Research Article

The Influence of Resveratrol on Conceiving of Repeat Breeder Cows

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Abstract | This study was designed to investigate whether resveratrol influences in repeat breeder cows on the conceiving. Treatment and control group consisted of 12 cows. Two doses of prostaglandin F2 alpha were administered intramuscularly 11 days apart. Treatment group was given 500 mg resveratrol once daily from the administration of the second prostaglandin F2alpha to the insemination day. Control group did not receive resveratrol supplement. The mean erythrocyte GSH-Px levels were nearly similar in both groups before resveratrol therapy. However, at post-therapy, mean GSH-Px concentrations in group T was significantly higher ($P<0.05$) than that of group C. Likewise, mean erythrocyte GSH-Px concentrations raised significantly ($P<0.05$) in group T after resveratrol therapy compared to pre-therapy ones. In group T, there were significant differences between values before and after application in both, conceiving and non-conceiving cows. Resveratrol therapy improved conception rate at a rate of 16.6 percent but the difference was statistically non-significant.

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Introduction

Failure to conceive is the main justification for a dairy cow to be removed from the production herd. Cows that have failed to conceive after at least three successive inseminations, even if they show regular estrus and appear clinically healthy, are assumed as repeat breeders (Taşal, 2011). The incidence of repeat breading in cows has been reported in the range from 5 to 32 percent (Verma *et al*., 2018). In states of repeat breeding, infertility is caused by either fertilization failure or early embryonic death (Wodaje and Mekuria, 2016). It has also been noted that oxidative stress is one of the main causes of embryonic mortality leading to repeat breeding syndrome in cows (Agarwal *et al*., 2005). Treatment procedures for repeat breeder cows are based on supporting of fertilization and of embryonic life. Local or parenteral antibiotics, antiseptics and hormones have been used in the treatment of repeat breading in order to eradicate factors conducive to this condition. The aetiology and pathophysiological complexity of the disease makes it difficult to obtain satisfactory results (Ahmed *et al*., 2013). Inconsistent results between applied treatments, the need for repetition of treatment, the happen probability of resistance against used drug, the high cost of treatment and the inability to use milk after antibiotic treatment are limiting factors in the treatment of repeat breading (Purohit, 2008). Therefore, despite the use of intensive treatment options in repeat breeder cows in recent years, it was impossible to achieve satisfactory improvement in conception rates. In parallel with ongoing research, new or modified treatment options have emerged, but the repeat breeding problem remains current and important. In ancient times, drugs made from plants were used in folk medicine. Nowadays, the harmful effects of synthetic drugs have led to renewed interest in herbal drugs. In recent years, there has been an increasing number of studies on the use of active components obtained from plants in the treatment of reproductive problems. Because of their antimicrobial, antioxidant and anti-inflammatory properties they are regarded as a promising alternative to conventional drugs (Yildiz, 2016). The formation and maintenance of pregnancy can be associated with an oxidative stress condition that can initiate and advance the steps of change that can lead to pregnancy loss. An efficient reduction-oxidation reaction system can provide an optimal medium for embryonic improvement by protecting the balance between the production of reactive oxygen species (ROS) and antioxidant enzymes (Yildiz, 2015). Therefore, therapeutic strategies such as antioxidant treatment of cows with fertility problems can minimize the harmful effects of ROS-induced oxidative stress. Resveratrol is a non-flavonoid polyphenol found in grapes. It has a pleiotropic effect, including antioxidant, anticancer, anti-aging, anti-bacterial, antifungal activity, anti-inflammatory, cardioprotective and neuroprotective actions (Ruan *et al*., 2021; Wang *et al*., 2021). The antioxidant, anti-proliferative and anti-inflammatory effects of resveratrol have been a subject of numerous studies. Resveratrol has been used as a culture medium supplement to increase the developmental potential of oocytes and embryos (Galeati and Spinaci, 2015; Silva *et al*., 2021; Sprícigo *et al*., 2017; Wang *et al*., 2014). In many studies, resveratrol is discussed as a potentially therapeutic element that prevents infertility due to ovarian aging-by-aging inhibition (Özcan *et al*., 2015). Injecting resveratrol into the womb of the cow during artificial insemination increases the rate of conception (Kim *et al*., 2020). However, clinical studies on the use of resveratrol for treatment of repeat breeding have not been performed in cows. Therefore, this study was designed to investigate whether resveratrol supplementation affects conception in repeat breeder cows.

Materials and Methods

The study conducted on 24 Holstein cows, which had not pregnant after three, or more inseminations, no clinically detectable reproductive disorder and no subclinical genital infections according to the White side test results using the procedure described by Anilkumar and Devanathan (Anilkumar and Devanathan, 1996). All animals were managed in two dairy farms with almost similar management conditions. The cows randomly divided into two groups having 12 cows as control group (group C) and treatment group (group T). Two doses of prostaglandin F2 alpha 11 days apart were administered intramuscularly to all cows used in the study (Öztürkler *et al*., 2001). All cows in both groups were then inseminated artificially 10 to 12 hours after standing heat. The treatment group was given orally 500 mg resveratrol (ResVitale) once daily from the administration of the second prostaglandin F2 alpha to the insemination day. The control group did not receive resveratrol supplement. For determination of erythrocyte glutathione peroxidase (GSH-Px) concentrations, blood samples were collected from each cow via the tail vein into heparinized glass tubes immediately before the insemination and the administration of the second prostaglandin F2 alpha. Collected blood samples were centrifuged at 3.000 × g at 4°C for 10 min to allocate the plasma from the erythrocytes. To obtain packed erythrocytes, the remaining erythrocytes were washed two times by a saline solution. To prepare erythrocyte hemolysates, 500 µL of packed erythrocytes were devastated with addition four volumes of cold redistilled water (Yildiz, 2015). The obtaining suspension was centrifuged two times, initially for 10 min in the tube centrifuge at 1.500 × g at 4°C and thereafter in an Eppendorf centrifuge at 5.000 × g for 5 min at 4°C. Clean supernatants was resulted as hemolysates to state GSH-Px. The lysate was stored at -20°C until analysis. The activity of erythrocyte GSH-Px was specified using the procedure formerly defined in Paglia and Valentine (Paglia and Valentine, 1967), and was indicated as U/gHb. Pregnancy diagnosis was made by rectal examination at 60th day after insemination.

Statistical analysis

Conception rate was compared with the relative risk between the groups. Levels of erythrocyte GSH-Px were compared using t-test to determine the effects of treatment. Differences of 0.05 or less in probability values were accepted as statistically significant.

Results and Discussion

Resveratrol is predicted to be a potential therapeutic agent to prevent infertility due to its antioxidant properties and its positive effect on improving follicles and producing high quality embryos. The supplement of resveratrol during in vitro maturation has been shown to have favourable impacts on in vitro embryo production in cows (Wang *et al*., 2014). Pre-treatment of blastocysts with 1.0 µM resveratrol has been reported to enhance pregnancy rates via embryo transfer in Japanese land cows (Hayashi *et al*., 2018). Kim *et al*. (2020) stating that a pregnancy rate was 21.0% higher than that in the control group when 1.0 µM resveratrol was directly injected into the uterus of cows during artificial insemination. However, clinical experiments including resveratrol therapy for infertility treatment have not been made in cows. In this present study, the treatment of repeat breeding cows once daily with 500 mg resveratrol from the second administration of prostaglandin F2alpha to the insemination day resulted into 41.6 percent conception rate (Table 1). Whereas, 25 percent of repeat breeder cows in control group became pregnant. Resveratrol therapy improved conception rate at a rate of 16.6 percent (5 in group T vs. 3 in group C; relative risk, 0.78; 95 percent confidence interval, 0.44 to 1.39; P=0.39) but the difference was statistically non-significant.

Resveratrol therapy of oocytes during in vitro maturation (IVM) has been declared to remedy embryo development and the quality of in vitro blastocysts manufactured in cattle (Sovernigo *et al*., 2017; Wang *et al*., 2014). Resveratrol is a sirtuin 1 (SIRT1) activator. SIRT1 is required for suitable oocyte maturation and embryonic development (Novakovic *et al*., 2022). The beneficial effects of RSV on embryonic development are due to the activation of SIRT1 molecules. Therefore, upregulation of SIRT1 by resveratrol administration may help improve the overall oxidative status of the uterus. The harmful effects of ROS stop embryonic development and lead to early embryonic death. Resveratrol has been detected to reduce intracellular ROS levels and improve GSH levels (Kwak *et al*., 2012). The resveratrol has been demonstrated to increase the quality of the oocytes, the number of blastocyst cells and hatching blastocyst rate after oocyte maturation and fertilization by decreasing the intracellular ROS level and elevating the GSH level (Wang *et al*., 2014). The principal role of GSH is to protect intracellular redox balance and eliminate ROS. Silva *et al*. (2021) stated that the GSH level serves as an indicator of embryo developmental competence. In present study, the mean erythrocyte GSH-Px levels were nearly similar in both groups before resveratrol therapy. However, at post-therapy, mean GSH-Px concentrations in group T was significantly higher ($P<0.05$) than that of group C (Table 2). Likewise, mean erythrocyte GSH-Px concentrations in group T increased significantly after resveratrol treatment compared to pretreatment ($P<0.05$). There were significant differences between pre- and post-treatment values in both pregnant and non-pregnant cows in group T.

Table 1: *The conception rates of cows in control group (group C) and treatment group (group T).*

Groups	n	Conception rate % (n)	Relative risk	95% Confidence interval	P values
Group C	12	25.0 (3)	Referent		
Group T	12	41.6 (5)	0.78	0.4359 to 1.3879	P= 0.39

Table 2: *Mean levels and standard deviations (±SD) of GSH-Px for control group and treatment group pre- and post-therapy.*

		Immediately pre-therapy		Imediately post-therapy	
Parameter	Pregnancy	Control group	Treatment group	Control group	Treatment group
GSH-Px (U/g Hg)	Pregnant	32.99 ± 1.72^{a}	33.88 ± 0.88^{a}	41.89 ± 1.84^{b}	65.71 ± 1.13^{c}
	Non-pregnant	31.31 ± 2.13^{a}	32.39 ± 1.06^{a}	38.24 ± 1.02^{b}	53.04 ± 0.67^{c}
	Total	31.73 ± 2.10^{a}	33.01 ± 2.50^{a}	39.57 ± 2.03^{b}	58.32 ± 6.8^{c}

^{a,b,c} *values with different superscript letters within a row differ significantly at* $P < 0.05$.

These results support the notion that oxidative stress plays a role in the etiopathogenesis of repeat breeding. GSH-Px has been demonstrated to be of great significance in embryonic development, owing to their antioxidant functions and regulatory effects on metabolic rate (Hostetler *et al*., 2003). Resveratrol supplementation as an external protection against oxidative stress can be an effective strategy for oocyte culture in vitro. However, in this study, the pregnancy rates with increasing GSH-Px activity were not significantly affected. Therefore, it is unclear whether the beneficial effects for embryo production observed in vitro due to the antioxidant effects of resveratrol would have a similar effect on clinical conception. These differences in results can be attributed to the resveratrol pattern applied and the different doses of resveratrol. It was demonstrated that resveratrol has a concentration-dependent effect oocyte maturation and on embryo survival (Kwak and Hyun, 2012). So in this study, conception may not directly affect due to the dose used in our model. Therefore, further studies investigating the dose dependent effect of resveratrol on pregnancy outcome could help identify the reasons underlying the pregnancy outcome.

Conclusions and Recommendations

In this study, it was determined that resveratrol treatment increased the conception rate by 16.6 percent, but this difference was not statistically significant. The treatment of repeat breeding cows once daily with 500 mg resveratrol from the second administration of prostaglandin F2 alpha to the insemination day appears to improve the conception insignificantly. Nonetheless, analysis of the treatment period and additional studies are required to achieve any conclusions.

Novelty Statement

Although many researchers have studied the effect of resveratrol on fertility *in vitro*, no clinical studies have been found on the effect of resveratrol on conception rate in repeat breeder cows. Therefore, this study analyzed in order to elucidate the antioxidant effect of resveratrol on conception rate in cows with repeat breeder, and further provide clinical for the research and development of phytotherapeutic agents for cows with repeat breeder.

Author's Contribution

All authors equally contributed to the work.

Conflict of interest

The authors have declared no conflict of interest.

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