation appear to be synchronised with periods of metabolic stress. Obesity levels reduced at 61-120d, 181-240d and 301-360d around the times of peak lactation [31], maximal foetal growth [32] and late gestation/ foaling when there may be a voluntary reduction in feed intake.

Both pregnancy and obesity in horses have previously been associated with hyperinsulinaemia and hyperleptinaemia [15-20,33] ; neither of which were identified in this study despite a high prevalence of obesity. Breed related differences in insulin dysregulation have been identified [23] and Thoroughbred horses are perceived to be less susceptible to insulin dysregulation and hyperinsulinemia. It should be acknowledged that the absence of fasting hyperinsulinemia does not exclude the possibility of insulin dysfunction as dynamic testing of insulin function has been shown to more accurately identify insulin dysfunction [34-36]. However repeated dynamic testing was not possible in pregnant mares on a commercial stud farm.

Insulin concentrations were significantly greater in the first trimester compared to later in gestation, which is in agreement with previous studies which identified decreasing basal insulin concentrations towards term [15,17]. Decreased pancreatic  $\beta$  cell sensitivity has been shown in late gestation which may divert available glucose towards to the fetus [15]. In contrast, in humans it is normal to see an approximate doubling of basal insulin concentration in late pregnancy [37] which is thought to be a response to increasing insulin resistance allowing increased glucose provision to the foetus.

There was a positive association of leptin concentration with both mare BCS and foal birthweight. Leptin concentration has been shown in non-pregnant equids to increase with increasing body condition score [18,20,33] and to alter the transport capacity of the placenta in other species [38]. It is therefore surprising that there was a negative correlation of leptin concentration in early and late gestation with birthweight with high leptin concentration at these times associated with a lower birthweight. Studies in overweight/ obese women have shown that the rate of change of leptin concentration in the second half of gestation is associated with reduced birthweight but that this effect is not seen in normal-weight women [39] and so there may be an impact of changing obesity levels on birthweight however it was beyond the scope of this study to analyse this impact. The significantly higher leptin concentration in mid-gestation than early or late gestation is different from a previous report in which leptin concentration increased over the course of gestation [40]. The difference between the two studies may be due to different management practices and therefore obesity levels, with the mares in this study under much more intensive management. In average weight humans, serum leptin concentration doubles during the course of gestation, with production and regulation by non-adipose tissue such as the placenta thought to be involved; however in obese humans serum leptin production decreases per unit adipose/ placental tissue in later gestation [39]. Thus, the changes in leptin concentration during equine gestation are different to that seen in pregnant humans.

Plasma triglyceride concentrations were significantly higher between 241-300 days gestation compared to other times, which is an expected adaptation of late gestation reflecting maternal

catabolism to provide nutrients for rapid foetal growth [41]. The fact that there was also a significantly smaller percentage of mares in the obese category in late gestation supports this. These findings highlight that increased energy reserves are mobilised by mares to meet the increased demands of late gestation and early lactation.

In this study foal birthweight was positively correlated with mare BCS. The strength of the correlation was weak which is likely to be due to the multifactorial influences on foetal growth including those such as placental function [42]. This finding is in contrast to previous studies which suggested that equine offspring size and development did not alter with maternal obesity or overnutrition [8-10]. In other mammalian species maternal obesity is associated with increased birthweight as obesity-associated metabolic alterations provide increased nutrition to the foetus [43]. In the present study, mares were fed by the stud aiming to maintain a stable BCS, whereas in all previous studies mares of normal BCS have been fed to induce obesity during gestation [8,44]. Obesity maintained throughout gestation may have a different impact on foetal growth compared to obesity developed during gestation as it has recently been suggested that the metabolic environment in early gestation plays a crucial role in developmental programming [45].

Foal birthweight in this study was slightly higher than those reported in recent studies of Thoroughbred foals [2,3] and was more than 10% higher than those reported in earlier studies [46] [1]. Increased foal birthweight may have important consequences. In other mammalian species increased birthweight is associated with both immediate effects such as alterations in body composition with increased fat and decreased muscle proportions [47] and long-term health effects including obesity, osteoarthritis, vascular dysfunction and metabolic dysfunction including type II diabetes mellitus [11] [12]. In horses, maternal nutrition and foetal overgrowth has been associated with altered insulin function in the neonatal period [13, 44, 48,49]. In equine embryo transfer models, alteration in cardiovascular function in the postnatal period has been associated with altered foetal

growth rate [50]. Recent studies have shown a higher occurrence of non-septic orthopaedic disease and conformational abnormalities in heavier Thoroughbred foals and also an association of altered insulin dynamics and the development of osteochondrosis in yearlings from mares fed a high plane of nutrition [3,13].

The main limitations of this study were the sample size and the tight clustering of body condition score of mares. The sample size reduced as mares were lost throughout the course of the study due to loss of pregnancy, movement to another stud farm or for behavioural reasons. It is likely there are strong genetic influences on foal size which may have been affected by interbreeding in this group of Thoroughbreds. Compared to studies of humans, there was a relatively small range of foal birthweights. Human literature defines the extremes of birthweight as large for gestational age, at or above the 90<sup>th</sup> centile, and small for gestational ages, at or below the 10<sup>th</sup> centile for the stage of gestation. When those criteria were applied to this data set, the 90<sup>th</sup> centile was similar to that obtained for a large group of foals in the UK, but the 10<sup>th</sup> centile was 4kg higher [3].

In conclusion, in this population of Thoroughbreds mares, 55% were obese but the incidence of fasting hyperinsulinaemia was low (1.5%). Maternal insulin concentrations decreased over the course of gestation, whilst leptin concentrations peaked in mid gestation and triglyceride concentrations increased towards late gestation. Mare BCS correlated with foal birthweight such that obese mares had heavier foals. Increased leptin concentration in early and late gestation was associated with decreased with foal birthweight. Further work is required to establish whether leptin concentration in late gestation could be used to predict foal birthweight and whether these findings are consistent in other equine populations.

### **Manufacturers' addresses**

*A.Insulin RIA, Coat-A-Count, Siemens, Camberley, Surrey, UK.*

*B. Glucose Colormetric Assay Kit, Cayman Chemical Company, MI 28108, USA*

*C. Multi-species Leptin RIA kit, EMD Millipore Corporation, MA 01821, USA*

*D. Bell Equine Veterinary Clinic, Kent, UK.*

*E. GraphPad Prism version 6.00 for Windows, GraphPad Software, La Jolla California USA,  
[www.graphpad.com](http://www.graphpad.com)”*

*F. R Core Team (2014). R: A language and environment for statistical computing. R Foundation  
for Statistical Computing, Vienna, Austria. URL <http://www.R-project.org>*

## **Figure Legends**

*Figure 1: Mean ( $\pm$  S.E.) serum insulin concentration in Thoroughbred mares (n=66) throughout gestation.*

*Bars denote a statistically significant difference.*

*Figure 2: Mean ( $\pm$  S.E.) plasma leptin concentration in Thoroughbred mares (n=66) throughout gestation.*

*\* denote a statistically significant difference.*

*Figure 3: Mean ( $\pm$  S.E.) plasma triglyceride concentration in Thoroughbred mares (n=66) throughout gestation.*

*\* denote a statistically significant difference.*

*Figure 4: Mean ( $\pm$ S.D.) mare weight (kg) over gestation (1 = 1-60 days, 2 = 61-120 days, 3 = 121-180 days, 4 = 181-240 days, 5 = 241 - 300 days, 6 = 310-360 days)*

Figure 5: Percentage of foals in each birthweight category (50-55, 55-60, 60-65, 65-70, 70-75kg) born to mares with a mean BCS 6 (left), BCS 7 (centre) and BCS 8 (right).

Bars denote a statistically significant difference.

Figure 1

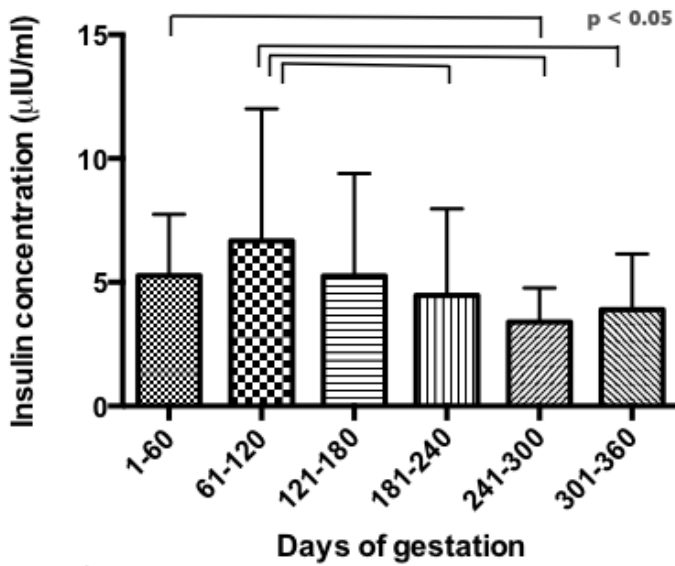


Figure 2

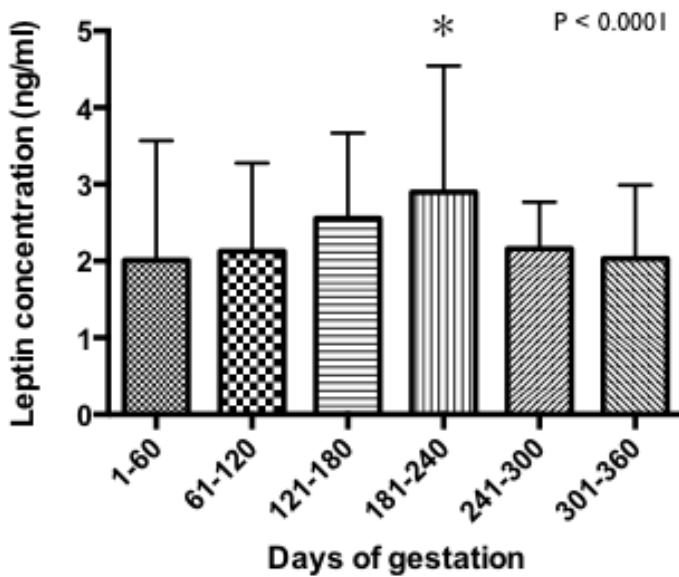


Figure 3

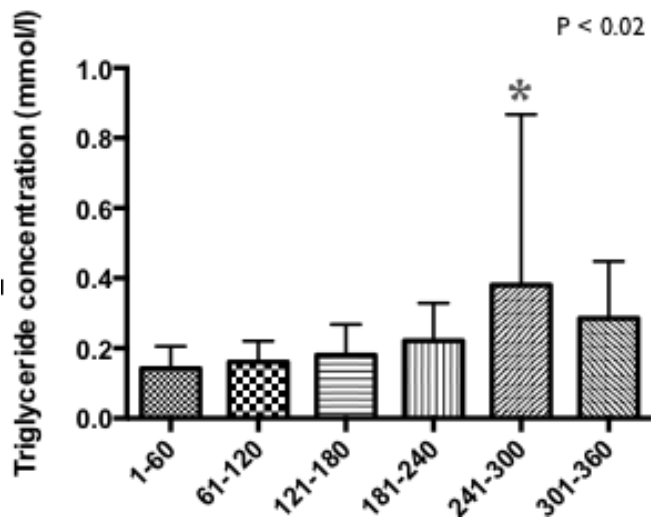


Figure 4

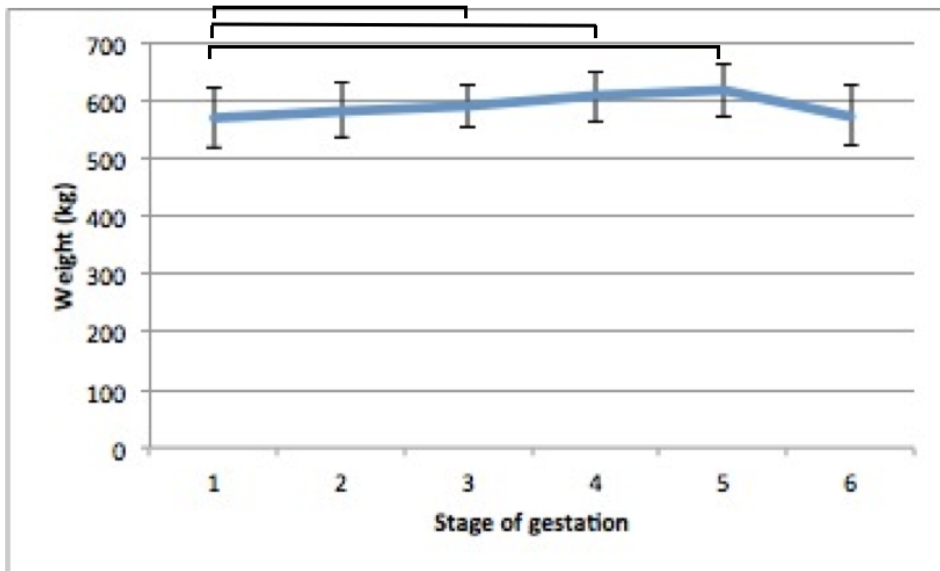
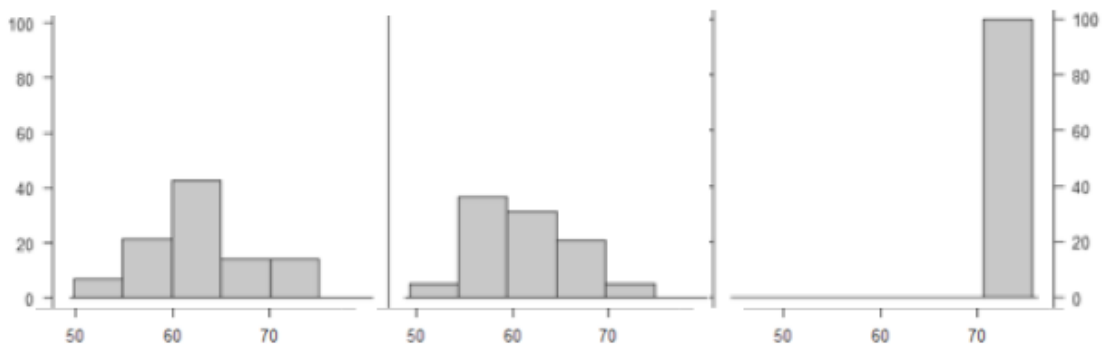


Figure 5



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