

2024

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Berrak Işık SOYTÜRK
bisiksoyturkk@gmail.com

Hüseyin ERDEM
erdemh@selcuk.edu.tr

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SOYTÜRK, Berrak Işık and ERDEM, Hüseyin (2024) "The effect of artificial insemination catheters covered with protective sheaths on conception rates in heifers," *Turkish Journal of Veterinary & Animal Sciences*: Vol. 48: No. 1, Article 8. <https://doi.org/10.55730/1300-0128.4339>
Available at: <https://journals.tubitak.gov.tr/veterinary/vol48/iss1/8>

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The effect of artificial insemination catheters covered with protective sheaths on conception rates in heifers

Berrak Işık SOYTÜRK^{ID}, Hüseyin ERDEM^{*ID}

Veterinary Obstetrics and Gynecology, Faculty of Veterinary Medicine, Selcuk University, Konya, Türkiye

Received: 10.05.2023

Accepted/Published Online: 25.01.2024

Final Version: 06.02.2024

Abstract: Artificial insemination is a biotechnological method widely practiced in cattle, first introduced in 1912. The present study investigated the effect of pierceable plastic sheaths used during artificial insemination of Holstein heifers on conception rates. A total of 110 heifers aged 15–18 months, detected to be in estrus based on observation, were included in the study. These heifers were randomly divided into two equal groups. The experimental group (n = 55) underwent insemination using an insemination catheter covered with a disposable, pierceable plastic sheath on day 0. Conversely, the control group (n = 55) underwent insemination using the standard conventional technique. Pregnancy was diagnosed on day 30 via ultrasonography. The conception rates were 56.4% and 50.9% in the experimental and control groups, respectively (p > 0.05). In conclusion, while the use of pierceable plastic sheaths to cover catheters during insemination made a positive contribution, it did not result in a statistically significant difference in conception rates. Therefore, the study suggests that the use of protective sheaths in inseminations using sexed semen or semen with outstanding genetic characteristics may positively impact conception rates in field conditions. However, this practice should be supported by microbiological and endocrinological examinations.

Key words: Heifer, catheter sheath, artificial insemination, conception rate

1. Introduction

Artificial insemination in cattle originated in Russia in 1912 and in Türkiye in 1925 [1]. The success of this biotechnological technique depends not only on semen-related factors such as collection, evaluation, and freezing but also on employing the appropriate insemination technique. A crucial aspect of sustainable animal husbandry is to ensure that pregnancy occurs within a certain time interval. In heifers, achieving pregnancy within the “breeding age” (15–18 months) is paramount. Delayed breeding in heifers leads to postponed lactation and calving, resulting in heightened input and depreciation costs for cattle farms.

Conception rates in heifers range from 45% to 74.4% [2-4]. These variations in conception rates stem from different stages of embryonic mortality: very early embryonic mortality (0–7 days), early embryonic mortality (7–24 days), late embryonic mortality (24–45 days), and fetal mortality (45–285 days) [5]. Employing meticulous artificial insemination procedures and adhering to hygiene protocols are crucial for achieving optimal fertility in cattle. However, artificial insemination carries the risk of introducing bacteria and debris from the vagina and vulva

into the uterus. A study addressing this concern utilized uterine lavage four hours after artificial insemination and observed an increase in the number of neutrophils [6]. Kaufmann et al. [6] reported that elevated neutrophil levels in the uterus suggest subclinical endometritis. One approach to prevent uterine contamination during artificial insemination is to use a protective sheath to cover the catheter. A previous study found no increase in conception rates during first services postpartum [7]; however, this study did not report conception rates achieved with subsequent inseminations. Conversely, a recent study reported that using a plastic sheath to cover the catheter during artificial insemination minimized bacterial contamination of the uterus and improved fertility in cows [8].

Bacterial contamination during artificial insemination can lead to inflammation of the uterus and subclinical endometritis, potentially disrupting the processes crucial for maternal recognition of pregnancy [9]. One method used to prevent or reduce such contamination during artificial insemination involves cleaning the vulva. Indeed, research indicates that inseminations performed when the vulva is contaminated with feces result in low fertility rates

* Correspondence: erdemh@selcuk.edu.tr

[10, 11]. In summary, the aim of this study is to investigate whether employing clean artificial insemination techniques, utilizing catheters covered with pierceable sheaths, could effectively enhance the conception rate in heifers.

2. Materials and methods

2.1. Animal materials and artificial insemination

The study involved 110 Holstein heifers aged between 15 and 18 months, all housed under the same conditions and receiving uniform care and feeding regimen. These heifers were housed in 250-head paddocks, and estrus detection was accomplished through visual observation. When heifers displaying signs of estrus were identified, they were moved to an examination paddock. Subsequently, their ovaries and uterus were examined via rectal palpation. Insemination was conducted during the examination upon observation of a Graafian follicle with an average diameter of 1.5 cm in the ovary, as well as indications of tone and edema in the uterus, along with hyperemia and moisture in the vaginal mucosa. Experienced veterinarians/veterinary technicians performed the inseminations using thawed semen from bulls known to be fertile. The study was conducted in July and August.

2.2. Study groups

The inseminated heifers were randomly divided into two groups. Experimental group (EG, n = 55): Unlike the heifers receiving standard insemination, those in the experimental group were inseminated using an insemination catheter covered with a pierceable plastic sheath. In this technique, the straw was inserted into the artificial insemination catheter, which was then covered with a plastic sheath. The outer portion of the catheter

was encased in a nylon sheath, featuring a tip that could be punctured with minimal force. This sheath served to prevent potential contamination of the catheter as it moved from the vulva-vagina to the cervix. Upon positioning the catheter against the portion of the cervix facing the vagina (orificium uteri externa), the tip of the outer sheath was punctured by advancing the catheter forward (Figure). Subsequently, the catheter was guided through the cervix and into the uterine body. At this stage, the plunger of the catheter was depressed to deposit the thawed semen from the straw into the uterus.

Control group (CG, n = 55): The heifers in this group were inseminated using the standard insemination technique routinely applied. In this technique, the straw was inserted into the artificial insemination catheter, which was then covered with a plastic sheath. Upon positioning the catheter against the part of the cervix facing the vagina (orificium uteri externa), it was advanced through the cervix into the uterine body. At this stage, the pistol of the catheter was pushed to deposit the thawed semen from the straw into the uterus.

2.3. Diagnosis of pregnancy

Cows were examined for pregnancy diagnosis using a real-time ultrasound device (6.0 MHz linear probe, Falcovet, Pie Medical, the Netherlands) on day 30 following artificial insemination. The identification of a hypoechoic embryo within an anechoic region in the uterus during the examination was considered a positive pregnancy.

2.4. Statistical analysis

In the present study, the pregnancy rates in the groups were subjected to statistical analysis using the chi-square (χ^2) test. The statistical analyses were performed using the SPSS 14.01 software suite.

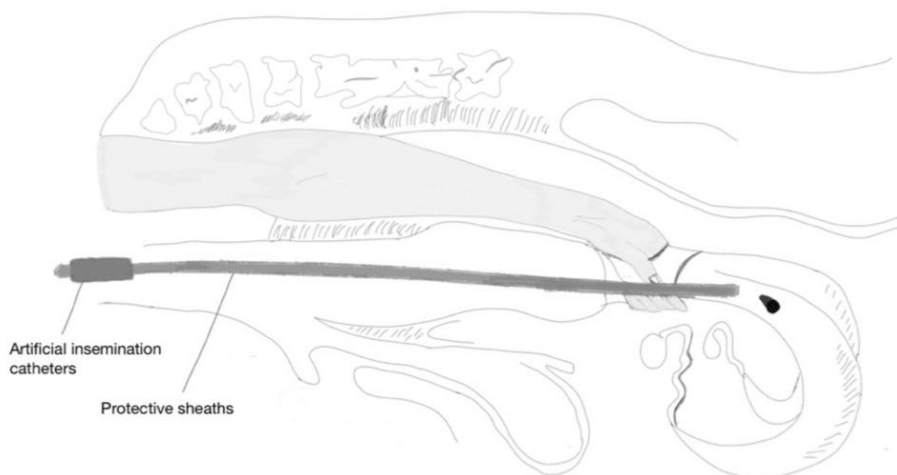


Figure. Perforation of the protective sheath when the catheter is placed on the cervix facing the vagina.

3. Results

The conception rates of heifers in the experimental and control groups are presented in Table 1. Conception was observed in 31 animals (56.4%) in the experimental group and in 28 animals (50.9%) in the control group.

Table 2 displays the number of animals observed to be in estrus and subsequently serviced a second time, as well as the number of animals showing no signs of estrus and thus not serviced again within the 30-day period, which concluded with the examination for pregnancy in both the experimental and control groups. Specifically, twenty animals in the experimental group and 21 animals in the control group received a second service.

4. Discussion

Artificial insemination currently stands as the prevailing biotechnological method employed in animal breeding to enhance yield per animal unit. However, despite its contribution to heightened milk production, artificial insemination practices have coincided with declining fertility over the last 50 years [12]. This trend has triggered a series of research endeavors aimed at optimizing fertility outcomes. The present study used a modified version of the standard artificial insemination procedure in Holstein heifers and investigated its effects on conception rates. This modified technique involved employing a pierceable plastic sheath to cover the insemination catheter. Notably, this practice did not incur any increase in labor or insemination costs.

During the embryonic process following fertilization in cows and heifers, approximately 40% of embryos are lost within the first 3 weeks due to developmental failure. These losses are attributed to several factors, including both specific and nonspecific uterine infections [13]. In the present study, although there was no statistical difference in the conception rates between the experimental and control groups, the experimental group exhibited a numerically higher conception rate. This finding holds

potential significance for insemination practices aimed at optimizing pregnancy outcomes, particularly when employing sexed semen or semen boasting superior genetic traits. The favorable outcomes observed in the experimental group likely stem from the prevention of environmental microorganisms from infiltrating the uterus. Consequently, the absence of inflammation and related inflammatory products may have facilitated the uninterrupted, normal development of embryos.

Endometritis can arise from various sources, including infectious agents (bacterial, viral), mechanical factors, thermal or chemical influences, as well as exposure to toxins. These inflammations may occur postpartum, following natural mating, artificial insemination, or the introduction of irritants into the uterus [14]. Hence, adhering to strict hygiene protocols during artificial insemination is paramount. As evidenced by our study, the hygiene practices during insemination significantly influence the conception rate. A notable difference observed in this study is the utilization of a pierceable protective sheath to cover the catheter until it reaches the portio vaginalis of the cervix. At this point, the sheath is pierced, allowing the catheter, free from vaginal contamination, to traverse the cervix and enter the uterus. Our findings indicate that this practice positively impacts the conception rate.

Artificial insemination encompasses a range of practices implemented before, during, and after the procedure to improve conception rates [15-17]. These practices may involve the administration of medications, hormones, and vitamins, which can potentially boost pregnancy rates by up to 25%. Such interventions primarily aim to promote normal embryo development or facilitate the secretion of adequate interferon tau, particularly in cases of poorly developing embryos during maternal recognition of pregnancy. In our study, a modified insemination technique was employed, resulting in a pregnancy rate of 56.4% in heifers in the experimental group (n = 55) and

Table 1. Conception rates in the experimental and control groups.

Group	Pregnant (n, %)
Experimental group (n = 55)	31 (56.4%)
Control group (n = 55)	28 (50.9%)

Table 2. Number of animals showing and not showing signs of estrus at the expected time after insemination in the experimental and control groups.

Group	Received a second service (n)	Had no signs of estrus and did not receive a second service (n)	Total (n)
Experimental group	20	4	24
Control group	21	6	27

50.9% in the control group (n = 55), with no statistical difference observed between them. However, there existed a 5.5% difference in conception rates between the experimental and control groups. As mentioned above, this difference may be attributed to the uterine infection status. Indeed, most reproductive tract infections are caused by nonadherence to hygiene protocols during artificial insemination. The introduction of the catheter into the uterus during insemination can disrupt the normal uterine environment, potentially facilitating the proliferation of pathogenic bacteria and leading to reproductive problems such as acute, chronic, and subclinical infections. Ideally, a healthy uterine environment should be free of infectious agents such as pyogenic bacteria, mycoplasmas, chlamydia, bovine viral diarrhoea (BVD), bovine herpesvirus (BHV), and Q fever [18]. Therefore, care must be taken when using insemination equipment.

Manipulating equipment during artificial insemination and embryo transfer, including the inadvertent contact of a contaminated artificial insemination or embryo transfer catheter with the uterus, can cause inflammation, leading to the secretion of prostaglandin F₂-alpha (PGF₂α). PGF₂α, known for its detrimental impact on embryo survival, ideally should not be present in the uterine lumen as it possesses embryotoxic properties [19-21]. In the present study, insemination procedures were conducted by experienced veterinarians/veterinary technicians. Therefore, it is plausible to surmise that any embryonic losses observed in the heifers were likely attributable to factors other than uterine manipulation.

Indeed, artificial insemination in cattle, typically conducted via the recto-vaginal method, involves navigating a catheter through the vulva, vagina, cervix, and ultimately into the uterus for semen deposition. This process poses a risk of introducing bacteria and tissue debris into the uterus at any point during catheter advancement [22, 23]. To mitigate this risk, catheter sheaths originally designed for contamination-free embryo transfer can be repurposed for artificial insemination procedures [24]. In the present study, employing this technique yielded positive results, albeit only in numerical terms. Considering that subclinical endometritis is recognized as a significant cause of infertility and/or subfertility in cattle [25], the adoption of protective sheaths may be advisable to enhance fertility.

Utilizing a clean artificial insemination technique involving pierceable plastic sheaths holds promise as an effective strategy for enhancing reproductive outcomes in dairy cattle [8]. In our study, despite no difference between the two groups in terms of the insemination period (July and August), care and feeding conditions, semen utilized, and estrus detection methods, the slightly higher

pregnancy rate achieved in the experimental group may be attributed to the prevention of uterine contamination. Furthermore, the inclusion of heifers as the study subjects likely contributed positively to the conception rate. Inflammatory products resulting from infection can detrimentally impact early embryonic development. Some studies have reported that early embryonic mortality represent a significant portion of all embryonic deaths [2, 12]. The modified insemination technique employed in the present study presents itself as a viable alternative strategy for preventing or reducing embryonic mortality, potentially yielding significant gains in reproductive success.

In cows, the calving to conception interval and the number of inseminations per pregnancy are the most important parameters of fertility. However, in heifers, the most critical fertility parameter is the number of inseminations per pregnancy. This is because each unsuccessful insemination represents a loss of at least 20 days. Therefore, ensuring pregnancy within a specific timeframe is crucial for heifers [26]. Although our study did not include data on the number of inseminations per pregnancy, the inseminations performed in July and August yielded satisfactory conception rates. It is worth noting that our study was conducted during the hottest months of the year in the region, and heat stress is widely acknowledged by researchers [27-29] as a significant contributor to early embryonic death.

5. Conclusion

In conclusion, while the implementation of a pierceable plastic sheath covering the insemination catheter did not cause a statistically significant difference in conception rates between heifers in the experimental and control groups, it did lead to a numerically higher conception rate in the experimental group. This finding holds potential significance, particularly for inseminations utilizing sexed semen or semen with superior genetic characteristics aimed at optimizing pregnancy outcomes. Additionally, the use of a pierceable plastic sheath covering the insemination catheter presents itself as a viable alternative strategy for increasing conception rates. However, this practice should be supported by microbiological and endocrinological examinations.

Funding organization

The study was supported by the Selçuk University Scientific Research Projects (SÜBAP).

Conflict of interest

The authors do not have any conflicts of interest.

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