https://doi.org/10.22319/rmcp.v10i4.4703

Review

Supplementation of ascorbic acid to improve fertility in dairy cattle. Review

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Abstract:

Ascorbic acid (vitamin C: VC) is an antioxidant that participates in the regulatory processes involved in the development of ovarian structures and fertility. However, supplementation of VC to dairy cattle to improve fertility has received little attention. However, reduced fertility in dairy cattle associated with high genetic merit for milk production and heat stress, which also diminish blood VC concentrations, suggest a potentially beneficial role for VC supplementation. The objectives of this review are to contribute to the current knowledge regarding the relationship between VC and fertility and to share many experiences that support the relevance of VC supplementation to improve dairy cattle reproductive performance.

Key words: Antioxidants, Ascorbic acid, Reproduction.

Received: 18/10/2017

Accepted: 11/07/2018

Introduction

The economic gains of a dairy farm increase as cattle reproductive efficiency improves. However, the historical decline in fertility of Holstein dairy cows hampers profitability, but at the same time offers a challenge to develop strategies to enhance reproductive performance. The cause of low fertility in modern Holstein dairy cattle is multifactorial. The main associated are the improvement in genetic merit to milk production, the inability to meet nutritional requirements, the adverse environmental conditions and the susceptibility to diseases that compromise oocyte and embryo viability[1].

The exact cause of low fertility is unknown, but oxidative stress could be implicated. Oxidative stress results when free radicals exceed the organism's antioxidant capacity[2]. Free radicals are molecules with an unpaired electron, highly reactive and normally produced in living aerobic organisms[3]. At a controlled production rate, they serve as molecular signals, but over production may result in a pathological process[4]. Sources of free radicals that may surpass the cow's antioxidant capacity include milk production yield and heat stress. High milk producers have higher blood concentrations of oxidative stress markers than those that produce less milk[5], and are also more susceptible to heat stress[6]. This is relevant because heat stress produces oxidative stress in dairy cattle[7]. Oxidative stress creates unfavorable intraoviductal conditions[8] that result in embryo death[9].

Oxidative stress is counteracted by antioxidants, which suppress the deleterious effect of free radicals by giving them one electron. One antioxidant that is relevant to mammalian reproduction is water soluble ascorbic acid (vitamin C, here after referred to as VC)[10]. The chemistry and biological functions of VC in cattle have been reviewed by others[11], and will therefore be not further addressed here. However, the impact of VC supplementation on dairy cattle fertility has been poorly studied, probably because bovines can synthetize their own VC in the liver from glucose[11], and thus have no need for external supplementation[12]. Nevertheless, the same factors that are blamed for disrupting fertility (high milk yield and heat stress) decreased blood VC concentration in dairy cattle[13,14]. It might be suspected that if VC is necessary for reproduction, a

diminished supply could affect fertility. Previous research has shown that supplementation of VC is advantageous to improving reproductive performance of repeat breeder cows[15] and dairy cattle under heat stress conditions[16]. It is important to consider that VC supplementation and impacts on dairy cattle fertility deserve more attention.

The objective of this review is to contribute to the current knowledge regarding the relationship between VC and fertility, and to share the experiences on the relevance of VC supplementation to improve dairy cattle reproductive performance.

Ovarian follicle and corpus luteum development

Vitamin C deficiency increases the number of atretic follicles[17]. However, supplementation attenuates follicular cell apoptosis[18], promotes primordial follicle activation[19], increases the population of growing follicles[20] and reduces those in atretic state[21]. These findings suggest that VC supports the development of healthy ovarian follicles.

The ovarian follicle is under constant structural remodeling. Its diameter increases up to 475 times from the primordial to the ovulatory size[22,23]. This increase in size implies a constant remodeling of the follicular basal lamina[24] and changing intrafollicular concentrations of VC, which are higher in smaller follicles[25]. The follicular basal lamina gives the follicle stability and serves as a molecule filter[24], but it needs increasing amounts of collagen as it increases in size[26]. Since VC is a cofactor in collagen synthesis[27], it is logical to assume that VC would be required in higher quantities in developing follicles. In fact, supplementation of VC improves follicle survival and increases the odds of a follicle reaching preovulatory size[28]. This could be explained by VC preventing follicular cell death and maintaining base membrane integrity as the follicle grows[18,29].

Under an environment with a regressing corpus luteum, the dominant follicle will reach the preovulatory state. At this stage, VC is needed for normal follicular steroidogenesis[30], which is accomplished by promoting the expression of key enzymes involved in steroidogenesis such as aromatase and P450 cholesterol side-chain cleavage[31]. However, as the follicle grows there is a reduction in the concentration of VC. Preovulatory follicle has lower intrafollicular concentrations of VC than large follicles from other stages of the estrus cycle[32]. This reduction may result from a higher intrafollicular concentration of IGF-I, which induces the uptake of VC by granulosa cells[33]. The LH surge also causes a reduction in VC concentrations[34], probably by increasing intrafollicular reactive oxygen species (ROS) concentrations[35].

The reduced intrafollicular concentrations of VC at the preovulatory stage may be part of the mechanism controlling ovulation. The collagen in the follicular basal lamina is reduced as the follicle grows, which makes it more expandable and easier to remodel[36]. The reduced intrafollicular concentrations of VC, together with degradation of collagen in preovulatory follicles, results in the weakening and rupture of the basal lamina, which are crucial events that can lead to preovulatory follicle rupture[37,38].

The number of pregnant women with luteal phase defects increases after supplementing VC, which likely worked by increasing corpus luteum progesterone[39]. Corpus luteum diameter[32] and concentration of progesterone[40] has been related to VC concentration. In addition, the content of VC is higher during the early stages of corpus luteum development[41], reaching the highest concentration, at least in bovines, on d 12 of the estrous cycle[42]. Furthermore, one key element in the relationship between VC and the corpus luteum is that this vitamin is required, as mentioned previously, for the synthesis of collagen, which is essential for corpus luteum development[43].

Vitamin C and fertility

Vitamin C improved fertility[44]. The enhancement in oocyte and embryo development could explain these results[45,46]. Unfortunately, the limited information available on this topic has been obtained mostly under *in vitro* conditions. In contrary, high doses of VC might harm both the oocyte (750 µM mL^{-1}) and embryo development (>200 µM in culture medium)[47,48], possibly resulting from a pro-oxidant effect of VC. The VC at low concentrations can act as an antioxidant while the opposite occurs at high concentrations, which may depend on the concentration of metal ions (iron)[49]. A pro-oxidant effect of VC could be expected as the concentration of metal ions increases[50]. The latter may be true under *in vitro* conditions, but it is unlikely to occur in living organisms[51].

Relationship between vitamin C and vitamin E

Vitamin C may control follicular development by interacting with other elements known to affect fertility. It is well accepted that after vitamin E fulfills its antioxidant activity, it can be reactivated by VC[52], which increases its availability[53]. Vitamin E deficiency disrupt follicle development, produces estrous cycle abnormalities and pregnancies loss[54]. It is not known exactly the blood concentration at which vitamin E can be considered as adequate or deficient in cattle. Vitamin E blood concentrations >1 µg mL^{-1} can considered as adequate, but there is not agreement on this topic[55]. In addition, it is unaware of any vitamin E recommendation for optimal reproduction

performance in cattle. However, previous work (see next section of this manuscript and reference 16), have shown that supplementation of 3,000 IU of vitamin E during a synchronized estrus is advantageous to improve fertility in dairy cattle.

The relationship between vitamins C and E in reproductive issues has received little attention. An antioxidant system, that includes vitamins C and E, is activated during ovarian steroidogenesis[42]. The supplementation with vitamin C (125 mg kg^{-1} d^{-1}) and E (75 mg kg^{-1} d^{-1}) to rats increases blood concentrations of testosterone, FSH and LH[56]. These higher concentrations of gonadotropins are in agreement with the fact that VC stimulates its secretion from pituitary[57]. Studies *in vitro* have shown a positive effect of vitamin C and E on oocyte quality and embryo development when supplemented separately, but not together[53,58,59]. Addition of vitamin C and E to the maturation medium impairs blastocyst occurrence rate by preventing the formation of the amount of ROS necessary for oocyte developmental competence[53]. This is acceptable because a tonic supply of ROS has proved to interrupt oocyte meiotic arrest[60]. However, it is unlikely that the situation described by Dalvit *et al*[53] also occur *in vivo* because supplementation of both vitamins has resulted in more pregnancies in dairy cattle (see next section of this manuscript and reference 16). In addition, an improvement in embryo quality after injecting superovulated cattle donors before estrus with two antioxidants, β-carotene and vitamin E, has been reported[61].

In vitro studies resemble conditions found under physiological conditions. However, contrary to *in vivo* conditions, *in vitro* systems are static, where metabolic activity, nutrient adsorption and storage, as well as waste disposal are limited by time and medium culture conditions. In addition, adaptation to changing conditions is faster in *in vivo* systems. Therefore, when supplementing vitamin C concomitant with vitamin E, the living organism choose between to storage, to excrete or to distribute them to where they are needed. This avoid possible harmful effects on cell biological process such as those affecting oocyte quality and embryo development.

Experiences supplementing vitamin C to dairy cattle

The evidence presented here supports a prominent role of VC on fertility. The first approach to evaluating the effect of VC on dairy cattle fertility was carried out on cows under heat stress conditions[16]. The results of this study revealed that injecting both vitamin C and E results in more pregnant cows than administering one or the other separately. In addition, no effect of vitamins supplementation was found on preovulatory follicle and corpus luteum size. These findings led to assume that the increased number of pregnant cows, obtained after supplementing both vitamins, was the result of cows carrying a healthier follicle, which eventually becomes a corpus luteum that produces more progesterone than that carried by non-supplemented cows. To prove this assumption a second trial was carried out (T2).

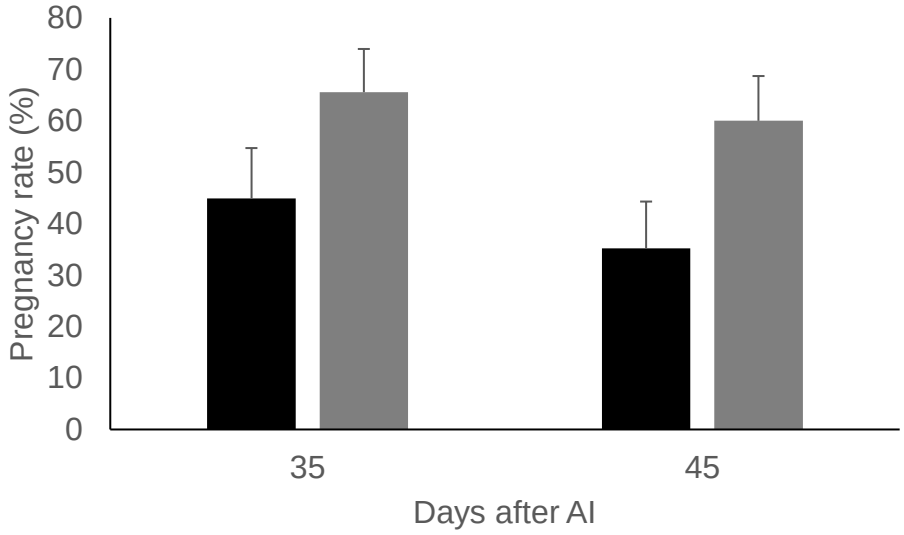
The general procedure, as well as justification of the doses and time of vitamins injections used in T2 is explained in detail elsewhere[16]. Briefly, the follicular wave of the cows was synchronized with a device containing 1.0 g of progesterone (Sincrogest®, Ourofino Agronegocio), inserted intravaginally for 8 d, and an intramuscular (i.m.) injection of 250 µg of GnRH analogue (GnRH, Sanfer). Estrus behavior was induced by an i.m. injection of 500 µg of cloprostenol (Celosil, MSD, Animal Health) at intravaginal device removal. Once the intravaginal device was withdrawn, the animals were constantly monitored by direct observation for signs of standing estrus. The cows were artificially inseminated 12 h after standing estrus with a single dose (approximately 20 x 10^6 spermatozoa) of semen from a single bull of proven fertility. Cows that received vitamins (n=32. Control group, n=28) were injected with a single i.m. injection of 3,000 IU of vitamin E ((±) α-tocopherol, Sigma-Aldrich)) on d-5 (day 0 is the day of intravaginal device removal) and subcutaneous (s.c.) injections with a total dose of 3,000 mg of VC (ascorbic acid, Q.P., Reasol) on d-5, immediately after estrus detection and 2 d after artificial insemination.

As depicted in Table 1, vitamin supplementation did not affect preovulatory follicle or corpus luteum size. In addition, no effect was noted on blood estradiol and progesterone production. However, in agreement with previous findings[16], pregnancy rate was higher (*P*=0.06) in cows injected with vitamins 45 d after artificial insemination (Figure 1).

Table 1: Least square means (±SE) for the effect of injecting vitamin C and E on ovarian structures size, estrus presentation and hormone concentrations in Holstein dairy cows

	Treatment		
Variable	**Control (n=28)**	**Vitamin Cand E (n=32)**	***P*-value**
Time to estrus, h	57.1±4.89	58.4±4.57	0.67
Diameter of the preovulatory follicle, mm	18.3±0.57	17.2±0.60	0.21
Estradiol concentration, pg mL^{-1}	45.1±3.12	46.8±3.26	0.71
Area of the corpus luteum, cm^2	6.9±0.39	6.7±0.37	0.74
Progesterone concentration, ng mL^{-1}	10.8±1.60	12.5±1.60	0.26

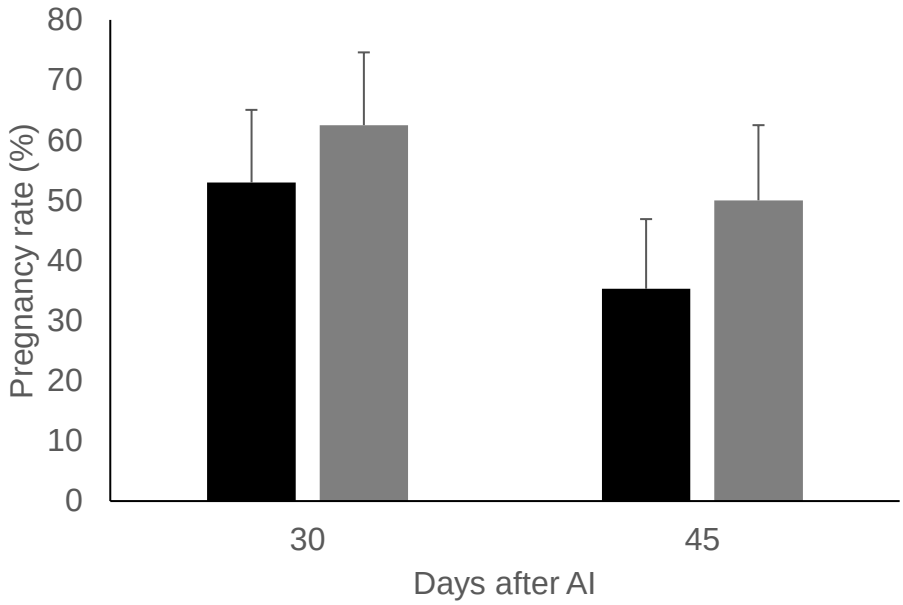
Figure 1: Pregnancy rate 35 and 45 days after AI in control group (black bars, n=28) and Holstein dairy cows injected with vitamins C and E (grey bars, n=32)



Estrus synchronization is a reproductive tool used in dairy cattle to improve fertility because it makes possible to control the onset of estrus. However, most technicians prefer to use fixed-time artificial insemination because it avoids the need for estrus detection. In addition, it is very convenient because all the cows are scheduled to be inseminated at the same time. Based on previous findings, it was decided to incorporate vitamin C and E injections to a fixed-time artificial insemination protocol (T3) to increase the number of pregnant cows. Briefly, cows were injected i.m. with 250 µg of GnRH analogue on d 0, 7 days after administering an i.m. injection of 500 µg of cloprostenol. A second dose of GnRH was given to cows 48 h after injecting cloprostenol. Insemination was performed 14 to 16 h after the second injection of GnRH. Injections of vitamins C and E were carried out as mentioned in T2, but the first injection of vitamin C and E was given 3 d after the first injection of GnRH. The second and third injections of VC were administered just after the second injection of GnRH and 2 d after artificial insemination.

The effect of vitamin C and E injections on preovulatory follicle diameter (16.8 ± 0.70 *vs* 16.2 ± 0.77 mm, for control group and cows injected with vitamins) and area of the corpus luteum (5.4 ± 0.48 *vs* 6.1 ± 0.50 cm^2, for control group and cows injected with vitamins) were not significant. Similar to previous results[20] and with T2, a greater percentage of cows were found to be pregnant 30 and 45 d after artificial insemination in the group of cows supplemented with vitamins than those in the control. However, the differences are not significant, most likely because of the small sample size used in T3 (cows injected with vitamins, n=16. Control group, n=17), Figure 2.

Figure 2: Pregnancy rate 30 and 45 days after AI in control group (black bars, n=17) and Holstein dairy cows injected with vitamins C and E (grey bars, n=16)



The results obtained show that VC injections in combination with vitamin E are a feasible way to improve dairy cattle fertility. This effect is not mediated by changes in preovulatory or corpus luteum size, nor by affecting estradiol or progesterone production. A likely explanation for the increased pregnancy rate in dairy cattle injected with vitamins C and E is that cows supplemented with vitamins produce better quality oocytes and embryos than those not supplemented.

Conclusions

In conclusion, contrary to current thought, evidence suggest that supplementation of vitamin C to dairy cattle improve fertility. However, there is a need to investigate the optimal dose and time of vitamin C supplementation to improve dairy cattle reproductive performance.

Literature cited:

1. Dobson H, Walker SL, Morris MJ, Routly JE, Smith RF. Why is it getting more difficult to successfully artificially inseminate dairy cows? Animal 2008;2:1104-1111.

2. Halliwell B, Whiteman M. Measuring reactive species and oxidative damage in vivo and in cell culture: how should you do it and what do the results mean? Brit J Pharmacol 2004;142:231-255.

3. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol 2007;39:44-84.

4. Rizzo A, Roscino MT, Binetti F, Sciorsci RL. Roles of reactive oxygen species in female reproduction. Reprod Domest Anim 2012;47:344-352.

5. Castillo C, Hernández J, López-Alonso M, Miranda M, Benedito JL. Values of plasma lipid hydroperoxides and total antioxidant status in healthy dairy cows: preliminary observations. Arch Tierz 2003;46: 227-233.

6. Gantner V, Bobic T, Gantner R, Gregic M, Kuterovac K, Novakovic J, Potocnik K. Differences in response to heat stress due to production level and breed of dairy cows. Int J Biometeorol 2017;1-11.

7. Bernabucci U, Ronchi B, Lacetera N, Nardone A. Markers of oxidative status in plasma and erythrocytes of transition dairy cows during hot season. J Dairy Sci 2002;85:2173-2179.

8. Takahashi M. Heat stress on reproductive function and fertility in mammals. Reprod Med Biol 2012;11:37-47.

9. Celi P, Merlo M, Da Dalt L, Stefani A, Barbato O, Gabai G. Relationship between late embryonic mortality and the increase in plasma advanced oxidized protein products (AOPP) in dairy cows. Reprod Fert Develop 2011; 23:527-533.

10. Luck MR, Jeyaseelan I, Scholes RA. Ascorbic acid and fertility. Biol Reprod 1995;52:262-266.

11. Ranjan R, Ranjan A, Dhaliwal GS, Patra RC. l-Ascorbic acid (vitamin C) supplementation to optimize health and reproduction in cattle. Vet Quart 2012;32:145-150.

12. NRC. National Research Council. Nutrient requirements of dairy cattle. 7th ed. Washington, DC, USA: National Academic Press; 2001.

13. Padilla L, Matsui T, Kamiya Y, Kamiya M, Tanaka M, Yano H. Heat stress decreases plasma vitamin C concentration in lactating cows. Livest 2006;101:300-304.

14. Jóźwik A, Strzałkowska N, Bagnicka E, Grzybek W, Krzyżewski J, Poławska E, Kołataj A, Horbańczuk JO. 2012. Relationship between milk yield, stage of lactation, and some blood serum metabolic parameters of dairy cows. Czech J Anim Sci 2012;57:353-360.

15. Phillips PH, Lardy HA, Boyer PD, Werner GM. The relationship of ascorbic acid to reproduction in the cow. J Dairy Sci 1941;24:153-158.

16. González Maldonado J, Santos RR, De Lara RR, Ramírez GV. Impacts of vitamin C and E injections on ovarian structures and fertility in Holstein cows under heat stress conditions. Turk J Vet Anim Sci 2017;41:345-350.

17. Kramer MM, Harman MT, Brill AK. Disturbances of reproduction and ovarian changes in the guinea-pig in relation to vitamin C deficiency. Am J Physiol 1933 106:611-622.

18. Thomas FH, Leask R, Srsen V, Riley SC, Spears N, Telfer EE. Effect of ascorbic acid on health and morphology of bovine preantral follicles during long-term culture. Reproduction 2001;122:487-495.

19. Andrade ER, van den Hurk R, Lisboa LA, Hertel MF, Melo-Sterza FA, Moreno K, et al. Effects of ascorbic acid on in vitro culture of bovine preantral follicles. Zygote 2012;20:379-388.

20. Al-Katib SR, Al-Azam AHA, Habeab SA. The effect of vitamin C on ovary of female white rats treated with kmno4. Histological & physiological study. Kufa J Vet Med Sci 2012;3:1-16.

21. Gürgen SG, Erdoğan D, Elmas C, Kaplanoğlu GT, Ozer C. Chemoprotective effect of ascorbic acid, α-tocopherol, and selenium on cyclophosphamide-induced toxicity in the rat ovarium. Nutrition 2013;29:777-784.

22. Braw-Tal R, Yossefi S. Studies in vivo and in vitro on the initiation of follicle growth in the bovine ovary. J Reprod Fert 1997;109:165-171.

23. Machado-Pfeifer LF, de Souza-Leal SCB, Schneider A, Schmitt E, Nunes-Corrêa M. Effect of the ovulatory follicle diameter and progesterone concentration on the pregnancy rate of fixed-time inseminated lactating beef cows. Rev Bras Zootec 2012;41:1004-1008.

24. Rodgers RJ, Irving-Rodgers HF, van Wezel IL. Extracellular matrix in ovarian follicles. Mol Cell Endocrinol 2000;163:73-79.

25. Meur SK, Sanwal PC, Yadav MC. Acorbic acid in buffalo ovary in relation to oestrus cycle. Indian J Biochem Bio 1999;36:134-135.

26. Haliloglu S, Erdem H, Serpek B, Tekeli T, Bulut Z. The relationship among vitamin C, beta-carotene, vitamin A, progesterone and oestradiol 17-beta concentrations in plasma and cyst fluid of Holstein cows with ovarian cyst. Reprod Domest Anim 2008;43:573-577.

27. Pinnell SR. Regulation of collagen biosynthesis by ascorbic acid: a review. Yale J Biol Med 1985;58:553-559.

28. Rose UM, Hanssen RGJM, Kloosterboer HJ. Development and characterization of an in vitro ovulation model using mouse ovarian follicles. Biol Reprod 1999;61:503-511.

29. Murray AA, Molinek MD, Baker SJ, Kojima FN, Smith MF, Hillier SG, Spears N. Role of ascorbic acid in promoting follicle integrity and survival in intact mouse ovarian follicles in vitro. Reproduction 2001;121:89-96.

30. Paszkowski T, Clarke RN. The Graafian follicle is a site of L-ascorbate accumulation. J Assist Reprod Gen 1999;16:41-45.

31. Wu X, Iguchi T, Itoh N, Okamoto K, Takagi T, Tanaka K, NakanishI T. Ascorbic acid transported by sodium-dependent vitamin C transporter 2 stimulates steroidogenesis in human choriocarcinoma cells. Endocrinology 2008;149:73-83.

32. Serpek B, Baspinar N, Haliloglu S, Erdem H. The relationship between ascorbic acid, oestradiol 17β and progesterone in plasma and in ovaries during the sexual cycle in cattle. Rev Med Vet 2001;152:253-260.

33. Behrman HR, Preston SL, Aten RF, Rinaudo P, Zreik TG. Hormone induction of ascorbic acid transport in immature granulosa cells. Endocrinology 1996;137:4316-4321.

34. Guarnaccia MM, Takami M, Jones EE, Preston SL, Behrman HR. Luteinizing hormone depletes ascorbic acid in preovulatory follicles. Fertil Steril 2000;74:969-963.

35. Yacobi K, Tsafriri A, Gross A. Luteinizing hormone-induced caspase activation in rat preovulatory follicles is coupled to mitochondrial steroidogenesis. Endocrinology 2007;148:1717-1726.

36. Rodgers RJ, Irving-Rodgers HF, Russell DL. Extracellular matrix of the developing ovarian follicle. Reproduction 2003;126:415-424.

37. Murdoch WJ. Regulation of collagenolysis and cell death by plasmin within the formative stigma of preovulatory ovine follicles. J. Reprod Fertil 1998;113:331-336.

38. Khan FA, Das GK. Follicular fluid nitric oxide and ascorbic acid concentrations in relation to follicle size, functional status and stage of estrous cycle in buffalo. Anim Reprod Sci 2011;125:62-68.

39. Henmi H, Endo T, Kitajima Y, Manase K, Hata H, Kudo R. Effects of ascorbic acid supplementation on serum progesterone levels in patients with a luteal phase defect. Fertil Steril 2003;80:459-461.

40. Miszkiel G, Skarzynski D, Bogacki M, Kotwica J. Concentrations of catecholamines, ascorbic acid, progesterone and oxytocin in the corpora lutea of cyclic and pregnant cattle. Reprod Nutr Dev 1999;39:509-516.

41. Luck MR, Zhao Y. Identification and measurement of collagen in the bovine corpus luteum and its relationship with ascorbic acid and tissue development. J Reprod Fertil 1993;99:647-652.

42. Rapoport R, Sklan D, Wolfenson D, Shaham-Albalancy A, Hanukoglu I. Antioxidant capacity is correlated with steroidogenic status of the corpus luteum during the bovine estrous cycle. Biochim Biophys Acta 1998;1380:133-140.

43. Jaglan P, Das GK, Kumar BV, Kumar R, Khan FA, Meur SK. Cyclical changes in collagen concentration in relation to growth and development of buffalo corpus luteum. Vet Res Commun 2010;34:511-518.

44. Yassein SK, Mahmoud M, Maghraby N, Ezzo O. Hot climate effects and their amelioration on some productive and reproductive traits in rabbit does. World Rabbit Sci 2008;16:173-181.

45. Ullah I, Jalali S, Khan H, Shami SA, Kiyani MM. Effect of L-ascorbic acid on Nili Ravi buffalo oocytes during in vitro maturation. Pak J Biol Sci 2006;9:2369-2374.

46. Hossein MS, Kim YW, Park SM, Koo OJ, Hashem MA, Bhandari DP, et al. Antioxidant favors the developmental competence of porcine parthenogenotes by reducing reactive oxygen species. Asian Austal J Anim Sci 2007;20:334-339.

47. Wang X, Falcone T, Attaran M, Goldberg JM, Agarwal A, Sharma RK. Vitamin C and vitamin E supplementation reduce oxidative stress-induced embryo toxicity and improve the blastocyst development rate. Fertil Steril 2002;78:1272-1277.

48. Nadri B, Zeinoaldini S, Kohram H. Ascorbic acid effects on in vitro maturation of mouse oocyte with or without cumulus cell. Afr J Biotechnol 2009;8:5627-5631.

49. Seo MY, Lee SM. Protective effect of low dose of ascorbic acid on hepatobiliary function in hepatic ischemia/reperfusion in rats. J Hepatol 202;36:72-77.

50. Buettner GR, Jurkiewicz BA. Catalytic metals, ascorbate and free radicals: combinations to avoid. Radiat Res 996;45:532-541.

51. Carr A, Frei B. Does vitamin C act as a pro-oxidant under physiological conditions?. FASEB J 1999;13:1007-1024.

52. Chauhan SS, Celi P, Ponnampalam EN, Leury BJ, Liu F, Dunshea FR. Antioxidant dynamics in the live animal and implications for ruminant health and product (meat/milk) quality: role of vitamin E and selenium. Anim Reprod Sci 2015;54: 1525-1536.

53. Dalvit G, Llanes SP, Descalzo A, Insani M, Beconi M, Cetica P. Effect of alpha-tocopherol and ascorbic acid on bovine oocyte in vitro maturation. Reprod Domest Anim 2005;40:93-97.

54. Martin AJP, Moore T. Some effects of prolonged vitamin-E deficiency in the rat. J Hyg 1939;39:643-650.

55. Sivertsen T, Øvernes G, Østerås O, Nymoen U, Lunder T. Plasma vitamin E and blood selenium concentrations in Norwegian dairy cows: regional differences and relations to feeding and health. Acta Vet Scand 2005;46:177–191.

56. Saki G, Jasemi M, Sarkaki AR, Fathollahi A. Effect of administration of vitamins C and E on fertilization capacity of rats exposed to noise stress. Noise Health 2013;15:194-198.

57. Karanth S, Yu WH, Walczewska A, Mastronardi CA, McCann SM. Ascorbic acid stimulates gonadotropin release by autocrine action by means of NO. Proc Natl Acad Sci USA 2001;98:11783-11788.

58. Olson SE, Seidel GE Jr. Culture of in vitro-produced bovine embryos with vitamin e improves development in vitro and after transfer to recipients. Biol Reprod 2000;62:248-252.

59. Miclea I, Păcală N, Zăhan M, Hettig A, Roman I, Miclea V. Influence of alpha-tocopherol and ascorbic acid on swine oocyte viability and maturation. B. UASVM Anim Sci Biotechol 2011;68:338-345.

60. Tripathi A, Khatun S, Pandey AN, Mishra SK, Chaube R, Shrivastav TG, Chaube SK. Intracellular levels of hydrogen peroxide and nitric oxide in oocytes at various stages of meiotic cell cycle and apoptosis. Free Radical Res 2009;43:287-294.

61. Sales JN, Dias LM, Viveiros AT, Pereira MN, Souza JC. Embryo production and quality of Holstein heifers and cows with beta-carotene and tocopherol. Anim Reprod Sci 2008;106:77-89.