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Theriogenology

Impact of Insemination Site Using Frozen-Thawed Semen on the Pregnancy Rate in Mares

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ABSTRACT

The aim of the current study was to study the effect of using frozen semen and the effect of the site of insemination on pregnancy rate in mares. Thirty-one mares were used. Two different artificial insemination protocols were carried out. In the first protocol, 12 mares were inseminated in the uterine body using cryopreserved-thawed semen of stallions with good fertility without adding additional extender using insemination pipette and insemination gun. In the second protocol, 19 mares were inseminated in the uterine horn ipsilateral to the ovary from which ovulation had occurred using cryopreserved-thawed semen of stallions of good fertility without adding additional extender using insemination pipette and insemination gun. Thirteen days after insemination, the mares were examined for diagnosis of pregnancy using ultrasonography. Results showed that pregnancy rate in case of insemination into the uterine horn was not significantly ($P > 0.05$) higher than insemination into the uterine body. In conclusion, artificial insemination of mares deeply into the uterine horn gave statistically better success nearly similar to insemination into the uterine body.

Keywords: Mares, Cryopreserved semen, Insemination and Pregnancy.

INTRODUCTION

Artificial insemination in equine does not have a big development as other species, that may be happened to avoid inbreeding problems and preserving the blood lines (Alvarez et al., 2014). Artificial insemination in equine using cryopreserved sperm provides an essential role for using proven stallions in the breeding program (Avanzi et al., 2015). Artificial insemination of mares using cryopreserved-thawed semen is carried out usually in a dose of 250 x 10^6 progressively motile sperm in addition to post-thawing motility equal to at least 35

% (Miller, 2008). Cryopreservation of stallion sperm may induce permanent capacitation like changes and lower the shelf life of sperm in the reproductive tract of mares (Watson et al., 2001). The oocyte may remain viable for long periods following ovulation (i.e., 8-16 hrs.), and so, longer examination intervals (reaches up to 15 hrs.) for detecting the ovulation can be carried out without lowering the fertility following post-ovulatory artificial insemination with cryopreserved-thawed semen (Newcombe and Cuervo-Arango, 2015; Lewis et al., 2015). Artificial insemination of horses with cryopreserved semen should be performed near to the time of occurrence of ovulation that is between 12 hours before and 6-12 hours after ovulation to give a better chance of fertilization (Sieme et al., 2003). When the dominant follicle grows to a specific size of 3.5 cm and the uterine edema decreases, the mares are given human chorionic gonadotropin (hCG) to arrange the time of occurrence of ovulation. After injection of hCG, 70-95% of mares ovulate within 24-48 hours (Tazawa et al., 2017). Artificial insemination of mares with frozen semen deeply into the uterine horn gives approximately thirty percent better success in the fertility rate than artificial insemination into the uterine body due to high number of sperms that reaches the site of fertilization in case of insemination in the uterine horn than uterine body (Govaere et al., 2014; Dascanio et al., 2021). There are several factors that limit using artificial insemination with frozen semen including increasing the number of ultrasonographic examinations used to detect the most accurate time for insemination (Squires et al., 2006). Diagnosis of pregnancy in mares can be carried out 9 days following ovulation as the embryonic vesicle has a diameter equal to 3 mm. However, most of the diagnosis of pregnancy in mares is performed between 12- and 14-days following ovulation as the embryonic vesicle has a diameter equals to 14-16 mm (Bergfelt and Adams, 2011). Artificial insemination of mares with cryopreserved semen is not popular in Egypt due to low pregnancy rate and high cost of the insemination dose, so the aim of the current study was to study the effect of using frozen thawed semen and the effect of site of insemination on the pregnancy rate in mares.

MATERIAL AND METHODS

This study was assessed and agreed by the Animal Care and Welfare Committee Ethics, University of Sadat City, Egypt.

Mares

Thirty-one non-lactating, cyclic mares (Arabian breed mares and foreign breed mares) with average age of (6-8 years) were housed in boxes, fed hay and balanced diet and the water was provided adlibitum. The study was carried out from March 2024 to March 2025**.** All mares had belonged to different private owners and were reared on different farms. All mares gave foals before and had no history of failure of reproduction and were normal clinically and gynecologically.

Insemination dose

Cryopreserved-thawed semen from ten Arabian stallions was used, with each insemination dose prepared locally using 8–10 straws, containing 400–500 million progressively motile sperm. The stallions were of proven fertility, with post-thaw motility ranging from 40% to 50% and

an average pregnancy rate per cycle of approximately 40%.

Experimental design:

This experiment was performed to study the effect of using frozen semen and effect of the site of insemination on the pregnancy rate.

Thirty-one gynecologically sound mares were used. During estrous, mares were evaluated using ultrasonography, when the size of the dominant follicle reached 35 mm in diameter, the mares were injected with human chorionic gonadotropin (Epifasi, EIPICO, 1500 IU) to stimulate the ovulation to occur then the mares were examined ultrasonographically every six hours beginning 18 hr. from injection of hCG to detect the ovulation and once the ovulation was observed, the mares were inseminated using cryopreserved semen. Two different artificial insemination protocols were carried out. In the first protocol, twelve mares were inseminated in the uterine body using cryopreserved-thawed semen of stallions (8-10 straws containing 400-500 million sperm) with good fertility without adding additional extender using insemination pipette (75 cm flexible long plastic artificial insemination pipette, Minitube pipette) and insemination gun. In the second protocol, nineteen mares were inseminated in the uterine horn ipsilateral to the ovary from which ovulation had occurred using cryopreserved-thawed semen of stallions (8-10 straws containing 400-500 million sperm) of good fertility without adding additional extender using insemination pipette and insemination gun. The insemination dose depends on the percent of progressive motile sperm and the sperm concentration. All inseminations in mares were performed by the same veterinarian.

Insemination protocol

Preparation of the mare was carried out through application of tail bandage and thorough cleaning of the external genitalia with povidine iodine and water then drying using cotthad occurredws were removed from liquid nitrogen tank and placed in water bath 37° C for 30 seconds and one straw was checked for its motility (post-thawing motility more than 35 %) then insemination either in the uterine body using vaginal technique by entering the insemination pipette till the uterine body and placing of straw through it with the insemination gun or in the uterine horn through rectally guided uterine horn ipsilateral to the ovary from which ovulation was occurred. All mares were injected oxytocin (Oxytocin, CHINA MEHECO, 30 IU, IM) following insemination to remove any fluid in the uterus that may affect the conception rate (Immonen and Cuervo-Arango, 2020).

Pregnancy diagnosis

Thirteen days after insemination, the mares were secured and examined for diagnosis of pregnancy using rectal ultrasonography (7.5 MHz) with linear array transducer to determine the pregnancy rate in inseminated mares (Bergfelt and Adams, 2011). (Figure 1)

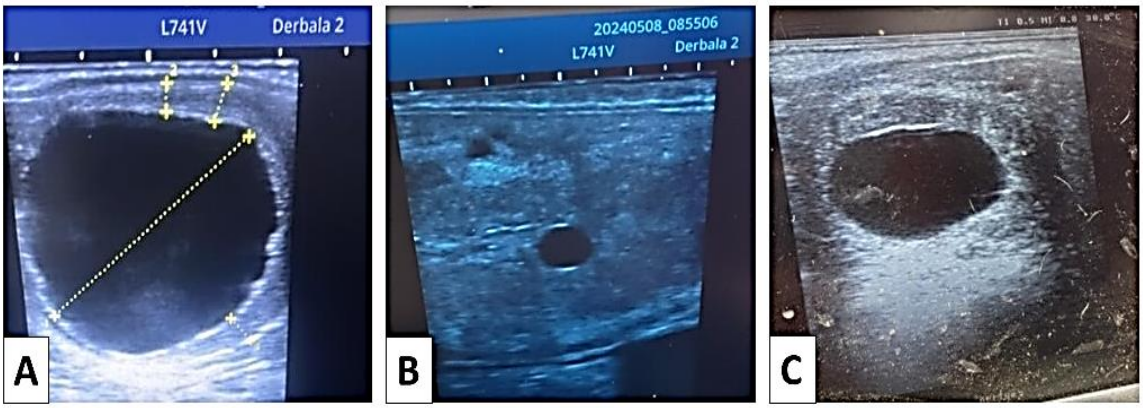

Figure 1. A. Ultrsonographic examination of mare ovary showing follicle 4.3 cm in diameter, **B**. Ultrasonographic examination of mare uterus showing pregnancy 13 days with spherical shape embryonic vesicle, **C**. Ultrasonographic examination of mare uterus showing pregnancy 17 days post insemination.

Statistical analysis

Conception rate data were arranged in a contingency table and the Fisher's exact test was conducted. Graphs and statistical analysis were performed in RStudio-2024.4.2.764 (Posit team, 2023) and R programming language v4.4.1 (R Core Team, 2023). Finally, results were represented as percentages, and the significance limit was set to $P<0.05$.

RESULTS

There was no significant difference ($P > 0.05$) in the conception rate between insemination either into the uterine horn or the uterine body. The conception rate in case of insemination into the uterine horn was 31.6%. The conception rate in case of insemination into the uterine body was 16.7%. as in Table (1) and Figure (2).

Table 1. Effect of the insemination site on the conception rate in mares.

Parameter	Uterine horn (n=19)	Uterine body (n=12)	P-value
Conception rate (%)	**31.6**	**16.7**	**0.43**

N.B. The results were analysed with the Fisher's exact test. Significance limit was set to $P<0.05$.

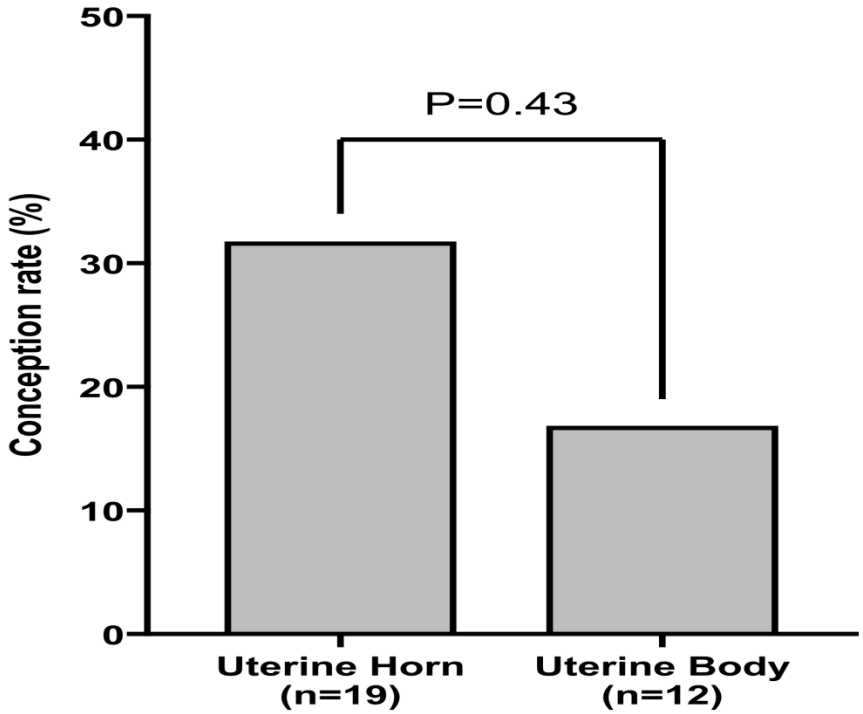

Figure 2. Effect of site of insemination on the conception rate.

DISCUSSION

Artificial insemination of mares was practised in modern reproduction of animals, especially when improvement of genetics was taken into account. The foaling percent that came to the world as a result of insemination with cryopreserved thawed semen reached about 90 % (Nath et al., 2010).

In the current study, the mares were examined 18 hr. after injection of the ovulating agent for determination of ovulation either every 6 hrs. or every 12 hrs. This method of examination did not affect the pregnancy rate which was similar to previous studies as (Newcombe et al., 2011) who mentioned that the interval of examination of mare to detect ovulation occurrence (16 hr. or 8 hrs. or 4 hrs.) would not affect the rate of pregnancy or embryonic losses.

In the present study, artificial insemination of mares with cryopreserved thawed semen resulted in pregnancy rate per cycle equal to 25 % which is nearly similar to previous study performed by (Clulow et al., 2008) who obtained a pregnancy rate equal to 27 % which is acceptable for population during using cryopreserved-thawed semen.

Artificial insemination of mares with cryopreserved-thawed semen deeply into the uterine horn resulted in conception rate that was not significantly (P> 0.05) higher than artificial insemination of mares with cryopreserved-thawed semen into the uterine horn. This result was not in a harmony with (Govaere et al., 2014) who demonstrated that insemination into the uterine body has approximately 30 % lower conception rate than insemination deeply into the uterine horn. This may be due to the difference in the number of sperm that reached the fertilization site which was high in case of insemination into the uterine horn and low in case of

insemination into the uterine body (Rigby et al., 2000). However, this result was not in agreement with (Squires et al., 2006) who mentioned that insemination of mares into the uterine body gave better results when compared with insemination of mares deeply into the uterine horn.

Artificial insemination of mares with cryopreserved-thawed semen deeply into the uterine horn resulted in conception rate equal to 31.6 %. This result was not in a harmony with (Metcalf, 2005) who found in his study that artificial insemination of mares with cryopreserved-thawed semen deeply into the uterine resulted in pregnancy rate equal to 47 %. This result of low conception rate may be occurred because artificial insemination of mares with frozen semen deeply in the uterine horn required manipulation of the horn of the uterus that may cause either irritation to the uterus or release of prostaglandin that affected the conception rate (Reger et al., 2003).

Artificial insemination of mares with cryopreserved-thawed semen into the uterine body resulted in conception rate equal to 16 %. This result was not in a harmony with (Govaere et al., 2014) who found in his study that artificial insemination of mares with cryopreserved-thawed semen into the uterine body resulted in pregnancy rate equalled to 40 %. This difference in the conception rate may be due to stallion semen quality, fertility, breeding status and management of the mare (Sieme et al., 2004).

CONCLUSION:

Although no statistically significant difference was found between the two insemination sites, the pregnancy rate following insemination into the uterine horn was nearly double that of insemination into the uterine body. This suggests that deep intrauterine insemination may provide a clinical advantage when using frozen-thawed semen in mares. However, due to the limited sample size, further research is recommended to validate these findings and optimize insemination techniques for enhanced reproductive success in equine practice.

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CONFLICTS OF INTEREST:

The authors declare no conflict of interest.

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