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Research Article

Effect of seminal plasma treatment on conception rate in ovsynch treated holstein cows

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ABSTRACT

The seminal plasma (SP) plays significant roles in fertilization processes including capacitation, acrosome reaction, and interaction between sperm and the oocyte. In addition, the SP provides an immunomodulatory effect by the cytokines that it contains, in the female reproductive tract. The aim of this study was to investigate the effect of intrauterine seminal plasma infusion on the success of artificial insemination (AI) in Holstein cows. In the study, a total of 60 multiparous Holstein cows were treated with the Ovsynch protocol (10 µg GnRH on day 0, 500 µg PGF2α on day 7, and 10 µg GnRH on day 9), and artificial insemination was performed 16-18 h after the second GnRH. The cows were categorized into four groups based on the number of AI during lactation period; Group-I (Control AI≤2): inseminated once or twice, Grup- II (SP+AI≤2): inseminated once or twice and infused intrauterine SP, Grup-III (Control AI≥3): inseminated thrice or more, Grup-IV (SP+AI≥3): inseminated thrice or more and infused intrauterine SP. SPs were obtained from 5 Holstein bulls. They were mixed and infused into the corpus uterine immediately before AI. Pregnancy diagnosis by ultrasonography was performed on the 32nd, 60th and 95th days after AI. The conception rates were found 53.3%, 60%, 26.6% and 40%, respectively, between the groups at the 32nd and 60th days after AI (P>0.05). The pregnancy loss was found only in two cows (25%) of Group I, on the day 95th (P>0.05). The days of lactation between the groups were 110±23.2, 104±28.2, 238±53 and 221±46.7, respectively, and were found to statistically significant (P<0.05). The results indicate that the application of seminal plasma is promising for pregnancy success in re-inseminated cows. However, it is considered that further studies based on proteomics or gene expression profiles of seminal plasma and uterine tissue in a large cow population are needed to verify this prediction.

INTRODUCTION

Holstein cows exhibit decreased reproductive efficiency, due to extensive breeding for heightened milk production over the years. The physiological stress induced by intensive feeding and high milk production is recognized to exert a negative impact on their fertility. Consequently, there is a surge in the occurrence of culls stemming from subpar reproductive performance and infertility among Holstein cows. (Abdalla et al., 2019; Leroy et al., 2008; Nanas et al., 2023; Wathes et al., 2009).

The liquid part of the ejaculate is known as seminal plasma. It is comprised of epididymal fluid and secretions from various glands in the male reproductive system, including the seminal vesicle, prostate, bulbourethral glands, and ampulla. Seminal plasma primarily consists of a specialized biochemical fluid containing nourishing and protective large molecules as well as trace elements that facilitate the transport of sperm during ejaculation. In fact, the majority of the components of seminal plasma adhere to the surface of the sperm and play a crucial role in fertilization processes such as capacitation, the acrosome reaction, and the interaction between the sperm and the oocyte (Juyena and Stelleta, 2012).

The seminal fluid, which contains cytokines and hormones, aids in maintaining pregnancy in the female reproductive system. Furthermore, it supports the function of sperm, their transportation to the fallopian tube, and fertilization. Additionally, inflammation due to seminal fluid in the endometrium leads to the production of embryokines, which play a role in regulating embryonic growth and development. It is moreover indicated to aid in maternal recognition of the embryo through regulation of the maternal immune response (Mateo-Otero et al., 2020; Rizo et al., 2019; Vera et al., 2003).

The main objective of bull stations is to produce as many straws of frozen semen as possible from ejaculates. Therefore, in the process of cryopreservation of bull semen, semen production centers tend to increase the dilution rate of the semen in line with developing cryopreservation techniques. This leads to a decrease in the seminal plasma/sperm ratio (Mokhtassi-Bidgoli et al., 2023). This is supported by the fact that the concentration of conventional bull motile sperm per straw which produced or imported into our country has been reduced from 7 million to 5 million as required by legal instructions in the last five years (Funk, 2006; Haygem, 2023; Vincent et al., 2014). A successful cryopreservation process requires the optimal ratio of spermatozoa, seminal plasma, and semen extender compounds. Research suggests that a certain rate of semen dilution does not impact semen quality and fertilization outcomes, but excessive dilution rates adversely affect semen quality and, consequently, fertilization success (Lone et al.,

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2020; Vera-Munoz et al., 2009).

Previous studies have suggested seminal plasma treatment improves the success of insemination or in vitro fertilization in humans and some laboratory animals (Bromfield et al., 2014; Saccone et al., 2019; O'leary et al., 2004). However, studies on this subject in dairy cattle are limited and results are variable (Badrakh et al., 2020; Ninpetch et al., 2022; Odhiambo et al., 2009; Ortiz et al., 2019). The aim of this study was to investigate the effect of seminal plasma treatment on conception rate in multiparous Holstein cows that are reproductively healthy and especially re-inseminated.

MATERIALS and METHODS

Animals and experimental design

Sixty Holstein multiparous cows (n= 60) were used in this study. The animals at the farm were housed under the same conditions. General health and reproductive examinations were performed before and during the study and healthy animals were included in the study. In the research, the cows were categorized into four groups based on the number of artificial insemination (AI) during the lactation period;

- Grup-I (Control AI≤2): Inseminated once or twice,

- Grup- II (SP+AI≤2): Inseminated once or twice and infused intrauterine seminal plasma (SP),

- Grup-III (Control AI≥3): Inseminated thrice or more,

- Grup-IV (SP+AI≥3): Inseminated thrice or more and infused intrauterine seminal plasma (SP).

Seminal plazma ve intrauterin infusion

Seminal plasma samples were obtained from 5 Holstein bulls of a commercial company. For this purpose, semen samples were collected by artificial vagina. Then semen samples were centrifuged at 800 rpm for 15 min and seminal plasma was separated (Ozturk et al., 2021). The separated seminal plasmas were pooled and then filled into 0.25ml straws and stored at -20˚C until use. Thawed seminal plasma samples were loaded in an AI gun and infused into the corpus uterine immediately before insemination.

Artificial insemination (AI) and pregnancy diagnosis

Ovysnch [day 0 GnRH (10 µg, buserelin acetate, Receptal, *İnterve*t, Türkiye), day 7 PGF2α (500 µg, cloprostenol, Estrumate, CEVA-DİF, Türkiye), day 9 GnRH)] protocol was used for synchronisation (Agaoglu et al., 2020). Cows were inseminated at a fixed time, 16-18 hours after the second GnRH administration, using frozen-thawed bull semen from the same batch. Pregnancy diagnosis by transrectal ultrasonography (7,5 MHz Linear prob, Hasvet® 838, Hasvet, Türkiye) was performed on the 32nd, 60th and 95th days after AI.

Statistical analysis

All data from the study were subjected to normality tests. Non-parametric values between groups were compared statistically using the Kruskal-Wallis test and the Mann-Whitney

Table 1. Days of lactation, conception rate, and pregnancy loss values in groups.

Group	n	Days of lactation	Conception rate (%)			Pregnancy loss (%)
			d 32	d 60	d 95	
Group I (Control AI≤2)	15	110±23.2^{A}	53.3 (8/15)	53.3 (8/15)	40 (6/15)	25 (2/8)
Group II (SP+AI≤2)	15	104±28.2^{A}	60 (9/15)	60 (9/15)	60 (9/15)	0
Group III (Control AI≥3)	15	238±53^{B}	26.6 (4/15)	26.6 (4/15)	26.6 (4/15)	0
Group IV (SP+AI≥3)	15	221±46.7^{B}	40 (6/15)	40 (6/15)	40 (6/15)	0

Grup-I (Control AI≤2): Inseminated once or twice. Grup- II (SP+AI≤2): Inseminated once or twice and infused intrauterine seminal plasma. Grup-III (Control AI≥3): Inseminated thrice or more. Grup-IV (SP+AI≥3): Inseminated thrice or more and infused intrauterine seminal plasma. A,B Means that different letters within the same column different (P<0.05).

U test. One-way analysis of variance (ANOVA) was used to calculate parametric values following the Tukey test for multiple comparisons. The confidence interval or *P* value<0.05 was considered statistically significant. The SPSS (Version 23) package program was utilized for the statistical analysis of the study.

RESULTS

The data obtained in the present study are shown in Table 1. The conception rates were found 53.3%, 60%, 26.6% and 40%, respectively, between the groups at the 32nd and 60th days after AI. Although the highest conception rate was found in Group II (60%), the difference between the groups was not statistically significant (*P*>0.05). The pregnancy loss was found only in two cows (25%) of Group I, on the day 95th. However, the differences in conception and pregnancy loss rates between groups were not statistically significant, on the day 95th. (*P*>0.05). The days of lactation between the groups were 110±23.2, 104±28.2, 238±53 and 221±46.7, respectively, and were found to statistically significant (*P*<0.05).

DISCUSSION

After fertilization, the zygote begins mitotic cell division. By the 8th day after fertilization, it reaches the blastocyst stage. During this period, the embryo is directed to implant in an area of the uterine endometrium where vascularization and nourishing uterine secretions are abundant. Maternal immune tolerance is essential for successful embryo implantation. This immune tolerance is mediated by cytokines, proteins that regulate cellular communication in the immune system. The main cytokines detected in bovine endometrium are insulin-like growth factor 1 (IGF1), epidermal growth factor (EGF), fibroblast growth factor 2 (FGF2), interleukins (IL-4, IL-6, IL-10), and transforming growth factor beta (TGF-β). These cytokines stimulate epithelial, stromal and endometrial cells and are effective in embryo attachment, implantation and placentation processes (Ealy eft al., 2021; Hirayama et al., 2015; Sugawara et al., 2010).

The composition of seminal plasma includes many proteins and cytokines of different molecular weights and intracellular or extracellular biological activation. Bovine seminal plasma proteins (BSP-1, BSP-2, BSP-3 and BSP-30), lactoferrin, clusterin, lipocalin-type prostaglandin D synthase, osteopontin, acidic seminal fluid protein (aSFP), ubiquitin and 25 kDa protein-beta (P25b) are the major seminal plasma proteins found in bull semen. These proteins also enable sperm transport and contribute to fertilisation stages such as capacitation, acrosome reaction and sperm-oocyte interaction (D'Amours et al., 2017; Kasimanickam et al., 2019; Westfalewicz et al., 2017) Interleukin-1β (IL-1β), interleukin-6 (IL-6), transforming growth factor-beta (TGF-beta), tumour necrosis factor-alpha (TNF-alpha), interferon-alpha and interferon-beta cytokines were also isolated from bull seminal plasma. These cytokines act synergistically or antagonistically with cytokines in the female genital tract and induce the expression of these cytokines in the uterine endometrium. The reactions of these immunological agents enable immune modulation by controlling the remodelling of uterine tissue and immune responses to antigens (Ibrahim et al., 2019; Mateo-Otero et al., 2020; Vera et al., 2003). Despite differences in seminal plasma composition and female genital anatomy, similar immunomodulatory effects were reported in various mammalian species, including humans, horses, sheep, rats, rabbits and mice. (Gardela et al., 2020; Portus et al., 2005; Schjenken & Robertson 2014). However, although recent literature studies in this field reveal the immune modulation effect of SP in the female reproductive tract, studies indicating its effect on fertilisation success are limited.

There are studies indicating the beneficial effect of SP in insemination and in vitro fertilisation applications in humans on pregnancy outcomes, as well as studies indicating that it has no statistically significant effect on pregnancy rates (Ata et al., 2018; Crawford et al., 2015; Friedler et al., 2013; Saccone et al., 2019). Studies in mice indicate a beneficial effect of seminal plasma on pregnancy rates. Bromfield et al. (2014) reported a significant decrease in vitro and in vivo fertilisation rates with spermatozoa from mice with surgical excision of the seminal vesicle gland. Similar results are also observed in the study conducted by Kawano et al. (2014).

Ortiz et al. (2019) investigated the effect of seminal plasma in first-service artificial insemination with conventional and sex-sorted semen in primiparous and multiparous Holstein cows and found no significant difference in pregnancy outcomes. In the same study, it was stated that the birth weights of calves born from the application of seminal plasma with sex-sorted semen were higher than the control group. Additionally, it suggested that seminal plasma may possess nutritive properties during the embryonic stage. The study in mice suggests that the similar effect of SP is not only limited to the embryonic or foetal period but also has a positive effect on postnatal offspring health (Bromfield et al., 2014). It is thought that the absence of foetal loss in Group-2 compared to Group-1 may be due to this immune modulation effect of SP in addition to many acquired factors. Odhiambo et al. (2009), in a study conducted with approximately two thousand beef and dairy cattle, reported that although SP applications increased pregnancy success numerically, there was no statistical difference between the groups. Badrakh et al. (2020) evaluated the effect of seminal plasma applications on endometrial epidermal growth factor and conception rate in repeat-breeders Holstein cows. According to this study, infusing SP into the vagina led to an increase in EGF levels about threefold more compared to infusing SP into the uterus and also it positively impacted conception rates. In the same study, it was reported that SP did not affect EGF and conception rates in fertile cows (Badrakh et al., 2020). Ninpetch et al. (2022), investigated the effect of seminal plasma on endometrial EGF and expression of leptin receptor (Ob-R). The authors noted that although SP treatment had no effect on OB levels, it normalized EGF levels in about half of the repeat breeder cows, resulting in a successful pregnancy after insemination (Ninpetch et al., 2022). The hypothesis of this study was to investigate the effect of seminal plasma in re-inseminated multiparous cows. Although conception rates were numerically higher in SP groups (Group-2 and Group-4), the difference between the groups was statistically insignificant. As a matter of fact, the scientific data obtained in this research are in parallel with the previous

studies performed.

In dairy cattle, the lactation begins calving and peak of lactation is usually observed during the period of 4-8 weeks postpartum. However, due to the negative energy balance, cows are at higher risk of developing diseases that affect fertility, such as metabolic or mastitis, during this period (Leroy et al., 2008; Nanas et al., 2023; Wathes et al., 2009). In order to reduce the risk of these diseases during the peak of lactation, voluntary waiting periods until the first service are considered a probable solution. In a study of Holstein cows treated with three different voluntary waiting periods (VWP-50 d, VWP-125 d, and VWP-200 d), it was stated that although in the VWP-200 d group became pregnant sooner after the VWP, there was no statistically significant difference in pregnancy rates between groups (Ma et al., 2022). A separate study treatment of two voluntary waiting periods (60d and 88d) reported no significant difference in pregnancy rates between treatment groups (Stangaferro et al., 2018). In the current study, the voluntary waiting period was treatment 90 days. Inseminations were carried out on day 110±23.2 and day 104±28.2 of lactation in groups I and II, respectively. In Groups III and IV, animals were inseminated on day 238±53 and day 221±46.7 of lactation, respectively, due to prior unsuccessful inseminations. In parallel with the mentioned studies, no statistically significant relationship was found between days of lactation and conception rate. The Ovsynch synchronization protocol that designed to synchronize estrus and ovulation is a widely used reproductive management tool in dairy cattle. Pregnancy rates achieved through the Ovsynch protocol in lactating dairy cows range from 32% to 76.92% (Birten et al., 2012; Fricke & Wiltbank, 2022). The pregnancy rates obtained in Group I are consistent with those found in previous studies. It is thought that non-congenital and non-infectious repeat breeding factors are responsible for the low pregnancy rates observed in Group III.

It has been stated that the composition of bull seminal plasma can be influenced by individuality, alimentation, age, frequency of semen collection, and season (Byrne ve ark., 2012; D'Amours ve ark., 2017; Kasimanickam ve ark., 2019; Peddinti ve ark., 2008). In order to avoid this situation, seminal plasma samples obtained from 5 different bulls were pooled and treatment in this study. Similarly, it is thought that the expression rates of immune agents in uterine tissue may vary depending on the individual, nutrition, number of lactations, and age, which could result in different reactions to the seminal plasma infusion (Agaoglu and Beceriklisoy, 2017; Davoodi et al., 2016; Herath, et al., 2009; Tanikawa et al., 2005). This study was carried out in a particular cattle population with limited data. However, the results obtained indicate that the application of seminal plasma is promising in terms of pregnancy success in re-inseminated or repeated breeder cows.

CONCLUSION

The AI success is important for the sustainability of cattle breeding. For this purpose, alternative treatment protocols with AI have been used for the conception of repeat breeders or infertile cows. It is evident from the obtained pregnancy results that the infusion of allogeneic seminal plasma does not have any adverse effects on inseminations that were performed with Holstein bull semen. Furthermore, it is thought that the seminal plasma treatment with routine insemination procedures in cases of repeat breeders or infertile cows will enhance the chances of achieving successful pregnancy outcomes. However, it is considered that further studies based on proteomics or gene expression profiles of seminal plasma and uterine tissue in a large cow population are needed to verify this prediction.

DECLARATIONS

Ethics Approval

This study was approved by Burdur Mehmet Akif Ersoy University Animal Experiments Local Ethics Committee at the meeting dated 08.06.2023 with the number of 1132 decisions.

Conflict of Interest

The authors have no conflicts of interest.

Author contribution

Idea, concept and design: İB

Data collection and analysis: İB, YÇ

Drafting of the manuscript: İB, YÇ

Critical review: YÇ

Data Availability

The data used to prepare this manuscript are available from the corresponding author when requested.

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Not applicable.

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