Comparative Study Concerning The Artificial Insemination Techniques In Mares

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Bulletin UASVM Veterinary Medicine 71(2) / 2014,
Print ISSN 1843-5270; Electronic ISSN 1843-5378
DOI:10.15835/buasvmcn-vm: 10694T

Abstract

The mare is a seasonal poliestric animal. The reproductive season lasts from April till September. For obtaining valuable offspring, the artificial insemination (AI) with frozen semen needs to be encouraged, because only a rigorous selection of the paternal line allows the accomplishment of this desideratum. This goal can also be obtained on the field with minimal costs. The main purpose of this work was to evaluate the efficiency of the artificial insemination with frozen semen in mares and to compare it to natural breeding.

This study was conducted from 2012 to 2014 on 45 adult mares of different breed and age. The total number of animals was divided in three groups: group I (n=8) was inseminated with frozen semen, group II (n=16) with refrigerated semen and group III (n=21) had natural cover. For each technique the following aspects were evaluated: the number of ultrasound examinations per cycle, the number of AI or natural covers per pregnancy and the pregnancy rate for each mare. For the lots with AI, the dynamic of the follicular growth, the dimensions of granulosa prior to ovulation and the number of the semen straws per gestation were also assessed.

The mares from Group I needed the most ultrasound examinations (n=11), compared to Group II (n=7) and Group III (n=8). The pregnancy was obtained after a single AI with frozen semen, 4 with refrigerated semen and 8 natural covers. The pregnancy rate was 62.5% for AI with frozen semen, 70.5% with refrigerated semen and 71.4% with natural breeding.

Since similar results have been obtained by using these three reproductive biotechnologies, the use of artificial insemination with frozen semen is the most recommended; mainly to benefit from the advantages that it offers.

Keywords: *artificial insemination, breeding, mare.*

INTRODUCTION

The mare is a seasonal poliestric animal. The reproductive season lasts from April till September. During the breeding season, the unpregnant mare presents repeated estrus cycles, which lasts 21 days with variations between 18 to 24 days. Sexual cycle is dependent on many external factors and individual factors, such as photoperiod day, state maintenance, nutrition, breed, age, conformation, normal functioning of the neuroendocrine system etc. During each sexual cycle ovulation occurs, the detection of which is very important

for reproductive management of the mare. If the fecundation does not take place, the oestrus is repeating (Morel and Mina, 2008). Estrus lasts 4-7 days, with a range of 2-12 days, usually lasting longer in the early breeding season. Ovulation takes place 24-48 hours before the end of the estrus so manifestations of heat being present up to 48 hours after ovulation. After gestation heats occur 4-10 days after birth, being called „foal heat”. After abortion, heat occurs at the delivery time. In case of early abortion, heat is occurring within a short time, 6-21 days (Groza et al., 2006).

In the last decades, in the countries where modern breeding technologies are applied, the artificial insemination is the most commonly used method, compared to natural covers. In Europe, more than 80% of the brood mares are artificially inseminated, while in Romania the natural covers persists as the main method. Even though the researches regarding the artificial insemination in equines started in the XIX century, the progress accomplished throughout the years was limited compared to other species, because, for a very long time this breeding method was not admitted for many breeds registers. More than that, the economical implication was minimal. Nowadays, almost all the herdbooks allow the use of this technology and encourage the breeders to focus on the development of more simple and efficient technological protocols and also to identify new technologies of freezing that can improve the fecundation capacity of the semen after defrosting. For obtaining valuable offspring, the artificial insemination (AI) with frozen semen needs to be encouraged, because only a rigorous selection of the paternal line allows the accomplishment of this desideratum. This goal can also be obtained on the field with minimal costs.

The main purpose of this work was to evaluate the efficiency of the artificial insemination with frozen semen in mares and to compare it to natural breeding. Most of the studies are focused only on one method. Detection time of ovulation is important since artificial insemination with fresh and chilled semen must be made 24 hours prior to ovulation and artificial insemination with frozen semen is made up to 12 hours before ovulation and no more than 6 hours after ovulation. Criteria by which one can predict the time of ovulation are: day of oestrus, intensity of oestrus signs, the presence of a mature follicle with a diameter greater than 35 mm with a diminished consistency, which has a less circular shape and tends to elongate towards the ovarian fossa and the follicular wall is thickening, the presence of echoic points in the antrum of the follicle, edematous endometrial folds and a relaxed cervix. (Pycock, 2008; Macpherson, 2010; McGregor, 2010; Brinsko et al., 2011)

One week prior to ovulation, the follicle increases by about 2-3 mm/day – from about 30 mm as it had 6 days prior to ovulation to about 45 mm on the day of ovulation) (Mair et al. 2013, Vogelsang, 2011). Knowing the previous

preovulatory diameter is very useful for predicting ovulation as mares tend to ovulate at the same follicular size (Pycock, 2008; Brinsko et al., 2011). The artificial insemination can be performed with fresh semen, refrigerated one or frozen semen. Different rules need to be respected for each technique. For the fresh semen the aim should be that each mare is inseminated once within 24-48 hours before ovulation. If the mare ovulates within 48 hours after insemination, there is no need to be inseminated again. Insemination dose is usually between 250-500 million sperm with forward movement and the volume varies between 10 to 30 mililiters. The semen should be placed in the body of the uterus not immediately after the cervix (Brinsko et al., 2011). Refrigeration of the semen, is required in order to prevent impairment of sperm when it is used for more than 6 hours after sampling. The veterinarian must ensure that only one mare is inseminated and that any unused semen will be destroyed. (Pycock, 2008). Refrigerated semen must be ordered when the dominant follicle size exceeds 35 mm and mare's characteristic signs of estrus are present. Ovulation induction is done when semen is collected and shipped. This ensures that most mares will ovulate within about 12 hours after insemination (LeBlanc, 2005). Frozen semen is stored and transported in liquid nitrogen containers that maintain a temperature of -190°C for several days or weeks, depending on the container. In general, an insemination dose consists of 4-8 straws of 0.5 ml. The straws are defrosted in warm water at 37°C for not more than 30 seconds (Barbacini, 2009). The frozen semen should be inseminated into the uterus 12 hours before the ovulation and up to 6-8 hours after ovulation (Macpherson, 2010).

MATERIALS AND METHODS

The aim of this study was to evaluate the effectiveness of artificial insemination with preserved semen in mare and to realize a comparative study with natural cover, the primary motivation being that very few studies compare the effectiveness of different reproductive biotechnologies applied to horses, most of them focusing only on one technique.

To assess the effectiveness of each technique, we were interested to determine the number of examinations per estrous cycle, the number of matings or artificial insemination necessary to

achieve pregnancy and the pregnancy rate for each category included in the study.

Study groups for artificial insemination we also proposed to evaluate the dynamics of follicular growth, preovulatory follicle granulosa size and the number of semen straws used to achieve pregnancy.

The studies were conducted during the breeding seasons of 2012, 2013 and 2014, at private breeders and at a national studfarm on a total of 45 mares of different breeds and age, which were divided into 3 groups: a group consisting of 8 mares which were artificially inseminated with frozen semen, a group of 16 mares that were inseminated with refrigerated semen and a group of 21 Lipizzaner mares that had natural cover at Beclean studfarm. The frozen material was imported from Hungary and Germany. Because semen doses were limited, a single insemination dose was used in maximum 6 hours after ovulation. The follicles larger than 25 mm have been monitored by ultrasound exam, and when the dominant follicle has exceeded the size of 35 mm, 3000 IU hCG (Chorulon®) were intravenously administered. 12 hours after the administration of the ovulatory agent, mares were examined every 6 hours to detect ovulation. This type of management was absolutely necessary to ensure that semen is deposited in the uterus up to a maximum 6 hours after ovulation.

For ultrasound examination of the genital tract, we used portable Mindray ultrasound machine

with 5MHz linear probe. In the obtained images we could assess ovarian activity by measuring follicle diameter, follicular wall thickness, appearance of uterus and detection of corpus luteum and pregnancy (Fig.1 and Fig.2).

The semen straws were maintained at -196°C in liquid nitrogen. One dose is composed out of 3 or 5 straws of 0,5 ml which contain 600 millions to 1 milliard spermatozoids. It is mandatory that after defrosting, the mobility of the spermatozoids to be at least 30%. Each dose contained all the identification data of the stallions. The artificial insemination was done by respecting the general protocol. 14 days later, an ultrasound exam was performed to check the presence of the embryo. The non pregnant mares were monitorized for the next estrus.

For the study group inseminated with refrigerated semen, in order to obtain an acceptable fertility is recommended that the material to be used within 24-48 hours after collection and insemination should take place in 24 hours prior to ovulation. The ovarian activity of mares was monitorized by transrectal and ultrasound exam. For the control and synchronization of ovulation in mares that presented a mature corpus luteum for more than 5 days, 7.5 mg of Luprostiol (Prosolvin®), a synthetic analogue of PGF2 α , have been administered intramuscularly. Estrus appeared in 2-4 days and ovulation occurred in 7-12 days. When the dominant follicle exceeded 35 mm size the semen was collected in syringes

Fig. 1. Uterus of estrus - characteristic appearance of endometrial folds

Fig. 2 Detection of 2 week pregnancy

and transported at 4°C. During the collection and shipment of semen the ovulation was induced with 3000 IU hCG (Chorulon®). This management has ensured us that most mares will ovulate about 12 hours after insemination and thus semen was used within 24 hours after harvesting. One dose of refrigerated semen contains 500 million spermatozooids and the analysis after shipment showed a motility of 75%. After insemination, ovarian activity was monitored, and if after 24 hours was found that ovulation did not occur, the mare was inseminated again.

For natural breeding, the detection of mares in heat was done individually with the help of a stallion. When the mare was ready, the natural cover took place every other day, until the mare refused the stallion. Unlike artificial insemination, for natural mating no hormonal treatment was used. was not called estrus or ovulation induction with hormone treatment, and transrectal and ultrasound examination was performed only in mares exhibiting atypical sexual behavior.

RESULTS AND DISCUSSIONS

For each mare taken into this study, an individual file was created that contained the name, age, breed, the last pregnancy, the controls, the ovulation, the insemination and the pregnancy rate. Also the size of follicles was monitored for each ovary, by ultrasound exam.

All mare inseminated in the breeding season of 2012 had foals in the spring of 2013. Out of these, only one mare was inseminated in the first estrus after foaling "foal heat", all the rest of them being inseminated in the second estrus cycle.

In 2014 breeding season mares were in the transition period and were not inseminated in the first heat cycle, but were examined daily during estrus until ovulation occurred first, which was denoted as day "0" of the new estrous cycle, and thus able to estimate when they come back in heat. The insemination is not recommended during the transition period because the ovaries develop several small follicles which often regress and not ovulate.

Mares were monitored for 1 to 3 estrous cycles. Because we disposed by a limited number of semen doses, an intensive monitoring of mares was necessary to be implemented, so that a total of 171 exams were performed with an average of 11 examinations per estrous cycle. A single

insemination per mare per estrus cycle was performed, always in maximum 6 hours after ovulation. Throughout the whole breeding season, mares were inseminated 1 to 3 times, requiring an average insemination with frozen semen to achieve pregnancy. Five mares out of eight were pregnant after one insemination in one estrus cycle, giving us a pregnancy rate of 62.50%. In one non pregnant mare the embryo was resorbed after pregnancy was diagnosed, but this was related with the corticosteroids treatment for respiratory problems.

The key element in this type of insemination consists in a precise measurement of the follicle (Fig. 3, 4, 5) in order to predict as accurate as possible the ovulation moment and can be correlated with the thickness of preovulatory follicle granulosa which varies between 2 and 5.2 cm (Fig. 6). Growth rate varied from 0.90 mm/day to 4.3 mm/day with an average of 2.3 mm/day (Fig. 3,4,5).

Several authors report a growth rate of the dominant follicle between 0.88mm/day to 5mm/day, with an average speed of 2.2 - 3 mm/day (Carnevale, 2008; Ginther et al., 2004; Vogelsang, 2011; Raz et al., 2012; Mair et al., 2013 Newcombe and Cuevo-Arango, 2013). Knowing the diameter of preovulatory follicle in the anterior eustrus is very useful for predicting ovulation as mares tend to ovulate at the same follicular size.

For the 16 mares that were inseminated with refrigerated semen, they were monitored during 1 to 4 estrus cycles, with a total of 210 examinations and an average of seven examinations per cycle. Two inseminations per cycle were done for each mare, with a total of 73 artificial inseminations. Out of the 16 mares included in this study group, 11 were pregnant, the pregnancy rate being 70.5%. Unsuccessful inseminations are related to old age of the mares, irregular functioning of the ovaries, uterine pathological formations or to missing the ovulation moment.

The study group that had natural covers (n=21) were supervised during a total of 42 estrous cycles and 320 examinations were done, with an average of 8 examinations per cycle. The breeding rate varied in between 1 and 13, with an average of 4 covers per mare. Out of 21 mares, 15 were pregnant with a pregnancy rate of 71.42%.

By comparing the three breeding technologies in mares, we noticed that most of the ultrasound

Fig.3. Preovulatory follicle

Fig. 4. Preovulatory follicle becomes less circular and elongates towards the ovulation fossa

Fig. 5. Thickened follicle granulosa (5.2 mm)

Fig. 6. Corpus hemorrhagicum

examinations were necessary for artificial insemination with frozen material (n=11), compared to refrigerated material (n=7) and natural covers (n=8). However, the group inseminated with frozen semen was monitored for a shorter time (1 to 3 estrus cycles) compared to the others (4 estrus cycles). For obtaining a pregnancy, only one insemination was sufficient for frozen material compared to refrigerated

one (n=4) and natural covers (n=8). The highest pregnancy rate was in natural covers (71.4%). More studies reported a pregnancy rate of 60-70% with refrigerated material for less than 24 hours at 5°C, but if the semen is refrigerated for more than 48 hours, the rate is reduced at half (Brinsko *et al.*, 2000). In the literature (Squires, 2005) there is not such a huge difference in the pregnancy rate of mares inseminated with frozen (56%) or

refrigerated material (54%). Most of the clinicians are focusing on using one insemination with frozen semen in maximum 6 hours after ovulation (Sieme et al., 2003) even though the pregnancy rate was higher when two inseminations were performed (Vidament et al., 1997; Samper, 2001; Reeger et al., 2003). Barbacini et al. (2005) showed that two inseminations realized before and after the ovulation has a similar efficiency with only one realized postovulatory.

Another key element is the quality of the semen and its capacity of preservation. Metcalf (2005) showed that the total volume of semen inseminated ranged from 0.5 ml to 5 ml. The total number of spermatozooids inseminated to a mare went from less than 100 to more than 800 million. Overall pregnancy rate was 68%. For mares inseminated with less than 200, 200-400, 400-600, 600-800 or more than 800 million spermatozooids with forward movement, the percentages of conception were 54%, 60.2%, 60%, 88.2% and 56.4%. Pregnancy rates were significantly increased when the mares were inseminated with 600-800 million spermatozooids, compared to less than 600 or more than 800 million spermatozooids.

CONCLUSIONS

As natural service, artificial insemination presents both advantages and disadvantages. The advantages of using semen preserved are: the possibility of maintaining the horse home, avoiding the stress and costs of transport of the mare and possibly of the foal, taking care of the mare at another farm, reducing the opportunities for mares and foals injury during transport; decreasing the chances of transmission of venereal diseases as diluents used for dilution and preservation of semen contain antibiotics; access to valuable stallions located far away with sportive activity or overloaded during the breeding season. Another advantage is a more efficient use of semen as from one ejaculate more insemination multi-doses can be obtained and more mares can stay pregnant in the breeding season. The genetic diversification, improvement and achieving the desired characters increase the value of the products.

Artificial insemination also presents some disadvantages: sperm fertility may be reduced after preservation. The total number of spermatozooids of one insemination dose needs to be higher for the refrigerated and frozen material in

order to insure a proper fertility after defrosting. The preservation techniques of the semen and insemination technique need to be well known, as non-compliance with harvesting, handling, processing semen and insemination technique may decrease pregnancy rates. An intensive management of mares is necessary to ensure that insemination is performed at the optimum time, in order to reduce as much as possible the cost and time invested in the insemination of mares.

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Acknowledgements: This paper was published under the frame of European Social Fund, Human Resources Development Operational Program 2007-2013, project no. POSDRU/159/1.5/S/136893.