

## EFFECT OF TOCOPHEROL AND SELENIUM ON THE PERFORMANCE OF BUSERELIN FOR ESTRUS INDUCTION IN LATE SEASON ANESTRUS MARES (*EQUUS CABALLUS*)

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### ABSTRACT

The aim of this study was to determine the effect of tocopherol and selenium on the performance of buserelin (GnRH analogue) for estrus induction in anestrus mares. A total of 18 seasonally anestrus mares were divided into three equal groups (n=6 in each) after ovarian scanning. Mares in group A (treatment group) were treated with tocopherol and selenium intramuscularly along with daily dose of buserelin. Mares which came into heat within ten-day buserelin protocol, further injections of buserelin for those mares were stopped. Mares in group B were treated with buserelin intramuscularly only for ten days. Mares which came into heat within ten-day buserelin protocol, further injections of buserelin were stopped. Blood/serum was collected from each experimental mare kept in different groups (A-C) at days 1, 11, 12, 13 and 14. Onset of estrus signs was 83.3, 33.3 and 0% in group A, B and C, respectively, that was significant ( $P<0.05$ ) among groups. Follicular dynamics were  $20.24\pm 3.00$ ,  $14.59\pm 2.24$  and  $10.35\pm 0.63$ mm in groups A, B and C, respectively with significant ( $P<0.05$ ) difference. Change in serum E2 contents was significantly ( $P<0.05$ ) different among groups A, B and C whereas progesterone concentration showed non-significant difference among groups.

**Keywords:** Mare, Estrus, Anestrus, Selenium, Tocopherol

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### 1. INTRODUCTION

Animals can be categorized as long-day (LD) breeders and short-day (SD) breeders based on when they are fertile relative to day length. LD breeders are fertile during spring when the days are longer, whereas SD breeders display reproductive behaviors during autumn when the day length shortens (Nishiwaki-Ohkawa and Yoshimura 2016). Other factors include age of mare (endometrosis), frequency and breeding time or AI (Morris and Allen 2002). In mares, the circannual rhythm of reproduction is mainly regulated by photoperiodic changes with the increase in day length preceding the onset of the breeding season (Nagy et al. 2000; Kwong et al. 2018). As the day light increases the pineal gland gets stimulus from the retina of eye and in response to this melatonin release will be suppressed which have negative feedback to GnRH secretion from hypothalamus, the increase secretion of GnRH from hypothalamus will trigger the gonadotropin from anterior pituitary and ultimately resumption of ovarian activity (Polasek et al. 2017; Rezende et al. 2018; Siemieniuch et al. 2019). During winter anestrus, the extended melatonin secretion during the long periods of darkness has an inhibitory effect on GnRH secretion which leads to reduction in gonadotropin secretion, luteinizing hormone (LH) and to a lesser extent follicle-stimulating hormone (FSH) (Nagy et al. 2000; Murphy 2019). Many other factors like nutrition, housing, management, climatic conditions and presence of stallion also affects the reproductive cyclicity of mare (Nagy et al. 2000). The continuous use of exogenous GnRH and its agonists can lead to decline in the pituitary receptiveness, due to decrease in its receptors. Moreover, failure of gonadal response to gonadotropin concurrently results in deficient steroidogenesis, anovulation, defective endometrial decidualization and implantation, abnormal fetal outcome and delayed parturition (Chatterjee et al. 2006). Tocopherol is naturally found in plant oils and it remains the necessary constituent of animals as well as human diet. Vitamin E has an important role in the cell membranes integrity although it is present in very small ratio about 0.01% of total lipid molecules in membrane. Moreover, it has low molar concentration in cell membrane but strongly works as a lipid soluble chain breaking antioxidant thus averting the peroxidation of lipids (Packer and Landvik 1989; Talukder et al. 2017). Tocopherol has a great influence on the reproductive cycle, as it may increase the reproductive performance of

mare and also increase conception rate in foal heat. It prevents the postpartum complications like retention of fetal membrane (Ishii et al. 2002).

Growth of granulosa cells is considered an important feature during the developmental process of follicles; i.e., the folliculogenesis. The proliferation of small primary follicles (with fewer granulosa cells) to maturing pre-ovulatory follicles (with many strata of cells) is the characteristic event in folliculogenesis (Basini and Tamanini 2000). The ability of Se to improve the immune response in farm animals is well documented (Chauhan et al. 2014). In animal experiments, it has been demonstrated that selenium might regulate the growth of the granulosa cells and  $17\beta$ -estradiol bio-synthesis in adult ovaries in vitro (Basini and Tamanini 2000). Recent studies have also elucidated that Se and selenoproteins levels are increased in large healthy follicles and might perform a vital antioxidant function during later growth and the proliferation of follicles (Ceko et al. 2015). Selenium contributes in maintaining the reproductive health of the mare as its deficiency can lead to ovarian cyst formation, early embryonic death and some other postpartum complications like retention of fetal membrane in mare (Zarczynska et al. 2013). Selenium and tocopherol play important role from the prevention of many problems regarding reproductive health like abortion, retained fetal membrane, infertility, and neonatal weakness. As vitamin E was discovered for its belongings on reproduction in rats, so reactive oxygen species disturbs many propagative processes like oocyte development, growth of embryo, pregnancy and spermatozoa growth (Finno and Valberg 2012).

## 2. MATERIALS AND METHODS

This research was conducted during the months of October and November. A total of 18 anestrus Thoroughbred Pak mares with age ranging from five to ten years were selected and placed in a separate paddock. All the mares were kept according to the farm protocol under same management conditions. Estimated average body weights of the mares were 750kg. All the mares were fed green hay, concentrate and forage that were grown in the local fields of the farm and some salt. The forages were not analyzed dietetically in this. Mares were divided into three groups (A-C) each having same number of mares (n=6). Mare in groups A and B were kept as experimental while the mares in group C were considered as untreated control animals. Mare in group A were treated with tocopherol (1.05g/animal), selenium (2.25mg/animal). Tocopherol and sodium selenite (Selevit®, Fatro, Italy) along with Buserelin (2.25ml/animal) intramuscularly (Table 1) was used in this experiment. In group A, first shot of Inj. Selevit® (15ml/mare IM) was administered on day 1 and then a second shot administered on after 3days. 3rd shot of Selevit® was administered along with 1st shot of Buserelin (GnRH analogue) at the dose rate of 2.5ml (10.50 $\mu$ g/mare) intramuscularly. Conceptal® 5ml packing containing 21 $\mu$ g of Buserelin. This administration of Buserelin was continued until the mares came to heat up to 10 consecutive days. While group B was only administered only with Buserelin @10.50 $\mu$ g/mare IM for up to 10 days until mares came into estrus. Group C was control group (Untreated).

### 2.1. Identification of mares in Estrus stage

For this purpose, teaser stallion of heavy horse breed was used to detect mares in estrus stage after the treatment. Mare in group A and B treated showed signs of estrus. Mares showing signs of estrus were palpated rectally and later on confirmed by ultrasonography for confirmation of estrus and follicular size.

### 2.2. Blood and serum Collection

Blood (10ml) sample was collected from the jugular vein of each experimental mare kept in different groups (A-C) at days 1, 11, 12, 13 and 14 of the trial for serum analysis. All the blood samples were obtained in glass test tubes without any anticoagulant. The serum was separated by centrifugation of blood. Serum estrogen (E2) and progesterone (P4) levels were measured after calibration and incubation with 125 I-labelled coated tubes with estrogen and progesterone antibodies. After aspirating the antibody coated incubated tubes the radioactivity is checked through radioimmunoassay. Curve is obtained from the interpretations of standard calibrators curve (Benabdelaziz et al. 2020).

### 2.3. Statistical Analysis

The collected data was subjected to one-way analysis of variance (ANOVA). Mean $\pm$ SD values in each group were compared by Tukey test using SPSS 20.0 computer software (Field 2009).

## 3. RESULTS

### 3.1. Effect of buserelin administration alone and in conjunction with tocopherol and selenium administration on the onset of estrus in mares

Out of 18 mares, 7 showed signs of estrus. In group A, B and C, 5, 2 and 0 mares showed signs of estrus respectively. A significantly greater (P<0.05) number of mares, given Buserelin and Selevit combination showed signs of estrus compared with control and those administered with Buserelin alone. There was no significant

difference ( $P > 0.05$ ) in number of mares that were administered Buserelin compared with control mares, for the onset of signs of estrus. Time for onset of estrus signs was also measured in days from first day of Buserelin treatment to the onset of first estrus sign. There was no significant difference in between mares given Selevit along with Buserelin in group A and mares treated with Buserelin alone in group B. Time for onset of estrus signs have significant difference ( $P < 0.05$ ) between group A and group C and group B and group C.

**Table 1:** Follicular size (mm) in different treatment groups

Treatment Groups	Experimental Days					Mean±SD
	1	5	6	7	8	
A	11.13	16.53	20.45	24.06	29.03	20.24±3.00a
B	11.25	12.9	14.96	16.5	17.31	14.59±2.24b
C	10.36	10.3	10.2	10.5	10.36	10.35±0.63c

Values bearing different alphabets in a column differ significantly ( $P < 0.05$ ).

### 3.2. Ultrasound examination to determine the follicular dynamics

All mares were scanned at different days e.g. day 1 (before start of treatment protocol) and day 5, day 6, day 7, and day 8 of Buserelin regime in all three groups through trans-rectal ultrasonography by using linear array transducer with 7.5 MHz frequency. The follicular dynamics was measured in all groups at different days.

Mean Values for overall follicular size (mm) at day 1, 5, 6, 7 and 8 for group A, B and C is given in Table 1. Change in overall follicular dynamics was significant ( $P < 0.05$ ) among group A, B and C as values were 20.24±3.00, 14.59±2.24 and 10.35±0.63, respectively. It was higher in group A, followed by groups B and C.

Mean values for overall estrogen level (pg/ml) at day 1, 5, 6, 7 and 8 for group A, B and C is given in Table 2. Changes in serum E2 contents were significantly ( $P < 0.05$ ) higher in group A (30.33±12.14pg/ml) followed by in group B (21.70±9.45pg/ml) and group C (15.08±1.19pg/ml). Mean values for overall progesterone level (ng/ml) at day 1, 5, 6, 7, and 8 for group A, B and C is given in Table 3. Overall progesterone level (ng/ml) pattern of serum P4 contents differ non-significantly among various groups.

**Table 2:** Estrogen levels (pg/ml) in different treatment groups

Treatment Groups	Experimental Days					Mean±SD
	1	5	6	7	8	
A	16.65	24.80	30.45	35.9	43.83	30.33±12.14 <sup>a</sup>
B	16.61	19.33	22.11	24.6	25.84	21.70±9.45 <sup>b</sup>
C	15.3	15.23	15.22	14.41	15.225	15.08±1.19 <sup>c</sup>

Values bearing different alphabets in a column differ significantly ( $P < 0.05$ ).

**Table 3:** Progesterone levels (ng/ml) in different treatment groups

Treatment Groups	Experimental Days					Mean±SD
	1	5	6	7	8	
A	0.21	0.22	0.20	0.22	0.20	0.21±0.02 <sup>a</sup>
B	0.21	0.22	0.20	0.21	0.21	0.21±0.02 <sup>a</sup>
C	0.21	0.21	0.21	0.21	0.21	0.21±0.02 <sup>a</sup>

Values bearing different alphabets in a column differ significantly ( $P < 0.05$ ).

## 4. DISCUSSION

Mares are seasonally polyestrous. Their breeding season starts when the day length is about 16 hours (Kooistra and Ginther 1975). When day light increases it suppress the pineal gland which produces melatonin during dark hours (Colquhoun et al. 1987). This melatonin suppression will stimulate the hypothalamus which secretes the GnRH that travels to pituitary gland and stimulate the production of FSH/LH. This FSH/LH travels through blood and acts on ovaries to stimulate the production and ovulation of follicles (Aurich 2011). While on the other hand mares showing anestrus when the day length is short, melatonin attains its peak by suppressing the hypothalamus and causing the decrease in GnRH production ultimately down regulating the FSH/LH concentrations. Decreased FSH/LH concentrations will lead to decreased follicular growth and leading to anestrus in mares (Donadeu and Watson 2007).

The current study was needed to conduct so that the reproductive performance of the mares can be increased during non-breeding season and to increase the number of foals per year in a stud which is the ultimate goal of breeding studs. So, this study was conducted in the months of October and November which are the non-breeding months for mares. Due to suppression of GnRH by increased level of melatonin, GnRH was administered exogenously

in treatment groups to bring the mares in estrus and one group was supplemented with tocopherol and selenium along with GnRH which enhances the GnRH performance in a group.

In the start of winter season, due to down regulation of melatonin, mares become quiescent and were supplemented with buserelin (a GnRH analogue) to enhance growth of quiescent follicles (Immonen and Cuervo-Arango 2020), a single administration of buserelin (40 $\mu$ g) was very effective for inducing ovulation in heavy draft mares. The timing of administration during the growth of the dominant follicle ( $\geq 45$ mm) and decrease in endogenous estrogen (and thus uterine edema) may explain its efficacy in inducing ovulation (Miki et al. 2016).

In present study, Estrus response was 83.3% and 28.6% in group A, B, respectively and showed significant ( $P < 0.05$ ) relation with each other and with control group mares. Meyers-Brown et al. (2013) said that mares in treatment group had developed  $\geq 35$ mm follicle within  $7.4 \pm 1.6$ -days of recombinant follicle stimulating hormone treatment, mares of control group (kept untreated) exhibit no significant development of follicles. It has been reported earlier that a single injection of 40  $\mu$ g buserelin when follicles are at least 45mm in diameter and uterine edema is decreased is effective for inducing ovulation (Miki et al. 2016).

Results revealed significantly ( $P < 0.05$ ) greater number of mares, given Buserelin and Selevit in combination showed signs of estrus compared with control and those administered with Buserelin alone. These findings for estrus induction rate are in agreement with earlier reports of Rezende et al. (2018) who reported administration of buserelin induce estrus in bitch during anestrus phase.

Mares show anestrus behavior during short days of the year. Melatonin is a dark/sleep hormone, in short days its secretion increases, causing gonadotropin releasing hormone down regulation (Dini et al. 2019). Decreased level of follicle stimulating hormone leads to decreased follicular growth & developments. Similarly, decreased level of luteinizing hormone fails to grow dominant follicles, explaining the reason of least follicular diameter in winter anestrus animals (Chankitisakul et al. 2017). O'Neil (2019) intended to find out that continuous or consistent treatment with different doses of estradiol (E2) increases pituitary response to gonadotropin releasing hormone in winter anestrus mares. At day 28, 35 mm follicle developed in, 0/6 control group, 6/6 gonadotropin releasing hormone group, 2/6 gonadotropin releasing hormone plus high estradiol group and 5/6 gonadotropin releasing hormone plus low estradiol group mares. Estrogen subcutaneous implants failed to increase response of mares to gonadotrophin releasing hormone. Camillo et al. (2014) reported the possibility of inducing ovulation in jennies between 24 and 48 h with a single subcutaneous injection of a very low dose of GnRH agonist buserelin: the minimum effective dose was 0.04mg.

In present study, because of buserelin and positive role of tocopherol for fertility, a significant ( $P \leq 0.05$ ) relation between group-A (mares treated with tocopherol and selenium along with buserelin), B (buserelin alone) and C (kept untreated) for estrus response, follicular dynamics and estrogen levels was seen. But the days for the onset of estrus from the start of buserelin treatment protocol was not significant as also endorsed by Meyers-Brown et al. (2017).  $\alpha$ -tocopherol supplementation of culture media significantly ( $P < 0.01$ ) increased the proportion of oocytes that reached metaphase II blastocyst rates compared to non- $\alpha$ -tocopherol supplemented media (Farzollahi et al. 2016). Thorson et al. (2014) reported that gonadotropin releasing hormone triggers secretion as well as synthesis of luteinizing hormone in absence of pituitary gland's refractoriness. Continuous administration of gonadotrophin releasing hormone is a highly acceptable option for winter anestrus mares.

Change in serum E2 contents was significant ( $P < 0.05$ ) among groups. These finding are supported by Se supplementation positively modulated the antioxidant status and reduced the rate of apoptosis in aging mice, thereby improving the in vitro developmental potential of embryos resulting from GV oocytes, potentially via modulation of expression of Gpx1, Gpx3, Gpx4, Selen of, p21, and Bcl-2 genes at the level of the ovaries. Based on these results, it is envisaged that Se deficiency can negatively impact ovarian function, and that Se supplementation at an appropriate level can substantiate ovarian function and female fertility (Qazi et al. 2020). These results are in consistent with previous reports that the treatment with 40 $\mu$ g buserelin when follicles are  $\geq 45$  mm in diameter may be used as an effective management tool to shorten the interval to ovulation in heavy draft mares and for improving reproductive efficiency in the case of reproductive failure (Miki et al. 2016). Karam et al. (2018) endorsed these findings by inducing estrus in anestrus mares using different regimes of gonadotrophin releasing hormone, estrogen and progesterone. After sequential hormonal therapies rate of estrus induction was about eighty one percent (21/26). A continuous significant increase in the serum concentration of estradiol was observed.

Overall progesterone level remains non-significant in all three groups. Mean $\pm$ SD of overall progesterone level (ng/ml) was  $0.21 \pm 0.02$ ,  $0.21 \pm 0.02$  and  $0.21 \pm 0.02$  in group A, group B and group C, respectively. This concept is supported by King et al. (1988) who studied the progesterone concentration in 20 seasonally acyclic mares. 14 out of 20 mares shows a significantly ( $P < 0.05$ ) decline in P4 concentration during transition as well as winter season. This decline in progesterone concentration is still not clear. Many researchers say that it may be due to decrease in LH and prolactin concentrations as the day light changes (Johnson 1986). But some says that cholinergic system of the mares inhibits the releases of progestins (P4) during the winter season (Kasson and Hsueh 1985).

**Conclusion:** In group-A, 83.3% mares came into heat during Buserelin+Tocopherol and selenium treatment which indicates that tocopherol and selenium increases the performance of buserelin in late season anestrus mares. In group-B, 33.3% mares came into heat during buserelin treatment at different days and no mare came into heat from group-C or control group. Onset of estrus varies between 6-7 days in responsive mares and maximum mares attained 30mm follicle size within 7 days of treatment.

**Author's Contribution:** Study concept and design were tailored by FA and LAL. Data were collected by: MAZ, SSUH, AI, MSI and AZ and analysis and interpretation of results were carried out by FA, LAL, RH and MSS. Manuscript was written by FA and MAZ. All authors reviewed and approved the final version of the manuscript.

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## REFERENCES

- Aurich C, 2011. Reproductive cycles of horses. *Animal Reproduction Science* 124: 220-228. <https://doi.org/10.1016/j.anireprosci.2011.02.005>.
- Basini G and Tamanini C, 2000. Selenium stimulates estradiol production in bovine granulosa cells: Possible involvement of nitric oxide. *Domestic Animal Endocrinology* 18: 1-17. [https://doi.org/10.1016/S0739-7240\(99\)00059-4](https://doi.org/10.1016/S0739-7240(99)00059-4)
- Benabdellaziz A, Boudjemai S, Khelili R, Besbaci M and Kaidi R, 2020. Development of in-house RIA kit for progesterone in cow's skim milk. *Journal of Immunoassay and Immunochemistry* 41: 195-207. <https://doi.org/10.1080/15321819.2019.1708386>
- Camillo F, Vannozi I, Tesi M, Sabalini C, Rota A, Paciullo E and Panzani D, 2014. Induction of ovulation with buserelin in jennies: In search of the minimum dose. *Animal Reproduction Science* 151: 56– 60. <https://doi.org/10.1016/j.anireprosci.2014.09.011>
- Ceko MJ, Hummitzsch K, Hatzirodos N, Bonner WM, Aitken JB, Russell DL, Lane M, Rodgers RJ and Harris HH, 2015. X-Ray fluorescence imaging and other analyses identify selenium and GPXI as important in female reproductive function. *Metallomics* 7: 71–82. <https://doi.org/10.1039/c4mt00228h>
- Chankitisakul V, Pitchayapipatkul J, Chuawongboon P, Rakwongrit D, Sakhong D, Boonkum W and Vongpralub T, 2017. Comparison of three superovulation protocols with or without GnRH treatment at the time of artificial insemination on ovarian response and embryo quality in Thai native heifers. *Tropical Animal Health and Production* 49: 633-639. <https://doi.org/10.1007/s11250-017-1243-6>.
- Chatterjee A, Rajikun MH, Chatterjee R and Ghosh S, 2006. Stress and how it affects reproduction. *Biomedical Research* 17: 1-6. <https://www.researchgate.net/publication/255637009>
- Chauhan SS, Celi P, Ponnampalam EP, Leury BJ, Liu F and Dunshea FR, 2014. Antioxidant dynamics in the live animal and implications for ruminant health and product (meat/milk) quality: role of vitamin E and selenium. *Animal Production Science* 54: 1525–1536. <https://doi.org/10.1071/AN14334>
- Colquhoun K, Eckersall P, Renton J and Douglas T, 1987. Control of breeding in the mare. *Equine Veterinary Journal* 19: 138-142. <https://doi.org/10.1111/j.2042-3306.1987.tb02610.x>
- Dini P, Ducheyne K, Lemahieu I, Wambacq W, Vandaele H and Daels P, 2019. Effect of environmental factors and changes in the body condition score on the onset of the breeding season in mares. *Reproduction in Domestic Animals* 54: 987-995. <https://doi.org/10.1111/rda.13452>
- Donadeu F and Watson E, 2007. Seasonal changes in ovarian activity: lessons learnt from the horse. *Animal Reproduction Science* 100: 225-242. <https://doi.org/10.1016/j.anireprosci.2006.12.001>.
- Farzollahi M, Tayefi-Nasrabadi H, Mohammadnejad D and Abedelahi, A, 2016. Supplementation of culture media with vitamin E improves mouse antral follicle maturation and embryo development from vitrified ovarian tissue. *Journal of Obstetrics and Gynaecology Research* 42: 526–535. <https://doi.org/10.1111/jog.12933>
- Field A, 2009. *Discovering Statistics using SPSS (And sex and drugs and rock 'n' roll)*. 3rd Ed, SAGE, London, UK.
- Finno C and Valberg S, 2012. A comparative review of vitamin E and associated equine disorders. *Journal of veterinary internal medicine* 26: 1251-1266. <https://doi.org/10.1111/j.1939-1676.2012.00994.x>
- Immonen I and Cuervo-Arango J, 2020. Effect of timing of postovulatory insemination relative to human chorionic gonadotropin/buserelin treatment with 1 straw of frozen-thawed semen on mare fertility. *Journal of Equine Veterinary Science* 87: 12-24. <https://doi.org/10.1016/j.jevs.2019.102900>
- Ishii M, Ogata H, Shimizu H, Takeuchi Y, Nozawa T, Yamamoto Y, Okamoto T, Shimamura T, Utsumi A and Jitsukawa T, 2002. Effects of vitamin E and selenium administration on pregnant, heavy draft mares on placental retention time and reproductive performance and on white muscle disease in their foals. *Journal of Equine Veterinary Science* 22: 213–220. <https://doi.org/10.1053/jevs.2002.34302>

- Johnson AL, 1986. Serum concentrations of prolactin, thyroxine and triiodothyronine relative to season and the estrous cycle in the mare. *Journal of Animal Science* 62: 1012-1020. <https://doi.org/10.2527/jas1986.6241012x>
- Karam KM, El-Belely MS, Ismail ST, Fadel MS and Abu Atiah EF, 2018. A new approach for estrus induction in Arabian mares suffering from ovarian inactivity. *Al-Qadisiyah Journal of Veterinary Medicine Sciences* 17: 142-146. <https://doi.org/10.29079/vol17iss1art490>
- Kasson B G and Hsueh AJ, 1985. Cholinergic inhibition of follicle-stimulating hormone induced progesterin production by cultured rat granulosa cells. *Biology of Reproduction* 33: 1158-1167. <https://doi.org/10.1095/biolreprod33.5.1158>
- King S, Neumann K, Nequin L and Weedman B, 1993. Time of onset and ovarian state prior to entry into winter anestrus. *Journal of Equine Veterinary Science* 13: 512-515. [https://doi.org/10.1016/S0737-0806\(07\)80266-8](https://doi.org/10.1016/S0737-0806(07)80266-8)
- Kooistra L and Ginther O, 1975. Effect of photoperiod on reproductive activity and hair in mares. *American Journal of Veterinary Research* 36: 1413-1419.
- Kwong GPS and Klein C, 2018. Deslorelin and naltrexone stimulate follicular development in mares during autumn transition and early anestrus. *Canadian Veterinary Journal* 60: 855-858.
- Meyers-Brown GA, McCue PM, Troedsson MH, Klein C, Zent W, Ferris RA, Lindholm AR, Scofield DB, Claes AN, Morganti M, Colgin MA, Wetzel RL, Peters A R and Roser JF, 2013. Induction of ovulation in seasonally anestrous mares under ambient lights using recombinant equine FSH (reFSH). *Theriogenology* 80: 456-462. <https://doi.org/10.1016/j.theriogenology.2013.04.029>
- Miki W, Oniyama H, Takeda N, Kimura Y, Haneda S, Matsui M, Taya K and Nambo Y, 2016. Effects of a single use of the GnRH analog buserelin on the induction of ovulation and endocrine profiles in heavy draft mares. *Journal of Equine Veterinary Science* 27: 149-156. <https://doi.org/10.1294/jes.27.149>
- Morris LHA and Allen WR, 2002. Reproductive efficiency of intensively managed thoroughbred mares in Newmarket. *Equine Veterinary Journal* 34: 51-60. <https://doi.org/10.2746/04251640277618122>
- Murphy B, 2019. Circadian and circannual regulation in the horse: Internal timing in an elite athlete. *Journal of Equine Veterinary Science* 76: 14-24. <https://doi.org/10.1016/j.jevs.2019.02.026>
- Nagy P, Guillaume D and Daels P, 2000. Seasonality in mares. *Animal Reproduction Science* 60: 245-262. [https://doi.org/10.1016/s0378-4320\(00\)00133-0](https://doi.org/10.1016/s0378-4320(00)00133-0)
- Nishiwaki-Ohkawa T and Yoshimura T, 2016. Molecular basis for regulating seasonal reproduction in vertebrates. *Journal of Endocrinology* 229: 117-127. <https://doi.org/10.1530/JOE-16-0066>
- O'Neil MM, Korthanke CM, Scarpa JO, Welsh TH, Cardoso RC and Williams GL, 2019. The Effects of estradiol-17beta in modulating pituitary-ovarian responsiveness to continuous infusion of gonadotropin-releasing hormone in winter anovulatory mares. *Journal of Equine Veterinary Science* 75: 93-103. <https://doi.org/10.1016/j.jevs.2019.01.013>
- Packer L and Landvik S, 1989. Vitamin E: Introduction to biochemistry and health benefits. *Annals of the New York Academy of Sciences* 570: 1-6. <https://doi.org/10.1111/j.1749-6632.1989.tb14903.x>
- Polasek TCM, Kozicki LE, Pedrosa VB, Weiss RR, Bertol MAF, Camargo CE and Talini R, 2017. Impact of a progesterone-releasing intravaginal device and inflammatory reaction on ovarian activity in embryo-recipient anestrus mares. *Theriogenology* 90: 175-184. <https://doi.org/10.1016/j.theriogenology.2016.11.005>
- Qazi IH, Cao Y, Yang H, Angel C, Pan B, Zhou G and Han H, 2020. Impact of dietary selenium on modulation of expression of several non-selenoprotein genes related to key ovarian functions, female fertility, and proteostasis: a transcriptome-based analysis of the aging mice ovaries. *Biological Trace Element Research*. <https://doi.org/10.1007/s12011-020-02192-x>
- Rezende RS, Eurides D, Barbosa CP, Lacerda M S, Sampaio RL and Gomes AL, 2018. Use of a GnRH synthetic analog (buserelin) for estrous induction in female dogs. *Brazilian Journal of Veterinary and Animal Science* 70: 656-660. <https://doi.org/10.1590/1678-4162-9587>
- Siemiński MJ, Kozdrowski R, Szóstek-Mioduchowska AZ, Raś A and Nowak M, 2019. Evidence for increased content of PGF 2 $\alpha$ , PGE 2, and 6-keto-PGF 1 $\alpha$  in endometrial tissue cultures from heavy draft mares in anestrus with endometritis. *Journal of Equine Veterinary Science* 77: 107-113. <https://doi.org/10.1016/j.jevs.2019.02.014>
- Talukder S, Kerrisk KL, Gabai G and Celi P, 2017. Role of oxidant-antioxidant balance in reproduction of domestic animals. *Animal Production Science* 57: 1588-1597. <https://doi.org/10.1071/AN15619>
- Thorson J, Allen C, Amstalden M and Williams G, 2014. Pharmacologic application of native GnRH in the winter anovulatory mare, I: Frequency of reversion to the anovulatory state following ovulation induction and cessation of treatment. *Theriogenology* 81: 579-586. <https://doi.org/10.1016/j.theriogenology.2013.11.018>
- Zarczynska K, Sobiech P, Radwinska J and Rekawek W, 2013. Effects of selenium on animal health. *Journal of Elementology* 329-340. <https://doi.org/10.5601/jelem.2013.18.2.12>