Effects of an obesogenic diet on the transitional phase in the horse

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Abstract
The purpose of this study was to determine the effects of an obesogenic diet on the autumn and spring transitional phase in the horse. Horses are a polyestrous species being fertile from spring until autumn and have an anovulatory period in the winter. Before and after winter mares go through a transitional phase.

For this experiment 14 (autumn transitional phase) and 13 (spring transitional phase) Shetland pony mares were used. The control group was fed a maintenance diet and the Fat group was fed a diet 200% of maintenance. The results do not show any significant differences between the two groups. A correlation is found between the duration of the autumn transitional phase and the weight gained during the experiment.

Introduction
Horses are a seasonal polyestrous species, which means that they are not reproductively active the all year-round [1,2]. In fact horses are long-day breeders, with their breeding season beginning in spring when the daylight, temperature and food availability increases. In the winter months horses show a period of ovarian inactivity also called "anestrous" characterized by low GnRH[3] production by the hypothalamus, which in turn causes reduced FSH[3,4] and LH[3,4] production in the hypophysis and absence of follicular growth in the ovaries[3]. Before and after this winter anovulatory phase, mares go through a "transitional phase"[3,5].

Spring transitional phase
During the spring transitional phase follicles do develop but ovulation does not take place and the developed follicles regress [6]. The fact that the follicle are able to develop is due to the relatively constant concentrations of mean plasma FSH and pituitary FSH content throughout the year. However since LH is necessary to stimulate ovulation and this hormone concentration is still very low during the transitional phase, ovulation of developed follicles does not occur[3,7]. The density of gonadotrophins in the pars tuberalis of the pituitary differs every season, being 4 to 5 times higher in cyclic mares than in transitional mares[3]. The transitional phase can be divided into "early"and "late" transition on the base of the number and size of the follicles present on the ovaries. The early transitional phase is characterized by the presence of only few follicles on the ovaries which do not exceed 16 or 17mm in diameter. The late transitional phase is characterized by the presence of many follicles on both ovaries and these follicles reach a diameter larger of 20-25mm before starting to regress[8]. In some mares the follicles will reach a size similar to the diameter of pre-ovulatory follicles [9].

Autumn transitional phase
Similarity to the spring transitional phase, during the autumn transitional phase there is suboptimal follicle development leading to acyclicity due to inadequate gonadotrophin stimulation. Pre-ovulatory FSH concentrations are prolonged and the FSH surge in dioestrus is reduced compared to ovulatory cycles in cyclic mares. Moreover LH secretion is impaired with a shortened LH surge, this leads to anovulation[3,10]. Gradually follicular growth is reduced to deep anestrus levels. During this period, follicular growth is minimal, only a few follicles have a diameter

of more than 15 mm and the maximal diameter of the largest follicle does not exceed 16 mm. No dominant follicle will develop. FSH surges and follicular waves can be distinguished throughout the anovulatory season[3,8].

Factors influencing the transitional phase

The duration of the transitional phase is influenced by different factors[1]. One of the main factors seems to be daylight. Light is converted into an endocrine signal in the pineal gland. The pineal gland secretes melatonin when it is dark, thus a longer day length leads to a decreased melatonin secretion and increased gonadotropin secretion, by the stimulation of GnRH secretion from the hypothalamus[1,8]. The hypothalamic–pituitary axis is restored gradually during the spring transitional phase allowing re-initiation of follicular growth and eventually ovulation.

As stated before, FSH levels and pulsatillity do not change between cyclic mares and anovulatory mares[10]. The key factor for restoring ovulation in anestrus mares is restoring the levels of LH and the LH surges. The increases in this LH pulsatility and mean concentrations during the spring transitional phase reinitiates the development of dominant-size follicles and eventually ovulation[8,11].

But not only melatonin is involved in seasonal changes in hypothalamic and pituitary activity, but also neurotransmitters such as opioids and catecholamines are involved[1,8].

A second important factor for the onset of ovulatory activity is nutrition and body condition[2,5,12]. The anovulatory period is shorter in mares which gain weight during early spring[1]. Henneke et al. (1984) describes that mares with a body condition score lower than 5.0 (scale from 1 to 9) have on average a longer interval to the first ovulation compared to mares with a body condition score above 5.0[1,13]. Kubiak et al. (1987) described that thin transitional mares (body fat <11,5%) receiving a high energy intake, present a shorter interval to first ovulation. However, in this study mares with a moderate (body fat of 11,5% to 15%) or fat (body fat >15%) body condition did not seem to benefit from the increased energy intake[12]. However the duration of the transitional phase of these thin mares receiving a high energy intake was still longer than the duration of the transitional phase of fat mares who were fed a maintenance diet [12]. In summary, the fat mares who were fed a maintenance diet had the shortest duration of the transitional phase. However increasing the food intake above maintenance has no effect on the duration of the transitional phase in mares with a good or fat body condition.

Salazar-Ortiz et al. (2011) reported that the duration of the winter anestrous was longer in mares who were fed restrictedly. In fact in their well-fed group, only 40% of the mares showed winter ovarian inactivity and this inactivity was shorter than in the food restricted group[5].

The mechanism in which the increased energy availability stimulates the ovarian activity is still unclear; however is seems to involve the hypothalamus[14,15]. Nutrients availability has a direct effect on the metabolism of the mare and in particular controls the production of hormones such as leptin, insulin, growth hormone (GH) and insulin-like growth factors (IGFs)[5,16].

Effects of leptin on reproduction

Leptin is a hormone produced by adipose tissue and is suggested to act as a signal between body fat and the hypothalamus[17]. The expression and secretion of leptin is highly correlated with the amount of body fat and adipocyte size[17,18]. Leptin regulates food

intake and satiety and also signals to the brain the body condition and adiposity, influencing reproduction[5].

White adipocytes secrete leptin in the circulation. It travels to the brain stimulating or inhibiting the release of neurotransmitters, such as neuropeptide Y. Neuropeptide Y inhibits food intake and stimulates physical activity in rodents. This results in stimulating other endocrine signals to inhibit leptin and to reduce the mass of adipose tissue. Leptin has a direct effect on metabolism and other peripheral tissues such as liver and adipocytes[17].

Insulin plays an important role on regulating leptin secretion. Hyperinsulinemia increases leptin levels for 3 to 5 hours in rodents and humans[17]. Peak levels of leptin are reached with the initiation of eating behavior. Insulin injections can also reinstate a peak in leptin levels. Fasting inhibits leptin production and release[17].

Gentry et al. (2002) found that mares fed with a nutrient restriction resulting in a low body condition score, resulted in a profound seasonal anovulatory period accompanied by lower leptin, IGF-I and prolactin concentrations. Mares with high body condition scores continued to cycle throughout the winter or had significant follicular activity on the ovaries. The leptin concentrations in mares with a high body condition score were higher than in the other group, although a wide variation was found in the group of well-fed mares[14].

Effects of GH and IGF on reproduction

GH secretion is influenced by nutritional intake and has an important effect on reproduction[5,19]. GH stimulates folliculogenesis and ovulation and is thus considered to be a 'co-gonadotrophin'[19,20]. Furthermore GH enhances the gonadotrophin responsiveness the ovaries[5,19]. The effect of GH on primordial follicles is the initiation of growth On primary and secondary follicles it stimulates growth . GH seems to stimulates the maturation of the oocyte in the antral stage follicle until it reaches the stage of a pre-ovulatory follicle[20].

Salazar-Ortiz et all. (2014) found that in well-fed mares, the concentration of GH and IGF-2 were lower, and IGF-1 was higher in plasma than mares who were fed with a restricted diet[21]. The IGF system plays an important role in ovarian follicular growth and follicular artresia[21]. IGF does so by intermediating indirect the actions of GH. Trough somatostatin IGF-1 has a negative feedback on GH. The IGF system initiates growth and atresia in primordial follicles and in antral follicles, IGF increases follicular sensitivity to gonadotrophins and oocyte maturation[20].

Body fat influences the plasma concentrations of GH and IGF-1. In the study of Salazar-Ortiz et all (2011) it is concluded that in fat mares the concentration of plasma GH is low and IGF-1 concentration is high[5].

Hypothesis

The purpose of this study is to evaluate the impact of an obesogenic diet on the transitional phase in the horse. The following hypotheses are tested in this study:

1) A high body condition and high fat percentage reduce the length of the transitional phase.
2) an increase in weight causes a reduced length of the transitional phase.

Material and methods

Animals and housing

In this experiment, 13 Shetland pony mares aged between 2,5 and 8 years were used. The experiment started in the autumn of 2014 and in February 2015. 14 ponies were used for the experiment in the autumn. The ponies were

individually housed but were able to have physical contact with each other. The ponies were allowed to exercise freely for 4h every other day. The lights in their stables were on 24 hours a day.

The 13 ponies were divided in two homogeneous groups on the base of their starting body condition score (BCS), 7 ponies in the fat group and 6 in the control group.

Feeding

The ponies were fed an individualized diet three times a day. The ponies in the control group were fed a maintenance diet composed of hay, concentrates and supplements to provide enough minerals and vitamins. The horses in the maintenance group were fed 85% of the digestible energy (DE) from hay and 15% DE from concentrates. The DE needed for maintenance was determined using the Dutch CVB book[22]. See table 1.0 for the composition of the concentrate used.

The ponies in the fat group were fed 200% of their DE requirements for maintenance. These ponies acquired 42,5% of DE from hay and 57,5% of DE from their concentrates. These ponies received the same amount of supplements as the ponies in the control group. The feeding regime was updated regularly when the ponies of the fat group gained weight . The leftovers of hay and concentrates were measured an noted down each morning.

91462 Pavo concentrate

Crude protein	10,90%
Crude fat	12,40%
Crude fibre	9,70%
Crude ash	6,60%
Calcium	0,86%
Phosphorus	0,40%
Sodium	0,65%
Potassium	0,63%
Magnesium	0,50%
Additives per kg	Vitamin E

Table 1.0: Composition of PAVO concentrate used for feeding of the ponies.

All ponies had a mineral lick in their stables.

Weighing and Body condition score

The ponies were weighed weekly. Each pony was weighted three times and the mean weight was used. The body condition score was also evaluated weekly by 2 separate observers. The body condition was graded with a number from 1 to 9, using the scale from Henneke et al. (1983)[23].

Reproductive status

The reproductive tract of all ponies was monitored using trans rectal ultrasonography (Esaote MyLab30, Netherlands) weekly. Whenever a follicle reached the pre-ovulatory size (35mm or bigger) the mare was monitored more frequently (every other day) to assess a possible ovulation. A mare was considered early-transitional when follicles reached a size of approximately 15mm to 20mm. When follicles reached the size larger than 20-25mm, the mare was considered to be late-transitional. When a corpus luteum was found, the mare ovulated.

Body fat composition

Body fat was measured once every month using ultrasonography (General Electronics Logiq7, Netherlands). Body fat was measured on 6 locations on the left side of the pony. These locations were shaved with clippers (Möser Arco, Germany) and cleaned with 70% alcohol solution and a paper tissue.

For the ultrasonography transmission gel (EZ-EM, NY, USA) was used. After the ultrasonography the locations were cleaned with chloorhexidine (Hibiscrub, Mölnlycke Health Care, Breda, Holland) dissolved in water.

The following locations were used for the body fat measurement:

- Retroperitionial (MP1): The probe is placed parallel and directly lateral of

the ventral midline, just caudally of the xiphisternum.

- Axillary(MP2): The location of the lateral thoracic vein is set on the location where it runs between the deep chest- and latissimus dorsi muscles. The measurement is done cranial of the first branch to the dorsal and ventral veins.
- Withers(MP3): The probe is placed perpendicular on the mid-line, just cranial from the highest point of the withers.
- Rib-eye(MP4): The fat in the 14e intercostal space, 20cm lateral of the dorsal mid-line is measured. The probe has to be placed parallel to the ribs.
- Body(MP5): The fat is measured between attachment of the tail and the tuber coxae. The probe is positioned on a 45 ° corner of the dorsal mid-line, pointing to the attachment of the tail.
- Tail-head(MP6): The probe is placed parallel at approximately 3cm lateral from the dorsal midline, directly cranial of the first hairs of the tail.

Measurements of the fat layer have been done from the middle of the screen, with exception from the withers and axillary images.

Statistical analysis

For the statistical analysis IBM SPSS statistics 20 was used. For testing the significance of differences between the fat and control group, an independent-samples t-test was used. For the correlation between the length of the transitional phase and the Body condition score, body fat percentage and weight gain, the Pearson correlation test was used.

Results

Autumn transitional phase

Six of the ponies were not included in the analysis since 1pony died during the experiment , 2 never show cyclicity and 3 produced repeatedly anovulatory hemorrhagic follicle throughout the year. Therefore only 8 ponies were included in the analysis; N=4 in the control group and N=4 in the fat group.

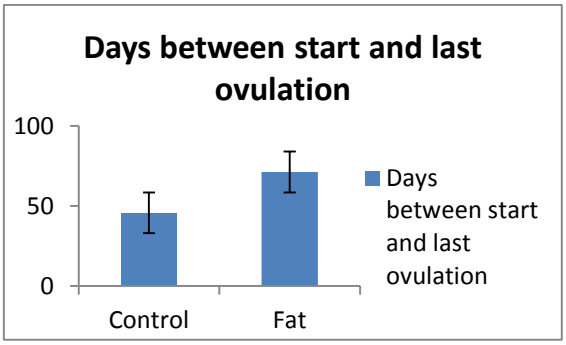


Fig 1. Bar graph showing the mean for days to transition and standard error.

The mean time between the start of the experiment and the last ovulation was 45,75 ± 8,864 days for the control group and 71,25 ± 2,016 days for the fat group. The difference between these groups were not significant (p=0,07)

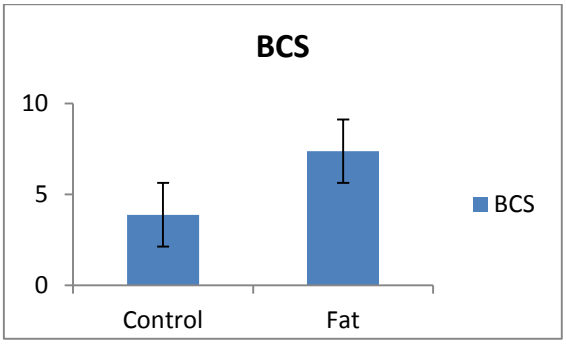


Fig 2. Bar graph showing the mean for BCS and standard error.

There was no significant difference in BCS between the control group and the fat group. The mean BCS for the control group at the end of this study was 3,875 ± 0,4270. The mean for the fat group was 7,375 ± 0,8004.

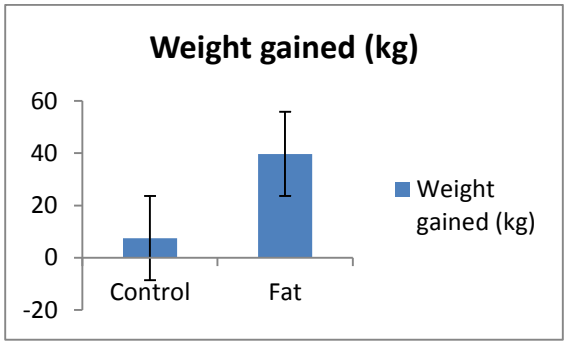


Fig 3. Bar graph showing the mean for Weight gain(kg) and standard error.

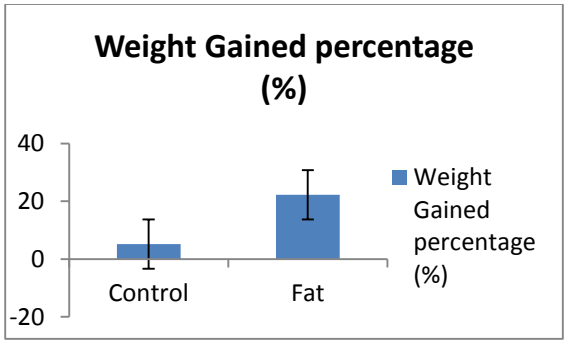


Fig 4. Bar graph showing the mean for Weight gain percentage and standard error.

There was no significant difference between the control and fat group for weight gained in kg or percentage.

The mean weight gained in the control group was 7,50 ± 1,32kg and 5,20 ± 1,36%. The mean weight gained in the fat group was 39,75 ± 3,326kg and 22,27 ± 0,788%.

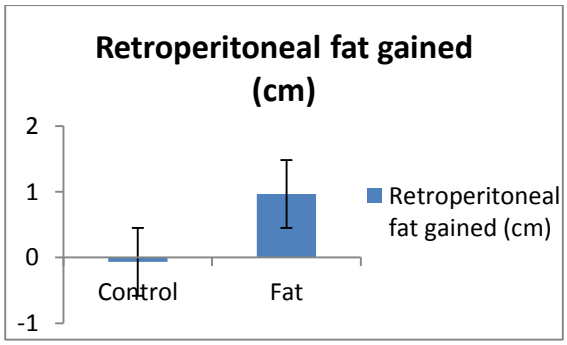


Fig 5. Bar graph showing the mean for retroperitoneal fat gain (cm) and standard error.

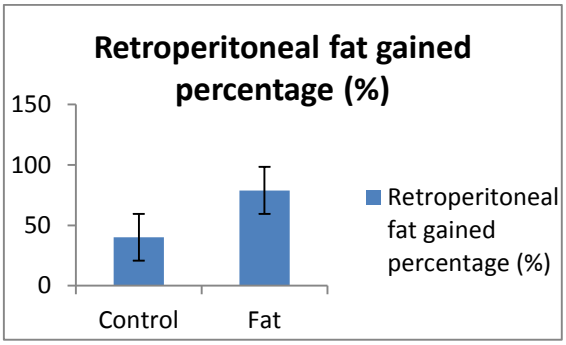


Fig 6. Bar graph showing the mean for retroperitoneal fat gain percentage (%) and standard error.

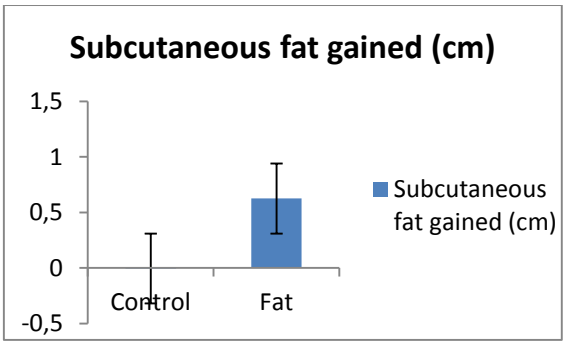


Fig 7. Bar graph showing the mean for subcutaneous fat gain (cm) and standard error.

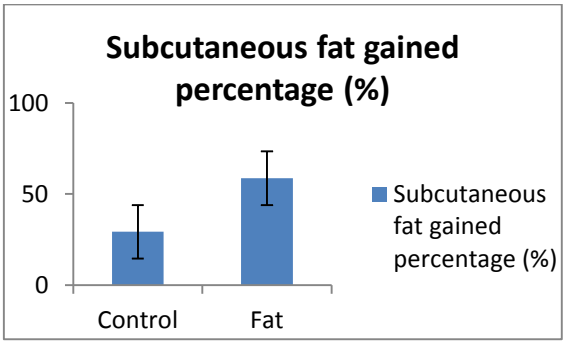


Fig 8. Bar graph showing the mean for subcutaneous fat gain (%) and standard error.

There was no significant difference between the control group and the fat group for retroperitoneal or subcutaneous fat gained.

The mean retroperitoneal fat gained in the control group was -0,065 ± 0,287cm and 40,1 ± 72,7%. In the fat group the mean retroperitoneal fat gained was 0,97 ± 0,32cm and 78,9 ± 27,4%.

The mean subcutaneous fat gained in the control group was -0,05 ± 0,23cm and 29,3 ± 38,5%. In the fat group the subcutaneous fat gained was 0,63 ± 0,38cm and 58,7 ± 32,0%.

Correlations

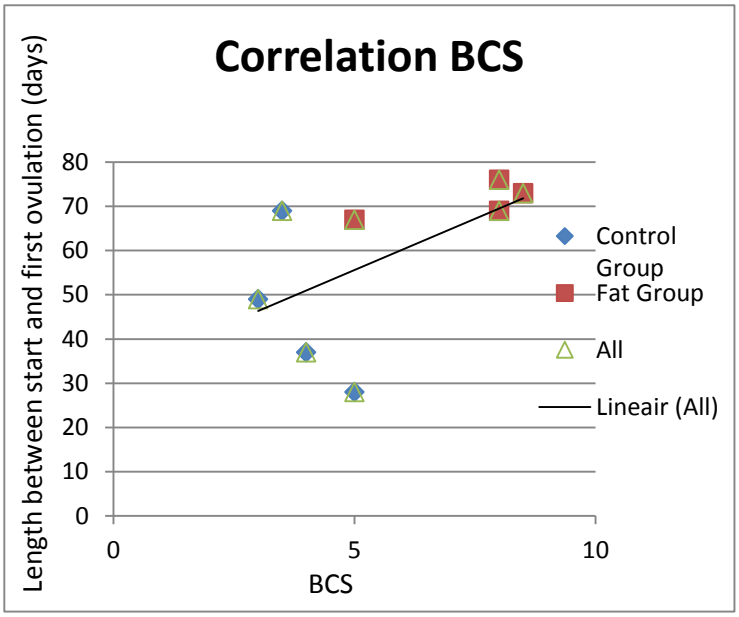
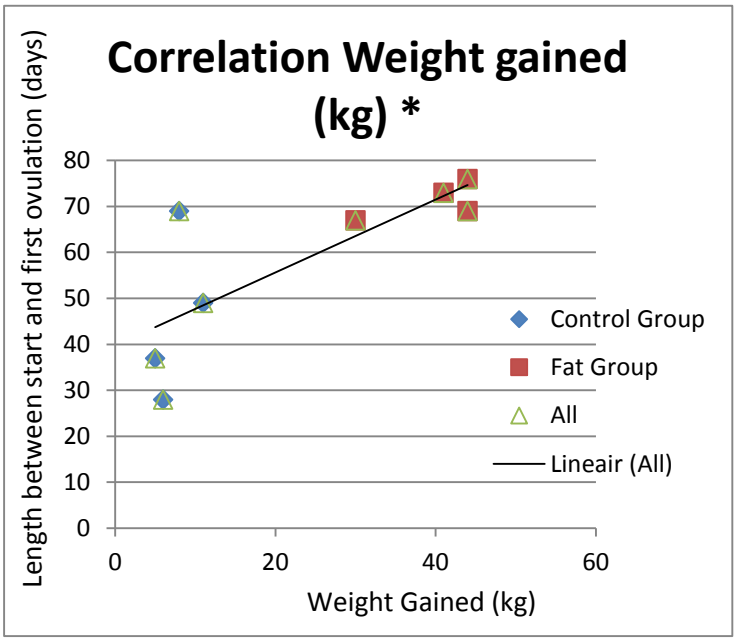


Fig 9. Scatterplot showing correlation between BCS and length between start experiment and first ovulation.



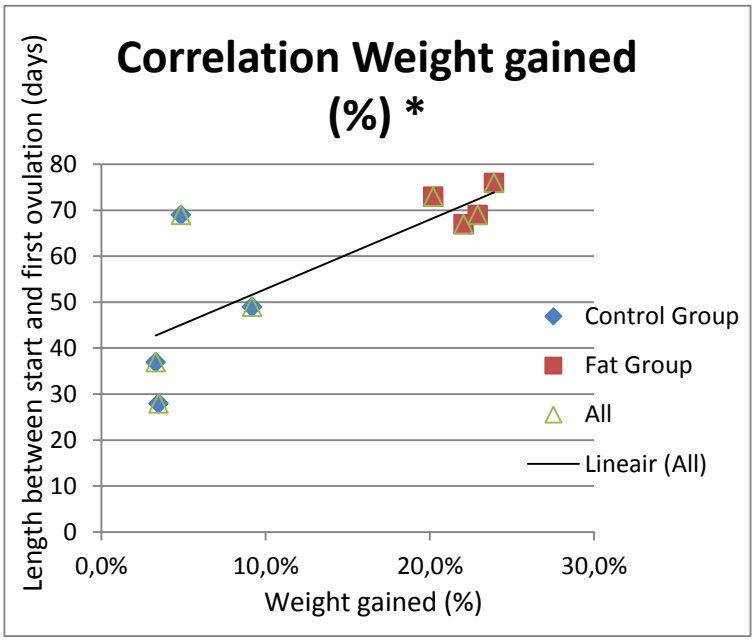
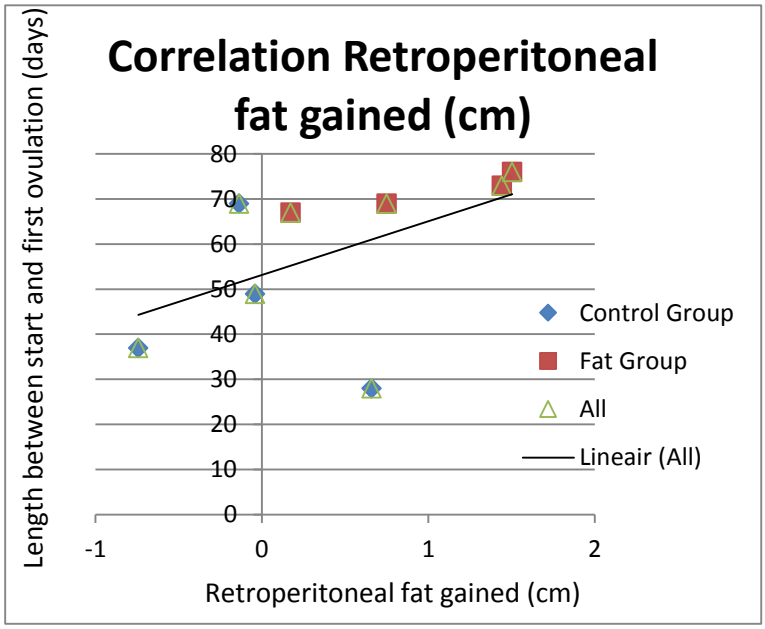


Fig 10 and 11. Scatterplots showing correlation between Weight gain (kg and percentage) and length between start experiment and first ovulation. *means a significant (P<0,05) correlation was found



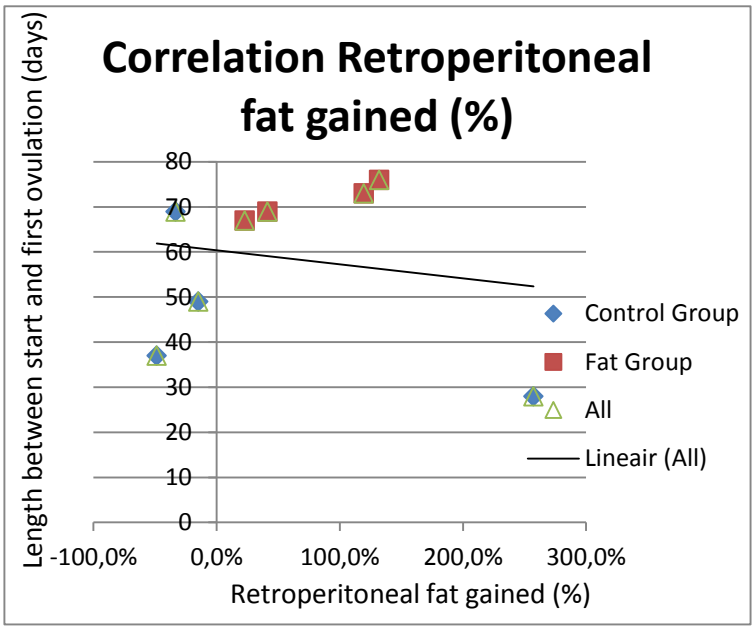
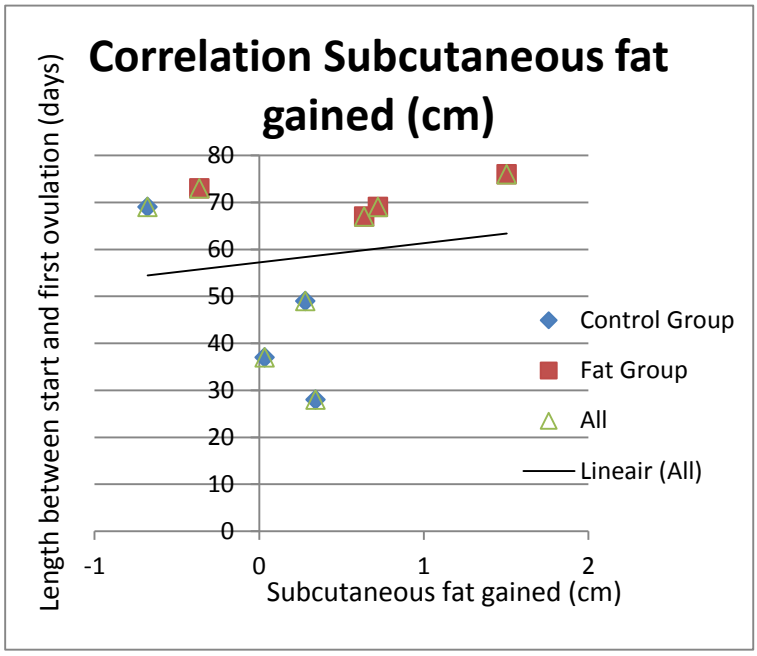


Fig 12 and 13. Scatterplots showing correlation between Retroperitoneal fat gained (cm and percentage) and length between start experiment and first ovulation.



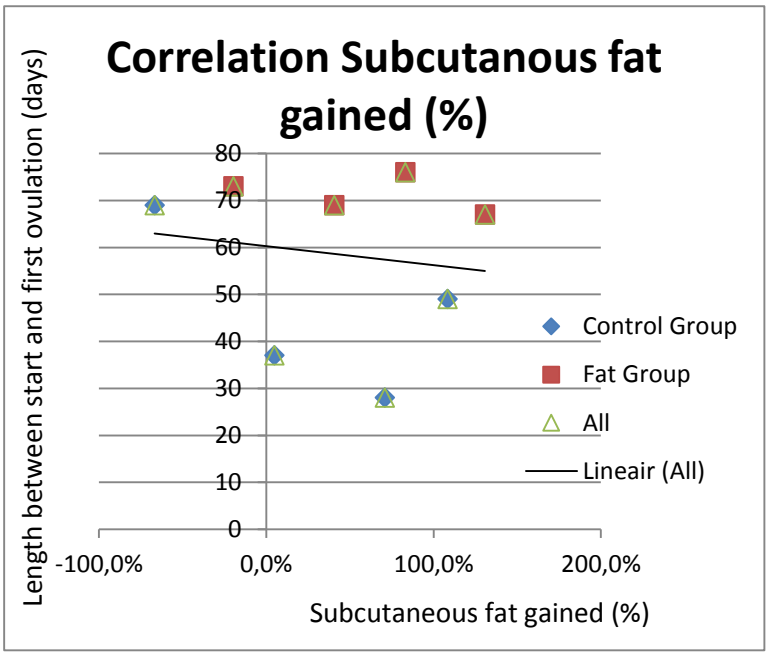


Fig 14 and 15. Scatterplots showing correlation between Subcutaneous fat gained (cm and percentage) and length between start experiment and first ovulation.

Using the Pearson correlation test, only two significant correlations were found. The correlations between weight gained (kg and percentage) and the time between the start of experiment and the last ovulation were 0,782 (P<0,05) and 0,781 (p<0,05) respectively.

		Days between start and last ovulation	BCS	Weight gained (kg)	Weight Gained percentage (%)	Retroperitoneal fat gained (cm)	Retroperitoneal fat gained percentage (%)	Subcutaneous fat gained (cm)	Subcutaneous fat gained percentage (%)
Days between start and last ovulation	Pearson Correlation	1	,568	,782*	,781*	,517	-,178	,152	-,151
	Sig. (2-tailed)		,142	,022	,022	,190	,674	,719	,721
	N	8	8	8	8	8	8	8	8

Table 2. Table showing the correlations between start of the experiment and last day of ovulation and the other variables.

Spring transitional phase

Three of the ponies were not included in the analysis since these three ponies were still transitional after finishing my research internship. Therefore only 10 ponies were included in the analysis; N=4 in the control group and N=6 in the fat group.

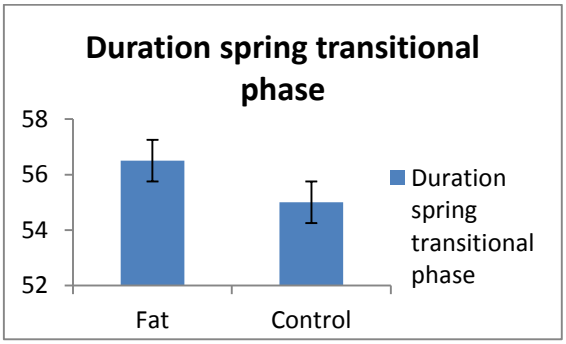


Fig 16. Bar graph showing the mean for the duration of the transitional phase.

The mean time between the start of the experiment and the last ovulation was 55,0 ± 4,0 days for the control group and 56,5 ± 3,45 days for the fat group. The difference between these groups were not significant.

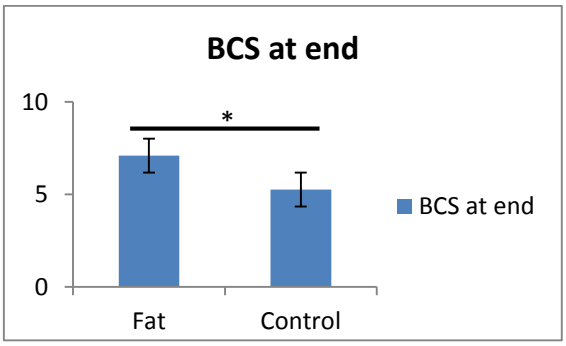


Fig 17. Bar graph showing the mean for BCS and standard error. *means a significant (P<0,05) difference was found.

There was a significant difference in BCS between the control group and the fat group (P<0,05). The mean BCS for the control group at the end of this study was 5,25 ± 0,25. The mean for the fat group was 7,083 ± 0,5974.

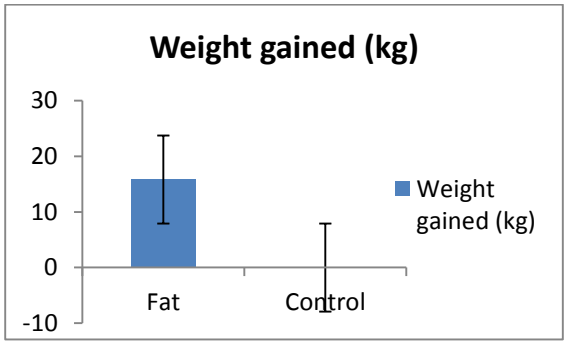


Fig 18. Bar graph showing the mean for Weight gain(kg) and standard error.

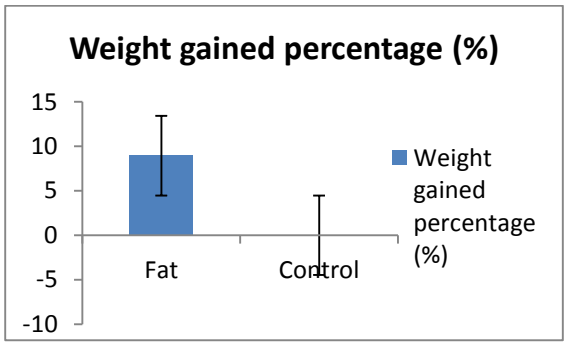


Fig 19. Bar graph showing the mean for Weight gain percentage and standard error.

There was no significant difference between the control and fat group for weight gained in kg or percentage.

The mean weight gained in the control group was 0 ± 2,858kg and 0 ± 1,620%. The mean weight gained in the fat group was 15,83 ± 3,851kg and 8,95 ± 2,459%.

Correlation

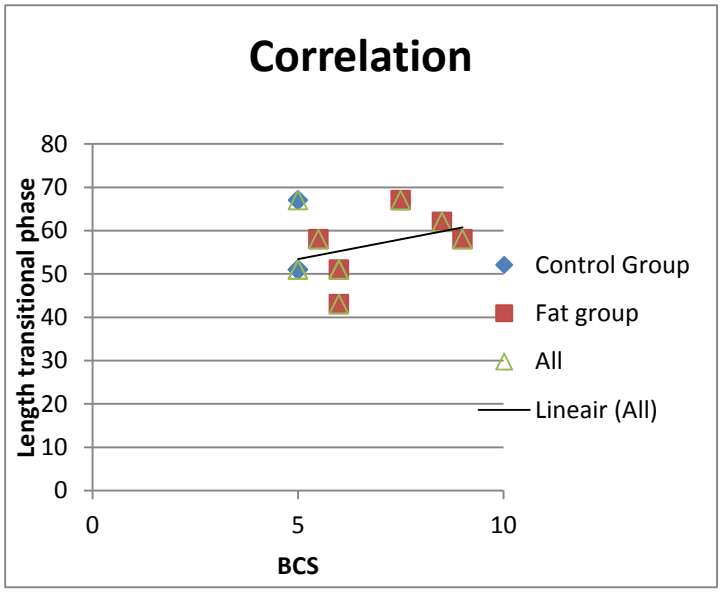
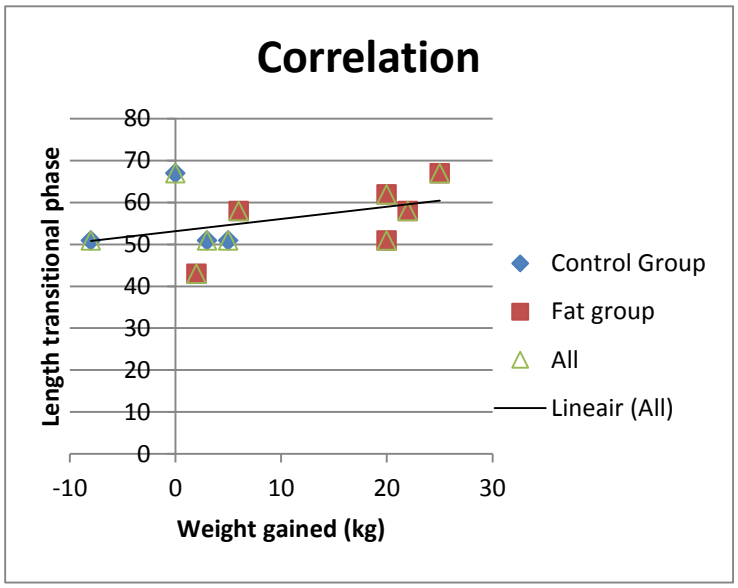


Fig 20. Scatterplots showing correlation between BCS and length of the transitional phase.



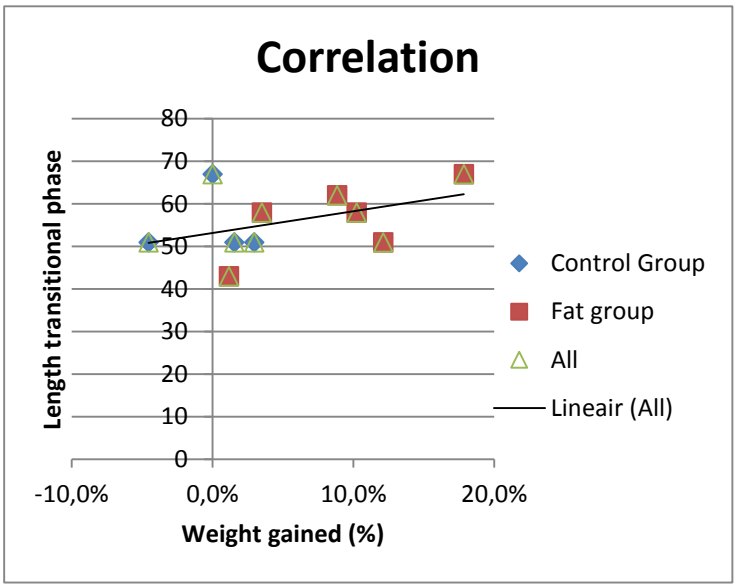


Fig 21 and 22. Scatterplots showing correlation between Weight gain (kg and percentage) and length of the transitional phase.

Using the Pearson correlation test, no significant correlations were found.

		Duration spring transitional phase	Weight gained (kg)	Weight gained percentage	BCS at end
Duration spring transitional phase	Pearson Correlation	1	,420	,435	,344
	Sig. (2-tailed)		,227	,209	,330
	N	10	10	10	10

Table 3. Table showing the correlations between start of the experiment and last day of ovulation and the other variables.

Conclusion and discussion

Autumn transitional phase

From this study it can be concluded that there is a positive correlation between weight gained and the length of the autumn transitional phase. Other correlations have not been proven by this study.

From the results no significant differences between the control and the fat group were found. For the results 8 out of 14 ponies from the experiment were used. 1 pony died during the experiment, 3 never showed cyclicity and 2 ponies showed hemorrhagic follicles (HM) making it impossible to determine the end of the autumn transitional phase in a reliable way. HM follicles are anovulatory follicles which tend to occur mostly during the early and late ovulatory season[24,25]; however some individuals can show them during the whole period of reproductive activity. The low number of ponies left for statistical analysis may be a contributing factor for not finding any significant differences between groups. The days of transition was almost significant at p=0,07 so larger groups could solve this problem.

One correlation has been found, between the time of the last ovulation and the gain of weight (kg and %). No correlation between BCS and start of the experiment and last ovulation day was found. However, there is a correlation between BCS and weight gained (kg and %) of P<0,01 and P<0,05 respectively. The BCS is still a subjective method and the measurement of fat percentage should be more reliable.

Light is also an important factor determining the length of the transitional phase. However in this study the animals were housed under conditions with light being on for 24/7. So this shouldn't be a disturbing factor for this experiment.

Kubiak et al. (1987) described that fat mares who were fed a maintenance diet had the shortest duration of the transitional phase. However increasing the food intake above maintenance has no effect on the duration of the transitional phase in mares with a good or fat body condition[12]. So probably the BCS score of the ponies did not differ enough between groups for a significant difference in this study. At the start of this experiment all the ponies had a BCS between 4 and 6 so according to Kubiak et al. (1987)[12] increasing food intake should not have an effect on the length of the transitional phase, since the ponies already have a good to high BCS.

Other studies that have been conducted on the transitional phase and correlation with diet used a different feeding strategy. Salazar-Ortiz et al. (2011) used three groups; a 'well-fed' group that maintained the mares in a good body condition, a 'restricted' group that used a diet calculated to keep the mares thin and a 'variable' group feeding both diets alternately for periods. The duration of this study was three years. A study with more groups differentiating between the BCS at the start of the study could give a more in depth sight on the relation between BCS, feeding and length of the transitional phase. To differentiate between the influence of food or a high BCS on the transitional phase an experiment with four groups could be performed. Two groups with a high BCS (7-9) feeding half of the group a maintenance diet and the other half an obesogenic diet and two groups with a low BCS (3-5) were half of the ponies will be fed a maintenance diet and the other half an obesogenic diet.

Spring transitional phase

From this study no correlation has been found between length of the transitional phase and Weight gain (Kg and %) and BCS.

No correlation between fat gained and length of the spring transitional phase was tested. This data was still being processed and unavailable for use in this statistical analysis.

10 out of 13 ponies were used for the statistical analysis. The other three ponies (2 from the control group and 1 from the fat group) were still transitional at the end of this research internship.

One significant difference for the BCS between the control and fat group was found. This may be due to the fact that at the start of the experiment there were already three ponies in the fat group with a BCS>7.

There was no significant difference between the groups for weight gain. One pony started the 200% maintenance diet later in this experiment because it was switched with a pony that did not fully eat the 200% maintenance diet. So the weight gain is relatively low for this pony. Another pony suffered from colic and could not eat the 200% maintenance diet at that moment. After the colic this pony had to gradually get used to eating the 200%maintenance diet again. This pony also suffered from sand in the intestines and frequently had leftovers of hay. This pony also gained relatively little weight.

References

1. Nagy P, Guillaume D, Daels P. Seasonality in mares. *Anim Reprod Sci*. 2000;60–61(0):245-262. doi: http://dx.doi.org.proxy.library.uu.nl/10.1016/S0378-4320(00)00133-0.

2. Van Niekerk FE. The effect of dietary protein on reproduction in the mare. III. ovarian and uterine changes during the anovulatory, transitional and ovulatory periods in the non-pregnant mare. *J S Afr Vet Med Assoc*. 1997;68(3):p. doi: savet_v68_n3_a5.

3. Aurich C. Reproductive cycles of horses. *Anim Reprod Sci*. 2011;124(3–4):220-228. doi: http://dx.doi.org.proxy.library.uu.nl/10.1016/j.anireprosci.2011.02.005.

4. Thompson D, Johnson L, St George R, Garza F. Concentrations of prolactin, luteinizing hormone and follicle stimulating hormone in pituitary and serum of horses: Effect of sex, season and reproductive state. *J Anim Sci*. 1986;63(3):854-860.

5. Salazar Ortiz J. Effects of nutritional cues on the duration of the winter anovulatory phase and on associated hormone levels in adult female welsh pony horses (equus caballus). *Reproductive biology and endocrinology*. 2011;9. doi: 10.1186/1477-7827-9-130.

6. Ginther OJ. Folliculogenesis during the transitional period and early ovulatory season in mares. *J Reprod Fertil*. 1990;90(1):311-20.

7. Alexander SL, Irvine CH. Effect of graded doses of gonadotrophin-releasing hormone on serum LH concentrations in mares in various reproductive states: Comparison with endogenously generated LH pulses. *J Endocrinol*. 1986;110(1):19-26.

8. Donadeu F, Watson E. Seasonal changes in ovarian activity: Lessons learnt from the horse. *Anim Reprod Sci*. 2007;100(3):225-242.

9. Ginther O, Gastal E, Gastal M, Beg M. Seasonal influence on equine follicle dynamics. *Anim Reprod*. 2004;1:31-44.

10. Irvine CH, Alexander SL, McKinnon AO. Reproductive hormone profiles in mares

during the autumn transition as determined by collection of jugular blood at 6 h intervals throughout ovulatory and anovulatory cycles. *J Reprod Fertil*. 2000;118(1):101-109.

11. Fitzgerald BP, Affleck KJ, Barrows SP, Murdoch WL, Barker KB, Loy RG. Changes in LH pulse frequency and amplitude in intact mares during the transition into the breeding season. *J Reprod Fertil*. 1987;79(2):485-493.

12. Kubiak JR. The influence of energy intake and percentage of body fat on the reproductive performance of nonpregnant mares. *Theriogenology*. 1987;28(5):587. doi: 10.1016/0093-691X(87)90275-5.

13. Henneke DR. Body condition during pregnancy and lactation and reproductive efficiency of mares. *Theriogenology*. 1984;21(6):897. doi: 10.1016/0093-691X(84)90383-2.

14. Gentry LR, Thompson DL, Gentry GT, Davis KA, Godke RA, Cartmill JA. The relationship between body condition, leptin, and reproductive and hormonal characteristics of mares during the seasonal anovulatory period. *J Anim Sci*. 2002;80(10):2695-703.

15. Gentry LR, Thompson Jr. DL, Gentry Jr. GT, Davis KA, Godke RA. High versus low body condition in mares: Interactions with responses to somatotropin, GnRH analog, and dexamethasone. *J Anim Sci*. 2002;80(12):3277-3285. Accessed 24 February 2015.

16. Fitzgerald BP, McManus CJ. Photoperiodic versus metabolic signals as determinants of seasonal anestrus in the mare. *Biol Reprod*. 2000;63(1):335-340. Accessed 24 February 2015.

17. Houseknecht KL, Baile CA, Matteri RL, Spurlock ME. The biology of leptin: A review. *J Anim Sci*. 1998;76(5):1405-20.

18. Buff PR, Dodds AC, Morrison CD, et al. Leptin in horses: Tissue localization and relationship between peripheral concentrations of leptin and body condition. *J Anim Sci*. 2002;80(11):2942-2948. Accessed 24 February 2015.

19. Hull KL, Harvey S. GH as a co-gonadotropin: The relevance of correlative changes in GH secretion and reproductive state. *J Endocrinol*. 2002;172(1):1-19.

20. Silva JRV, Figueiredo JR, van den Hurk R. Involvement of growth hormone (GH) and insulin-like growth factor (IGF) system in ovarian folliculogenesis. *Theriogenology*. 2009;71(8):1193-1208. doi: http://dx.doi.org/10.1016/j.theriogenology.2008.12.015.

21. Salazar Ortiz J. The influence of nutrition on the insulin-like growth factor system and the concentrations of growth hormone, glucose, insulin, gonadotropins and progesterone in ovarian follicular fluid and plasma from adult female horses (equus caballus). *Reproductive biology and endocrinology*. 2014;12(1). doi: 10.1186/1477-7827-12-72.

22. CVB PD. CVB tabellenboek voeding paarden en pony's. 2013.

23. Henneke D, Potter G, Kreider J, Yeates B. Relationship between condition score, physical measurements and body fat percentage in mares. *Equine Vet J*. 1983;15(4):371-372.

24. Ginther OJ, Pierson RA. Regular and irregular characteristics of ovulation and the interovulatory interval in mares. *Journal of Equine Veterinary Science*. 1989;9(1):4-12. doi: http://dx.doi.org.proxy.library.uu.nl/10.1016/S0737-0806(89)80110-8.

25. Ginther OJ, Gastal EL, Gastal MO, Beg MA. Incidence, endocrinology, vascularity, and morphology of hemorrhagic anovulatory follicles in mares. *Journal of Equine Veterinary Science*. 2007;27(3):130-139. doi: http://dx.doi.org.proxy.library.uu.nl/10.1016/j.jevs.2007.01.009.