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Dedication

I'm dedicating this project to **ALLAH** Almighty, who made me and gave me strength, ideas, wisdom, knowledge, and understanding. He has strengthened me throughout this program, and I have only flown on His wings.

This study is dedicated from the bottom of my heart to my wonderful **parents**, who have given me so much, who gave me hope and strength when I wanted to give up, and who continue to be a source of moral, emotional, and financial support.

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Fatma

Dedication

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Ouiam

Abbreviation list

AI : Artificiel Insemination

AV : Artificiel Vagina

CASA : Computer Assisted Sperm Analyzer

LO: left ovary

RO : right ovary

SCA : Sperm Class Analyzers

VET : Veterinarian

YO: Year Old

SF: Small Follicle

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Abstract

The aim of our study is to provide valuable insights and recommendations for optimizing the artificial insemination process, ultimately contributing to the improvement of breeding outcomes in mares in Tiaret.

We have inseminated artificially 7mares during the mating season (from February to June 2023) at the veterinary institute of Ibn-Khaldun University Tiaret. Semen was collected from stallion using an artificial vagina. Mares were inseminated each 24 - 48h once the follicular diameter reaches 35mm.

The results obtained in our study show that the main diameter of pre-ovulatory follicle at the last insemination was **41.43**mm. Conception rate is**85.71%**. The index of insemination is very interesting. Its value is **1.43.** The rate of the mares diagnosed pregnant is **66.66%**after the first cycle and **16.66%**after both the second and the third cycle.

In conclusion, according to our results, artificial insemination can improve breeding outcomes in mares in Tiaret.

Final year project focuses on the study of artificial insemination in mares. Artificial insemination is a widely used assisted reproductive technique in the equine industry to enhance breeding and genetic selection of horses.

In this project, we examine the various procedures employed in artificial insemination in mares, by concentrating on the key factors that influence the success of this technique. We explore aspects such as stallion selection, semen preparation, different insemination methods and ovulation timing.

By describing the standard operating procedure for artificial insemination in the mare using fresh and diluted semen, relying on insemination per vagina technique in order to obtain positive results (pregnant mares).

Through artificial insemination a mare and stallion are allowed to be mated that under normal circumstances could not be covered naturally.

In conclusion, this study provides a comprehensive overview of artificial insemination in mares, emphasizing essential aspects of this assisted reproductive process. The gathered information can be utilized to enhance artificial insemination practices among horse breeders, aiming to optimize conception and fertility rates in mares.

ملخص

الهدف من دراستنا هو تقديم توضيحات وتوصيات قيمة لتطوير عملية التلقيح الاصطناعي الذي يساهم بدوره في اعطاء فرصة كبيرة لتحسين الكفاءة الإنتاجية للخيول والإنتاج يكون بشكل موثوق أكثر وبسلامة أكبر .

قمنا بتلقيح 7أفراساصناعيًا خلال موسم التزاوج (من فبراير إلى يونيو 2023) في المعهد البيطري بجامعة ابن خلدون بتيارت. تم جمع السائل المنوي من الفحل باستخدام مهبل اصناعي. حيث تم تلقيح الأفراس كل 24-48 ساعة بمجرد وصول قطر الجريب 35 مم..

تظهر النتائج التي تم الحصول عليها في دراستنا أن القطر الرئيسي للجريب ما قبل التبويض في التلقيح كان 41.43 ملم. في حين ان معدل الحمل قدر بنسبة 85.71٪. علما ان مؤشر التلقيح مثير جدا للاهتمام. قيمته قدرت ب 1.43 . وبلغ معدل الأفراس التي تم تشخيص حملها ب 66.66٪ بعد الدورة الأولى و 16.66٪ بعد كل من الدورة الثانية والثالثة.

في الاخير و وفقًا لنتائجنا ، تاكدنا من أن التلقيح الصناعي يؤدي إلى تحسين العمليات الإنتاجية عند الأفراس على مستوى ولاية تيارت .

يركز مشروعنا البحثي على دراسة التلقيح الصناعي فهذا الاخيرعبارة عن تقنية مساعدة على الإنجاب مستخدمة على نطاق واسع في تكاثرالخيول للتعزيز و التحسين الوراثي..

في هذا المشروع ، ندرس كذلك الإجراءات المختلفة المستخدمة في التلقيح الاصطناعي للأفراس ، من خلال التركيز على العوامل الرئيسية التي تؤثر على نجاح هذه التقنية. نستكشف جوانب مثل اختيار الفحل وإعداد السائل المنوي وطرق التلقيح المختلفة وتوقيت الإباضة.

من خلال وصف إجراءات التشغيل المعيارية للتلقيح الاصطناعي في الفرس باستخدام السائل المنوي الطازج والمخفف ، والاعتماد على وسيلة التلقيح بالمهبل للحصول على نتائج إيجابية (الأفراس الحامل).

في الختام ، تقدم هذه الدراسة نظرة عامة شاملة على التلقيح الاصطناعي في الأفراس ، مع التركيز على الجوانب الأساسية لهذه العملية الإنجابية المساعدة. يمكن استخدام المعلومات التي تم جمعها لتعزيز ممارسات التلقيح الاصطناعي بين مربي الخيول ، بهدف تحسين معدلات الحمل والخصوبة في الأفراس.

Résumé

Le but de notre étude est de fournir des informations précieuses et des recommandations pour optimiser le processus d'insémination artificielle, contribuant finalement à l'amélioration des résultats d'élevage des juments à Tiaret.

Nous avons inséminé artificiellement 7 juments pendant la saison de reproduction (de février à juin 2023) à l'institut vétérinaire de l'université Ibn-Khaldoun de Tiaret. Le sperme a été collecté sur un étalon à l'aide d'un vagin artificiel. Les juments ont été inséminées toutes les 24 à 48h une fois que le diamètre folliculaire a atteint 35 mm.

Les résultats obtenus dans notre étude montrent que le diamètre principal du follicule pré-ovulatoire à la dernière insémination était de 41,43 mm. Le taux de conception est de 85,71 %. L'indice d'insémination est très intéressant. Sa valeur est de 1,43. Le taux de juments diagnostiquées gestantes est de 66,66 % après le premier cycle et de 16,66 % après le deuxième et le troisième cycle.

En conclusion, selon nos résultats, l'insémination artificielle peut améliorer les résultats d'élevage des juments à Tiaret.

Le projet de fin d'étude qui sur l'insémination artificielle chez les juments. L'insémination artificielle est une technique de reproduction assistée largement utilisée dans l'industrie équine pour améliorer l'élevage et la sélection génétique des chevaux.

Dans ce projet, nous examinons les différentes procédures utilisées dans l'insémination artificielle chez les juments, en nous concentrant sur les facteurs clés qui influencent le succès de cette technique. Nous explorons des aspects tels que la sélection des étalons, la préparation de la semence, les différentes méthodes d'insémination et le moment de l'ovulation.

En décrivant les procédures de l'insémination artificielle chez la jument à l'aide de semence fraîche et diluée, s'appuyant sur la technique d'insémination par voie vaginale pour obtenir des résultats positifs (juments gestantes).

Grâce à l'insémination artificielle, une jument et un étalon sont autorisés à être accouplés qui, dans des circonstances normales, ne pourraient pas être saillis naturellement.

En conclusion, cette étude permet de mieux comprendre l'insémination artificielle chez les juments, en focalisant sur les aspects essentiels de ce processus de reproduction assistée. Les informations recueillies peuvent être utilisées pour améliorer les pratiques d'insémination artificielle chez les éleveurs de chevaux, dans le but d'optimiser les taux de conception et de fertilité des juments.

Summary

Experimental part

Introduction

In the field of equine reproduction, artificial insemination has revolutionized breeding techniques and contributed to the genetic improvement of horse populations worldwide.Artificial insemination is widely used in equine breeding as it allows for the extensive use of valuable stallions for selection(Pagl et al., 2006 ; Gmel et al., 2021).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 (Morar et al., 2014).

Not only does it provide the opportunity to breed with high-performing athletes, but AI also reduces the risk of serious injury to both mares and stallions(Gmel et al., 2021).

Furthermore, artificial insemination with fresh, cooled, or frozen semen has become one of the most commonly used assisted reproductive techniques in the global equine industry. For the fresh semen the aim should be that each mare is inseminated once within 24-48hours before ovulation (Morar et al., 2014).

Artificial insemination has not always been readily accepted in the equine industry. In the past, the use of artificial insemination in horses faced resistance from some horse breeding associations. However, this situation has changed over time, and artificial insemination has become an integral part of horse breeding practices globally. In recent years, the availability and advancements in artificial insemination techniques have greatly influenced genetic progress in the equine industry (Rečková et al., 2022).

By addressing these problematic aspects, this research aims to provide valuable insights and recommendations for optimizing the artificial insemination process, ultimately contributing to the improvement of breeding outcomes in mares in Tiaret.

1).History:

According to historical accounts, the first reference to the use of AI in horses is reputed to be in **Arabic** texts dating from as early as **1322**.

The story, apocryphal or not, is that one of two feuding **sheikhs** stole semen from his enemy's prize stallion for use on one of his mares. No details are given on the methods used but the operation was reported to have been successful (**Perry**, **1945**, **1968**). In **1677**, **Antonvan Leeuwenhoek** and **JohanHamm** first identified spermatozoa using a microscope.

They subsequently were described as "animalcula – innumerable" minute bodies with the power of active forward motion. However, it took another **100** years before artificial insemination was documented for the first time. This report was of the work done in 1780 by the Italian physiologist **Spallanzani** who, after some encouraging successes with amphibians, attempted the insemination of a bitch with freshly collected semen, kept at room temperature prior to immediate insemination directly into the uterus. This procedure was successful and resulted in the birth of three puppies. He later went on to evaluate his technique in horses (**Perry**, **1945**, **1968**; **Varner**, **1986**). Work by **Rossi** and **Branchi** in **1782** verified**Spallanzani**'s successes (**Perry, 1968**). **Spallanzani** was able, during the course of his research, to establish that it was the sperm (he termed them spermatic vermiculi) that had the power of fertilization, rather than the associated fluid (seminal plasma) which he removed by filtration. Initial experiments into semen cooling and, therefore, prolonging of their life span were also conducted by **Spallanzani** . He used snow to freeze the 'spermatic vermiculi' and found that though they became motionless when in contact with the snow, they did not die, and could be revived by warming. Before the late **19**th century, AI in all species remained largely of scientific concern, until interest in the technique grew from commercial lay personnel. Programs to apply the use of AI to livestock on a more commercial basis began to be developed in **Russia** and **China** during the late **19**th century

Towards the end of the **19**th century there were reports of the use of AI in several European countries (**Boyle**, **1992**).While doing so, **Walter Heape**, at the University of **Pennsylvania**, was building upon his recent success in the insemination of bitches (**Heape, 1897**) and reported the successful use of AI in mares on a number of farms (**Perry, 1945**). The method was being

developed in France as well. Spearheaded by a vet, **Repiquet,** who advised on the use of AI in Europe for the first time, to overcome infertility **(Perry, 1945**). During this period Professor **Hoffman** of **Stuttgart** produced a report, A Description, Instruments and Techniques for AI as a Supplement to Natural Service, which, as the title suggests, described the use of AI as an additional safeguard after natural service, in order to try to improve the low conception rates that are characteristic of the horse. For this work the mare's semen was collected post coitum, from the depression in the lower and inseminated into the mare's uterus using a specially designed syringe. Vaginal wall, by means of a speculum or spoon. Cow's milk was used to dilute the semen. Professor **Hoffman** only used the technique in addition to natural service or as a back-up to natural service, rather than in isolation. He did not investigate the use of semen in mares that had not already been covered naturally (**Perry, 1968**).

Later, researchers looked into the use of sponges to gather semen deposited in the vagina du ring natural service.Despite some success was recorded, their use turned out to be ineffective due to high contamination rates from vaginal fluids, germs, and debris, as well as low spermatozoa re covery rates (**Boyle, 1992**).When the 19th Century came to a close **Lideman**(in **1895**) and **Izmailov** and **Enisherlov** (in **1896**) were using AI for the mass production of horses in **Russia**.

Chaechowski in **1894** and **Kaldrovics** in **1902** in the **Ukraine** and **Hungary** were using similar techniques, respectively, for the mass production of horses on large studs on a commercial basis (**Chaechowski, 1894; Tischner, 1992b**). In **1902Sand** and **Stribolt** reported at the Northern Livestock Conference in **Copenhagen** a **50%** success rate (four pregnancies out of eight mares inseminated) for equine AI and identified a use for AI in the increased commercial use of valuable stallions (**McKinnon and Voss, 1993**). This view marked the realization of the potential of the technique to improve farm livestock breeding efficiency rather than just as a treatment for infertility. This objective was first given substantial consideration in Russia at the beginning of the **20**th century when the first equine AI programs were organized by the **pioneer I.I. Ivanoff**, on Russian government-controlled stud farms, from **1899** onwards (**Berliner, 1947; Gordon, 1983**). At this time, many Russian studs employed AI as a means of serving their mares, but results were highly variable. It was reported that in inseminations carried out by **Ivanoff** himself conception rates equivalent to those of natural service were obtained. In one particular piece of work carried out by **Ivanoff, 39** mares were inseminated, out of which **31** became pregnant; this compared very

favourably with a similarly kept group of mares that were covered by natural service, in which only **10** out of **23** conceived (**Ivanoff, 1922; Perry, 1945, 1968**).

Apart from research, these laboratories trained veterinary surgeons and AI practitioners. In the years leading up to the First World War, **300–400** trained AI practitioners went out from these laboratories to work on stud farms, and so significantly increased the number of artificially bred horses in Russia at this time. After the war a central experimental station was set up in Russia under the directorship of **Ivanoff**. These series of laboratories were directly responsible for the development of the modern artificial vagina in **1930**, and for the proliferation of collection techniques including the use of the dummy mare (**Olbrycht, 1935; Perry, 1945, 1968; Tischner, 1992a**). In 1938, 120,000 mares were reported to have been covered by AI in Russia. This figure significantly increased in the following years to an average of **300,000–400,000** mares covered by AI per year.

Along with this significant interest in Russia, other countries – Japan in particular – started to develop an interest in the use of AI. Between 1913 and **1917, 323** Japanese mares were covered by AI (**Perry, 1968**). Interest was also developing in China, where **600,000** mares were inseminated in **1959**, with a reported pregnancy rate of **61%**. In **1960**, China's two most popular stallions were used to inseminate **4415** and **3039** mares, respectively, with reported pregnancy rates of **76.9%** and **68.1%,** respectively (**Cheng, 1962**). Between the two world wars there was also extensive use of AI in European and Balkan countries (**Boyle, 1992**). Around this period and immediately after the Second World War, Hanover was particularly prominent in its use and development of AI techniques. The development of AI for horses in the **USA** was slower than that in Europe, with significant work there being carried out on the use of AI in cattle, and in **1938** the first AI organization was established in the **USA** for cattle (**Foote, 1982**). In **1938** only **50** calves were born per sire per year by AI but, by **1981**, this figure had increased to **50,000**. Unfortunately, there was no synchronous development in the use of AI in goats, swine, sheep or horses. The stated reasons included management problems, prejudice, economic viability and less well developed technology.

By the **1930**s the techniques for using AI in horses were well established and its use was spreading worldwide.

Shideler, R.K. (1993a) History. In: McKinnon, A.O. and Voss, E.L. (eds) Equine Reproduction.

Boyle, M.S. (1992) Artificial Insemination in the horse. Annales de Zootechnie 41(3–4), 311–318.
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Foote, R.H. (1978) General principles and basic techniques involved in synchronisation of oestrus in cattle. Proceedings of the 7th Technical Conference on AI and Reproduction. NAAB, Madison, pp. 55–61.

Heape, W. (1897) The artificial insemination of mammals and subsequent fertility on impregnation of their ova. Proceedings of the Royal Society of London, 61, 52.

2).Advantages & Disadvantages of Artificial Insemination

2.) a. Advantages of AI :

- Disease prevention. this is achieved by avoiding skin –to skin contact, such as occurs during natural service.

- The herpes virus, which causes horse coital exthanema, and infectious equine metritis, which is brought on by bacteria, are themain illnesses it guards against.
  Additionally, antibiotics may be administered to the semen at the time of collection or insemi nation to help minimize or completely avoid contaminating the mare with any kind of bacteri a prior to breeding it.

- Decreases chances of injury. Semen is generally only collected every other day in an AI program, so there is much less chance for injury. In addition, semen can be collected on a phantom and a mare in heat may not even be needed. Not only does this almost eliminate injury to the mare (if she didn't want to get served) and stallion but also it dramatically reduces the chances of injury to those staff involved in the breeding shed. It may be in future that farms could be in legal trouble when staff is hurt and AI could have been used.

- Semen can be collected from stallions with problems. Each year stallions are either injured or have trouble breeding due to inherent libido (sexual behavior) problems. Because semen only needs to be collected every other day it reduces the effects of breeding pressure and injuries.

- Each time sperm is collected, it is examined. With natural service we are flying a bit blind because it is not until pregnancy rates are established that we can assumethe semen was OK at the time of breeding. With AI we can look and measure parameters related to fertility every time we collect. One way to look at reproduction is to say that when we feel the testicles we are "feeling the future" and when looking at semen we are "looking at history" as one represents potential and the other the recent event of semen production.

- Prevents excessive use stallions.

- Helps more mares have offspring. AI lets us divide the semen up into as many doses as he is capable of giving in an ejaculate. Typically that is around 10-15 doses on an every other day collection schedule.

- Permits breeding of mares with problems. Each year mares are presented for natural service that may not be psychologically ready for service. Sometimes this is due to the mare's own agitation and sometimes it may be because she has a 'foal at foot' and becomes worried about her foal (foal proud).

- Permits use of older valuable stallions. As stallions get older their sperm numbers decrease. This typically begins at around 13 years old. Many stallions are already exhibiting quite obvious sperm reduction by the time they are 16 - 17 and many are almost infertile by the time they are 19 - 20 years old. AI allows deposition of the correct number of sperm to be made for each mare and removes the guesswork.

- Allows mares to be bred at the best time for conception. Because we can store semen either cooled or frozen mares can be bred when they are most suited for conception. In a natural service program this would necessitate breeding the stallion as many as 5-6 times per day. Most stallions would not handle a breeding schedule like that for very long.

2).b. Disadvantages of AI

- It requires specialized equipment. An artificial vagina (AV), thermometers, warmed containers and equipment non-spermicidal gel and equipment to measure motility (warmed stage microscope) and sperm concentration are all necessary for AI to be practiced properly. This is expensive and a well-equipped laboratory may cost in vicinity of $40 000 just for the equipment (without the building and fittings).
- Professional expertise must be acquired. Personnel need to know how to make the AV so that it is right for the stallion and then how to collect and process the semen properly.
- Problems may result from improper AI application. You would be surprised to learn just how often the wrong lubricating gel is used in the AV and that there have been weeks before anyone realized that the pregnancy rates were disastrous.[1]

3).Sexual and anatomy physiology reminder

3).a. REPRODUCTIVE ANATOMY OF THE MARE:

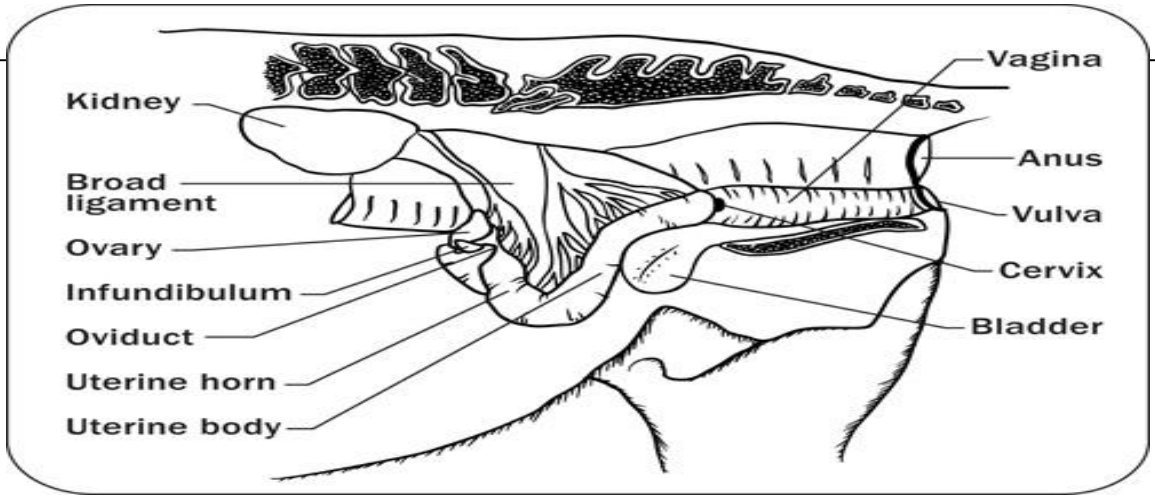

Figure 01: Sagittal view of the mare reproductive structures (Pickett and Voss, 1972).

[1]https://www.petsupplies4less.com/Pet-Pointers-Horse-Care-Articles-Advantages-Disadvantages-of-Artificial-Insemination.html

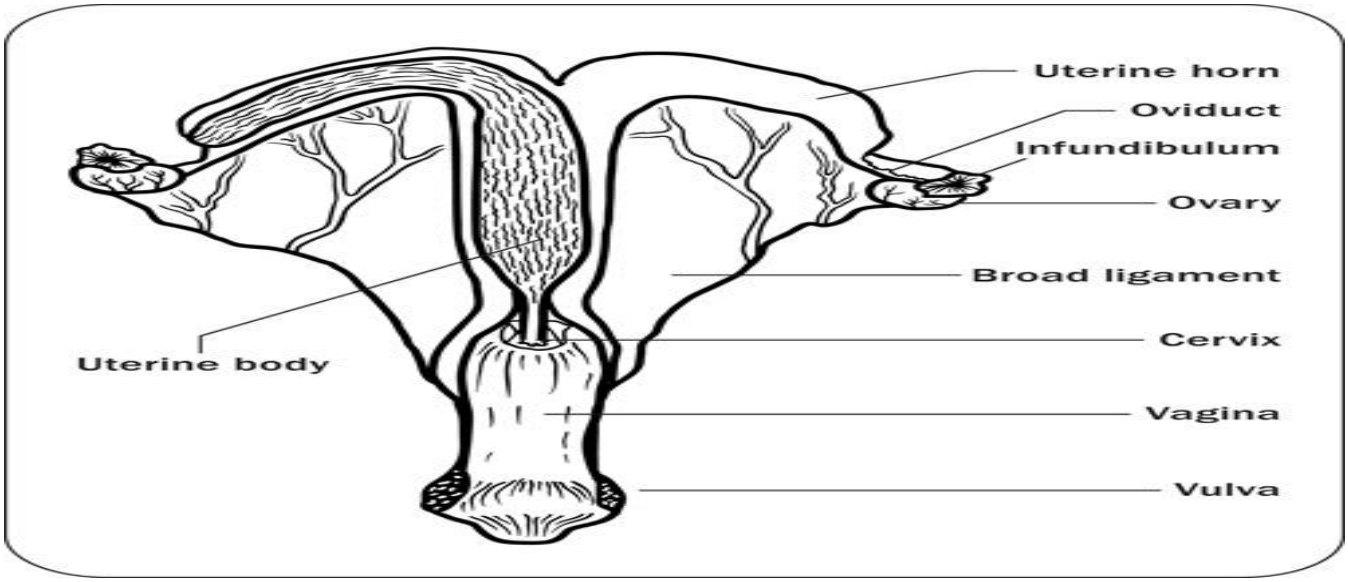

Diagram showing the basic elements of the reproductive tract (dorsal view). Indicates uterine body, uterine horn, oviduct, infundibulum, ovary, broad ligament, cervix, vagina and vulva**(Pickett and Voss, 1972).**

Figure 02: **Frontal view of the mare reproductive structures (Pickett and Voss, 1972).**

3).b. <u>THE REPRODUCTIVE CYCLE OF THE MARE</u>:

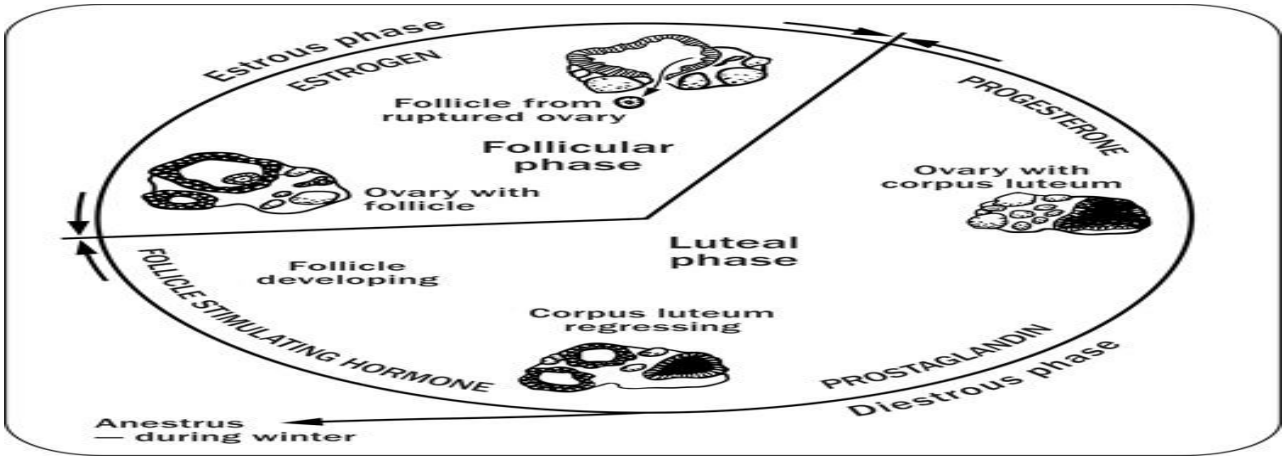

Figure 03: Breeding cycle of a mare (Pickett and Voss, 1972).

The mare has both a reproductive season and a non-reproductive season. Regulated by light. The non-reproductive season, known as anestrus is when there's minimal natural light, like in fall and winter. In the spring, when the number of daylight hours starts to increase, the reproductive season begins. In the spring, when the number of daylight hours starts to increase, the reproductive season begins.

Two other periods are known as the spring and autumn transitional stages. One occurs just before the mare becomes reproductively active in the spring and the other occurs just before anestrus in the winter. During these periods mares are generally erratic in their cycles and sexual behavior. The spring transition period coincides with increased daylight hours, increased grass growth and ambient temperatures. As the season progresses cycles become regular the mare's breeding cycle is on average 21 days, but can vary greatly between individual. For a period of around five to seven days within the 21-day cycle the mare is 'in estrus', 'in heat', 'in season', 'horsing', or 'receptive' to the stallion. For the other 14 to 16 days the mare is 'in diestrus', 'out of season' or 'not receptive' to the stallion**(Pickett and Voss, 1972)**.

4). Seasonality:

As in the mare, reproductive performance of the stallion is affected by season, regulated by the influence of daylight hours on hormones. During the breeding season, April through August, stallions are more productive than in the fall and winter months in several ways (Dickie, 1995):

- Greater seminal volume: during the summer months, the average mature stallion will produce approximately 8 billion sperm cells per day, with production being proportional to the size of the testes.

- Increased sexual response: the reaction time of stallions as influenced by season. During the breeding season, it took less than 2 min for the stallions to become sexually stimulated, mount and enter the artificial vagina. In the wintertime, the reaction time was in excess of 10 min (figure 4). Therefore, when training the young stallion to breed, it is best to start in the normal breeding season.

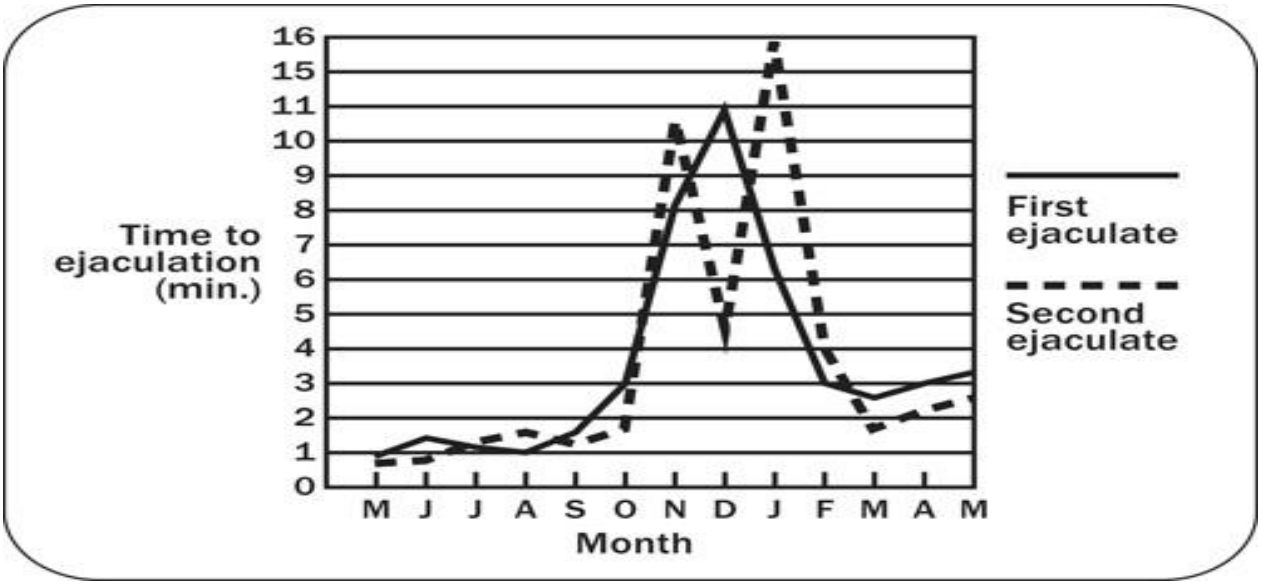

Figure 04: Effect of season on sexual behavior by time to ejaculation (Pickett and Voss, 1972).

- **Increased ability to ejaculate - Figure 04** shows the seasonal influence on ability to ejaculate. In the normal breeding season, just over one mount per ejaculate was required, but in the non-breeding season, over 2.5 mounts per ejaculate was required on average.

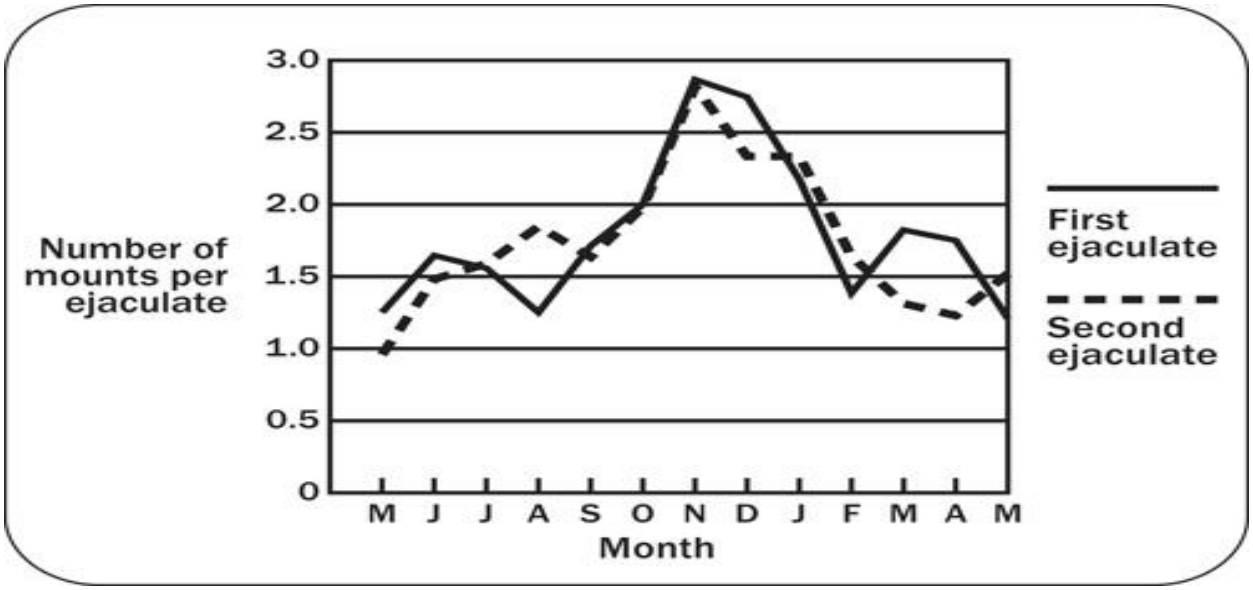

Figure 05: Effect of season on sexual behavior by number of mounts required per ejaculation. Source: Pickett and Voss

Graph showing the effect of season on sexual behavior as measured by number of mounts required per ejaculation. Number of mounts per ejaculate, on y-axis. Time of year by month, on the x-axis. Number of mounts required for ejaculation peaks at less than 3 around November for first and second ejaculate. Number of mounts required for ejaculation lowest in May for first ejaculate. Number of mounts required for ejaculation lowest around April and May for second ejaculate.

Pickett, B.W., and J.L. Voss. 1972. Reproductive management of the stallion. Proc. 18th Annual Convention. American Association of Equine Practitioners, p. 501.

5).Hormonal control of sexual activity

As spring approaches, the pituitary gland is stimulated by increased daylight to enhance follicle-stimulating hormone production. **Follicle-stimulating hormone** is released into the blood stream and travels to the ovaries to initiate development of a follicle containing an ovum. The developing follicle produces estrogens, which are released into the blood stream. Estrogens perform a variety of tasks in the body. When blood estrogen reaches a certain level, a surge of luteinizing hormone is released from the pituitary gland into the blood stream. Estrogens are responsible for the clinical signs of estrus and act on the oviducts, uterus and cervix to prepare the reproductive tract for pregnancy. The surge of **luteinizing hormone** causes the follicle on the ovary to rupture, resulting in ovulation. As the follicle develops on the ovary, the ovum (egg) inside the follicle undergoes a number of changes to become capable of being fertilized by the sperm. The follicle contains a viscous fluid and when the follicular wall ruptures, this fluid flows out, carrying the ovum with it. The cavity left by the ruptured follicle becomes engorged with blood to form a corpus hemorrhagicum.

The corpus hemorrhagicum luteinizes to form the corpus luteum, sometimes called the yellow body.

As the corpus luteum develops, it starts to produce **progesterone**, which influences the pituitary gland and reproductive tract. The feedback of progesterone via the blood stream inhibits the release of luteinizing hormone. Under the influence of progesterone, the mare will not show estrus. Progesterone function is to maintain the pregnancy by maintaining a uterine environment conducive to fetal development.

If the mare does not conceive, the corpus luteum remains functional for about 12-14 days. At this time, prostaglandin is released from the endometrium (inner lining of the uterus). **Prostaglandin** has a luteolytic effect - it acts on the corpus luteum via the bloodstream, causing it to regress. As the corpus luteum regresses, progesterone levels are reduced, resulting in the removal of the inhibition to luteinizing hormone secretion. The cycle starts over again.

If the mare conceives, hormonal activities are essentially the same as for the 12-14 days post-ovulation. Pregnancy recognition is stimulated by the action of the developing embryo migrating throughout the uterus; this action inhibits prostaglandin release. The result is an antiluteolytic effect, so the corpus luteum remains functional, progesterone levels are maintained and the pregnancy is continued.

Somewhere between Days 25 and 30 of gestation, the corpus luteum starts to regress, resulting in declining blood progesterone levels. If the progesterone level were to continue to decrease, the pregnancy would be terminated. However, a compensatory system has evolved that is unique to the mare. Between the 25th and 36th day of gestation, a girdle-like band of special cells develops around the fetal sac. On about Day 37 of gestation, this band detaches from the fetal membranes and invades the endometrial wall where these cells undergo tremendous enlargement and structural change. These cells clump to form the endometrial cups that secrete the hormone **equine chorionic gonadotropin**. Equine chorionic gonadotropin reaches the ovaries via the blood stream, stimulating secondary follicular development and luteinization. The secondary corpus luteum produces progesterone, as does the primary corpus luteum to Day 130 to 150 of gestation. From about Day 80 of pregnancy to term, adequate progesterone levels are maintained by special areas of the uterus and fetal membranes, to sustain the pregnancy.

The pregnant mare foals (parturition) at 340 days ±20, post-breeding. Initiation of parturition is very complex and not completely understood, but the fetus probably plays a role in initiating the process. Mechanical stimuli occur from distension of the uterus, which brings about an increased sensitivity of the uterus to the hormones estrogen and Oxytocin. At the end of pregnancy, the uterus becomes active and the cervix dilates. **Oxytocin**, released by the pituitary gland, causes the muscles of the uterus to contract and expel the fetus (foal). (Dickie;1995).[2]

[2] Pickett, B.W., and J.L. Voss. 1972. Reproductive management of the stallion. Proc. 18th Annual Convention. American Association of Equine Practitioners, p. 501.

Chapter II : Semen collection

1). Alternative Methods of Semen Collection

1).a. Manual stimulation

Manual stimulation may be employed to provide extra (or the sole means of) stimulation and encouragement to ejaculate.

This might be performed with a warm towel (45–50°C) at the base of the shaft of the penis or on the glans penis.

Stallions that are unable to mount Due to an injury or anxiety, a teaser mare or dummy could aid from manual stimulation.

The semen ejaculated may be collected into an AV as per the normal collection procedure or even into a clean, sterile plastic bag placed over the penis (McDonnell and Love, 1990; Love, 1992).

Semen collected at ejaculation resulting from manual stimulation is reported to contain spermatozoa of comparable characteristics to those produced by natural ejaculation (McDonnell and Turner, 1994).

1).b. Collection without mounting

Semen may be collected using an AV but in the absence of a teaser mare or dummy for mounting.

1).c. Electro-ejaculation

Electro-ejaculation is the electrical stimulation of the musculature surrounding the vas deferens, urethra, accessory glands and the base of the penis. This stimulation is the result of a probe placed in the rectum of the stallion.

Equine Artificial Insemination pdf. (McDonnell and Love, 1990; Love, 1992). Schumacher and Riddell (1986). (J.M. Parlevliet, The Netherlands, 1998, personal communication).

1).d. Condom or breeder's bag method

- Breeder's bag is a heavy rubber material. It has to be washed of , rinsed with distilled water finally with normal saline. For reuse ,it should be sterilized properly.
- The stallion's **penis** should be washed from smegma or dirt removed and dried.
- The bag is applied over the erect penis, squeeze out the air and applied rubber bands.
- The stallion is allowed to mount; the ejaculate is collected in a pre warmed sterile tube. Bottle and kept in a water bath of 35^{o} c for further processing.[3]

2) <u>Artificial vagina</u>

For collecting a stallion's semen a dummy or phantom mare may be used as a mount for a stallion during semen collection.It's quite simple to train stallions to ride a breeding phantom to make collection much safer and more consistent. The stallion comfort should be taken into consideration when designing the phantom, and should mimic the same angle as the mare. If a jump mare is used, she should be in good standing heat and have her tail wrapped and perineal area washed. Restrain the mare to prevent injury to the stallion or personnel assisting.

To collect the semen for analysis, an artificial vagina is used, multiple AV are available: the Colorado, Missouri and Japanese models. The selection is determined by management and stallion preference. To prepare the artificial vagina, fill it with water to obtain proper temperature and pressure. Some models use a combination of water and air to get the proper pressure. Because of stallions' individual natures, water temperature preference, pressure and lubrication will vary. Therefore, it is important to prepare the AV to the personal preference for the stallion being collected..

[3]Ecoursesonline.iasri.res.in/mod/page/view.php?id=74794 Jun 2012.

2).a. The different types of artificial vaginas for semen collection:

Figure06: Colorado (The CSU model AV)

Rigid plastic case and handle with padded ends, outer latex rubber liner that forms the water jacket, inner latex rubber liner and cone, collection bottle and filter, and insulating bag for placement over the end of assembled unit.[4]

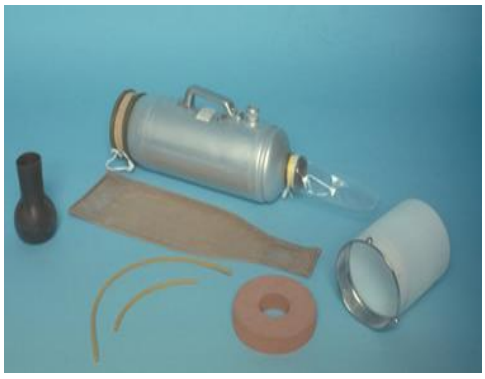

Figure07: Japanese model

Aluminum case with rigid handle, latex rubber liner, rubber bands for attaching the rubber liner to the aluminum case, collection bag and attached rubber band, insulating receptacle for the end of the artificial vagina, and rubber cushion (doughnut) for placement at the inside front of artificial vagina. The black rubber attachment can be used around the collection bag if desired.[5]

[4]From Varner DD, Schumacher J, Blanchard TL, et al: *Diseases and management of breeding stallions,* Goleta, CA, 1991, American Veterinary Publications, 119.

[5]From Varner DD, Schumacher J, Blanchard TL, et al: *Diseases and management of breeding stallions,* Goleta, CA, 1991, American Veterinary Publications, 120.

Figure08: Missouri model

Leather carrying case, double-layered latex rubber water jacket with attached latex rubber cone, bottle adapter, and collection bottle with semen filter inside. Disposable bottle liners *(shown)* can be used inside most collection bottles.[6]

2).b. Preparation of Artificial Vaginas for Semen Collection

Immediately before semen collection is attempted, the water jacket of the AV is usually filled with 45°C to 50°C water to provide an internal AV temperature of 44°C to 48°C.Providing an AV temperature above that of the body seems to aid in penile stimulation and facilitates ejaculation. Occasionally, some stallions may respond more favorably to semen collection with an AV if its luminal temperature is 50°C to 55°C. Sperm can be permanently damaged.

Pressure of the AV should be adjusted to provide uniformly good contact around the penis, without interfering with penile penetration. Proper AV pressure accommodates expansion of the penis to full erection. Full insertion of the penis into the AV during the first penile thrust and then maintenance of the penis in this fully inserted position is important; otherwise, the glans penis dilates and may be too large to permit full penile penetration into the AV. The result is extended contact of ejaculated semen with the AV liner and elevated temperature en route to the semen receptacle or ejaculatory failure from inadequate penile stimulation. Both temperature and water pressure in the AV should be maintained relatively constant during semen collection to promote consistent stallion performance and maximal sperm harvest.

The inner surface of the AV should be lubricated with a sterile, no spermicidal lubricant before penile insertion.

The collection receptacle should be maintained at body temperature during semen collection and transport to the laboratory to prevent cold shock to the sperm before they are placed in a protective extender. Semen should also be protected from light.

[6]From Varner DD, Schumacher J, Blanchard TL, et al: *Diseases and management of breeding stallions,* Goleta, CA, 1991, American Veterinary Publications, 118

To maximize the number of sperm available from each semen collection, an appropriate filter should be incorporated into the collection receptacle. The filter allows most of the gel-free fractions to pass into the seminal receptacle but traps the gel (which is presented in the final fractions of an ejaculate).

New Technologies for Agriculture Extension grant no. 2020-41595-30123 from the USDA National Institute of Food and Agriculture.

3)Collection Procedure

Multiple methods of semen collection are used the majority of which involve the use of an AV. The procedures for the correct use of an AV are varied and depend on the type of AV being used and individual stallion preference. Regardless of the specific techniques that are chosen, the following principles apply to the collection procedure. The aim when using an AV is to mimic the natural vagina and it is important that, among other things, the positioning of the AV during collection is appropriate.

Events involved in semen collection using a jump mare:

A) introduction or teasing of the stallion and mare.

B) AV ready for use.

C) Guiding the stallion's penis into the AV and the correct positioning of the handlers all on the same side of the mare and stallion.

D) Slightly inclined positioning of the AV to mimic the natural position of the mare's vagina.

E) Dismount of the stallion and slow removal of the AV, ensuring all semen is collected.

F) After dismount the stallion should be turned away from the mare to reduce the chance of injury.[7]

[7](Rousset et al. 1987 ; Thompson et al ; 2004) ; (Ball 2014).

After removing the gel fraction and placing the ejaculate into an incubator at 37 °C, the semen must be measured to determine its volume. Usually, the ejaculate volume is measured with prewarmed, sterile graduated cylinders. However, the most practical and accurate method for volume measurement is based on weighing the ejaculate on a scale, assuming an equivalence of 1 g to 1 mL.

- **Gross Evaluation**: As soon as the semen sample has arrived in the laboratory the first process is to filter the sample to remove the gel fraction by using a filter paper , detritus and sloughed epithelial cells.
- **Appearance**: Once the filtered sample is in the measuring cylinder, its color and consistency can be assessed. This is normally done by eye but requires microscopic examination for confirmation. A good sample should appear milky white in color, though it may range from watery to creamy, depending upon the spermatozoan concentration within the sample (Kuklin, 1983).
- . **Volume**: the volume is reduced for pure blooded horse (30-50ml) than for heavy breed (120-150ml)(Fauquenot, 1987).
- **Sperm concentration** : 100 à 200 millions of spz / ml on average.
- **Sperm motility**: 75 % on average.(Besse, 1993).
- **Osmolarity**: The normal osmolarity range should be between 290 and 310 mOsm (Pickett et al., 1976, 1989).
- **Seminal PH fluid**:(6.9-7.7).(Davies Morel, 1993; Oba et al., 1993).
- **Longevity**: Longevity is related to fertility of the stallion.

  (Fauquenot, 1987). (Besse, 1993). Pickett et al, 1976, 1989). (Davies Morel, 1993; Oba et al., 1993).

- **CASA system for sperm analysis**

A conventional semen analysis, as it is made in a Reproductive biology clinic or general laboratory, tries to find out the quality of the sample. For that purpose, the microscopic characteristics must be evaluated: the concentration of the spermatozoa in the seminal liquid, their motility, morphology, vitality, and more recently, the presence of DNA fragmentation is evaluated.

All with the aim to discover if a patient is fertile, and therefore capable of natural reproduction, or by the contrary, the microscopic analysis must be done starting with the sperm count with the use of SCA® **CASA** System for semen analysis allows the accurate, repetitive and automatic assessment of the following sperm parameters: **motility**, **concentration**, **morphology**, **DNA fragmentation**, **vitality**, **acrosome reaction** and **leukocytes**.hemocytometer, where the fixed and diluted sample is placed and would need an Assisted Reproduction technique to help.

Traditionally, a well- trained laboratory technician makes the analysis, starting by the macroscopic visualization of the sample: the color, odor, Ph, complete Liquefaction. All description data must be recorded (https://www.micropticsl.com/).

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Motility and concentration:

Progressive sperm motility is extensively known to be related to **pregnancy rates**. Motility must be assessed as soon as possible after liquefaction of the sample, preferably at 30 minutes, but in any case within one hour after ejaculation,

as **WHO recommends**, to decrease the effects of dehydratation, Ph or changes in temperature.

Morphology:

Morphology isa module that permits the automatic **morphometry** analysis and **morphology** classification of animal stained sperm samples observed in brightfield microscopy.

There are enormous variation in the sperm morphology, which make its assessment quite complex, but several studies have recovered spermatozoa from female reproductive tract, especially in postcoital endoncervical mucus, and from the surface of zona pellucida and have helped to define the appearance of potentially fertilizing, that is, morphologically normal, spermazoa in some species.

Vitality

SCA® Vitality is a module that automatically provides the percentage of **live** and **dead** spermatozoa in animal semen sample.

Sperm Vitality is estimated by assessing the **membrane integrity** of the cells, and is fundamental for samples having less than 40% progressive motile spermatozoa. The percentage of the viable cells normally exceeds that of motile cells.

Vitality assessment can be performed with **Vitality slides SCA** (in bright field) or with **FluoVit** kit (under fluorescence).

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Acrosome reaction:

This test measures in the first instance the **percentage intact acrosomes** of washed/diluted sperm samples. The next step is that we use a biological substitute to induce the **acrosome reaction** such as Ca++ ionophore.

DNA Fragmentatiion :

SCA® DNA Fragmentation is a module for the counting of DNA fragmented and non-fragmented spermatozoa, in animal samples treated with kits based on the **Sperm Chromatin Dispersion (SCD)**.

DNA fragmentation represents a distinguish mark of **apoptosis**, and is usually associated with structural changes in cellular morphology.

Leukocytes :

SCA® Leukocytes is a module for the automatic analysis of animal **peroxidase-positive leukocytes**, in samples treated with the **Leukocytes slides SCA**.

A semen sample is mixed with a specific substrate for the peroxidase enzyme. If there is peroxidase, the substrate containing hydrogen peroxide will be reduced. At the same time, diaminobenzidine (DAB) will be oxidized and turn into an insoluble brown products.

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1. Sperm dilution:

Dilution rate with all insemination is an important consideration. Inadequate dilution will not provide adequate support for spermatozoa. On the other hand, excessive dilution has been reported to be associated with depressed fertilization rates (**Katila, 1997**).Minimum semen: extender ratio of 1:1 has been recommended (**Brinsko and Varner, 1992).**Other workers have also demonstrated an effect of dilution rate on spermatozoan survival. Semen dilution rates of **1:2**, **1:5**, **1:10** and **1:20** were compared and resulted in survival rates of **41.2%, 54.9%, 57.2%** and **56.4%,** respectively. In general, semen for use fresh is diluted in a ratio of between **1:2** and **1:4,** depending upon the concentration of the raw semen sample. (Katila, 1997). (Brinsko and Varner, 1992).

2. Dilution method:

The composition of extenders varies enormously, but they are normally based upon milk or egg yolk products plus antibiotics.

The horse semen dilution can effectively prolong the viability time of horse sperms, improve the preservation quality and survival rate of the sperms and improve the plasma membrane integrity and the acrosomal integrity of the sperms in order to improve the fertility rate of mare.

Skim milk not only can be for sperm that provides energy matter and large protein material but it can also prevent sperm from suffering cold strike at temperature-fall period.

Before use, extender is put in to preheating in 37°C of water-baths, when use, mixes with horse seminal fluid.

In general, pasteurized skimmed milk plus antibiotic provides or satisfies all these requirements. Fine tuning of extenders with the addition of various extra components is normally carried out to perfect the final result.

Equine artificial insemination. (**Katila, 1997**).**(Brinsko and Varner, 1992).**

Partly Skim milk extender Volume =2. (Semen volume after filtration)

Table 01 : Average nutritional values for100ml

Component	Quantity
lipids including	1.5 g
saturated fatty acids	1
carbohydrates	4.5g
including sugars	4.5g
Proteins	3g
Salt	0.1g
Calcium	120mg
Vit D3	0.75µg
B1	0.17mg
B2	0.21mg
B3	2.4mg
B5	0.9mg
B6	0.21 µg
B8	7.5 µg
B9	30 µg
B12	0.38 µg
E	1.8mg

Fresh semen: mares are inseminated within 30 minutes of collection because seminal plasma is toxic to sperm after this time.

3. Storage temperature:

Sperm can be used fresh, refrigerated gradually at 12-14°C (within 24 to 96 hours) or more often 4°C (0-5°C, up to 8 days) or frozen in nitrogen liquid. Diluted sperm can be stored in a refrigerator set at 7°C (Dusssauge,1963; Magistini, 1990).

Chapter V: Preparation of the mare

Once the intended mare has been proved appropriate for AI after rectal palpation, ultrasonography or bacterial culturing her preparation must be geared towards achieving maximum conception rates. The correct timing of insemination relative to ovulation is of paramount importance. Insemination may be carried out in naturally cycling mares that have been identified as being in estrus by observation, by teasing with a stallion or by the use of ultrasonography and rectal palpation.

1.Physical preparation of the mare

Once the timing of insemination has been determined, any necessary swabs have been taken, all paperwork has been completed or is ready for completion and the semen is available, the mare should be prepared for insemination largely as for natural covering and according to the minimum contamination techniques.

Ideally, she should be bridled and restrained in stocks. If stocks are not available she may be backed up into a stable doorway, though this gives less protection to the practitioner. The mare's perineal area should be thoroughly washed and rinsed to remove any antibacterial agents that may be spermicidal or act as an irritant of the genital area. The area should then be dried with a clean, dry disposable towel. During washing, particular attention should be paid to the removal of all fecal contamination. The mare's tail should be bandaged and covered with a sterile wrap, and tied or held out of the way to prevent contamination. All equipment should be non-toxic and sterile and ideally disposable.

Figure 09: preparation of the mare

Prior to insemination, a mare should be prepared as for natural service. This must include washing the perineal area

Insemination volume

The volume of inseminate used appears to have less of an effect on pregnancy rates than the number of spermatozoa .The volumes of inseminate used may vary from 0.5 to 100 ml, though commonly they range from 10 to 30 ml for fresh semen, 30–60 ml for chilled semen and 0.5–5 ml for frozen semen.

Methods of Insemination

Once the mare and semen have been prepared there are two main methods of insemination, which involve the guiding of the insemination pipette through the cervix into the uterus via an arm placed either in the vagina (per vagina) or in the rectum (per rectum) of the mare. Whichever system is used, it is very important that all the equipment is warm, clean, dry and sterile. It is also important, throughout the whole process of preparing the semen and during insemination, that all equipment is maintained at 37°C to avoid cold shock. Semen should also be protected from exposure to air and sunlight.

1. Insemination per vagina

The inseminator's arm should be covered in a sterile insemination glove. The insemination pipette is then placed in the center of the hand. A small amount of lubricant may be placed on the knuckle of the hand, avoiding contact with the insemination pipette. The amount of lubricant should be minimized, as evidence suggests that some kinds of lubricant may be detrimental to motility and hence viability. The inseminator then places the hand, plus pipette, into the vaginaand proceeds up towards the cranial part of the vagina near the cervix. The opening of the cervix is located using the index finger, and the insemination pipette is then guided in through the cervix and into the uterus. The pipette should ideally be pushed into the uterus far enough to allow at least 2 cm to protrude into the lumen. This may be ascertained if a small elastic band has been placed around the insemination pipette about 5 cm from the end. Estimating the size of the cervix to be 2–3 cm, then the pipette will be appropriately placed within the uterus when the elastic band can be felt against the cervix.

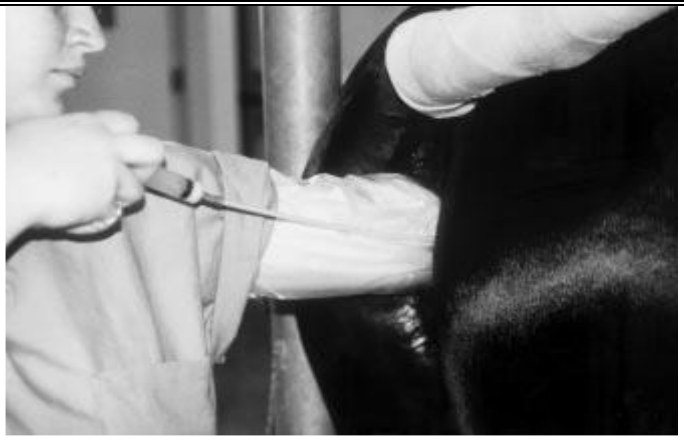

Figure10: Insemination per vagina

2. Insemination per rectum

An alternative but less popular method of insemination is to guide the pipette per rectum. (This is the preferred method in cattle AI.) The inseminator places the gloved hand and arm into the rectum of the mare, as for rectal palpation. Once the inseminator has located the cervix by feeling through the rectum wall, the insemination pipette is placed into the mare's vagina and guided up and through the cervix by means of the hand within the rectum.

Insemination per rectum reduces the risk of contamination of the reproductive tract as the arm is not introduced into the vagina and only a relatively small breach of the natural physical seals occurs, due to the small size of the insemination pipette. However, it is more difficult to locate and manipulate the cervix per rectum. It is largely for this reason that the preferred method of insemination in the mare is guiding the pipette per vagina. Some pipettes have a flexible tip, which allows direction into either of the uterine horns in the belief that deposition of the semen into the horn ipsilateral to the ovulating ovary will improve success rates.

Figure11: hand lubrication before insemination

Laparoscopic AI:

Laparoscopic AI, though in theory possible in the mare, is not practiced except for research, as the cervix and uterus are easily accessible via the vagina **(J. Baumber, California, 1998, personal communication**). Once the pipette is in place, the syringe, pre-loaded with semen, is attached to the end of the pipette and the plunger is slowly depressed, pushing the semen out into the uterus. It is important that all the inseminated sperm is deposited:

In the preparation of the pipette, it is advantageous to ensure that a small volume of air is included, which can be pushed out after the semen to ensure that the pipette is fully emptied. If inseminating per vagina it is suggested that manual closing of the cervix for a few seconds after insemination will help to reduce any loss of semen back through into the vagina (**S. Revell, Wales, 1999, personal communication**). Once inseminated, the mare should be removed from the stocks and returned to her normal environment. Mares that have been inseminated require no different or special care than mares that have been covered naturally. Due to the often high value of such mares and the semen inseminated, close monitoring postinsemination is often practiced. An inseminated mare might be examined at **24** h post insemination to ensure that she has indeed ovulated. If she has not, and enough semen remains, she is often re-inseminated. A Caslick operation may be carried out if the mare's perineal conformation indicates that one is necessary.

Equine artificial insemination **(J. Baumber, California, 1998, personal communication**). (**S. Revell, Wales, 1999, personal communication**).

Factors Affecting the Success of Artificial Insemination :

Several factors affect the success of AI, including the method of storage, and the volume and dose rate of semen inseminated. Two other considerations have a significant effect on pregnancy rates: the timing and frequency, along with the total number of inseminations per estrous cycle.[10]

[10]Equine artificial insemination book 1999.

Work time: during the reproduction season.

A. Materials and methods:

- **Animals:**

Our study was carried out during the mating season (from February to June 2023) at the veterinary institute of Ibn-Khaldun University of Tiaret on 7 mares that came to be bred either by natural service or by artificial insemination .So, we have chosen only mares that we have inseminated artificially. Stallions were kept at the veterinary institute and used for semen collection once mares will be inseminated.

- **Materials:**

Semen collection was done using a Colorado artificial vagina .Ejaculates were assessed by CASA (Sperm Class Analyzer® CASA System)and ejaculates handling and mares' insemination was done using water bath, sterile insemination probe, glove , and syringe. Genital tract of mares was examined by rectal palpation and ultrasonography.

- **Methods:**

B.Preparation of the mare

Once the intended mare has been proved appropriate for AI, her preparation must be geared towards achieving maximum conception rates. The correct timing of insemination relative to ovulation is of paramount importance. Insemination may be carried out in naturally cycling mares that have been identified as being in estrus by observation, by teasing with a stallion or by the use of ultrasonography and rectal palpation.

- **Physical preparation of the mare :**

The mare should be prepared foe insemination largely as for natural covering and according to the minimum contamination techniques.

It was bridled and restrained in stocks.

The mare's perineal area was thoroughly washed and rinsed to remove any antibacterial that may be spermicidal or act as an irritant of the genital area. The area was then dried with a clean, dry disposable towel. During washing, particular attention should e paid to the removal of all fecal contamination. The mare's tail was bandaged and covered, tied or held out of the way to prevent contamination.

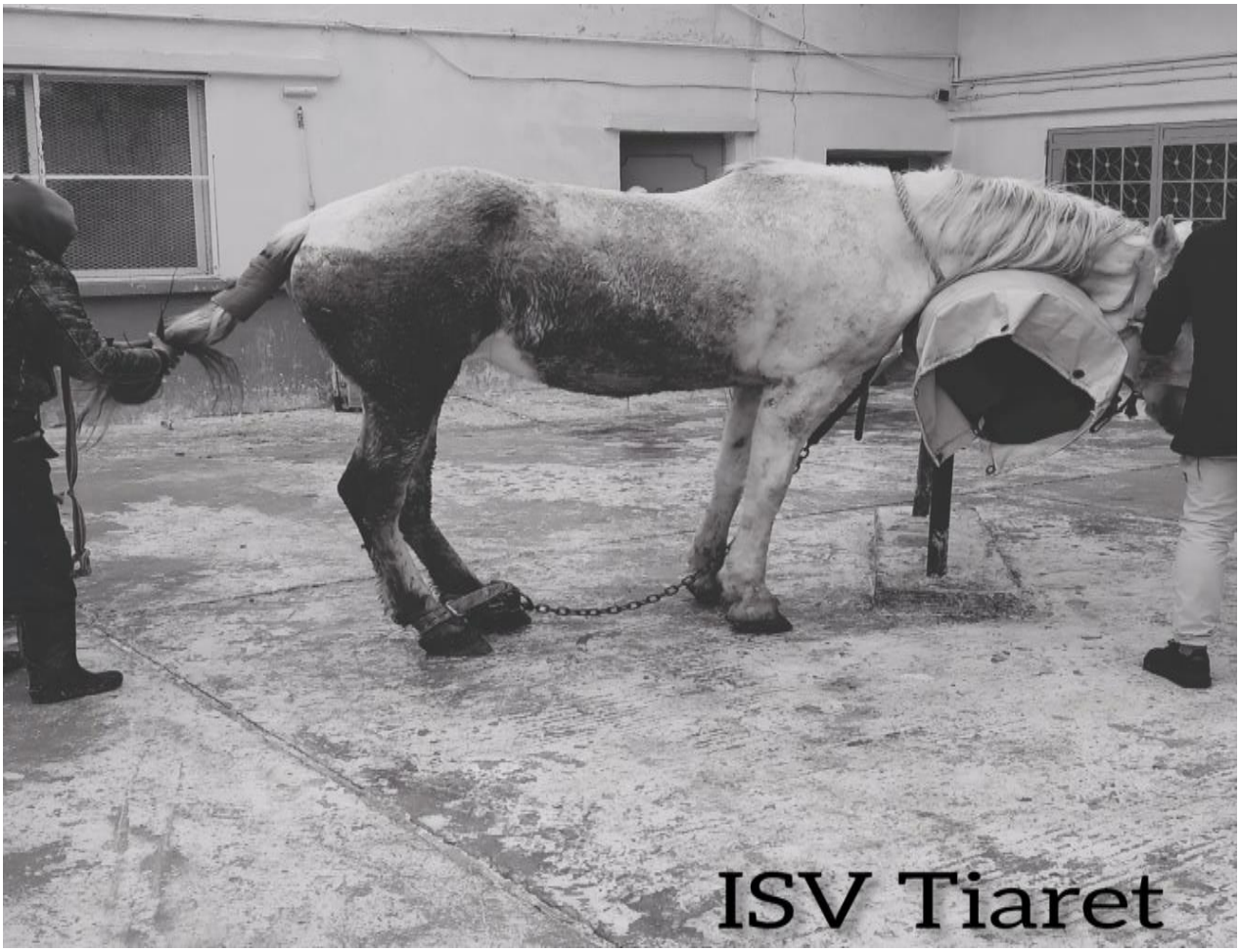

Figure 12: preparation of the mare

C. Semen collection

The artificial vagina was filled with warm water to obtain proper temperature (44-4/8°C) and pressure on stallion penis then, it was lubricated by applying a sufficient amount of Vaseline. In order for the stallion to ejaculate successfully, adequate sexual stimulation is required .This was achieved by presenting the stallion to a mare in heat.

After each use, the AV should be cleaned and dried with paper towel.

Figure13/14: cleaning and drying the AV

Figure 15: drying from the outside the AV

Semen collection was proceeded on this way:

-AV preparation.

-presentation of stallion to a mare in heat.

-Guiding the stallion's penis into the Av once stallion mounts the mare.

-The AV is slightly inclined to mimic the natural position of the mare's vagina.

-Dismount the stallion and slowly remove the AV, making sure all semen is collected and that stallion should be turned away from the mare to reduce the chance of injury.

Figure16/17: presentation of stallion to a mare in heat

D. Semen evaluation and preparation

Using a filter paper, the gel fraction was removed .The ejaculate was placed into an incubator at 37°C .The semen volume must be measured by a prewarmed, sterile graduated cylinder.

Figure 18: semen filtration

Djawed semen was evaluated by the responsible of CASA (see Appendix)

Semen dilution

40ml was sufficient enough to inseminate the mares (40ml for each mare).

Partly Skim milk extender Volume =2. (Semen volume after filtration)

Dissolving the materials in water, autoclaving, cooling, mixing the obtained mixture with heated, filtered and cooled skim milk or adding the antibiotics, glycerol, and sealing to obtain the horse semen dilution.

Before use, dilution is put in to preheating in 37 DEG C of water-baths, when use, mixes with horse seminal fluid.

Fresh semen: mares are inseminated within 30 minutes of collection because seminal plasma is toxic to sperm after this time.

Mares are inseminated within 30 minutes of harvest,

E. Method of Insemination

- **. Insemination per vagina**

The insemination per vagina is the method that we practiced in our thesis.

The inseminator's arm should be covered in a sterile insemination glove. The insemination pipette is then placed in the center of the hand. A small amount of lubricant may be placed on the knuckle of the hand, avoiding contact with the insemination pipette. Then, the inseminator places the hand, plus pipette, into the vagina and proceeds up towards the cranial part of the vagina near the cervix .The opening of the cervix is located using the index finger, and the insemination pipette is then guided through the cervix into the uterus. The pipette should ideally be pushed deeply into the uterus.

The syringe containing the semen is placed on the pipette.At the point, the plunger is pressed and the instrument is withdrawn. Mares were inseminated each 24 - 48h once the follicular diameter reach 35mm.

Figure 19: insemination per vagina

Results

The results obtained in our study (appendix 2) show that the mean diameter of pre-ovulatory follicle at the last insemination was **41.43**mm.

Conception rate was **85.71%** which**6** mares among 7 inseminated were diagnosed pregnant by ultrasonography **14**days after the date of insemination. On the other hand, index of insemination is very interesting. It value is **1.43.**

66.66% of the mares were diagnosed pregnant after the first ovulation.

16.66% of the mares were pregnant after the second ovulation.

16.66% of the mares were pregnant after the third ovulation.

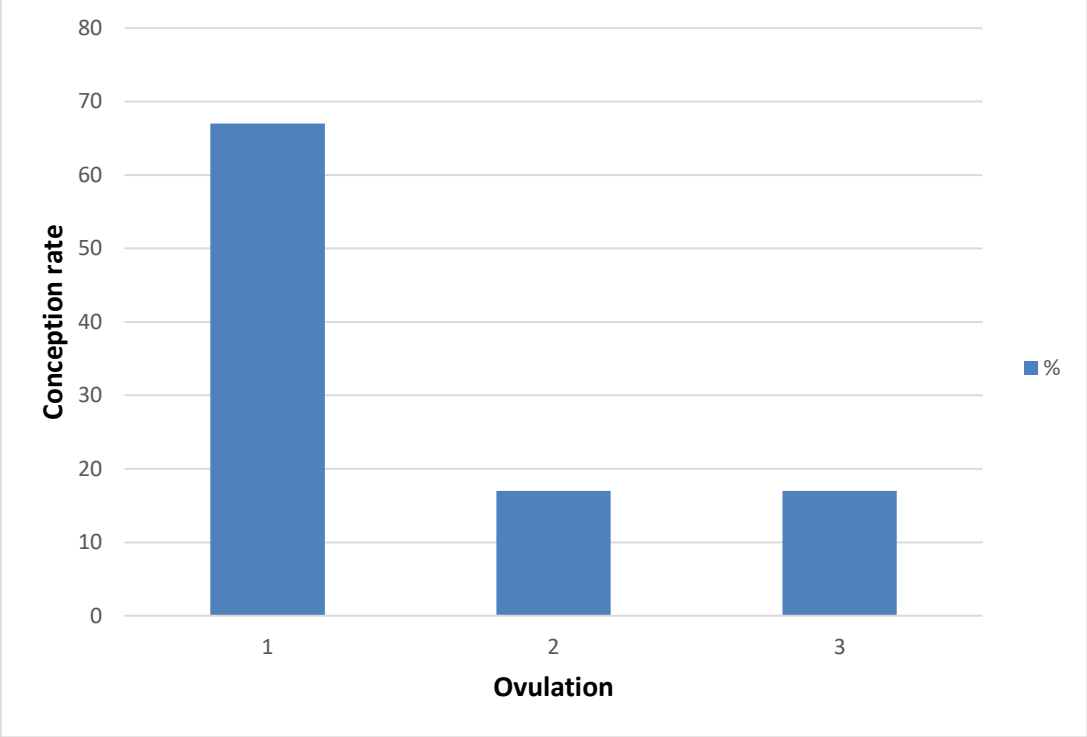

Figure 20: diagram represent the conception rate during ovulation

Mare	pre-ovulatory follicle diameter	AI date	Date of ovulation	results
01	F45	04/17/2023	04/18/2023 2nd cycle	ECHO+
02	F47	03/21/2023	03/22/2023 1st cycle	ECHO+
03	F38	03/18/2023	03/20/2023 1st cycle	ECHO+
04	F48	05/13/2023	05/14/2023 1st cycle	ECHO +
05	F33	05/12/2023	////// 1st cycle	ECHO-
06	F41	05/10/2023	05/11/2023 1st cycle	ECHO+
07	F38	04/28/2023	04/30/2023 3rd cycle	ECHO+

Table 02 : table represent ovulation, insemination day, and follicular diameter during estrus mare's cycle.

Discussion

As with natural service, the longevity of the spermatozoa within the female tract and the duration of the viability of the ovum determine the optimum timing of insemination. Significant variation exists in spermatozoan longevity (Watson and Nikolakopoulos, 1996). It has been reported to vary from 24 h to 7 days, whereas ovum viability is much shorter (in the region of 8 h) (Hunter, 1990).

Bearing this in mind, as with natural service, good success rates are normally achieved with insemination every 48 h while estrus lasts, or until the mare has ovulated (Watson and Nikolakopoulos, 1996).

Such mares are likely to ovulate within the next 48 h and so immediate insemination is recommended.

The impact of several factors on fertility commonly recognized as relevant to the success of artificial insemination with fresh or diluted semen:

Frequency of insemination per cycle, semen dose, AI site and techniques and the optimal timing of AI compared to ovulation which is not less important than the previous factors.

40ml per insemination was sufficient enough to achieve positive results by the intrauterine technique.

The number of AIs does not seem to have a profound effect on how a pregnancy turns out in the end contrasted with the ideal moment of AI relative to ovulation which applying insemination 48^{h} or 24^{h} before ovulation contribute to obtaining satisfactory results.

Conclusion

The practice of artificial insemination in mares has revolutionized the field of equine reproduction, offering numerous benefits and opportunities for horse breeders and enthusiasts. Through the use of advanced reproductive techniques, such as fresh or chilled semen, frozen semen, and assisted reproductive technologies, breeders have gained greater control over the genetic diversity and quality of offspring. Artificial insemination allows for the utilization of superior stallions regardless of geographical constraints, expanding the options available for mare owners and promoting the preservation of valuable equine bloodlines.

Moreover, artificial insemination has proven to be a time-saving and cost-effective method compared to natural breeding, enabling breeders to maximize their resources and achieve higher reproductive success rates. It offers increased convenience and flexibility in managing the breeding process, allowing mare owners to optimize their breeding programs and enhance the overall efficiency of their operations.

However, it is essential to recognize that artificial insemination should be approached with care and attention to ensure the welfare of the mares involved. Proper handling and expertise are crucial to minimize any potential risks or complications associated with the procedure. Maintaining a thorough understanding of reproductive physiology, proper timing, and the use of appropriate techniques are vital for success with interest rate increased by 85.71%.

As technology and research continue to advance, the field of artificial insemination for mares is expected to evolve further, offering even more opportunities for breeders. Future developments may include improvements in semen preservation techniques and advancements in assisted reproductive technologies, ultimately leading to even higher success rates and improved genetic progress.

All in all, artificial insemination in mares has transformed the breeding landscape, offering numerous advantages in terms of genetic selection, convenience, and efficiency. By embracing and furthering our understanding of this reproductive technique, breeders can contribute to the improvement and preservation of equine breeds while ensuring the welfare and reproductive health of the mares involved.

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Annexes

1. **Mare n°1**

- **Age : 06yearold**
- **Breed : ARABIAN BERBER**
- **Color : Dapple-gray**
- **GAVE BIRTH IN 2022**

DATE	LO	RO	UTERUS	PATHOLOGY	TREATMENT	OBSERVATION
03 /21/2023	SF	F43/48	IN HEAT	ENDOMETRITIS	OXYTOCYN INJECTION + UTERINE LAVAGE	To inseminate
03/22/2023		ovulation				AI with Fresh semen (40ML) OPPONTMENT AFTER 14 DAY
04/06/2023	SF	Corpus luteum			PGF2α (1ml)	Appointment :04/10/2023
04/10/2023			IN HEAT			AI WITH DJAWED
04/11/2023	SF	F28	IN HEAT			Appointment :04/13/2023
04/13/2023	SF	F35	IN HEAT			IA 04/15/2023
04/15/2023			IN HEAT			AI WITH DJAWED
04/16/2023	SF	F45	IN HEAT			Appointment:04/17/2023
04/17/2023			IN HEAT			AI WITH RESH SEMEN (20ml) DJAWED
04/18/2023		OVULATION				Appointment:04/30/2023
04/30/2023		ECHO+				13 DAYS OF PREGNANCY

Annexes

2.Mare n°02:

- **Age : 8 YO**
- **BREED :ARABIAN BERBER**
- **COLOR : Bay**

Date	LO	RO	UTERUS	PATHOLOGY	TREATMENT	OBSERVATION
03/09/2023		SF				Appointment:03/14/2023
03/14/2023	F38/37	SF	ESTRUS	LIQUID IN UTERUS		Appointment:15/03/2023
03/15/2023			IN HEAT			AI WITH DILUTED SEMEN (80 ML) Djawed
03/18/2023			IN HEAT			AI
03/20/2023	OVULATON					Appointment:04/04/2023
04/04/2023			ECHO+			Appointment AFTER 15 DAYS

Annexes

3.Mare n° 03

- **Age : 03 YO**
- **Breed : ARABIAN BERBER**
- **Color : Grey**

Date	LO	RO	UTERUS	PATHOLOGY	TREATMENT	OBSERVATION
04/26/2023	SF	SF	IN HEAT			Appointment AFTER 7 DAYS
05/02/2023	SF	SF	PROESTRUS			Appointment:05/07/2023
05/07/2023	F25	F25				Appointment:05/09/2023
05/09/2023	SF	F32/32				Appointment AFTER 48 H
05/11/2023	SF	F38/48	IN HEAT			AI 05/13/2023
05/13/2023			IN HEAT			AI WITH FRESH SEMEN (20ML) DJAWED
05/14/2023	SF	OVULATION	DI ESTRUS			Appointment15DAYSAFTER
05/28/2023			ECHO+			

Annexes

4.MARE n° 04

- **Age : 7YO**
- **Breed : ARABIAN BERBER**
- **Color : Grey**

DATE	LO	RO	UTERUS	PATHOLOGY	TREATMENT	OBSERVATION
03/21/203	SF	F22/21	IN HEAT			Appointment:03/26/2023
03/26/2023	SF	F27/35	IN HEAT			Appointment:03/30/2023
03/30/2023	F30	SF	IN HEAT			Appointment:04/02/2023
04/02/20223	F33/25	SF	IN HEAT			Appointment:04/04/2023
04/04/2023	OVULATION	SF				PGF2 INJECTION 04/11/2023
04/11/2023	F20	SF			PGF2α(1ml) in IM	Appointment AFTER 48^{H}
04/30/2023	CJ	PF20			PGF2(IM) 1ML	Appointment:05/04/2023
05/04/2023	SF	SF	PROESTRUS			Appointment :05/07/2023
05/07/2023	SF	F34	IN HEAT			Appointment:05/09/2023
05/09/2023	SF	F38	IN HEAT			AI WITH DILUTED SEMEN (40ML)DJAWED
05/10/2023	SF	F39/33	IN HEAT			To inseminate 05/11/2023
05/11/2023						AI (40 ML) diluted semen DJAWED
05/12/2023			IN HEAT			AI WITH DILUTED SEMEN (40ML)DJAWED
05/27/2023			ECHO-			

Annexes

5.Mare n° 5

- **Age : 6 YO**
- **Breed : ARABIAN BERBER**

DATE	LO	RO	UTERUS	PATHOLOGY	TREATMENT	OBSERVATION
04/26/2023	SF	CJ			PGF2 (IM) 1ML	Appointment:04/30/2023
04/30/2023	SF	SF	PROESTRUS			Appointment:05/04/2023
05/04/2023	F24/34	SF	IN HEAT			Appointment:05/07/2023
05/07/2023	F30/32	SF	IN HEAT			
05/08/2023	F42/28	SF	IN HEAT			TO INSEMINATE AFTER 24^{H}
05/09/2023			IN HEAT			AI WITH DELUTED SEMEN (40ML) DJAWED
05/10/2023	F41/40	SF	IN HEAT			AI WITH FRESH SEMEN
05/11/2023	OVULATION	SF				OPP 05/25/2023
05/25/2023			ECHO+			

Annexes

6.Mare n° 6

- **Age: 8 yo**
- **Breed :ARABIAN BERBER**
- **Color : Grey**
-

Date	LO	RO	UTERUS	PATHOLOGY	TREATMENT	Observation
03/05/2023	F30/35	SF	IN HEAT			TO INSEMINATE WITH DJAWED (40 ML)
03/09/2023	F33	SF	IN HEAT			Appointment:03/12/2023
03/12/2023			IN HEAT			AI WITH FRESH SEMEN DJAWED (40ML)
03/13/2023			IN HEAT			AI WITH FRESH SEMEN
03/14/2023	OVULATION					Appointment:03/28/2023
03/28/2023	SF	SF	IN HEAT			Appointment:02/04/2023
04/02/2023	SF	F40	IN HEAT			TO INSEMINATE 04/03/2023
04/03/2023			IN HEAT			AI (DJAWED SEMEN)40 ML
04/06/2023		OVULATION				Appointment:04/19/2023
04/19/2023						Appointment:04/24/2023
04/24/2023			IN HEAT			AI
04/26/2023			IN HEAT			AI
04/27/2023	F38	SF	IN HEAT			TO INSEMINATE 04/28/2023
04/28/2023			IN HEAT			AI
04/30/2023	OVULATION	SF				Appointment: AFTER 14 DAYS
05/11/2023	CJ	SF	ECHO+			Appointment: AFTER 20 DAYS

Annexes

7. Mare n° 07

- **Age: 8 yo**
- **Breed :ARABIAN BERBER**
- **Color : Grey**

Date	LO	RO	UTERUS	PATHOLOGY	TREATMENT	Observation
03/05/2023	F30/35	PF	IN HEAT			TO INSEMINATE WITH DJAWED (40 ML)
03/09/2023	F33	PF	IN HEAT			Appointment:03/12/2023
03/12/2023			IN HEAT			AI WITH FRESH SEMEN DJAWED (40ML)
03/13/2023			IN HEAT			AI WITH FRESH SEMEN
03/14/2023	OVULATION					Appointment:03/28/2023
03/28/2023	PF	PF	IN HEAT			Appointment:02/04/2023
04/02/2023	PF	F40	IN HEAT			TO INSEMINATE 04/03/2023
04/03/2023			IN HEAT			AI (DJAWED SEMEN)40 ML
04/06/2023		OVULATION				Appointment:04/19/2023
04/19/2023						Appointment:04/24/2023
04/24/2023			IN HEAT			AI
04/26/2023			IN HEAT			AI
04/27/2023	F38	PF	IN HEAT			TO INSEMINATE 04/28/2023
04/28/2023			IN HEAT			AI
04/30/2023	OVULATION	PF				Appointment: AFTER 14 DAYS
05/11/2023	CJ	PF	ECHO+			Appointment: AFTER 20 DAYS